RESEARCH PAPER

A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices

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Abstract

A quantitative method for the determination of per- and polyfluoroalkyl substances (PFAS) using liquid chromatography (LC) tandem mass spectrometry (MS/MS) was developed and applied to aqueous wastewater, surface water, and drinking water samples. Fifty-three PFAS from 14 compound classes (including many contaminants of emerging concern) were measured using a single analytical method. After solid-phase extraction using weak anion exchange cartridges, method detection limits in water ranged from 0.28 to 18 ng/L and method quantitation limits ranged from 0.35 to 26 ng/L. Method accuracy ranged from 70 to 127% for 49 of the 53 extracted PFAS, with the remaining four between 66 and 138%. Method precision ranged from 2 to 28% RSD, with 49 out of the 53 PFAS being below < 20%. In addition to quantifying > 50 PFAS, many of which are currently unregulated in the environment and not included in typical analytical lists, this method has efficiency advantages over other similar methods as it utilizes a single chromatographic separation with a shorter runtime (14 min), while maintaining method accuracy and stability and the separation of branched and linear PFAS isomers. The method was applied to wastewater influent and effluent; surface water from a river, wetland, and lake; and drinking water samples to survey PFAS contamination in Australian aqueous matrices. The compound classes FTCAs, FOSAAs, PFPAs, and diPAPs were detected for the first time in Australian WWTPs and the method was used to quantify PFAS concentrations from 0.60 to 193 ng/L. The range of compound classes detected and different PFAS signatures between sample locations demonstrate the need for expanded quantitation lists when investigating PFAS, especially newer classes in aqueous environmental samples.

Keywords PFAS · Wastewater · Surface water · Drinking water · LC-MS/MS

Introduction

The contamination of the environment with per- and polyfluoroalkyl substances (PFAS) is a serious concern to regulators, scientists, and the public worldwide, due to their ubiquitous presence, persistence, toxicity, and potentially bioaccumulative properties [1–3]. To better understand the environmental fate, ecological impacts, and potential human exposure pathways, it is necessary to develop robust analytical techniques that can accurately and precisely quantify these pollutants at trace levels. Appropriate analytical techniques, and the fundamental data that they generate, will allow scientists to characterize PFAS environmental behavior, and help regulators make informed assessments of their use in modern society.

Historically, many studies have measured only a few perfluoroalkyl acids (PFAAs), with a focus on perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), which have since been phased out of production in North American (PFOS, by 3M, and PFOA, by DuPont) [4, 5]. Currently, PFOS and its salts are listed under Annex B of the Stockholm Convention for Persistent Organic Pollutants [6] and PFOA and perfluorohexane sulfonate (PFHxS) have been proposed for inclusion [7]. Drinking water guidelines have

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been set using these compounds such as the US EPA health advisory (70 ng/L PFOS + PFOA) [8] and the Australian Government health-based guidance values (PFOS 70 ng/L, PFHxS 70 ng/L, and PFOA 560 ng/L) [9]. However, the current industry practice has been to phase out these compounds and then replace them with structurally similar PFAS that are currently not routinely monitored or present on regulatory guideline lists [5, 10]. Therefore, there are now 4730 known PFAS [11] that have found many commercial uses [4, 5].

There is a need for quantitation of many unregulated and emerging PFAS in aqueous environmental matrices. In wastewater, many PFAAs have been detected worldwide with quantitation limits in the low to sub nanogram per liter range [12]. More recent studies have measured unregulated compound classes such as perfluoroalkane sulfonamides (FASAs), fluorotelomer alcohols (FTOHs), and fluorotelomer phosphate diesters (diPAPs) at similar concentrations to PFAAs and with similar limits of quantitation [12–14]. Some PFAS undergo transformation in wastewater treatment plants and the environment; therefore, PFAS precursors, transformation intermediates, and stable end products need to be included in quantitative methods [15–17].

In China, emerging compounds such as the chlorinated perfluoroether sulfonates (Cl-PFESAs) have been detected in wastewater treatment plants and the environment at similar levels as the compound they have replaced, PFOS [18, 19]. Furthermore, in the USA, the perfluoroalkyl ether carboxylic acids (PFECAs) have been measured at approximately eight times the summed concentration of PFCAs and PFSAs downstream of a PFAS manufacturer in North Carolina [20]. These perfluoroalkyl ether compounds have not yet been included in PFAS quantitation methods incorporating a broad range of PFAS classes.

Published analytical method papers for the determination of PFAS in aqueous environmental matrices that include compounds outside of the typically employed US EPA method 537.1 [21] list of compounds (PFCAs, PFSAs, and FOSAAs) are not widely abundant. Two recently published papers were for 29 compounds (27 PFAS, 10-min runtime, 5-μL injection and using an LC-MS/MS and 2 PFAS, 12-min runtime, 5-μL injection and using a liquid chromatograph coupled to a quadropole ion trap mass spectrometer, LC-QTRAP) [22] and 52 compounds (47 PFAS, 27-min runtime, 50-μL injection and using an LC-QTRAP in MS/MS mode and 5 PFAS, 17-min runtime, 20-μL injection and using an LC-QTRAP in MS/MS mode) [23]. Method quantitation limits in water after solid-phase extraction for these methods ranged from 4 to 10 ng/L for 6 PFAS compound classes [22] and 0.3–102 ng/L for 12 PFAS compound classes [23]. In both cases, a single analytical method was not appropriate and a second analytical method for a smaller number of PFAS was employed. Although both methods were extensively validated and robust, neither was validated using methods such as US

EPAs 40 CFR Part 136 Appendix B Revision 2 [24]. Calculation of MDLs using 40 CFR Part 136 Appendix B Revision 2 does have some limitations; however, it is employed by many commercial laboratories and was therefore considered the most applicable validation technique for our study.

The aim of our study was to screen the available PFAS analytical standards (including legacy and emerging compounds) for inclusion in a single quantitative analytical method using LC-MS/MS. The chromatographic separation was optimized for the greatest number of PFAS from the screened compound list, with reasonable runtime (≤ 15 min, with satisfactory chromatography and PFAS branched and linear isomer separation), accuracy, high sensitivity, and robustness. An extraction method was then validated using 40 CFR Part 136 Appendix B Revision 2 with MDLs below, or comparable with, currently existing PFAS analytical methods and applied to Australian environmental aqueous samples. This method can serve as an initial quantitation and screening method in environmental aqueous samples and then can be augmented with smaller boutique PFAS lists by individual laboratories if required.

The main advantages of the developed method are efficiency gains over existing methods through a runtime ≤ 15 min, detection limits equivalent to or below existing techniques, improvements in method accuracy and robustness, inclusion of emerging PFAS such as the perfluoroalkyl ethers among others, and a single solid-phase extraction and analytical method validated using 40 CFR Part 136 Appendix B Revision 2.

Materials and methods

Instrument configuration and background contamination elimination

In this study, we have validated a single extraction and analytical technique for the quantitation of 53 legacy and emerging PFAS in aqueous matrices using isotope dilution analytical methodology. Analysis was performed on an Agilent Technologies 1290 II infinity liquid chromatograph (LC) coupled with an Agilent Technologies 6495B tandem mass spectrometer (MS/MS). A suitable surrogate compound for each PFAS was determined using the most accurate response during method validation and set as a mass-labeled compound from a similar class and/or close elution time (Fig. 1). To improve analytical efficiency and increase practical applicability, the method included a range of legacy and emerging PFAS in a single chromatographic separation.

