Application Note

Environmental

Robust and Reproducible Monitoring of PFAS in Treated Wastewater by Direct Injection on 6495D LC/MS

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Abstract

This study demonstrates the Agilent 6495D liquid chromatography/mass spectrometry (LC/MS) robustness with direct injections of industrial wastewater monitoring 38 per- and polyfluoroalkyl substances (PFAS) in a 17-minute analysis. The widespread contamination of PFAS in the environment has shown a need for an accurate and robust method for wastewater testing. Direct injection mitigates multistep sample preparation and allows for higher throughput of samples. The method presents reproducible quantitation with RSD < 20% for the 38 PFAS by analyzing 150 direct injections of wastewater at 10 ng/L. All 38 analytes demonstrated linearity with $R^2 \geq 0.995$ between the concentration of 0.5 to 100 ng/L. Method detection limits of the 38 PFAS were calculated between the range of 0.25 to 3.4 ng/L. To further demonstrate the sensitivity of the 6495D, these 38 PFAS were also spiked into the wastewater at 1 ng/L. Despite the presence of some incurred PFAS, we managed to show that more than 30 PFAS could be detected at 1 ng/L in the wastewater samples.

Introduction

PFAS is a class of synthetic chemicals that are resistant to heat, water, grease, and oil. Exposure to this class of chemicals is widespread and their ability to move and persist in the environment makes them difficult to clean up. Found in everyday household and workplace products such as drinking water, food packaging, fish caught from compromised environments, and personal care products is an indication of how widespread the use of PFAS and its contamination in the environment.

Traditional techniques of analysis to enhance sensitivity incorporate the use of solid phase extraction, which allows enrichment of the sample before injection. Increased awareness of emerging contaminants has necessitated the need for higher throughput methods, some bypassing the sample preparation step, to decrease sample analysis time. Direct injection analysis can save time by removing the need to prepare samples but still providing robustness and reproducibility to justify its capabilities and improved efficiency.

Experimental

Reagents and chemicals

LC/MS grade solvents and analytical reagents were used for this study.

Standards and solutions

PFAS standards and isotopically labeled surrogate standards were procured from the University of Melbourne as individual standards and prepared into a stock standard mix before being diluted for the calibration curve and spiking. The calibration curve and method detection limit (MDL) spikes at 1 ng/L were prepared in water. Serial dilutions were performed to prepare six calibration concentrations.

Sample preparation

A single aliquot of treated wastewater was spiked with 38 PFAS analytes (listed in Table 1) at 10 ng/L and nine isotopically labeled surrogate standards (listed in Table 2). The spiked wastewater was then diluted 1:1 with methanol and aliquoted to analytical vials prior to analysis. Additional spikes at 1 ng/L were prepared in wastewater.

Table 1. 38 PFAS compounds.

Analyte	CAS No.
PFBA	375-22-4
PFPeA	2706-90-3
PFHxA	307-24-4
PFHpA	375-85-9
PFOA	335-67-1
PFNA	375-95-1
PFDA	335-76-2
PFBS	375-73-5
PFPeS	2706-91-4
PFHxS	355-46-4
PFHpS	375-92-8
PFOS	1763-23-1
PFNS	98789-57-2
PFDS	335-77-3
HFPO-DA (Gen X)	62037-80-3
ADONA	958445-44-8
PFMOPrA	377-73-1
PFMOBA	863090-89-5
PFecHS	133201-07-7

Analyte	CAS No.
3:3 FTCA	356-02-5
5:3 FTCA	914637-49-3
7:3 FTCA	812-70-4
PFEESA	113507-82-7
6:2 Cl-PFESA	756426-58-1
8:2 Cl-PFESA	763051-92-9
4:2 FTSA	757124-72-4
6:2 FTSA	27619-97-2
8:2 FTSA	39108-34-4
FBSA	30334-69-1
FHxSA	41997-13-1
MeFOSA	31506-32-8
EtFOSA	4151-50-2
NMeFOSAA	2355-31-9
NEtFOSAA	2991-50-6
6:2-FTAB	34455-29-3
PFUnA	2058-94-8
PFUnDS	749786-16-1
PFDoA	307-55-1

Table 2. Nine Internal standards.

