SIMULTANEOUS MONITORING OF MATRIX INTERFERENTS DURING THE ANALYSIS OF PERFLUORINATED COMPOUNDS IN ENVIRONMENTAL WATERS AND BIOTA BY UPLC®/MS/MS WITH A NOVEL DUAL SCAN-MRM APPROACH.

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Abstract

The potential of a new acquisition mode in LC/MS/MS instrumentation applied to the analysis of perfluorinated compounds (PFCs) is discussed. Matrix background monitoring during the quantitation of PFCs using a novel dual scan-MRM mode of operation was performed. This was utilised in combination with rapid UPLC separation for the analysis of environmentally significant samples such as tap water, surface water, river water and salmon liver. Dual scan-MRM acquisitions allowed correlations between background matrix components and analytical problems to be observed, particulary for humic and fulvic substances in environmental waters and indigenous bile acids in salmon liver.

Introduction

Perfluorinated compounds (PFCs) have been determined over the last ten years in an array of matrices by various techniques with liquid chromatography tandem quadrupole mass spectrometers (LC/MS/MS) featuring heavily.[1] More recently UPLC has been introduced as a technique utilised in the analysis of PFCs and has offered rapid analysis whilst preserving separations.[2] The ability of laboratories to successfully measure PFCs in various matrices has improved greatly in recent times, largely due to improvements in labelled standard availability highlighted in recent inter laboratory studies.[3,4] These studies also attribute the continuous improvement in data quality to advances in instrumental technology. Advances in LC/MS/MS instrumental performance have largely been focussed on Multiple Reaction Monitoring (MRM) sensitivity to satisfy the need for increasingly lower detection limits. While this is clearly a priority for this type of instrumentation there has previously been limitations in acquiring important qualitative information from a sample in a single injection. This information can be of high value when analysing ultra trace level contaminants in difficult sample matrices such as environmental waters and biological tissues when trying to further improve quality of methods and subsequent data.

Materials and Methods

Perfluorobutanesulfonate (PFBuS) tetrabutylammonium salt (>98%), PFOS potassium salt (>98%), perfluorodecanoic acid (PFDA; >97%), and perfluorohexanoic acid (PFHxA, >97%) were purchased from Fluka. Perfluoroheptanoic acid (PFHpA, 99%), perfluorononaoic acid (PFNA, 97%), perfluorooctanoic acid (PFOA, 96%), perfluoroundecanoic acid (PFUnDA, 95%), perfluorododecanoic acid (PFDoDA; 95%) were purchased from Aldrich (Steinheim, Germany, and Milwaukee, WI, USA). *1H,1H,2H,2H*-PFOS (THPFOS, purity unknown), and perfluorohexanesulfonate (PFHxS, 98%) were purchased from Interchim (Montlucon, France). $^{13}C_4$-labeled PFOA, $^{13}C_4$-labeled PFOS and $^{13}C_5$-labeled PFNA were from Wellington Laboratories (Guelph, Ontario, Canada).

Environmental water samples were obtained from Lake Mariestadssjön, River Svartån and various drinking water sources in Sweden. Salmon liver were from unknown locations in Norway.

Water samples were stored at 4°C until analysis and filtered through glass microfiber filters (Whatman, Schleicher and Schuell, Maidstone, UK). Extraction was performed by solid-phase extraction using Oasis WAX (Waters Corp., Milford USA) according to standard method ISO 25101[5] Detailed method description for salmon liver extraction are found elsewhere.[6] In short, acetonitrile extraction was followed by clean-up using Oasis WAX and dispersive carbon (Supelclean ENVI-Carb 120/400 mesh, Supelco Bellefonte, PA).

Sample extracts and standard solutions were prepared so that the solvent composition was 40:60 MeOH:H_20 with 2 mM ammonium acetate.

The analytical UPLC/MS/MS system used was a Waters Xevo™ TQ MS (Waters Corp, Milford USA) operated in ESI negative dual scan-MRM mode. This system was fitted with a PFC column kit (Waters Corp, Milford USA) which removes system PFCs. A flow rate of 0.65 mL min^{-1} was utilised with an ACQUITY® BEH C_{18} 1.7µm, 2.1 x 50 mm UPLC column at 50°C. 10 µl injections of samples and standards were made and a gradient elution of analytes performed. Initial mobile phase composition was 75 % (98:2) 2mM ammonium acetate $_{(aq)}$:MeOH : 25% MeOH (+ 2mM ammonium acetate) held for 0.5 min then to 85% MeOH (+ 2mM ammonium acetate) at 5 min, then increasing MeOH (+ 2mM ammonium acetate) to 100% at 5.1min held until 6.6 min after which column was re-equilibrated to initial conditions.