Setup of PFAS analytical methods can be difficult due to the prevalence of background contamination in all stages of extraction and analysis (including the LC-MS/MS system). The LC system was modified to control background PFAS contamination [25]. A delay column (Zorbax Eclipse Plus
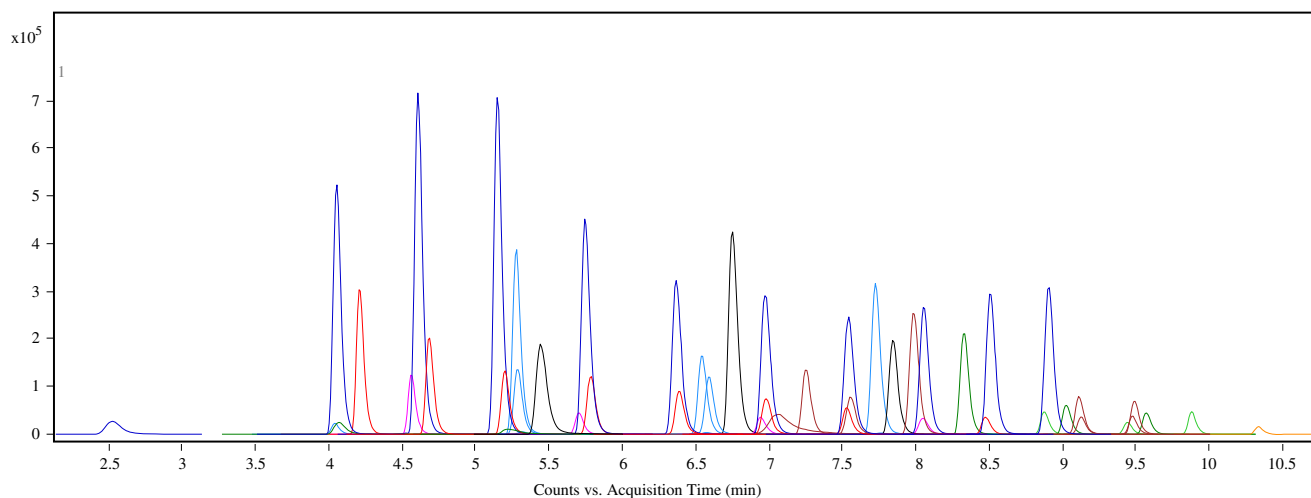
Fig. 1 Chromatogram of 53 compound simultaneous analytical method from 2-μL injection of 50 ng/mL standard. PFAS grouped by class listed in Table 1: dark blue (PFCA), red (PFSA), pink (*n*:2 FTS), light blue (*n*:2 FTCA, *n*:3 FTCA, and *n*:2 FTUCA), black (PFECA and Cl-PFESA), maroon (FASA, FASAA, and FOSE), green (PFPA, diPAP, and PFPiA), orange (diSAmPAP)

C18 RRHD, 4.6 × 50 mm, 3.5 μm, Agilent Technologies, USA) was installed between the solvent mixer and injector module. Peek tubing and steel solvent filters were installed in the needle wash system to replace ethylene tetrafluoroethylene (ETFE) lines and glass/polytetrafluoroethylene (PTFE) solvent filters. To reduce contamination due to sorption after injection, the needle wash procedure consisted of a 10-s wash with 50:50 ultrapure water:MeOH followed by a 10-s needle seat backflush using 90:10 ultrapure water:MeOH (an instrument blank chromatogram is included in the Electronic Supplementary Material, ESM).

For the duration of the study, MeOH batches were routinely monitored for background contamination. Each injection series included instrument blanks (the gradient program with no injection) and MeOH blank injections to monitor for background contamination. Each extraction batch of 12 samples contained at least one method blank to monitor any extraction-related background contamination. All method blanks were below MDL and time-delayed instrument contamination peaks (due to use of the delay column) were routinely monitored to eliminate instrument blank contamination (a chromatogram displaying the delayed instrument peak for PFBS is included in Fig. S1 in ESM).

Chromatographic separation was optimized for peak shape, and minimum 15 scans per peak. Analysis was performed in dynamic multiple reaction monitoring (dMRM), using a 2-μL injection, and in negative mode using an ESI source. A 2-μL injection was used to reduce possible injection volume variations as it was 10% of the installed injector loop (20 μL) in the injector module. Sufficient sensitivity was achieved on the MS with this injection volume, and hence additional injection volume was not needed which could have introduced more matrix and potentially increase the frequency of MS cleaning.

Chemicals and standards

Fifty-three PFAS analytical standards plus 21 isotopically labeled analogues from 14 compound classes were purchased from Wellington Laboratories (Ontario, Canada). The full compound list, including abbreviation, compound class, and CAS identifier, is found in Table 1. Compounds from the classes fluorotelomer phosphate monoesters (PAPs) and fluorotelomer alcohols (FOTHs) were considered for inclusion in the initial method but were removed during analytical method development due to the need for separate analytical methods. Standards were purchased as solutions of 50 μg/mL in methanol except for the saturated fluorotelomer carboxylic acids (2 μg/mL) and 8:2 FTCA (50 μg/mL), and the unsaturated fluorotelomer carboxylic acids and isotopically labeled 8:2 FTUCA (50 μg/mL) in isopropanol. The solvents methanol (MeOH, LC-MS grade, Honeywell, USA, and LiChrosolv hypergrade, Merck Millipore, Australia) and ultrapure water (pH 8, Merck Millipore, Australia) were tested for PFAS contamination prior to use. Ammonium hydroxide solution (28% in H_2O, ≥ 99.99%), sodium acetate, glacial acetic acid, and ammonium acetate (≥ 99.99%) were purchased from Sigma-Aldrich (Australia).

Stock solutions of 100 ng/mL for native and surrogate PFAS (except for FTCAs and FTUCAs) were prepared in MeOH for spiking. A separate set of stock native and surrogate solutions at a concentration of 2000 and 100 ng/mL in isopropanol were prepared for FTCAs, FTUCAs, and mass-labeled surrogates 8:2 FTCA-13C2 and 8:2 FTUCA-13C2.

