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Article Fast HILIC Method for Separation and Quantification of Non-Volatile Aromatic Compounds and Monosaccharides from Willow (Salix sp.) Bark Extract

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Abstract: Willow bark water extracts contain a mixture of chemically heterogeneous compounds. Fast screening techniques of the extracts are often needed to obtain information on the profile of bioactive and/or other valuable components in the extract. This is, however, a challenging task due to the different chemical structures of the components. Willow bark extract from the hybrid Karin contains several bioactive compounds such as aromatic picein, triandrin, and (+)-catechin. Willow bark extract also contains significant amounts of the monosaccharides fructose and glucose. Here, we demonstrate the applicability of hydrophilic interaction liquid chromatography, coupled with evaporative light scattering and ultraviolet detectors, for the simultaneous separation and quantification of major aromatic compounds and monosaccharides from the willow bark extract. The ternary eluent mixture consisting of acetonitrile, water, and methanol enabled the baseline separation of the main components in the extract in a short analysis time, which makes this method ideal for fast screening of the plant extracts and investigating the purity of fractionated bioactive compounds.

Keywords: separation; hilic; evaporative light scattering detector; aromatic compounds; monosaccharide; willow bark extract

1. Introduction

Bioactive secondary metabolites derived from lignocellulosic biomass can replace petroleum-based materials in many fields, including pharmaceutical and biochemical industries. One highly productive source for lignocellulosic biomass is cultivated willow (Salix sp.). Debarked wood can be used in the production of bioethanol and sugars. Willow bark contains several bioactive compounds that have been used for clinical purposes (e.g., as pain relief) since ancient times [1]. These bioactivities are most commonly related to the secondary metabolite salicin, which is a phenolic glycoside containing salicyl alcohol and β -D-glucopyranose moiety. Willow bark also contains several other aromatic bioactive compounds such as picein, catechin, and triandrin [2,3] (Scheme 1), as well as a large amount of other extractives. The aromatic component picein was demonstrated to play a role as a stress regulator against the outbreak of spruce budworm [4] and to inhibit the growth of neuroblastoma cells [5]. Triandrin, a phenolic glycoside, was reported to play a role as a stress-response modifier in regulating the adaptogenic activity of a plant [6]. A flavonoid catechin may have antibacterial and anticarcinogenic functions [7]. Due to the abundance of the above mentioned compounds, willow bark is a promising feedstock, both for biomedical purposes and for the production of aromatic compounds for various applications.



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Scheme 1. Structures of major aromatic components in *Salix* Karin bark water extract: (1) picein, (2) (+)-catechin, and (3) triandrin.

Plant water extracts contain a mixture of chemically heterogeneous compounds. Thus, characterizing these compounds from a mixture is a challenging task. The water extract of willow bark from the hybrid Karin is reported to be rich in aromatic compounds and monosaccharides. These include picein, (+)-catechin, triandrin, glucose, and fructose. Gas chromatographic methods (gas chromatography coupled to mass spectrometry (GC-MS) and gas chromatography coupled to flame ionization detector (GC-FID)) were used for separation, identification, and quantification of these compounds after trimethylsilylation [2]. In addition to gas chromatographic characterization methods, HPLC has also been used for the characterization of phenolic compounds from willow bark water extract [3,8]. In these studies, reversed-phase columns have been used for the separation of phenolic compounds from willow bark extract, together with MS detection for compound identification. Low-molar-mass carbohydrates can also be separated and quantified with GC-FID, or alternatively using ion chromatography [9]. High-performance anion-exchange chromatography coupled with pulse-amperometric detection (HPAEC-PAD), a variant of ion chromatography, is nowadays the gold standard method for characterization of monoand oligosaccharides due to the high sensitivity of PAD detection for carbohydrates [10,11]. Ion exclusion chromatography and hydrophilic interaction liquid chromatography (HILIC) separations with refractive index (RI) detection and evaporative light scattering detection (ELSD) have also been used for the characterization of mono- and oligosaccharides [12,13]. The HILIC technique seems promising for simultaneous separation of both polar carbohydrates and semi-polar aromatic compounds. The exact separation mechanism of HILIC has not been established, but it combines the characteristics of normal-phase, reversedphase, and ion chromatography. The stationary phase in HILIC columns are polar and it is assumed that water, which is used in the eluent (commonly \geq 3% in eluent), forms a thin layer on the surface of the stationary phase. Retention is facilitated by the distribution of analytes between the water layer and organic solvent in eluent (commonly \geq 80% in eluent). Ionic interactions may also contribute to the retention [14].

