THE IMPLEMENTATION OF A SCREENING WORKFLOW FOR ION MOBILITY QUADRUOPOLE TIME-OF-FLIGHT MASS SPECTROMETRIC ANALYSIS OF PFOS ISOMERS

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Introduction

Perfluoroalkyl substances (PFASs) were widely used in surfactants for industrial processes and consumer commodities such as non-stick cookware, textiles and carpeting[1]. Due to detection in the environment and biota and observations of persistence[1-3], perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) have been identified as perfluoroalkyl compounds of particular interest for monitoring and legislation. PFASs are formed by either electrochemical fluorination or telomerization[2]. The former process resulting in a mixture of branched, linear and short chain perfluoroalkyl compound forms[2]. In the case of PFOS and PFOA, numerous branched structures have been determined. These structurally different isomers have varied response factors in tandem mass spectrometry, making their differentiation important for accurate determinations[4]. Existing knowledge of unique product ion patterns associated with the different branched forms allow for their specific identification in samples. However, under nominal mass conditions as in tandem quadrupole analyses, the biological matrix interference taurodeoxycholate (TDCA) undergoes the same common PFOS transition of $499 \rightarrow 80$[5]. Additionally, chromatographic co-elutions of 3/4/5-PFOS and the complexity of matrices in which PFOS are routinely analyzed results in complicated data interpretation.

Here we propose the use of ion mobility quadrupole time-of-flight mass spectrometry (IM-qTOF-MS) and a screening approach to address these challenges in PFOS analysis. Exact mass information is obtained from the use of qTOF MS detection, for both precursor and product ions. Once established, product ions and retention time (RT) were used here in a database to mine the full scan TOF MS data and make identifications in technical blends and biological matrices. Additionally, the use of ion mobility separations provided additional characterization of PFOS. Ion mobility is an orthogonal separation technique which separates ions based on their size, shape and charge. Ions traverse through a gas filled drift cell, and are assigned a drift time based on their travel time. Drift time can be used as a unique experimental characteristic of an ion, and in many cases can differentiate between isobaric compounds that co-elute chromatographically. In this work we show the utility of ion mobility separations for PFOS branched isomers identified in the aforementioned samples. We also illustrate the ability for this complex raw data to be treated using a database screening approach to make identifications of PFOS in sample analyses.

Materials and methods

A Waters ACQUITY I-Class UPLC system was coupled to a Synapt G2-S (Waters Corporation, Milford, USA) using negative electrospray ionization for the determination of PFOA, PFOS and co-eluting isobaric interferences. A Waters ACQUITY UPLC BEH C18 column (100 mm x 2.1 mm, 1.7 µm particle size) was used for chromatographic separation. A gradient of mobile phases of water, methanol and acetonitrile with 2 mM ammonium acetate added were delivered at 0.45 mL/min at 45 °C with a desolvation temperature of 550 °C. Injection volume was 10 µL. All solvents used were of HPLC grade. Linear, monomethyl and dimethyl branched isomers used for confirmation of experimentally identified isomers were obtained from the Wellington Laboratories (Guelph, Canada).

The mass spectrometer was operated in the ion mobility separation (IMS) mode employing an alternting collision ramp from 35-75 eV for collecting high energy and 4eV for collecting low energy mass spectral information (MS^e). Data was collected within the m/z range of 50-600 at an acquisition rate of 10

spectra/second. For electrospray ionization a capillary voltage of 2.3 kV and a cone voltage of 15 V were used. Both N_2 and CO_2 were used as mobility drift gases applying both static wave velocity of 550 m/s and ramp ranging of 400-550 m/s and wave height of 40 V. Instrument mass accuracy was calibrated using sodium formate. For mobility data polyalanine was used for calibrant. Masslynx 4.1 (Waters Corporation, Milford, MA, USA) was used for data collection and instrumental control, and UNIFI Research Edition 1.6.5 (Waters Corporation, Milford, MA, USA) was used for data processing.

