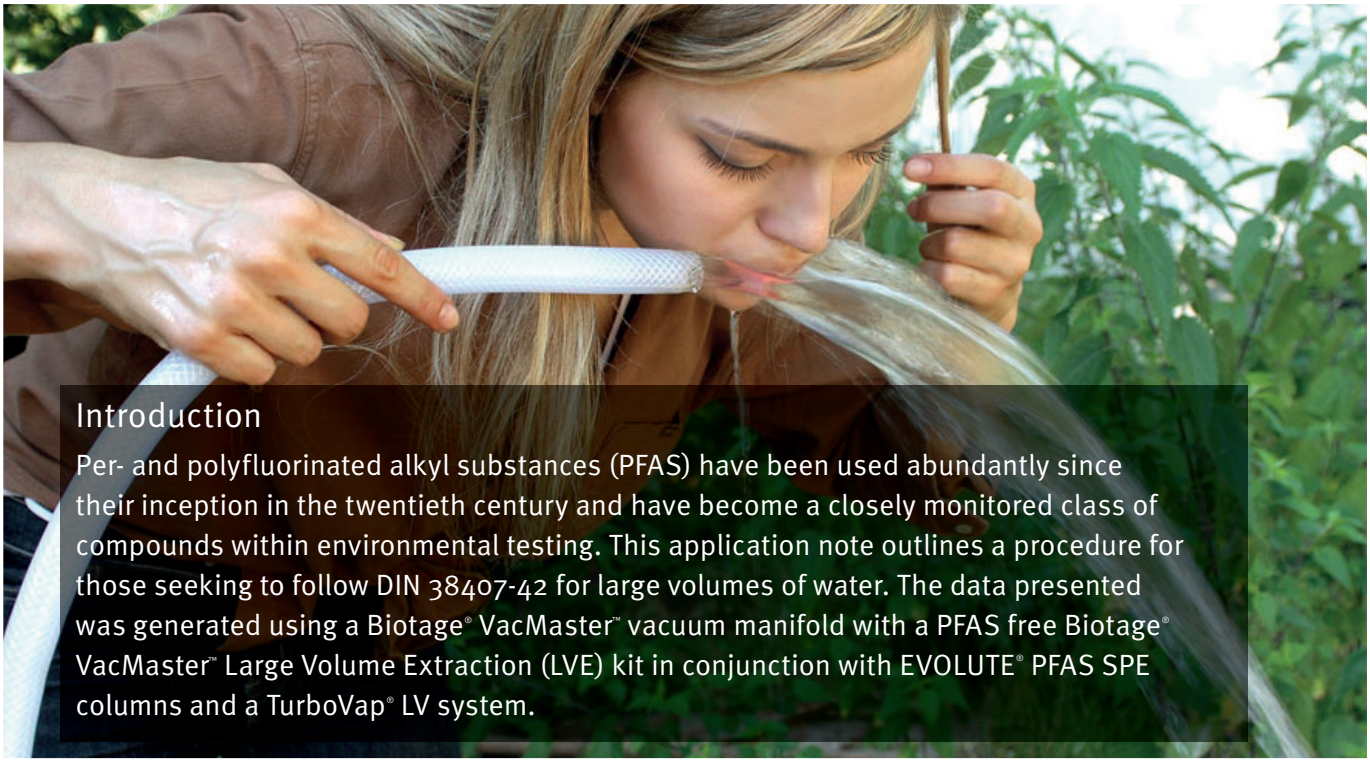
Manual Extraction of PFAS in Drinking Water Following DIN 38407-42

Authors

William Jones, Biotage, Salem, NH, USA
Matthew Harden, Biotage, Salem, NH, USA



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 follow DIN 38407-42 for large volumes of water. The data presented was generated using a Biotage® VacMaster™ vacuum manifold with a PFAS free Biotage® VacMaster™ Large Volume Extraction (LVE) kit in conjunction with EVOLUTE® PFAS SPE columns and a TurboVap® LV system.

Equipment and Materials Used

Biotage

- Biotage® VacMaster™ 20 Sample Processing Station (with 15 mL rack), p/n 121-2015ML, fitted with polypropylene (PFAS free) stopcocks (p/n 121-0009-PP)
- Biotage® VacMaster™ LVE Kit (PFAS) for 1, 3, 6 mL SPE Columns (p/n 121-2190)
- EVOLUTE® PFAS 500 mg/6 mL SPE Columns, p/n 614-0050-CP
- EVOLUTE® PFAS 150 mg/6 mL SPE Columns, p/n 614-0015-CP
- TurboVap® LV Automated Solvent Evaporation System, p/n 415000
- TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes), p/n 414964

Wellington Laboratories

- ISO 21675:2019 Labelled Stock Solution, 1.2 mL, p/n ISO 21675-LSS
- ISO 21675:2019 Native Stock Solution, 1.2 mL, p/n ISO 21675-NSS

Agilent

- InfinityLab PFC Delay Column, 4.6 x 30 mm, p/n 5062-8100
- ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 x 50 mm, 1.8 µm, p/n 959757-902

Sigma-Aldrich

- Ammonium Acetate, ACS Reagent Grade ≥ 97%, p/n 238074-25G
- Acetic Acid, Glacial, ReagentPlus®, ≥ 99% p/n A6283

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

Analytes

Table 1. Listing of Target Analytes and Internal Standards.

Target Analyte	Acronym	CAS
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluoroundecanoic acid[1]	PFUnA	2058-94-8
Perfluorododecanoic acid[1]	PFDoA	307-55-1
Perfluoroheptanesulfonic acid[1]	PFHpS	375-92-8
Perfluorodecanesulfonic acid[1]	PFDS	335-77-3
1H,1H,2H,2H-perfluorooctanesulfonic acid[1]	H4PFOS	27619-97-2
Internal Standard		
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA	
Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	$^{13}C_3$-PFHxS	
Sodium perfluoro-1-[1,2,3-$^{18}O_2$]hexanesulfonate	$^{18}O_2$-PFHxS	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	
Perfluoro-[1,2-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA	
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	^{13}C4-PFOS	
Perfluoro-n-[$^{13}C_5$]nonanoic acid	$^{13}C_5$-PFNA	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA	

[1]Target compounds are from the expanded list given in DIN 38407-42 Section 2.1.

