Application note | AN003317

Environmental

Demonstrating sample preparation with automation and LC-MS/MS quantitation of PFAS following EPA Method 533

Workflow to ensure compliance with EPA's National Primary Drinking Water Regulation

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Keywords

EPA Method 533, per- and polyfluorinated alkyl substances (PFAS), National Primary Drinking Water Regulation (NPDWR), environmental contaminants, solid phase extraction, Vanquish Flex Binary UHPLC, TSQ Quantis Plus triple quadrupole mass spectrometer, Dionex AutoTrace 280 PFAS, Chromeleon CDS

Goal

To confidently measure PFAS, particularly the six PFAS regulated by the US EPA in the NPDWR, from 250 mL drinking water samples at 2 parts per trillion (ng/L) using EPA Method 533 by automated off-line solid phase extraction followed by LC-MS/MS on the Thermo Scientific™ TSQ Quantis™ Plus mass spectrometer.

Introduction

In April 2024, the US Environmental Protection Agency (EPA) finalized a rule to regulate certain PFAS in drinking water. This PFAS National Primary Drinking Water Regulation[1] (NPDWR) establishes legally enforceable Maximum Contaminant Levels (MCL) of 4 ng/L, or parts per trillion (ppt), for PFOA and PFOS. It also sets MCLs of 10 ppt for PFHxS, PFNA, and HFPO-DA (also known as GenX) for these compounds. These MCLs apply to all public water systems (PWS) that serve an average of at least 25 people for at least 60 days per year. Additionally, the NPDWR will regulate PFHxS, PFNA, HFPO-DA, and PFBS through a Hazard Index (HI), in which the sum of the ratios of the measured PFAS concentrations to the EPA set health-based water concentrations must be less than 1.

To comply with the NPDWR, initial monitoring of PFAS concentrations in public drinking water systems must be completed three years after the rule has been promulgated, or by the middle of 2027. Monitoring must be done on a quarterly basis for surface water systems or for large groundwater systems (i.e., serving greater than 10,000) and semi-annually for groundwater systems serving under 10,000. For ongoing compliance after 2027, quarterly monitoring must continue unless all regulated PFAS are below the target MCLs for four consecutive quarters. Annual monitoring can be implemented for ongoing compliance if the previous conditions are met, and as long as new sample results are below the MCLs for any regulated PFAS, otherwise quarterly monitoring must resume. Further, if three consecutive annual monitoring samples are below the rule trigger levels of 50% of MCLs (e.g., 2 ppt for PFOA) and below 0.5 for the Hazard Index for the regulated PFAS, then monitoring can be further reduced to triennial sample measurements.[2]

Section VII, p. 32605 of the NPDWR stipulates the two EPA methods approved to support the PFAS monitoring requirements are EPA Method 533 and Method 537.1, Version 2.0.[3] These methods, which use solid phase extraction (SPE) of 250 mL drinking water samples followed by LC-MS/MS for measuring PFAS, have undergone multi-laboratory validation and have been used for UCMR5 data monitoring of PFAS in water since 2023. Both EPA methods contain the six regulated PFAS in the NPDWR. Laboratories that want to monitor drinking water samples for NPDWR compliance will have to use either one of these two EPA methods and be able to achieve minimum reporting levels (MRLs) at 2 ppt, for at least PFOA and PFOS, to meet the rule trigger levels for ongoing compliance monitoring.

This application note will focus on applying Thermo Scientific™ analytical systems to readily achieve and potentially exceed the current NPDWR rule trigger levels by following EPA Method 533.[4] Reagent water samples were spiked at 2 ppt (ng/L) with 25 target PFAS, which included the six regulated PFAS in the NPDWR, processed with SPE using the Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS system, and subsequently analyzed by LC-MS/MS on the Thermo Scientific™ Vanquish™ Flex Binary UPHLC system with the Thermo Scientific™ TSQ Quantis™ Plus mass spectrometer.

Experimental

Consumables

A list of supplies used in this application note is included in Table A1 of the Appendix section.

Sample preparation

All calibration standard solutions and PFAS fortified water samples were prepared from primary dilution standards (PDS) received from Wellington Laboratories (Guelph, Ontario, Canada). PDS solutions were stored at 4 °C until needed. Prior to making calibration standards or fortified water samples, the PDS solutions were removed from 4 °C storage and allowed to come to room temperature for at least one hour.

Isotope Performance PDS and Isotope Dilution Analogue PDS were diluted 10-fold and 5-fold, respectively, with 80% methanol prior to preparation of LC-MS/MS calibration standards or to fortifying 250 mL water samples. This was done due to the sensitivity of the TSQ Quantis Plus mass spectrometer, which did not require the concentrations recommended in sections 7.15 and 7.16 of EPA Method 533 for adequate response factors and to accurately measure SPE recovery values. Additionally, the lower concentrations used for these isotopically labeled standards limit any observed response from any residual unlabeled PFAS to near or below the limits of detection (LODs) on the TSQ Quantis Plus mass spectrometer. Finally, because of the decreased concentrations required for the Isotope Performance and Isotope Dilution Analogue standards, the overall cost per sample of running this method is reduced.

A total of ten calibration standards were prepared according to section 7.17.5 in EPA Method 533. Table A2 lists the target PFAS concentrations of the calibration standards and their drinking water equivalent concentration prior to extraction. Several of the calibration standards were below the target MRL of 2 ng/L, or 500 ng/L extraction concentration (i.e., calibration standard 5). This is to demonstrate that the TSQ Quantis Plus mass spectrometer has the capability of achieving significantly lower MRLs than is required by the current PFAS NPDWR. Prepared solution for calibration standard 3 at 125 ng/L was also used as Continuing Calibration Check (CCC) standards in this assay. The CCC standard at the beginning of any analysis batch must be at or below the concentration of the target MRL, as required in section 10.4.

Prior to preparation of Laboratory Reagent Blanks (LRBs) and Laboratory Fortified Blanks (LFBs), all supplies coming in to contact with these water samples were rinsed twice with UHPLC-MS grade methanol and allowed to air dry. Reagent water aliquots of 250 mL were measured with a polypropylene graduated cylinder and then added to high-density polyethylene (HDPE) bottles that contained 0.25 g ammonium acetate. On the day of sample extraction, 2 ng/L LFB samples were prepared by adding 20 µL of 25 ng/mL PFAS standard made from 1:20 dilution of the EPA 533 Native Analyte Primary Dilution Standard (Wellington Laboratories). All LFB and LRB samples were spiked with 20 µL of 1:5 dilution of Isotope Dilution Analogue PDS solution as described above.

Solid phase extraction (SPE) was accomplished following section 11.4 of EPA Method 533 on the AutoTrace 280 PFAS system.

The system was programmed and conditioned as described in Thermo Fisher Scientific Application Note 73883.[5] Briefly, program 1 was used to condition the weak anion exchange (WAX) cartridges, load the water samples, transfer sample bottle rinses, and dry cartridges with nitrogen. Program 2 was used for the elution steps. Program 3 was for cleaning the sample lines of the AutoTrace 280 PFAS system after sample processing.

