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BIOMONITORING OF GC AND LC AMENABLE ENVIRONMENTAL CONTAMINANTS USING A SINGLE MS PLATFORM

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Introduction

Monitoring of environmental contaminants in biological samples is typically performed by targeted analysis using a tandem quadrupole system on separate GC and LC platforms in order to cover the expansive range of compounds. Use of multiple systems can add complexity to the analysis and increase turnaround times for samples to be reported. The work that will be presented shows the analysis of common environmental contaminants in a biomonitoring context on a single high resolution mass spectrometric (HRMS) platform. The compounds of interest include:
•GC amenable compounds: Halogenated pesticides, brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo dioxins/furans (PCDD/Fs)
•LC amenable compounds: Perfluoroalkyl substances (PFASs), HBCDD/TBBP-A and phosphorus-based flame retardants (OPFRs)

Materials and methods

The analyses were performed on a QTof instrument with universal ionization source architecture. The system was operated in LC electrospray (ESI) mode and GC atmospheric pressure chemical ionization mode (APGC), allowing GC and LC amenable compounds to be analyzed on a single MS system. Sample preparation incorporated multiple approaches, based on the nature of the compounds being analyzed and the chormatographic system (LC or GC) employed. In the case of PFASs and PCDD/Fs/BDEs, previously described sample preparation methods were used for initial biological sample assessment[1,2]. For the LC analysis, a BEH C18 50mm column (Waters Corporation) and ESI^- was used for the PFASs analysis, and 60m DB-5 column nitrogen (N_2) carrier gas was used for the PCDD/Fs, PBDEs and PCBs. A single injection approach was taken for the latter compounds, thus the reason to use a 60m column. N_2 carrier gas was implemented after initial method developement and the GC program updated using a method calculator; using an atmospheric source, N_2 proved to be able to flow at a faster rate then helium and thus retain appropriate resolution. HRMS data was acquired using alternating high and low collision energy states across the full analytical mass range of 50-1200 Da, such that product ions were also generated. Product ions were chromatographically aligned with the precursor ions, and this information was useful for both known compounds of interest and in identifying unknown compounds. The data independent analysis allowed targeted analytes to be extracted out with narrow mass extraction windows. Since the targets are selected after the data is acquired, it can be further interrogated for emerging contaminants of interest in the form of an updated target list. Alternately, spectral peaks that have not been identified can be elucidated through the use of elemental composition and structural assingment where available. Quantitative analysis for identified compounds was performed with standards. In cases where a low-level (sub-ppb) contaminant has been identified in the initial screening analysis a more selective acquisition method of the QTof is utilized to achieve lower reporting limits. In this mode the instrument was operated in a targeted acquisition and the pusher frequency of the system was optimized to increase sensitivity and selectivity for the target compound which allows lower reporting limits to be achieved in matrix samples.

Results and discussion

Preliminary results indicate detection limits at relevant levels for most analytes in solvent standard, based on peak-to-peak signal-to-noise measurement of 3:1, and two examples are provided here. Specifically, for the LC analysis, PFOS was observed with a level of detection in solvent standard of 0.01 ng/mL and limit of quantification, based on a signal-to-noise ratio of 10:1, of 0.05 ng/mL. In the case of the GC analysis, 2,3,7,8-TCDD was detectable at 0.5 ng/mL, the concentration level used in EPA 1613 method calibration standard 1 (CS1)[3]. Following assessment of instrumental performance with solvent standards,

samples of biological origin were analyzed for each chromatographic approach, with identifications made in both sample sets. Contrasted with the most commonly utilized methods for POPs analysis such as single ion response (SIR) for magnetic sector MS, or multi-reaction monitoring (MRM) on tandem quadrupole MS, data is acquired in a non-directed manner using this approach. As a result, the searched masses provided exact mass full spectral information including isotopic distribution patterns for both precursors and product ions. Therefore, criteria for identification in samples included retention time, mass accuracy (mass errors were below 1 ppm in both examples shown here, though a tolerance of 5 ppm was used), isotopic fidelity and product ion assignment. Figure 1 and 2 show BDE 47 and PFOS results using GC and LC injections, respectively, as solvent standards and in biological matrix samples using this approach.

For the compounds requiring increased sensitivity, implementing a targeted enhancement acquisition mode as a separate injection was found to increase sensitivity by at least 2x, thus affording cleaner spectrum and lower detection limits within the required levels. This was achieved by selecting the predominant product ion mass in the acquisition method, and then following collision induced dissociation (CID) in the collision cell, executing an increases pusher frequency to increase the signal of the targeted mass. Also, a cleaner spectrum is afforded by this approach which results in greater selectivity in matrix particularly. Full spectral acquisition is still attained using this approach, as a separate channel in the same injection and compared to the previous injection that did not feature targeted signal enhancement.

In addition to interrogating data for the initial targeted analytes, unassigned spectral peaks were interrogated in two ways. The first approach involved updating the search list with emerging compound structural files, such as newer classes of PFASs, including fluorotelomer-based compound classes, perfluoroalkyl sulfonamide amino carboxylates and perfluoroalkyl sulfonamido amines[4]. Metabolites could also be searched for using a list of known transformations on compounds in the search list. The second approach, used primarily to focus on unknown halogenated compounds, reduced the peak list to unassigned spectral peaks in the samples that contained a Cl or Br isotope distribution pattern. These masses were then subjected to elemental composition and online database searching. Product ions were also used both as a possible common factor with known compounds in the original search and as confirmation of a proposed compound structure, if available. Using both approaches, proposed identifications were made on a small number of observed masses. Steps to confirm the identifications of some of the new PFASs include tracking response across multiple injections and consideration of chemical characteristics (if available) of the proposed compound relative to the observed chromatographic measurement.

