Application Note

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Analysis of >50 Legacy and Emerging PFAS in Water Using the Agilent 6495B Triple Quadrupole LC/MS

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Abstract

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, and toxicity.[1-3] Robust analytical techniques that can accurately and precisely quantify these pollutants at trace levels are necessary for understanding their environmental fate, ecological impacts, and impacts on public health. Appropriate analytical techniques and the fundamental data they generate allow scientists and regulators to make informed assessments of PFAS use in modern society.

This Application Note describes a sensitive and reliable method for the simultaneous quantitation of 53 legacy and emerging PFAS from 14 compounds classes. The method uses isotope dilution on an Agilent 1290 Infinity II LC coupled to an Agilent 6495B triple quadrupole LC/MS.[4]

Introduction

PFAS are a diverse family of fluorinated synthetic chemicals used as surfactants and polymers for a wide variety of industrial and commercial applications since the 1950s.[5,6] The most common applications include aqueous-film firefighting foams (AFFFs), textile protection surface coating for cooking implements, and food contact paper.[7,8] For many years, PFAS were thought to be inert and nontoxic, and therefore were widely used with little thought for environmental dispersal or ecological impact. It was not until 2001 that the extent of PFAS global contamination was first demonstrated for perfluorooctane sulfonate (PFOS; $C_8F_{17}SO_3H$)[3] and perfluorooctanoic acid (PFOA; $C_7H_{15}COOH$). Since then, PFAS have been detected in almost every wildlife sample measured[9], ubiquitously in humans throughout the world[10], and most environmental compartments, including pristine locations[11]. The list of known PFAS has expanded to over 4,800 compounds, some of which will transform to the problematic perfluorosulfonic acids (PFSAs) and perfluorocarboxylic acids (PFCAs) in the environment[12].

Liquid chromatography coupled to tandem quadrupole mass spectrometry (LC/MS/MS) with electrospray ionization (ESI) has been the most commonly used instrumental technique for quantifying PFAS. The most common approach for extracting PFAS from aqueous matrices is solid phase extraction (SPE) using a weak anion exchange resin. These approaches are the recommended techniques for U.S. EPA[13] and ASTM analytical methodologies.

This Application Note used a single extraction and analytical technique for the quantitation of 53 legacy and emerging PFAS in aqueous matrices using isotope dilution analytical methodology. The analysis was performed on an Agilent 1290 Infinity II liquid chromatograph (LC) coupled with an Agilent 6495B tandem mass spectrometer (MS/MS).

Experimental

Reagents and standards

PFAS analytical standards including 21 isotopically labeled analogs were purchased from Wellington Laboratories (Ontario, Canada). Methanol (MeOH, LC/MS grade, Honeywell, USA, LiChrosolv hypergrade, Merck Millipore, Australia) and ultrapure water (pH 8, Merck Millipore, Australia) were tested for PFAS contamination before 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).

Water extraction

Water samples were collected in 250 mL polypropylene containers, filtered (1 µm glass fiber) before being spiked with 5 ng of isotopically labeled standards. Extraction was performed using a weak anion exchange cartridge (6 mL, 150 mg WAX), preconditioned with 4 mL of 0.1% (v/v) ammonium hydroxide in MeOH, 4 mL of MeOH, and 4 mL of ultrapure water. Samples were loaded 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 minutes. SPE cartridges before elution with 2 mL of MeOH (used to rinse the sample bottle) and 4 mL of 0.1% (v/v) ammonium hydroxide in MeOH. Extracts were evaporated to 500 µL under a gentle stream of nitrogen (at 25 °C) and reconstituted to 1 mL in MeOH.

LC/MS analysis

LC operating conditions

Separation was achieved using an Agilent ZORBAX Eclipse Plus RRHD C18 column (2.1 × 50 mm, 1.8 µm,) with a guard column attached (Agilent ZORBAX 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 minutes was diverted to waste:

Time (min)	%B
0	10
0.5	10
2.5	55
9	90
9.5	100
11.5	100
11.6	10
14	10

Total run time (injection to injection) was approximately 15 minutes, an improvement over existing methods measuring 46 PFAS in 27 minutes.[14]

To control background contamination from the system, a delay column (Agilent ZORBAX Eclipse Plus C18 RRHD, 4.6 × 50 mm, 3.5 µm) was installed between the solvent mixer and autosampler module. PEEK tubing and stainless-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-second wash with 50:50 ultrapure water:MeOH followed by a 10-second needle seat backflush using 90:10 ultrapure water:MeOH.