In this study, the HILIC method was developed for fast and simultaneous separation of both phenolic compounds, as well as monosaccharides, from willow bark extract. In the HILIC method, filtration of the sample is the only sample preparation step, which makes this method more convenient than GC, in which derivatization is needed prior to analysis. The HILIC method can be used for the screening of plant extracts containing both phenolic compounds and monosaccharides, as well as investigating the purity of the isolated bioactive compounds.

2. Materials and Methods

2.1. Materials

Triandrin was synthesized by Dr. Yuki Tobimatsu [15] in the laboratory of Prof. John Ralph at the University of Wisconsin-Madison. Picein, (+)-catechin, glucose, and fructose were purchased from Sigma Aldrich (St. Louis, MO, USA). The standard stock solutions were prepared in Milli-Q water with the concentration of 1 mg/mL. The calibration curve used for quantification in the HILIC method ranged from 0.05 to 0.5 mg/mL. HPLC-grade acetonitrile, methanol, 2-propanol, and tetrahydrofuran were from Riedel-de Haën (Honeywell, Seelze, Germany). Ammonia solution and glacial acetic acid were from Merck (Darmstadt, Germany). Willow bark extract from the hybrid Karin was prepared by extracting willow bark

using water in a microwave reactor, as described earlier [2]. Willow bark extract solution was prepared by dissolving 100 mg of freeze-dried extract in 10 mL of water.

2.2. HILIC-UV/ELSD

The HPLC instrument consisted of an Agilent 1260 system including a binary pump, autosampler, column oven, diode array detector (DAD), and evaporative light scattering detector (Agilent 1290 ELSD). Phenomenex Luna[®] Omega Sugar 3 μ m (250 × 4.6 mm) and Waters XBridge®Amide (150 × 2.1) columns were used for separation. The ELSD nebulizer temperature was 60 °C and evaporation temperature was 80 °C. A wavelength of 260 nm was monitored with the DAD detector. The columns were thermoregulated to 40 °C and the injection volume was 5 μ L.

3. Results and Discussion

3.1. Optimization of Eluent Composition

Picein, triandrin, fructose, and glucose standard compounds were used for HILIC optimization with the Phenomenex Luna® Omega Sugar column. This column has proved to be useful in carbohydrate separations [16,17] and was thus selected for the eluent composition optimization studies. The use of an ELSD detector for non-absorbing carbohydrates allows for the use of gradient elution, but our initial aim was to develop an isocratic method that could also be used with a refractive index detector wherever the ELSD detector is not available. Already, the preliminary trials showed that the separation of picein and triandrin from each other is the most challenging task. The method optimization for isocratic separation was started by testing the eluent composition of 80/20 (v/v; all the eluents prepared in this work were v/v) ACN/water, which is recommended by the column manufacturer. Increasing the ACN content to 83% resulted in the near baseline separation between picein and triandrin (Figure 1). This preliminary method (ACN/water 83/17) was recently used for the screening of separated fractions from willow bark extract [18]. Further increase in ACN content in the eluent increased the resolution between picein and triadrin, but the retention time of the monosaccharides increased also, and the total analysis time was prolonged. Additionally, the glucose peak area was diminished drastically when the ACN content was above 83%.



Figure 1. ELSD signals for picein ($t_r = 3.45$ and 3.49 min), triandrin ($t_r = 3.66$ and 3.77 min), fructose ($t_r = 7.11$ and 8.09 min), and glucose ($t_r = 8.41$ and 9.80 min) using the eluents: A = water, B = ACN (A/B = 17/83) and A = 30% MeOH in water, B = ACN (A/B = 20/80). The flow-rate was 1.3 mL/min and the injection volume 5 μ L. The use of MeOH in aqueous eluent clearly increased the resolution between the picein and triandrin, while the retention time of fructose and glucose decreased. The column used was Phenomenex Luna Omega Sugar (please see Materials and Methods section for further details).

