Extraction of Polyfluorinated Compounds in Water Using EVOLUTE® WAX Prior to LC-MS/MS Analysis

This application note describes a Biotage polymer-based mixed-mode weak anion exchange SPE protocol for the extraction of a range of polyfluorinated (PFA) compounds in water prior to LC-MS/MS detection.

Introduction

The method described in this application achieves high reproducible recoveries for a number of common PFA compounds in water, chemical structures are shown in **Figure 1**. The method was tested against a commonly performed DIN 38407-42 method[1] for these compounds as an alternative extraction technique.

Analytes

Perfluorooctanoic acid (PFOA)
Perfluorooctanesulfonic acid (PFOS)
Perfluorohexanoic acid (PFHxA)
Perfluoroheptanoic acid (PFHpA)
Perfluorodecanoic acid (PFDA)
Perfluorononanoic acid (PFNA)
Perfluorobutanesulfonic acid (PFBuS)
Perfluorohexanesulfonic acid (PFHxS)

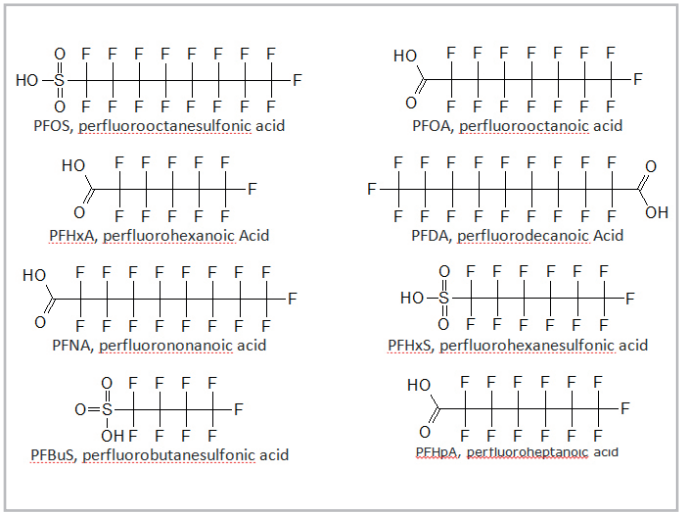

Figure 1. Structure of of target polyfluorinated compounds

Sample Preparation Procedure

Analytes were prepared at a concentration of 50 pg μL^{-1} in 18.2 MΩ cm water and stored at approximately 4 °C prior to use. A 20 µL aliquot of this solution was diluted to 1 L in 18.2 MΩ cm water to give a spiked concentration of 1 ng L^{-1}. No internal standards were used in this method.

Solid Phase Extraction

Format:	**EVOLUTE® WAX 50 mg/3 mL solid phase extraction cartridges, Part Number 614-0005-B; with 150 mL reservoirs, connected using PE column adapters**
Condition:	Condition column with methanol (2 mL)
Equilibration:	Equilibrate column with 18.2 MΩ cm water (2 mL)
Sample Loading:	Load untreated water (100 mL) at approximately 6 mL min^{-1}
Wash 1:	Elute interferences with 18.2 MΩ cm water (2 mL)
Wash 2:	Elute interferences with 1% formic acid (2 mL)
Wash 3:	Elute interferences with methanol (2 mL)
Elution:	Elute analytes with 1% concentrated ammonia solution (28-30% v/v) in methanol (3 x 0.8 mL)
Post Elution:	Dry the eluate in a stream of air or nitrogen using a SPE Dry 96 (40 °C, 20 to 40 L min^{-1}) or TurboVap® LV (15 bar at 40 °C for 1 hr). Reconstitute in 120 µL 40% methanol : 60% water

HPLC Conditions

Instrument: Nexera UHPLC system (Shimadzu Europe Gmbh, Germany)

Column: ACE Excel 1.7 C18 100 x 2.1 mm (Hichrom Ltd., UK)

Mobile Phase:

A: water: acetonitrile (9:1 v/v) containing 2mM ammonium acetate and 0.1% (v/v) formic acid