Scanning method for dual scan-MRM was MS2 scanning ESI –ve 50-650*m/z* with a scan time of 0.2 s. Simultaneously acquired MRM acquisition conditions are shown in Table 1

Table 1 MRM acquisition parameters

RT (min)	PFC	Precursor m/z	Product m/z	Cone (V)	Collision Energy (V)
1.67	PFBuS	299	80	40	30
		299	99	40	31
2.37	PFHxA	313	119	16	17
		313	269	16	10
3.05	PFHpA	363	119	16	17
		363	169	16	19
		363	319	16	10
3.13	PFHxS	399	80	45	33
		399	99	45	31
3.52	THPFOS	427	80	42	30
		427	407	42	19
3.55	PFOA	413	169	16	19
		413	219	16	17
		413	369	16	10
3.55	13CPFOA	417	372	16	10
3.94	PFNA	463	169	16	19
		463	219	16	17
3.94	13CPFNA	468	423	16	10
3.97	PFOS	499	80	60	39
		499	99	60	38
3.97	13CPFOS	503	80	60	39
4.27	PFDA	513	219	16	17
		513	469	16	10
4.56	PFUnDA	563	319	16	17
		563	519	16	10
4.80	PFDoDA	613	169	16	22
		613	569	16	10

Results and Discussion

Rapid UPLC separations of PFCs were achieved with PFDoDA eluting at 4.8 minutes. This allowed for a high sample throughput on the analytical system. The flow rate used in the analysis was 0.65 mL min^{-1} which is within the optimum range for UPLC. This helped reduce chromatographic band broadening and resulting in peak widths of approximately 3 s for all compounds. Source design improvements have enabled optimum UPLC efficiency at higher flow rates to be utilised without adversely affecting instrumental sensitivity.

After quantitation of Multiple Reaction Monitoring (MRM) transitions simultaneously acquired full scan Total Ion Current (TIC) chromatograms of all samples were reviewed to observe regions of potential matrix effects and to investigate possible sources of interference. Figure 1a shows overlaid and normalised dual scan-MRM chromatograms for detected native PFCs from a Lake Mariestadssjön sample. Dual scan-MRM acquisitions allowed enough sensitivity for low level detections (0.23 ng L^{-1} to 1.30 ng L^{-1}) of many of the native PFCs as well as providing information about the complexity of the each sample matrix. Figure 1b is a combined full scan spectrum taken from 0-1 minutes in the chromatogram. This spectrum is characteristic of humic and fulvic substances often found as the principal matrix component in environmental samples[7]. These substances contribute to the majority of the ion current in the sample and have been shown to cause matrix suppression in electrospray ionisation[8] likely due to the larger charge distribution upon ionisation. These humic and fulvic substances also appear to significantly increase the background noise level for PFBuS and could therefore effect detection limits. The ability to observe the elution region for these substances in each sample at the same time as acquiring MRM transitions for target PFCs allows for greater confidence when good chromatographic separations are achieved and for appropriate action to be taken where problems occur.

