Application Note

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Targeted PFAS Analysis in Industrial Wastewater Using the Agilent 6475 Triple Quadrupole LC/MS System

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Abstract

This application note focuses on the regulatory requirements from industrial manufacturers regarding the quantitative analysis of per- and polyfluoroalkyl substances (PFAS) in wastewater matrices. Going beyond the scope of the US Environmental Protection Agency (EPA) Method 1633, a comprehensive target list comprising 71 native and 37 labeled PFAS compounds was thoroughly investigated and analyzed with aqueous wastewater samples. The sample extraction methodology was performed using Agilent Bond Elut PFAS WAX SPE cartridges. The quantitation of PFAS was executed using an Agilent 1290 Infinity II liquid chromatograph (LC) system coupled with an Agilent 6475 triple quadrupole mass spectrometer (LC/TQ). The analytical performance parameters, including linearity, sensitivity, accuracy, and precision, were verified. Furthermore, detailed discussions were conducted on the analysis results of the wastewater samples.

Introduction

PFAS have emerged as significant environmental contaminants due to their widespread applications. These compounds, characterized by their stable carbon-fluorine bonds, exhibit remarkable resistance to degradation processes. This resistance leads to their persistence in the environment and potential accumulation in surface waters. In industrial settings, PFAS has utility in processes such as fire-fighting foam production, metal plating, and semiconductor manufacturing, among others.[1-4] Consequently, industrial wastewater streams become significant sources of PFAS contamination, posing potential harmful exposure to surrounding ecosystems.[4] The manufacture and use of a variety of PFAS are being banned or restricted under the European Union persistent organic pollutants (EU POPs) regulation and the registration, evaluation, authorization, and restriction of chemicals (REACH).[5] The US EPA issued Method 1633 in January 2024, a standard analytical protocol for detection and quantitation of PFAS in many matrices, including wastewater.[6]

Accurate quantitation of PFAS in industrial wastewater is essential for regulatory compliance, environmental monitoring, and risk assessment purposes. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) has emerged as a powerful analytical technique for the precise identification and quantification of PFAS compounds in complex matrices such as industrial wastewater.

In this application note, a comprehensive approach is presented for the quantitation of emerging and legacy PFAS in industrial wastewater. It uses a 1290 Infinity II LC connected to a 6475 LC/TQ for analysis. The note details the methodology, analytical parameters, and the results obtained. The acquisition method is based on the Agilent PFAS MRM Database covering 100 plus native and isotopically labeled PFAS. In this study, solid phase extraction (SPE) using a Bond Elut PFAS WAX cartridge is applied for the sample preparation based on EPA Method 1633. This application note aims to contribute to the ongoing efforts in understanding and mitigating the environmental impact of PFAS contamination in industrial wastewater.

Experimental

Chemicals and standards

All the chemicals and solvents used for this study were LC/MS grade. Ultrapure water was used from a Milli-Q water system (Merck Millipore, U.S.).

Native and isotopically labeled PFAS analytical standards were purchased as individual stock solutions, solution mixes, or powdered standards from Wellington Laboratories Inc. (Guelph, ON, Canada) and Toronto Research Chemicals (Toronto, ON, Canada). Calibration standards preparation followed the procedure as indicated in the workflow guide in the Agilent PFAS eMethod solution (part number G5285AA).

Instrumentation

The Agilent 1290 Infinity II LC system operating conditions and 6475 LC/TQ instrument parameters are detailed in Table 1, which is referred to as Agilent PFAS MRM Database. An Agilent polyfluorinated compound (PFC)-free HPLC conversion kit (part number 5004-0006) was installed instead of a standard LC to minimize PFAS contamination from solvents and LC flow path. Data processing was performed using Agilent MassHunter LC/MS Acquisition software version 12.0 and Quantitative Analysis software version 12.0.

Table 1. LC operating conditions and MS source parameters.