After SPE was completed, each polypropylene sample collection tube was placed into a heated block set at 55 °C, and extracts were evaporated to dryness under a gentle stream of ultra-high purity nitrogen. Samples were reconstituted to a total volume of 1 mL 80% methanol, which included 20 µL of 1:10 dilution of Isotope Performance PDS and vortexed. An aliquot of each solution was transferred to polypropylene autosampler vials and analyzed by LC-MS/MS.

Liquid chromatography

The Thermo Scientific Vanquish Flex Binary UHPLC system was used for all LC separations. To limit interferences from affecting the quantitative performance of PFAS analytes, the Vanquish UHPLC system was retrofitted with the Thermo Scientific™ PFAS Upgrade Kit (P/N 80100-62144), which includes PEEK™ tubing and a Thermo Scientific™ Hypersil GOLD™ C18 PFAS Delay column (P/N 25002-054630). Additionally, to help mitigate LC peak fronting of PFBA or other early eluting PFAS targets, a strong solvent loop (P/N 6036.2200) was added in-line between the autosampler injection valve and the analytical column.

For preparation of the mobile phases, it is strongly recommended to use the highest grade solvents and reagents possible to reduce any possible PFAS background interferences. Table A1 lists the chemicals used for this application note.

The LC method parameters used for all LC-MS/MS experiments are presented in Table A3.

Mass spectrometry

The TSQ Quantis Plus mass spectrometer was used for quantitative detection of PFAS target compounds, isotope dilution analogues, and isotope performance standards. Table A4 provides the ion source and mass analyzer settings used on the TSQ Quantis Plus mass spectrometer. Analyte detection was accomplished using Timed SRM, whereby the target compounds were measured only during a data collection window around the retention times of those compounds. Table A5 presents the SRM table for all measured PFAS. Note that while EPA Method 533 does not require confirming ion transitions, they were collected, if available, for all target PFAS and for the three x:2FTS Isotope Dilution Analogues (where x = 4,6,8). As allowed in section 4.4.4 of EPA Method 533, the alternate product ion at *m/z* 81 was used for quantitative measurements for the three telomer sulfonates used as Isotope Dilution Analogues. This was to ensure better quantitative accuracy for the corresponding unlabeled x:2FTS compounds, especially at higher concentrations.

Data analysis and reporting

All LC-MS/MS data were acquired and processed using Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) 7.2 software.

Results and discussion

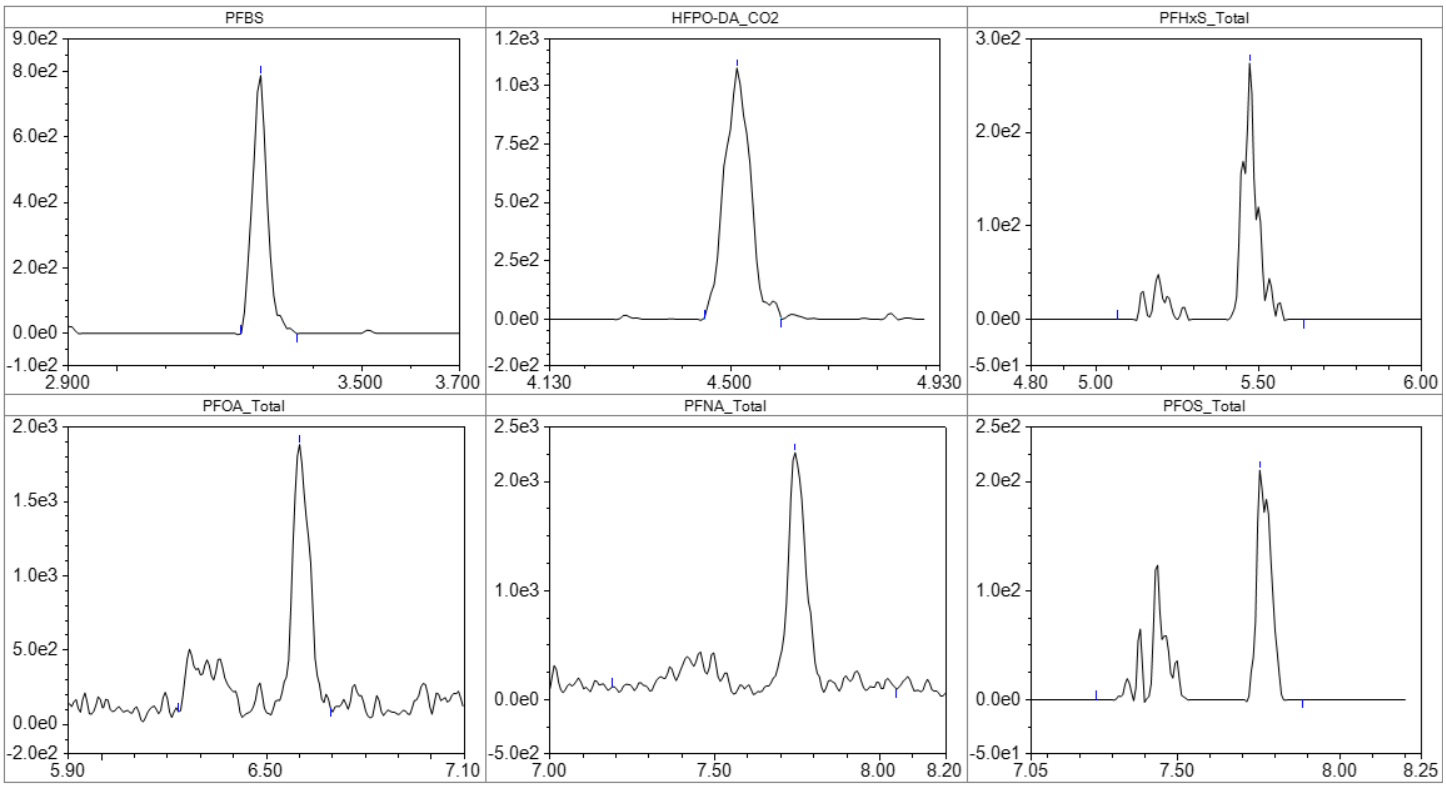
Calibration data

A total of ten calibration standards were prepared, from 25 ng/L to 25,000 ng/L for 25 target PFAS compounds, as described in the Experimental section. Data from the standard solutions were analyzed on the TSQ Quantis Plus mass spectrometer and were quantified per the requirements in section 10.3 of EPA Method 533. All targets were fit to linear regression curves, weighted 1/x and forced through zero. All compounds met the calibration acceptance criteria described in section 10.3.5 down to 25 ng/L, with the exception of PFBA, which was outside the 50–150% true value at that concentration. PFBA did meet the acceptance criteria at the lowest calibration standard of 50 ng/L.