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Solution Preparation

Ammonia/Methanol Solution

1. Add 400 µL of NH_4OH for every 100 mL of methanol to a clean beaker.
2. Agitate to homogenize.
3. Prepare new solution daily.

Acetate Buffer

1. Measure out 499.5 mL of reagent water in a clean beaker.
2. Add 0.193 g of NH_4Ac.
3. Sonicate the solution for 5 minutes until the salt is fully dissolved.
4. Add 570 µL of glacial acetic acid.
5. Agitate to homogenize the solution.

Working Spiking Solution

1. Dilute 100 µL of the native stock solution with 900 µL of methanol to achieve a 10 ppt solution.

Summary of SPE Method

SPE Column Format

EVOLUTE® PFAS 500 mg/6 mL or EVOLUTE® PFAS 150 mg/6 mL

Sample Pre-Treatment

Adjust the pH of each sample to 3 using glacial acetic acid. Add targets and internal standards.

Conditioning

Condition each column with 0.1 % NH_4OH in methanol (10 mL) followed by methanol (10 mL).

Equilibration

Equilibrate each column with reagent water (10 mL).

Sample Loading

Load sample at a flow rate of 5 mL/min.

Wash

Rinse the sample container with acetate buffer solution (10 mL) and load onto the column. Repeat using reagent water (10 mL).

Dry

Dry the column for 5 minutes at a flow rate of 5 mL/min.

Elution

Rinse the sample container with methanol (5 mL) and use to elute the analytes from the column at a flow rate of 2 mL/min. Repeat using 0.1 % NH_4OH in methanol (5 mL).

Post Extraction

Concentrate the extract to a volume of 1 mL. Add IS and mix prior to analysis.

Note: EVOLUTE® PFAS 60 mg/3 mL columns may be used however sample and solvent volumes should be adjusted. Refer to DIN 38407-42 for the appropriate amounts.

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Sample Preparation Procedure

1. Clean all parts of the Biotage® VacMaster™ system per the procedure given in Appendix A.
2. Set up and fill new sample containers with water; 250–500 mL are typical for this method.
3. Add glacial acetic acid to each of the sample containers to reduce the pH to 3 (approximately 100 µL for 250 mL sample volumes and 200 µL for 500 mL sample volumes).
4. Verify the pH of the sample is 3 using pH paper. To reduce the possibility of contamination, a duplicate volume was collected and adjusted to the appropriate pH and the same volume of acid was added to the sample container.
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 20 µL of the undiluted Labeled Stock Solution to each of the sample containers. If desired, fortify a sample using target analytes: the addition of 125 µL or 37.5 µL of the native stock solution will yield either 50 ppt or 15 ppt concentrations respectively, while the addition of 50 µL of the working spiking solution will yield a 2 ppt concentration. If the mixes used were different than the ones outlined in this note, adjust the concentration or spiking amounts as needed.
7. Load the desired EVOLUTE® PFAS columns onto the Biotage® VacMaster™. Seal any unused positions using VacMaster Port Sealing Plugs (p/n 121-0005)
8. Rinse each column with 10 mL of 0.1 % NH_4OH in methanol and apply vacuum at 10 mL/min to pull it to waste. Do not allow the sorbent to go dry.
9. Rinse each column with 10 mL of methanol and apply vacuum at 10 mL/min to pull it to waste. Do not allow the sorbent to go dry.
10. Rinse each column with 10 mL of reagent water and apply vacuum at 10 mL/min to send it to waste. Do not allow the water level to drop below the top of the packing.
11. Using the Biotage® 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.
12. Load the samples onto the columns using a flow rate of 5 mL/min.
13. Once the sample has been fully loaded, rinse the sample containers using 10 mL of acetate buffer solution, swirl to ensure the full rinsing of the container, and load the aliquot onto the column at a rate of 5 mL/min.
14. Rinse the sample containers using 10 mL of reagent water, swirl to ensure the full rinsing of the container, and load the aliquot onto the column at a rate of 5 mL/min.
15. Dry the column for 5 minutes at a rate of 5 mL/min.
16. Load 15 mL centrifuge tubes into the rack corresponding to each of the column positions and load into the VacMaster.
17. Rinse each sample container using 5 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.
18. Rinse each sample container using 5 mL of 0.1% NH_4OH in methanol and swirl to ensure the full rinsing of the container. Load the aliquot through the appropriate column and collect at a dropwise rate.
19. 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.
20. Transfer the centrifuge tubes to the TurboVap® LV system and concentrate the samples to just under 1 mL using nitrogen according to the parameters in Table 2.
21. Bring the final extract to 1 mL and transfer to an autosampler vial.
22. 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:	1.5 L/min for 20 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.

LC-MS/MS Conditions

Agilent 1290 Infinity II LC System

- 1290 Infinity II Multicolumn Thermostat, G7116B
- 1290 Infinity II Multisampler, G7167B
- 1290 Infinity II High Speed Pump, G7120A
- InfinityLab PFC-free HPLC Conversion Kit, 5004-0006

Columns

- InfinityLab PFC Delay Column, 4.6 x 30 mm, p/n 5062-8100
- ZORBAX RRHD Eclipse Plus C18, 95 Å, 2.1 x 50 mm, 1.8 µm, p/n 959757-902

Mobile Phases

- A: 20 mM Ammonium Acetate in Water
- B: Methanol

Table 3. LC Gradient.