B: water: acetonitrile (1:9 v/v) containing 2mM ammonium acetate and 0.1% (v/v) formic acid

Flow Rate: 0.5 mL min^{-1}

Injection: 10 µL

Gradient

Initial to 0.5 min hold at 25 % B
0.5 to 3.5 min linear ramp to 70 % B
3.5 to 4 min linear ramp to 100 % B
4 to 5.5 min hold at 100 % B
5.5 to 6 min linear ramp to 25 % B
6 to 9 min hold at 25 % B

Total run time 9 min
(MS acquisition during initial 4.5 mins only)

Column Temperaure: 40 °C

Sample Temperaure: 20 °C

Table 1. Typical retention times for PFA analytes

Analyte	Retention Time (min)
Perfluorohexanoic acid (PFHxA)	2.2
Perfluorobutanesulfonic acid (PFBuS)	2.2
Perfluoroheptanoic acid (PFHpA)	2.4
Perfluorooctanoic acid (PFOA)	3.0
Perfluorohexanesulfonic acid (PFHxS)	3.1
Perfluorononanoic acid (PFNA)	3.4
Perfluorodecanoic acid (PFDA)	3.7
Perfluorooctanesulfonic acid (PFOS)	3.9

MS Conditions

Instrument: Triple Quad 5500 (AB Sciex, Framingham USA)

Curtain gas: 40

IonSpray voltage: -2400

Temperature: 450

Ion source gas 1 (GS1): 40

Ion source gas 2 (GS2): 50

Collision gas: 7

Entrance potential, EP: -5.0

Setting time: 50 ms

Pause between mass ranges: 5.0 ms

Table 2. Mass Spectrometer properties for selected PFA analytes

Analyte	Transition (m/z)	DP (V)	CE (V)	CXP (V)
Perfluorohexanoic acid (PFHxA)	313.0>268.9	-40.0	-11.0	-14.0
Perfluorobutanesulfonic acid (PFBuS)	299.0>79.9	-75.0	-67.0	-8.0
Perfluoroheptanoic acid (PFHpA)	363.0>319.0	-40.0	-13.0	-14.0
Perfluorooctanoic acid (PFOA)	413.0>368.9	-55.0	-5.0	-20.0
Perfluorohexanesulfonic acid (PFHxS)	399.0>80.0	-100	-85.0	-8.0
Perfluorononanoic acid (PFNA)	463.0>419.0	-50.0	-13.0	-26.0
Perfluorodecanoic acid (PFDA)	513.0>469.0	-58.0	-15.0	-27.0
Perfluorooctanesulfonic acid (PFOS)	499.0>80.0	-100	-120	-10.0

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Method Evaluation

Method linearity was determined by spiking known amounts of PFAs into 18.2 MΩ cm water. Calibration lines were constructed by spiking 100 µL of PFA stocks at variable concentrations into 250 mL 18.2 MΩ cm water giving final concentrations of 0.1, 0.5, 2, 4 and 6 ng L^{-1}. The samples were extracted in duplicate using the method detailed above to give duplicate calibration curves. A 1/x weighting was applied and the regression coefficient (r) was determined using Analyst software.

Method LOQ was determined from the lowest matrix-spiked calibration standard giving a peak to peak signal:noise ›10:1.

Performance of the method was assessed against an established DIN 38407-42 method, summarized in **Table 3**. Extraction recoveries of analytes spiked in 18.2 MΩ cm water at 1 ng L^{-1} were compared. The extracts were also compared against a pure standard to determine extract cleanliness.

The effect of loading volume on the method was investigated. The Biotage method was performed in duplicate using loading volumes of 2 and 100 mL for identical analyte loading quantities and their extraction recoveries compared.

