

APPLICATIONS

Analysis of Perfluorinated Compounds (PFCs) in Aqueous Matrices, Evaluating Various Online SPE Sorbents by LC-MS/MS

Introduction

The analysis of perfluorinated compounds (PFCs) in aqueous matrices, especially drinking water and ground water, has received much attention in recent years. The US EPA has issued health advisories specifically for PFOA and PFOS and the occurrence data from the third Unregulated Contaminant Monitoring Rule (UCMR3) indicates a continuing need for this specific analysis. Using EPA method 537, a 250 mL water sample is fortified with surrogates and passed through a solid phase extraction (SPE) cartridge containing polystyrenedivinylbenzene (SDVB)¹. This extraction is very robust and very sensitive to the low ng/L levels using only 2-10 μ L injections.

However, method 537 does take a considerable amount of time to perform the SPE because the methanol extract has to be evaporated to dryness and then reconstituted. Online SPE has been shown to be an excellent technique for analyzing aqueous matrices because it bypasses the "offline" SPE time mentioned previously, increases sample throughput significantly⁴, and requires a much smaller sample volume. Additionally, it can be just as sensitive due to the larger injection volume. The performance of three different online SPE sorbents was evaluated for this application; Strata[®] C18-E, Strata-X, and Strata-X-AW. Four PFC analyte classes were evaluated; Perfluoroalkyl acids (PFAAs), Perfluorosulfonates (PFSAs), Perfluorosulfonamidoacetic acids (FOSAAs), and Fluorotelomersulfonates (FTSs). We present data on the pros and cons of each sorbent as well as sensitivity, accuracy, and precision.

Materials and Methods

Materials

LC Column:	Kinetex [®] 5 μ m EVO C18 100A
Dimensions:	100 x 2.1 mm
Part No.:	00D-4633-AN
Guard Column:	SecurityGuard [™] ULTRA Cartridges
Part No.:	AJO-9298
Online SPE:	Strata-X-AW 33 μ m Polymeric Weak Anion-Exchange
Dimensions:	20 x 2.0 mm
Part No.:	00M-S038-B0-CB
Online SPE Cartridge Holder:	20 mm Cartridge Holder
Part No.:	CHO-5845
Sample Filters:	Phenex [™] Glass Fiber 1.2 μ m 28 mm
Part No.:	AF0-8515-12

Sample Preparation Procedure

1. Samples are collected in polypropylene bottles and preserved with 0.5 g/L Trizma[®].
2. A 10 mL aliquot is spiked with surrogates at a concentration of 50 ng/L.
3. If necessary, filter using a 10 mL syringe fitted to a 1.2 μ m glass fiber syringe filter.
4. The filtered sample is spiked with internal standard at 50 ng/L.
5. The filtered sample is loaded and analyzed using a 5.0 mL injection volume.
6. The online SPE is completely automated; it includes a sample wash step (2.1 to 4.1 min) to wash Trizma preservative from the media.

Instrumentation

LC Pump 1: Thermo Accela[®] 1200, Flow 300 μ L/min
 LC Pump 2: Thermo Accela 600 (see table for flow rates)
 Autosampler Parameters: Transfer Time 250 sec,
 Elution Time 290 sec

LC-MS/MS Conditions

Column:	Kinetex [®] 5 μ m EVO C18 100A
Dimensions:	100 x 2.1 mm
Part No.:	00D-4633-AN
Mobile Phase:	A: 0.4 % Ammonium Hydroxide in Water B: Methanol
Gradient:	Time (min) % B
	0 90
	3.1 20
	4.5 20
	6.1 90
	11 90
	14 90
Flow Rate:	0.30 mL/min
Temperature:	Ambient
Detector:	Thermo TSQ Quantum [®] Ultra QQQ (MS/MS)
HPLC System:	Thermo Accela 1250
Injection:	5.0 mL