Figure 1 shows example SRM chromatograms for the six PFAS in the NPDWR at both 25 and 50 ng/L, which is equivalent to 0.1 and 0.2 ng/L in 250 mL drinking water samples prior to extraction. Note that the branched and linear isomers are observed and quantified as “Total” PFAS for PFHxS, PFOA, PFNA, and PFOS. Data for these calibration standards indicate that the TSQ Quantis Plus mass spectrometer has sufficient sensitivity to potentially quantify these six PFAS targets at concentrations significantly below 1 ng/L following EPA Method 533. Hence, the TSQ Quantis Plus mass spectrometer could, if necessary, achieve MRLs much lower than currently required by the PFAS NPDWR.

Since the water samples in this assay were analyzed over two days, continuing calibration check (CCC) standards were analyzed according to section 10.4 of EPA Method 533. The total number of extracted samples per day was always less than ten; therefore, the only CCC standard concentration analyzed was at 125 ng/L, or 0.5 ng/L water sample equivalent, which is below the target MRL concentration of 2 ng/L. For the three CCC standard injections at 125 ng/L analyzed over two days (i.e., end of acquisition sequence on day 1; beginning and end of sequence on day 2), the overwhelming majority of target PFAS compounds had relative percent differences less than 10% from true value. The largest relative percent difference for all target PFAS was 16.5%, well within the required 50% relative difference criteria for the CCC at or below the MRL. Further, the relative percent difference for the isotope dilution analogues were all below 5.6%, which is well below the 30% relative difference acceptance criteria.

A



B

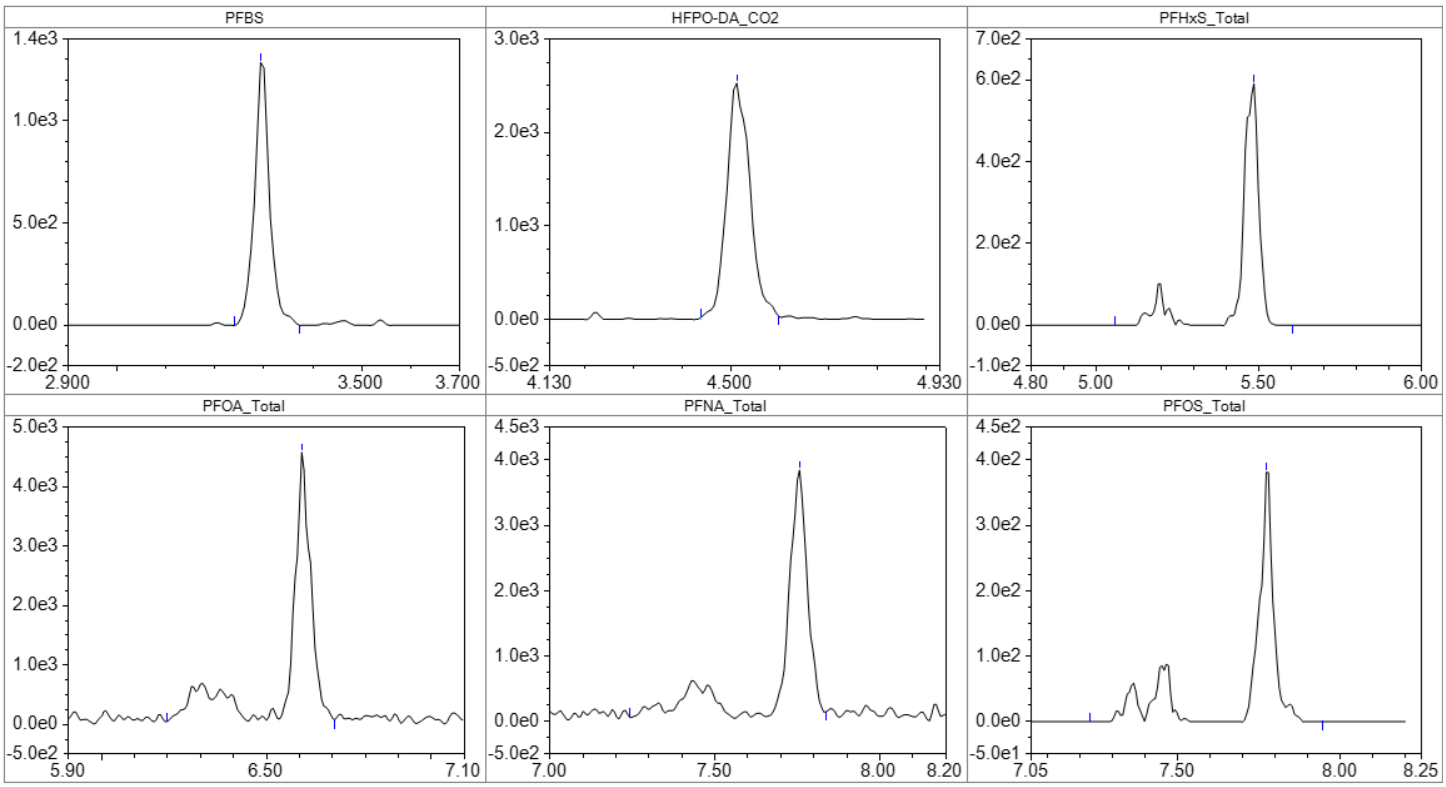
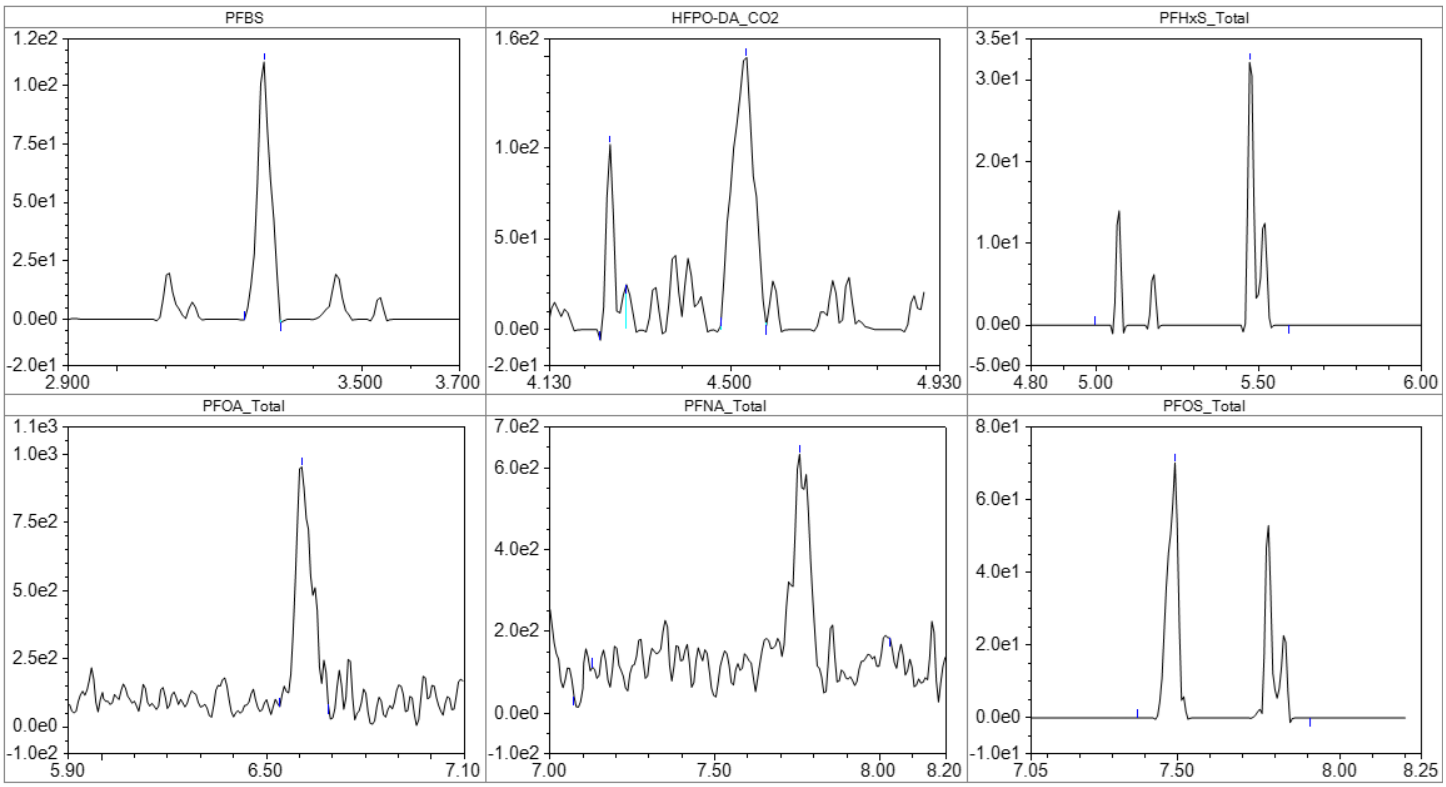