Table 3. Side by side comparison of the Biotage and DIN 38407-42 extraction methods

Extraction Step	Biotage method	DIN 38407-42 method
Condition	2 mL methanol	2 mL methanol
Equilibrate	2 mL water	2 mL water
Sample Load	100 mL water spiked at 1 ng L^{-1}	100 mL water spiked at 1 ng L^{-1}
Wash 1	2 mL water	2 mL water
Wash 2	2 mL 1 % formic acid	2 mL 1 % formic acid in acetonitrile : acetone (1:1)
Wash 3	2 mL Methanol	2 mL Methanol
Elution	3 x 0.8 mL 1 % ammonia solution in methanol	3 x 0.8 mL 0.1 % ammonia solution in methanol

Results

The extracted ion chromatograms in **Figure 2** demonstrate good chromatography at 1 ng L^{-1} from a spiked extraction of 100 mL 18.2 MΩ cm water. The analytes demonstrate acceptable linearity over the calibrated range of 0.1 ng L^{-1} to 6 ng L^{-1} as demonstrated by the calibration curves in **Figure 3**. The correlation coefficients and LOQ are tabulated in **Table 4**.

Table 4. PFA linearity coefficients and LOQ

Analyte	Coefficient (r)	LOQ (ng L^{-1})	S/N (peak-to-peak)
Perfluorohexanoic acid (PFHxA)	0.997	0.1	47
Perfluorobutanesulfonic acid (PFBuS)	0.998	0.1	92
Perfluoroheptanoic acid (PFHpA)	0.993	0.1	27
Perfluorooctanoic acid (PFOA)	0.990	0.1	21
Perfluorohexanesulfonic acid (PFHxS)	0.994	0.1	37
Perfluorononanoic acid (PFNA)	0.987	0.1	53
Perfluorodecanoic acid (PFDA)	0.978	0.1	35
Perfluorooctanesulfonic acid (PFOS)	0.984	0.1	43

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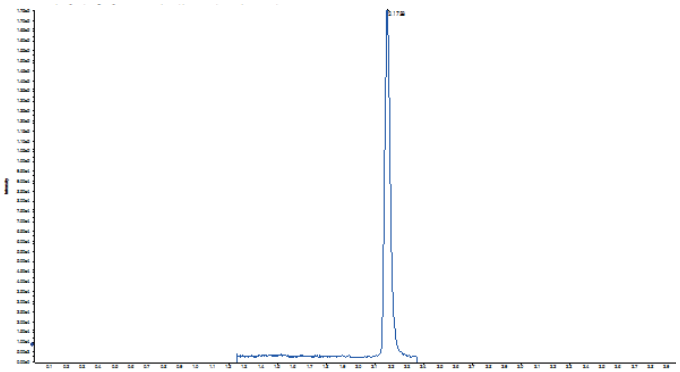
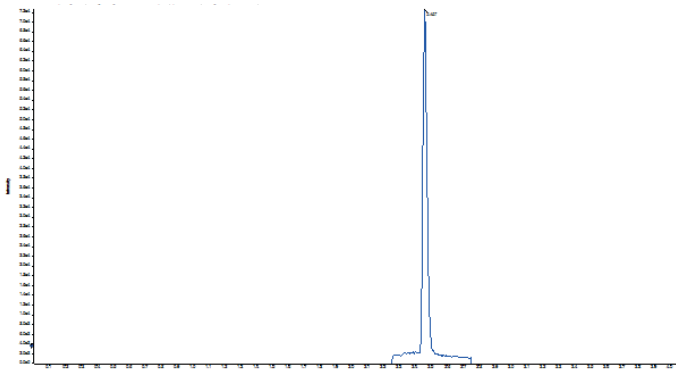
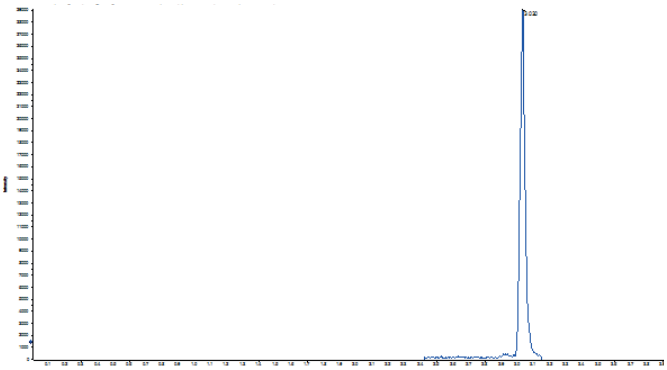


