Manual Extraction of PFAS in Drinking Water in Compliance with EPA Method 537.1

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Introduction

Per- and polyfluorinated alkyl substances (PFAS) have been used abundantly since their inception in the twentieth century and have become a closely monitored class of compounds within environmental testing. This application note outlines a procedure for those seeking to become compliant with EPA Method 537.1. The data presented was generated using a Biotage® VacMaster™ vacuum manifold with a Large Volume Extraction (LVE) kit designed for PFAS work in conjunction with ISOLUTE® 101 SPE columns and a TurboVap® LV system. This note will demonstrate that the solution outlined is capable of generating data which exceeds the requirements outlined within the 537.1 method.

Equipment and Materials Used

Biotage:

- Biotage® VacMaster™-20 Sample Processing Station (with 16 mm rack), p/n 121-2016, fitted with polypropylene stopcocks (p/n 121-0009-PP)
- Biotage® VacMaster™ LVE Kit (PFAS) 1, 3, 6 mL SPE Column (p/n 121-2190)
- ISOLUTE® 101 500 mg/6 mL SPE Columns, p/n 101-0050-C
- TurboVap® LV Automated Solvent Evaporation System, p/n 415000
- TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes), p/n 414964

Wellington Laboratories:

- USEPA Method 537.1 PDSL (linear) stocks, 1.2 mL, p/n EPA-537PDSL-R1
- USEPA Method 537 Internal Standard Mix, 1.2 mL, p/n EPA-537IS
- USEPA Method 537.1 Surrogate Mix, 1.2 mL, p/n EPA-537SS-R1

Restek:

- PFAS Delay Column, 5 µm, 50 x 2.1 mm, p/n 27854
- Raptor C18 LC Column, 5 µm, 50 x 2.1 mm, p/n 9304552

Sigma-Aldrich:

- Ammonium Acetate, ACS Reagent Grade ≥ 97%, p/n 238074-25G

Honeywell:

- Water, ACS Certified, HPLC Grade, p/n AH365-4
- Methanol, Burdick & Jackson™, LC-MS Grade, p/n LC230-4

VWR:

- 15 mL Polypropylene Centrifuge Tubes with Caps, p/n 21008-670
- 250 mL Polypropylene Wide Mouth Bottles, p/n 414004-125

ACROS Organics:

- Tris(hydroxymethyl)aminomethane, p/n 140505000

Analytes

Table 1.
Listing of Target Analytes, Surrogate Standards, and Internal Standards.

Target Analyte	Acronym	CASRN
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorohexanoic acid	PFHxA	307-24-4
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6b
Perfluorohexanesulfonic acid	PFHxS	355-46-4
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4e
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanoic acid	PFNA	375-95-1
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1d
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9c
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTA	376-06-7
Surrogate Standard	**Acronym**	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA	
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-NEtFOSAA	
Internal Standard	**Acronym**	
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA	
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	$^{13}C_4$-PFOS	
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-NMeFOSAA	

Summary of SPE Method

SPE Column Format
ISOLUTE® 101 500 mg/6 mL p/n 101-0050-C

Sample Pre-Treatment
Add 0.94 g of tris(hydroxymethyl)aminomethane and 660 µL of concentrated HCl to each 250 mL sample, check pH is 7 ± 0.5. Add surrogate standards.

Conditioning
Condition each column with methanol (15 mL)

Equilibration
Equilibrate each column with reagent water (18 mL + 3 mL)

Sample Loading
Load sample (250 mL) at a flow rate of 15 mL/min

Wash
Rinse the column with reagent water (sample bottle rinsate, 2 x 7.5 mL)

Dry
Dry the column for 5 minutes using a vacuum of -10 to -15 in. Hg.

Elution
Elute the analytes with methanol (sample bottle rinsate 2 x 4 mL)

Post Extraction
Evaporate to dryness and reconstitute in methanol/water (96/4, v/v, 990 µL). Add IS and mix prior to analysis.

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Sample Preparation Procedure

1. Clean all parts of the VacMaster™ system per the procedure given in Appendix A.
2. Set up and fill new sample containers with 250 mL of water.
3. Add 0.94 g of tris(hydroxymethyl)aminomethane and 660 µL of concentrated HCl to each of the sample containers.
4. Verify the pH of the sample is 7 ± 0.5.
5. Prepare for the determination of the initial sample volume by either marking the level of the sample on the container or by weighing the sample container.
6. Add 10 µL of the undiluted Surrogate Standard Mix to each of the sample containers. If the mixes used were different than the ones outlined in this note, adjust the concentration or spiking amounts as needed.
7. Using the VacMaster™ LVE Kit, place one end of the cleaned tubing into the bottom of each of the sample containers, and secure in position using the clips provided.
8. Load ISOLUTE® 101 columns onto the VacMaster™. Seal any unused positions using VacMaster™ Port Sealing Plugs (p/n 121-0005)
9. Rinse each column with 15 mL of methanol and apply vacuum to pull it to waste. Do not allow the sorbent to go dry.
10. Rinse each column with 18 mL of reagent water and apply vacuum to send it to waste. Do not allow the water level to drop below the top of the packing.
11. Add 3 mL of reagent water to each tube and attach the column adapters to the columns.
12. Load the samples onto the columns using a flow rate of 15 mL/min.
13. Once the sample has been fully loaded, rinse the sample containers using 7.5 mL of reagent water, swirl to ensure the full rinsing of the container, and load the aliquot onto the column. Repeat this step one additional time.
14. Dry the column for 5 minutes using a vacuum of -10 to -15 in. Hg.
15. Load 15 mL centrifuge tubes into the rack corresponding to each of the column positions and load into the VacMaster™.
16. Rinse each sample container using 4 mL of methanol and swirl to ensure the full rinsing of the container. Load the aliquot through the appropriate column and collect at a dropwise rate. Repeat this step one additional time.
17. Determine the initial sample volume by either using a graduated cylinder and filling the sample container to the original mark or by taking an additional weight of the container.
18. Transfer the centrifuge tubes to the TurboVap® LV system and concentrate the samples under nitrogen according to the parameters in Table 2.
19. Reconstitute each extract using 990 µL of 96% methanol/4% water within the centrifuge tubes.
20. Add 10 µL of the internal standard mix and mix thoroughly.
21. Transfer each extract to a 1 mL storage vial.
22. Transfer a 300 µL aliquot of each extract to an autosampler vial.
23. Load the extract onto a calibrated LC-MS/MS system and process using the conditions given in the below sections.

Table 2. TurboVap® LV Concentration Protocol.

Bath Temp	60 °C
Evaporation Mode	Method (Ramp Gradient)
Manifold Setup	48 positions
Rack Row Height	120 mm*
Step 1:	2.5 L/min for 15 min
Step 2:	3.0 L/min for 15 min
Step 3:	3.5 L/min for 45 min

*The nozzle position was adjusted such that it was as far to the right as possible to give the user a clear view of the vortex within the tube.

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LC-MS/MS Conditions

Shimadzu Nexera X2 UHPLC System

- CBM-20A Communications Controller
- DGU-20A5R Degassing Unit
- LC-30AD Solvent Delivery Unit
- SIL-30AC Autosampler
- CTO-30A Column Over
- SPD-20A UV-Vis Detector

Columns

- Restek PFAS Delay Column, 5 µm, 50 x 2.1 mm, p/n 27854
- Restek Raptor C18 LC Column, 5 µm, 50 x 2.1 mm, p/n 9304552

Mobile Phases

A: 20 mM Ammonium Acetate in Water

B: Methanol

Table 3. LC Gradient.

