Application note | 004442

Water safety

Anticipating emerging regulations: Direct injection LC-MS/MS quantification of C1–C4 ultrashort-chain PFAS in drinking water using the TSQ Altis Plus EFOX Edition

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Keywords

PFAS, TFA, ultrashort-chain PFAS, HILIC, Hypersil GOLD, LC-MS/MS, TSQ Altis Plus EFOX edition, mass spectrometer, dual-CID, MS3-like, Chromeleon, drinking water, Vanquish Duo, dual stage reaction monitoring, DSRM

Application benefits

- Robust quantification of ultrashort-chain PFAS at low ng/L levels
- Improved retention and separation of highly polar PFAS such as TFA
- Dual-CID fragmentation capability enabling MS^3-like selectivity through sequential ion activation and dissociation
- Compatible with routine drinking water monitoring workflows
- Ready for future regulatory expansion

Objective of this application note

This application note presents a comprehensive and forward-looking direct injection LC-MS/MS method for the quantification of ultrashort-chain PFAS (C1–C4) in drinking water. Using the Thermo Scientific™ TSQ Altis™ Plus EFOX Edition Triple Quadrupole Mass Spectrometer combined with a Thermo Scientific™ Hypersil GOLD™ HILIC column and the Thermo Scientific™ Vanquish™ Duo UHPLC system, the method overcomes retention and sensitivity challenges associated with highly polar PFAS, delivering excellent quantitative performance to support routine monitoring and anticipated regulatory expansion.

Introduction

Ultrashort-chain PFAS—emerging regulatory and analytical priorities

Per- and polyfluoroalkyl substances (PFAS) are a large group of persistent anthropogenic chemicals that have been widely used in industrial and consumer applications for decades. Growing awareness of their environmental persistence, mobility, and potential health impacts has led to increasingly stringent drinking water regulations worldwide.[1,2] While regulatory attention has historically focused on long- and short-chain PFAS,[3,4] ultrashort-chain PFAS (USC-PFAS) emerge as contaminants of concern due to their high-water solubility and mobility. In this work, USC-PFAS are defined as C1–C4 perfluorinated and polyfluorinated compounds, including acids, sulfonates, and selected neutral precursors relevant for drinking water monitoring. Their short carbon chain length results in high polarity, low sorption potential, and rapid environmental transport, while also making them difficult to remove by conventional treatment processes.[5] Reliable quantification of USC-PFAS at the ultra-trace levels at which they are typically present requires exceptional analytical sensitivity, selectivity, and robustness.

However, the analysis of USC-PFAS remains technically challenging. Their high polarity leads to weak retention on conventional reversed-phase columns, early elution near the void volume, and increased susceptibility to matrix effects and ion suppression. To address these challenges, this application note presents a sensitive and robust LC-MS/MS method for the quantification of USC-PFAS (C1–C4) in drinking water using a Hypersil GOLD HILIC column coupled to a Vanquish UHPLC system and a TSQ Altis Plus EFOX Edition Triple Quadrupole Mass Spectrometer.

Enhanced chromatographic retention achieved through HILIC separation, combined with highly selective detection using selected reaction monitoring (SRM) and dual stage reaction monitoring (DSRM) generated through dual-CID fragmentation, enables accurate quantification at low ng/L levels. Internal standard (IS) correction (i.e., isotope dilution) further compensates for matrix effects and signal suppression commonly observed for highly polar PFAS. The method demonstrates excellent linearity, precision, and robustness across a broad concentration range and is well-suited for routine monitoring of emerging USC-PFAS, including challenging analytes such as trifluoroacetic acid (TFA), in drinking water matrices.

Experiment

LC-MS/MS system configuration

The LC setup consists of a Thermo Scientific™ Vanquish™ Duo Flex UHPLC Binary Pump, a Thermo Scientific™ Vanquish™ Dual Split Sampler, and a Thermo Scientific™ Vanquish™ Column Compartment. The TSQ Altis Plus EFOX Edition triple quadrupole mass detector was used in SRM mode and dual-CID (MS^3-like) for negative ionizing USC-PFAS. Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software 7.3.2 was used for full control of the LC-MS/MS configuration, data acquisition, and processing of all data required for compound quantification. The software supports guided, efficient, and compliant data review and reporting. The hardware configuration schematics can be seen in Figure 1 and Table 1.

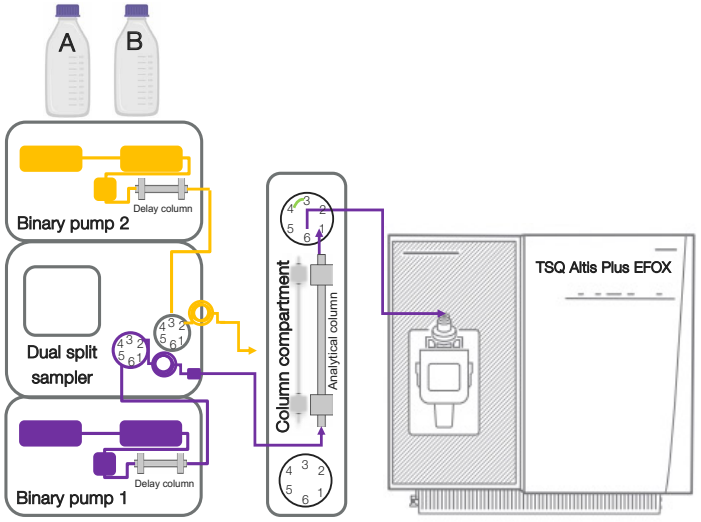

Figure 1. LC-MS/MS configuration consisting of a Vanquish HPLC system and a TSQ Altis Plus EFOX Triple Quadrupole Mass Spectrometer for USC-PFAS analysis. The dual-channel Environmental and Food Organic Xenobiotics (EFOX) configuration includes a second LC pathway available for complementary PFAS analysis (not utilized for the results shown here).

Table 1. References of configuration utilized for USC-PFAS analysis.

Part No.	Instrument
VF-P10-A-01	Vanquish Flex Binary UHPLC Pump
VF-A40-A-03	Vanquish Dual Split Sampler
VH-C10-A-03	Vanquish Column Compartment H
B51006700	TSQ Altis Plus EFOX Edition Triple Quadrupole Mass Spectrometer
CHROMELEON7	Chromeleon CDS

Target analytes and IS

Table 2 describes all the USC-PFAS from C1 to C4 analyzed in this method. Although not routinely regulated, PBSF, PFBSAm, and TFSI were included to demonstrate selectivity and chromatographic versatility on USC-PFAS.

Table 2. List of USC-PFAS targeted.