Time (min)	%A	%B
0.50	95.00	5.00
3.00	60.00	40.00
16.00	20.00	80.00
18.00	20.00	80.00
20.00	5.00	95.00
20.50	0.00	100.00
25.00	0.00	100.00
26.00	5.00	95.00

- Flow Rate: 0.2 mL/min
- Injection Volume: 5 µL
- Column Temperature: 50 ˚C

Agilent 6470 MS/MS, G6470B

- Gas Temperature: 230 ˚C
- Gas Flow: 4 L/min
- Nebulizer: 20 psi
- Sheath Gas Temperature: 375 ˚C
- Sheath Gas Flow: 12 L/min
- Capillary Voltage (Positive): 3500 V
- Capillary Voltage (Negative): 3500 V
- Nozzle Voltage (Positive): 500 V
- Nozzle Voltage (Negative): 0 V

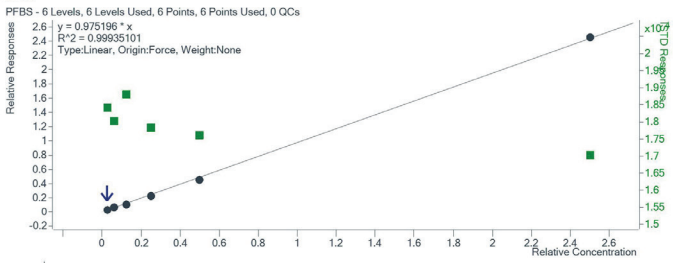
For a complete listing of MRM Transitions, see Appendix B

Results

System Calibration

For the work being done here, a total of six points were used in the calibration covering a range of 0.2-20 ppt. The lowest three points were below the calculated MRL. The curve was forced through zero and achieved excellent linearity across the calibration range.

PFBS



PFHxS

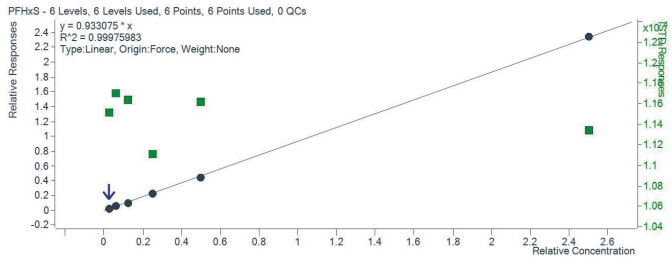


Figure 1. Calibration curves for PFBS and PFHxS. Calibration curves for the remaining target analytes in Table 1 are shown in Appendix C.

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

A target MRL of 2 ng/L was selected and at least seven replicate laboratory fortified blanks (LFBs) were created and run at that concentration. Figure 2 below illustrates the results of this test for both the 150 mg and 500 mg columns using 250 mL sample volumes; all compounds were recovered within 15% of the spiked amount and had less than 10% C_V.

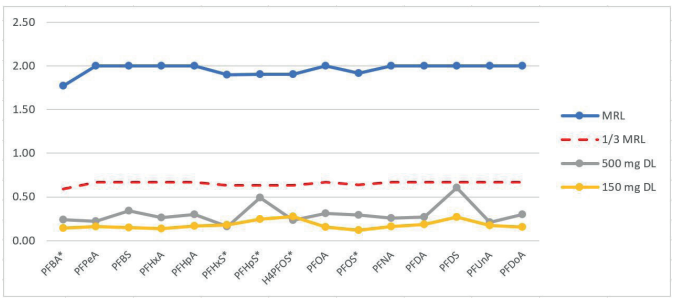


Figure 2. MRL and DL Recoveries. Those compounds with an asterisk were used in salt form.

The data for individual compounds is shown in Appendix D.

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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 methanol 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 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, and run it on the analytical system. By separating the process into three 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 given in Appendix E and selected data are shown below in Figures 3–5.

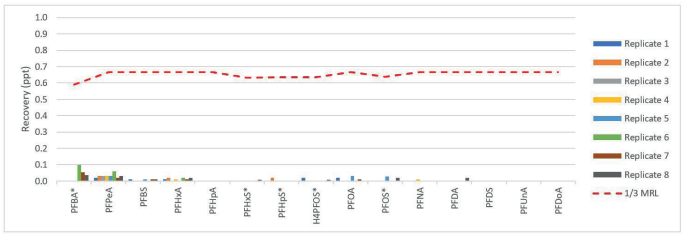

Figure 3. Contribution of the TurboVap® LV to the PFAS Background. Those compounds with an asterisk were used in salt form.

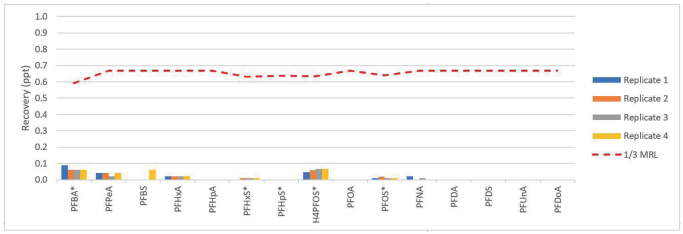

Figure 4. PFAS Background for full LRB using EVOLUTE® PFAS 500 mg/6 mL columns. Those compounds with an asterisk were used in salt form.

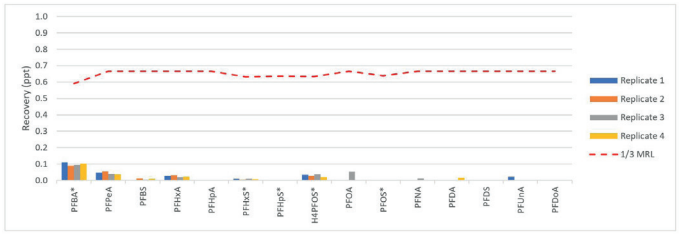

Figure 5. PFAS Background for full LRB using EVOLUTE® PFAS 150 mg/6 mL columns. Those compounds with an asterisk were used in salt form.

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 tests (which includes the Biotage® VacMaster™ manifold, PFAS Free Large Volume Loading Kit, and the EVOLUTE® 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 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, four LFB samples were prepared at concentrations of 15 ppt. The data is given in Appendix F and illustrated in Figures 6 and 7.