Time (min)	%A	%B
0.01	95	5
0.10	45	55
4.50	1	99
4.95	1	99
5.00	95	5
6.75	Stop	

Flow Rate

0.6 mL/min

Injection Volume

10 µL

Column Temperature

35 °C

AB SCIEX Triple Quad™ 5500

Note: The PFAS conversion kit from SCIEX was installed and used for this testing with the exception of the included UHPLC columns.

Curtain Gas

35

Collision Gas

8

Ion Spray Voltage

-4500

Temperature

350 °C

Ion Source Gas 1

50

Ion Source Gas 2

50

Polarity

Negative

For a complete listing of MRM Transitions, see Appendix B

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Results

System Calibration

For the work being done here, a total of nine points were used in the calibration covering a range of 0.2-100 ppt in the sample. The lowest three points were below the calculated MRL. The curve was forced through zero as required by method 537.1 and achieved excellent linearity across the calibration range.

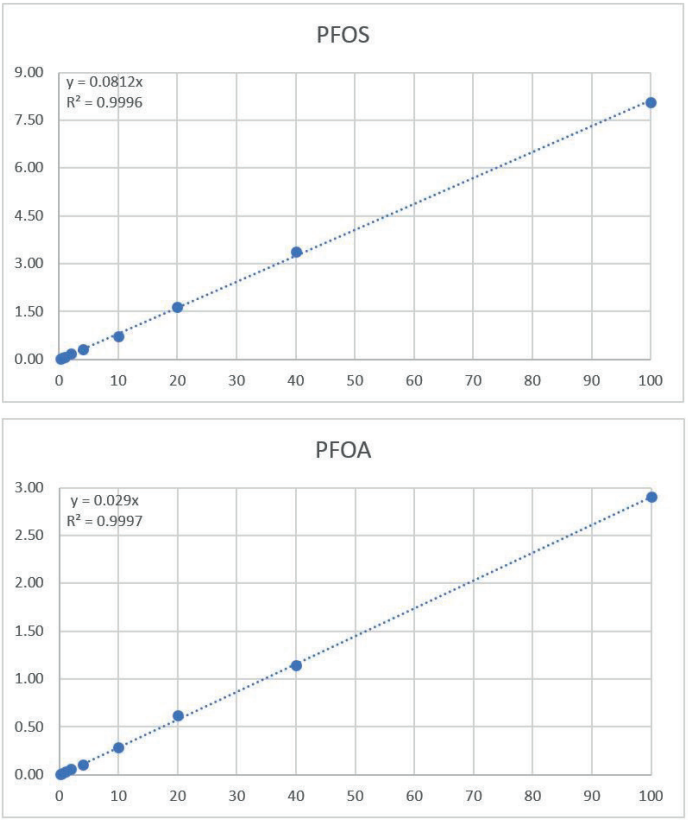

Figure 1. Calibration curves for PFOS and PFOA. Calibration curves for the remaining target analytes in Table 1 are shown in Appendix B.

Demonstration of Peak Asymmetry Factor

Method 537.1 only requires that the first two eluting peaks on a single mid-level calibration standard have their peak asymmetry factors calculated and that the results of this calculation must fall within a range of 0.8-1.5. For the work done here, the scope of the investigation was broadened and six of the early eluting compounds were examined across all calibration levels. The results are given in Table 4 and shown graphically in Figure 2.

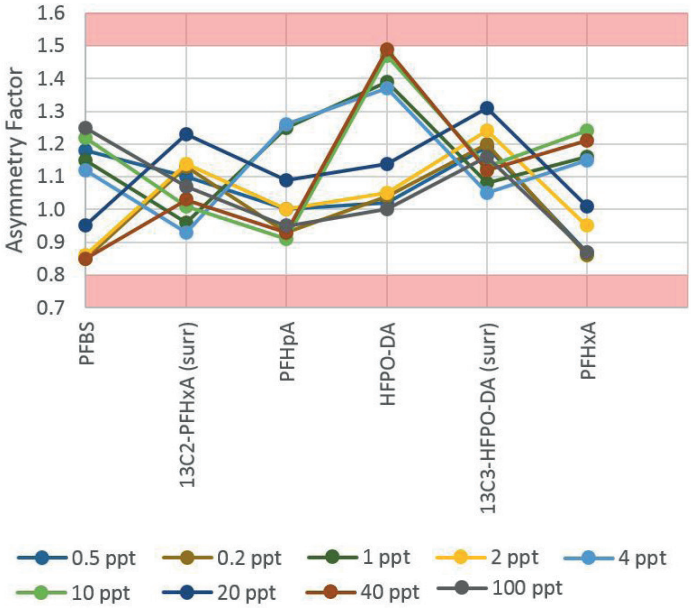

Figure 2. Peak asymmetry factor with the range of acceptance shown in white.

Each of the first six eluting compounds across all calibration levels were able to meet and exceed the asymmetry factor requirements proving that the chromatographic conditions are acceptable.

Determination of the Minimum Reporting Level (MRL) and Detection Limits (DL)

A target MRL of 2 ng/L was selected and seven replicate laboratory fortified blanks (LFBs) were created and ran at that concentration. Table 5 below lists the recoveries of the replicates, showing the averages and deviations for each compound along-side the calculated half-range for the prediction interval of results (HR_{PIR}), the upper and lower bounds for the PIR, and the resulting DL calculations. It should be noted that the constant used to calculate the HR_{PIR} and the DL was adjusted to accommodate the increased degrees of freedom for this experiment.

Table 4. Peak Asymmetry Factor.

	Retention Time (min.)	Concentration								
		0.2 ppt	0.5 ppt	1 ppt	2 ppt	4 ppt	10 ppt	20 ppt	40 ppt	100 ppt
PFBS	1.06	0.85	1.18	1.15	0.86	1.12	1.22	0.95	0.85	1.25
$^{13}C_2$-PFHxA (surr)	1.21	1.13	1.10	0.96	1.14	0.93	1.01	1.23	1.03	1.07
PFHpA	1.21	0.93	1.00	1.25	1.00	1.26	0.91	1.09	0.93	0.95
HFPO-DA	1.27	1.04	1.02	1.39	1.05	1.37	1.47	1.14	1.49	1.00
$^{13}C_3$-HFPO-DA (surr)	1.28	1.20	1.19	1.08	1.24	1.05	1.13	1.31	1.12	1.16
PFHxA	1.45	0.86	0.87	1.16	0.95	1.15	1.24	1.01	1.21	0.87

Table 5. MRL and DL Recoveries and Calculations.