Figure 1. Chromatograms for calibration standards at (A) 25 and (B) 50 ng/L for target PFAS in US EPA NPDWR on the TSQ Quantis Plus mass spectrometer

A



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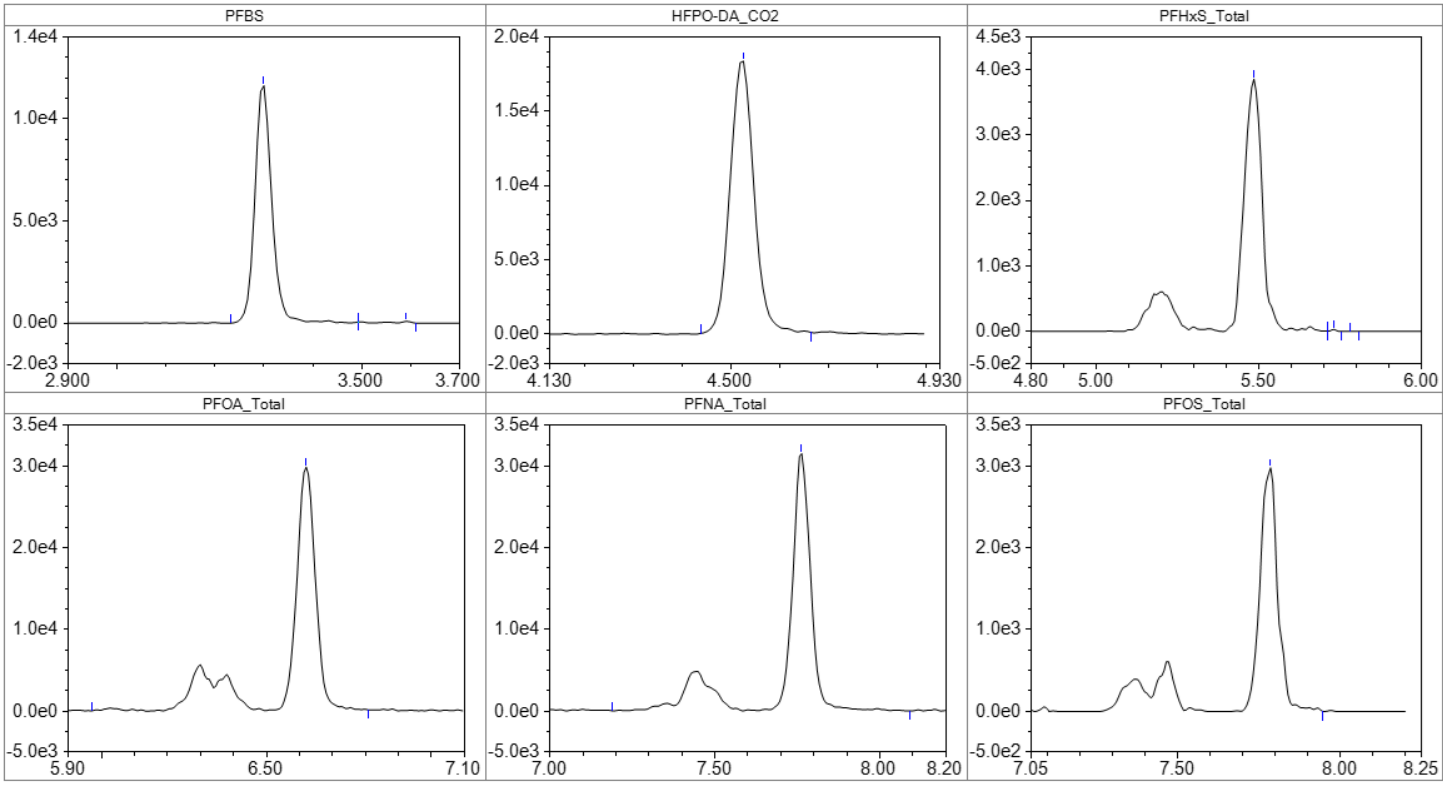


Figure 2. Example chromatograms for (A) LRB and (B) 2 ng/L LFB for target PFAS in US EPA NPDWR on the TSQ Quantis Plus mass spectrometer

MRL confirmation at 2 ng/L

To meet the requirements of the PFAS NPDWR and based on the monitoring trigger levels at 50% MCLs for PFOA and PFOS, the target MRL concentration was set at 2 ng/L. A total of seven LFB samples were prepared at 2 ng/L along with multiple LRB samples to be analyzed over two days. Sample extractions were accomplished on the AutoTrace 280 PFAS system, which allowed automation of the SPE process. Prior to running the MRL confirmation experiments, six LRBs were extracted using all channels of the AutoTrace 280 PFAS and then analyzed by LC-MS/MS (Figure 2). All PFAS showed responses below 0.20 ng/L, with the exception of PFBA, which had LRB concentrations of 0.44–1.30 ng/L (data not shown). Except for PFBA, this easily exceeded the criteria for LRB concentrations below 1/3 the concentration of the target MRLs (i.e., 0.67 ng/L), indicating the AutoTrace 280 PFAS system is well suited for analyzing water samples using EPA Method 533.