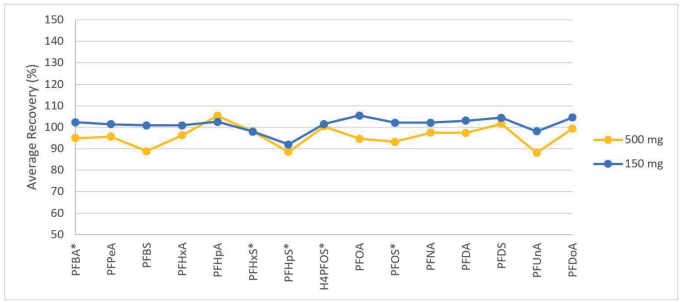

Figure 6. Initial Demonstration of Accuracy (15 ng/L, n=4). Those compounds with an asterisk were used in salt form.

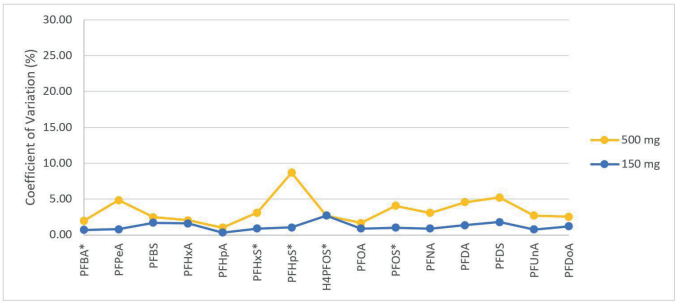

Figure 7. Initial Demonstration of Precision (15 ng/L, n=4). Those compounds with an asterisk were used in salt form.

The results show that the average recovery for each target analyte was within 15% of the nominal value and that the coefficient of variation (C_V) for each analyte fell under 10% on average.

Examination of System Carryover

To simulate an influent sample, four LFB samples were created with concentrations which were above the range of the calibration curve. These samples were extracted, and the cleanup procedure given in Appendix A was run three times. To ensure that the system background was adequately reduced, a set of four LRB samples were extracted immediately afterwards and analyzed. The LRB data is presented in Appendix G and illustrated in Figure 8.

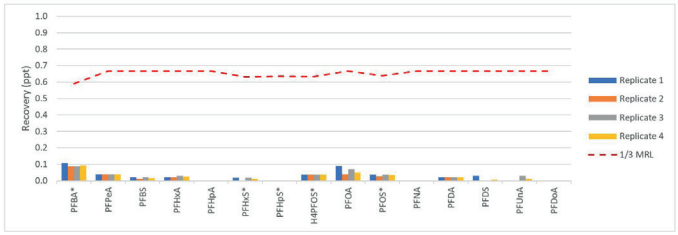

Figure 8. Results of carryover study following four, 50 ng/L LFB samples using EVOLUTE® PFAS 500 mg/6 mL columns. Those compounds with an asterisk were used in salt form.

The graph shown in Figure 8 shows a clear indication that the cleaning procedure in Appendix A was successful in reducing the background of PFAS compounds to below the 1/3 MRL limit. For further reductions in the background, additional cleaning steps could be employed.

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 DIN 38407-42. This application note has shown that the VacMaster™ vacuum manifold with PFAS free accessories, EVOLUTE® PFAS SPE columns and the TurboVap® LV can be used to easily meet and exceed the demands of the method.

Ordering Information

Part Number	Description	Qty
121-2015ML	Biotage® VacMaster™ 20 Sample Processing Station With 15 mm Rack	1
121-2190	Biotage® VacMaster™ LVE Kit (PFAS) for 1, 3, 6 mL SPE Columns	1
121-0009-PP	Polypropylene (PFAS) Stopcocks	10
614-0050-CP	EVOLUTE® PFAS 500 mg/6 mL columns	30
614-0015-CP	EVOLUTE® PFAS 150 mg/6 mL columns	30
614-0006-BP	EVOLUTE® PFAS 60 mg/3 mL columns	50
415000	TurboVap® LV Automated Solvent Evaporation System	1
414964	TurboVap® LV Multi Rack (48 Positions, 10–20 mm Tubes)	1

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Appendix A

Biotage® VacMaster™ Cleaning Procedure

For the best results, it is recommended that this procedure be completed before the use of the 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

Table 4. MRM Transitions for Agilent 6470 MS/MS.