	Conc.	1	2	3	4	5	6	7	Average	Std. Dev.	HR_{PIR}	Lower PIR	Upper PIR	DL
	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(ng/L)	(%)	(%)	(ng/L)
PFBS*	1.77	1.69	1.97	1.82	1.87	2.27	1.99	2.09	1.96	0.19	0.70	62.9	133.1	0.57
PFHxA	2.0	2.00	2.18	2.22	2.03	2.05	2.23	2.04	2.11	0.10	0.36	87.1	123.5	0.29
HFPO-DA	2.0	1.82	2.03	2.24	2.05	2.05	2.09	1.85	2.02	0.14	0.53	74.5	127.4	0.43
PFHxS*	1.89	1.86	2.08	1.99	1.70	1.98	1.84	2.12	1.94	0.15	0.54	69.9	123.9	0.44
ADONA*	1.89	2.16	2.27	2.49	2.20	2.24	2.44	2.29	2.30	0.12	0.46	92.2	137.7	0.37
PFHpA	2.0	1.91	2.26	2.41	2.12	2.01	2.22	2.20	2.16	0.17	0.62	77.2	138.9	0.50
PFOA	2.0	1.92	2.22	2.22	2.12	2.17	2.19	1.98	2.12	0.12	0.44	83.7	127.9	0.36
PFOS*	1.91	2.02	2.02	2.11	1.83	2.08	1.99	2.10	2.02	0.09	0.35	83.7	118.5	0.28
PFNA	2.0	1.93	2.14	2.27	2.20	2.21	2.22	2.01	2.14	0.12	0.46	83.9	130.3	0.37
9Cl-PF3ONS*	1.86	1.89	2.01	2.18	2.03	2.09	2.12	2.05	2.05	0.09	0.35	85.5	120.0	0.28
PFDA	2.0	1.80	2.07	2.31	2.14	2.05	2.10	1.90	2.05	0.16	0.61	72.1	133.2	0.49
PFUnA	2.0	1.91	1.99	2.18	1.84	1.98	2.06	1.92	1.98	0.11	0.41	78.8	119.7	0.33
NEtFOSAA	2.0	1.87	2.03	1.95	1.97	1.93	2.23	1.58	1.94	0.19	0.72	61.1	132.6	0.58
11Cl-PF3OUdS*	1.88	1.86	1.82	1.94	1.75	2.03	1.92	1.96	1.89	0.09	0.35	77.2	112.3	0.28
NMeFOSAA	2.0	1.75	2.02	1.90	1.76	2.03	1.97	1.57	1.86	0.17	0.64	60.8	124.9	0.52
PFDoA	2.0	2.17	1.94	2.11	1.92	2.01	2.13	1.81	2.01	0.13	0.49	76.1	125.1	0.40
PFTrDA	2.0	1.70	1.89	1.95	1.67	1.85	1.87	1.67	1.80	0.12	0.44	68.2	111.7	0.35
PFTA	2.0	1.75	1.94	1.98	1.69	1.67	1.97	1.63	1.80	0.15	0.56	62.2	118.2	0.45

*Analytes were used in salt form and calculated concentrations were corrected to compensate where needed.

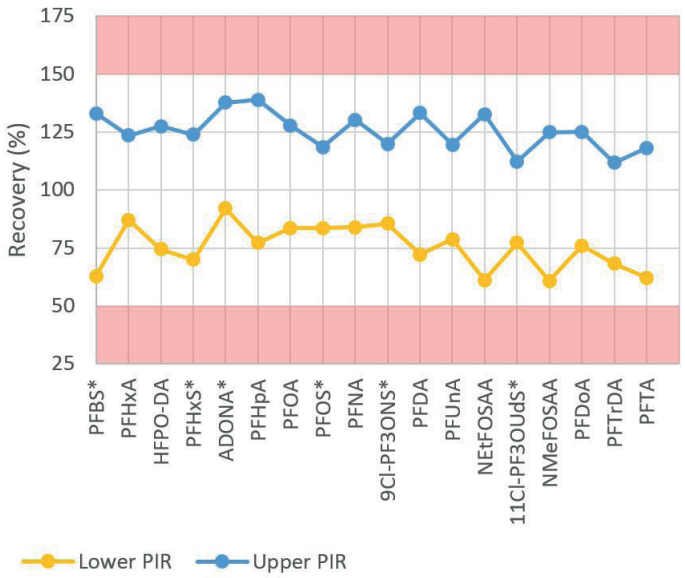

Figure 3. Upper and Lower calculated PIR limits with the range of acceptance shown in white. Those compounds with an asterisk were used in salt form.

Based on the data obtained, the calculated Upper and Lower PIR were all well within the specified boundaries and the MRL concentrations given in Table 5 are all deemed acceptable. The DL was calculated from this data set and is found to be lower than the DL given in Table 5 of EPA Method 537.1.

Demonstration of Low System Background

An investigation into the background of the complete process was done in three steps. The first step was to run blank injections of a mixture of 96% methanol/4% water on the analytical system (system blank). The second step was to load centrifuge tubes containing a similar volume of methanol as would result from the extraction process onto the evaporation system, allowing them to concentrate to dryness, be reconstituted, and then run on the analytical system (evaporation blank). The third and final step was to create a full Laboratory Reagent Blank (LRB), extract and concentrate it, reconstitute it, and run it on the analytical system. By separating the process into three separate steps it becomes easier to determine what, if any, contribution to the overall background each of the steps has. The result of these tests are shown in Table 6 and selected data are shown graphically in Figures 4 and 5.

Table 6. Results of PFAS Background Study (recoveries in ng/L).

	TurboVap® LV						Laboratory Reagent Blank							
Replicate	**1**	**2**	**3**	**4**	**5**	**6**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
PFBS*	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.011	0.020	0.019	0.042	0.033	0.017	0.021	0.011
PFHxA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.084	0.082	0.083	0.089	0.094	0.139	0.087	0.090
HFPO-DA	N.D.	N.D.	N.D.	0.002	0.006	N.D.	0.032	0.024	0.031	0.028	0.038	0.032	0.026	0.036
PFHxS*	N.D.	N.D.	N.D.	N.D.	0.061	N.D.	0.034	0.019	0.042	0.031	0.032	0.050	0.030	0.034
ADONA*	N.D.	N.D.	N.D.	N.D.	N.D.	0.004	0.032	0.031	0.034	0.031	0.031	0.032	0.028	0.040
PFHpA	N.D.	0.030	0.012	0.031	0.024	0.012	0.050	0.069	0.064	0.067	0.054	0.065	0.057	0.068
PFOA	0.073	0.069	0.050	0.071	0.071	0.063	0.082	0.083	0.085	0.076	0.081	0.088	0.086	0.102
PFOS*	0.143	0.143	0.115	0.145	0.130	0.137	0.086	0.091	0.086	0.091	0.097	0.110	0.097	0.095
PFNA	N.D.	0.015	0.013	0.005	0.014	N.D.	0.030	0.039	0.032	0.038	0.052	0.038	0.034	0.037
9Cl-PF3ONS*	N.D.	0.004	N.D.	0.005	0.002	0.003	0.029	0.033	0.029	0.031	0.037	0.035	0.033	0.032
PFDA	N.D.	N.D.	N.D.	0.005	0.013	N.D.	0.046	0.050	0.045	0.045	0.048	0.051	0.037	0.097
PFUnA	N.D.	N.D.	N.D.	0.026	N.D.	0.012	0.032	0.025	0.027	0.027	0.025	0.034	0.029	0.035
NEtFOSAA	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.026	0.025	0.025	0.020	0.029	0.032	0.021	0.032
11Cl-PF3OUdS*	N.D.	0.006	N.D.	N.D.	0.003	N.D.	0.028	0.029	0.028	0.030	0.039	0.033	0.030	0.030
NMeFOSAA	N.D.	N.D.	N.D.	N.D.	N.D.	0.013	0.048	0.044	0.044	0.041	0.044	0.047	0.039	0.034
PFDoA	N.D.	0.004	N.D.	0.007	0.008	0.007	0.022	0.031	0.027	0.028	0.025	0.025	0.027	0.029
PFTrDA	N.D.	0.003	N.D.	0.013	0.014	N.D.	0.022	0.028	0.025	0.026	0.023	0.021	0.018	0.025
PFTA	N.D.	N.D.	N.D.	N.D.	0.004	N.D.	0.020	0.019	0.020	0.020	0.020	0.018	0.014	0.024

*Analytes were used in salt form and calculated concentrations were corrected to compensate where needed.