Section 9.1.4.2 of EPA Method 533 discusses the calculations used to confirm the MRLs. In addition to determining the mean concentration and standard deviation of the target PFAS compounds, the half-range for the Prediction Interval of Results (PIR) must be calculated. Based on the standard deviation of the seven LFB samples, these values are used to calculate Upper and Lower PIR limits for all PFAS targets. The acceptance criteria to confirm the MRLs is 150% and 50% for the Upper and Lower PIR limits, respectively.

Using EPA Method-specific reporting templates in Chromeleon 7 CDS, these values are readily determined. Figure 3 presents a chart for all target PFAS using the MRL data collected at 2 ng/L. The blue spheres are the mean percent accuracy values for 25 target PFAS in EPA Method 533, which range from 74.5% to 116.8%. The orange and gray bars represent the calculated Upper and Lower PIR Limit percentages, respectively, for each target PFAS. Except for PFBA and PFHxA, all target PFAS are within the Upper and Lower PIR limits. PFBA and PFHxA are slightly above the 150% Upper PIR Limit owing to elevated amounts (e.g., 0.38–0.78 ng/L) of those PFAS in the LRB samples on the second day of MRL experiments. Nevertheless, the 2 ng/L MRL is confirmed for all other PFAS targets, particularly for the six PFAS under the NPDWR.

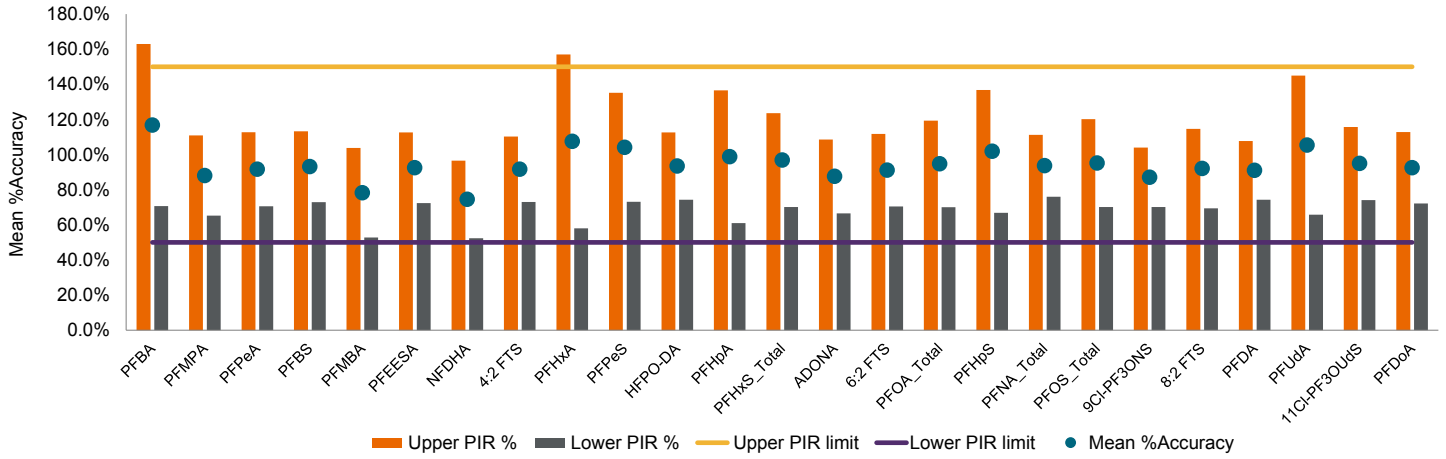


Figure 3. Chart of MRL confirmation data for target PFAS in EPA Method 533 at 2 ng/L

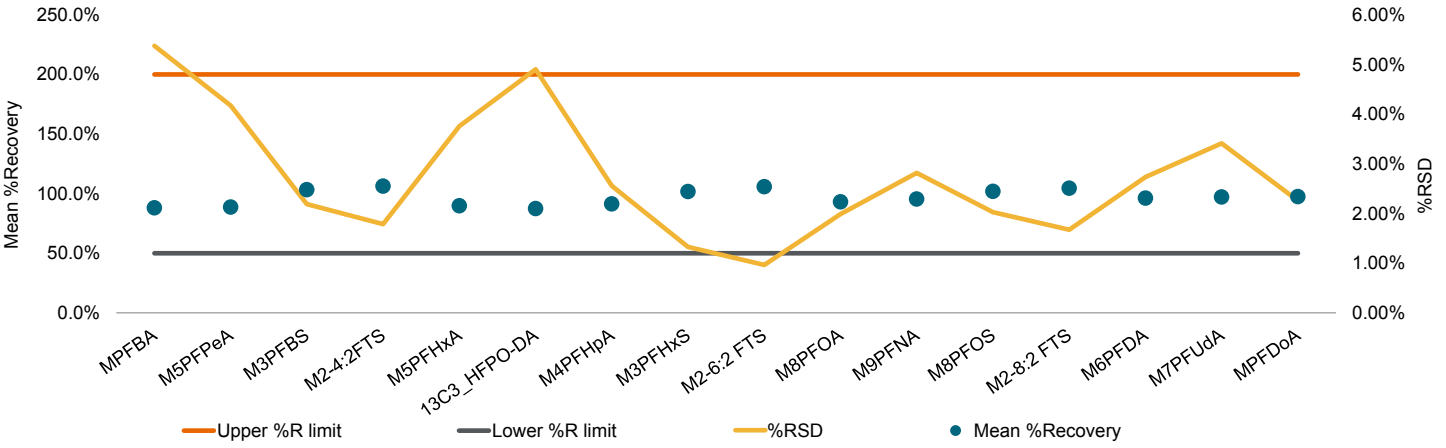


Figure 4. Chart of mean percent recovery and %RSDs for Isotope Dilution Analogues from MRL samples. %Recovery data are plotted against the primary (left) axis; %RSD values are plotted against the secondary (right) axis.

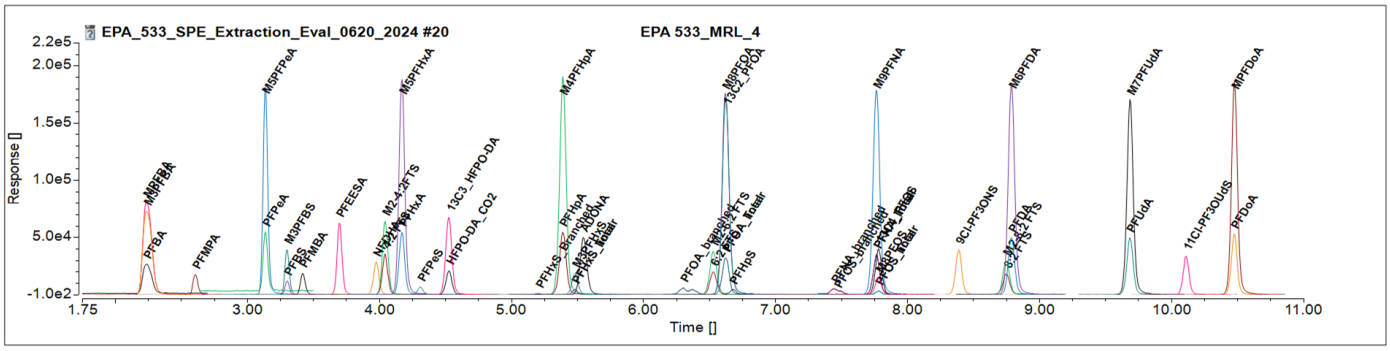
Quantitation Report

Sequence Details

Sequence Name:	**EPA_533_SPE_Extraction_Eval_0620_2024**	*Created On:*	**22/May/24 17:29:30**
Directory:	**McHale\PFAS\EPA 533**	*Created By:*	**Orbitrap Eclipse**
Data Vault:	**ChromeleonLocal**	*Updated On:*	**19/Jul/24 11:08:30**
No. of Injections:	**36**	*Updated By:*	**kevin.mchale**
Calibration Source:	**EPA_533_SPE_Extraction_Eval_0620_2024**		