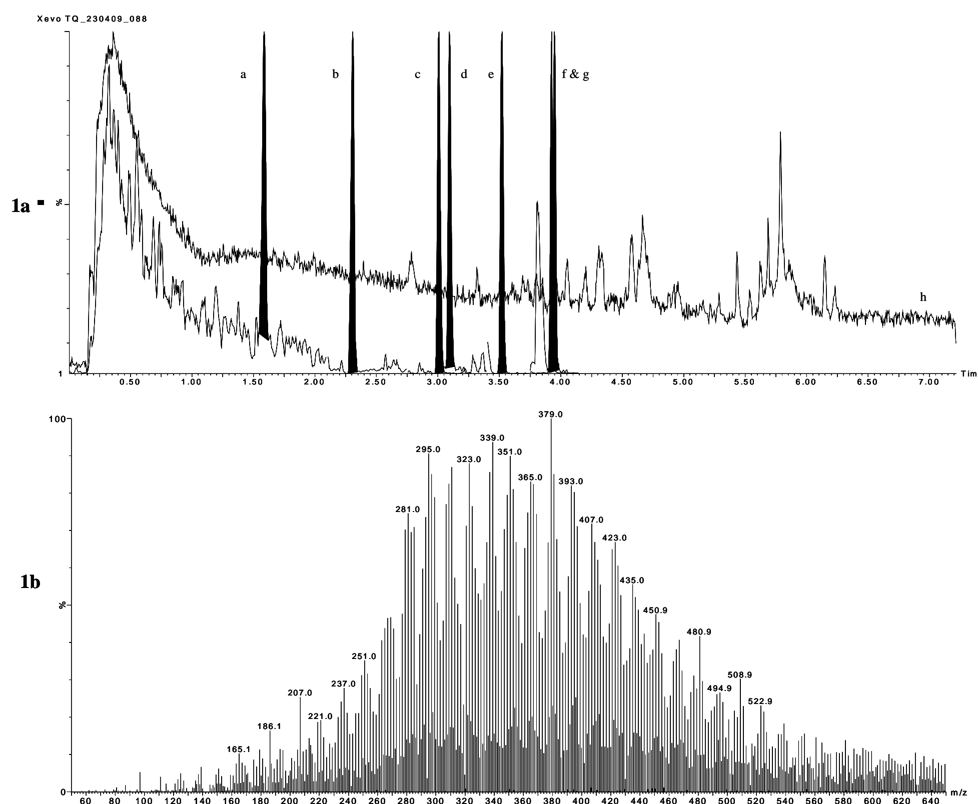

Figure 1 (1a) Dual Scan-MRM overlaid chromatograms of a non-fortified Lake Mariestadssjön sample. Detected PFC MRM chromatograms (normalised) (a) PFBuS 0.23 ng L^{-1} (b) PFHxA 0.41 ng L^{-1} (c) PFHpA 0.69 ng L^{-1} (d) PFHxS 0.42 ng L^{-1} (e) PFOA 1.30 ng L^{-1} (f&g) PFNA 0.45 ng L^{-1} & PFOS 1.30 ng L^{-1} and (h) Full scan 50-650 *m/z* TIC (1b) Combined spectrum 0-1 mins showing presence of humic and fulvic substances.

In the case of more complex samples such as fish liver, dual-scan-MRM data was available to troubleshoot observed analytical problems. Dual scan-MRM acquisitions of a non-fortified salmon liver sample revealed some difficulties with the analysis, retention time shifting as well as PFOS interference on the 499>80 transition. Figure 2 shows the overlaid dual-scan MRM experiment for a non-fortified salmon liver sample with the MRM transitions for PFOS extracted. The full scan TIC chromatogram indicated that two extremely concentrated matrix components elute in a critical region in the chromatogram, one of which eluting between 3.82 mins and 3.96 mins (b in Figure 2) interfering with PFOS 499 >80 transition. Mass spectra from these components indicated these as possible derivatives of taurocholate bile acids.

Additional evidence for these compounds were obtained using product ion scanning (Figure 3) which indicated the presence of deoxytaurocholate isomers co-eluting with PFOS. This component is a known interference for human serum analyses and using 499>99 transition allows for more accurate quantitation when using ^{13}C labelled standards.[9] The other abundant matrix component eluting between 3.00 and 3.64 min (a in Figure 2) with a 514 m/z pseudo-molecular ion was also investigated using product ion scanning. The product ion spectrum from this component strongly correlated with that from deoxytaurocholate and subsequent literature searching indicates this component to be one or more isomers of taurocholate[10].

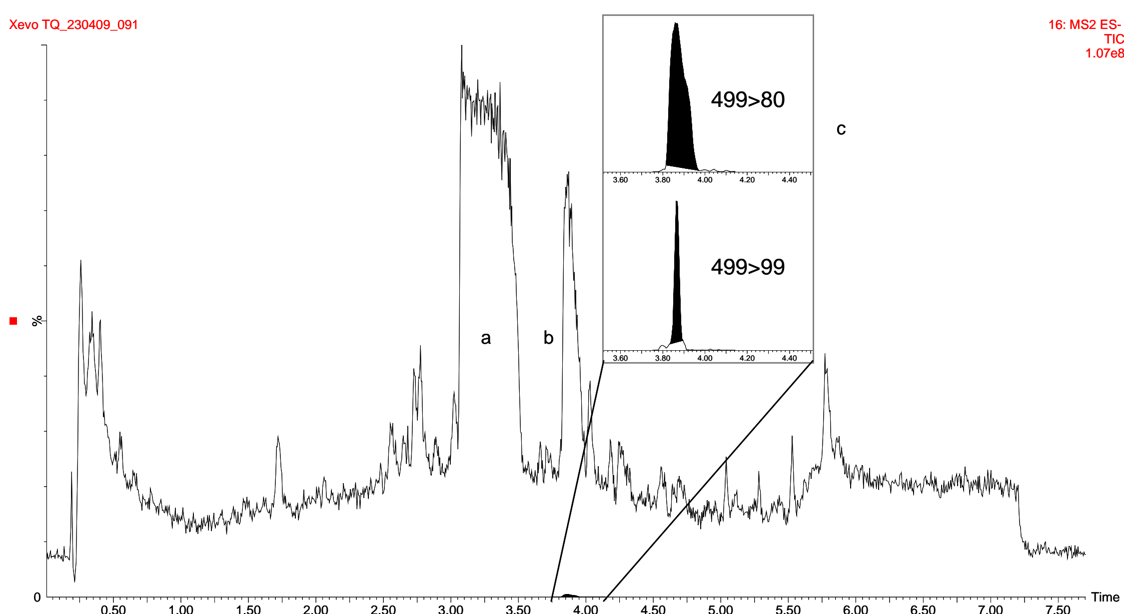

Figure 2 Dual Scan-MRM overlaid chromatograms of a non-fortified salmon liver sample. Full scan 50-650 *m/z* TIC indicating likely matrix components (a) taurocholate and (b) deoxytaurocholate with inset (c) PFOS MRMs showing 499 > 80 interference