NOTE: Where "N.D." is indicative of the inability of the target peak to be separated from the system background.

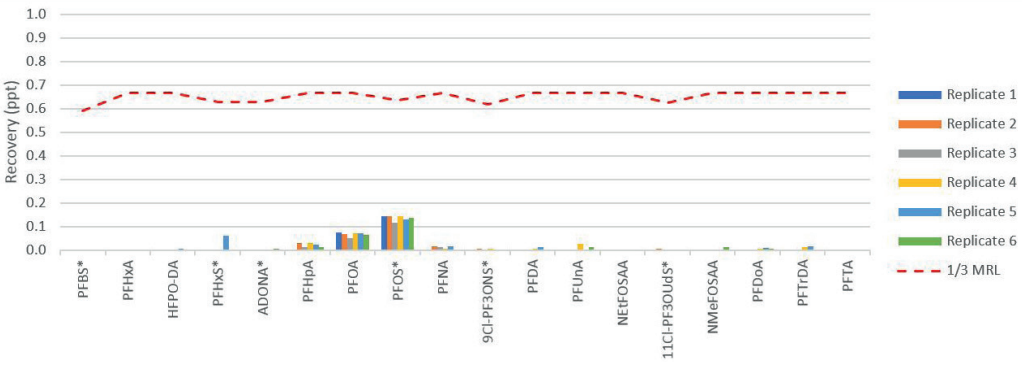

Figure 4. Contribution of the TurboVap® LV to the PFAS Background. Those compounds with an asterisk were used in salt form.

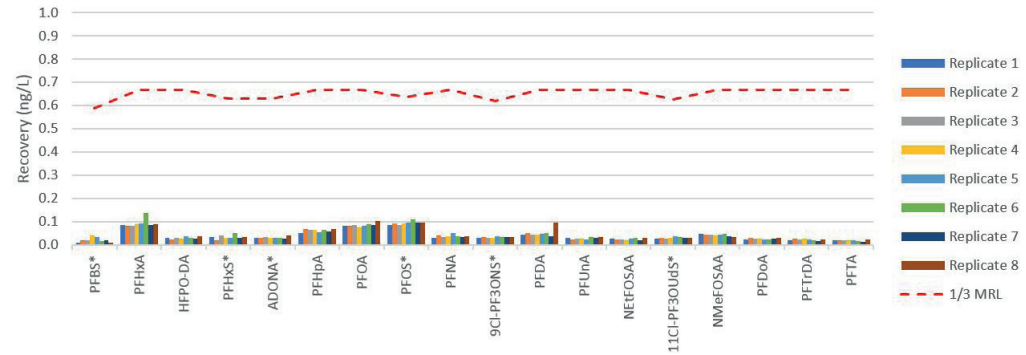

Figure 5. PFAS Background for full LRB. Those compounds with an asterisk were used in salt form.

For those results which were generated using only the analytical system, all target analytes were N.D. (unable to be separated from the noise in the baseline) and so were not listed out in the previous tables.

When examining the data resulting for both the TurboVap® LV and the full LRB (which includes the Biotage® VacMaster™ manifold, Large Volume Loading Kit, and the ISOLUTE® 101 cartridges as well as the TurboVap® LV) there are clear indications of the presence of a PFAS background. However, even at the highest concentrations detected, all levels are much lower than the 1/3 MRL limit specified within EPA Method 537.1 indicating that the background is acceptable and will not interfere with future sample runs.

Initial Demonstration of Precision and Accuracy (IDP, IDA)

To determine the precision and accuracy of the sample preparation process, eight LFB samples were prepared at concentrations of 20 ppt. The data is shown in Table 7 and illustrated in Figures 6 and 7.

The results show that the average recovery for each target analyte was within 15% of the nominal value; falling well within the criteria of ± 30% stated within Method 537.1. Additionally, the relative standard deviation (RSD) for each analyte fell under 11% on average; again being much less than the 20% requirement set forth within the method.

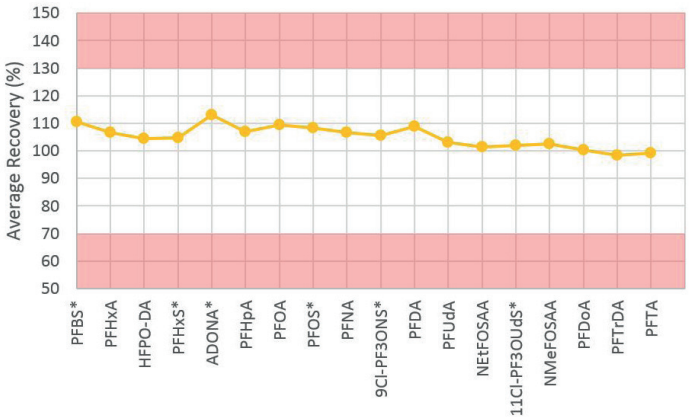

Figure 6. Initial Demonstration of Accuracy with range of acceptance shown in white (20 ng/L, n=8). Those compounds with an asterisk were used in salt form.

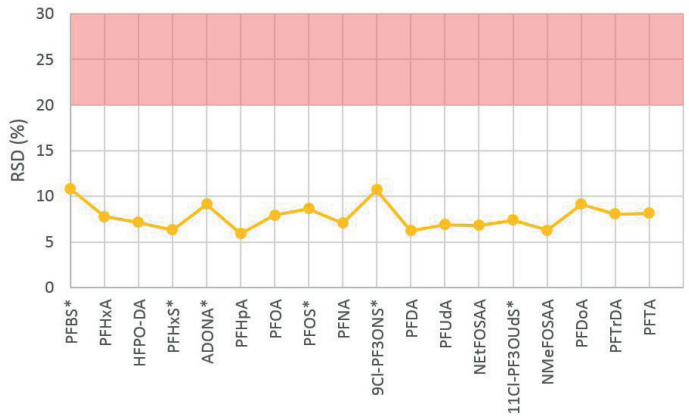

Figure 7. Initial Demonstration of Precision with range of acceptance shown in white (20 ng/L, n=8). Those compounds with an asterisk were used in salt form.

Table 7. Results of IDP and IDA (20 ng/L, n=8).