Table 1 Compounds measured in this study with CAS identifier. Compounds were categorized as legacy (L) or emerging (E) using data presented in Xiao [26] and Wang et al. [10] and further categorized as replacement for C8 compounds (R) and transformation intermediates (T) according to known transformation pathways outlined in Liu and Mejia Avendaño [27] and Ruan et al. [28]

Compound class	Compound name	Acronym	CAS No.	Category
Perfluoroalkyl carboxylic acid (PFCA)	Perfluorobutanoic acid	PFBA	375-22-4	L, R
	Perfluoropentanoic acid	PFPeA	2706-90-3	L
	Perfluorohexanoic acid	PFHxA	307-24-4	L, R
	Perfluoroheptanoic acid	PFHpA	375-85-9	L
	Perfluorooctanoic acid	PFOA	335-67-1	L
	Perfluorononanoic acid	PFNA	375-95-1	L
	Perfluorodecanoic acid	PFDA	335-76-2	L
	Perfluoroundecanoic acid	PFUnA	2058-94-8	L
	Perfluorododecanoic acid	PFDoA	307-55-1	L
	Perfluorotridecanoic acid	PFTrA	72629-94-8	L
	Perfluorotetradecanoic acid	PFTeA	376-06-7	L
Perfluoroalkyl sulfonic acid (PFSA)	Perfluorobutane sulfonic acid	PFBS	375-73-5	L, R
	Perfluoropentane sulfonic acid	PFPeS	2706-91-4	L
	Perfluorohexane sulfonic acid	PFHxS	355-46-4	L
	Perfluoroheptane sulfonic acid	PFHpS	375-92-8	L
	Perfluorooctane sulfonic acid	PFOS	1763-23-1	L
	Perfluorononane sulfonic acid	PFNS	474511-07-4	L
	Perfluorodecane sulfonic acid	PFDS	335-77-3	L
	Perfluorododecane sulfonic acid	PFDoS	79780-39-5	L
Perfluoroether carboxylic acid (PFECA)	ADONA	ADONA	958445-44-8	E, R
Fluorotelomer carboxylic acid (*n*:2 FTCA)	6:2 Fluorotelomer carboxylic acid	6:2 FTCA	53826-12-3	T, R
	8:2 Fluorotelomer carboxylic acid	8:2 FTCA	27854-31-5	T
	10:2 Fluorotelomer carboxylic acid	10:2 FTCA	53826-13-4	T
Fluorotelomer unsaturated carboxylic acid (*n*:2 FTUCA)	6:2 Fluorotelomer unsaturated carboxylic acid	6:2 FTUCA	70887-88-6	T
	8:2 Fluorotelomer unsaturated carboxylic acid	8:2 FTUCA	70887-84-2	T
	10:2 Fluorotelomer unsaturated carboxylic acid	10:2 FTUCA	70887-94-4	T
Fluorotelomer carboxylic acid (*n*:3 FTCA)	3:3 Fluorotelomer carboxylic acid	3:3 FTCA	356-27-4	T
	5:3 Fluorotelomer carboxylic acid	5:3 FTCA	914637-49-3	T
	7:3 Fluorotelomer carboxylic acid	7:3 FTCA	812-70-4	T
Perfluoro phosphonic acid (PFPA)	Perfluorohexyl phosphonic acid	PFHxPA	40143-76-8	E
	Perfluorooctyl phosphonic acid	PFOPA	40143-78-0	E
	Perfluorodecyl phosphonic acid	PFDPA	52299-26-0	E
Fluorotelomer phosphate diester (diPAP)	6:2 Fluorotelomer phosphate diester	6:2 diPAP	57677-95-9	E
	6:2/8:2 Fluorotelomer phosphate diester	6:2/8:2 diPAP	943913-15-3	E
	8:2 Fluorotelomer phosphate diester	8:2 diPAP	678-41-1	E
Chlorinated perfluoroether sulfonate (Cl-PFESA)	6:2 Chlorinated perfluoroether sulfonic acid (F-53B)	6:2 Cl-PFE-SA	73606-19-6	E, R
	8:2 Chlorinated perfluoroether sulfonic acid	8:2 Cl-PFE-SA	83329-89-9	E, R
Fluorotelomer sulfonic acid (FTS)	4:2 Fluorotelomer sulfonic acid	4:2 FTS	757124-72-4	L
	6:2 Fluorotelomer sulfonic acid	6:2 FTS	27619-97-2	L, R
	8:2 Fluorotelomer sulfonic acid	8:2 FTS	39108-34-4	L
	10:2 Fluorotelomer sulfonic acid	10:2 FTS	120226-60-0	L
Perfluoroalkane sulfonamide (FASA)	Perfluorooctane sulfonamide	FOSA	754-91-6	L, T

Table 1 (continued)

Compound class	Compound name	Acronym	CAS No.	Category
	N-Methyl perfluorooctane sulfonamide	MeFOSA	31506-32-8	L, T
	N-Ethyl perfluorooctane sulfonamide	EtFOSA	4151-50-2	L, T
Perfluoroalkane sulfonamido acetic acid (FASAA)	Perfluorooctane sulfonamido acetic acid	FOSAA	2806-24-8	L, T
	N-Methyl perfluorooctane sulfonamido acetic acid	MeFOSAA	2355-31-9	L, T
	N-Ethyl perfluorooctane sulfonamido acetic acid	EtFOSAA	2991-50-6	L, T
Perfluoroalkane sulfonamido ethanol (FASE)	N-Methyl perfluorooctane sulfonamido ethanol	MeFOSE	24448-09-7	L, T
	N-Ethyl perfluorooctane sulfonamido ethanol	EtFOSE	1691-99-2	L, T
Disubstituted perfluoro phosphinic acid (PFPiA)	6:6 Perfluorophosphinic acid	6:6 PFPiA	40143-77-9	E
	6:8 Perfluorophosphinic acid	6:8 PFPiA	610800-34-5	E
	8:8 Perfluorophosphinic acid	8:8 PFPiA	40143-79-1	E
Perfluorooctane sulfonamido-ethanol-based phosphate diester (diSAmPAP)	Perfluorooctane sulfonamido-ethanol-based phosphate diester	diSAmPAP	30381-98-7	E

Data processing and statistical analysis

LC-MS/MS quantitation data were processed using MassHunter QQQ quantitative analysis software (version 08.00, Agilent Technologies, USA). Raw data were processed and formatted in R [29], and data visualizations were produced using Microsoft Excel (Microsoft Corporation).

Linear calibration curves were constructed for six to nine levels by gravimetric dilution of 100 ng/mL native compound mixture in MeOH with lowest concentrations ranging from 0.05 to 1 ng/mL (example calibration curves are presented in ESM). Each calibration standard had a surrogate concentration of 5 ng/mL to match the expected concentration of 5 ng/mL in final extracts.

Solid-phase extraction

Some extraction techniques have been moving towards online solid-phase extraction approaches, with recent quantitation limits of 0.2–5 ng/L for 12 PFCAs and PFSAs [30]; however, online solid-phase extraction techniques have not yet been applied to expanded PFAS quantitation lists, which require specialist LC equipment and extensive validation procedures to find appropriate sorbents for the entire range of PFAS compound classes. As such, offline solid-phase extraction (SPE) was deemed most appropriate for this study and performed using methods similar to our previously published method [31]. Briefly, 250-mL samples were first spiked with isotopically labeled PFAS at a concentration of 20 ng/L and extracted using Oasis weak anion exchange cartridges (6 mL, 150 mg WAX, Waters Corporation). SPE cartridges were conditioned sequentially with 4 mL 0.1% (*v/v*) ammonium hydroxide in methanol, 4 mL methanol, and 4 mL ultrapure water. The entire sample was passed through the cartridge under vacuum at approximately one drop per second, washed with 4 mL of a pH 4 buffer (sodium acetate/acetic acid), and dried under vacuum for 10 min. SPE cartridges were eluted using 2 mL MeOH that was used to rinse the sample bottle, followed by 4 mL of 0.1% (*v/v*) ammonium hydroxide in methanol. Extracts were evaporated to 500 μL under a gentle stream of nitrogen (at 25 °C) and reconstituted to 1 mL in MeOH and split into a polypropylene cryovial for storage and a polypropylene chromatography vial with polyethylene lid for analysis.