Injection Details

Injection Name:	**EPA 533_MRL_4**	*Injection Volume:*	**5.00**
Analysis Type:	**Method Validation**	*Dilution Factor:*	**1.0000**
Injection Type:	**Unknown**	*Sample Weight:*	**250.0000**
Injection Date/Time:	**20/Jun/24 22:43**	*Processing Method:*	**EPA533_Internal_Calib_GCMS Enviro Plugin**
Vial Number:	**R:C6**	*Instrument Method:*	**EPA 533_SSL_v5**



Isotope Performance Standards

CASRN	Compound MS Quantitation	Ret.Time min	Quantitation Ion (m/z)	Area counts*min	S/N peak-to-peak	Amount ng/L	Manually Integrated?	Conf. Peak Result
	M3PFBA	2.24	668.960	5,707.8	165.5	2000.000		n.a.
	13C2_PFOA	6.62	370.000	10,646.2	435.5	2000.000		n.a.
	13C4_PFOS	7.78	80.000	2,317.6	392.7	6000.000		n.a.

Isotope Dilution Analogues

CASRN	Compound MS Quantitation	Ret. Time min	Quantitation Ion (m/z)	Area counts*min	S/N peak-to-peak	Amount ng/L	Manually Integrated?	Conf. Peak Result	Spiked Amt. ng/L MS Quantitation	%Recovery MS Quantitation
	MPFBA	2.23	668.960	6,250.6	220.2	7.33		n.a.	8.0000	91.6
	M5PFPeA	3.13	668.960	7,699.1	988.0	7.36		n.a.	8.0000	92.0
	M3PFBS	3.30	307.000	1,600.9	574.0	8.34		n.a.	8.0000	104.2
	M2-4:2FTS	4.04	81.000	3,310.3	478.0	34.40			32.0000	107.5
	M5PFHxA	4.17	270.000	9,835.3	1427.9	7.50		n.a.	8.0000	93.8
	13C3_HFPO-DA	4.53	169.000	3,776.5	498.8	7.30		n.a.	8.0000	91.3
	M4PFHpA	5.39	668.960	11,142.9	542.3	7.54		n.a.	8.0000	94.3
	M3PFHxS	5.48	668.960	1,065.1	322.7	8.15		n.a.	8.0000	101.9
	M2-6:2 FTS	6.53	81.000	2,388.1	373.8	33.92		n.a.	32.0000	106.0
	M8PFOA	6.62	668.960	10,942.2	657.9	7.56		n.a.	8.0000	94.5
	M9PFNA	7.76	668.960	10,880.8	602.0	7.92		n.a.	8.0000	99.0
	M8PFOS	7.78	80.000	857.8	97.4	8.42		n.a.	8.0000	105.2
	M2-8:2 FTS	8.74	81.000	1,833.6	466.2	33.48		n.a.	32.0000	104.6
	M6PFDA	8.79	668.960	10,873.2	386.5	7.90		n.a.	8.0000	98.7
	M7PFUdA	9.68	569.000	9,580.9	373.5	8.09		n.a.	8.0000	101.1
	MPFDoA	10.48	569.000	10,070.9	505.1	8.04		n.a.	8.0000	100.5

Target Compounds

CASRN	Compound MS Quantitation	Ret.Time min	Quantitation Ion (m/z)	Area counts*min	S/N peak-to-peak	Amount ng/L	Manually Integrated?	Amount > CRQL?	Conf. Ion m/z MS Quantitation	Conf. Ratio %	Conf. Peak Result
375-22-4	PFBA	2.24	668.960	2,007.7	70.2	2.31		Yes	n.a.	n.a.	n.a.
377-73-1	PFMPA	2.61	85.000	797.8	589.6	1.92		Yes	n.a.	n.a.	n.a.
2706-90-3	PFPeA	3.14	307.000	2,171.0	85.0	1.98		Yes	n.a.	n.a.	n.a.
375-73-5	PFBS	3.30	80.000	491.9	342.7	2.01		Yes	n.a.	n.a.	n.a.
863090-89-5	PFMBA	3.42	85.000	839.0	284.0	1.73		Yes	n.a.	n.a.	n.a.
113507-82-7	PFEESA	3.69	135.000	2,859.4	693.1	1.97		Yes	83.000	4.9	
151772-58-6	NFDHA	3.97	201.000	1,431.0	213.6	1.62		Yes	85.000	26.5	
757124-72-4	4:2 FTS	4.04	307.000	1,715.8	175.3	1.94		Yes	81.000	14.3	
307-24-4	PFHxA	4.17	269.000	2,826.8	353.7	2.10		Yes	119.000	3.3	
2706-91-4	PFPeS	4.31	80.000	360.3	498.4	2.10		Yes	99.000	75.3	
13252-13-6	HFPO-DA_CO2	4.53	169.000	1,170.0	338.8	2.04		Yes	n.a.	n.a.	n.a.
	PFHxS_Branched	5.20	80.000	67.6	243.1	0.45			n.a.	n.a.	n.a.
375-85-9	PFHpA	5.39	319.000	3,255.3	230.4	1.94		Yes	169.000	18.7	
355-46-4	PFHxS_linear	5.48	80.000	252.1	4387.9	1.70		Yes	n.a.	n.a.	n.a.
	PFHxS_Total	5.48	80.000	323.5	997.2	2.17		Yes	n.a.	n.a.	n.a.
919005-14-4	ADONA	5.54	251.000	3,103.3	675.9	1.90		Yes	85.000	16.6	
	PFOA_branched	6.30	369.000	685.5	54.5	0.67		Yes	n.a.	n.a.	n.a.
27619-97-2	6:2 FTS	6.53	407.000	1,236.6	91.7	1.96		Yes	81.000	13.5	
335-64-1	PFOA_linear	6.62	369.000	2,039.7	93.8	1.34		Yes	169.000	23.4	
	PFOA_Total	6.62	369.000	2,809.3	298.2	2.08		Yes	n.a.	n.a.	n.a.
375-92-8	PFHpS	6.67	80.000	284.2	135.0	2.33		Yes	99.000	68.1	
	PFNA_branched	7.44	419.000	548.5	34.7	0.67		Yes	n.a.	n.a.	n.a.
	PFOS_branched	7.46	80.000	77.3	29.0	0.48			n.a.	n.a.	n.a.
375-95-1	PFNA_linear	7.76	419.000	2,059.4	166.2	1.25		Yes	219.000	20.7	
	PFNA_Total	7.76	419.000	2,694.6	261.5	1.98		Yes	n.a.	n.a.	n.a.
1763-23-1	PFOS_linear	7.78	80.000	194.1	575.6	1.60		Yes	n.a.	n.a.	n.a.
	PFOS_Total	7.78	80.000	271.0	849.9	2.08		Yes	n.a.	n.a.	n.a.
756426-58-1	9Cl-PF3ONS	8.39	351.000	2,206.8	330.1	1.89		Yes	353.000	31.2	
39108-34-4	8:2 FTS	8.75	668.960	1,099.8	76.6	2.02		Yes	n.a.	n.a.	n.a.
335-76-2	PFDA	8.79	469.000	2,949.1	335.9	1.94		Yes	n.a.	n.a.	n.a.
2058-94-8	PFUdA	9.68	518.970	2,843.3	289.5	2.17		Yes	n.a.	n.a.	n.a.
763051-92-9	11Cl-PF3OUdS	10.11	451.000	1,784.8	437.6	2.05		Yes	453.000	31.9	
307-55-1	PFDoA	10.47	569.000	2,813.1	335.4	2.02		Yes	n.a.	n.a.	n.a.

