Determination Of Perfluorinated Alkyl Substances (PFAS) In Sewage Treatment Plant Effluents And Biosolids By Liquid Chromatography – Tandem Mass Spectrometry

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Introduction

Perfluoro alkyl substances are specialty chemicals having a polar moiety attached to a non-polar alkyl chain composed principally of C-F bonds. Their wide spread use stems from their stability under extreme heat and chemical stress and ability to reduce surface tension imparting oil and water repellency. These chemicals are used in a variety of applications including industrial polymers (Teflon™), paper coatings, stain repellents (ScotchGard™, Stainmaster™) and aqueous film forming foams (AFFF).

Perfluoro alkyl substances (PFAS) are toxic, bioaccumulative, persistent and undergo long range transport qualifying them as persistent organic pollutants (POPs) under the United Nations Environmental Programme definition. These chemicals bind to proteins in the blood, influencing hormone feedback systems, causing a multitude of toxicological effects from reproduction problems such as postnatal deaths to changes in cholesterol/triglyceride levels and cell membrane permeability and thyroid/liver tumours[1]. 3M Company announced in May of 2000 that it would begin to voluntarily phase-out perfluorinated type surfactants based on initial toxicity and epidemiology data[2]. Animal studies showed that perfluorooctane sulfonate concentrations in liver and serum increase dramatically with exposure[3]. Bioconcentration factors in fish can exceed 100,000x. The magnitude of the bioconcentration factor increases with increased alkyl chain length[4]. Once present in biota or ecosystems, PFAS are extremely persistent. Perfluorooctane sulfonate for example, has an estimated mean half-life of 4-8 years in humans and over 1000 years in the environment[5]. Using toxicity data and European Chemicals Bureau (ECB) protocols, maximum permissible concentrations (iMAC) for perfluorooctane sulfonate (PFOS, $C_8F_{17}SO_3^-$) and perflurooctanoic acid (PFOA, $C_7F_{15}CO_2^-$) in surface waters of 5 ug/L and 300 ug/L respectively have been calculated[6]. A Drinking Water Health Advisory of 1 ug/L for PFOS can be derived using United States Environmental Protection Agency criteria[7].

PFAS have been detected in human serum, various surface waters, sediments and dust in both industrialized and remote locations throughout the world[8,9,10,11]. The presence of PFOS and PFOA in biota from the Arctic indicates that some mechanism for long range transport or in-situ formation from precursors such as fluorotelomer alcohols exists[12]. Even though the occurrence of these compounds in the environment has been established, there is limited information on the actual sources of these chemicals to the environment. Previous studies have identified sewage treatment plant effluents and biosolids as a source of various contaminants to the environment including PFAS[13]. In this study, sewage treatment plant (STP) biosolids and final effluents were tested for the presence of perfluorinated alkyl substances.

Material and Methods

The detection and measurement of perfluorinated alkyl compounds is challenging because of their volatility, polarity and surface-active properties. Issues involving sample/standard/extract handling, compound isolation from environmental matrices and laboratory background contamination (sample preparation and instrumentation) makes analytical testing complex[14]. The non-volatile nature of PFAS dictates the use of liquid chromatography (HPLC) separation/instrument introduction techniques. All sample preparation was done using polypropylene or polyethylene labwares to minimize PFAS losses as well as avoid contamination associated with Teflon™.

PFAS and 7H-dodecafluoroheptanoic acid were obtained as crystals (> 98% purity) or solutions from Sigma-Aldrich (Oakville, Ontario Canada), SynQuest Labs (Alachua, Florida USA), Oakwood Products (West Columbia, South

Carolina USA) and Chiron AS (Trondheim, Norway). Solutions of $^{13}C_2$ labelled PFOA and PFDA were purchased from Perkin Elmer Life Sciences (Boston, Massachusetts USA) and Wellington Laboratories (Guelph, Ontario Canada). PFAS crystals were weighed and dissolved in methanol (Fisher, Toronto Canada). Standard solutions were stored in high density polyethylene bottles in the dark at -15°C.

Biosolid samples were processed using a modified Hansen method[15]. Samples (~ 5 g) were air dried, weighed and reconstituted with distilled water. After addition of surrogate standard (7H-dodecafluoroheptanoic acid), 0.25 M sodium carbonate and 0.50 M tetrabutylammonium hydrogen sulfate (ion pairing reagent) solutions, samples were homogenized. Slurries were then extracted twice with 5mL aliquots of methyl tert-butyl ether (MTBE). MTBE extracts were isolated by centrifuging and decanting, combined, evaporated to dryness under a gentle stream of nitrogen and reconstituted in 1mL of methanol. Final extracts were filtered through 0.2um nylon syringe filters and stored in the dark at 4°C until instrumental analysis.