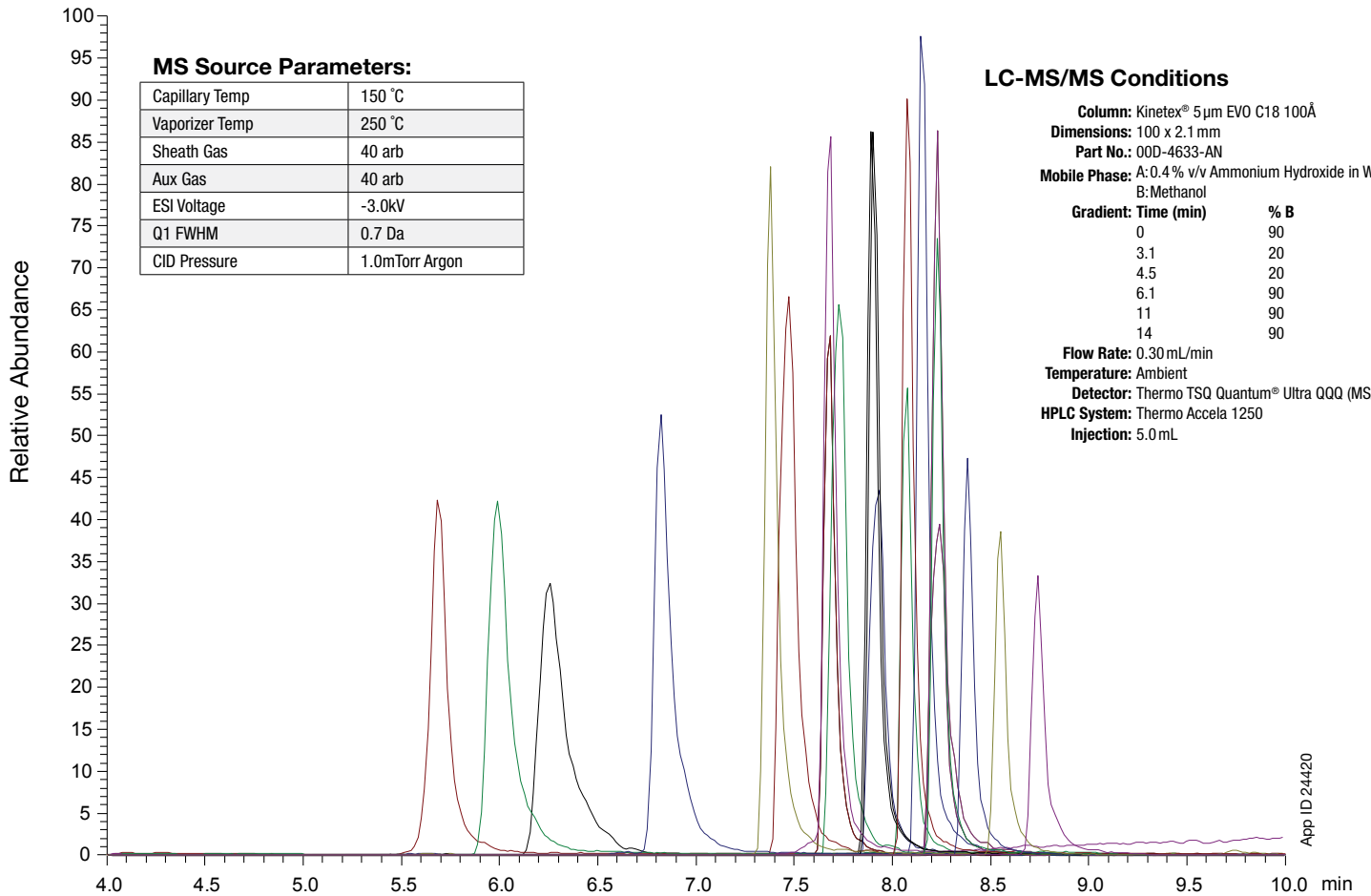
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 Ярославль (4852)69-52-93



LC Gradient (pump 1)

Time	Water	MeOH	0.4% NH ₃
0.00	0	90	10
3.10	20	20	60
4.50	20	20	60
6.10	0	90	10
11.00	0	90	10
14.00	0	90	10

Note: To decrease PFOA contributed by the eluent system, MeOH is kept at 90% while loading the online SPE with sample and subsequently brought down to 20% 1 min prior to online SPE elution.