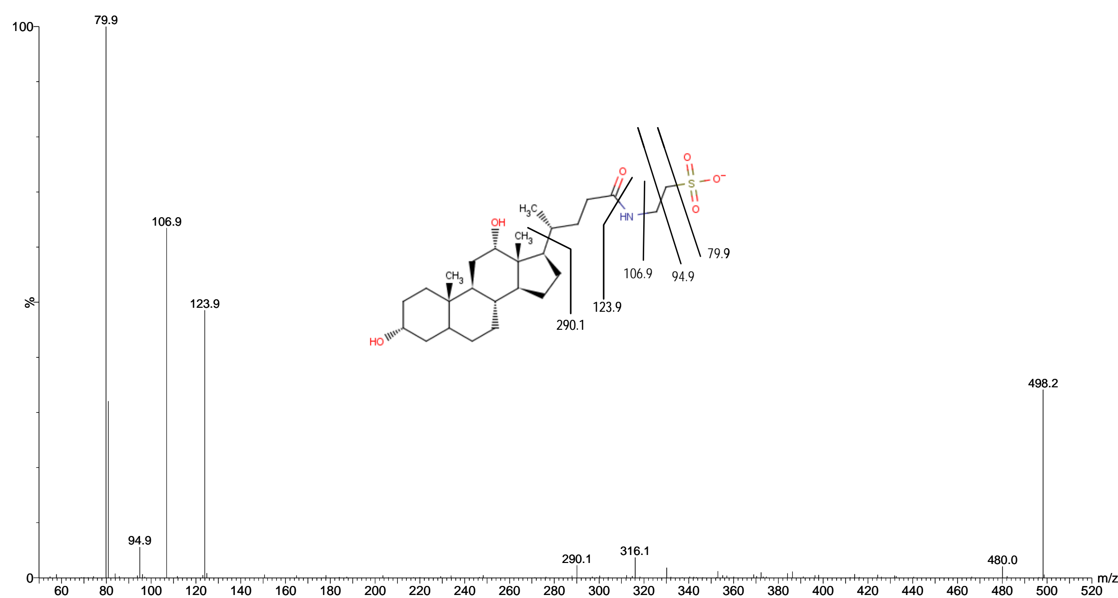

Figure 3 MS/MS product ion scan of 498 *m/z* indicating presence of deoxycholate

No direct interference with any of the targeted PFCs was noted from taurocholate but it is thought that this along with deoxycholate is responsible for adverse retention times (as detailed in Table 2) due to stationary phase saturation. The subsequent dilution of this sample and re-analysis improved retention time for the majority of target analytes as loading on the analytical column is reduced. Dilution of matrix components is not an ideal approach as errors are introduced into the analysis and detection limits are compromised.

Table 2 Retention times of PFCs in salmon liver extract and 5x diluted salmon liver extract compared with a solvent standard (STD). (a) Elution region of major matrix components taurocholate and deoxytaurocholate. ND=not detected, *ND due to retention time shift out of acquisition window.

	Retention Time (min)		
	STD	**Salmon Liver**	**Salmon Liver Diluted**
PFBuS	1.67	1.67	1.67
PFHxA	2.36	2.35	2.36
PFHpA (a)	3.04	3.00	3.04
PFHxS (a)	3.13	3.06	3.13
THPFOS (a)	3.52	ND	ND
PFOA (a)	3.54	ND*	3.44
^{13}CPFOA (a)	3.54	ND*	3.45
PFNA (a)	3.94	4.00	3.94
^{13}CPFNA (a)	3.94	3.86	3.93
PFOS (a)	3.96	3.87	3.94
^{13}CPFOS (a)	3.96	3.87	3.95
PFDA	4.27	4.26	4.27
PFUnDA	4.55	4.55	ND
PFDoDA	4.80	ND	ND

There has been successful separation for the PFOS interferent deoxycholate using an ion exchange column[11] However; it would be preferable to remove these matrix components before instrumental analysis ideally on or before sample extraction. The presence of an amide group could allow for a hydrolysis of both of these components either by chemical means (acid/base hydrolysis) or possibly using enzymes. Due to their stability PFCs may be resistant to the hydrolytic conditions need for these matrix bile acids if chemical means are necessary.

Further work is required to manage the negative effects of matrix in PFC analysis but continuously monitoring sample background using a dual-scan MRM approach can lead to more information about the challenges of each individual sample. This is a novel intra-sample QC check that has the potential to help improve quality within PFC analysis and is a possibility brought by next generation instrumentation.

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