Journal of Chromatography A, 1572 (2018) 90–99

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Investigation of isomeric structures in a commercial mixture of naphthenic acids using ultrahigh pressure liquid chromatography coupled to hybrid traveling wave ion mobility-time of flight mass spectrometry

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ARTICLE INFO

Article history:
Received 22 June 2018
Received in revised form 13 August 2018
Accepted 23 August 2018
Available online 24 August 2018

Keywords:
Classical naphthenic acids
Commercial mixture
UPLC/TWIM-TOF-MS

ABSTRACT

The separation of isomeric naphthenic acids (NAs) is of high importance to obtain more detailed and therefore useful information to understand their fate, transport, toxicity and potential removal treatment methodologies. In the current study, the capabilities of the ultrahigh pressure liquid chromatography/traveling wave ion mobility-time of flight mass spectrometry (UPLC/TWIM-TOF-MS) to separate and study different isomeric structures of NAs were investigated. Fifty seven standard compounds belonging to different chemical families of classical NAs were analyzed to obtain their experimental drift times in addition to chromatographic retention time and mass-to-charge information (*m*/*z*). These acyclic and cyclic molecules yielded ions with collision cross section (CCS) values ranging from 110 to 210 Å^2. The feasibility of the UPLC/TWIM-TOF-MS method to provide a higher degree of confidence in the identification of isomeric structures was evaluated by analyzing the commercial (Sigma-Aldrich) NAs mixture. Identification and structure confirmation of several alicyclic compounds of similar *m*/*z* in the NAs mixture were possible by this method. For instance, the presence of previously tentatively identified compounds by the ultrahigh pressure liquid chromatography/quadrupole time of flight mass spectrometry (UPLC/QTOF-MS), such as 4-*tert*-butylcyclohexanoic acid, 4-dicyclohexylacetic acid or 3,5-dimethyladamantane-1-carboxylic acid, was confirmed. Combining ion mobility separation with UPLC/TOF-MS gives a higher level of selectivity to the overall method by selective interrogation of specific retention time, mass-to-charge and mobility regions. However, there are cases where it is not possible to resolve many similar molecules such as acyclic isomeric compounds in commercial NAs mixture by this technique. This was likely due to the very small CCS area differences among the structural isomeric species. Considerable improvements in the ion mobility resolution and separation will be required for this technique to be able to resolve isomeric species with slight differences in physicochemical properties (e.g., size and structure).

1. Introduction

There is growing interest in research providing a better understanding of environmental and health impacts of naphthenic acids (NAs), which are complex mixtures of petroleum-derived carboxylic acids [1]. This broad group of organic acids are classically defined as the family of alkyl-substituted saturated cyclic and acyclic carboxylic acids with general formula $C_nH_{2n+Z}O_2$, where *n* represents the carbon number, and *Z* specifies the degree of unsaturation or number of cyclic rings in a series [1,2]. For each *n* and *Z* combination, there are numerous isomers with poorly defined structures [3]. These surfactant-like compounds are the most significant environmental contaminants present in oil sands process-affected water (OSPW), resulting from petroleum extraction from oil sands deposits [4,5]. The classic NAs remain relevant as a major component of OSPW, and given the similarities in structural characteristics between sub-classes of oxygenated NAs and some estrogens, are significant as potential toxic components [1,6–9]. Thus, increased attention has been given to characterization, monitoring and understanding of their fate in the environment [1,10,11]. For example, research on source identification has demonstrated the possible migration of naphthenic acids from oil sands tailings to groundwater adjacent to those tailings ponds [12]. NAs are also identified in ambient air particulate matter collected in close

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https://doi.org/10.1016/j.chroma.2018.08.052
0021-9673/

proximity to oil sands processing activities in the Athabasca oil sands region (AOSR) [13]. There are a wide variety of analytical methods developed for the semi-quantification of total naphthenic acids in OSPW and other environmental samples [2,14,15]. Recommended methods are mass spectrometry methods based on time-of-flight mass spectrometry (TOF-MS), Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and Orbitrap mass spectrometry, with or without on-line chromatography [13,16–20]. However, the structural elucidation and the differentiation of the positional isomers of NAs by these techniques are still a challenge due to their compositional complexity. Since the reactivity of compounds depends on their structure, it is essential to distinguish and characterize structural isomers in NAs to understand their fate and transport, toxicity and potential treatment methodologies [21].

Recently, ion mobility technologies combined with mass spectrometry (IM-MS), especially coupled inline to UPLC/QTOF-MS, have rapidly evolved as a powerful analytical technique that shows great applications potential for structural elucidation and positional isomer separation in complex mixtures including oil and petroleum samples [22–24]. IM-MS is not only able to differentiate between m/z of the ions, but also separates rapidly (ms) ionic species on the basis of differences in their physical size and shape in the gas phase. As the ions move through a neutral drift gas, typically helium (He) under the influence of a weak electric field, they provide specific information on the ionic configuration and potential structural confirmation of these ionic species [25,26]. Therefore, isomers with identical masses but a different collisional cross section (CCS) can be distinguished [27,28]. The CCS is a unique physicochemical property of a molecule, at a particular condition, that provides a descriptor of the ion shape and facilitates the elucidation of its structure by measuring the time required for an ion to traverse the ion mobility cell filled with an inert gas (drift time) [29]. In combination with chromatography, analytes can be characterized by three orthogonal separation techniques, by obtaining a retention time, a CCS value and an accurate mass for each analyte, which significantly improves the overall peak capacity and specificity [30]. This can be particularly beneficial in the analysis of complex mixtures, like NAs, and in the separation of isomeric compounds [29,31]. Recently, the use of UPLC/IM-TOF-MS was reported to achieve two-dimensional (2D) separation map (drift vs. retention times) of NAs in unprocessed and ozonated commercial Merichem NAs and OSPW matrices. The method was able to differentiate between isobaric compounds (compounds with the same nominal mass but different formula) with a mass difference of 3.4 m Da that could not be resolved by TOF-MS [21,32]. More recently, Huang et al. reported the successful use of UPLC/IM-TOF-MS for the analysis of NAs in fractions of pH-dependent sequential liquid-liquid extractions from OSPW [33]. Ion mobility separation allowed the differentiation of O_yS-NAs from O_x-NAs species in OSPW via drift time versus retention time. However, the separation of NAs isomers has not been thoroughly discussed in these studies.