Cpd Name	ISTD?	Prec Ion	MS1 Res	Prod Ion	MS2 Res	Frag (V)	CE (V)	Cell Acc (V)	Ret Time (min)	Ret Window	Polarity
H4PFOS	No	427	Unit/Enh (6490)	406.8	Unit/Enh (6490)	125	24	5	13.3	1.37	Negative
H4PFOS	No	427	Unit/Enh (6490)	80.9	Unit/Enh (6490)	125	40	5	13.3	1.37	Negative
C3-PFHxS	No	402	Unit/Enh (6490)	80	Unit/Enh (6490)	100	49	5	11.7	1.5	Negative
C4-PFBA	No	217	Unit/Enh (6490)	172	Unit/Enh (6490)	60	8	5	5.1	1.45	Negative
C4-PFHpA	No	367	Unit/Enh (6490)	322	Unit/Enh (6490)	72	0	5	11.6	1.2	Negative
C5-PFHxA	No	318	Unit/Enh (6490)	273	Unit/Enh (6490)	70	8	5	9.7	1.14	Negative
C5-PFPeA	No	268	Unit/Enh (6490)	223	Unit/Enh (6490)	60	20	5	7.4	1.55	Negative
C6-PFDA	No	519	Unit/Enh (6490)	474	Unit/Enh (6490)	81	4	5	16.2	1.65	Negative
C8-PFOA	No	421	Unit/Enh (6490)	376	Unit/Enh (6490)	80	8	5	13.4	1.38	Negative
C8-PFOS	No	507	Unit/Enh (6490)	80	Unit/Enh (6490)	100	50	5	15	1.53	Negative
C9-PFNA	No	472	Unit/Enh (6490)	427	Unit/Enh (6490)	66	4	5	14.9	1.52	Negative
PFBA	No	213	Unit/Enh (6490)	168.9	Unit/Enh (6490)	60	8	5	5.1	1.48	Negative
PFBS	No	298.9	Unit/Enh (6490)	98.9	Unit/Enh (6490)	100	29	5	7.9	1.41	Negative
PFBS	No	298.9	Unit/Enh (6490)	80	Unit/Enh (6490)	100	45	5	7.9	1.41	Negative
PFDA	No	513	Unit/Enh (6490)	469	Unit/Enh (6490)	81	4	5	16.2	1.65	Negative
PFDA	No	513	Unit/Enh (6490)	218.7	Unit/Enh (6490)	100	16	5	16.2	1.65	Negative
PFDoA	No	613	Unit/Enh (6490)	569	Unit/Enh (6490)	79	5	5	18.2	1.84	Negative
PFDoA	No	613	Unit/Enh (6490)	268.7	Unit/Enh (6490)	100	20	5	18.2	1.84	Negative
PFDS	No	599	Unit/Enh (6490)	99	Unit/Enh (6490)	100	40	5	17.15	1.75	Negative
PFDS	No	599	Unit/Enh (6490)	80	Unit/Enh (6490)	100	40	5	17.15	1.75	Negative
PFHpA	No	362.9	Unit/Enh (6490)	319	Unit/Enh (6490)	72	0	5	11.6	1.2	Negative
PFHpA	No	362.9	Unit/Enh (6490)	169	Unit/Enh (6490)	72	12	5	11.6	1.2	Negative
PFHpS	No	448.9	Unit/Enh (6490)	98.7	Unit/Enh (6490)	100	44	5	13.5	1.39	Negative
PFHpS	No	448.9	Unit/Enh (6490)	79.7	Unit/Enh (6490)	100	52	5	13.5	1.39	Negative
PFHxA	No	313	Unit/Enh (6490)	268.9	Unit/Enh (6490)	70	8	5	9.7	1.12	Negative
PFHxA	No	313	Unit/Enh (6490)	119	Unit/Enh (6490)	70	18	5	9.7	1.12	Negative
PFHxS	No	398.9	Unit/Enh (6490)	99	Unit/Enh (6490)	100	45	5	11.7	1.5	Negative
PFHxS	No	398.9	Unit/Enh (6490)	80	Unit/Enh (6490)	100	49	5	11.7	1.5	Negative
PFNA	No	463	Unit/Enh (6490)	419	Unit/Enh (6490)	66	4	5	14.9	1.52	Negative
PFNA	No	463	Unit/Enh (6490)	219	Unit/Enh (6490)	66	17	5	14.9	1.52	Negative
PFOA	No	413	Unit/Enh (6490)	369	Unit/Enh (6490)	69	4	5	13.4	1.38	Negative
PFOA	No	413	Unit/Enh (6490)	169	Unit/Enh (6490)	69	12	5	13.4	1.38	Negative
PFOS	No	498.9	Unit/Enh (6490)	99	Unit/Enh (6490)	100	50	5	15	1.53	Negative
PFOS	No	498.9	Unit/Enh (6490)	80	Unit/Enh (6490)	100	50	5	15	1.53	Negative
PFPeA	No	263	Unit/Enh (6490)	218.9	Unit/Enh (6490)	60	8	5	7.4	1.77	Negative
PFUnA	No	563	Unit/Enh (6490)	519	Unit/Enh (6490)	73	5	5	17.2	1.75	Negative
PFUnA	No	563	Unit/Enh (6490)	269	Unit/Enh (6490)	100	20	5	17.2	1.75	Negative

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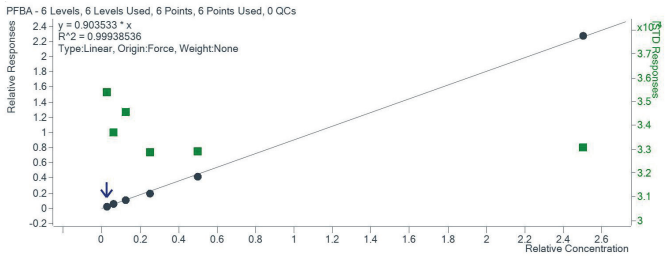
Appendix C

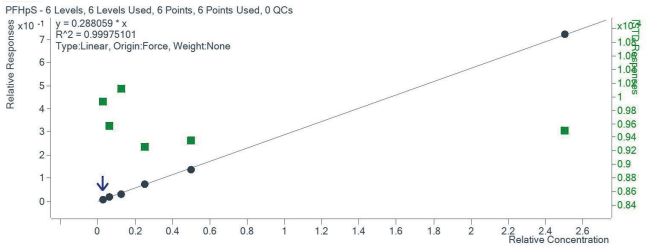
Calibration Curves

Figure 9. Calibration curves for the target analytes in Table 1, covering a concentration range of 0.2-20 ppt.