Carbon number	USC-PFAS	CAS number	Chemical name	Formula
C1	TFMS (or PFMeS)	1493-13-6	Trifluoromethanesulfonic acid	CF3SO3H
C2	TFA	76-05-1	Trifluoroacetic acid	C2HF3O2
C2	PFEtS	354-88-1	Perfluoroethanesulfonic acid	C2HF5O3S
C2	TFSI	82113-65-3	Trifluoromethanesulfonimide	C2HF6NO4S2
C2	DFA	381-73-7	Difluoroacetic acid	C2H2F2O2
C3	PFPrA	422-64-0	Perfluoropropionic acid	C3HF5O2
C3	PFMOAA	674-13-5	Perfluoro-2-methoxyacetic acid	C3HF5O3
C3	PFPrS	423-41-6	Perfluoropropanesulfonic acid	C3HF7O3S
C3	2,3,3,3-TeFPrA	359-49-9	2,3,3,3-tetrafluoro-n-propanoic acid	C3H2O2F4
C3	2,2,3,3-TeFPrA	756-09-2	2,2,3,3-tetrafluoro-n-propanoic acid	C3H2O2F4
C4	PFBA	375-22-4	Perfluorobutanoic acid	C4HF7O2
C4	PFBSAm (or FBSA)	30334-69-1	Perfluorobutane sulfonamide	C4F9SO2NH2
C4	PBSF	375-72-4	Perfluorobutanesulfonyl fluoride	C4F10SO2
C4	PFMPA	377-73-1	Perfluoro-3-methoxypropanoic acid	C4HF7O3

Isotopically labeled IS are essential for accurate USC-PFAS analysis, as they correct matrix effects, recovery losses, and LC-MS/MS variability at ultra-trace levels. The list of IS used is given in Table 3.

Table 3. List of isotopically labeled IS for USC-PFAS analysis.

Carbon number	Isotopically labeled IS	CAS number	Chemical name	Formula
C2	^{13}C2-TFA	1794767-05-7	Sodium trifluoroacetate ^{13}C2	^{13}C2F3NaO2
C3	^{13}C3-PFPrA	422-64-0 NR	Perfluoropropanoic acid ^{13}C3	^{13}C3HF5O2
C4	^{13}C4-PFBA	1017281-29-6	Perfluorobutanoic acid ^{13}C4	^{13}C4HF7O2
C4	^{13}C3-PFBS	2708218-84-0	Perfluorobutanesulfonic acid ^{13}C3	^{13}C3CF9O3SNa

Standards, reagents, and contamination control

LC-MS grade acetonitrile and water were verified to be PFAS-free, and the volatile buffer compatible with HILIC separation and negative ESI. Polypropylene labware was used throughout the method, and PTFE-containing components were avoided. System blanks and solvent blanks were included in every analytical batch to monitor background contamination. The list of the LC-MS reagents is given in Table 4.

Table 4. List of recommended reagents.

Reagent	Part No.	Supplier	Dedicated for
Acetonitrile, UHPLC-MS	A9561	Thermo Fisher Scientific	Mobile phase (LC)
Water, UHPLC-MS	W8-1	Thermo Fisher Scientific	Mobile phase (LC)
Isopropanol, Fisher Chemical™ Optima™ LC/MS grade	A461-500	Fisher Scientific channel	Mobile phase (LC) Rear seal wash solution (LC)
Ammonium formate, 99%	401152500	Thermo Fisher Scientific	Mobile phase (LC)
Methanol, UHPLC-MS	A4581	Thermo Fisher Scientific	AS needle rinse solution (LC)
Ammonium hydroxide, 25% NH3, 99.99%	087903.AK	Thermo Fisher Scientific	AS needle rinse solution (LC)

Sample preparation

Calibration solutions were prepared in acetonitrile with concentrations ranging from 5 to 5,000 ng/L, with IS concentration at 1,000 ng/L to correct for any possible extraction and matrix effects.[6]

The method was evaluated using drinking water samples. To avoid any sample contamination, sample preparation consists solely of a two-fold dilution of the samples in acetonitrile. The dilution of the water samples with ACN lowers the elution strength of the sample solvent and therefore represents the best compromise for direct injection between maintaining sensitivity and excellent chromatographic peak shape for USC-PFAS and providing a robust method suitable for routine laboratories.[7]

HILIC LC conditions

Chromatographic separation was performed using a Hypersil GOLD HILIC 1.9 µm column, selected for its ability to retain highly polar acidic compounds such as TFA. Ammonium formate 20 mM in water was chosen to enhance interactions and to stabilize the retention time of the compounds.[8,9] A short gradient and fast re-equilibration time were optimized to enable high-throughput analysis while maintaining chromatographic performance. These conditions allow all analytes to elute after the void volume with excellent peak shape and reduced matrix interference. LC parameters are reported in Table 5.

Table 5. LC method parameters.

Parameter	Value
Analytical column	Hypersil GOLD HILIC 100 x 2.1 mm, 1.9 µm (Part No. 26502-102130)
Delay column	Hypersil GOLD HILIC 50 x 2.1 mm, 1.9 µm (Part No. 26502-052130)
Column temperature	40 °C
Injection volume	5 µL
Autosampler temperature	25 °C
Run time	15 min
Flow	400 µL/min
Mobile phase A	Ammonium formate 20 mM in water
Mobile phase B	Acetonitrile 95%/Isopropanol 5% (V/V)

MS/MS conditions (TSQ Altis Plus EFOX Edition mass spectrometer)

The TSQ Altis Plus EFOX Edition mass spectrometer uses SRM to maximize sensitivity and robust ion source conditions for long-term stability. Detection is performed using negative-ion HESI for reliable and high-performance quantitation (Table 6).

Table 6. Ion source conditions.

Parameter	Value
Spray voltage negative	300 V
Sheath gas	80
Aux gas	15
Sweep gas	1
Ion transfer tube temp	250 °C
Vaporizer temp	300 °C

The built-in dual-CID capability of the TSQ Altis Plus EFOX Edition mass spectrometer further strengthens compound confirmation for selected analytes when required, providing added confidence in trace-level detection. All SRM and DSRM transitions are reported in Table 7.

Table 7. SRM and DSRM transitions.

Compound	CAS number	Retention time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	S-CID voltage (V)
PFBSAm	30334-69-1	3.76	78	64	24	67	76
			298	78	27	89	–
TFSI	82113-65-3	4.19	147	78	15	77	69
			280	147	25	68	–
PBSF	375-72-4	4.33	299	80	35	111	–
			299	99	31	111	–
^{13}C3-PFBS	2708218-84-0	4.33	302	80	35	91	–
			302	99	32	91	–
PFPrS	423-41-6	4.36	249	80	31	67	–
			249	99	29	67	–
PFEtS	354-88-1	4.41	199	80	29	93	–
			199	99	26	93	–
TFMS	1493-13-6	4.5	149	80	24	64	–
			149	99	26	64	–
PFMPA	377-73-1	4.74	229	85	13	30	–
			229	185	6	30	–
PFBA	375-22-4	4.86	169	19	27	51	0
			213	169	9	30	–
^{13}C4-PFBA	1017281-29-6	4.86	217	19	36	33	–
			217	172	9	33	–
PFMOAA	674-13-5	5.09	179	85	13	30	–
			179	135	5	30	–
PFPrA	422-64-0	5.2	163	69	37	30	–
			163	119	10	30	–
^{13}C3-PFPrA	422-64-0 NR	5.2	121	70	26	30	29
			166	121	11	30	–
2,2,3,3-TeFPrA	756-09-2	5.88	145	19	26	32	–
			145	81	19	32	–
TFA	76-05-1	6.26	113	69	12	30	–
			113	113	10	30	–
^{13}C2-TFA	1794767-05-7	6.26	115	19	34	30	–
			115	70	12	30	–
2,3,3,3-TeFPrA	359-49-9	6.96	145	63	13	31	–
			145	81	20	31	–
DFA	381-73-7	9.3	95	51	12	38	–
			95	77	17	38	–

Results and discussion

Chromatographic performance

The Hypersil GOLD HILIC column with optimized LC conditions helped ensure reproducible retention and sharp peaks for USC-PFAS, including clear separation of TFA (Figure 2) from the solvent front, while minimizing matrix co-elution and enhancing quantitative robustness. For better visualization of the chromatogram, the DFA compound with a retention time exceeding 9 minutes is not shown.