Optimization of triple quadrupole mass spectrometer

Tandem mass spectrometer (MS/MS) conditions were optimized for each compound through experiments varying MS/MS parameters using multiple injections of a 500 ng/mL analytical standard (Table 2). The compound responses were then compared, and the method parameters optimized to give the best response for the largest range of compounds included in the method. In 8 of the 53 compounds, only one ion transition was present with the method instrument parameters; therefore, similar to previously reported methods [23], only one ion transition was used for quantitation. For compounds where two or more transition ions were present, the transition with the highest response was set as the quantifier and the others set as qualifier ions in the dynamic multiple reaction monitoring (dMRM) method.

MS/MS parameters most suitable for the 53 compounds were as follows: gas temperature 250 °C, gas flow 11 L/min, nebulizer 25 psi, sheath gas temp 375 °C, sheath gas flow 11 L/min,

Table 2 Analytical method details for simultaneous quantitation of 53 PFAS. Data for product ion displayed as quantifier (qualifier). Relative response of qualifier ion to quantifier ion (Rel. Resp.) is displayed as a percentage

Compound	Prec (m/z)	Prod (m/z)	CE (V)	Rel. resp. (%)	R_t (min)	Surrogate
PFBA	213	169	6	–	2.54	PFBA-13C3
PFPeA	263	219	6	–	4.05	PFPeA-13C3
PFHxA	313	269 (119)	6 (22)	4.0	4.61	PFHxA-13C2
PFHpA	363	318.9 (168.9)	6 (18)	20	5.15	PFOA-13C8
PFOA	413	368.9 (169)	6 (18)	30	5.74	PFOA-13C8
PFNA	463	418.9 (218.9)	10 (18)	28	6.37	PFDA-13C2
PFDA	512.9	469 (268.9)	6 (18)	21	6.97	PFDA-13C2
PFUnA	563	518.9 (268.9)	12 (16)	21	7.54	PFDA-13C2
PFDoA	612.9	569 (319)	14 (22)	14	8.05	PFDoA-13C2
PFTrA	663	618.9 (168.9)	14 (34)	14	8.51	PFTeA-13C2
PFTeA	712.9	668.9 (168.9)	10 (38)	10	8.91	PFTeA-13C2
ADONA	377	250.9 (85)	12 (36)	35	5.44	PFOA-13C8
6:2 FTCA	377	292.9 (63.1)	16 (4)	34	5.32	8:2 FTCA-13C2
8:2 FTCA	477	393 (63.1)	8 (8)	47	6.57	8:2 FTCA-13C2
10:2 FTCA	577	492.9 (63.1)	8 (4)	77	7.76	8:2 FTCA-13C2
6:2 FTUCA	357	292.9 (242.9)	20 (40)	7.6	5.28	8:2 FTUCA-13C2
8:2 FTUCA	457	393.1 (242.9)	28 (42)	2.1	6.55	8:2 FTUCA-13C2
10:2 FTUCA	563	492.9 (242.9)	12 (44)	2.6	7.72	8:2 FTUCA-13C2
3:3 FTCA	241	177 (117.1)	4 (36)	86	4.05	PFPeA-13C3
5:3 FTCA	341	237 (217)	12 (28)	89	5.29	PFOA-13C8
7:3 FTCA	441	336.9 (316.9)	8 (24)	82	6.59	PFOA-13C8
PFBS	299	99 (80)	44 (36)	250	4.21	PFBS-13C2
PFPeS	348.9	80 (99)	40 (36)	46	4.69	PFHxS-13C3
PFHxS	399	80 (99)	48 (44)	47	5.2	PFHxS-13C3
PFHpS	449	80 (99)	50 (46)	46	5.79	PFOS-13C4
PFOS	498.9	80 (99)	56 (56)	43	6.39	PFOS-13C4
PFNS	548.9	80 (98.9)	76 (48)	65	6.98	PFOS-13C4
PFDS	598.9	80 (98.9)	60 (60)	42	7.53	PFOS-13C4
PFDoS	698.9	80 (98.9)	64 (60)	62	8.47	PFTeA-13C2
6:2 Cl-PFESA	530.9	350.9 (98.9, 83)	28 (28, 32)	1.5, 3.9	6.75	PFOS-13C4
8:2 Cl-PFESA	630.9	451 (98.9, 83)	32 (32, 42)	2.7, 5.5	7.85	PFOS-13C4
4:2 FTS	327	307 (81)	16 (44)	48	4.56	6:2 FTS-13C
6:2 FTS	426.9	407 (81)	28 (44)	54	5.71	6:2 FTS-13C
8:2 FTS	526.9	507 (80)	32 (52)	39	6.94	6:2 FTS-13C
10:2 FTS	627	607 (80.1)	36 (56)	32	8.05	PFOS-13C4
FOSA	497.9	78	38	–	7.99	PFOS-13C4
MeFOSA	512	169 (218.9)	28 (28)	82	9.11	EtFOSA-D5
EtFOSA	526	169 (218.9)	32 (28)	95	9.49	EtFOSA-D5
FOSAA	556	498 (78)	32 (48)	40	7.06	EtFOSAA-D5
MeFOSAA	570	418.9 (512, 168.9)	20 (20, 32)	45, 33	7.26	EtFOSAA-D5
EtFOSAA	584	418.9 (526, 168.9)	20 (20, 36)	76, 33	7.56	EtFOSAA-D5
MeFOSE	616	59.2	16	–	9.13	EtFOSE-D9
EtFOSE	630	59.2	44	–	9.48	EtFOSE-D9
PFHxPA	398.9	79	56	–	4.07	PFOPA-Cl
PFOPA	498.9	79	44	–	5.23	PFOPA-Cl
PFDPA	598.9	79	40	–	6.47	PFOPA-Cl
6:2 diPAP	789	97 (79)	40 (76)	64	8.88	PFTeA-13C2
6:2/8:2 diPAP	889	97 (442.9, 79)	40 (20, 80)	53, 63	9.45	8:2 diPAP-13C4
8:2 diPAP	989	97.1 (79.1)	36 (72)	52	9.88	8:2 diPAP-13C4
6:6 PFPiA	700.9	400.9 (63.1)	56 (60)	14	8.33	PFTeA-13C2
6:8 PFPiA	800.9	400.9 (501, 63.1)	68 (64, 76)	79, 39	9.03	8:2 diPAP-13C4
8:8 PFPiA	900.9	500.9 (63.1)	76 (80)	31	9.57	8:2 diPAP-13C4
diSAmPAP	1203	525.9 (168.9)	48 (72)	9.5	10.34	8:2 diPAP-13C4

capillary voltage 2500 V, high-pressure ifunnel RF 90 V, and low-pressure ifunnel RF 60 V. Separation was achieved using a Zorbax Eclipse Plus RRHD C18 column (2.1 × 50 mm, 1.8 μm, Agilent Technologies, USA) with a guard column attached (Eclipse Plus C18, 2.1 × 5 mm, 1.8 μm). Gradient elution with the solvents 5 mM ammonium acetate in ultrapure water (A) and

MeOH (B) at 400 μL/min was performed and the first 1.5 min was diverted to waste ($t_0 = 10\%$ B; $t_{0.5} = 10\%$ B; $t_{2.5} = 55\%$ B; $t_9 = 90\%$ B; $t_{9.5} = 100\%$ B; $t_{11.5} = 100\%$ B; $t_{11.6} = 10\%$ B; $t_{14} = 10\%$ B). Total runtime (injection to injection) was approximately 15 min, an improvement over existing methods measuring 46 PFAS in 27 min [23].