Agilent 1290 Infinity II LC Conditions	
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 × 100 mm, 1.8 µm, 1200 bar pressure limit (p/n 959758-902)
UHPLC Guard	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm, 1.8 µm, 1200 bar pressure limit, UHPLC guard (p/n 821725-901)
Column Temperature	55 °C
Injection Volume	5 µL
Autosampler Temperature	5 °C
Mobile Phase A	5 mM Ammonium acetate in water
Mobile Phase B	Methanol
Mobile Phase Flow Rate	0.4 mL/min
Gradient	Time (min) %A %B 0.00 85 15 1.00 85 15 1.50 45 55 5.50 30 70 7.00 20 80 12.00 0 100 14.40 0 100 14.50 85 15
Stop Time	14.5 minutes
Post Time	2.5 minutes
Needle Wash	Multiwash
Wash Solvent 1 (S1)	15:85 Methanol:water
Wash Solvent 2 (S2)	1:1 Acetonitrile:2-propanol
Agilent 6475 MS Parameters	
Ion Source	Agilent AJS ESI
Polarity	Negative
Q1 and Q3 Resolution	Unit
Cycle Time	580 ms
Gas Temperature	230 °C
Gas Flow	6 L/min
Nebulizer	20 psi
Sheath Gas Temperature	375 °C
Sheath Gas Flow	12 L/min
Capillary (Negative)	2,500 V
Nozzle Voltage	0 V

Sample extraction

The industrial wastewater sample used for this study was provided by a local agency. The sample preparation procedure following EPA 1633 is outlined in Figure 1. First, 10 mL of unpreserved wastewater samples were taken into a 15 mL polypropylene (PP) conical tube and fortified with surrogates as extracted internal standards (EIS). The sample pH was adjusted to between 6 and 7 with ammonium hydroxide or acetic acid before loading the SPE cartridges. To prepare matrix spike quality control (QC) samples, an appropriate amount of native PFAS mix solution was added into wastewater samples to make low spike QCs (LSQ) and high spike QCs (HSQ). A matrix blank was prepared without the addition of native PFAS standards. The entire process including SPE setup, cartridge conditioning, sample loading and eluting, Carbon S cleanup, concentration, and reconstitution steps are detailed in Figure 1.

The final sample preconcentration factor was 20-fold. Importantly, non-extracted internal standards (NIS) were added post-preparation but before injection and were used to report EIS recoveries as surrogates.[6]

1. Prepare the sample	– Place 10 mL of sample into 15 mL Falcon tube. – Spike the surrogate mix (EIS) into sample tube and vortex well. – Check that the sample pH is within 6 to 7, adjusting using ammonium hydroxide or acetic acid if necessary.
2. Setup SPE	– Connect the SPE manifold to the vacuum trap. – Place the sample tube in the collection rack. – Rotate to waste position. – Assemble the stopcock, WAX cartridge (part number 5610-2152), adaptor, and sample reservoir (12 mL, part number 12131010).
3. Condition SPE	– Add 5 mL of 1% methanolic ammonium hydroxide. – Add 5 mL of 0.3 M formic acid. – Drain to waste by gravity and close the stopper when the liquid is just above the sorbent.
4. Load the sample	– Load 10 mL of samples into the reservoir. – Adjust the vacuum and stopcock to achieve a flow rate of ~2 mL/min.
5. Rinse sample tube and reservoir	– Add 2 × 5 mL of water. – Add 2.5 mL of 1:1 0.1 M formic acid:methanol. – Dry the cartridge under vacuum.
6. Elute the sample	– Rotate to the sample collect position. – Rinse the sample tube and reservoir with 5 mL of methanolic ammonium hydroxide. – Transfer to the SPE cartridge. – Flute the cartridge by gravity and dry it under vacuum.
7. Carbon S cleanup	– Add 25 µL of acetic acid to the sample eluate tube and vortex well. – Setup the carbon S cartridge (part number 5610-2082) onto the positive pressure manifold (PPM). – Pass the eluate through the cartridge and dry it. – Vortex the sample collection tube.
8. Concentrate and reconstitute	– Dry the sample tube under a gentle nitrogen steam in a water bath at 50 to 55 °C. – Reconstitute to a final 0.5 mL with methanol/water and NIS mix. – Vortex well.
9. Centrifuge and analyze	– Centrifuge the sample tube at 3,000 rcf at 10 °C for 5 minutes. – Transfer the final extract to a 250 µL-PP vial for LC/TQ analysis. – Store any remaining solution at 4 °C.

Figure 1. Wastewater sample extraction procedure.

Results and discussion

Initial calibration performance

A series of calibration solutions containing the native PFAS (target analytes), surrogates (EIS), and isotope performance standards (NIS) were used to establish the initial calibration of the analytical instrument. The concentrations of the target analytes in the solutions were varied to encompass the working range of the instrument, while the concentrations of the EIS and NIS remained constant. The relative standard error (RSE) ≤ 20% was attained for each targeted analyte using a minimum of seven contiguous calibration standards. The accuracy and precision of each calibration standard conformed to the typical acceptable range of 70 to 130% and ≤ 20% (n = 3), respectively. Figure 2 shows the initial calibration graphs for four representative analytes: PFPeS, PFHxPA, PFMBA, and PFBS.