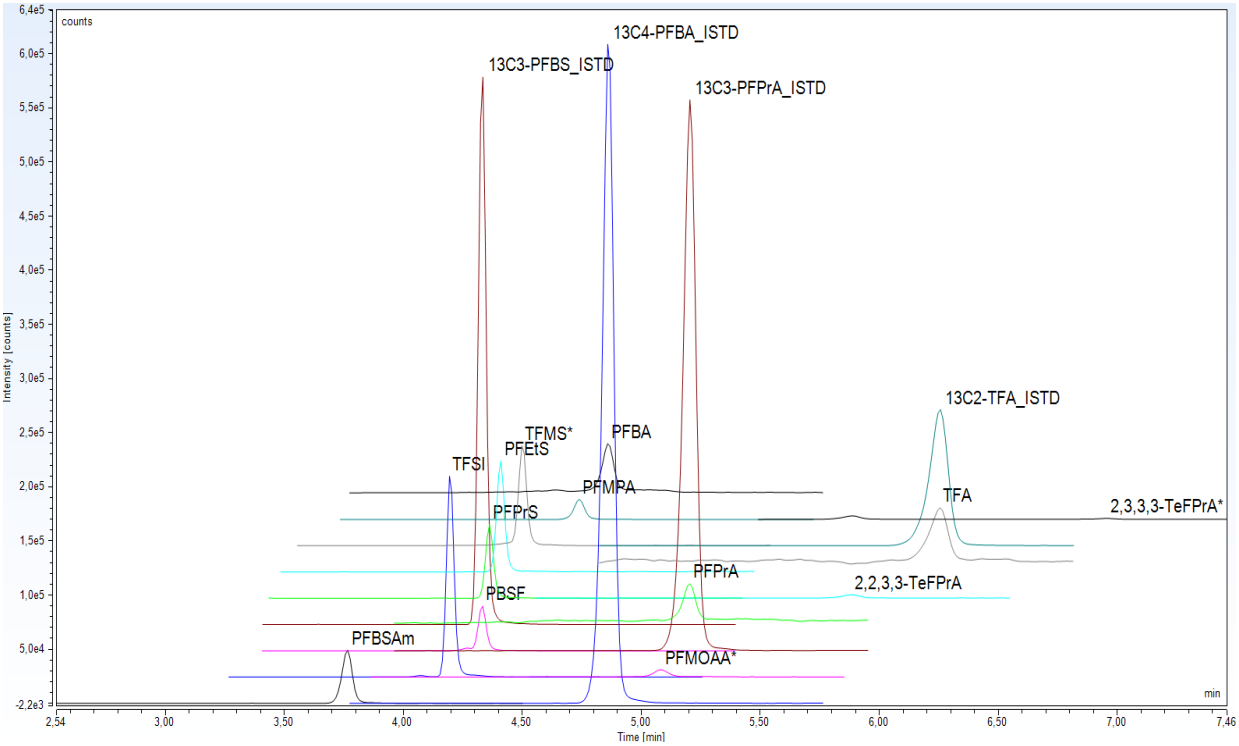

Figure 2. The Hypersil Gold HILIC column provides excellent separation and peak shape for all USC-PFAS, as shown here for a standard mixture at 500 ng/L (also containing isotopically labeled standards).

Dual-CID fragmentation

On Thermo Scientific™ TSQ™ triple quadrupole systems, TFSI (CAS 82113-65-3) exhibits two highly complementary SRM transitions that leverage both conventional MS/MS and dual-CID capabilities (Figure 3).

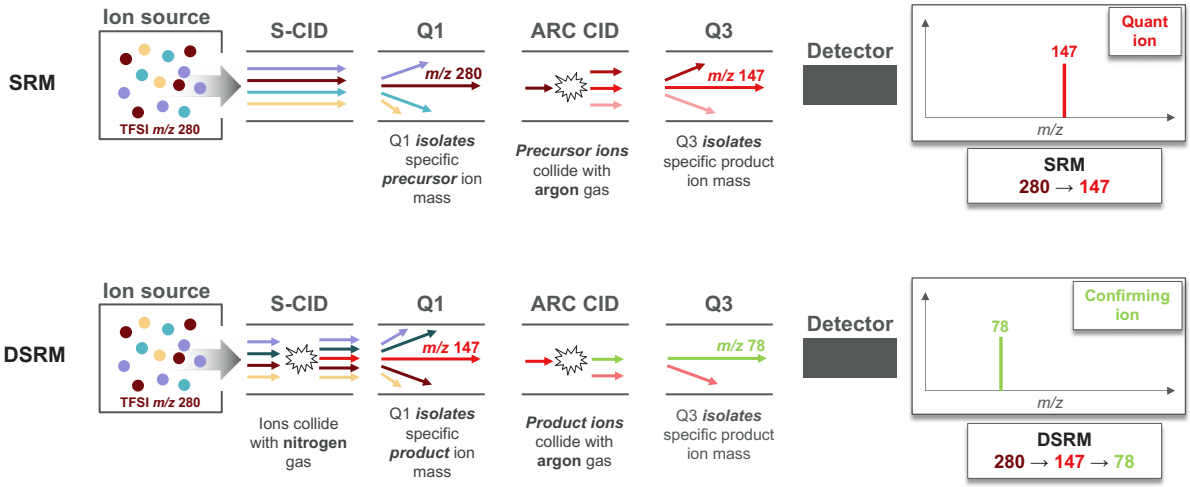

Figure 3. DSRM provides enhanced selectivity compared to SRM by employing multiple fragmentation steps to generate new product ions not observed with SRM. Here is an example of using SRM and DSRM to fragment TFSI.

During the conventional SRM transitions, the TFSI ion fragments in Q2 (via ARC-CID) to form stable product ions:

- *m/z* 280→147 for quantitation
- *m/z* 280→211 for confirmation

Similarly, TFSI also fragments efficiently by S-CID prior to Q1 to produce a strong ion at *m/z* 147. This ion is isolated in Q1 and further fragmented in Q2 (via ARC-CID) to generate *m/z* 147→78. This mechanism corresponds to a dual-CID (MS^3-like) on the TSQ Altis Plus EFOX Edition mass spectrometer.

This strategy provides a more intense confirmation ion than conventional MS/MS (Figure 4).