Instrument detection limits

An instrument detection limit (IDL) was calculated for all compounds by performing replicate injections ($n = 10$) of 2 μL at the lowest concentration with a peak signal to noise > 5 using the following formula:

$$IDL = t_{(n-1,\ \ 1-\alpha=0.99)} \times \frac{RSD}{100} \times \text{mass injected,}$$

where

$t_{(n-1,\ \ 1-\alpha = 0.99)}$	Student's t value for the single tailed 99th percentile 0.99 level for $n-1$ degrees of freedom;
RSD	relative standard deviation calculated using peak area for replicate injections; and
mass injected	total mass on-column in fg.

Instrument precision was assessed by calculating the relative standard deviation of 10 injections of a 5 ng/mL standard in MeOH in 1 day (inter-day) and for replicate injections ($n = 6$) of a 5 ng/mL standard over three consecutive days (intra-day).

Method detection limits and method quantitation limits

The method detection limits (MDLs) were calculated using the US EPA method outlined in 40 CFR Part 136 Appendix B Revision 2 [24] over three consecutive days. Seven 250-mL aliquots of ultrapure water were spiked at 5 ng/L for each PFAS, except for FTCAs, FOSEs, and PFDPA which were spiked at 20 ng/L. Spiked samples were subjected to the outlined SPE procedure. Seven blank samples in ultrapure water were extracted concurrently and no PFAS peak of corresponding retention time and mass transitions with the required qualification ratio were present. The method accuracy was assessed using the mean surrogate-corrected value from those same seven spiked replicates and compared with the expected concentration. MDL$_s$ was calculated using the formula outlined in US EPA [24]:

$$MDL_s = t_{(n-1,\ \ 1-\alpha=0.99)}S_S,$$

where

$t_{(n-1,\ \ 1-\alpha = 0.99)}$	Student's t value for the single tailed 99th percentile 0.99 level for $n-1$ degrees of freedom; and
S_S	sample standard deviation of replicate spiked sample analyses relative standard deviation.

Method quantitation limits (MQLs) were set as 3.963 times the sample standard deviation for the seven extracted replicates used in calculating the MDL.

Matrix recovery and assessment

Six 250-mL aliquots of a filtered composite wastewater sample were spiked with native PFAS and isotopically labeled surrogates (matrix spike samples) at a concentration of 20 ng/L (5 ng spiked). Two 250-mL aliquots from the same composite sample were spiked with 5 ng of isotopically labeled surrogates (matrix blank samples). Two spiked ultrapure water samples and ultrapure water blanks were prepared similarly (lab control samples and method blank samples, respectively). All matrix recovery samples were extracted using the outlined SPE protocol. To determine the matrix effect, the mean matrix blank concentration was subtracted from the matrix spike sample extract concentration then compared with a 5 ng/mL concentration prepared in methanol.

Application to environmental samples

Prior to sampling, wide-mouth polypropylene bottles (Nalgene, Australia) were rinsed with MeOH then ultrapure water ($\times$ 3) to remove any potential PFAS contamination. During sampling, bottles were rinsed with site water before being filled. Samples were transported on ice and stored at 4 °C until analysis. Samples were filtered using 1-μm glass fiber filters (Merck Millipore, Australia), before extraction and analysis using the outlined SPE procedure. Most compounds analyzed in this method will have negligible sorption to glass fiber filters at low concentrations; however, diPAPs and PFPiAs can sorb $> 25\%$ to glass fiber filters introducing negative bias in measurement [32]. As unfiltered samples easily clog SPE frits that then need to be replaced, it was decided to filter samples before SPE and surrogate spiking.

Twenty-four-hour composite influent and effluent samples were collected at three Australian wastewater treatment plants (WWTPs A, B, and C) with primarily domestic influent sources. Three surface water samples were collected from an upstream river and downstream wetland and lake at a known Victorian AFFF contamination site. Drinking water was collected from three faucets located in Melbourne, Victoria. An injection internal standard (2 ng in 20 μL MeOH of PFOS-13C8) was added to 180 μL of spiked matrix samples, QA/QC samples, environmental samples, and calibration standards as per Quality Systems Manual (QSM) 5.1 [33] and met the required quality control criteria (ESM Fig. S6).

Results and discussion

Instrument detection limit and accuracy

Instrument detection limits (IDLs) ranged from 2.5 to 469 fg on-column for all compounds (Table 3). Calculated IDLs were below 10 fg on-column for 22 compounds from the classes PFCAs, PFSAs, FTSs, FOSAAs, and Cl-PFAESs and the compounds FOSA, diSAmPAP, and ADONA. For the PFCAs, PFSAs, FTUCAs, PFPAs, FTSs, and FASAs, IDLs increased with compound molecular mass. The IDLs of the n:2 FTCAs were 1–2 orders of magnitude higher than other PFAS due to the sensitivity loss from the increased sheath gas temperatures, but still much lower than expected detection levels for this study. Furthermore, the high IDL of PFDPA (311 fg on-column) was due to poor chromatography as no pH modifier was included in the method. IDLs determined in this study were below limits of 50–5000 fg on-column reported in a method measuring 52 PFAS using two chromatographic separations [23].

Intra-day variability was assessed by calculating the relative standard deviation (RSD) for replicate injections of a 5 ng/mL standard and was < 15% for all compounds except FTCAs (16–24%) (ESM Fig. S2). In many cases, RSD of intra-day replicate injections was < 10%. The relative standard deviation of replicate injections over 3 days (inter-day) was similarly < 15% for all compounds except FTCAs (16–22%).

The n:2 FTCAs displayed lower instrument sensitivity when included in the current method, which may account for the high RSD at 5 ng/mL. During MS/MS optimization, it was determined that n:2 FTCAs and FOSEs sensitivity was improved by lowering sheath gas temperatures. In the published method by Gremmel et al. [23], the reduction in sensitivity for FOSEs was a result of the instability of acetate adducts in the ion source, and these compounds were subsequently separated into a second method with FTOHs. However, in our method, the efficiency benefits of including the n:2 FTCAs and FOSEs in one chromatographic method was deemed of greater importance than the sensitivity gains of using two separate analytical methods.

Method detection limits and method quantitation limits

Method detection limits (MDLs) were calculated using ultra-pure water spiked with known concentrations of 5 ng/L or 20 ng/L and extracted using SPE protocol described earlier (n = 7). The MDLs ranged from 0.28 to 18 ng/L and method quantitation limits (MQLs) from 0.35 to 26 ng/L (Table 4). The comparison of detection limits between published studies is complicated, as many extraction parameters vary such as compounds measured, surrogates used, extraction method, injection volume, mass spectrometer conditions, and MDL

calculation method, among others. Furthermore, the analytical method was optimized to have high sensitivity for the greatest number of compounds. By optimizing for some groups of PFAS over the n:2 FTCAs, FOSEs, and PFDPA, there is a resulting sensitivity reduction. However, the MDLs and MQLs of n:2 FTCAs, FOSEs, and PFDPA were an improvement over many previous methods and at levels adequate for application to current guideline limits.