MS/MS parameters

MS/MS conditions were optimized using the Optimizer tool in Agilent MassHunter software for each compound, and Table 2 presents the best response for the largest range of compounds included in the method.

Target analytes were determined by retention time and two ion transitions using Agilent MassHunter Quantitative Analysis software. For each compound, one transition was used for quantitation, and a second transition used for qualitative confirmation.

Positive identification of analytes in samples was dependent on three criteria:

- The signal-to-noise (S/N) ratio must exceed 3:1
- The retention time must be within ±5% of those determined from analytical standards
- The abundance ratio between quantitative and qualitative ion transitions must be within ±30% of the ratios measured in standards.

Table 1. Agilent 6495B MS parameters.

Parameter	Value
Mass Spectrometer	Agilent 6495B with electrospray ionization (ESI) operated in multiple reaction monitoring mode (MRM)
Ionization Mode	Negative
Gas Temperature	250 °C; 11 L/min
Nebulizer	25 psi
Sheath Gas	375 °C; 11 L/min
Capillary Voltage	2500 V
High Pressure iFunnel RF	90 V
Low Pressure iFunnel RF	60 V

Table 2. Agilent 6495B LC/MS/MS acquisition parameters.

Compound	Precursor (*m/z*)	Product (*m/z*)	CE (V)	RT (min)	Surrogate
PFBA	213	169	6	2.68	PFBA-$^{13}C_3$
PFPeA	263	219	6	4.21	PFPeA-$^{13}C_3$
PFHxA	313	269 (119)	6 (22)	4.82	PFHxA-$^{13}C_2$
PFHpA	363	318.9 (168.9)	6 (18)	5.45	PFOA-$^{13}C_8$
PFOA	413	368.9 (169)	6 (18)	6.11	PFOA-$^{13}C_8$
PFNA	463	418.9 (218.9)	10 (18)	6.79	PFDA-$^{13}C_2$
PFDA	512.9	469 (268.9)	6 (18)	7.44	PFDA-$^{13}C_2$
PFUnA	563	518.9 (268.9)	12 (16)	8.03	PFDA-$^{13}C_2$
PFDoA	612.9	569 (319)	14 (22)	8.56	PFDoA-$^{13}C_2$
PFTrA	663	618.9 (168.9)	14 (34)	9.03	PFTeA-$^{13}C_2$
PFTeA	712.9	668.9 (168.9)	10 (38)	9.42	PFTeA-$^{13}C_2$
ADONA	377	250.9 (85)	12 (36)	5.54	PFOA-$^{13}C_8$
6:2 FTCA	377	292.9 (63.1)	16 (4)	5.63	8:2 FTCA-$^{13}C_2$
8:2 FTCA	477	393 (63.1)	8 (8)	7.01	8:2 FTCA-$^{13}C_2$
10:2 FTCA	577	492.9 (63.1)	8 (4)	8.25	8:2 FTCA-$^{13}C_2$
6:2 FTUCA	357	292.9 (242.9)	20 (40)	5.60	8:2 FTUCA-$^{13}C_2$
8:2 FTUCA	457	393.1 (242.9)	28 (42)	6.98	8:2 FTUCA-$^{13}C_2$
10:2 FTUCA	563	492.9 (242.9)	12 (44)	8.22	8:2 FTUCA-$^{13}C_2$
3:3 FTCA	241	177 (117.1)	4 (36)	4.18	PFPeA-$^{13}C_3$
5:3 FTCA	341	237 (217)	12 (28)	5.56	PFOA-$^{13}C_8$
7:3 FTCA	441	336.9 (316.9)	8 (24)	6.97	PFOA-$^{13}C_8$
PFHxPA	398.9	79	56	4.22	PFOPA-Cl
PFOPA	498.9	79	44	5.50	PFOPA-Cl
PFDPA	598.9	79	40	6.88	PFOPA-Cl
6:2 diPAP	789	442.9 (97, 79)	20 (40, 76)	9.38	PFTeA-$^{13}C_2$
6:2/8:2 diPAP	889	97 (442.9, 79)	40 (20, 80)	9.95	8:2 diPAP-$^{13}C_4$
8:2 diPAP	989	543 (97.1, 79.1)	20 (36, 72)	10.39	8:2 diPAP-$^{13}C_4$