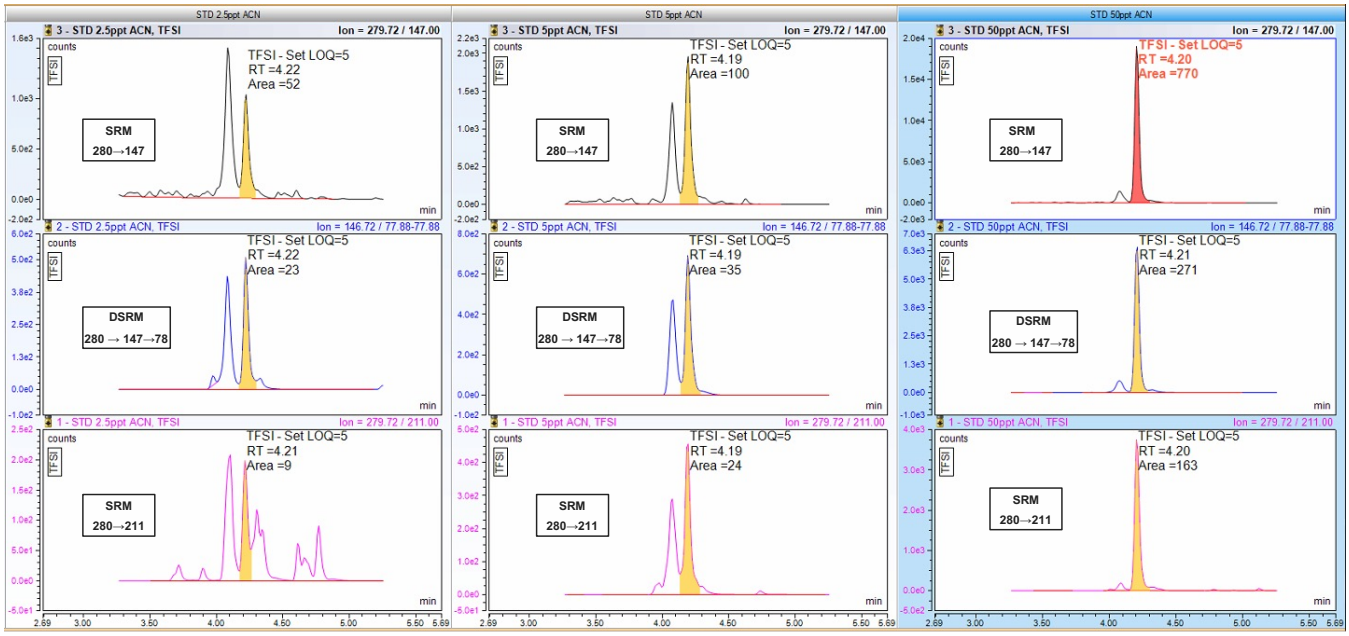

Figure 4. The combination of both SRM and DSRM expands the ability to generate multiple confirming ions in addition to a quantitation ion. Here we show an example for TFSI, where SRM is used to generate the quantitation ion (top row) and a confirming ion (bottom row) while an additional confirming ion is generated by DSRM (middle row). All three ions are observed across three concentrations of 2.5 ng/L (left), 5 ng/L (middle), and 50 ng/L (right).

The combination of these SRM and DSRM transitions fully leverages the selectivity and sensitivity of Thermo Scientific™ TSQ™ triple quadrupole technology, enhancing identification confidence, improving quantitative accuracy, and ensuring robust performance for routine PFAS analysis at ultra-trace concentration levels. The dual-CID capability was also applied to the enhanced confirmation ions of the following compounds: PFBSAm and PFBA.

Linearity

The results of the linearity study are consistent across several curves. Each curve met the validation criteria (R^2 >0.990 and a relative deviation ≤40% at the LOQ and less than 20% for other levels). As summarized in Table 8, using a 5 µL injection volume, LOQs between 5 ng/L and 100 ng/L were achieved for all analytes, and 500 ng/L for TFA due to background limitation.

Table 8 Calibration results for USC-PFAS.

Compound	CAS	ISTD	Cal. type	Solvent LOQ (ng/L)	Range (ng/L)	R^2
PFPrS	423-41-6	13C3-PFBS_ISTD	Quad, with offset, 1/A	5	5–5000	1.000
TFMS	1493-13-6	13C3-PFBS_ISTD	Quad, with offset, 1/A	5	5–5000	0.999
TFSI	82113-65-3	13C3-PFBS_ISTD	Quad, with offset, 1/A	5	5–5000	1.000
PFBSAm	30334-69-1	13C3-PFBS_ISTD	Quad, with offset, 1/A	5	5–5000	1.000
PFEtS	354-88-1	13C3-PFBS_ISTD	Quad, with offset, 1/AA	5	5–5000	1.000
PBSF	375-72-4	13C3-PFBS_ISTD	Quad, with offset, 1/A	25	25–5000	0.999
PFBA	375-22-4	13C4-PFBA_ISTD	Quad, with offset, 1/A	25	25–5000	1.000
PFMPA	377-73-1	13C4-PFBA_ISTD	Quad, with offset, 1/A	25	25–5000	0.999
PFPrA	422-64-0	13C3-PFPrA_ISTD	Quad, with offset, 1/A	50	50–5000	0.999
PFMOAA	674-13-5	13C4-PFBA_ISTD	Quad, with offset, 1/A	50	50–5000	0.998
2,2,3,3-TeFPrA	756-09-2	13C2-TFA_ISTD	Quad, with offset, 1/A	50	50–5000	0.998
2,3,3,3-TeFPrA	359-49-9	13C2-TFA_ISTD	Quad, with offset, 1/A	100	100–5000	0.997
DFA	381-73-7	13C2-TFA_ISTD	Quad, with offset, 1/A	100	100–5000	0.993
TFA	76-05-1	13C2-TFA_ISTD	Quad, with offset, 1/A	500	500–50000	1.000

The calibration curves for all analyzed compounds are given in Figure 5.

