Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography A, 1181 (2008) 51–59
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Development of a comprehensive two-dimensional hydrophilic interaction chromatography/quadrupole time-of-flight mass spectrometry system and its application in separation and identification of saponins from *Quillaja saponaria*

Yuan Wang, Xin Lu, Guowang Xu *

National Chromatographic R & A Center, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian 116023, China

Received 13 November 2007; received in revised form 11 December 2007; accepted 13 December 2007
Available online 23 December 2007

Abstract

Hydrophilic interaction chromatography (HILIC) was used in two dimensions in a comprehensive two-dimensional HILIC hyphenated with a quadrupole time-of-flight mass spectrometry (HILIC × HILIC-Q-TOF-MS) system for the analysis of complex samples of hydrophilic compounds. A TSKgel Amide-80 column was employed as the first dimension, and a short PolyHydroxyethyl A column was as the second dimension. The column system showed moderate orthogonality at defined operational conditions. A high speed Q-TOF-MS detector as a third complementary dimension significantly improved the peak capacity. The separation capability of the developed HILIC × HILIC-Q-TOF-MS system was tested by separating an extract from *Quillaja saponaria*. The major components, quillaja saponins, in the extract were well identified by means of $[M-H]^-$ ions, characteristic product ions, and their two-dimensional retention behaviors. Several pairs of isomers, which were often co-eluted on conventional LC–MS methods and had similar fragmentation characteristics in MS/MS spectra, were well separated on the two-dimensional system based on their different hydrophilicity. The developed comprehensive two-dimensional HILIC system demonstrates unique selectivity for hydrophilic compounds and satisfactory peak capacity and resolution for analogues by making sufficient use of two-dimensional separation plane.

Keywords: HILIC; Comprehensive two-dimensional liquid chromatography; Orthogonality; Quillaja saponins

1. Introduction

Comprehensive two-dimensional liquid chromatography (LC × LC) is a powerful technique for separation of complex samples. In this technique, the eluate from the first dimension is collected as fractions which are all separated individually in the second dimension. Due to the different separation mechanisms of the two dimensions, higher resolution is expected compared to conventional one-dimensional LC. This technique has been proven feasible not only to separate analytes but also as an aid in identification, characterization, and quality control [1]. As first defined by Bushey and Jorgenson [2], the two separation mechanisms should be as different from each other as possible to obtain a comprehensive two-dimensional separation of the entire sample. Schoenmakers et al. [3] proposed that a two-dimensional separation can be called comprehensive if every part of sample is subjected to two different separations, equal percentages (either 100% or lower) of all sample components pass through both columns and eventually reach the detector, and the separation (resolution) obtained in the first dimension essentially remained. Several operational conditions should also be considered to build a successful LC × LC system, such as matched flow rates and dimensions of columns with sampling loops' volume and modulation frequency, miscibility and eluent strength of mobile phases in both dimensions.

By carefully configuring the column system, an LC × LC separation plane can offer a much higher theoretical peak capacity (PC_T) than the common one-dimensional HPLC. It is well known that the PC_T of an LC × LC system is the product of the PC_T of each dimension [4]. Theoretically, a high peak capacity of

* Corresponding author. Tel.: +86 411 84379530; fax: +86 411 84379559.
E-mail address: xugw@dicp.ac.cn (G. Xu).

0021-9673/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2007.12.034

a comprehensive two-dimensional LC system can be obtained by using an ultra long first dimensional column, very short second dimensional column, very fast modulation frequency and long separation time [5]. An improved peak capacity can be expected by optimizing column systems [6,7] and introducing more operation parameters for specific compounds [8,9]. Meanwhile, the use of mass spectrometry (MS) as the detection method in comprehensive two-dimensional systems introduces an additional separation dimension and improves the total peak capacity of the system [10–13].

Early studies in LC × LC were focused on the separation of proteins or peptides by means of SEC (size-exclusion chromatography) × RPLC (reversed-phase liquid chromatography), or IEC (ion-exchange chromatography) × RPLC [14–18]. In 1998, Murphy et al. [19] proposed using normal-phase LC (NPLC) and RPLC to build two-dimensional LC. But the miscibility of the two sets of mobile phases was a severe problem. Recently, the NPLC × RPLC or RPLC × NPLC systems have been used to separate (co)oligomers [20,21], lipids [22], pharmaceutical samples [23] and other hydrophobic compounds [24,25]. The miscibility problem of mobile phases between the two dimensions was resolved by decreasing the sampling volume and increasing the flow rate on the second dimensional column. Other reported applications included PEG (polyethylene glycol)-coated silica × RPLC and PEG-coated silica × carbon-coated zirconia for phenolic antioxidants [26], zirconia × RPLC for polymers [27], RPLC × RPLC for natural products [28], and silver-ion column × RPLC for lipids [10]. In these studies, the combination of various stationary phases in the LC × LC system extended its application to wider scope of samples.