Compound	Precursor (*m/z*)	Product (*m/z*)	CE (V)	RT (min)	Surrogate
PFBS	299	99 (80)	44 (36)	4.35	PFBS-$^{13}C_2$
PFPeS	348.9	80 (99)	40 (36)	4.89	PFHxS-$^{13}C_3$
PFHxS	399	80 (99, 119)	48 (44, 44)	5.49	PFHxS-$^{13}C_3$
PFHpS	449	80 (99)	50 (46)	6.15	PFOS-$^{13}C_4$
PFOS	498.9	80 (99)	56 (56)	6.80	PFOS-$^{13}C_4$
PFNS	548.9	80 (98.9)	76 (48)	7.44	PFOS-$^{13}C_4$
PFDS	598.9	80 (98.9)	60 (60)	8.01	PFOS-$^{13}C_4$
PFDoS	698.9	80 (98.9)	64 (60)	8.99	PFTeA-$^{13}C_2$
6:2 Cl-PFESA	530.9	350.9 (98.9, 83)	28 (28, 32)	7.19	PFOS-$^{13}C_4$
8:2 Cl-PFESA	630.9	451 (98.9, 83)	32 (32, 42)	8.33	PFOS-$^{13}C_4$
4:2 FTS	327	307 (81, 80)	16 (44, 32)	4.76	6:2 FTS-^{13}C
6:2 FTS	426.9	407 (81, 80)	28 (44, 44)	6.07	6:2 FTS-^{13}C
8:2 FTS	526.9	507 (80)	32 (52)	7.41	6:2 FTS-^{13}C
10:2 FTS	627	607 (80.1)	36 (56)	8.56	PFOS-$^{13}C_4$
FOSA	497.9	78	38	8.07	PFOS-$^{13}C_4$
MeFOSA	512	169 (218.9)	28 (28)	9.15	EtFOSA-D5
EtFOSA	526	169 (218.9)	32 (28)	9.52	EtFOSA-D5
FOSAA	556	498 (78)	32 (48)	7.35	EtFOSAA-D5
MeFOSAA	570	418.9 (512, 168.9)	20 (20, 32)	7.73	EtFOSAA-D5
EtFOSAA	584	418.9 (526, 168.9)	20 (20, 36)	8.03	EtFOSAA-D5
MeFOSE	616	59.2	16	9.16	EtFOSE-D9
EtFOSE	630	59.2	44	9.51	EtFOSE-D9
6:6 PFPiA	700.9	400.9 (63.1)	56 (60)	8.81	PFTeA-$^{13}C_2$
6:8 PFPiA	800.9	400.9 (501, 63.1)	68 (64, 76)	9.51	8:2 diPAP-$^{13}C_4$
8:8 PFPiA	900.9	500.9 (63.1)	76 (80)	10.06	8:2 diPAP-$^{13}C_4$
diSAmPAP	1203	525.9 (168.9)	48 (72)	10.65	8:2 diPAP-$^{13}C_4$

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 or close elution time (Table 2).

Results and discussion

Analytical performance

Instrument detection limits (IDLs) ranged from 2.5 to 469 fg on column for all compounds. Calculated IDLs were below 10 fg on column for 22 compounds from the classes PFCAs, PFSAs, FTSs, FOSAAs, 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 method detection limits (MDLs) for the 53 PFAS were calculated based on the US EPA's 40 CFR Part 136 Appendix B Revision 2.[15] Briefly, 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 and extracted using SPE protocol described earlier. The MDLs ranged from 0.28 to 18 ng/L and method quantification limits (MQLs) from 0.35 to 26 ng/L with 46 PFAS having quantification levels below 5 ng/L using a single analytical method (Table 3, Figure 1).

Table 3. SPE extraction MDL, 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 seven 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.