Figure 5. Example Quantitation Report from Chromeleon CDS for EPA Method 533

Recovery and precision data

Section 9.2.5 of EPA Method 533 discusses the calculation and method requirements for percent recovery (%R) of the isotope dilution analogues. Recovery and precision data are presented in Figure 4 using the same seven LFB samples that were used for MRL confirmation, in which the isotope dilution analogues were fortified at 32 ng/L for labeled x:2FTS compounds and 8 ng/L for all others. Mean percent recovery values are plotted as blue spheres against the primary y-axis. %R values were measured to be 87.5–105.7%, which are well within the acceptance range of 50–200%. In fact, all isotope dilution analogues are within the acceptance criteria for target PFAS of 70–130% at concentrations above the MRL as discussed in section 9.2.3.2 of EPA Method 533.

Relative standard deviations (%RSDs) were calculated from the measured concentrations of isotope dilution analogues for the seven LFBs, which are plotted on the secondary y-axis of Figure 4. All %RSDs were below 5.5%, with 11 of 16 isotope dilution analogues below 3%, which is well below the 20% requirement for precision described in section 9.1.2 of EPA Method 533. These data demonstrate that the AutoTrace 280 PFAS system yields high precision for the recovery of PFAS at low ng/L levels in water samples, providing confidence that the AutoTrace 280 PFAS system can be used for processing samples in an automated fashion.

Reports for EPA Methods in Chromeleon CDS

There is a great deal of data that needs to be evaluated for the initial demonstration of capability and for ongoing quality control of the results generated for published EPA-based analytical methods. Chromeleon CDS provides a Report Designer with templates that are specifically tailored to chromatography-based EPA methods, including EPA 533. Derived from the GC-MS Environmental Analysis Extension package, these Chromeleon CDS templates are provided to cover the range of results needed to comply with all reporting criteria in EPA Method 533. However, these templates still provide a degree of flexibility to allow user modifications if needed to suit their own laboratory needs.

Figure 5 presents an example of the Quantitation Report from Chromeleon 7 CDS. This report provides an injection summary of LC-MS/MS data, including a chromatogram, areas for isotope performance standards, %Recovery for isotope dilution analogues, and calculated amounts for PFAS targets in addition to S/N values and retention times for all components.

Other provided report templates include Calibration and Check Standard reports with %Difference and limit flagging for calibration and CCC standards, respectively, an Isotope Dilution Analogue Recovery report, a MRL Confirmation report for calculating Upper and Lower PIR values, a Method Validation report to ensure demonstration of precision and accuracy from seven midpoint LFB samples, amongst others.

Conclusions

Data presented here clearly demonstrate that the Vanquish Flex Binary UHPLC system with the TSQ Quantis Plus mass spectrometer can easily quantify target PFAS at MRLs of 2 ng/L following EPA Method 533, and in particular, the six targets regulated in the PFAS NPDWR. Based on calibration standards data on the TSQ Quantis Plus mass spectrometer, the limitation for achieving MRLs below 1 ng/L using EPA Method 533 is from PFAS cross-contaminations during sample handling and sample preparation, as observed herein with PFBA and PFHxA.

By providing automation and precise sample delivery, the Dionex AutoTrace 280 PFAS system is well suited for SPE of 250 mL water samples at low to sub ng/L levels required for PFAS monitoring for the NPDWR.

Utilizing Chromeleon CDS for LC-MS/MS data collection and for reporting sample results yields a streamlined mechanism for ensuring all analytical analysis procedures of EPA Method 533 are attained.

When combined, this Thermo Fisher Scientific solution provides a comprehensive means to readily implement and consistently achieve the US EPA monitoring requirements of the PFAS NPDWR.

References

1. US EPA PFAS National Primary Drink Water Regulation, April 2024. https://www.epa.gov/sdwa/and-polyfluoroalkyl-substances-pfas
2. Fact Sheet: PFAS NPDWR Monitoring and Reporting. https://www.epa.gov/system/files/documents/2024-04/pfas-npdwr_fact-sheet_monitoring_4.8.24_0.pdf
3. Federal Register Notice: Final PFAS National Primary Drinking Water Regulation. https://www.federalregister.gov/d/2024-07773
4. Method 533: Determination of Per- and Polyfluoroalkyl Substances in Drinking Water by Isotope Dilution Anion Exchange Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry. https://www.epa.gov/sites/default/files/2019-12/documents/method-533-815b19020.pdf
5. Thermo Scientific Application Note 73883: Determination of per-and polyfluorinated alkyl substances (PFAS) in drinking water – Using automated solid phase extraction and LC-MS/MS for U.S. EPA Method 533. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-73883-lc-ms-spe-per-pfas-drinking-water-an73883-en.pdf

Appendix

Table A1. Supplies used for EPA Method 533. All products are from Thermo Fisher Scientific unless specifically noted.