Perfluorohexanoic acid (PFHxA)



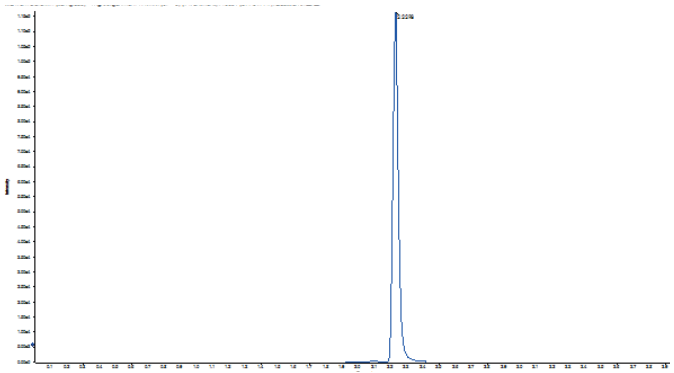
Perfluoroheptanoic acid (PFHpA)



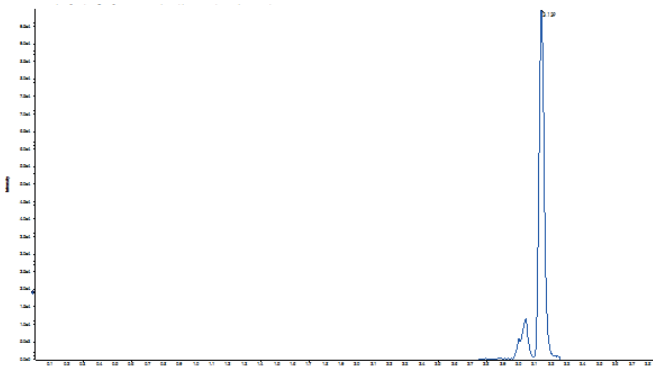
Perfluorooctanoic acid (PFOA)

Perfluorononanoic acid (PFNA)

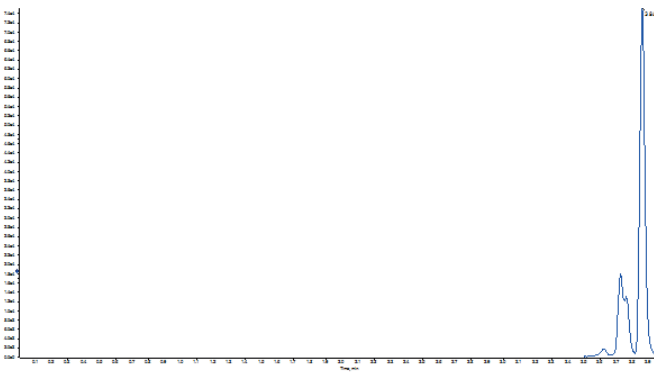
Perfluorodecanoic acid (PFDA)



Perfluorobutanesulfonic acid (PFBuS)



Perfluorohexanesulfonic acid (PFHxS)



Perfluorooctanesulfonic acid (PFOS)

Figure 2. Representative extracted ion chromatograms of PFA compounds at 1 ng L^{-1} (extracted from 100 mL 18.2 MΩ cm water)