It has been reported that HILIC separation can be enhanced by adding organic modifiers to the eluent. In some cases, it is possible to replace water with organic protic solvent [19,20]. To improve the resolution between picein and triandrin, we decided to test different organic modifiers, namely 2-propanol, tetrahydrofuran, and methanol, in the water phase (pump A being a mixture of organic modifier and water, and pump B always consisting of 100% acetonitrile). In addition, the effect of pH (pH 4.5) was tested, but mild lowering of the pH had no effect to the retention of the analytes. The preliminary trials with organic modifiers showed that 2-propanol and tetrahydrofuran did not increase the resolution between picein and triandrin (results not shown). Methanol, however, seemed promising, and thus a mixture of picein and triandrin as well as mixture of fructose and glucose were separated using different methanol contents in the eluent A (20%, 30%, 40%, 50%, 60%, and 80% of methanol in water; eluent B was ACN and A/B = 20/80). The corresponding ACN/water/MeOH ratios are 80/16/4, 80/14/6, 80/12/8, 80/10/10, 80/8/12, and 80/4/16. As shown in Figures 1 and 2, the resolution between picein and triandrin increased drastically when methanol content was increased. A resolution close to 1.5 was achieved when 20% methanol was added in the water phase. In addition, the other two compounds, fructose and glucose, eluted earlier when methanol was added to eluent A. The evolution of the retention time of the latest eluting component, glucose, is also presented in Figure 2. The retention time for glucose was below 9 min when a low concentration of methanol (20% and 30%) was used, but increased to over 15 min when 80% methanol was added in eluent A. The peak area of glucose decreased as a function of increasing methanol content, and the peak became almost undetectable when the methanol content was 80%. Solubility issues might explain the low peak area of glucose when high organic content in the eluent was used. Glucose is known to be sparingly soluble in alcohols such as methanol and ethanol [21]. According to the graph (Figure 2) in which the resolution between picein and triandrin, retention time of glucose, and peak area of glucose are presented as a function of methanol content in eluent A, 30% of methanol in the aqueous phase was considered the most optimal for the separation of phenolic glycosides and monosaccharides. An even lower methanol content than 30% would have separated the standard compounds well, but because the method is aimed at the separation of willow bark extracts with tens of different compounds, conditions that enabled baseline separation between the two phenolic glycosides were chosen for the further studies.



Figure 2. Optimization of eluent conditions (detection ELSD). We chose 30% of MeOH in eluent A (eluent B was ACN, A/B = 20/80) for further studies.

As discussed in the literature [14], the retention mechanism of HILIC is not unambiguously understood; therefore, unambiguous explanation of the effect of methanol cannot be given here. In HILIC separations, water is the strongest eluent while acetonitrile is the most common weak eluent. Methanol is slightly weaker than water [22]. In HILIC, it is assumed that water forms a thin layer on the silica surface of the column stationary phase. It can also be speculated that methanol would be part of the aqueous layer that forms on top of the silica surface. The presence of methanol in the water layer might explain the behavior observed here. The aromatic compounds can have more interactions with a column stationary phase when part of the water is replaced with an organic modifier. Thus, the addition of methanol increases the resolution between the two aromatic compounds. Monosaccharides likely have interactions with water, and when methanol is embedded in the water layer, monosaccharides have less interaction with the water layer and thus the retention times of fructose and glucose decrease [22]. The ternary eluent consisting of ACN, water, and methanol seems promising mobile phase in HILIC, and could also be tested for other applications. To date, the eluents containing three components have not been widely used in HILIC separations.

As a comparison with the Phenomenex Luna[®] Omega Sugar column, another HILIC column, Waters XBridge[®] Amide, was tested with the optimized method to see if similar retention behavior was observed for picein, triandrin, fructose, and glucose standard compounds. Only partial separation between picein and triandrin was obtained (results not shown). The difference between the retention behaviors likely arose from differences in the stationary phases. Both columns contain amide groups, but exact knowledge of the stationary phases is not available. Apparently, the Luna[®] Omega Sugar column stationary phase also contains polyols.

