Journal of Chromatography A, 1444 (2016) 123–128

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Supercritical fluid chromatography coupled with in-source atmospheric pressure ionization hydrogen/deuterium exchange mass spectrometry for compound speciation

Yunju Cho[a], Man-Ho Choi[b], Byungjoo Kim[c,**], Sunghwan Kim[a,d,*]

[a] Kyungpook National University, Department of Chemistry, Daegu 41566, Republic of Korea
[b] Future Convergence Research Division, Korea Institute of Science and Technology, Seoul 02792, Republic of Korea
[c] Division of Organic Analysis, Korea Research Institute of Standards and Science, Daejeon 34113, Republic of Korea
[d] Green Nano Center, Department of Chemistry, Daegu 41566, Republic of Korea

ARTICLE INFO

Article history:
Received 26 November 2015
Received in revised form 27 February 2016
Accepted 6 March 2016
Available online 19 March 2016

Keywords:
Supercritical fluid chromatography
High resolution mass spectrometry
Hydrogen/deuterium exchange

ABSTRACT

An experimental setup for the speciation of compounds by hydrogen/deuterium exchange (HDX) with atmospheric pressure ionization while performing chromatographic separation is presented. The proposed experimental setup combines the high performance supercritical fluid chromatography (SFC) system that can be readily used as an inlet for mass spectrometry (MS) and atmospheric pressure photo ionization (APPI) or atmospheric pressure chemical ionization (APCI) HDX. This combination overcomes the limitation of an approach using conventional liquid chromatography (LC) by minimizing the amount of deuterium solvents used for separation. In the SFC separation, supercritical CO_2 was used as a major component of the mobile phase, and methanol was used as a minor co-solvent. By using deuterated methanol (CH_3OD), AP HDX was achieved during SFC separation. To prove the concept, thirty one nitrogen- and/or oxygen-containing standard compounds were analyzed by SFC-AP HDX MS. The compounds were successfully speciated from the obtained SFC-MS spectra. The exchange ions were observed with as low as 1% of CH_3OD in the mobile phase, and separation could be performed within approximately 20 min using approximately 0.24 mL of CH_3OD. The results showed that SFC separation and APPI/APCI HDX could be successfully performed using the suggested method.

1. Introduction

One of the important roles of mass spectrometry (MS) is to identify molecular structures, and hydrogen/deuterium exchange (HDX) combined with mass spectrometry (MS) has been used as a dynamic tool to achieve this goal. HDX can be performed in solution or in gas phase [1–7]. Especially HDX can be performed in the ionization source (in-source HDX). In-source HDX does not require special experimental setup and can be done by switching to deuterium containing solvent or reagent gas. Therefore, it is quick and convent way of performing HDX. HDX with chemical ionization (CI) was reported to be useful for the identification of the labile hydrogens bonded to heteroatoms [1,2]. Mass spectra of electrospray ionization (ESI) HDX were used to study various compounds including proteins [3–5,8–16]. Recently, atmospheric pressure photo ionization (APPI) and atmospheric pressure chemical ionization (APCI) coupled to HDX MS and the usefulness of these combined methods for elucidating the structure of compounds was explored [17–20].

In most modern applications, MS with atmospheric pressure ionization is coupled with chromatographic separation. In particular, liquid chromatography coupled with MS (LC–MS) is one of the most important tools used for protein, metabolite, drug, and hormone analyses [11,21–26]. Therefore, the combination of chromatographic separation and HDX is reasonably expected to be a powerful tool for simultaneously obtaining mass and structural information.

However, despite its potential, the combination of chromatographic separation and in-source HDX coupled with atmospheric-pressure ionization has not been widely used and only limited examples are available [27,28]. The key reason for the lack of this combination is attributable to the cost of analysis. To perform HDX experiments, the use of solvents with exchangeable hydrogen must be minimized because the presence of a protic solvent can induce back-exchange of HDX products [29]. This phenomenon means that use of solvent must be limited to aprotic solvents or deuterated

* Corresponding author at: Kyungpook National University, Department of Chemistry, Daegu 41566, Republic of Korea.
** Corresponding author.
E-mail addresses: byungjoo@kriss.re.kr (B. Kim), sunghwank@knu.ac.kr (S. Kim).