Final effluent samples were processed entirely in Mini-UniPrep^{TM} polypropylene syringeless filter devices (Whatman, Florham Park, New Jersey USA). Samples were diluted 1:1 with methanol, containing labelled internal standards ($^{13}C_2$-PFOA and $^{13}C_2$-PFDA), in the 750uL polypropylene autosampler vial housing and then forced through a 0.2um polypropylene filter media by compressing the vial housing and polypropylene-capped filter housing together. The autosampler vial / filter assembly was then placed directly in the LC autosampler for injection and LC-MS/MS analysis.

Instrumental analysis of biosolid extracts was done using a Waters LC - Micromass Quattro Micro^{TM} triple quadrupole mass spectrometer (Beverly, Massachusetts USA). Liquid chromatographic separations were performed on a 4mm x 2.0mm Phenomenex SecurityGuard^{TM} C18 guard column and 50mm x 2.1mm x 4um Jones Genesis^{TM} C8 analytical column (Chromatographic Specialties, Brockville, Ontario Canada) using a water/methanol (+ 10mM ammonium acetate) gradient at 200µL/minute. Twenty (20) microliters of extract was injected. The mass spectrometer was operated in negative electrospray ionization (ESI) MRM mode. Typical source/mass spectrometer conditions were used: nebulizer gas – 600L/hr N_2 at 210°C; source temperature – 150°C; capillary voltage – 2.75kV, collision gas – 5 x 10^{-3}mBar argon. Q1 and Q3 resolution were set at unit resolution - 0.7amu FWHM. Capillary cone voltage and collision energy were optimized for each MRM transition (Table 1).

Instrumental analysis of final effluent samples was done using an Agilent 1100^{TM} LC coupled to an Applied Biosystems/Sciex 4000QTrap^{TM} triple quadrupole mass spectrometer (Concord, Ontario Canada). Liquid chromatographic separations were performed on a 4mm x 2.0mm Phenomenex SecurityGuard^{TM} C18 guard column and 50mm x 2.1mm x 4um Jones Genesis^{TM} C8 analytical column using a 20:80 water/methanol (+ 10mM ammonium acetate) mobile phase at 250µL/minute. One hundred (100) microliters of extract was injected. The mass spectrometer was operated in negative electrospray ionization (ESI) MRM mode. Typical instrument settings were used: nebulizer gas (N_2) – 45; desolvation gas (N_2) – 60 at 400°C; curtain gas (N_2) – 10; interface heater – 100°C; needle voltage – -4.5kV; entrance potential – -10; collision gas (N_2) – 7. Q1 and Q3 resolution were set at unit resolution - 0.7amu FWHM. Declustering potential, collision energy and collision cell exit potential were optimized for each MRM transition (Table 2).

The MRM transitions used for quantification were $[M-H]^-$® $[SO3]^-$ or $[FSO3]^-$ for perfluorinated sulfonates and $[M-H]^-$® $[M-COOH]^-$ for perfluorinated carboxylates (Tables 1 and 2). Quantitation was done by external standard multipoint calibration. $^{13}C_2$ labelled PFOA and PFDA were added to extracts before injection in order to allow compensation for matrix effects and instrument variability. Reagent blanks and spikes were processed with each batch of samples.

Results and Discussion

In general, surface and potable water sources are likely to have part-per-trillion (ng/L) or lower PFAS levels. To minimize both target compound losses and contamination problems at these levels direct sample introduction would be advantageous. The LC-4000QTrap^{TM} MS/MS had sufficient sensitivity to directly inject water samples and achieve 1 - 4 ng/L (fg/uL) detection limits for individual PFAS. In order to routinely inject water, especially surface and

effluent samples, into an LC-MS/MS without performance loss and unscheduled maintenance / plugging problems, samples must be filtered prior to injection. During the course of investigating filtration options it was observed there was preferential adsorption of individual PFAS on nylon syringe filters. This was not observed at high fg/uL or pg/uL concentrations but evident at low fg/uL concentrations. Two steps were taken to combat the phenomena; 1) polypropylene filters were substituted for nylon filters and 2) water extracts were diluted 50:50 with methanol to decrease the tendency of PFAS to adsorb to the filters or sample particulates. Addition of methanol to the water sample also allowed the introduction of $^{13}C_2$ labelled PFAS which could be used to compensate for system variability and matrix effects. Whatman Mini-UniPrep^{TM} filtering devices simplified sample preparation further by allowing dilution, filtration and injection to be done in one vessel with no sample transfer.