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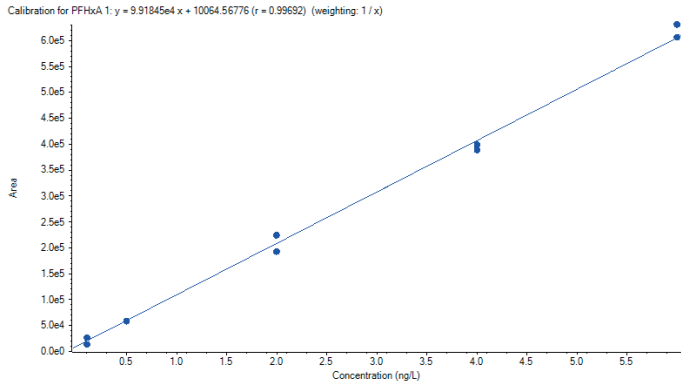
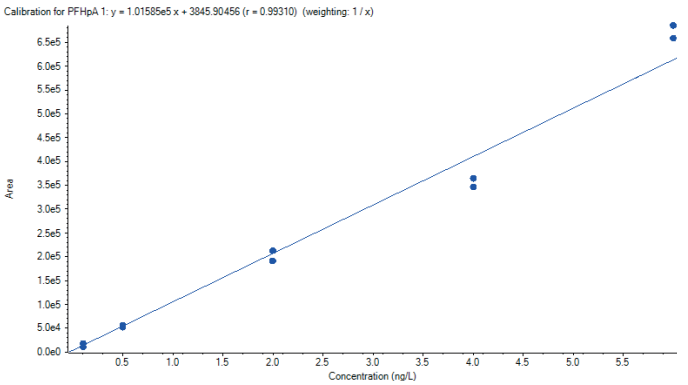
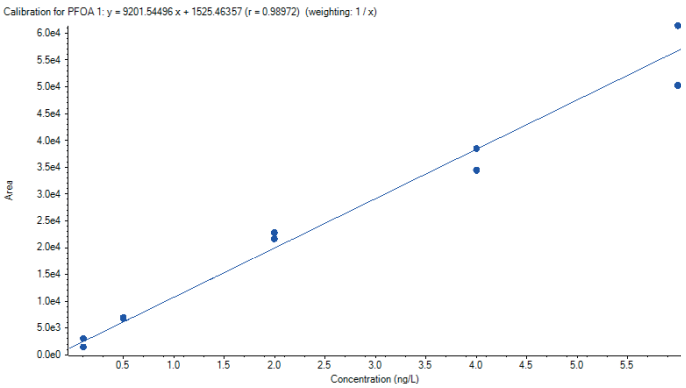


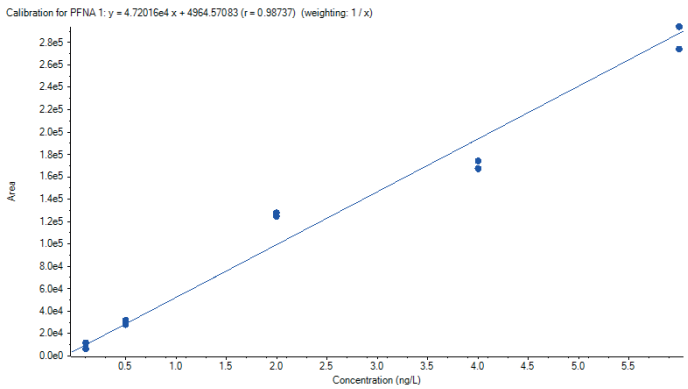
Perfluorohexanoic acid (PFHxA)



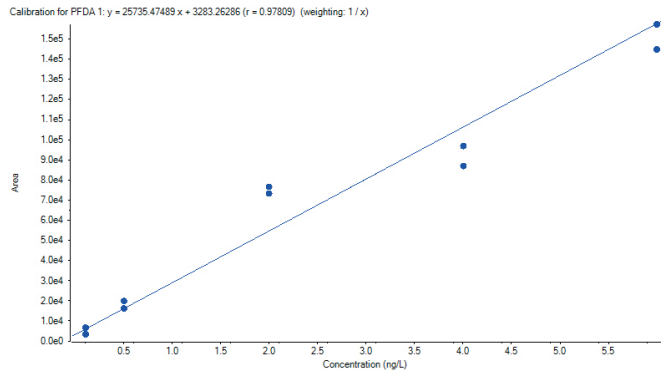
Perfluoroheptanoic acid (PFHpA)