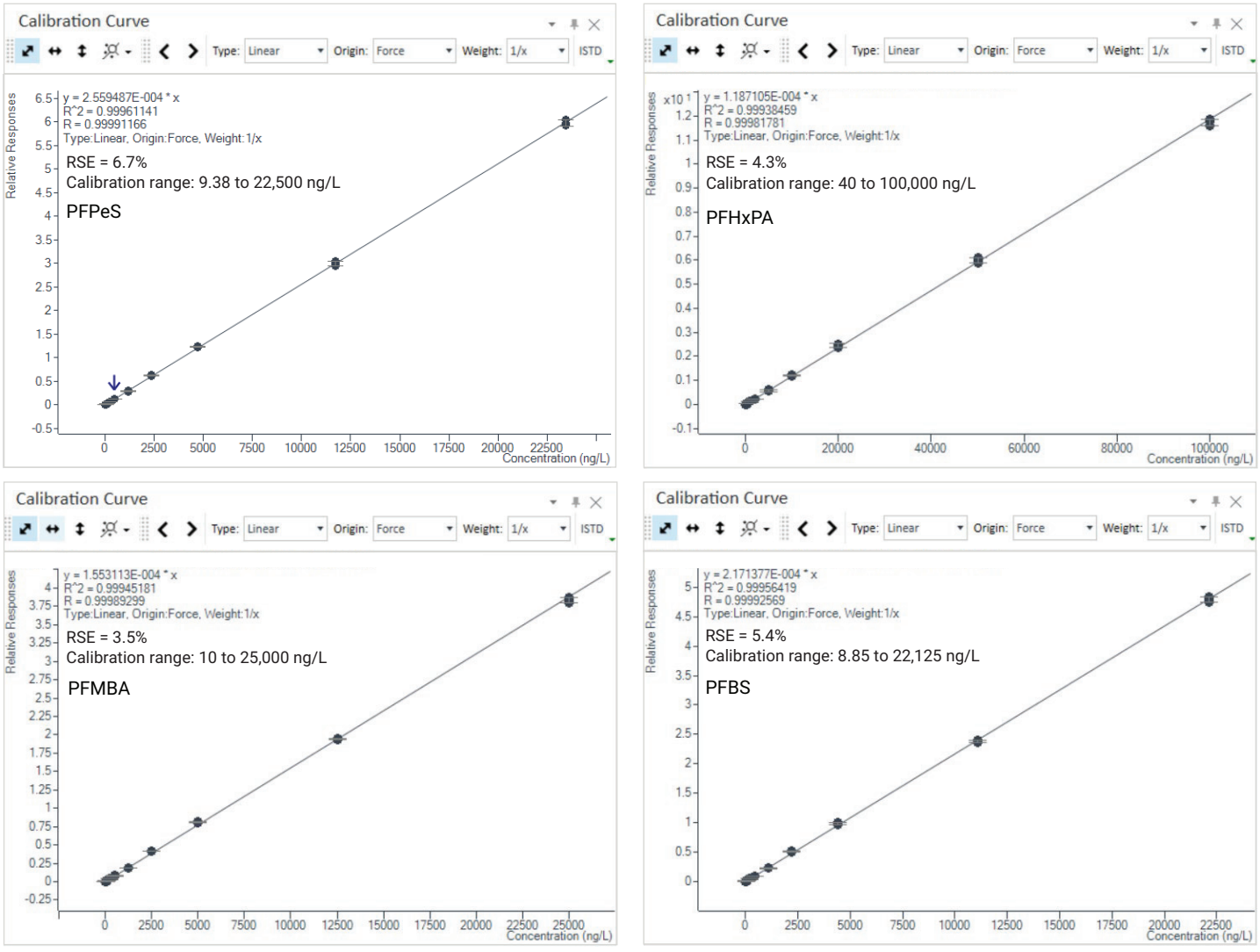

Figure 2. Linear calibration curves for PFPeS, PFHxPA, PFMBA, and PFBS (three injections per level).