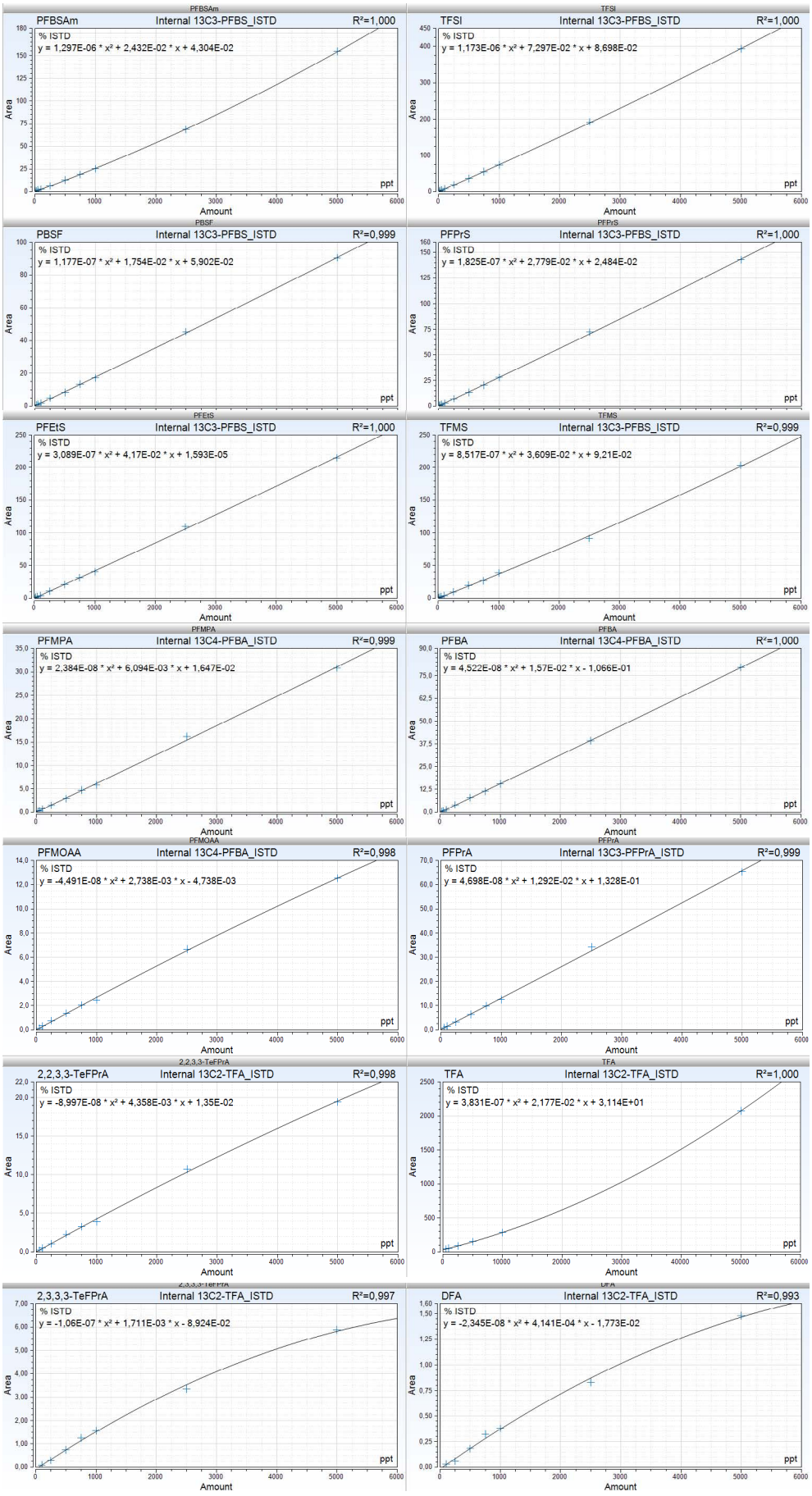

Figure 5. Calibration curves from 5 ng/L to 5,000 ng/L and 0.5 to 50 µg/L for TFA.

Table 9. Calibration performance summary for USC-PFAS, including number of calibration levels, LOQ, coefficient of determination (R^2), and percent deviation between target and measured concentrations.

Relative amount deviation (%)														
Peak name	#points	Method LOQ (ng/L)	R^2	STD 5 ng/L	STD 10 ng/L	STD 25 ng/L	STD 50 ng/L	STD 100 ng/L	STD 250 ng/L	STD 500 ng/L	STD 750 ng/L	STD 1,000 ng/L	STD 2,500 ng/L	STD 5,000 ng/L
2,2,3,3-TeFPrA	8	50	0.998	–	–	–	-6	11	-5	5	1	-8	4	-1
2,3,3,3-TeFPrA	7	100	0.997	–	–	–	–	3	-11	0	10	3	-6	2
PBSF	9	25	0.999	–	–	-3	-1	-1	9	-4	1	-4	1	0
PFBA	9	25	1.000	–	–	-7	4	-2	4	3	-2	0	0	0
PFBSAm (or FBSA)	11	5	1.000	-18	-2	-3	13	6	5	3	-1	-2	-1	0
PFEtS	11	5	1.000	-15	-10	5	-2	-8	1	-1	0	-3	3	-1
PFMOAA	8	50	0.998	–	–	–	-22	16	10	1	1	-8	2	0
PFMPA	9	25	0.999	–	–	6	-3	8	-6	-6	2	-5	5	-1
PFPrA	8	50	0.999	–	–	–	4	5	-4	-4	1	-5	4	-1
PFPrS	11	5	1.000	14	-9	3	1	-8	2	-3	-2	0	2	0
TFMS	11	5	0.999	0	-11	-2	3	5	2	5	-3	5	-4	1
TFSI	11	5	1.000	6	-11	4	-2	3	3	-3	0	-1	1	0

Relative amount deviation (%)									
Peak name	#points	Method LOQ (µg/L)	R^2	TFA 0.5 µg/L	TFA 1 µg/L	TFA 2.5 µg/L	TFA 5 µg/L	TFA 10 µg/L	TFA 50 µg/L
TFA	6	0.5	1.000	-3	2	1	1	-1	0

Sensitivity

The chromatograms below illustrate examples of USC-PFAS at their LOQ (Figures 6–9). The overlays correspond to the quantitation and confirmation ions.