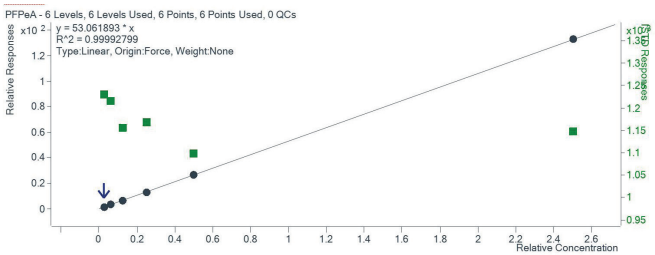
PFBA



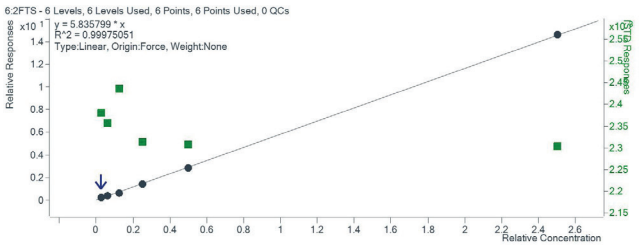
PFHpS



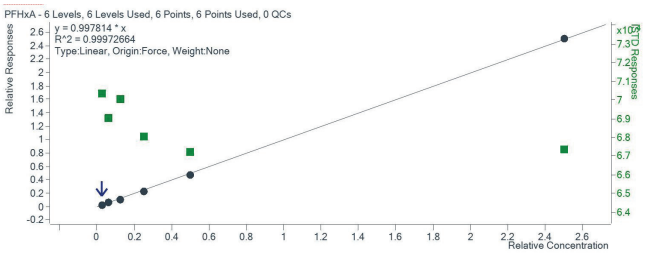
PFPeA



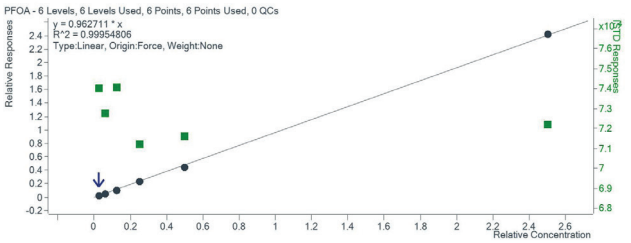
H4PFOS



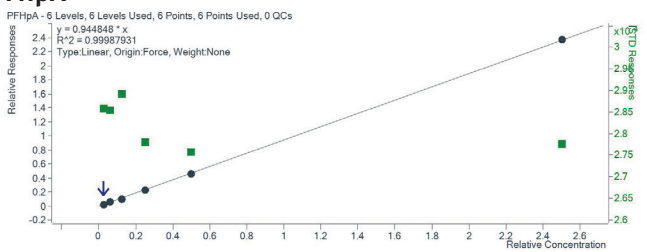
PFHxA



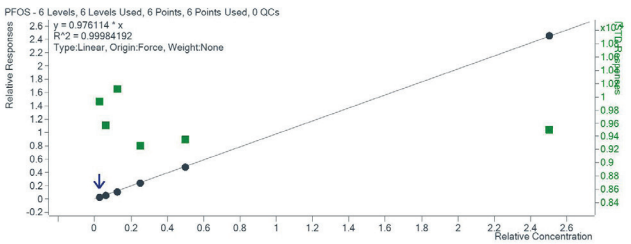
PFOA



PFHpA



PFOS



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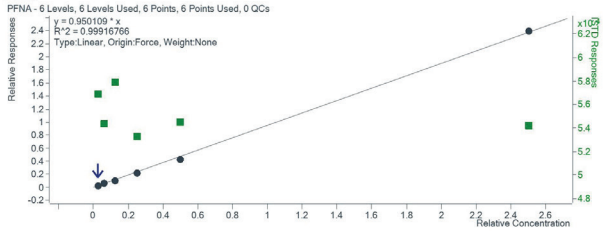
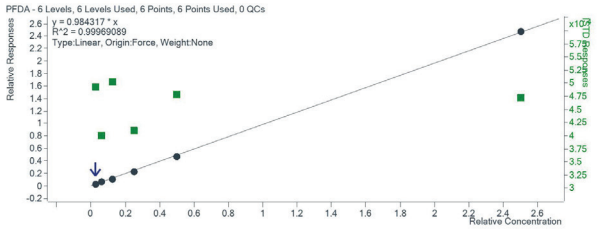
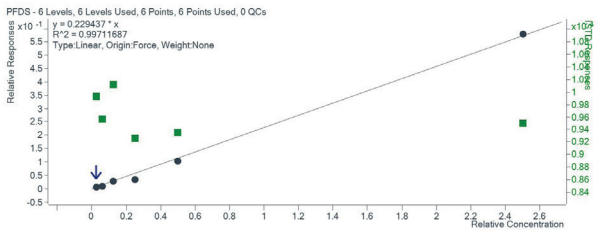
PFNA



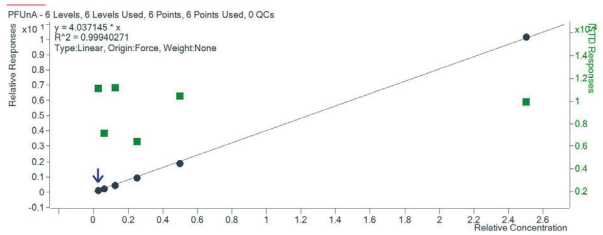
PFDA



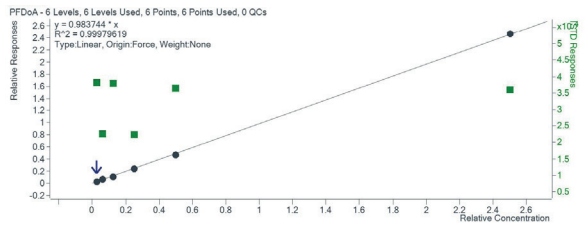
PFDS



PFUnA



PFDoA



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Appendix D

MRL and DL Data

Table 5. MRL and DL Recoveries for EVOLUTE® PFAS 500 mg/6 mL columns.