All of the samples were initially dissolved in water and diluted with ACN (1:2). The addition of the weaker solvent ACN was necessary in order to avoid peak splitting. Solvent mismatch is typical, especially in HILIC where the amount of organic solvent in eluent is high [23]. Pure organic solvent is rarely the best option, however, if the analytes are highly polar. In our case, peak splitting was observed for phenolic glycosides when the injection solvent was pure water. After adding 50% of ACN, splitting was not observed.

3.2. Quantification of Aromatic Compounds and Monosaccharides by HILIC-UV/ELSD

As pointed out in the Introduction, our aim was to develop a simple and fast screening method for simultaneous quantification of the main aromatic compounds and monosaccharides from willow bark extract. Since monosaccharides do not contain chromophores, ELSD was added to the detector train, in addition to UV (Figure 3). ELSD, a quasi-universal detector, has several advantages over the more commonly used refractive index detector. To name a few, ELSD has fairly similar response factors for structurally similar compounds, it is compatible with most of the solvents and modifiers, it can be used with solvent gradients, and it is not sensitive to pulsation of the HPLC pump [24]. One disadvantage of ELSD is that, in the case of most applications, the detector response is non-linear. The peak area (A) correlates with the mass of the analyte (m) as follows:

$$A = a \times m^b \tag{1}$$

where *a* and *b* are coefficients depending the ELSD instrumentation. Similar behavior was also observed here; exponential behavior was evident both for aromatic compounds and for monosaccharides (examples of ELSD calibration curves are presented in Figure S1). Aromatic compounds can also be detected with UV and, as shown in Table 1, the limit of quantification (LOQ) decreased a hundredfold if UV was used instead of ELSD. The sensitivity of ELSD was, however, better compared with the RI detector for which the LOQ was in the order of few hundred mg/L (150 for fructose and 300 mg/L for glucose with a 10 μ L injection) (personal communication with Jari Heinonen).



Figure 3. Chromatogram (UV at 260 nm and ELSD signals) of willow bark extract. Eluent used was A = 30% MeOH in water, B = ACN (A/B = 20/80).

Table 1. Limit of detection (LOD) and limit of quantification (LOQ) presented as mg/l for main compounds in willow bark extract.

	ELSD		UV		
	LOD	LOQ	LOD	LOQ	
Picein	5	10	0.01	0.05	
Triandrin	5	10	0.01	0.05	
Fructose	10	50	n.a.	n.a.	
Glucose	25	125	n.a.	n.a.	

LOD = 2 × detector noise, LOQ = 5 × detector noise for UV (260 nm), LOQ for ELSD determined experimentally (please note the non-linear response of ELSD); 5 μ L injections were used in systems containing UV and ELDS; Phenomenex Luna[®] Omega Sugar columns were used in all analyses; n.a. = not applicable.

As shown in Figure 3, picein, triandrin, fructose, and glucose are the main components in willow bark extract. Other components, such as (+)-catechin, are also present. The combination of both a UV detector and ELSD is powerful, especially in cases in which the amount of aromatic compounds is very low (the sensitivity of UV significantly higher than ELSD). In this study, the standard 10 mm flow cell was used, but sensitivity can be increased if needed by changing the 10 mm flow cell to a longer 60 mm flow cell.

The quantified amounts for picein, triandrin, fructose, and glucose for willow bark extract (calculated using ELSD signals) are presented in Table 2. The most prevalent component in the extract was glucose, followed by fructose and picein. The results for the UV-absorbing components picein and triandrin, obtained with ELSD and a UV detector, agreed well with each other, being within 5%. The results obtained with the HILIC method were validated using GC-FID for aromatic compounds and HPAEC-PAD for monosaccharides (Table S1). The HILIC results are in accordance with the results obtained with these established methods. Day-to-day variation in the ELSD response was observed, and thus the addition of a suitable internal standard would be feasible if the method were to be used routinely for quantification.

Table 2. The amounts of main components (mean \pm SD) in willow bark extract presented as mg/g.