Forty-nine of the 53 extracted PFAS had mean accuracies within the acceptable range of 70 to 130%. The exceptions were 10:2 FTCA (67%, RSD 28%), 10:2 FTS (66%, RSD 13% RSD), and 8:8 PFPiA (138%, RSD 12%) and were likely due to a lack of matched mass labeled surrogate. Furthermore, the analytical protocol had high precision, with RSD <20% for 49 of 53 compounds; the exceptions were 8:2 FTCA (RSD 23%), 7:3 FTCA (RSD 20%), PFOPA (RSD 26%), and 10:2 FTCA (28 %).

Analysis of wastewater samples

Composite wastewater samples (n = 6) were spiked with a known amount of PFAS to determine matrix impacts. Of the 53 compounds included in this method, 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%.

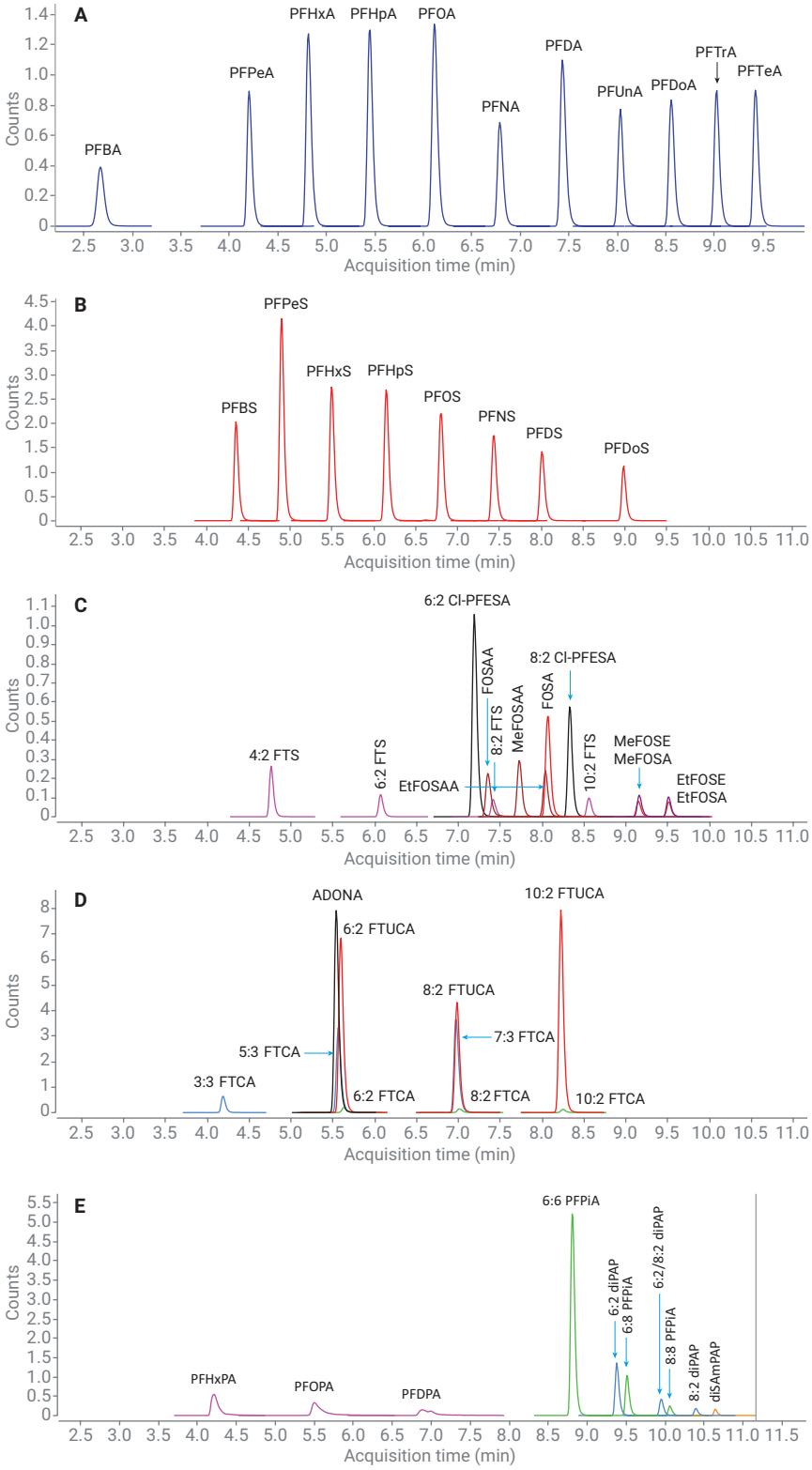

Figure 1. Example chromatograms from a 5 ng/mL PFAS-spiked methanol standard for: PFCAs (A); PFSAs (B); FTSs, Cl-PFESAs, FASAs, FASAAs, and FOSEs (C); n:3 FTCAs, n:2 FTCAs, n:2 FTUCAs, and ADONA (D); PFPAs, PFPIAs, diPAPS, and diSAmPAP (E).