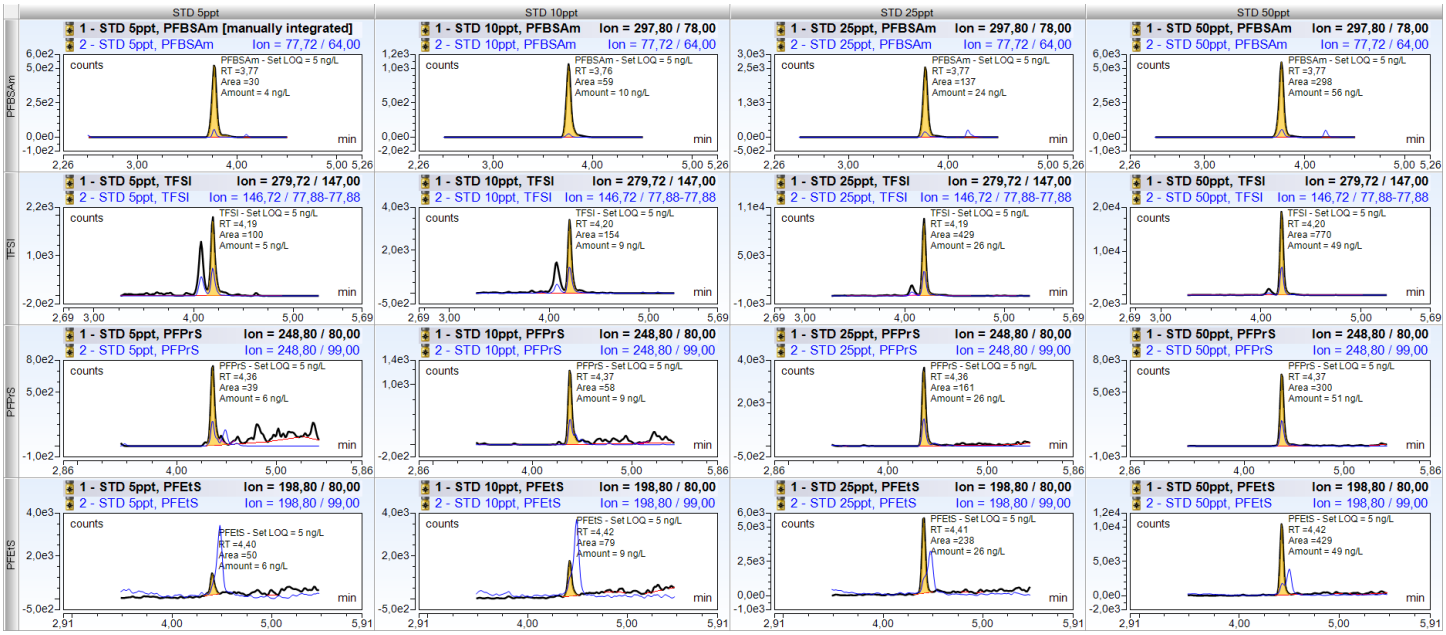

Figure 6. Overlaid SRM chromatograms at the LOQ (5 ng/L), 10, 25, and 50 ng/L for PFBSAm, TFSI, PFPrS, and PFEtS.

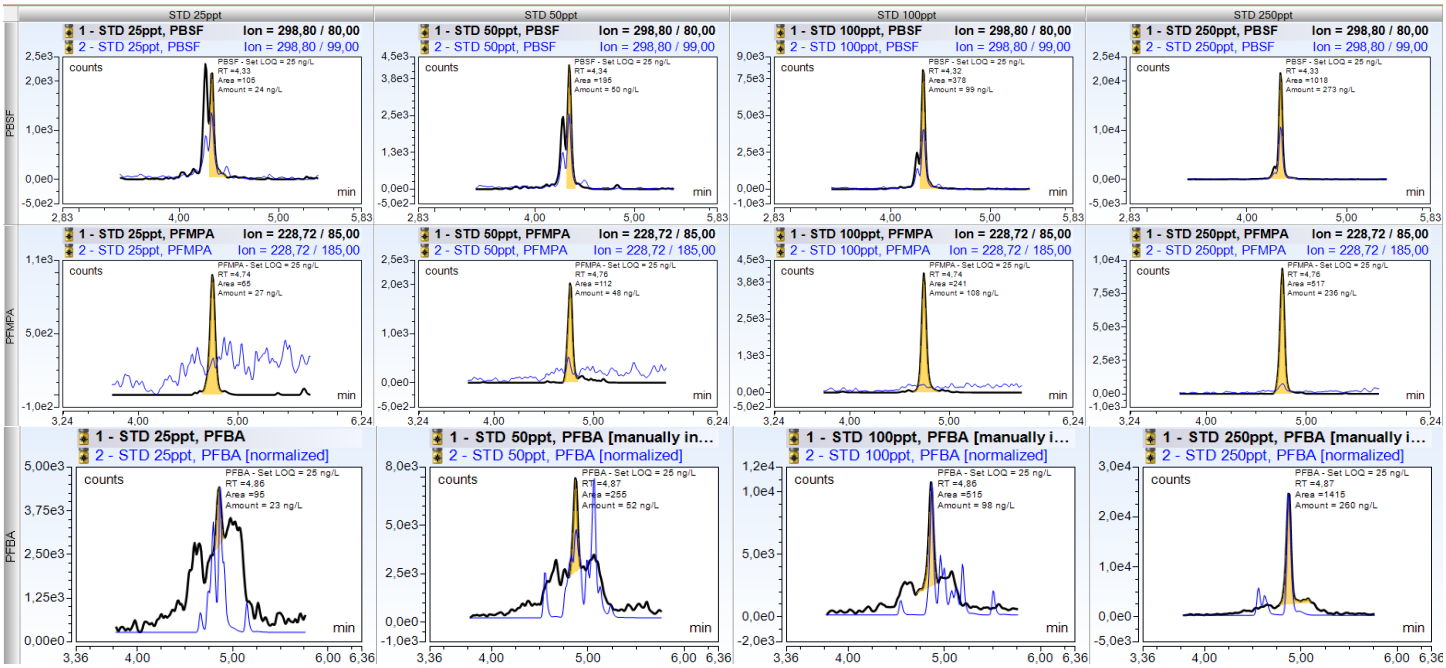

Figure 7. Overlaid SRM chromatograms at the LOQ (25 ng/L), 50, 100, and 250 ng/L for PBSF, PFMPA, and PFBA.

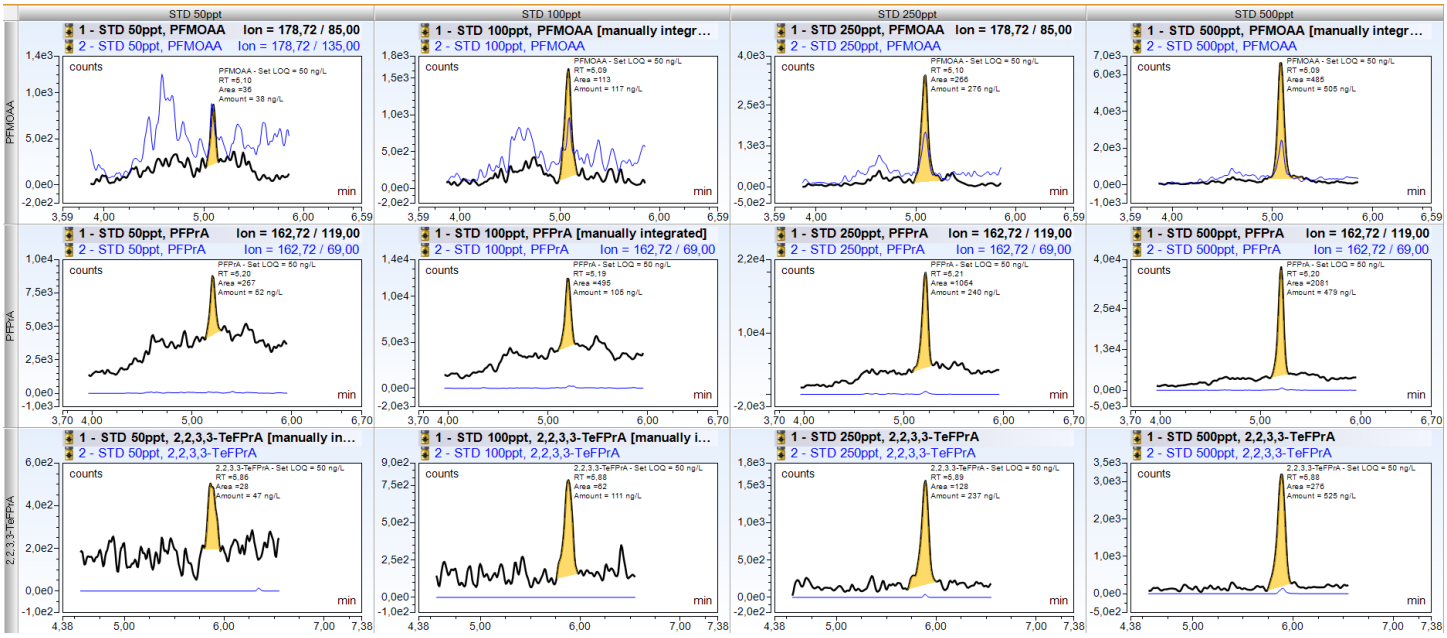

Figure 8. Overlaid SRM chromatograms at the LOQ (50 ng/L), 100, 250, and 500 ng/L for PFMOAA, PFPrA, and 2,2,3,3-TeFPrA.