Method sensitivity

During the pilot study, the unspiked industrial wastewater (matrix blank) was screened and detected with many of the positive native PFAS. Thus, the matrix was not suitable to be used for the evaluation of method detection limit (MDL).[7] In this study, method sensitivity was assessed based on the limit of quantitation (LOQ), which was set at or above the concentration of the lowest initial calibration standard. This met the performance criteria as described in EPA Method 1633.[6] The LOQs for all target analytes were summarized in Table 2, while the highlighted compounds in green were included in EPA 1633. As shown in Table 2, LOQs obtained in this study were all lower or within pooled ranges of LOQ values for aqueous matrices listed in EPA 1633. These results demonstrate the sensitivity of the analytical method using the 6475 LC/TQ.

Method accuracy and precision

Method accuracy and precision were evaluated based on QC recoveries and the %RSD of recoveries, respectively. Triplicate preparations of low spike QCs (LSQ, concentration range from 0.0125 to 0.125 µg/kg) and high spike QCs (HSQ, concentration range from 0.25 to 2.5 µg/kg) were performed following the entire sample extraction procedure. The measured concentration of each analyte in QCs was corrected by subtracting the native level present in the unspiked wastewater sample. The method recovery was calculated based on the mean percent recovery, while the method precision was assessed from the %RSD of recoveries.

Table 2. Analytical result summary.

No.	Compound	PFAS Group	CAS Number	Surrogate	LOQ (ng/L)	Pooled Ranges of LOQ in EPA 1633 (ng/L)	LSQ		HSQ	
							Recovery	Precision (n = 3)	Recovery	Precision (n = 3)
1	PFUnDA	PFCA	2058-94-8	$^{13}C_7$-PFUnDA	2.5	1 to 4	113%	2%	86%	2%
2	PFTrDA	PFCA	72629-94-8	$^{13}C_2$-PFDoDA	1	1 to 4	104%	3%	81%	4%
3	PFTDA	PFCA	376-06-7	$^{13}C_2$-PFTDA	1	1 to 4	103%	3%	91%	7%
4	PFPeS	PFSA	2706-91-4	$^{13}C_3$-PFHxS	0.938	1 to 4	111%	1%	90%	2%
5	PFPeA	PFCA	2706-90-3	$^{13}C_5$-PFPeA	1	2 to 8	108%	1%	91%	1%
6	PFOSA	FASA	754-91-6	$^{13}C_8$-PFOSA	1	1 to 4	128%	10%	98%	6%
7	PFOS	PFSA	1763-23-1	$^{13}C_8$-PFOS	0.73	1 to 4	76%	1%	91%	3%
8	PFOPA	PFPA	40143-78-0	Cl-PFOPA	10	NA	100%	3%	78%	3%
9	PFODA	PFCA	16517-11-6	$^{13}C_2$-PFHxDA	1	NA	87%	4%	87%	14%
10	PFOA	PFCA	335-67-1	$^{13}C_8$-PFOA	1	1 to 4	84%	2%	99%	2%
11	PFNS	PFSA	68259-12-1	$^{13}C_8$-PFOS	2.4	1 to 4	111%	1%	90%	3%
12	PFNA	PFCA	375-95-1	$^{13}C_9$-PFNA	1	1 to 4	76%	2%	92%	1%
13	PFMPA	PFECA	377-73-1	$^{13}C_4$-PFBA	1	4 to 16	87%	1%	93%	2%
14	PFMBA	PFECA	863090-89-5	$^{13}C_5$-PFPeA	1	4 to 15	114%	1%	110%	2%
15	PFHxS	PFSA	355-46-4	$^{13}C_3$-PFHxS	0.74	1 to 4	79%	2%	84%	2%
16	PFHxPA	PFPA	40143-76-8	Cl-PFOPA	4	NA	72%	2%	119%	5%
17	PFHxDA	PFCA	67905-19-5	$^{13}C_2$-PFHxDA	1	NA	86%	4%	84%	13%
18	PFHxA	PFCA	307-24-4	$^{13}C_5$-PFHxA	1	1 to 4	76%	1%	70%	1%
19	PFHpS	PFSA	375-92-8	$^{13}C_8$-PFOS	0.952	1 to 4	111%	1%	89%	2%
20	PFHpA	PFCA	375-85-9	$^{13}C_4$-PFHpA	1	1 to 4	97%	1%	112%	2%
21	PFEESA	PFESA	113507-82-7	$^{13}C_3$-PFBS	0.89	2 to 8	104%	1%	83%	2%
22	PFDS	PFSA	335-77-3	$^{13}C_8$-PFOS	0.964	1 to 4	108%	3%	88%	4%
23	PFDPA	PFPA	52299-26-0	Cl-PFOPA	10	NA	85%	8%	83%	2%
24	PFDoS	PFSA	79780-39-5	$^{13}C_8$-PFOS	2.42	1 to 4	91%	2%	83%	6%
25	PFDoDA	PFCA	307-55-1	$^{13}C_2$-PFDoDA	1	1 to 4	108%	3%	85%	2%