A water/methanol gradient was used for liquid chromatographic (LC) separate of PFAS in biosolids and fish extract testing with good success. The advantage of gradient elution is in the sharpness of the peaks and separation of target compounds from unwanted interferences (reduced matrix effects). Usually PFAS levels in biosolids and fish extracts are relatively high – pg/uL. When gradient elution methods are used for PFAS separation in low level (fg/uL) applications, PFOA contamination is observed. At low methanol content PFOA present in the LC mobile phase and leaching out of Teflon^{TM} instrument parts (seals, lubricants, etc.) accumulates on the analytical column eluting during the gradient program along with any PFOA present in the injected sample. The low level PFOA contamination, equivalent to ~ 5-20 fg/uL, is not an issue for samples at the pg/uL level, but for low level direct injection it can cause problems. Many factors influence the extent of the PFOA contamination but it is not consistent enough for blank subtraction purposes. PFAS separations can be accomplished in 5-8 minutes with peak widths of 10-25 seconds and adequate sensitivity using an isocratic mobile phase. The use of an isocratic separation results in a constant bleed of the PFOA (consistent elevated baseline) over which any PFOA contribution from an injection can be observed and quantified with accuracy/precision. The low matrix background in most waters combined with no sample concentration during preparation allows the majority of interferences to elute off the LC column before the PFAS peaks.

Combining minimal sample manipulation, $^{13}C_2$ labelled compound addition and isocratic LC separation results in a fast robust low level analytical method. Analysis of nine water samples fortified at 5ng/L and 50ng/L gave average PFOS / PFOA results of 4.6 / 5.3 ng/L (RSD <15%) and 48.4 / 47.0 ng/L (RSD < 5%) respectively. PFOS and PFOA results produced by the direct injection and traditional C18 solid phase extraction (SPE) methods for duplicate naturally contaminated surface waters were comparable.

The direct sample introduction LC-MS/MS method developed for part-per-trillion testing of waters was applied to the testing of STP final effluents. PFOS and PFOA were detected in all the STP final effluents tested at concentrations of 17 – 100 ng/L and 10 – 34 ng/L respectively (Table 3). It appears that some PFAS survive the STP treatment process and are discharged into ambient surface waters.

Sewage treatment plant and paper fibre biosolids were also analysed. PFAS were detected in all biosolids tested at parts-per-billion levels (Table 3). Biosolids PFAS profiles were dominated by PFOS but differences in PFAS patterns could be observed between sources (Figure 1). Paper fibre biosolids had much higher percentages of the perfluorocarboxylic acids. No statements with regard to the correlation between PFAS content of STP final effluents and STP biosolids could be made because samples were obtained from the same locations but at different dates. Biosolid samples were also analysed by GC-HRMS for other persistent organic pollutants (POPs) including polychlorinated dioxins and furans (PCDD/Fs), dioxin-like polychlorinated biphenyls (DLPCBs) and brominated diphenyl ethers (BDEs). No correlation between the POPs and PFAS results could be determined although PFAS appear to contribute significantly to the POPs content of the STP biosolids (Figure 2).

Several perfluorinated alkyl substances were detected in STP final effluents and biosolids collected at several Ontario, Canada locations. Further investigation is required to establish both the PFAS levels and their fate during sewage treatment processes and to assess environmental and human health impacts.

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Table 1 – Waters (Micromass) Quattro Micro MS/MS Acquisition Parameters					
Compound (alkyl chain length)	**Acronym**	**MRM Transitions**		**Cone Voltage** (V)	**Collision Energy** (eV)
		Parent Ion (m/z)	**Product Ion** (m/z)		
Perfluorohexane sulfonate (6)	PFHxS	399	99	45	35
Perfluorooctane sulfonate (8)	PFOS	499	99	55	45
Perfluoroheptanoic acid (7)	PFHpA	363	319	14	12
Perfluorooctanoic acid (8)	PFOA	413	369	15	12
Perfluorononanoic acid (9)	PFNA	463	419	16	12
Perfluorodecanoic acid (10)	PFDA	513	469	17	14
Perfluoroundecanoic acid (11)	PFUA	563	519	17	14
Perfluorododecanoic acid (12)	PFDoA	613	569	17	14
Perfluorotetradecanoic acid (14)	PFTeA	713	669	17	14
7H-Dodecafluoroheptanoic acid (7)	7H-PFHpA	345	281	14	12