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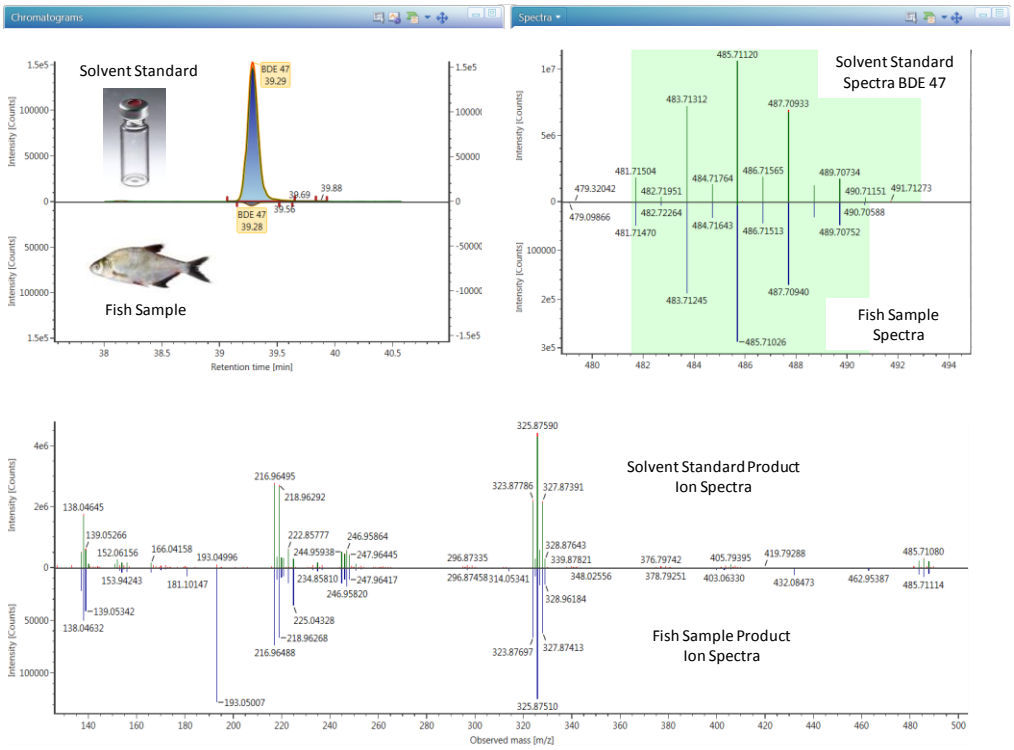

Figure 1: Binary comparison of reference to fish sample chromatogram and spectra for BDE 47, analyzed by APGC. Data shown includes an extracted ion chromatogram (XIC) of the $M^{\bullet+}$ ion in solvent standard and fish sample, where retention times (RT) are aligned, precursor ion spectrum showing the full isotopic distribution pattern for the four Br containing congener, and product ion spectrum for both. The y-axes are scaled by normalized intensity for the XICs, and then relative intensities for the spectra.

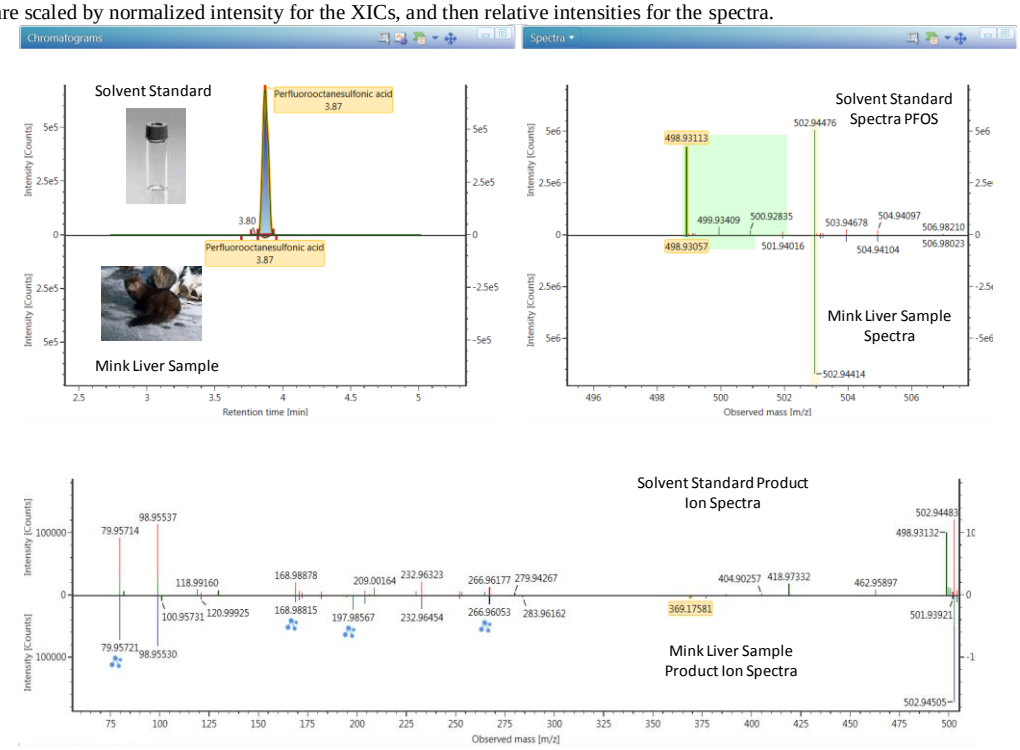

Figure 2: Binary comparison of reference to mink liver of perfluorooctanesulfonic acid (PFOS) analyzed by LC. The same visualization of the data was used as described for Figure 1.