No.	Compound	PFAS Group	CAS Number	Surrogate	LOQ (ng/L)	Pooled Ranges of LOQ in EPA 1633 (ng/L)	LSQ		HSQ	
							Recovery	Precision (n = 3)	Recovery	Precision (n = 3)
26	PFDA	PFCA	335-76-2	$^{13}C_6$-PFDA	1	1 to 4	120%	2%	88%	2%
27	PFBS	PFSA	375-73-5	$^{13}C_3$-PFBS	0.885	1 to 4	116%	1%	83%	2%
28	PFBPA	PFPA	52299-24-8	Cl-PFOPA	4	NA	91%	6%	88%	8%
29	PFBA	PFCA	375-22-4	$^{13}C_4$-PFBA	2.5	4 to 16	99%	0%	92%	1%
30	P5MeODIOXOAc	PFECA	1190931-41-9	$^{13}C_3$-HFPO-DA	2.5	NA	128%	7%	100%	3%
31	N-MeFOSAA	FASAA	2355-31-9	$^{2}H_3$-N-MeFOSAA	0.76	1 to 4	81%	2%	86%	4%
32	N-MeFOSA	FASA	31506-32-8	$^{2}H_3$-N-MeFOSA	1	1 to 4	114%	32%	88%	16%
33	NFDHA	PFECA	151772-58-6	$^{13}C_5$-PFHxA	1	2 to 7	115%	1%	103%	2%
34	N-EtFOSAA	FASAA	2991-50-6	$^{2}H_5$-N-EtFOSAA	0.775	1 to 4	82%	3%	74%	5%
35	N-EtFOSA	FASA	4151-50-2	$^{2}H_5$-N-EtFOSA	1	1 to 4	57%	28%	99%	17%
36	MeFOSE	FASE	24448-09-7	$^{2}H_7$-MeFOSE	4	10 to 40	91%	11%	80%	12%
37	MeFHxSA	FASA	68259-15-4	$^{13}C_8$-PFOSA	2.25	NA	51%	19%	58%	23%
38	MeFBSA	FASA	68298-12-4	$^{13}C_8$-PFOSA	4	NA	57%	29%	51%	12%
39	HFPO-TA	PFECA	13252-14-7	$^{13}C_9$-PFNA	0.95	NA	96%	4%	82%	3%
40	HFPO-DA	PFECA	13252-13-6	$^{13}C_3$-HFPO-DA	1	2 to 8	109%	3%	87%	4%
41	FOSAA	FASAA	2806-24-8	$^{2}H_3$-N-MeFOSAA	1	NA	92%	10%	102%	13%
42	FHxSA	FASA	41997-13-1	$^{13}C_8$-PFOS	1	NA	54%	31%	117%	6%
43	FDSA	FASA	NA	$^{13}C_8$-PFOSA	1	NA	116%	8%	83%	11%
44	FBSA	FASA	30334-69-1	$^{13}C_3$-PFHxS	1	NA	57%	33%	113%	5%
45	EtFOSE	FASE	1691-99-2	$^{2}H_9$-EtFOSE	4	10 to 40	59%	40%	94%	4%
46	DONA	PFECA	919005-14-4	$^{13}C_4$-PFHpA	0.945	2 to 8	97%	1%	80%	1%
47	diSAmPAP	SAmPAP	2965-52-8	$(^{13}C_2)_2$-8:2 diPAP	2.45	NA	97%	5%	100%	13%
48	Cl-PFHxPA	PFPA	NA	Cl-PFOPA	4	NA	74%	2%	98%	6%
49	9Cl-PF3ONS	PFESA	756426-58-1	$^{13}C_8$-PFOS	2.3	4 to 15	95%	2%	78%	3%
50	8:8 PFPi	PFPiA	40143-79-1	$(^{13}C_2)_2$-6:2 diPAP	2.4	NA	124%	5%	110%	7%
51	8:3 FTCA	FTCA	34598-33-9	$^{13}C_6$-PFDA	2.5	NA	49%	17%	77%	12%
52	8:2 FTUCA	FTUCA	70887-84-2	$^{13}C_2$-8:2 FTUCA	1	NA	113%	14%	96%	14%
53	8:2 FTSA	FTSA	39108-34-4	$^{13}C_2$-8:2 FTSA	0.958	4 to 15	107%	1%	87%	2%
54	8:2 FTCA	FTCA	27854-31-5	$^{13}C_2$-8:2 FTCA	10	NA	108%	11%	109%	12%
55	8:2 diPAP	diPAP	678-41-1	$(^{13}C_2)_2$-8:2 diPAP	0.978	NA	89%	5%	108%	13%
56	7:3 FTCA	FTCA	812-70-4	$^{13}C_2$-8:2 FTUCA	2.5	25 to 100	79%	16%	85%	15%
57	6:8 PFPi	PFPiA	610800-34-5	$(^{13}C_2)_2$-6:2 diPAP	4.86	NA	66%	6%	108%	3%
58	6:6 PFPi	PFPiA	40143-77-9	$^{13}C_2$-PFDoDA	0.97	NA	87%	4%	99%	4%
59	6:2/8:2 diPAP	diPAP	943913-15-3	$(^{13}C_2)_2$-6:2 diPAP	0.975	NA	115%	3%	98%	9%
60	6:2 FTUCA	FTUCA	70887-88-6	$^{13}C_2$-6:2 FTUCA	1	NA	125%	13%	96%	12%
61	6:2 FTSA	FTSA	27619-97-2	$^{13}C_2$-6:2 FTSA	0.948	4 to 15	112%	1%	87%	3%
62	6:2 FTCA	FTCA	53826-12-3	$^{13}C_2$-6:2 FTCA	25	NA	105%	12%	112%	11%
63	6:2 diPAP	diPAP	57677-95-9	$(^{13}C_2)_2$-6:2 diPAP	0.97	NA	114%	3%	96%	4%
64	5:3 FTCA	FTCA	914637-49-3	$^{13}C_2$-6:2 FTUCA	2.5	25 to 100	78%	18%	88%	15%
65	4-PFecHS	PFSA	646-83-3	$^{13}C_8$-PFOS	2.3	NA	105%	2%	86%	2%
66	4:2 FTSA	FTSA	757124-72-4	$^{13}C_2$-4:2 FTSA	0.934	4 to 15	108%	2%	86%	2%