MDLs and MQLs calculated in this study for PFCAs (MDL, 0.28–1.4 ng/L; MQL, 0.35–1.8 ng/L) and PFSAs (MDL, 0.49–1.4 ng/L; MQL, 0.62–1.8 ng/L) were sufficient for regulatory limits in drinking water [8, 9]. The PFCA and PFSA MDLs and MQLs were comparable with or lower than those reported in Gremmel et al. [23] (LOD: PFCAs 0.2–2.6 ng/L, PFSAs 0.1–0.3 ng/L and LOQ: PFCAs 0.4–5.2 ng/L, PFSAs 0.4–1.7 ng/L) and Gros et al. [34] (0.25–1.5 and 0.25–3.1 ng/L, respectively) and MRLs reported in Procopio et al. [35] (5 ng/L for PFCAs and PFSAs, except PFBA 10 ng/L).

MDLs for the diPAPs, FTSs, FOSAs, FOSAAs, and FASEs (0.8–1.9, 0.60–2.7, 0.76–4.0, 1.4–3.2, and 2.9–4.9 ng/L, respectively) were similar to those determined in a method quantifying a similar number of PFAS (1.2, 0.3–1.4, 0.9–4.4, 1.7–1.8, and 5.5 ng/L, respectively) [23]. MDLs for the replacement compounds ADONA, 6:2 Cl-PFESA, and 8:2 Cl-PFESA (0.82, 1.3, and 1.1 ng/L, respectively) were comparable with the MDLs calculated for the compounds they have been used as replacements for PFOA and PFOS (0.28 and 0.8 ng/L, respectively).

The extraction method accuracy and precision were reproducible and met expected standards. Forty-nine of the 53 extracted PFAS had mean accuracies of 70–126%, except 10:2 FTCA (67%, RSD 28%), 10:2 FTS (66%, RSD 13%), and 8:8 PFPiA (138%, RSD 12%). Most extracted PFAS displayed high precision with RSD < 20%, except 8:2 FTCA (RSD 23%), 7:3 FTCA (RSD 20%), PFOPA (RSD 26%), and 10:2 FTCA (RSD 28%).

Matrix assessment results

Of the 53 compounds tested, 47 PFAS had mean surrogate-corrected recovery rates from spiked wastewater (n = 6) between 80 and 120%, five had recoveries between 120 and 130% (MeFOSA, 4:2 FTS, PFHxPA, 6:2 diPAP, and 6:6 PFPiA), and 8:8 PFPiA had a mean recovery of 134% (Fig. 2). The accuracy of matrix recoveries in our method is an improvement over previously reported methods for a similar number of PFAS (52 PFAS using two analytical methods on a single extract, 53–490%) [23]. For the compounds MeFOSA, 4:2 FTS, PFHxPA, and 6:2 diPAP, C13- or Cl-labeled forms are available but were not included in this study. Future work employing a more appropriate surrogate may improve matrix recovery accuracy for these

Table 3 Instrument detection limits (IDLs)

Compound	IDL (fg on-column)	Compound	IDL (fg on-column)
PFBA	3.1	PFBS	2.5
PFPeA	4.6	PFPeS	3.9
PFHxA	2.9	PFHxS	2.7
PFHpA	3.8	PFHpS	3.6
PFOA	6.4	PFOS	3.1
PFNA	5.5	PFNS	17
PFDA	12	PFDS	8.8
PFUnA	5.4	PFDoS	14
PFDoA	25	6:2 Cl-PFESA	7.9
PFTrA	23	8:2 Cl-PFESA	9.2
PFTeA	26	4:2 FTS	4.1
ADONA	3.1	6:2 FTS	4.2
6:2 FTCA	436	8:2 FTS	16
8:2 FTCA	469	10:2 FTS	21
10:2 FTCA	320	FOSA	7.2
6:2 FTUCA	46	MeFOSA	11
8:2 FTUCA	59	EtFOSA	20
10:2 FTUCA	40	FOSAA	15
3:3 FTCA	33	MeFOSAA	8.2
5:3 FTCA	18	EtFOSAA	9
7:3 FTCA	72	MeFOSE	73
PFHxPA	19	EtFOSE	28
PFOPA	115	6:6 PFPiA	14
PFDPA	311	6:8 PFPiA	41
6:2 diPAP	18	8:8 PFPiA	23
6:2/8:2 diPAP	12	diSAmPAP	7.7
8:2 diPAP	17		

IDL was calculated using replicate injections ($n = 10$) of 10 fg/μL, 25 fg/μL, 50 fg/μL, 250 fg/μL, 500 fg/μL, or 900 fg/μL. Intra- and inter-day variability was assessed using repeat injections of a 5 ng/mL standard in methanol over 1 day and on repeat over three consecutive days, and results are contained in Electronic Supplementary Material, ESM

compounds. To the authors' knowledge, there is no available mass-labeled PFPiA analogue available. Quantitation of PFPiAs using this method will come with some inherent overestimation that must then be included in the error calculation and reported for PFPiA quantitation results.

The 19 PFCAs and PFSAs had recoveries between 95 and 105% for all compounds except PFHpA, PFTrA, and PFDS (109, 86, and 89%, respectively). Interferences were found for the PFBS sulfite radical transition ($299 > 80$ m/z); therefore, the more selective FSO_s^- transition ($299 > 99$ m/z) was used for quantitation of PFBS; this has been previously reported in SPE wastewater analytical methods [23]. In wastewater matrix samples, there was an interfering peak with the same transition as PFPeA ($263 > 219$ m/z). Therefore, for quantitation of PFPeA in environmental samples, analyte peaks that did not match the retention time of PFPeA-13C3 by $\pm 2\%$ were assigned <MDL.

For the compound PFDoS ($R_t = 8.47$ min), a surrogate of similar retention time (PFTeA-13C2, $R_t = 8.91$ min) was more suitable than a PFAS with the same functional group (PFOS-13C4, $R_t = 6.39$ min). PFDoS and PFTeA-13C2 have 12 and 13 fully fluorinated carbons, respectively, attached to a functional group (PFDoS: sulfonate and PFTeA: carboxylic acid), and both compounds had similar SPE efficiencies (determined by absolute recoveries of PFDoS at 58% and PFTeA-13C2 at 59%). Therefore, for PFDoS, the coeluting matrix components had a greater effect than the functional group on ionization behavior, so a compound of similar retention time and extraction efficiency was deemed a more appropriate surrogate. For some of the other compounds in the method, this was also the case.