	Picein	Triandrin	(+)-catechin	Fructose	Glucose
Willow bark extract	46 ± 2	36 ± 1	32 ± 2	56 ± 9	114 ± 15

ELSD traces were used for quantification except for (+)-catechin (UV detector at 260 nm was used for (+)-catechin due to better resolution between (+)-catechin and oxidized catechin obtained with UV than ELSD).

4. Conclusions

Characterization of complex plant extracts is commonly time-consuming due to the need for various analytical approaches for structurally different compounds. Here, the HILIC method was developed for willow bark extract, which is known to be rich in aromatic compounds, especially picein and triandrin, as well as the monosaccharides fructose and glucose. These compounds are commonly quantified using gas chromatography after a laborious derivatization procedure. HILIC coupled with ELSD and UV detectors proved to be a fast separation and quantification method for both aromatic compounds and monosaccharides. Ternary eluent consisting of ACN, water, and methanol (80/14/6) resulted in baseline separation between picein and triandrin, and short retention times for fructose and glucose. This minimized the time needed for the analysis. ELSD allowed for the quantification of aromatic compounds and monosaccharides at a level of 10–125 ppm. HILIC can be considered a promising separation technique for plant extract screening containing both polar carbohydrates and semi-polar aromatic compounds.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10 .3390/app11093808/s1, Figure S1: Calibration curves for glucose and picein obtained by HILIC-ELSD. Table S1: The amounts of picein, triandrin, (+)-catechin, fructose, and glucose in willow bark extract presented as mg/g.

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References

- 1. Hedner, T.; Everts, B. The early clinical history of salicylates in rheumatology and pain. *Clin. Rheumatol.* **1998**, *17*, 17–25. [CrossRef]
- Dou, J.; Xu, W.; Koivisto, J.J.; Mobley, J.K.; Padmakshan, V.; Kögler, M.; Xu, C.; Willför, S.; Ralph, J.; Vuorinen, V. Characteristics of hot water extracts from the bark of cultivated willow (*Salix* sp.). ACS Sustain. Chem. Eng. 2018, 6, 5566–5573. [CrossRef]
- 3. Noleto-Dias, C.; Wu, W.; Bellisai, A.; Macalpine, W.; Beale, H.M.; Ward, L.J. Phenylalkanoid glycosides (non-salicinoids) from wood chips of *Salix triandra* × *dasyclados* hybrid willow. *Molecules* **2019**, *24*, 1152. [CrossRef] [PubMed]
- Mageroy, M.H.; Parent, G.; Germanos, G.; Giguère, I.; Delvas, N.; Maaroufi, H.; Bauce, É.; Bohlmann, J.; Mackay, J.J. Expression of the β-glucosidase gene Pgβglu-1 underpins natural resistance of white spruce against spruce budworm. *Plant J.* 2015, *81*, 68–80. [CrossRef]
- Kesari, K.K.; Dhasmana, A.; Shandilya, S.; Prabhakar, N.; Shaukat, A.; Dou, J.; Rosenholm, J.M.; Vuorinen, T.; Ruokolainen, J. Plant-derived natural biomolecule picein attenuates menadione induced oxidative stress on neuroblastoma cell mitochondria. *Antioxidants* 2020, *9*, 552. [CrossRef]
- 6. Kurkin, V.A. Phenylpropanoids as the biologically active compounds of the medicinal plants and phytopharmaceuticals. *Adv. Biol. Chem.* **2013**, *3*, 26–28. [CrossRef]
- Gadkari, P.V.; Balaraman, M. Catechins: Sources, extraction and encapsulation: A review. Food Bioprod. Process. 2015, 93, 122–138. [CrossRef]