	Conc. (ng/L)	1 (ng/L)	2 (ng/L)	3 (ng/L)	4 (ng/L)	5 (ng/L)	6 (ng/L)	7 (ng/L)	$\bar{x}$ (ng/L)	$\bar{x}$ (%)	s (ng/L)	C_V (%)	DL (ng/L)
PFBA*	1.77	1.67	1.71	1.67	1.68	1.81	1.60	1.80	1.71	96	0.08	4.43	0.24
PFPeA	2.00	1.85	1.83	1.92	1.97	1.88	1.75	1.91	1.87	94	0.07	3.81	0.22
PFBS	2.00	1.73	1.90	1.82	1.70	1.73	1.65	1.95	1.78	89	0.11	6.12	0.34
PFHxA	2.00	1.80	1.92	1.89	1.80	1.98	1.75	1.92	1.87	93	0.08	4.50	0.26
PFHpA	2.00	1.96	2.06	2.04	1.94	2.01	1.80	2.08	1.99	99	0.09	4.75	0.30
PFHxS*	1.90	1.85	1.78	1.77	1.70	1.82	1.73	1.77	1.77	94	0.05	2.84	0.16
PFHpS*	1.91	1.90	1.90	1.86	1.65	1.92	1.51	1.73	1.78	93	0.16	8.79	0.49
H4PFOS*	1.90	1.80	1.87	1.88	1.86	1.87	1.68	1.86	1.83	96	0.07	4.02	0.23
PFOA	2.00	1.80	1.86	1.93	1.91	1.94	1.67	1.94	1.86	93	0.10	5.34	0.31
PFOS*	1.92	1.96	1.89	1.79	1.89	1.92	1.69	1.89	1.86	97	0.09	4.98	0.29
PFNA	2.00	1.88	1.92	1.95	1.90	1.94	1.79	2.07	1.92	96	0.08	4.30	0.26
PFDA	2.00	1.87	1.89	1.87	1.83	2.01	1.79	2.01	1.89	95	0.09	4.55	0.27
PFDS	2.00	2.10	2.06	1.95	2.14	1.70	1.64	1.93	1.93	97	0.19	9.98	0.61
PFUnA	2.00	1.89	1.91	1.90	1.81	1.88	1.75	1.94	1.87	93	0.07	3.51	0.21
PFDoA	2.00	1.85	1.96	1.96	1.81	2.00	1.75	1.96	1.90	95	0.10	5.04	0.30

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

Table 6. MRL and DL Recoveries for EVOLUTE® PFAS 150 mg/6 mL columns.

	Conc. (ng/L)	1 (ng/L)	2 (ng/L)	3 (ng/L)	4 (ng/L)	5 (ng/L)	6 (ng/L)	7 (ng/L)	8 (ng/L)	$\bar{x}$ (ng/L)	$\bar{x}$ (%)	s (ng/L)	C_V (%)	DL (ng/L)
PFBA*	1.77	1.88	1.83	1.87	1.94	1.83	1.86	1.79	1.83	1.85	104	0.05	2.56	0.14
PFPeA	2.00	1.96	1.92	1.96	2.05	1.97	1.93	1.86	1.96	1.95	98	0.05	2.79	0.16
PFBS	2.00	1.87	1.76	1.84	1.73	1.77	1.81	1.73	1.75	1.78	89	0.05	2.83	0.15
PFHxA	2.00	2.09	2.00	2.04	2.10	2.03	2.09	1.98	2.08	2.05	103	0.05	2.20	0.14
PFHpA	2.00	2.08	1.96	1.98	1.91	2.04	1.95	2.01	2.01	1.99	100	0.06	2.76	0.17
PFHxS*	1.90	1.75	1.83	1.80	1.85	1.66	1.74	1.77	1.74	1.77	93	0.06	3.34	0.18
PFHpS*	1.91	1.77	1.78	1.85	1.79	1.64	1.66	1.69	1.64	1.73	91	0.08	4.69	0.24
H4PFOS*	1.90	1.91	1.84	1.97	1.92	2.05	2.06	2.01	2.11	1.98	104	0.09	4.58	0.27
PFOA	2.00	1.96	1.99	2.01	2.08	1.97	2.05	1.91	1.97	1.99	100	0.05	2.59	0.15
PFOS*	1.92	2.00	1.89	1.93	2.01	1.95	1.95	1.98	1.96	1.96	102	0.04	1.99	0.12
PFNA	2.00	2.08	1.94	2.02	2.01	2.04	2.08	2.11	2.02	2.03	102	0.05	2.59	0.16
PFDA	2.00	1.99	1.89	1.96	1.99	1.91	2.03	2.07	2.04	1.98	99	0.06	3.12	0.19
PFDS	2.00	1.92	2.08	1.81	2.04	2.01	1.93	2.04	1.90	1.97	98	0.09	4.61	0.27
PFUnA	2.00	2.09	1.97	1.93	1.95	1.89	1.97	1.95	1.98	1.97	98	0.06	2.94	0.17
PFDoA	2.00	2.08	1.91	2.02	2.05	2.02	2.03	2.04	2.04	2.02	101	0.05	2.52	0.15

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

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Appendix E

PFAS Background Study

Table 7. Results of PFAS Background Study for EVOLUTE® PFAS 500 mg/6 mL columns (recoveries in ng/L).

	TurboVap® LV								Laboratory Reagent Blanks			
Replicate	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**1**	**2**	**3**	**4**
PFBA*	0.00	0.00	0.00	0.00	0.00	0.10	0.05	0.04	0.09	0.06	0.06	0.06
PFPeA	0.02	0.03	0.03	0.03	0.03	0.06	0.02	0.03	0.04	0.04	0.02	0.04
PFBS	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.06
PFHxA	0.01	0.02	0.00	0.01	0.00	0.02	0.01	0.02	0.02	0.02	0.02	0.02
PFHpA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFHxS*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01
PFHpS*	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H4PFOS*	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.06	0.07	0.07
PFOA	0.02	0.00	0.00	0.00	0.03	0.00	0.01	0.00	0.00	0.00	0.00	0.00
PFOS*	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.02	0.01	0.02	0.01	0.01
PFNA	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.02	0.00	0.01	0.00
PFDA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
PFDS	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFUnA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFDoA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

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

Table 8. Results of PFAS Background Study for EVOLUTE® PFAS 150 mg/6 mL columns (recoveries in ng/L).