The PFOS replacement compounds 6:2 Cl-PFESA and 8:2 Cl-PFESA displayed good surrogate-corrected recoveries using PFOS-13C4 as a surrogate compound (98 and 93%, respectively). The PFOA replacement compound ADONA

Table 4 SPE method detection limit (MDL), method quantitation limit (MQL), and extraction method accuracy and precision data

Compound	MDL (ng/L)	MQL (ng/L)	Extraction method accuracy (%)	Method precision (RSD %)
PFBA	0.59	0.75	93%	4%
PFPeA	0.71	0.89	92%	5%
PFHxA	0.87	1.1	90%	6%
PFHpA	0.84	1.1	96%	6%
PFOA	0.28	0.35	93%	2%
PFNA	0.61	0.77	98%	4%
PFDA	0.71	0.89	98%	4%
PFUnA	0.80	1.0	85%	6%
PFDoA	1.2	1.5	93%	8%
PFTrA	1.4	1.8	78%	12%
PFTeA	0.67	0.84	93%	5%
PFBS	0.49	0.62	89%	3%
PFPeS	1.2	1.5	100%	9%
PFHxS	0.69	0.88	91%	5%
PFHpS	0.79	1.0	99%	6%
PFOS	0.78	1.0	95%	5%
PFNS	1.0	1.3	87%	7%
PFDS	1.1	1.3	83%	8%
PFDoS	1.4	1.8	72%	13%
ADONA	0.82	1.0	88%	6%
6:2 FTCA	13	17	103%	16%
8:2 FTCA	16	19	92%	23%
10:2 FTCA	17	21	67%	28%
6:2 FTUCA	1.7	2.1	121%	9%
8:2 FTUCA	1.6	2.0	111%	10%
10:2 FTUCA	2.8	3.6	87%	19%
3:3 FTCA	1.4	1.7	118%	7%
5:3 FTCA	1.8	2.3	103%	11%
7:3 FTCA	2.4	3.1	75%	20%
PFHxPA	2.9	3.4	104%	17%
PFOPA	4.6	5.8	100%	26%
PFDPA	18	26	82%	10%
6:2 diPAP	1.9	2.4	81%	14%
6:2/8:2 diPAP	1.9	2.4	123%	11%
8:2 diPAP	0.83	1.1	93%	6%
6:2 Cl-PFESA	1.3	1.7	88%	9%
8:2 Cl-PFESA	1.1	1.4	80%	9%
4:2 FTS	2.7	3.4	93%	16%
6:2 FTS	0.56	0.7	90%	4%
8:2 FTS	1.3	1.7	87%	9%
10:2 FTS	1.4	1.8	66%	13%
FOSA	0.76	1.0	70%	7%
MeFOSA	4.0	5.0	127%	18%
EtFOSA	2.1	2.7	80%	19%
FOSAA	3.2	4.0	91%	17%
MeFOSAA	1.4	1.7	106%	8%
EtFOSAA	1.5	1.9	93%	10%
MeFOSE	2.9	3.7	96%	5%
EtFOSE	4.9	6.2	93%	9%
6:6 PFPiA	1.2	1.5	74%	10%
6:8 PFPiA	1.8	2.3	95%	12%
8:8 PFPiA	3.1	4.0	138%	11%
diSAmPAP	3.3	3.0	76%	19%

MDL was determined by 7 replicate extractions of 5 ng/L spike into ultrapure water for all compounds except FTCAs, FASEs, and PFDPA which were spiked at 20 ng/L. Ultrapure water blanks ($n = 7$) were extracted alongside method validation samples. Method accuracy was expressed as the mean recovery of method validation samples for the expected concentration as a percentage and relative standard deviation

displayed acceptable surrogate-corrected recoveries using PFOA-13C8 as a surrogate compound (92%). This information is useful for future method application in environmental samples as there are no currently available commercial mass-labeled analogues of these emerging PFAS.

Environmental aqueous sample analysis

Twenty-one of the 53 analyzed PFAS were detected in at least one sample (Table 5). PFOA was the only compounded detected in 100% of samples, followed by PFBA, PFHxA, 6:2
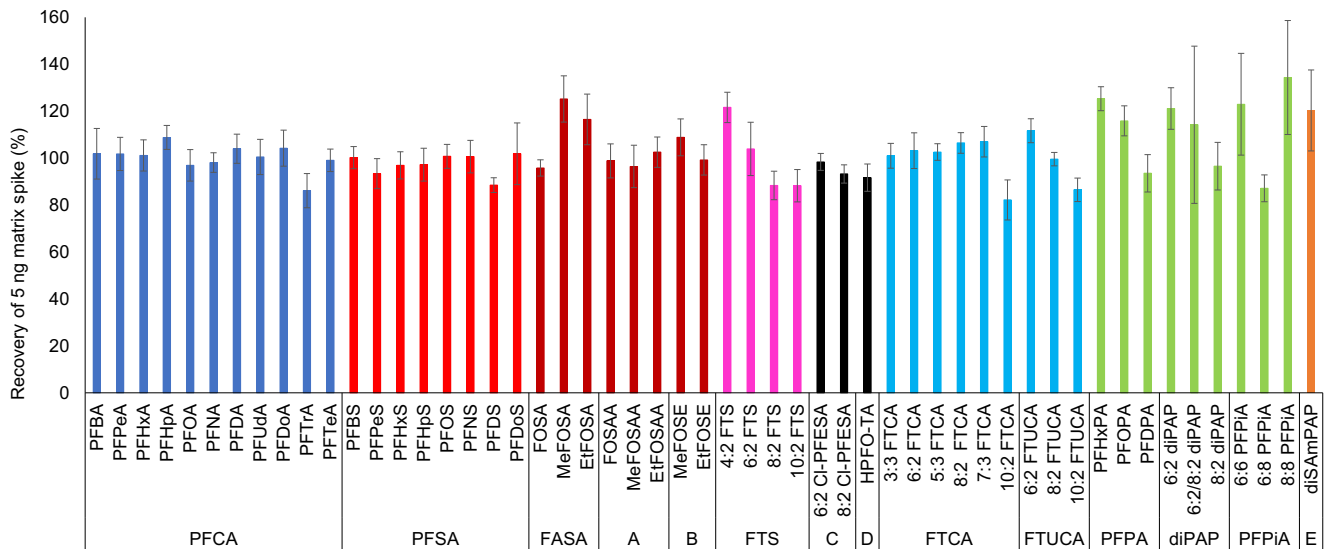
Fig. 2 Surrogate-corrected recovery results from 250-mL wastewater aliquots ($n = 6$) spiked with 5 ng of each PFAS and concentrated to 1 mL using SPE then compared with 5 ng spiked into 1 mL of methanol. Error bars represent one standard deviation. PFAS are grouped by class listed: dark blue (PFCA), red (PFSA), maroon (FASA, FASAA: A, and FOSE: B), pink (n:2 FTS), black (Cl-PFESA: C, and PFECA: D), light blue (n:2 FTCA, n:3 FTCA, and n:2 FTUCA), green (PFPA, diPAP, and PFPiA), and orange (diSAmPAP: E)

Table 5 PFAS concentrations and MDLs (ng/L) for environmental aqueous samples of influent (IN) and effluent (EFF) at three WWTPs (A, B, C), surface water (SW) at three sites, and drinking water (DW) from three locations in Melbourne, Victoria. Colors represent a heatmap for each individual site (one site per column), increasing in intensity from yellow (low) to green (high). Values marked with an asterisk (*) are estimated concentrations, as they were less than calculated MQL but greater than MDL