No.	Compound	PFAS Group	CAS Number	Surrogate	LOQ (ng/L)	Pooled Ranges of LOQ in EPA 1633 (ng/L)	LSQ		HSQ	
							Recovery	Precision (n = 3)	Recovery	Precision (n = 3)
67	3:3 FTCA	FTCA	356-02-5	$^{13}C_5$-PFPeA	5	5 to 20	98%	12%	97%	9%
68	11Cl-PF3OUdS	PFESA	763051-92-9	$^{13}C_8$-PFOS	0.945	4 to 15	90%	1%	72%	3%
69	10:2 FTUCA	FTUCA	70887-94-4	$^{13}C_2$-10:2 FTUCA	1	NA	122%	13%	101%	8%
70	10:2 FTSA	FTSA	120226-60-0	$^{13}C_2$-8:2 FTSA	0.964	NA	110%	2%	86%	4%
71	10:2 FTCA	FTCA	53826-13-4	$^{13}C_2$-10:2 FTCA	50	NA	84%	7%	95%	8%

NA: Not Applicable
Green highlighted cells are targets listed in EPA 1633

Table 2 lists the recovery values and %RSD for all target analytes. For LSQ samples, 70 out of 71 analytes met recovery of 50 to 130%. For HSQ samples, all target analytes met this condition, which demonstrated the excellent efficiency of the WAX cartridge used for PFAS extraction from industrial wastewater samples in this study. For 40 analytes regulated in EPA 1633, HSQ recoveries fell between 70 to 110% with good precision of ≤ 17%. The recovery and precision distribution for 40 compounds from the list in EPA 1633 is illustrated in Figure 3. The results indicated the reproducibility of each technical preparation and thus confirm the accuracy and reliability of the workflow that was developed for PFAS analysis in the industrial wastewater sample matrix.