http://dx.doi.org/10.1016/j.chroma.2016.03.011
0021-9673/

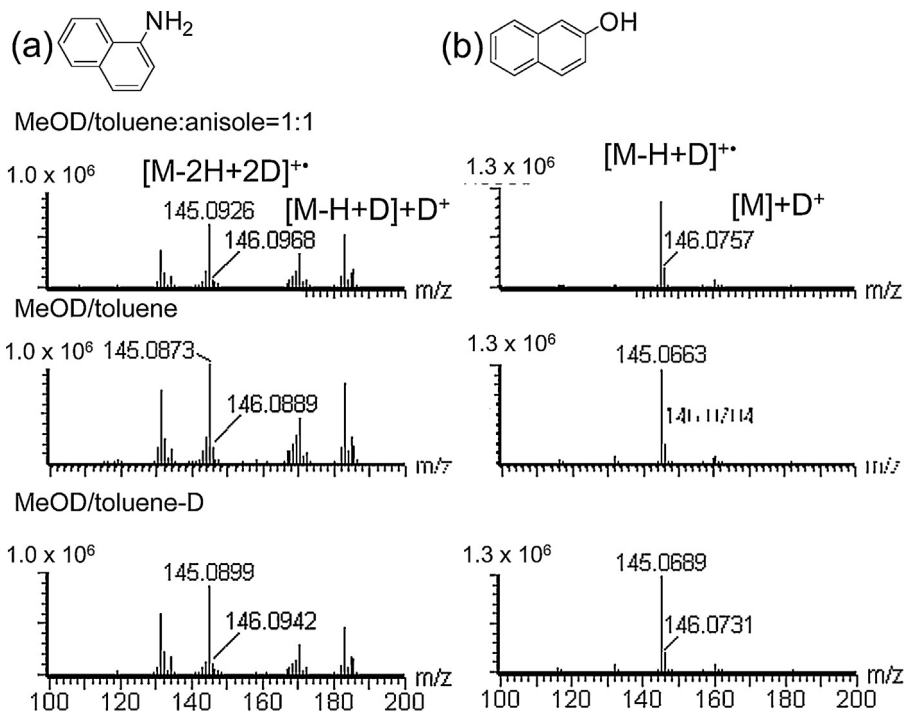

Fig. 1. Spectra of 1-naphthylamine and 2-naphthol obtained using a toluene and anisole mixture (top), toluene (middle), and toluene-D (bottom) as dopants.

solvents such as D_2O or CH_3OD for chromatography. However, performing separations using only aprotic solvents will limit choice of solvents especially for the reverse phase separation. In addition, deuterated solvents are expensive; thus, filling LC bottles with deuterated solvents is not practical.

To circumvent this problem, a chromatography technique that does not involve the use of large amounts of protic solvents can be utilized. A high performance supercritical fluid chromatography (SFC) system that can be readily connected to a mass spectrometer was developed and applied [30].

In SFC, supercritical CO_2 is typically used as a major component of the mobile phase. Therefore, back exchange can be minimized. In fact, SFC has been combined with HDX occurring in solution phase to minimize back exchange [31]. However, combining SFC with in-source HDX such as APPI HDX or APCI HDX has not been reported before. Therefore, in this study, the potential of combining SFC and in source API HDX has been explored. We believe that this is the first study reporting combination of SFC and in-source HDX/MS to identify structures of aromatic compounds.

Table 1
Solvent program used for SFC separation of (a) nitrogen and oxygen containing aromatic compounds and (b) steroid hormones.

(a)

Time (min)	CO_2 (%)	MeOD (%)	Flow Rate (mL/min)
0.0	100	0	1
0.5	100	0	1
1.0	99	1	1
3.0	98	2	1
5.0	98	2	1
7.0	95	5	1
9.0	92	8	1
20.0	80	20	1

(b)

Time (min)	CO_2 (%)	MeOD (%)	Flow Rate (mL/min)
0.0	95	5	1
2.0	92	8	1
13.0	80	20	1
20.0	80	20	1
25.0	100	0	1

2. Material and methods

2.1. Sample preparation

31 standard compounds and solvents used in this study were obtained from Sigma-Aldrich (St. Louis, MO) and used without further purification. All of the standard samples were listed in Supplementary material section (Table S1–S3). The elution time in the UV chromatograms and concentration of the compounds used for the SFC were also listed in the table. A group of compounds was dissolved in toluene and injected into SFC column.

2.2. SFC separation

The experimental setup for SFC APPI/APCI HDX-MS is presented in Fig. S1. An ACQUITY UPC^2 manufactured by Waters (Milford, MA) was used as the SFC system. For SFC separation, supercritical CO_2 was used as the major mobile phase solvent, and methanol was used as a co-solvent. Deuterated methanol (CH_3OD) was used as a co-solvent for HDX in this study. The solvent program used in this study for SFC separation is presented in Table 1. The separation was done in less than 15 min but the solvent program was up to 20 min to clean up and re-equilibrate the column. The SFC system was equipped with a PDA detector. A BEH 2- Ethylpyridine (EP) column (100 mm L × 3 mm ID, 1.7 μm d_p from Waters) was used for separation of 12 nitrogen-, and 10 oxygen-containing organic compounds. For 9 steroid hormone samples, BEH silica column (100 mm L × 3 mm ID, 1.7 μm d_p from Waters) was used. Column oven was operated at a temperature of 55 °C during running time of 20 min.

2.3. Notations for exchanged peaks

The notation for exchanged peak used in the previous work was employed in this paper [18]. Briefly explained, $M^{\bullet+}$ designates a