Internal Standards	
4:2 FTSA IS	PFBS IS
6:2 FTSA IS	PFDoA IS
8:2 FTSA IS	PFHxS IS
HFPO-DA IS	PFOS IS
NEtFOSAA IS	

Instrumentation

Liquid chromatography system:

- Agilent 1290 Infinity II Bio-LC High-Speed Pump (G7132A)
- Agilent 1290 Infinity II Bio-LC Multisampler (G7137B)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B)

Mass spectrometry system:

- Agilent 6495D Triple Quadrupole LC/MS (G6495D)
- Agilent Jet Stream (AJS) Source

A PFC free kit, together with a delay column, was also used.

Method

Optimization of multiple reaction monitoring (MRM) transitions for analytes and ISTDs was carried out using Auto MRM Optimizer in the Agilent MassHunter Acquisition software version 12.1 to provide optimal sensitivity and selectivity. The data were processed using the Agilent MassHunter qualitative analysis software version 12.0 and quantitative analysis software version 12.1.

Table 3. Instrument acquisition polarity.

Compound	Polarity	Compound	Polarity
PFBA	Negative	3:3 FTCA	Negative
PFPeA	Negative	5:3 FTCA	Negative
PFHxA	Negative	7:3 FTCA	Negative
PFHpA	Negative	PFEESA	Negative
PFOA	Negative	6:2 Cl-PFESA	Negative
PFNA	Negative	8:2 Cl-PFESA	Negative
PFDA	Negative	4:2 FTSA	Negative
PFBS	Negative	6:2 FTSA	Negative
PFPeS	Negative	8:2 FTSA	Negative
PFHxS	Negative	FBSA	Negative
PFHpS	Negative	FHxSA	Negative
PFOS	Negative	MeFOSA	Negative
PFNS	Negative	EtFOSA	Negative
PFDS	Negative	NMeFOSAA	Negative
HFPO-DA (Gen X)	Negative	NEtFOSAA	Negative
ADONA	Negative	6:2-FTAB	Positive
PFMOPrA	Negative	PFUnA	Negative
PFMOBA	Negative	PFUnDS	Negative
PFecHS	Negative	PFDoA	Negative

Table 4. Agilent 1290 Infinity II LC parameters.

Parameter	Description
Column	Agilent InfinityLab Poroshell AQ-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-742)
Column Temperature	45 °C
Mobile Phase	A) Water with 5 mM ammonium acetate B) Methanol
Flow Rate	0.5 mL/min
Gradient Program	Time (min) %B Flow rate (mL/min) 0 20 0.5 0.5 20 0.5 2.5 50 0.5 7.0 72 0.5 10.0 90 0.5 14.4 90 0.5 14.41 20 0.7 16.0 20 0.7 16.05 20 0.5
Stop Time	1 min
Injection Volume	20 µL

Table 5. Agilent 6495 triple quadrupole LC/MS and source parameters.

Parameter	Description
Drying Gas Temperature	250 °C
Drying Gas Flow	12 L/min
Sheath Gas Temperature	370 °C
Sheath Gas Flow	12 L/min
Nebulizer	30 psi
Capillary Voltage	3,000 V (+) 2,350 (–)
Nozzle Voltage	0 V (+)
Measurement Mode and Polarity	dMRM, positive and negative

Results and discussion

The sensitivity of the method was calculated with ten spikes of water at 1 ng/L resulting in the method detection limit. The calculated concentration was prior to dilution of methanol and demonstrates that all but three analytes were equal or less than 1 ng/L detection limits, the analytes with greater than 1 ng/L MDL were 3:3 FTCA and 8:2 FTSA (Table 6).

Table 6. Calculated MDLs.

Analyte Name	MDL (ng/L)	Analyte Name	MDL (ng/L)
PFBA	0.31	PFOA	0.50
PFMOPrA	0.33	PFOS	0.54
PFPeA	0.44	PFNA	0.32
3:3 FTCA	3.37	FHxSA	0.43
PFBS	0.30	7:3 FTCA	0.46
PFMOBA	0.40	6:2 Cl-PFESA	0.62
PFEESA	0.36	6:2 FTAB	0.56
4:2 FTSA	0.35	PFNS	0.68
PFHxA	0.45	PFDA	0.38
PFPeS	0.44	8:2 FTSA	1.02
HFPO-DA (Gen X)	0.41	NMeFOSAA	0.26
FBSA	0.31	PFDS	0.32
PFHpA	0.53	PFUnA	0.48
PFHxS	0.47	NEtFOSAA	0.37
ADONA	0.44	8:2 Cl-PFESA	0.58
5:3 FTCA	0.54	PFUnDS	0.75
PFecHS	0.54	PFDoA	0.62
6:2 FTSA	1.00	MeFOSA	0.68
PFHpS	0.52	EtFOSA	0.68

The reproducibility and robustness of the method was measured by 150 direct injections of wastewater spiked at 10 ng/L. The RSDs calculated on all 150 injections were within the range of 4 to 19% (Table 7 and Figure 1). Acceptable ranges for environmental testing are typically less than 20%.