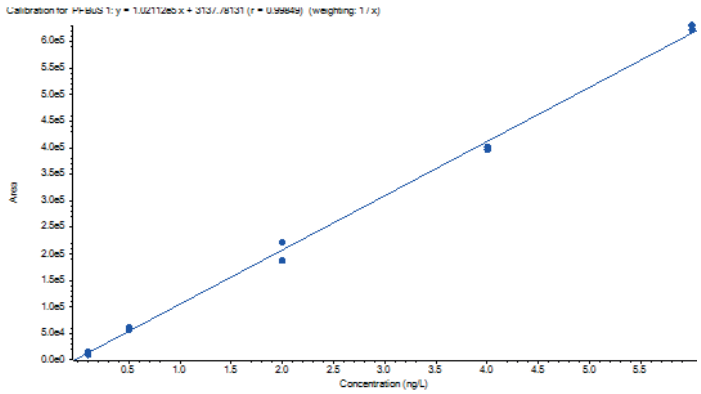
Perfluorooctanoic acid (PFOA)



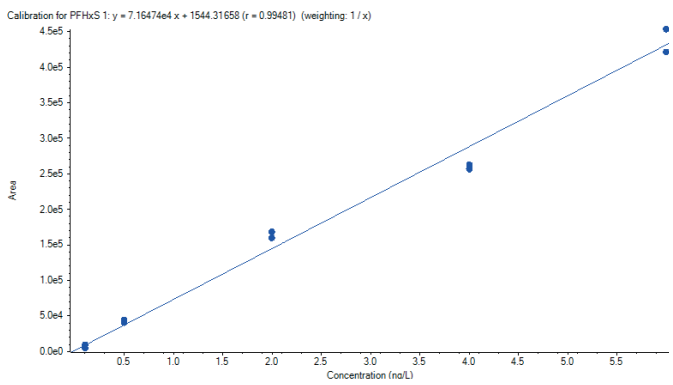
Perfluorononanoic acid (PFNA)



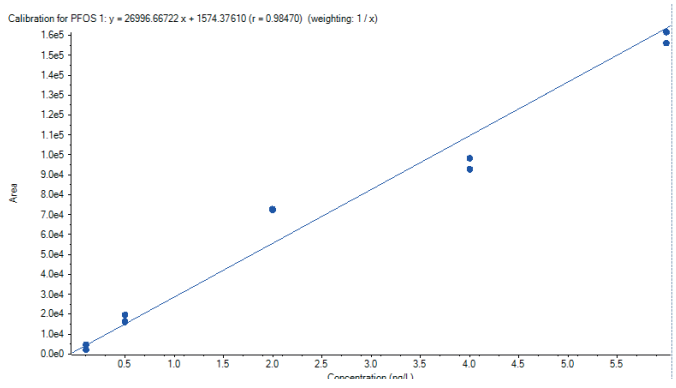
Perfluorodecanoic acid (PFDA)



Perfluorobutanesulfonic acid (PFBuS)



Perfluorohexanesulfonic acid (PFHxS)



Perfluorooctanesulfonic acid (PFOS)

Figure 2. Calibration curves of PFA compounds spiked in 100 mL 18.2 MΩ cm water

The Biotage method detailed in **Table 3** gave improved extraction recoveries for the majority of the PFA suite as demonstrated in **Table 5**; using the DIN method demonstrates lower recoveries of sulfonated PFAs. Assay performance and sensitivity were assessed by comparing the signal from a pure solution of mixed analytes to a blank (100 mL) water sample that had been extracted and over-spiked with an identical level of analytes prior to evaporation. **Table 6** demonstrates using EVOLUTE® WAX with an appropriate extraction method gives cleaner sample extracts. Any drop in the signal responses moving from the pure to extracted over-spiked sample were due to: co-extraction of analytes that cause ion suppression, evaporation losses, or binding of the analyte to collection tube walls.