Replicate	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)	Average (%)	Std. Dev. (%)	RSD (%)
PFBS*	94.5	108.1	107.5	114.8	127.2	119.8	113.1	120.6	110.5	11.9	10.8
PFHxA	92.3	110.1	107.2	111.4	112.4	101.7	106.5	118.4	106.7	8.3	7.8
HFPO-DA	91.4	106.6	106.2	109.2	109.0	100.6	102.7	114.6	104.5	7.4	7.1
PFHxS*	94.3	107.3	104.0	105.9	112.2	108.0	105.4	110.2	104.7	6.6	6.3
ADONA*	95.5	118.2	112.6	119.4	120.1	103.3	111.9	115.9	113.2	10.3	9.1
PFHpA	96.4	107.0	108.3	109.2	113.3	97.0	109.7	117.5	106.9	6.3	5.9
PFOA	95.0	109.4	110.9	116.5	115.8	104.3	106.9	118.2	109.5	8.7	7.9
PFOS*	93.2	112.5	106.5	117.4	112.4	108.5	115.3	117.2	108.4	9.3	8.6
PFNA	93.9	110.5	106.1	110.2	112.6	97.0	106.5	115.6	106.7	7.5	7.0
9Cl-PF3ONS*	88.5	103.3	104.5	118.1	113.1	102.0	106.7	112.6	105.5	11.3	10.7
PFDA	98.0	107.6	110.0	113.6	115.0	99.7	108.1	109.4	108.8	6.7	6.2
PFUdA	90.9	105.6	103.3	105.9	109.3	93.3	98.5	113.7	103.0	7.1	6.9
NEtFOSAA	90.3	101.9	101.1	108.9	104.7	103.6	111.1	110.9	101.4	6.9	6.8
11Cl-PF3OUdS*	90.2	102.4	100.9	107.8	109.1	99.4	108.4	109.9	102.1	7.5	7.3
NMeFOSAA	92.0	102.0	103.3	108.0	107.3	106.5	107.5	112.7	102.5	6.4	6.2
PFDoA	86.4	103.2	96.6	109.7	106.1	90.0	98.2	109.0	100.4	9.2	9.1
PFTrDA	84.4	100.5	100.4	103.1	103.2	94.0	98.5	110.6	98.3	7.9	8.0
PFTA	86.0	99.2	99.9	103.2	107.4	90.9	97.2	104.5	99.1	8.0	8.1

*Analytes were used in salt form and calculated concentrations were corrected to compensate where needed.

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Examination of System Carryover

To simulate an influent sample, eight LFB samples were created with concentrations which were much higher that the calibration curve. These samples were split into two groups of four. The first group of four samples were extracted and, immediately following them, a LRB was extracted. For the second group, again four samples were extracted followed by a set of four LRB samples however, between the two extractions the cleaning procedure given in Appendix A was run three times. The sets of LFB and LRB samples were analyzed and the data for the LRB runs is given in Table 8 and illustrated in Figure 8.

The graph shown in Figure 8 indicates that without a cleaning procedure between influent and effluent sample extractions, carryover should be expected for some of the PFAS compounds contained within the test. Additionally, while the cleaning procedure did reduce the background to a point below the 1/3 MRL limit on average for all compounds, there is one instance where the compound NEtFOSAA was found to be above the limit. This indicates that further cleaning should be done in the future for influent samples of this concentration.

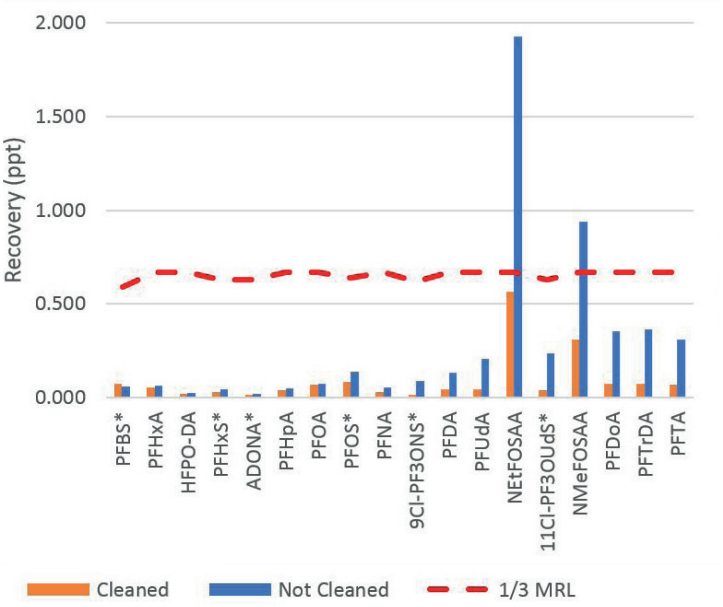

Figure 8. Results of carryover study following eight, 400 ng/L LFB samples; Four LRB samples extracted without cleaning procedure and four with the cleaning procedure in Appendix A. Those compounds with an asterisk were used in salt form.

Table 8. Results of carryover study following eight, 400 ng/L LFB samples; Four LRB samples extracted without cleaning procedure and four with the cleaning procedure in Appendix A.

Replicate	Not Cleaned					Cleaned				
	1 (ppt)	2 (ppt)	3 (ppt)	4 (ppt)	Average (ppt)	1 (ppt)	2 (ppt)	3 (ppt)	4 (ppt)	Average (ppt)
PFBS*	0.038	0.079	0.033	0.082	0.058	0.061	0.133	0.033	0.064	0.073
PFHxA	0.061	0.071	0.058	0.052	0.061	0.062	0.042	0.058	0.051	0.053
HFPO-DA	0.018	0.039	0.022	0.010	0.022	0.007	0.012	0.027	0.019	0.016
PFHxS*	0.033	0.048	0.059	0.043	0.046	0.021	0.029	0.030	0.029	0.027
ADONA*	0.023	0.020	0.020	0.010	0.018	0.011	0.011	0.014	0.011	0.012
PFHpA	0.047	0.047	0.033	0.063	0.048	0.023	0.054	0.035	0.034	0.037
PFOA	0.064	0.091	0.067	0.065	0.071	0.061	0.072	0.074	0.064	0.068
PFOS*	0.178	0.124	0.085	0.161	0.137	0.088	0.071	0.082	0.085	0.081
PFNA	0.063	0.070	0.029	0.061	0.056	0.018	0.027	0.036	0.025	0.027
9Cl-PF3ONS*	0.158	0.062	0.020	0.112	0.088	0.023	0.011	0.016	0.015	0.016
PFDA	0.199	0.096	0.050	0.175	0.130	0.058	0.032	0.040	0.045	0.044
PFUdA	0.364	0.168	0.068	0.230	0.208	0.037	0.054	0.047	0.037	0.044
NEtFOSAA	2.949	1.997	1.216	1.544	1.926	0.410	0.740	0.563	0.556	0.567
11Cl-PF3OUdS*	0.455	0.207	0.061	0.223	0.236	0.030	0.068	0.033	0.030	0.040
NMeFOSAA	1.544	0.996	0.518	0.704	0.940	0.298	0.384	0.253	0.310	0.311
PFDoA	0.612	0.327	0.161	0.321	0.355	0.050	0.097	0.080	0.068	0.074
PFTrDA	0.625	0.335	0.172	0.331	0.366	0.053	0.087	0.080	0.067	0.072
PFTA	0.506	0.280	0.158	0.291	0.309	0.051	0.074	0.086	0.068	0.070

*Analytes were used in salt form and calculated concentrations were corrected to compensate where needed.