In the current study, the potential of the ion mobility separation combined with the previously developed UPLC/QTOF-MS method [13] was investigated to study different isomeric NAs structures. Traveling wave ion mobility mass spectrometry (TWIM-MS) was used to systematically evaluate some key relationships between structures and drift times by analyzing NA compounds with moderate analytical complexity. A database including retention times, accurate masses and CCS values for over 50 acyclic and cyclic NAs was generated. Finally, the feasibility of the UPLC/TWIM-TOF-MS method to provide a higher degree of confidence in the identification of isomeric structures was evaluated by analyzing the commercial (Sigma-Aldrich) NAs mixture. Advantages and limitations of the developed methodology are also discussed.

2. Experimental

2.1. Chemicals and standards

Unless stated otherwise, all materials were obtained from Sigma-Aldrich (Oakville, ON, Canada). All chemicals and solvents were of analytical grade or higher purity. 1-adamantane carboxylic acid ($C_{11}H_{16}O_2$; $Z=-6$) was supplied by Acros Organics (New Jersey, USA). LiChrosolv LC/MS grade acetonitrile (ACN) and ammonium hydroxide (≥20%; OmniTrace Ultra, EMD) were purchased from VWR International (Mississauga, ON, Canada). Optima LC–/MS grade formic acid (≥ 99.5%) was supplied by Fisher Scientific (Ottawa, ON, Canada). Individual stock solutions of organic acids (1000 mg/L) were prepared by dissolving each acid in alkaline methanol (1% (v/v) NH_4OH). A mixed standard solution (5 μg/mL) prepared in 1% (v/v) aqueous NH_4OH was used for further dilutions. The Sigma-Aldrich NA STD mix (Technical; NA STD mix; cat. no. 70340; lot no. BCBC9959 V) was dissolved in alkaline methanol (1% (v/v) NH_4OH) to make a 10,000 mg/L. This stock solution was further diluted with 1% (v/v) aqueous NH_4OH before analysis. Unless otherwise noted, all solutions were prepared using double deionized (DDI) water (18 MΩ·cm; Milli-Q Reference A + System; Bedford, MA, USA).

2.2. UPLC/TWIM-TOF-MS analysis

All measurements were carried out using a Waters Acquity UPLC® I-Class system (Waters, Milford, MA, USA) consisting of a membrane degasser, a binary ultrahigh-pressure gradient pump, an autosampler, and a column thermostat. Chromatographic separation was carried out on an Acquity UPLC HSS Cyano (100 mm × 2.1 mm; i.d., 1.8 μm) column with compatible guard column, also from Waters, thermostated at 30 ± 0.1 °C. A short UPLC BEH C_{18} column (50 mm × 2.1 mm; i.d., 1.7 μm) was installed between the Tee-mixer valve assembly and the sample injection port used for on-line mobile phase cleanup [13]. Briefly, a gradient elution was carried out using (A) 0.1% (v/v) formic acid in DDI and (B) pure ACN at the flow rate of 0.4 mL/min. The following linear gradient was used: 0 min, 2.5% B, 0.2 min; step at a slope of 9.75% B per min for 10 min. Between the runs, the column was flushed with 100% B for 4 min (column cleaning) and then equilibrated for 2 min using 2.5% B. The injection volume was set at 7.5 μL.

The UPLC was coupled to a Synapt G2-Si HDMS spectrometer (Waters), which is a hybrid quadrupole time-of-flight (QTOF) mass spectrometer with an inline traveling-wave ion mobility spectrometer (TWIM-MS) between the quadruple and the TOF. The combined Z-spray/LockSpray ion source was operated in negative ion electrospray ionization (ESI-) mode under the following specific conditions: capillary voltage, 2.5 kV; cone voltage, 30 V; source offset, 80 V; source temperature, 120 °C; desolvation gas temperature, 450°C; desolvation gas flow, 1000 L/h, and the nebulizer pressure 6.5 bar. Nitrogen gas (purity 99.9%), generated from a pressurized nitrogen generator (Parker Hannifin, Tewksbury, MA, USA), was used as the nebulizer and the desolvation gas. Mass accuracy of less than 2 ppm (RMS) was obtained with a lock-mass compound (leucine enkephalin; 0.2 ng/μL in 50:50 ACN/0.2% aqueous formic acid) infused at a flow rate of 10 μL/min via the lockspray ionization source and detected as $[M-H]^-$ at m/z of 554.2615. The TOF-MS spectra were obtained in "V" mode operating at a resolution of >30,000 full width at half maximum (m/z of 554). The mass range acquired was 50-1200 m/z at rate of 2 scan/s.

For the IM separation, the traveling wave (T-wave) IM cell consists of 3 consecutive sections including the trap, IM and transfer assemblies. Both T-wave trap and transfer assemblies were filled with argon gas at a constant pressure of 2.3×10^{-2} mbar, and operated at wave height and velocity of 4 V and 300 m/s, respectively.