Table 7. Calculated RSD% of 150 direct injections of wastewater.

Analyte	RSD (n = 150)	Analyte	RSD (n = 150)
PFBA	6.93%	PFOA	9.45%
PFMOPrA	5.70%	PFOS	8.77%
PFPeA	6.19%	PFNA	8.03%
3:3 FTCA	18.64%	FHxSA	6.80%
PFBS	6.88%	7:3 FTCA	4.92%
PFMOBA	5.96%	6:2 Cl-PFESA	10.32%
PFEESA	5.89%	6:2 FTAB	10.06%
4:2 FTSA	12.03%	PFNS	9.96%
PFHxA	5.99%	PFDA	10.52%
PFPeS	6.27%	8:2 FTSA	15.34%
HFPO-DA (Gen X)	6.93%	NMeFOSAA	9.72%
FBSA	5.71%	PFDS	10.85%
PFHpA	5.96%	PFUnA	11.20%
PFHxS	6.52%	NEtFOSAA	9.35%
ADONA	6.18%	8:2 Cl-PFESA	12.51%
5:3 FTCA	4.76%	PFUnDS	14.16%
PFecHS	6.55%	PFDoA	14.56%
6:2 FTSA	13.59%	MeFOSA	13.56%
PFHpS	6.42%	EtFOSA	17.79%

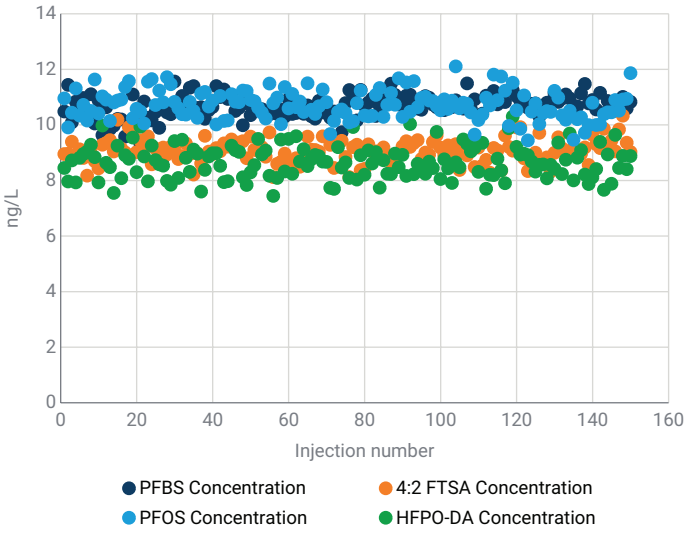

Figure 1. Calculated concentration over 150 injections for 10 ng/L wastewater spikes.

The column chosen for this application was a 4.6 mm inner diameter AQ-C18 column, which can withstand 100% aqueous conditions. This allowed for better resolving capabilities when dealing with large panels of PFAS at varying concentrations. Calibration curve concentrations were measured between 0.5 to 100 ng/L and displayed greater than 0.995 R^2 for all 38 analytes (Figures 2 to 4). The detection of PFAS in spikes at 1 ng/L in wastewater were observed clearly for more than 30 PFAS while displaying the clean baseline of a blank overlay. Figure 5 shows an example of seven PFAS chromatograms together with the matrix blank at 1 ppt in wastewater.