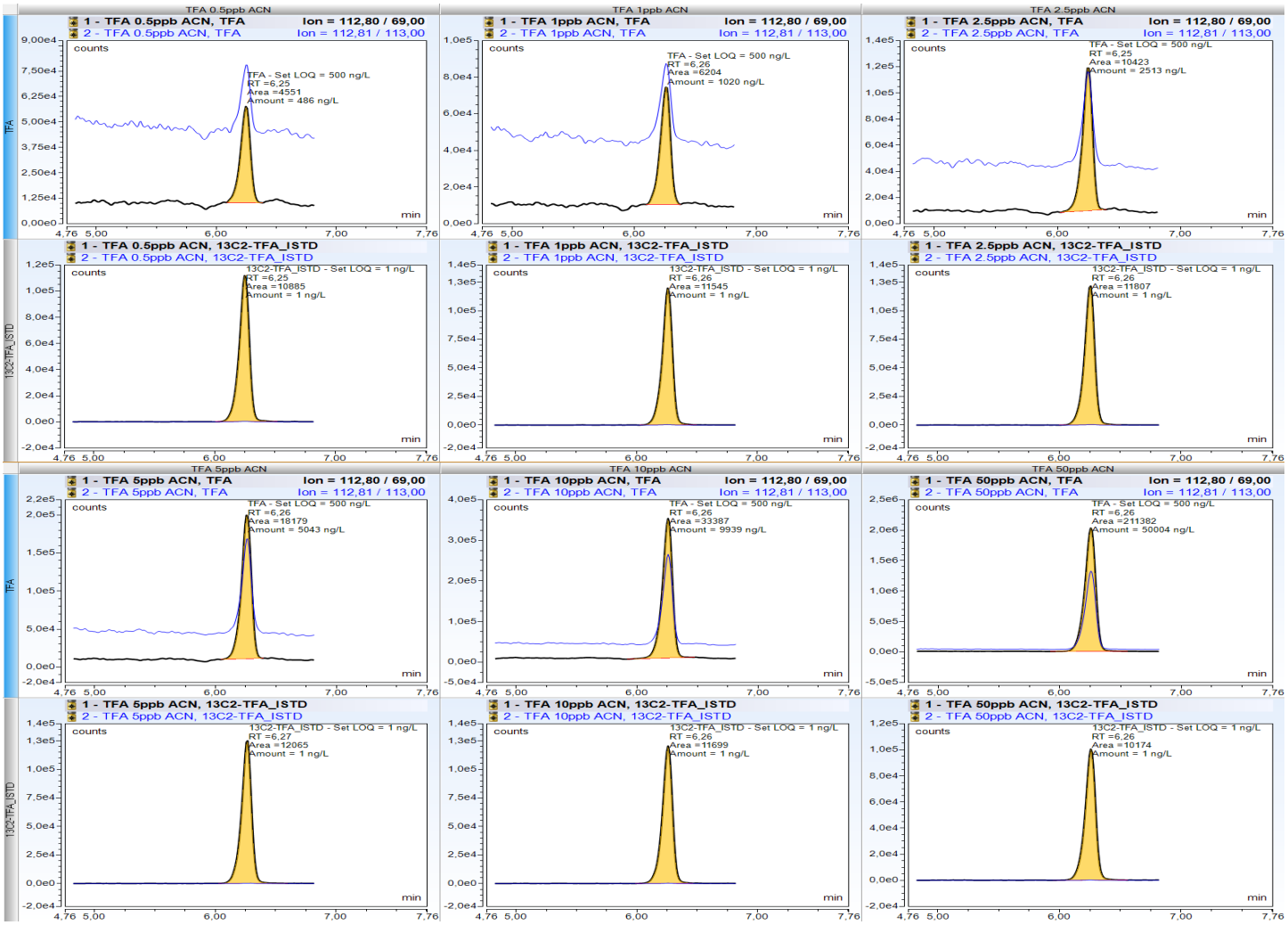

Figure 9. Overlaid SRM chromatograms from the LOQ (0.5 µg/L) to 50 µg/L for TFA, and 13C2-TFA IS.

While the TSQ Altis Plus EFOX Edition mass spectrometer can detect lower LOQs, due to the ubiquitous nature of PFAS in the lab environment, LOQs were limited to the background level of the USC-PFAS in our lab environment. LOQs should be set in accordance with the USC-PFAS level in the end-user laboratory atmosphere. It is acknowledged that some laboratories may experience high levels of air contamination with TFA or PFBA. Other background compounds such as PFPrA or PFBS due to consumables, pipette tips, or reagent contamination, can also be observed occasionally.

Sample results

Six representative drinking water samples were analyzed, including two commercially available mineral waters, three tap water samples, and one sample from a water fountain. All drinking water samples were diluted 1:1 (v/v) with acetonitrile prior to injection to mitigate salt-related ion suppression.

Despite matrix dilution, approximately 80% signal attenuation was observed for 13C3-PFPrA in real samples (Figure 10). Indeed, mineral and tap waters introduce significant amounts of Na^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, etc. These species can elute as a concentrated “salt plug” and cause suppression effect over a narrow retention time window. As 13C3-PFPrA elutes within this window, its signal is impacted.

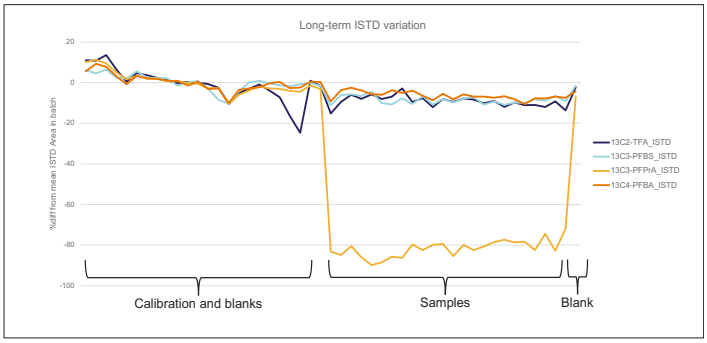

Figure 10. ISTD signal variation during a sequence of 50 injections.

The PFPrA has the same retention time and undergoes the same ionization suppression effect as 13C3-PFPrA. Thus, PFPrA is optimally corrected by 13C3-PFPrA IS. Other PFAS cannot be corrected by 13C3-PFPrA; they are corrected by other IS that do not undergo this ionization suppression effect.

The method quantification limit (MQL) was calculated by applying the overall dilution factor used during sample preparation. Table 10 describes the MQL and the quantitative results obtained on different drinking water samples.