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Conclusion

With the scrutiny being given to the presence of PFAS compounds in the environment, it is essential to find reliable products which can meet the requirements of EPA Method 537.1. This application note has shown that the Biotage® VacMaster™ vacuum manifold with accessories, ISOLUTE® 101 SPE columns and the TurboVap® LV can be used to easily meet and exceed the demands of the method.

Ordering Information

Part Number	Description	Quantity
121-2016	Biotage® VacMaster™-20 Sample Processing Station (with 16 mm rack)	1
121-2190	Biotage® VacMaster™ LVE Kit (PFAS) for 1, 3, 6 mL SPE Column	1
121-0009-PP	Biotage® VacMaster™ PP (PFAS) Stopcock	10
120-1111	ISOLUTE® Column Adapters (PFAS) 1, 3, 6 mL Columns	10
101-0050-C	ISOLUTE® 101 SPE Columns, 500 mg/6 mL	30
415000	TurboVap® LV Automated Solvent Evaporation System	1
414964	TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes)	1

Appendix A

Biotage® VacMaster™ Cleaning Procedure

For the best results, it is recommended that this procedure be completed before the use of the Biotage® VacMaster™ each day and at the end of each extraction prior to proceeding with the next set of samples.

1. Ensure that a column and column adapter is installed onto each VacMaster™ position slated to be cleaned.
2. Fill a clean beaker with 50 mL of methanol and place no more than four of the LVE Kit lines into the beaker.
3. Apply vacuum to the manifold and pull the methanol through the positions into the waste container.
4. Remove the column and discard.
5. Using methanol in a squeeze bottle, clean the exterior of the LVE Kit's lines, the column adapters, the stopcock, and the metal cannula. Discard all rinsate.
6. Repeat this up to three times for all positions which require cleaning.

Note: In situations where the previous sample was highly concentrated, the above cleaning procedure may need to be repeated multiple times. If there is concern regarding potential carryover contamination regardless of the cleaning procedure, a laboratory reagent blank should be run in that position to ensure its cleanliness.

Appendix B

MRM Transitions

	Q1 Mass (Da)	Q3 Mass (Da)	Time (Min)	ID	DP (Volts)	CE (Volts)	CXP (Volts)
1	314.900	269.900	1.14	MPFHxA	-40.000	-14.000	-11.000
2	314.900	119.000	1.14	MPFHxA	-40.000	-26.000	-7.000
3	514.900	429.900	2.33	MPFDA	-60.000	-16.000	-19.000
4	514.900	219.500	2.33	MPFDA	-60.000	-26.000	-11.000
5	588.900	419.000	2.64	d5-N-EtFOSAA	-165.000	-28.000	-17.000
6	588.900	531.000	2.64	d5-N-EtFOSAA	-165.000	-28.000	-17.000
7	256.900	168.900	1.19	M3HFPO-DA	-90.000	-10.000	-9.000
8	256.000	184.900	1.19	M3HFPO-DA	-90.000	-24.000	-11.000
9	414.900	370.000	1.64	IS-M2PFOA	-55.000	-14.000	-5.000
10	414.900	169.500	1.64	IS-M2PFOA	-55.000	-26.000	-9.000
11	572.900	419.000	2.48	IS-d3-N-MeFOSA	-120.000	-28.000	-7.000
12	572.900	483.000	2.48	IS-d3-N-MeFOSA	-120.000	-22.000	-7.000
13	502.300	80.000	1.98	IS-MPFOS	-90.000	-110.000	-11.000
14	502.300	98.800	1.98	IS-MPFOS	-90.000	-98.000	-9.000
15	312.900	268.900	1.13	PFHxA	-45.000	-14.000	-11.000
16	312.900	118.900	1.13	PFHxA	-45.000	-28.000	-7.000
17	362.900	318.900	1.35	PFHpA	-55.000	-14.000	-7.000
18	362.900	168.900	1.35	PFHpA	-55.000	-24.000	-9.000
19	412.900	369.000	1.64	PFOA	-45.000	-16.000	-5.000
20	412.900	168.900	1.64	PFOA	-45.000	-24.000	-9.000
21	462.900	419.000	1.98	PFNA	-55.000	-16.000	-9.000
22	462.900	218.900	1.98	PFNA	-55.000	-24.000	-3.000
23	512.900	469.000	2.33	PFDA	-55.000	-16.000	-9.000
24	512.900	218.900	2.33	PFDA	-55.000	-26.000	-7.000
25	562.900	519.000	2.65	PFUdA	-65.000	-18.000	-9.000
26	562.900	268.900	2.65	PFUdA	-65.000	-26.000	-7.000
27	612.900	569.000	2.95	PFDoA	-70.000	-18.000	-11.000
28	612.900	318.900	2.95	PFDoA	-70.000	-28.000	-5.000
29	662.800	619.000	3.20	PFTrDA	-65.000	-20.000	-7.000
30	662.800	169.000	3.20	PFTrDA	-65.000	-36.000	-7.000
31	712.800	669.000	3.43	PFTeDA	-75.000	-20.000	-7.000
32	712.800	169.000	3.43	PFTeDA	-75.000	-36.000	-14.000
33	569.900	419.000	2.47	N-MeFOSAA	-90.000	-28.000	-7.000
34	569.900	483.000	2.47	N-MeFOSAA	-90.000	-22.000	-17.000
35	583.900	419.000	2.63	N-EtFOSAA	-90.000	-28.000	-7.000
36	583.900	526.000	2.63	N-EtFOSAA	-90.000	-28.000	-7.000
37	284.900	168.900	1.18	HFPO-DA	-80.000	-10.000	-7.000
38	284.900	184.900	1.18	HFPO-DA	-80.000	-22.000	-9.000
39	298.300	79.900	1.00	L-PFBS	-75.000	-62.000	-11.000
40	298.300	98.900	1.00	L-PFBS	-75.000	-36.000	-5.000
41	398.400	80.000	1.36	L-PFHxS	-110.000	-86.000	-11.000
42	398.400	99.000	1.36	L-PFHxS	-110.000	-42.000	-5.000
43	498.600	79.900	1.98	L-PFOS	-255.000	-110.000	-11.000
44	498.800	99.000	1.98	L-PFOS	-255.000	-94.000	-5.000
45	376.900	250.900	1.38	ADONA	-45.000	-16.000	-9.000
46	376.900	64.900	1.38	ADONA	-45.000	-36.000	-7.000
47	530.800	350.900	2.16	9Cl-PF3ONS	-90.000	-36.000	-7.000
48	530.800	83.000	2.16	9Cl-PF3ONS	-90.000	-68.000	-7.000
49	630.800	450.900	2.79	11Cl-PF3OUdS	-125.000	-42.000	-13.000
50	630.800	82.900	2.79	11Cl-PF3OUdS	-125.000	-88.000	-13.000