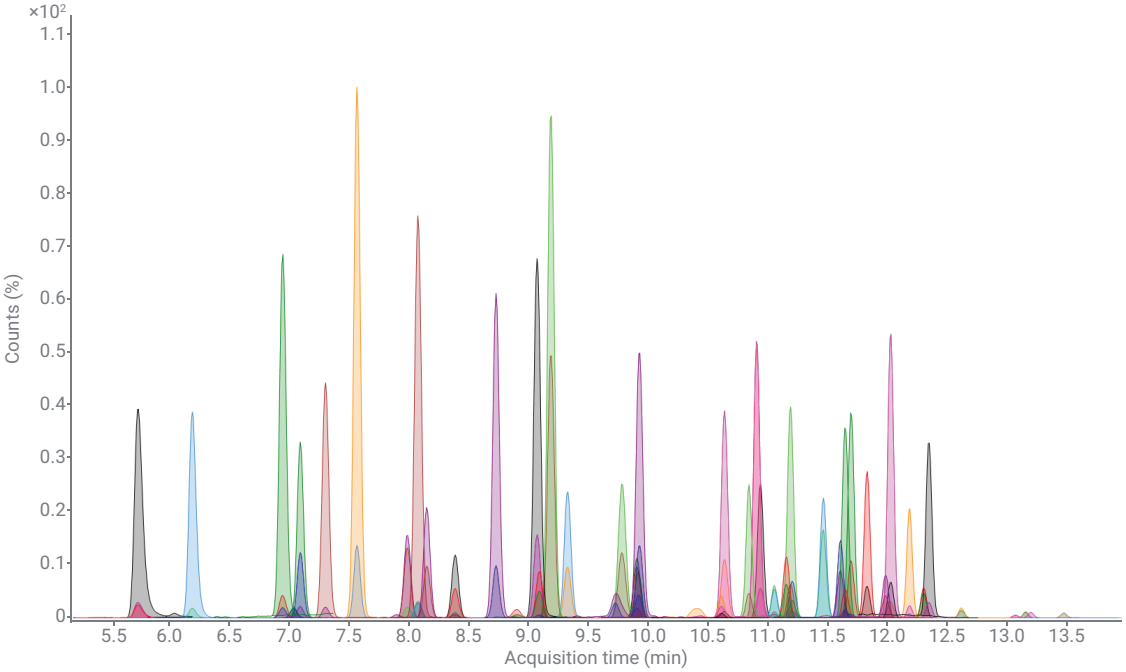

Figure 2. The 100 ng/L standard.

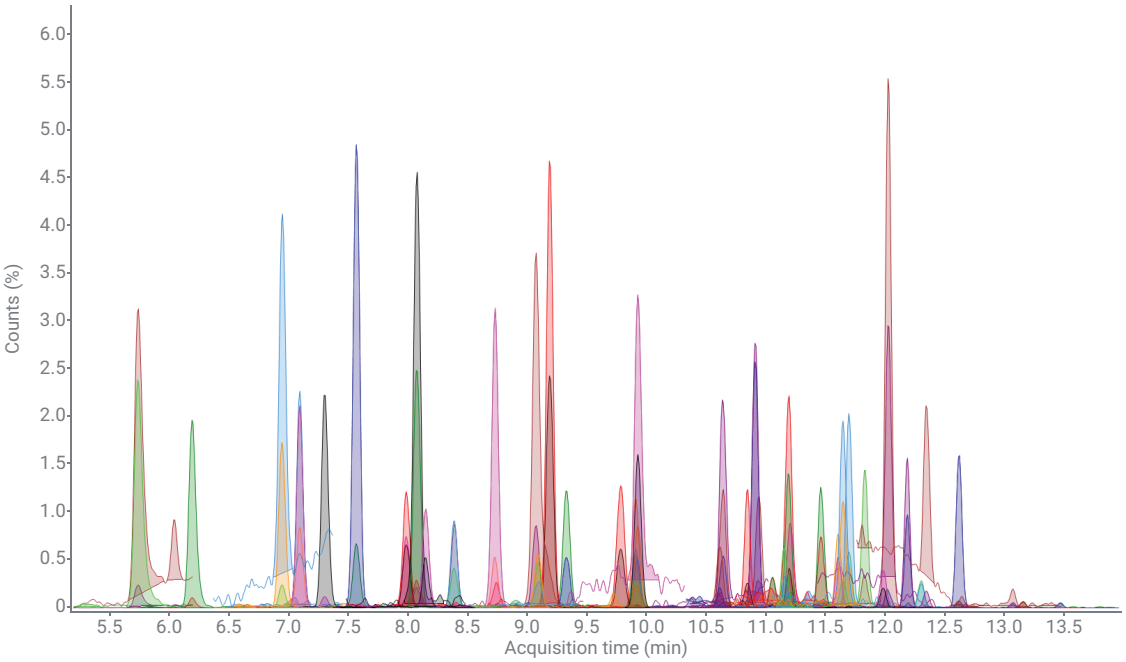

Figure 3. The 5 ng/L calibration standard.