The method was applied to influent and effluent samples from three Australian wastewater treatment plants (WWTPs). Twenty-one PFAS were detected in wastewater samples at concentrations ranging from <MDL to 56 ng/L (Figure 2). The application of this method allowed for discrimination of PFAS signatures between individual wastewater treatment plants and sample locations within these wastewater treatment plants. Several emerging PFAS such as diPAPs were also detected.

Details of the occurrence of PFAS in WWTPs in water and biosolids can be found in published literature.[4]

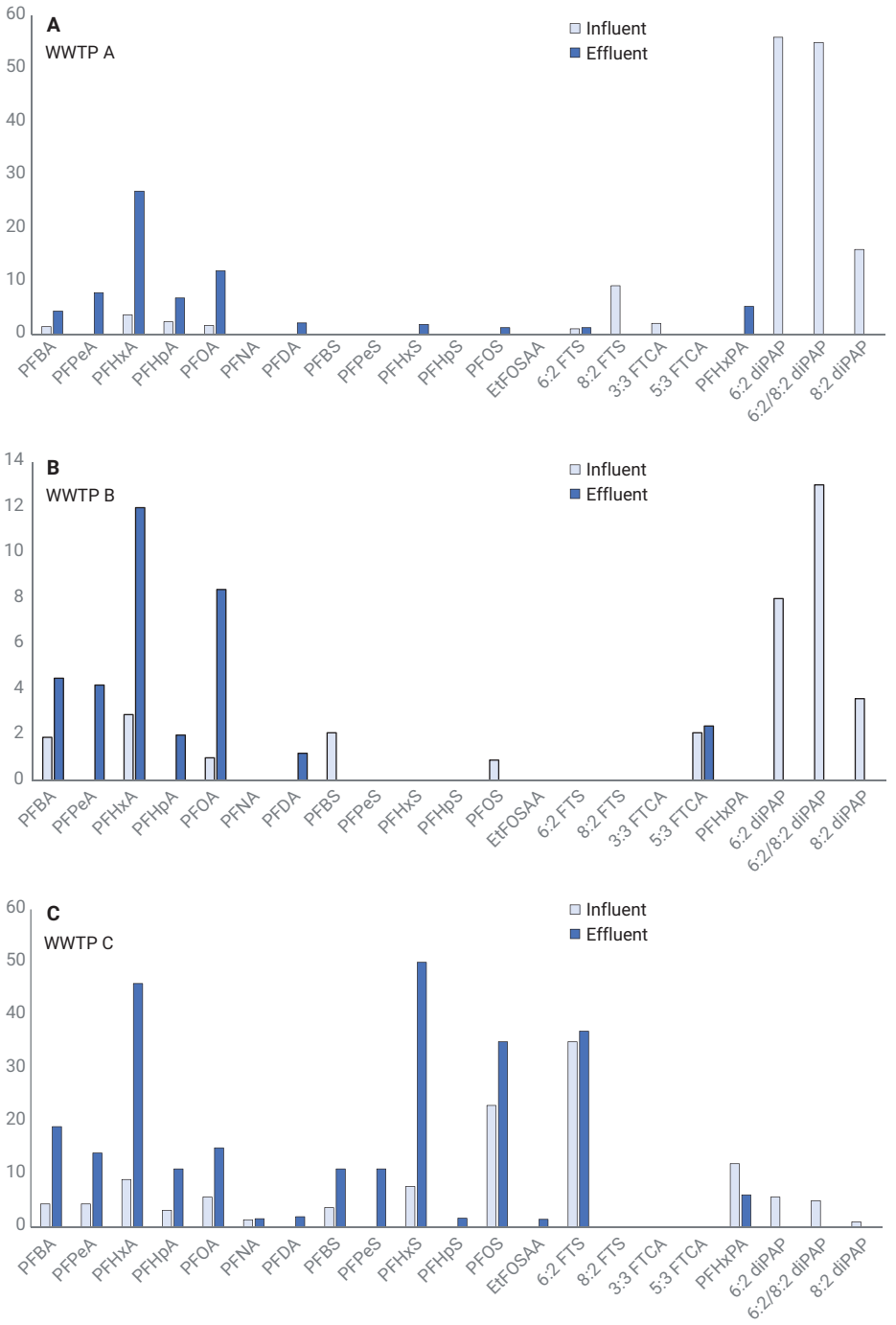

Figure 2. Influent and effluent concentrations measured in three Australian wastewater treatment plants.

Conclusion

This Application Note presents the simultaneous analysis of 53 PFAS from 14 compound classes using the Agilent 6495B triple quadrupole LC/MS.

The 6495B triple quadrupole LC/MS was demonstrated to provide reliable and robust quantification of legacy and emerging PFAS from 14 compound classes. An analytical approach for quantifying these substances from water samples is presented with low ng/L method quantification limits.

Good peak shapes were achieved for all analytes at low and sub-ng/L concentrations to provide excellent sensitivity while providing robustness to analyze several wastewater samples. The SPE protocol delivered good recoveries for all analytes with typically low RSDs across repeated analyses.

References

1. Houde, M.; *et al.* Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (Tursiops truncatus) food web. *Environ. Sci. Technol*. **2006**, *40(13)*, 4138-4144.
2. Ahrens, L.; Bundschuh, M. Fate and effects of poly- and perfluoroalkyl substances in the aquatic environment: A review. *Environ. Toxicol. Chem*. **2014**, *33*, 1921–1929.
3. Giesy, J. P.; Kannan, K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol*. **2001**, *35(7)*, 1339–1342.