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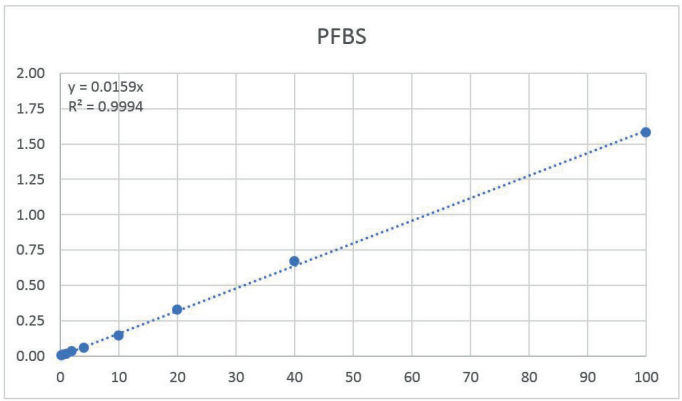
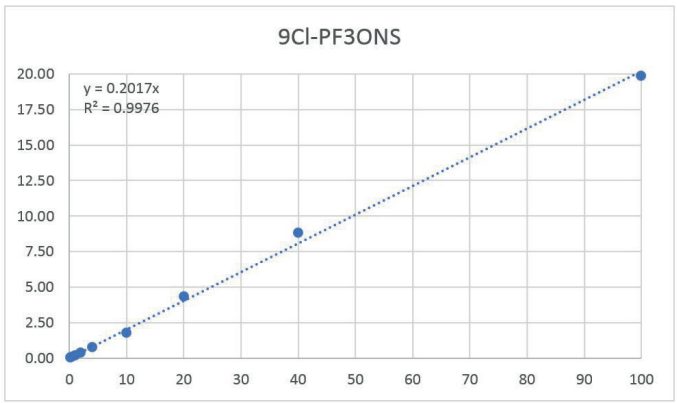
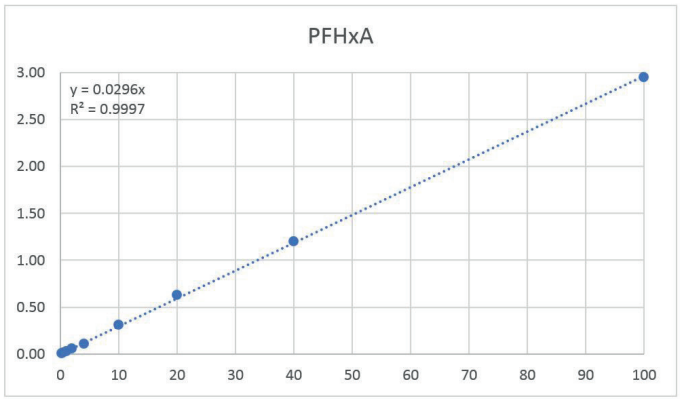
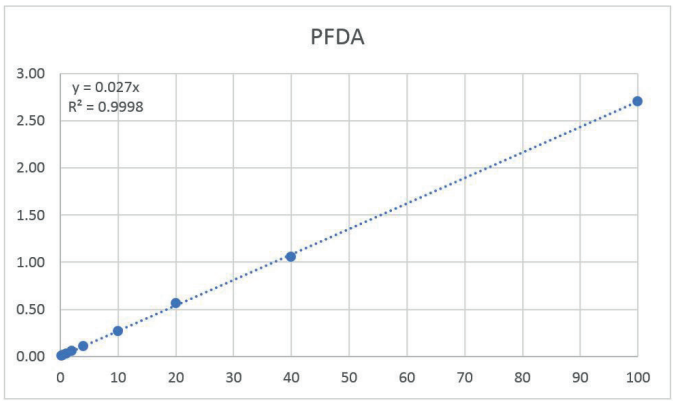
Appendix C