An 8-point calibration curve, ranging from 0.93-186 ng/mL, was prepared from individual PFOS/PFOA isomer solutions as well as a mixture of all isomers. Signal-to-noise ratios were assessed for the curve, and used to determine LODs and LOQs. Matrix samples consisted of mink liver extract prepared according to Kärrman et al.[6], with minor modifications. Homogenization of liver samples was performed using Ultra-Turrax (IKA) after which a sub-sample of 1g liver was taken for analysis. Labeled ^{13}C-internal standards were added followed by 10 mL of acetonitrile. After vortex mixing, sonication and centrifugation (10000 x g, 30 min) the supernatant of two repeated extractions were reduced in volume to 10 mL. With an addition of 25 mL the samples were solid phase extracted on Oasis WAX solid phase cartridge (Waters, Milford, MA, USA) previously conditioned with 4 mL methanol followed by 4 mL water. After sample loading the WAX cartridge was washed with 4 mL 25 mM sodium acetate (pH 4) and 4 mL 40v% methanol in water, followed by drying using vacuum suction. The SPE cartridges were eluted with 8 mL of methanol (discarded) followed by 2 mL 2% ammonium hydroxide in methanol for collection of PFASs. Further clean-up was performed with 50 mg ENVI-Carb (Supelclean, 120/400 mesh, Supelco (Bellefonte, PA)) and 100 µL acetic acid, followed by vortex mixing and filtration through a 0.2 µm GHP membrane (Pall, East Hills, NY, USA). Final volume was set to 500 µL including labeled recovery standard 7HPFHpA and 300 µL 2 mM ammonium acetate in water. A technical mixture of PFOS potassium salts (98%) from Fluka (Steinheim, Germany) was dissolved in methanol and also analyzed.

Results and discussion

A screening database was created for PFOS branched isomers 1/3/4/5/6/4,4/3,5/4,5/5,5-PFOS based on observed retention times and unique product ions found using solvent standards. Product ion structures were proposed based on high energy spectrum peaks, as shown in Figure 1 for 3-PFOS. Upon the addition of this information to the database, it was used for the investigation of PFOS branched isomers in samples. Identifications of an isomer in the liver extracts as well as technical blend were filtered by the software to include only isomers which had a positively identified product ion. Several isomers in each sample were identified in this manner, and quantitative assesment was also performed where applicable.

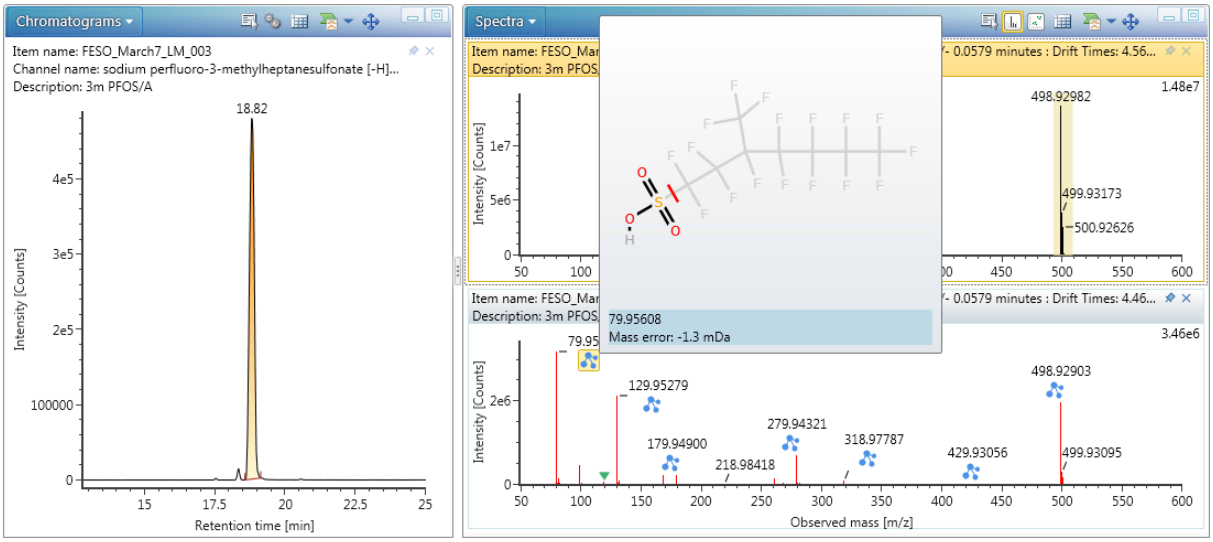

Figure 1. Extracted ion chromatogram and spectrum for both low energy (top) and high energy (bottom) collision states. Using the structure, proposed product ions were deduced from the observed spectral peaks, as indicated by the blue molecule icons.