Comparison Against an Established Method

Table 5. Extraction recovery and precision of the Biotage and DIN 38407-42 methods

	Extraction Method		DIN 38407-42 Method	
	Extraction Recovery	% RSD	Extraction Recovery	% RSD
Perfluorooctanesulfonic acid (PFOS)	100	1.3	82	9.3
Perfluorooctanoic acid (PFOA)	98	17.8	102	9.7
Perfluorohexanoic acid (PFHxA)	108	10.3	105	19.5
Perfluoroheptanoic acid (PFHpA)	82	9.8	not determined	
Perfluorodecanoic acid (PFDA)	91	13.8	87	13.6
Perfluorononanoic acid (PFNA)	98	11.8	98	9.1
Perfluorobutanesulfonic acid (PFBuS)	109	4.5	57	3.6
Perfluorohexanesulfonic acid (PFHxS)	109	4.7	75	6.9

Table 6. Losses of signal from over spiked water extracts compared to pure solution

Sample Cleanliness	% of signal in reconstituted over-spiked sample compared to pure solution system suitability	
	Biotage Method	DIN 38407-42 Method
perfluorooctanesulfonic acid (PFOS)	83	26
perfluorooctanoic acid (PFOA)	137	81
perfluorohexanoic acid (PFHxA)	97	59
perfluoroheptanoic acid (PFHpA)	83	not determined
perfluorodecanoic acid (PFDA)	109	36
perfluorononanoic acid (PFNA)	126	49
perfluorobutanesulfonic acid (PFBuS)	108	67
perfluorohexanesulfonic acid (PFHxS)	101	42

The Biotage method generates samples that are significantly cleaner than the DIN 38407-42 method. Many of the analytes from the over-spiked DIN 38407-42 extraction give MRM transitions that yield a peak area less than half of the diluted pure solution equivalent.

Effect of Loading Sample Volume on Extraction Recovery

Table 7 demonstrates consistent extraction recoveries were obtained when loading 0.1 ng of analytes in either 2 mL or 100 mL volumes. The similarity of the extraction recoveries demonstrate that if required the sample volume could be increased to improve sensitivity without any significant deterioration in extraction recovery.

Table 7. Extraction recoveries of the Biotage methods when loading 0.1 ng in 2 and 100 mL

Extraction Recoveries	PFBuS	PFHxA	PFHxS	PFHpA	PFNA	PFOA	PFOS
2 mL water load	105	106	108	N/A	96	102	97
100 mL water load	109	107	108	N/A	98	97	100

Additional Notes

1. The method detailed in this application note lists a selection of PFA compounds that can be extracted, demonstrating method applicability. System changes were made to reduce the background level of fluorinated compounds, replacing the solvent delivery tubing of the LC pumps with non PTFE alternatives. Some PFA compounds e.g. heptafluorobutyric acid were not measured because the LC-MS system was not sufficiently adapted to remove background levels. The results above demonstrate other PFA compounds would be expected to have similar method performance metrics when analyzed using a system with low PFA background levels.
2. Additional options to reduce PFA background levels include: thorough purging/flushing of the LC-MS system with methanol prior to analysis; thorough cleaning/rinsing of glassware whilst ensuring it has not been in contact with phosphate-containing detergents; use of plastic vessels in contact with pre/post extracted samples; retrofitting non-PTFE polymeric tubing in all parts of the LC-MS system; installation of a PFA-scavenger column between the pumps and the autosampler.

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Ordering Information

Part Number	Description	Quantity
614-0005-B	EVOLUTE® WAX 50 mg/3 mL SPE cartridges	50
120-1110-J	Reservoirs, standard grade, 150 mL	25
120-1101	ISOLUTE® PE Column Adapters 1,3,6 mL columns	10
121-2010	Biotage® VacMaster™-20 Sample Processing Station (with 10 mm rack)	1
SD-9600-DHS-EU	Biotage® SPE Dry Sample Concentrator System 220/240 V	1
SD-9600-DHS-NA	Biotage® SPE Dry Sample Concentrator System 100/120 V	1
C103198	TurboVap® LV, Evaporator 100/120V	1
C103199	TurboVap® LV, Evaporator 220/240V	1

References

1. DIN 38407-42: 2011-03; determination of selected polyfluorinated compounds (PFCs) in water - method using HPLC-MS / MS after solid-liquid extraction; Berlin, Germany.

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