Table 10. Quantitative results on the six drinking water samples. Results are given in ng/L in the samples (n.d. for non-detected compound).

Compound name	MQL (ng/L)	Mineral water 1 conc. (ng/L)	Mineral water 2 conc. (ng/L)	Tap water 1 conc. (ng/L)	Tap water 2 conc. (ng/L)	Tap water 2 conc. (ng/L)	Water fountain conc. (ng/L)
2,2,3,3-TeFPrA	100	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2,3,3,3-TeFPrA	200	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
DFA	200	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PBSF	50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFBA	50	<50	<50	<50	<50	<50	<50
PFBSAm	10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
PFEtS	10	<10	n.d.	<10	n.d.	<10	<10
PFMOAA	100	n.d.	n.d.	<100	n.d.	<100	<100
PFMPA	50	<50	n.d.	<50	<50	n.d.	n.d.
PFPrA	100	126	<100	n.d.	<100	<100	<100
PFPrS	10	<10	n.d.	<10	<10	n.d.	n.d.
TFA	1,000	<1,000	<1,000	1,906	1,496	<1,000	2,351
TFMS	10	n.d.	n.d.	63	<10	<10	n.d.
TFSI	10	n.d.	n.d.	n.d.	<10	n.d.	n.d.

Most USC-PFAS were not detected in the various samples. PFBA and PFPrA were detected at levels of a few tens of ng/L in most drinking water samples, but their concentrations remained below the MQL. Only bottled water sample 1 showed a PFPrA concentration exceeding the MQL. TFA was detected in all samples, with a concentration exceeding the MQL of 1,000 ppt in three of the tap water samples.

Method accuracy

To check the method accuracy, three different concentration levels were prepared and spiked to the bottled and tap water samples. The spike recoveries are given in Table 11.

Table 11. Spike recoveries of 10 ng/L, 100 ng/L, and 1,000 ng/L in six drinking water samples.

	Drinking water 10 ng/L spike		Drinking water 100 ng/L spike		Drinking water 1000 ng/L spike	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
PFBSAm	77	21	61	21	58	19
PFEtS	104	16	97	8	105	6
PFPrS	109	14	101	7	105	5
TFMS	98	33	109	13	99	7
TFSI	99	24	96	7	102	5
PBSF	–	–	94	11	96	3
PFBA	–	–	81	23	92	9
PFMPA	–	–	97	9	95	5
2,2,3,3-TeFPrA	–	–	113	35	105	8
PFMOAA	–	–	122	18	106	9
PFPrA	–	–	108	10	100	22
2,3,3,3-TeFPrA	–	–	–	–	83	11
TFA	–	–	–	–	91	26

The repeatability of the spiking experiment was assessed by performing (n = 6) independent spikes at a fixed concentration level. Except for the PFBSAm compound all the recovery values were between ±20% of the target concentration. The RSD of recovery values was below 30%, indicating good precision of the method.

Method robustness

Method robustness was evaluated by periodic injections of a fortified matrix at concentrations close to the compounds' MQLs. A total of 10 replicate injections (R1–R10) were performed every 10 matrix samples (bottled and tap water), resulting in several hundred injections over the analytical sequence.

As shown in Table 12, retention times for all compounds remained highly stable throughout the sequence, demonstrating excellent chromatographic reproducibility. The RSD of the calculated concentrations across the 10 replicate injections was below 20% for all USC-PFAS. These results demonstrate robust performance under routine batch conditions for real drinking water matrices.

PFPrA results were excluded from this robustness experiment due to laboratory background contamination observed for this analyte.

Table 12. Quantitative performance evaluation of target USC-PFAS and related compounds: MQL, retention time, and matrix spike accuracy/ precision (n = 10).

Compound	MQL ng/L	Retention time		Matrix spike ng/L	Concentration											
		Average R1 to R10 (min)	RSD% (n=10)		R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Average R1 to R10 (ng/L)	RSD (n=10)
PFBSAm	10	3.8	0.5	10	7.9	8.1	7.1	7.3	7	6.8	7.8	7.3	7.1	5.5	7.2	10.2
TFSI	10	4.2	0.1	10	12.5	9.2	10.9	10.3	11.7	10.3	10.3	10.6	9.4	10.6	10.6	9.3
PFPrS	10	4.4	0.01	10	10.8	11.1	11.9	11.2	10.1	7.8	12.9	10.5	10.7	11.4	10.8	12.1
PFEtS	10	4.4	0.01	10	12.7	10.1	12	9.8	10.4	10.8	13	10.5	10.7	13.8	11.4	12.1
TFMS	10	4.5	0.1	10	14.2	15.8	13.5	12.9	12.7	13.7	13.8	14.8	16.2	14.7	14.2	8
PBSF	50	4.3	0.01	100	103.7	106.8	96.1	97.4	94.1	89.8	96.3	98.2	100.6	103.7	98.7	5.2
PFMPA	50	4.8	0.1	100	96.9	101.6	111.7	94	96.6	109	88.6	95.9	94.7	109.7	99.9	7.8
PFBA	50	4.9	0.1	100	119.2	114.2	122.9	123.4	125.5	117.8	126.7	123.4	111.8	113.5	119.9	4.4
PFMOAA	100	5.1	0.01	100	132.4	99.7	121.9	127.7	123.1	123.1	133	126.3	133.7	124.4	124.5	7.9
2,2,3,3-TeFPrA	100	5.9	0.1	100	122.3	126.2	121.6	128.7	116.3	120.1	136.3	105.9	102.8	141.4	122.2	9.9
2,3,3,3-TeFPrA	200	6.8	3.5	100	77.9	80.8	75.3	80	85.7	72.3	76	78.5	81.3	91.4	79.9	6.8
DFA	200	9.2	0.1	1000	1033	1003	1040	875	925	1037	971	889	858	1032	966.4	7.6
TFA	1000	6.3	0.1	1000	846	801	822	947	798	827	858	808	924	838	846.7	6

In this robustness test the HILIC column exhibited stable performance over extended analytical sequences, with minimal carryover and low background interference, supporting its suitability for routine laboratory implementation.

Conclusion

This application note:

- Demonstrates a reliable and forward-looking LC-MS/MS method for analyzing USC-PFAS in drinking water with a Vanquish UHPLC system coupled with a TSQ Altis Plus EFOX Edition Triple Quadrupole Mass Spectrometer
- Applies HILIC chromatography to significantly improve retention and separation of highly polar PFAS, overcoming limitations of conventional reversed-phase methods
- Employs highly selective SRM detection with DSRM transitions generated by dual-CID fragmentation (MS^3-like)
- Enables robust quantification of challenging analytes such as TFA
- Achieves excellent sensitivity, accuracy, and long-term stability at low ng/L concentrations
- Maintains performance in water matrices affected by inorganic salt-related ion suppression
- Provides a scalable and regulation-ready solution suitable for routine environmental monitoring laboratories addressing current and emerging USC-PFAS requirements
- Leverages the unique EFOX Edition configuration to seamlessly integrate USC-PFAS and conventional PFAS analysis on one high-performance platform thanks to the Vanquish Duo UHPLC system

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