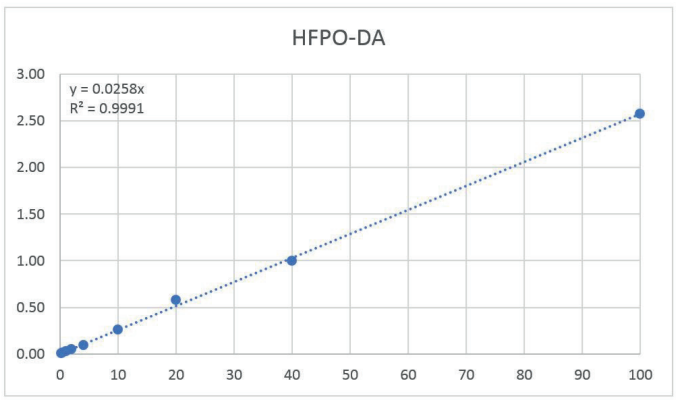
Calibration Curves

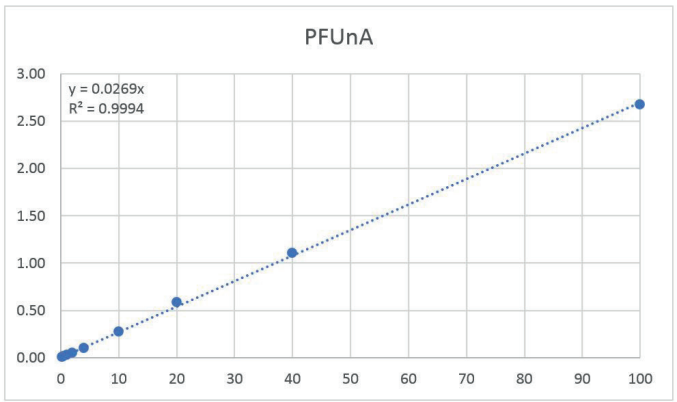


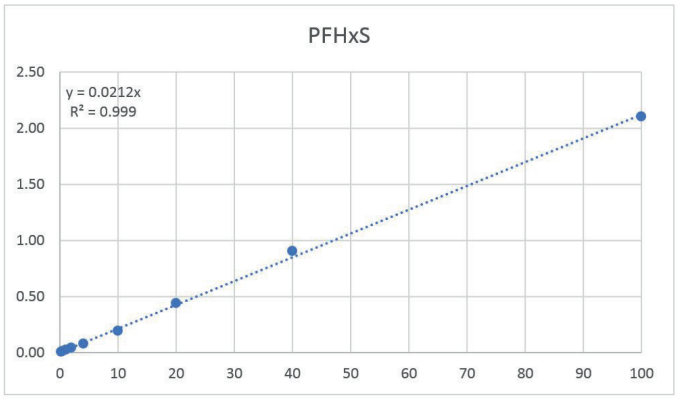


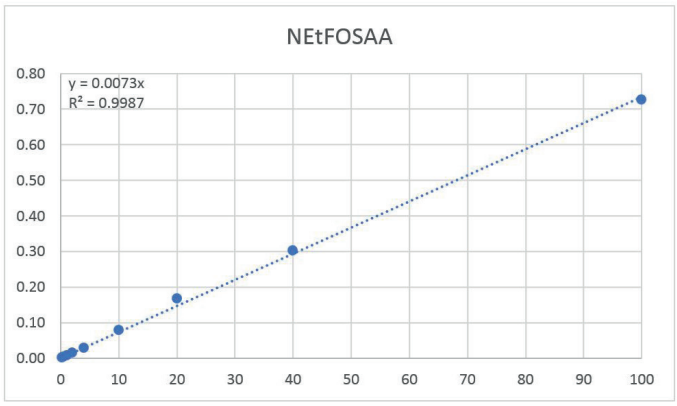


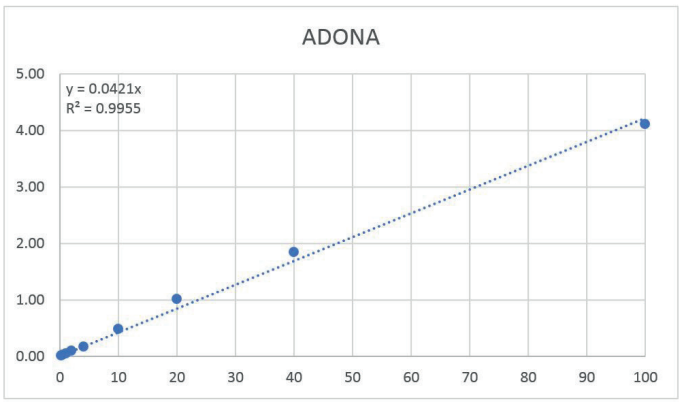


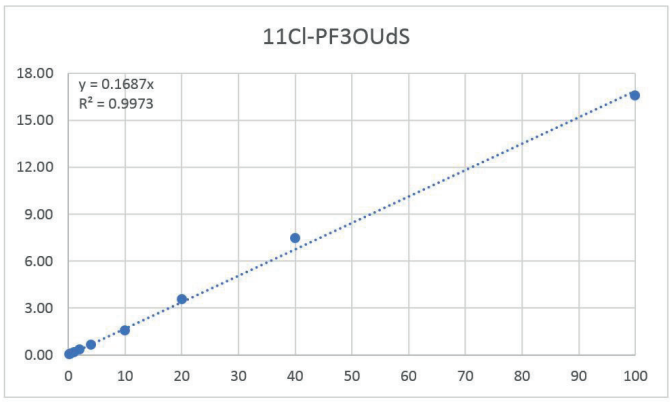


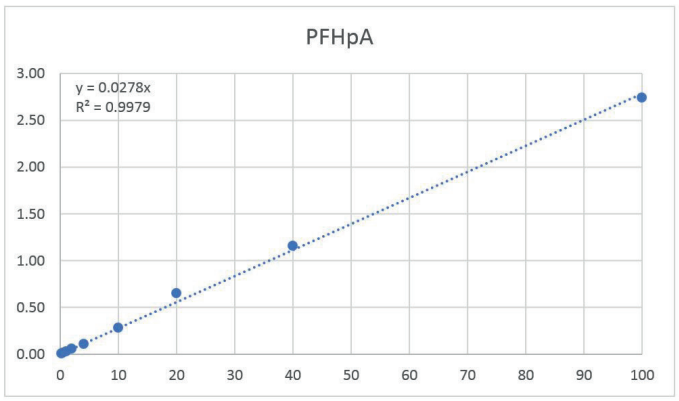


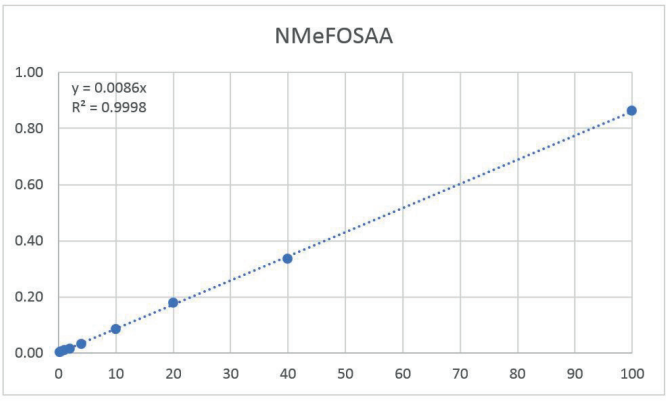












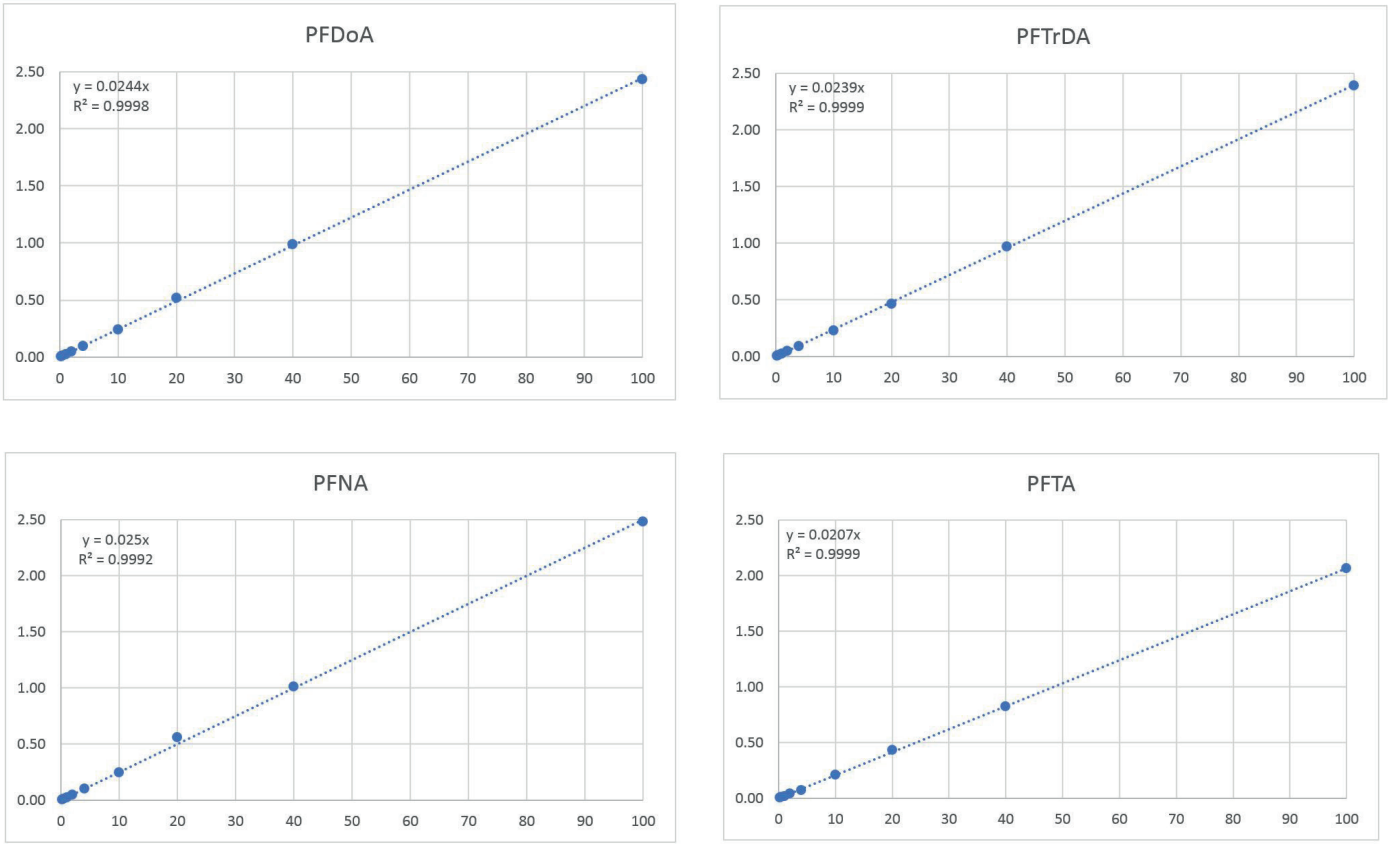


Figure 9. Calibration curves for the target analytes in Table 1, covering a concentration range of 0.2-100 ppt.

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