Table 2 – Applied Biosystems (MDS Sciex) 4000QTrap MS/MS Acquisition Settings						
Compound (alkyl chain length)	**Acronym**	**MRM Transitions**		**Decluster Potential**	**Collision Energy**	**Collision Cell Exit Potential**
		Parent Ion (m/z)	**Product Ion** (m/z)			
Perfluorohexane sulfonate (6)	PFHxS	399	99	- 87	- 57	- 10
Perfluorooctane sulfonate (8)	PFOS	499	99	- 103	- 75	- 9
Perfluorodecane sulfonate (10)	PFDS	599	99	- 97	- 87	- 9
Perfluorooctane sulphonamide (8)	PFOSA	498	78	- 84	- 79	- 11
Perfluoroheptanoic acid (7)	PFHpA	363	319	- 43	- 14	- 8
Perfluorooctanoic acid (8)	PFOA	413	369	- 44	- 14	- 9
Perfluorononanoic acid (9)	PFNA	463	419	- 44	- 16	- 9
Perfluorodecanoic acid (10)	PFDA	513	469	- 54	- 15	- 9
Perfluoroundecanoic acid (11)	PFUA	563	519	- 49	- 16	- 9
Perfluorododecanoic acid (12)	PFDoA	613	569	- 50	-18	- 11
C13-Perfluorooctanoic acid (8)	$^{13}C_2$-PFOA	415	370	- 44	- 14	- 8
C13-Perfluorodecanoic acid (10)	$^{13}C_2$-PFDA	515	470	- 55	- 16	- 13

Table 3 – Sewage Treatment Plant Final Effluent and Biosolids Results									
	PFHxS	**PFOS**	**PFOSA**	**PFHpA**	**PFOA**	**PFNA**	**PFDA**	**PFUA**	**PFDoA**
Final Effluents - ng/L									
TM STP	ND	100	ND	ND	33	ND	ND	ND	ND
GRK STP	ND	25	ND	ND	10	ND	ND	ND	ND
K WPCP	ND	17	ND	ND	34	ND	ND	ND	ND
Limit of Detection (LOD)	4	1	1	2	1	2	4	2	2
Biosolids - ng/g dry weight									
GRK STP (TP20)	4.0	600		3.6	0.7	1.1	5.2		
TM STP (TP21)	1.3	350		ND	0.7	2.4	5.1		
NF STP (TP23)	2.5	72		3.2	0.9	1.9	2.5		
HW STP (TP24)	ND	120		ND	ND	0.4	3.1		
Paper Fibre (PF15)	ND	2.7		0.6	ND	1.6	2.7		
Paper Fibre (PF16)	0.4	1.4		4.6	ND	4.8	4.1		
Paper Sludge (PS17)	0.6	28		6.4	0.8	10	13		
Paper Compost (PC18)	4.5	460		35	13	23	99		
Limit of Detection (LOD)	0.2	0.1		0.2	0.1	0.2	0.2		

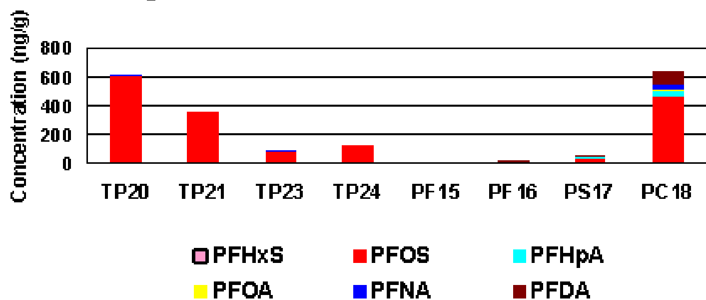
Figure 1 - Biosolids PFAS Profiles
Concentration (ng/g)
800
600
400
200
0
TP20
TP21
TP23
TP24
PF15
PF16
PS17
PC18
PFHxS
PFOS
PFHpA
PFOA
PFNA
PFDA

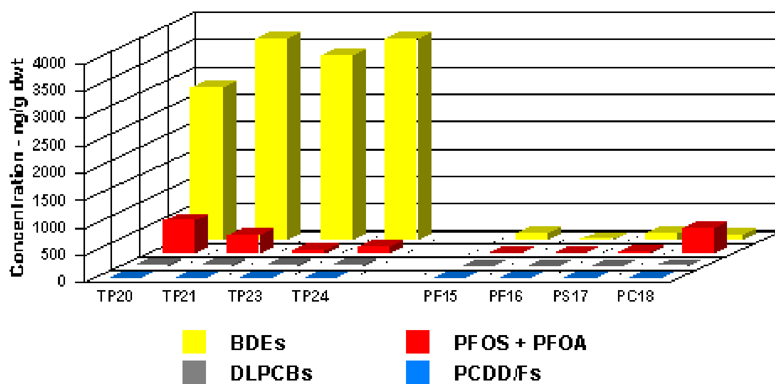
Figure 2 - Persistent Organic Pollutants Levels in Biosolids
Concentration - ng/g dwt
4000
3500
3000
2500
2000
1500
1000
500
0
TP20
TP21
TP23
TP24
PF15
PF16
PS17
PC18
BDEs
PFOS + PFOA
DLPCBs
PCDD/Fs

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