Compound	MDL	WWTP A - IN	WWTP A - EFF	WWTP B - IN	WWTP B - EFF	WWTP C - IN	WWTP C - EFF	SW-1	SW-2	SW-3	DW-1	DW-2	DW-3
PFBA	0.59	1.5	4.4	1.9	4.5	4.4	19	2.2	31	2.5	<MDL	0.79	0.71*
PFPeA	0.71	<MDL	7.9	<MDL	4.2	4.4	14	<MDL	30	<MDL	<MDL	<MDL	<MDL
PFHxA	0.87	3.7	27	2.9	12	9	46	2.3	186	2.1	<MDL	<MDL	1.2
PFHpA	0.84	2.4	6.9	<MDL	2.0	3.2	11	<MDL	12	<MDL	<MDL	<MDL	<MDL
PFOA	0.28	1.7	12	1.0	8.4	5.7	15	3.3	8.4	2.5	0.79	1.8	0.58
PFNA	0.61	<MDL	<MDL	<MDL	<MDL	1.4	1.6	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFDA	0.71	<MDL	2.2	<MDL	1.2	<MDL	2.0	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFBS	0.49	<MDL	<MDL	2.1	<MDL	3.7	11	<MDL	21	1.0	<MDL	<MDL	<MDL
PFPeS	1.2	<MDL	<MDL	<MDL	<MDL	<MDL	11	<MDL	26	<MDL	<MDL	<MDL	<MDL
PFHxS	0.69	<MDL	1.9	<MDL	<MDL	7.7	50	0.95	193	3.4	<MDL	<MDL	<MDL
PFHpS	0.79	<MDL	<MDL	<MDL	<MDL	<MDL	1.7	<MDL	5.4	<MDL	<MDL	<MDL	<MDL
PFOS	0.8	<MDL	1.3	0.90*	<MDL	23	35	2.9	137	4.3	<MDL	<MDL	<MDL
EtFOSAA	1.5	<MDL	<MDL	<MDL	<MDL	<MDL	1.5*	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
6:2 FTS	0.56	1.1	1.3	<MDL	<MDL	35	37	9.1	8.7	3.1	<MDL	4.3	2.0
8:2 FTS	1.3	9.2	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
3:3 FTCA	1.4	2.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
5:3 FTCA	1.8	<MDL	<MDL	2.1*	2.4	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
PFHxPA	2.9	<MDL	5.3	<MDL	<MDL	12	6.1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
6:2 diPAP	1.9	56	<MDL	8.0	<MDL	5.7	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
6:2/8:2 diPAP	1.9	55	<MDL	13	<MDL	5.0	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
8:2 diPAP	0.83	16	<MDL	3.6	<MDL	1.0	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL

FTS, PFOS, PFHpA, and PFHxS (detected in 92, 83, 75, 58, 50, and 50%, respectively). When the optimized and validated method was applied to environmental samples, separation of branched and linear isomers was achieved. The concentrations of PFOS, PFHxS, PFHpS, and PFOA are reported as the sum of branched plus linear isomers.

Compounds from the classes PFCAs, FTCAs, PFSAs, FTSs, FOSAAs, PFPAs, and diPAPs were all detected in WWTP aqueous samples. PFBA, PFHxA, PFOA, PFDA, PFBS, and PFOS were detected in 100% of WWTP samples. At WWTPs A and B, diPAPs were present at the highest concentration in influent (from 3.6 to 56 ng/L), whereas PFOA and PFHxA were preset and at the highest concentration in effluent (from 8.4 to 27 ng/L). The high concentrations of diPAPs in influent were a result of 6:2 diPAP and 6:2/8:2 diPAP. At both WWTPs, FTCAs were detected, which have been demonstrated as intermediate products of diPAP transformation to final PFCA end products such as PFHxA and PFOA [36]. The similar PFAS signature may indicate that a similar source of diPAPs exists, and that diPAP transformation and sorption to WWTP solids are occurring as treatment progresses in WWTPs A and B.

A different signature of PFAS was quantified at WWTP C. In influent, 6:2 FTS, PFOS, and PFHxPA were measured at the highest concentrations (35, 23, and 12 ng/L, respectively). In effluent, PFHxS, PFHxA, 6:2 FTS, and PFOS were measured at the highest concentrations (50, 46, 37, and 35 ng/L, respectively). In all measured sample sites, the compound EtFOSAA was only detected in effluent from WWTP C. FOSAAs have been previously demonstrated as a PFOS transformation intermediate [37] and, similar to diPAPs, FTSs have been demonstrated to transform to final stable PFCAs [15]. The sensitivity and broad range of PFAS measured in this method have many advantages for wastewater aqueous sample analysis over previously published methods, as the difference in PFAS signatures within and between domestic wastewater treatment plants was able to be established.

In surface water samples, PFBA, PFHxA, PFOA, PFHxS, PFOS, and 6:2 FTS were quantified at all three locations. The sample site on the river upstream (SW-1) and lake downstream (SW-3) of the AFFF contamination area displayed similar PFAS concentrations (< 10 ng/L for all quantified PFAS). At SW-2, the concentrations of PFHxS, PFHxA, and PFOS were at the highest concentration reported in this study (193, 186, and 137 ng/L, respectively) and likely linked to the AFFF contamination area. SW-2 was sampled from a fully enclosed wetland downstream of the AFFF contamination area. In contrast, SW-3 was sampled from a site that is part of a chain of large, connected, semi-enclosed lakes that form an estuarine lagoon system. Both SW-2 and SW-3 are downstream of the AFFF contamination area; however, significant dilution at SW-3 may explain the lower PFAS concentrations measured relative to SW-2.

In drinking water samples, PFBA, PFHxA, PFOA, and 6:2 FTS were present at concentrations ranging from 0.79 to 4.3 ng/L. Similar concentrations have been previously reported for two locations near Melbourne: Parkdale (PFOA and PFOS, detected at <LOQ) and Footscray (PFHxA, PFHpA, PFOA, PFBS, PFHxS, and PFOS, detected, range from <LOQ to 1.21 ng/L) [38].

Conclusions

A method for the quantitation of 53 legacy and emerging PFAS using a single chromatographic separation and extraction method was successfully developed and applied to aqueous samples. Application of this method will provide comprehensive data for PFAS mass fluxes within WWTPs and the aquatic environment. The 14 compound classes quantified in the method included both legacy and emerging PFAS. As data on emerging PFAS are limited, this method will provide a useful tool to collect baseline data on these substances.

The single injection method has efficiency advantages over other large PFAS compound list methods as the runtime is almost halved while maintaining analytical method accuracy and stability and separation of branched and linear PFAS isomers. Instrument detection limits (IDLs) ranged from 2.5 to 469 fg on-column and were much lower than those reported for similar methods. The method detection limits (MDLs) determined using US EPA method 40 CFR Part 136 Appendix B Revision 2 ranged from 0.28 to 18 ng/L. These MDLs are adequate for application to PFAS analysis of drinking water and environmental aqueous samples.

The method was successfully validated in WWTP aqueous matrices and applied to influent and effluent from three Australian WWTPs, surface water and drinking water samples which displayed a wide range of PFAS concentrations (0.6–193 ng/L). The compound classes FTCAs, FOSAAs, PFPAs, and diPAPs were detected for the first time in Australian WWTPs. The range of compound classes detected and different PFAS signatures between sample locations demonstrate the need for expanded quantitation lists when investigating PFAS in aqueous environmental samples.

Compliance with ethical standards

Conflict of interest T. Anumol and J. Pyke are employees of Agilent Technologies Inc, who supplied the instrumentation used for LC-MS/MS analysis. B. Clarke has an LC-MS/MS demonstration instrument from Agilent Technologies Inc operating within his laboratory. T. Coggan receives scholarship funding from the Australian government Research Training Program and Water Research Australia. J. Shimeta declares that he has no conflicts of interest.

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