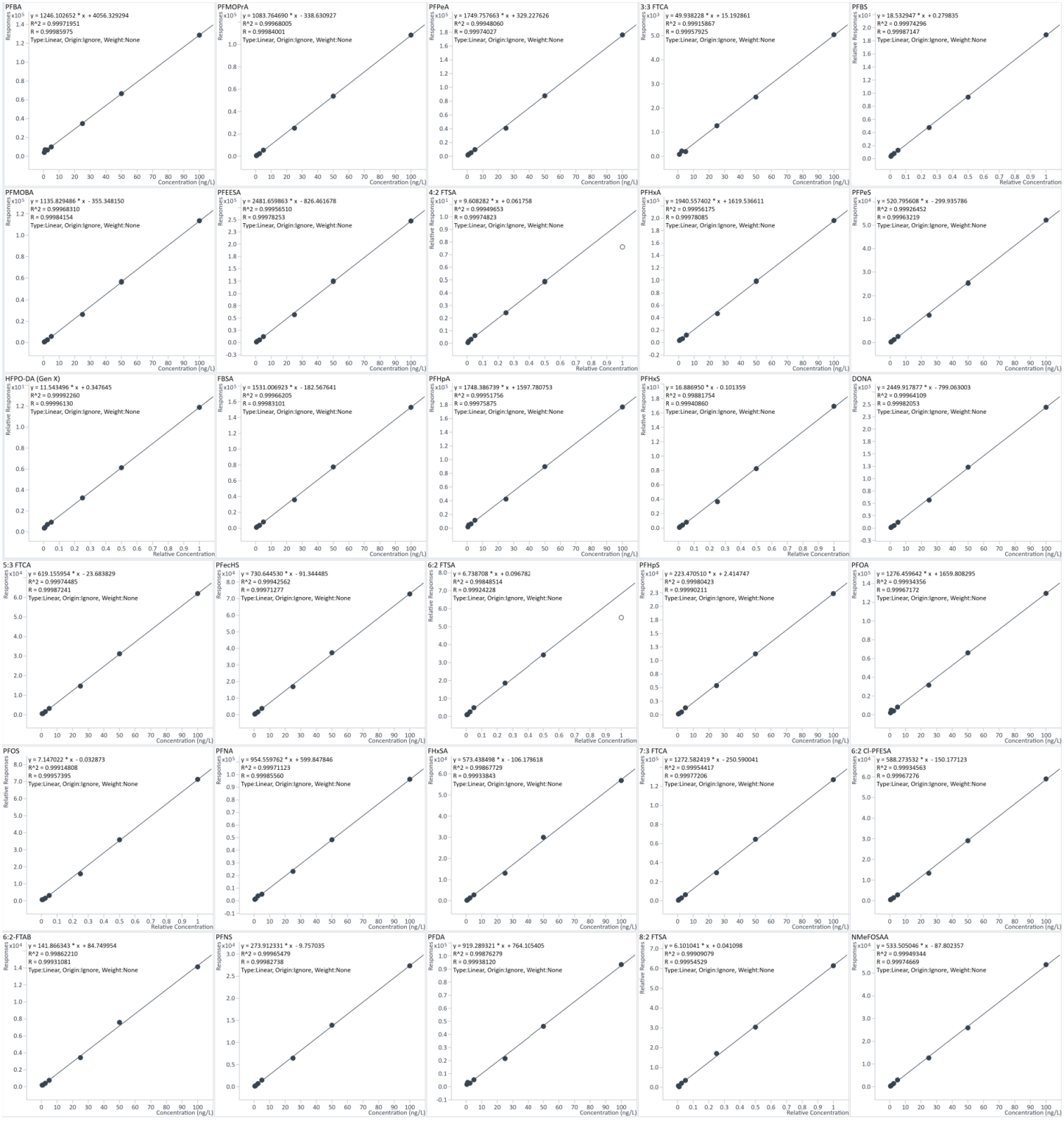

Figure 4. The 0.5 to 100 ng/L calibration curves. (Continued on the next page).