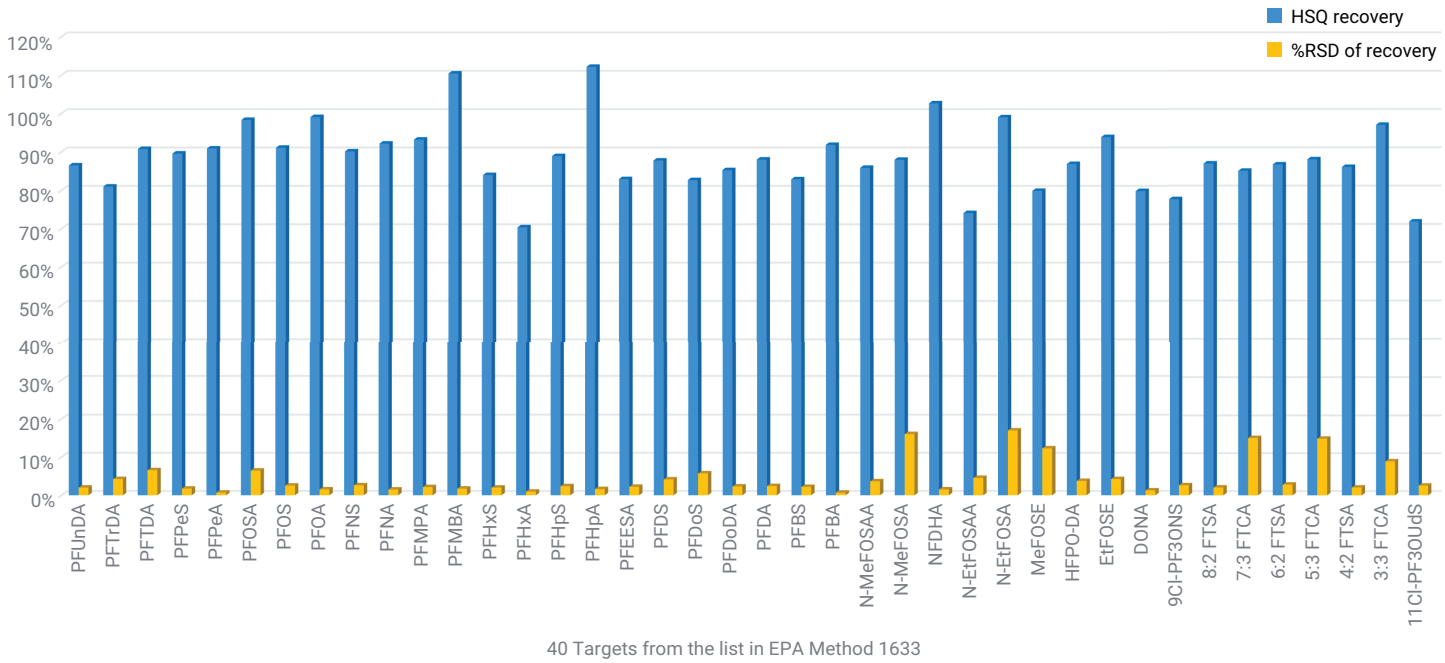

Figure 3. The recovery and precision distribution for 40 targets from the list in EPA 1633.

Wastewater analysis results

The concentration of native PFAS present in industrial wastewater samples was measured. To ensure the reliability of the analytical results, duplicate preparations of wastewater samples were performed with the addition of surrogates followed by the entire extraction process and LC/TQ detection. Figure 4 illustrates the chromatogram of compounds determined above LOQ level in the extract of wastewater samples. Over 10 native PFAS targets (such as HFPO-DA, PFBA, PFBS, PFDA, PFDoDA, PFHpA, PFMBA, PFNA, PFOA, PFOS, PFPeA, and PFUnDA, most of which are currently banned or restricted in regulations such as the EPA, POPs, REACH, etc.) were found to be above LOQ levels in wastewater samples. Recovery results of these targets using spiked QC samples were between 76 and 120% with RSD ≤ 5%. The recovery and recovery repeatability values confirmed the reliability of the newly developed method for PFAS analysis in industrial wastewater.