4. A single analytical method for the determination of 53 legacy and emerging per- and polyfluoroalkyl substances (PFAS) in aqueous matrices. DOI : 10.1007/s00216-019-01829-8
5. Paul, A. G.; Jones, K. C.; Sweetman, A. J. A First global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environ. Sci. Technol*. **2009**, *43(2)*, 386–392.
6. Prevedouros, K.; *et al.* Sources, fate and transport of perfluorocarboxylates. *Environ. Sci. Technol*. **2006**, *40(1)*, 32–44.
7. Buck, R. C.; *et al.* Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Int. Env. Assess. Man*. **2011**, *7(4)*, 513–541.
8. Rao, N.; Baker, B. Textile Finishes and Fluorosurfactants. In *Organofluorine Chemistry*, Banks, R. E.; Smart, B. E.; Tatlow, J. C., Eds. Springer US: 1994; pp 321-338.
9. Giesy, J. P.; *et al.* Aquatic toxicology of perfluorinated chemicals. In *Reviews of Environmental Contamination and Toxicology*, Springer New York: 2010; Vol. 202, pp 1–52.
10. Toms, L.-M. L.; *et al.* Polyfluoroalkyl chemicals in pooled blood serum from infants, children, and adults in Australia. *Environ. Sci. Technol.* **2009**, *43(11)*, 4194–4199.
11. Lindstrom, A. B.; Strynar, M. J.; Libelo, E. L., Polyfluorinated compounds: past, present, and future. *Environ. Sci. Technol*. **2011**, *45(19)*, 7954–7961.
12. Wang, Z.; *et al.* A never-ending story of per- and polyfluoroalkyl substances (PFAS)? *Environ. Sci. Technol*. **2017**, *51(5)*, 2508–2518.
13. US EPA EPA 822-R-16-005 Drinking Water Health Advisory for Perfluorooctanoic Acid (PFOA); United States Environmental Protection Agency: Wasington, USA, **2016**.
14. Gremmel, C.; Frömel, T.; Knepper, T. P. HPLC–MS/MS methods for the determination of 52 perfluoroalkyl and polyfluoroalkyl substances in aqueous samples. *Analytical and Bioanalytical Chemistry* **2016**, 1–13.
15. US EPA Definition and Procedure for the Determination of the Method Detection Limit, Revision 2; **2016**.

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