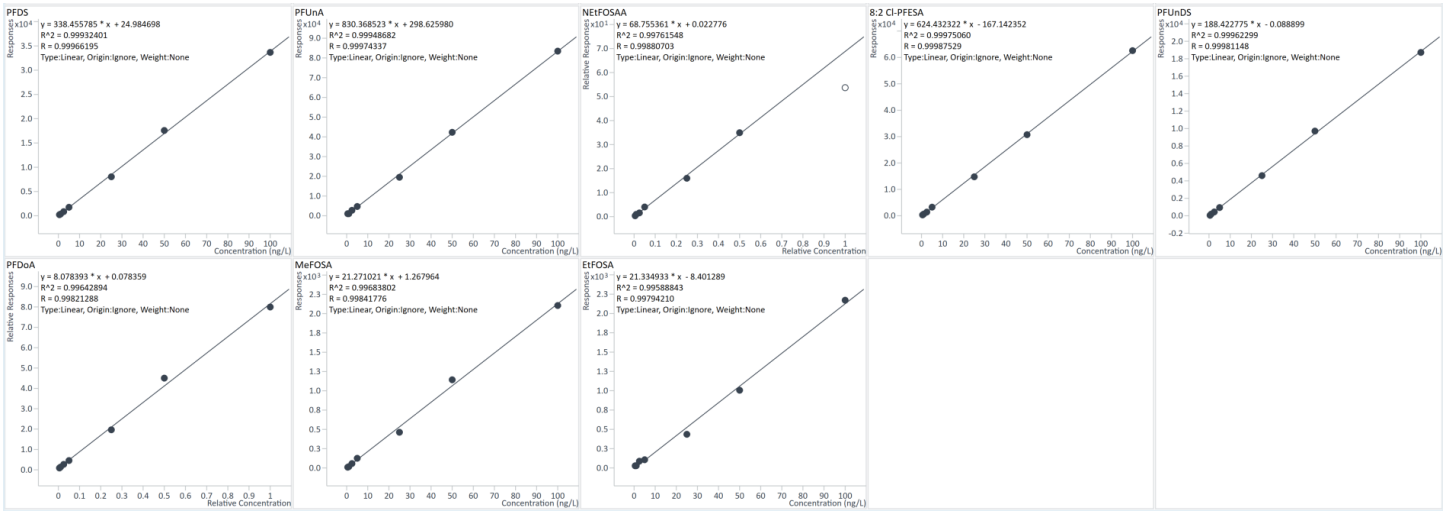

Figure 4. The 0.5 to 100 ng/L calibration curves. (Continued from the previous page).

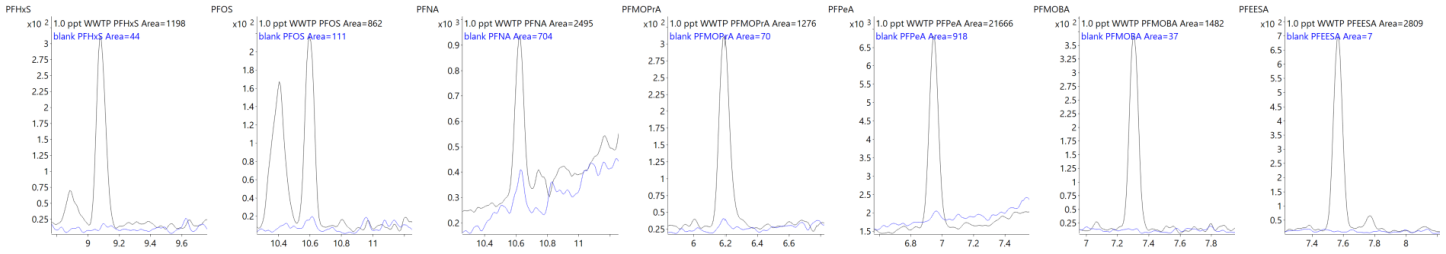

Figure 5. Chromatograms of 1 ng/L wastewater spike with blank overlay.

Conclusion

The increased awareness of PFAS globally has meant that regulations are constantly being adopted and larger amounts of samples at lower limits are required. By incorporating the direct injection technique on the Agilent 6495D LC/MS we get a combination of high sample throughput while providing excellent analytical sensitivity. Also, increasing throughput and reducing consumables cost creates increased value in the instrument. The method displays a clean baseline and sensitivity with the observation of 1 ng/L detection in spiked wastewater. Reproducibility and robustness were measured between the concentrations of 0.5 to 100 ng/L on the 6495D LC/MS. The system was able to detect 38 PFAS between 0.25 to 3.4 ng/L with RSD < 20% over 150 direct injections, providing confidence in the data for a complex matrix like wastewater.

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