	TurboVap® LV				Laboratory Reagent Blanks			
Replicate	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
PFBA*	0.06	0.02	0.01	0.00	0.11	0.09	0.09	0.10
PFPeA	0.03	0.03	0.03	0.02	0.05	0.06	0.04	0.04
PFBS	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.01
PFHxA	0.02	0.02	0.01	0.00	0.03	0.03	0.02	0.02
PFHpA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PFHxS*	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.01
PFHpS*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
H4PFOS*	0.01	0.00	0.00	0.00	0.03	0.03	0.04	0.02
PFOA	0.00	0.02	0.03	0.00	0.00	0.00	0.05	0.00
PFOS*	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
PFNA	0.00	0.01	0.00	0.00	0.00	0.00	0.01	0.00
PFDA	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.02
PFDS	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
PFUnA	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00
PFDoA	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00

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

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Appendix F

IDP and IDA Data

Table 9. Results of IDP and IDA for EVOLUTE® PFAS 500 mg/6 mL columns (15 ng/L, n=4).

Replicate	1 (%)	2 (%)	3 (%)	4 (%)	$\bar{x}$ (%)	s (%)	C_V (%)
PFBA*	93.97	93.52	97.66	94.56	94.93	1.87	1.97
PFPeA	91.10	99.84	99.34	92.16	95.61	4.62	4.83
PFBS	88.73	88.20	91.76	86.48	88.79	2.20	2.47
PFHxA	94.83	95.12	99.13	95.85	96.23	1.98	2.06
PFHpA	104.38	106.43	104.54	106.18	105.38	1.07	1.02
PFHxS*	95.20	99.75	101.57	95.96	98.12	3.04	3.10
PFHpS*	89.31	87.18	98.22	79.57	88.57	7.67	8.66
H4PFOS*	96.43	102.56	100.83	101.53	100.34	2.70	2.69
PFOA	94.28	94.77	96.59	92.79	94.61	1.56	1.65
PFOS*	91.58	93.17	98.48	89.66	93.22	3.79	4.06
PFNA	93.76	98.27	100.96	96.89	97.47	2.99	3.07
PFDA	96.19	91.59	101.20	100.51	97.38	4.45	4.57
PFDS	96.50	102.88	108.47	98.56	101.60	5.29	5.21
PFUnA	88.89	85.05	87.96	90.74	88.16	2.37	2.69
PFDoA	96.65	99.00	102.74	98.79	99.30	2.53	2.55

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

Table 10. Results of IDP and IDA for EVOLUTE® PFAS 150 mg/6 mL columns (15 ng/L, n=4).

Replicate	1 (%)	2 (%)	3 (%)	4 (%)	$\bar{x}$ (%)	s (%)	C_V (%)
PFBA*	102.37	101.69	101.80	103.26	102.28	0.72	0.70
PFPeA	101.89	102.16	101.32	100.30	101.42	0.82	0.81
PFBS	99.30	102.15	99.56	102.63	100.91	1.72	1.71
PFHxA	100.58	103.25	99.56	100.27	100.92	1.61	1.60
PFHpA	102.78	102.88	102.64	102.10	102.60	0.35	0.34
PFHxS*	97.07	98.52	98.87	97.40	97.96	0.86	0.88
PFHpS*	91.57	92.08	90.92	93.18	91.94	0.95	1.04
H4PFOS*	99.40	99.12	104.89	102.46	101.47	2.74	2.70
PFOA	104.83	105.70	104.59	106.65	105.44	0.94	0.89
PFOS*	101.18	103.31	101.24	102.55	102.07	1.04	1.02
PFNA	102.52	103.28	101.34	101.57	102.18	0.90	0.88
PFDA	102.85	104.63	101.29	103.65	103.10	1.41	1.37
PFDS	105.46	102.18	103.54	106.38	104.39	1.89	1.81
PFUnA	98.55	97.67	97.27	98.90	98.10	0.76	0.77
PFDoA	105.74	103.29	103.54	105.36	104.48	1.25	1.19

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

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Appendix G

Carryover Data

Table 11. Results of carryover study following four, 50 ng/L LFB samples using EVOLUTE® PFAS 500 mg/6 mL columns.

Replicate	1 (ng/L)	2 (ng/L)	3 (ng/L)	4 (ng/L)	$\bar{x}$ (ng/L)
PFBA*	0.11	0.09	0.09	0.09	0.09
PFPeA	0.04	0.04	0.04	0.04	0.04
PFBS	0.02	0.01	0.02	0.01	0.02
PFHxA	0.02	0.02	0.03	0.03	0.03
PFHpA	0.00	0.00	0.00	0.00	0.00
PFHxS*	0.02	0.00	0.02	0.01	0.01
PFHpS*	0.00	0.00	0.00	0.00	0.00
H4PFOS*	0.04	0.04	0.04	0.04	0.04
PFOA	0.09	0.04	0.07	0.00	0.05
PFOS*	0.04	0.03	0.04	0.04	0.04
PFNA	0.00	0.00	0.00	0.00	0.00
PFDA	0.02	0.02	0.02	0.02	0.02
PFDS	0.03	0.00	0.00	0.00	0.01
PFUnA	0.00	0.00	0.03	0.01	0.01
PFDoA	0.00	0.00	0.00	0.00	0.00

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

EUROPE
Main Office: +46 18 565900
Fax: +46 18 591922
Order Tel: +46 18 565710
Order Fax: +46 18 565705
order@biotage.com
Support Tel: +46 18 56 59 11
Support Fax: + 46 18 56 57 11
eu-1-pointsupport@biotage.com

NORTH & LATIN AMERICA
Main Office: +1 704 654 4900
Toll Free: +1 800 446 4752
Fax: +1 704 654 4917
Order Tel: +1 800 446 4752
Order Fax: +1 704 654 4917
ordermailbox@biotage.com
Support Tel: +1 800 446 4752
us-1-pointsupport@biotage.com

JAPAN
Tel: +81 3 5627 3123
Fax: +81 3 5627 3121
jp_order@biotage.com
jp-1-pointsupport@biotage.com

CHINA
Tel: +86 21 68162810
Fax: +86 21 68162829
cn_order@biotage.com
cn-1-pointsupport@biotage.com

KOREA
Tel: +82 31 706 8500
Fax: +82 31 706 8510
korea_info@biotage.com
kr-1-pointsupport@biotage.com

INDIA
Tel: +91 11 45653772
india@biotage.com

Distributors in other regions are listed on www.biotage.com

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