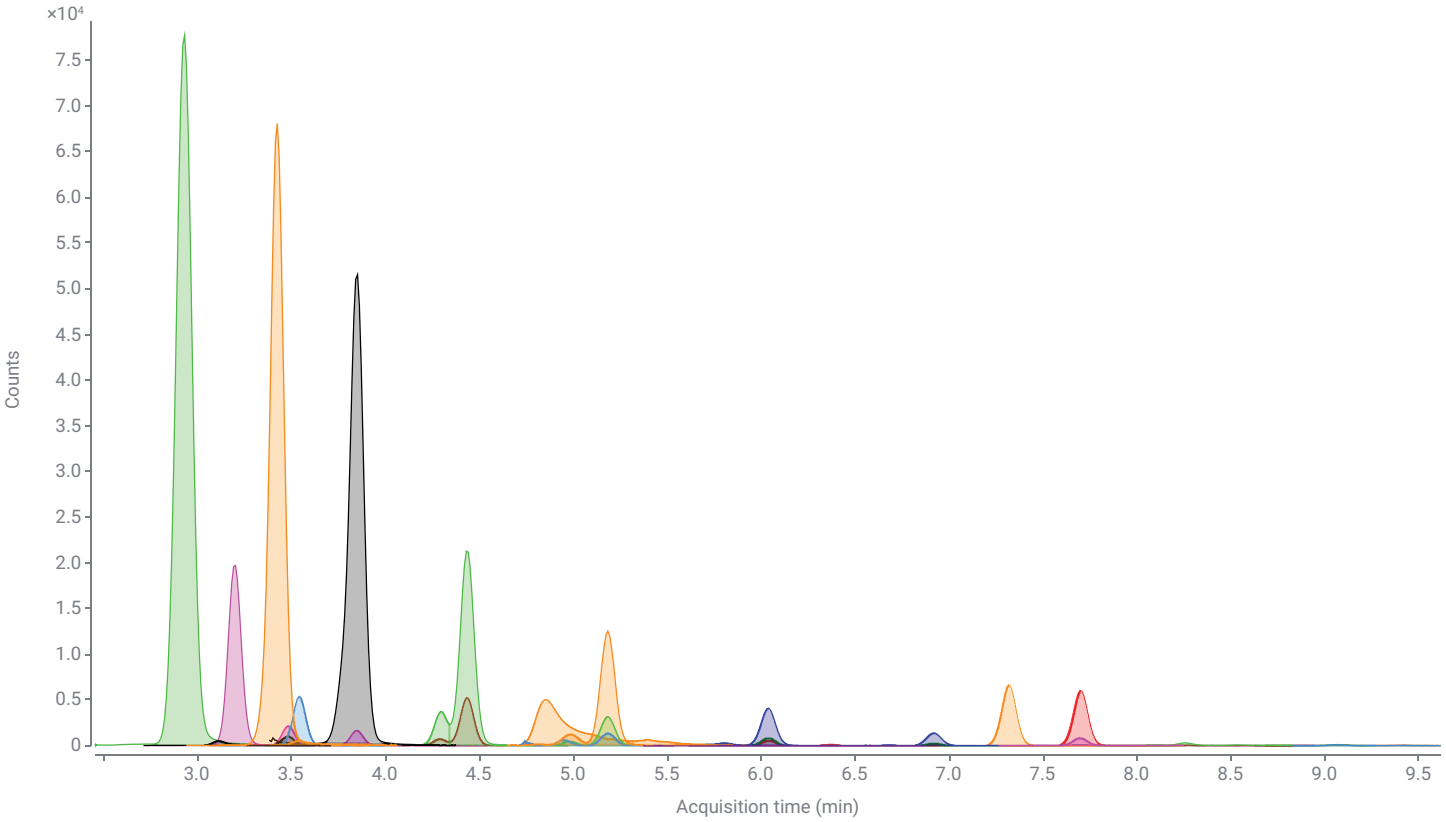

Figure 4. The MRM chromatogram of the unspiked wastewater extract (matrix blank).

Conclusion

This study focused on the quantitative analysis of PFAS in industrial wastewater, one of the significant sources of PFAS contamination, using the Agilent 1290 Infinity II LC coupled to the Agilent 6475 LC/TQ system. The comprehensive acquisition method with 108 PFAS (including native and labeled) was deployed based on the Agilent PFAS MRM Database (part number G1736AA) and eMethod (part number G5285AA).

The SPE-based sample preparation was performed using the Agilent Bond Elut PFAS WAX cartridge followed by Carbon S cleanup, with the addition of EIS and NIS as described in EPA Method 1633. Calibration performance, LOQ, spiked QC recoveries, and reproducibility were evaluated for the workflow. The LOQ ≤ 5 ng/L was achieved for 93% of analytes, and the HQC recovery met 50 to 130% with an %RSD ≤ 20 for 99% of compounds. These results demonstrate the high selectivity and sensitivity of the analytical workflow using the Agilent 6475 LC/TQ. The system offers an end-to-end solution for industrial manufacturers who need to monitor/control the PFAS contaminant level in the wastewater before discharge to the environment.

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