Item	Product	Part number
PFAS delay column	Thermo Scientific™ Hypersil GOLD™, 3.0 x 50 mm, 1.9 um	25002-054630
Analytical column	Thermo Scientific™ Acclaim™ 120 C18, 2.1 x 50 mm, 2.2 um	068981
Mobile phase chemicals	Water, UHPLC-MS grade, 1 L, Thermo Scientific™	W8-1
	Methanol, UHPLC-MS grade, 1 L, Thermo Scientific™	A458-1
	Ammonium acetate, Optima™ LC/MS grade, 50 g, Fisher Chemical™	A114-50
	Acetic acid, Optima™ LC/MS grade, 1 mL ampoules, Fisher Chemical™	A113-10X1AMP
SPE reagents	Ammonium acetate, HPLC grade, 500 g, Fisher Chemical™	A639-500
	Ammonium hydroxide, ACS Grade, 28-30%, 100 mL, Sigma-Aldrich	221228-100ML-A
	Sodium phosphate dibasic heptahydrate, ACS grade, 500 g	5373-500
	Sodium phosphate monobasic monohydrate, ACS grade, 500 g	5369-500
SPE cartridges	Agilent™ Bond Elut™ PFAS, WAX, 500 mg/6 mL, 30/pk	5610-2152
Collection tubes	Corning™ Falcon™ round-bottom polypropylene tubes with cap, 14 mL	14-959-11B
Autosampler vials	Polypropylene, 1.5 mL, screw-top, Level 1	6ESV9-1PP
Autosampler caps	Polypropylene caps, 9 mm, screw-thread	C5000-50

Table A2. Calibration standard concentrations of target PFAS for the TSQ Quantis Plus mass spectrometer. Concentration in bold was also used as Continuing Calibration Check standard.

Cal. std.	Calibration std. PFAS conc. (ng/L)	Equivalent drinking water conc. (ng/L)
1	25	0.1
2	50	0.2
3	**125**	0.5
4	250	1
5	500	2
6	1250	5
7	2500	10
8	5000	20
9	12500	50
10	25000	100

Table A3. LC method parameters

Parameter	Value
Analytical column	Acclaim 120 C18, 2.1 x 50 mm, 2.2 um
Delay column	Hypersil GOLD, 3.0 x 50 mm, 1.9 um
Column temperature	40 °C
Injection volume	5 µL
Autosampler temperature	20 °C
Mobile phase	H_2O + 2% MeOH + 2 mM ammonium acetate + 0.1% acetic acid MeOH + 2% H_2O + 2 mM ammonium acetate + 0.1% acetic acid
Flow rate	0.4 mL/min
Gradient	
Time (min)	%B
0.0	20
1.0	50
10.0	85
11.5	100
13.0	100
13.2	20
15.75	20

Table A4. TSQ Quantis Plus mass spectrometer general settings

Parameter	Value
Ion source	H-ESI
Polarity	Negative
Spray voltage	-1,000 V
Sheath gas	50 a.u.
Aux gas	12 a.u.
Sweep gas	0.5 a.u.
Ion transfer tube temperature	250 °C
Vaporizer temperature	225 °C
Q1, Q3 resolution	0.7 FWHM
CID gas	2.5 mTorr argon
SRM cycle time	0.4 s

Table A5. Timed SRM transitions for PFAS measured for EPA Method 533 on the TSQ Quantis Plus mass spectrometer.
Compounds highlighted in blue and in purple are for isotope performance standards and isotope dilution analogues, respectively. Product *m/z* in bold are the quantifier ions.

Compound	Start time (min)	End time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF lens (V)
PFBA	1.1	2.6	213	**169**	9	72
M3PFBA	1.1	2.6	216	**172**	9	72
MPFBA	1.1	2.6	217	**172**	9	72
PFMPA	2	2.9	229	**85**	10.5	72
PFPeA	2.4	3.4	263	**219**	8.5	77
M5PFPeA	2.4	3.4	268	**223**	8.5	77
PFMBA	2.8	3.7	279	**85**	10.5	80
PFBS	2.6	3.6	298.94	**80**	32	190
PFBS	2.6	3.6	298.94	99	29	190
M3PFBS	2.6	3.6	302	**80**	32	190
PFEESA	3	4	314.95	83	19	135
PFEESA	3	4	314.95	**135**	22	135
NFDHA	3.4	4.3	295	85	22	63
NFDHA	3.4	4.3	295	**201**	8	63
4:2FTS	3.4	4.3	327	81	28	160
4:2FTS	3.4	4.3	327	**307**	20	160
M2-4:2FTS	3.4	4.3	329	**81**	28	160
M2-4:2FTS	3.4	4.3	329	309	20	160
PFHxA	3.5	4.5	313	119	19	92
PFHxA	3.5	4.5	313	**269**	9	92
M5PFHxA	3.5	4.5	318	**273**	9	92
PFPeS	3.6	4.6	348.94	**80**	35	200
PFPeS	3.6	4.6	348.94	99	32	200
HFPO-DA_CO2	3.9	4.8	285	**169**	7	80
HFPO-DA_CO2	3.9	4.8	285	185	17	80
13C3-HFPO-DA	3.9	4.8	287	**169**	7	80
PFHpA	4.7	5.7	363	169	17	102
PFHpA	4.7	5.7	363	**319**	9.5	102
M4PFHpA	4.7	5.7	367	**322**	9.5	102
PFHxS	4.5	5.9	398.94	**80**	38	220

Table A5 (continued)

Compound	Start time (min)	End time (min)	Precursor (*m/z*)	Product (*m/z*)	Collision Energy (V)	RF lens (V)
PFHxS	4.5	5.9	398.94	99	34	220
M3PFHxS	4.8	5.9	402	**80**	38	220
ADONA	4.9	5.9	377	85	22	94
ADONA	4.9	5.9	377	**251**	10	94
6:2FTS	5.9	6.9	427	81	30	195
6:2FTS	5.9	6.9	427	**407**	22.5	195
M2-6:2FTS	5.9	6.9	429	**81**	30	195
M2-6:2FTS	5.9	6.9	429	409	22.5	195
PFOA	5.5	7	413	169	17	114
PFOA	5.5	7	413	**369**	10	114
13C2_PFOA	6	7	415	**370**	10	114
M8PFOA	6	7	421	**376**	10	114
PFHpS	5.9	7.1	448.93	**80**	40	240
PFHpS	5.9	7.1	448.93	99	37	240
PFOS	6.6	8.1	498.93	**80**	46	270
PFOS	6.6	8.1	498.93	99	40	270
PFNA	6.6	8.1	463	219	17	122
PFNA	6.6	8.1	463	**419**	10.5	122
M9PFNA	7.1	8.1	472	427	10.5	122
13C4_PFOS	7	8.1	503	**80**	46	240
M8PFOS	7	8.1	507	**80**	46	240
9Cl-PF3ONS	7.8	8.8	530.9	**351**	25	175
9Cl-PF3ONS_37Cl	7.8	8.8	532.9	353	25	175
8:2FTS	8	9	527	81	33	220
8:2FTS	8	9	527	**507**	26	220
M2-8:2FTS	8	9	529	**81**	33	220
M2-8:2FTS	8	9	529	509	26	220
PFDA	8	9.1	512.96	269	17	138
PFDA	8	9.1	512.96	**469**	11	138
M6PFDA	8	9.1	519	**474**	11	138
PFUdA	9	10.1	562.96	269	18	151
PFUdA	9	10.1	562.96	**518.97**	11	151
M7PFUdA	9	10.1	570	**525**	11	151
11Cl-PF3OUdS	9.4	10.5	630.9	**451**	27	163
11Cl-PF3OUdS_37Cl	9.4	10.5	632.9	453	27	163
PFDoA	9.9	12	612.95	169	25	163
PFDoA	9.9	12	612.95	**569**	11.5	163
MPFDoA	9.9	12	615	**570**	11.5	163

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