In addition to compound screening, interogation of data rich IM-qTOF-MS spectra provided a means to differentiate the matrix component TDCA from PFOS isomers. Through their separation in the drift cell, an additional level of differentiation is possible for these chromatographically co-eluting compounds. Using software capable of separating ions which do not share the same drift time, TDCA peaks are removed from the spectrum for 1-PFOS (Figure 2). This results in a spectrum free of interfering peaks that often occur in the analysis of complex samples. Drift time of the precursor is also shared by product ions when they are fragmented after the drift cell, so the same approach can be taken with the high energy spectrum. Generation of drift time corrected spectrum is easily viewed by simply indicating which view is desired in the spectra display toolbar. Investigation of drift time separations of chromatographically co-eluting 3/4/5-PFOS were also undertaken.

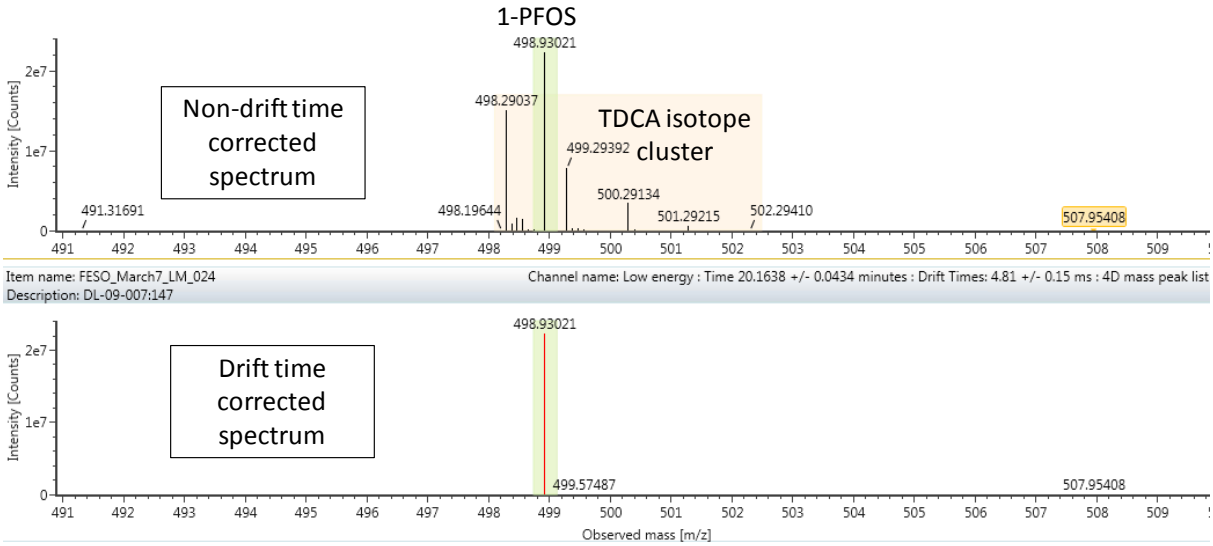

Figure 2: Spectrum for the same chromatographic peak, 1-PFOS, with interference from TDCA evident in the top spectrum. The bottom spectrum is from the same chromatographic peak and injection, but with the removal of any ions that do not share the same drift time as 1-PFOS.

Implementation of the software-enabled routine screening approach described in this work has provided further access to exact mass and ion mobility mass spectral data for the analysis of PFOS. Upon processing the raw data with a user created database, the highly informative UPLC/IM-qTOF-MS data can be assesed thoroughly from a quantitative and qualitative viewpoint. Ion mobility information provides another component of analytical separation, and is of particular utility in complex matrices. Further implementation of the screening approach proposed here would be of interest and utility for environmental contaminant analyses.

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References:

1. Buck, R.C., Franklin J., Berger U., Conder J.M., Cousins I.T., de Voogt P., Jensen A.A., Kannan K., Mabury S.A., van Leeuwen S.P.J. (2011); *Integr. Environ. Assess. Manag.* 7:513-541
2. Houde M., Martin J.W., Letcher R.J, Solomon K.R., Muir D.C.G. (2006) *Environ. Sci. Tech.*40:3463-3473
3. Olsen, G.W., Church T.R., Miller J.P., Burris J.M., Hansen K.J., Lundberg J.K., Armitage J.B., Herron R.M., Medhdizadehkashi Z., Nobeiletti J.B. (2003) *Environ. Health Pers.* 111:1892-1901

4. Riddell N., Arsenault G., Benskin J.P., Chittim B., Martin J.W., McAlees A., McCrindle R. (2009) *Environ. Sci. Technol.* 43:7902-7908
5. Benskin J., Bataineh M., Martin J. (2007) *Anal. Chem.* 79:6455-6464
6. Kärrman A., Domingo J., Llebaria X., Nadal M., Bigas E., van Bavel B., Lindström G. (2010) *Environ. Sci. Pol. Res.* 17:750-758