Hydrophilic interaction chromatography offers a unique separation mode for hydrophilic samples other than NPLC or RPLC [29]. In HILIC, the elution is promoted by polar mobile phases, which is similar to NPLC, but frequently used mobile phases are water and water-soluble modifiers. Commonly, the proportion of water in the mobile phase ranges from 5 to 40% [30]. The highly volatile organic solvents in the mobile phases for HILIC provide increased electrospray ionization mass spectrometry (ESI-MS) sensitivity [31]. Recently, HILIC has been widely used in the separation and detection of strong hydrophilic compounds, such as pharmaceuticals [32], peptides [33], and some small polar compounds in bio-samples [34,35]. Meanwhile, it has been revealed that HILIC was highly orthogonal to RPLC [36–38]. However, it is difficult to build a HILIC × RPLC system because of the incompatibility of the mobile phases. Namely, the relatively weak mobile phase for HILIC is rather strong for RPLC (or *vice versa*), making the "column head focus" in the second dimension difficult to be realized. In 2006, Jandera et al. [39] reported a RPLC × NPLC (HILIC) system for the separation of water-soluble polymers. However, its mobile phase (dichloromethane:ethanol:water = 98.5:1.477:0.023) used in HILIC is more like classical NPLC.

On the other hand, HILIC columns with different functional groups can differ in selectivity and capacity, making it possible to build a comprehensive two-dimensional system for separation of complex mixtures of hydrophilic compounds, which is one of the challenging topics in the era of "-omics". However, to the best of our knowledge, no such HILIC × HILIC system has been reported before. In this study, a novel HILIC × HILIC-Q-TOF-MS system was established. The orthogonality between the two dimensions was investigated. The up-to-date Q-TOF-MS system is expected to provide an additional complementary dimension to the HILIC × HILIC combination and enhanced peak capacity. The hydrophilic compounds, quillaja saponins, in an extract from *Quillaja saponaria* were used to test the developed system.

Quillaja saponins are heterogenous mixture of molecules varying both in their aglycone and sugar moieties. The main aglycone (sapogenin) moiety are the triterpenes, such as quillaic acid (Q) [40] and phytolaccinic acid (P) (Fig. 1). The C-3 and C-28 positions of the aglycone are substituted with sugar moieties (R^A and R^B in Fig. 1). These sugar moieties are further extended by monosaccharides, oligosaccharides or fatty acid chain-arabinose at particular positions (R^0–R^4 in Fig. 1). More than 60 quillaja saponins have been isolated and identified from different origins. Many of them are structurally similar, and the commonly used HPLC-MS method cannot offer sufficient separation for such complex mixtures [41]. The abundance of various sugars, including glucose (Glc), glucuronic acid, galactose (Gla), xylose (Xyl), apiose (Api), rhamnose (Rha), fucose (Fuc) and arabinose (Ara), make the quillaja saponins highly hydrophilic and suitable for separation by the HILIC × HILIC-Q-TOF-MS system.

2. Experimental

2.1. Chemicals and materials

HPLC-grade acetonitrile (ACN), methanol (MeOH), formic acid, acetic acid (HAc), and ammonium acetate (NH_4HAc) were purchased from Tedia (Fairfield, OH, USA). Distilled water was purified with a Milli-Q water purification system (Millipore, Bedford, MA, USA). *Q. saponaria* extract was purchased from Aldrich (Sigma–Aldrich, Steinheim, Germany), of which the sapogenin content was 10%. It was dissolved in MeOH/water (1/1, v/v) at 100 mg/mL and filtered with 0.22 μm nylon membrane before injection.

2.2. HILIC × HILIC-MS equipment and conditions

The HILIC × HILIC-Q-TOF-MS system was composed of commercial available modules. An Agilent 1200 chromatography system was used for the first dimension, including binary gradient solvent delivery pumps (Pump 1 and Pump 2), solvent degasser and auto sampler, controlled by an Agilent MassHunter Workstation (Agilent Technologies, Palo Alto, CA, USA). The second dimension system was from Shimadzu (Kyoto, Japan): model LC-20AB solvent delivery pumps (Pump 3 and Pump 4) equipped with a DGU-A5 degasser and controlled by LC Solution software (ver. 1.3). The synchronization of the two dimensions was triggered by injection signal from the auto sampler. An electronically controlled 10-port dual-position valve (7725i, Rheodyne, Rohnert park, CA, USA) equipped with two 100 μL sampling loops was used as the interface between the