- Kammerer, B.; Kahlich, R.; Biegert, C.; Gleiter, C.H.; Heide, L. HPLC-MS/MS analysis of willow bark extracts contained in pharmaceutical preparations. *Phytochem. Anal.* 2005, *16*, 470–478. [CrossRef] [PubMed]
- 9. Brummer, Y.; Cui, S.W. Understanding carbohydrate analysis. In *Food Carbohydrates—Chemistry, Physical Properties, and Applications;* Cui, S.W., Ed.; CRC Press, Taylor & Francis Group: Boca Raton, FL, USA, 2005; pp. 67–104.
- 10. Lee, Y.C. Carbohydrate analyses with high-performance anion-exchange chromatography. J. Chromatogr. A **1996**, 720, 137–149. [CrossRef]
- 11. Corradini, C.; Cavazza, A.; Bignardi, C. High-performance anion-exchange chromatography coupled with pulsed electrochemical detection as a powerful tool to evaluate carbohydrates of food interest: Principles and applications. *Int. J. Carbohydr. Chem.* **2012**, 2012, 487564. [CrossRef]
- 12. Fu, Q.; Liang, T.; Li, Z.; Xu, X.; Ke, Y.; Jin, Y.; Liang, X. Separation of carbohydrates using hydrophilic interaction liquid chromatography. *Carbohydr Res.* 2013, *379*, 13–17. [CrossRef] [PubMed]
- Juvonen, M.; Kotiranta, M.; Jokela, J.; Tuomainen, P.; Tenkanen, M. Identification and structural analysis of cereal arabinoxylanderived oligosaccharides by negative ionization HILIC-MS/MS. *Food Chem.* 2019, 275, 176–185. [CrossRef] [PubMed]
- 14. Buszewski, B.; Noga, S. Hydrophilic interaction liquid chromatography (HILIC)—A powerful separation technique. *Anal. Bioanal. Chem.* **2012**, *402*, 231–247. [CrossRef] [PubMed]
- Takano, T.; Tobimatsu, Y.; Hosoya, T.; Hattori, T.; Ohnishi, J.; Takano, M.; Kamitakahara, H.; Nakatsubo, F. Studies on the dehydrogenative polymerizations of monolignol β-glycosides. Part 1. Syntheses of monolignol β-glycosides, (*E*)-isoconiferin, (*E*)-isosyringin, and (*E*)-triandrin. *J. Wood Chem. Technol.* 2006, 26, 215–229. [CrossRef]
- 16. Pauliuc, D.; Dranca, F.; Oroian, M. Antioxidant activity, total phenolic content, individual phenolics and physicochemical parameters puitability for Romanian honey authentication. *Foods* **2020**, *9*, 306. [CrossRef] [PubMed]
- Multari, S.; Carafa, I.; Barp, L.; Caruso, M.; Licciardello, C.; Larcher, R.; Tuohy, K.; Martens, S. Effects of *Lactobacillus* spp. on the phytochemical composition of juices from two varieties of *Citrus sinensis* L. Osbeck: 'Tarocco' and 'Washington navel'. *LWT Food Sci. Technol.* 2020, *125*, 109205. [CrossRef]
- 18. Dou, J.; Heinonen, J.; Vuorinen, T.; Xu, C.; Sainio, T. Chromatographic recovery and purification of natural phytochemicals from underappreciated willow bark water extracts. *Sep. Purif. Technol.* **2021**, *261*, 118247. [CrossRef]
- 19. Hao, Z.; Xiao, B.; Weng, N. Impact of column temperature and mobile phase components on selectivity of hydrophilic interaction chromatography (HILIC). J. Sep. Sci. 2008, 31, 1449–1464. [CrossRef]
- 20. Soukup, J.; Jandera, P. Comparison of nonaqueous hydrophilic interaction chromatography with aqueous normal-phase chromatography on hydrosilated silica-based stationary phases. *J. Sep. Sci.* **2013**, *36*, 2753–2759. [CrossRef] [PubMed]
- 21. Peres, A.M.; Macedo, E.A. A modified UNIFAC model for the calculation of thermodynamic properties of aqueous and nonaqueous solutions containing sugars. *Fluid Phase Equilib.* **1997**, *139*, 47–74. [CrossRef]
- Greco, G.; Letzel, T. Main interactions and influences of the chromatographic parameters in HILIC separations. *J. Chromatogr. Sci.* 2013, 51, 684–693. [CrossRef] [PubMed]
- Olsen, B.A.; Pack, B.W. (Eds.) Hydrophilic Interaction Chromatogaphy—A Guide for Practitioners; John Wiley & Sons Inc.: Hoboken, NJ, USA, 2013.
- Megoulas, N.C.; Koupparis, M.A. Twenty years of evaporative light scattering detection. *Crit. Rev. Anal. Chem.* 2005, 35, 301–316. [CrossRef]