Online SPE Program (pump 2)

Time	Water	MeOH	ACN	Flow mL/min	Comments
0.00	100	0	0	2.5	Sample Loading
2.00	100	0	0	2.5	Sample Loading
2.10	100	0	0	2.5	SPE Wash
4.10	100	0	0	2.5	SPE Wash
4.11	30	70	0	0	Idle
9.00	30	70	0	0	Idle
9.01	0	0	100	2.0	ACN Wash
9.49	0	0	100	2.0	ACN Wash
9.50	2.0	98	0	3.0	MeOH Wash
11.50	2.0	98	0	3.0	MeOH Wash
11.51	100	0	0	3.0	Cond: Water
14.00	100	0	0	3.0	Cond: Water

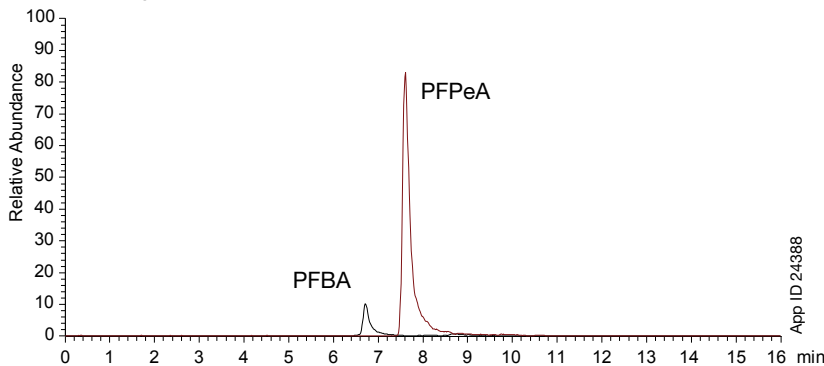
Methanol (MeOH); Acetonitrile (ACN); Ammonia (NH₃); Ammonium Hydroxide (NH₄OH); Ammonium Acetate (NH₄OAc)

Table 1.
Summary of online SPE and HPLC conditions that were investigated and their performance

Options	Column	Strata [®] SPE Sorbent	Sample pH	SPE Conditioning pH	Eluent*	PFBA / PFPeA %	Shape
1	Kinetex [®] EVO C18 5 μm 100 x 2.1 mm	X-AW	Trizma (pH=7)	neutral	0.24-0.04% NH ₃	100	excellent
2	Kinetex EVO C18 5 μm 50 x 2.1 mm	X-AW	neutral	neutral	0.04% NH ₃	106	very poor
3	Kinetex EVO C18 5 μm 50 x 2.1 mm	X-AW	neutral	neutral	0.24-0.04% NH ₃	76	OK
4	Kinetex EVO C18 5 μm 50 x 2.1 mm	X-AW	acidic	neutral	0.02% Formic Acid	13	OK
5	Luna [®] Omega C18 1.6 μm 50 x 2.1 mm	C18	neutral	neutral	2 mM NH ₄ OAc	<1	—
6	Luna Omega C18 1.6 μm 50 x 2.1 mm	C18	acidic (pH=2)	acidic (pH=2)	0.02% Formic Acid	22	very poor
7	Luna Omega C18 1.6 μm 50 x 2.1 mm	C18	acidic (pH=2)	acidic (pH=2)	2 mM NH ₄ OAc	11	OK
8	Luna Omega C18 1.6 μm 50 x 2.1 mm	C18	neutral	acidic (pH=2)	2 mM NH ₄ OAc	11	OK
9	Luna Omega C18 1.6 μm 50 x 2.1 mm	X	neutral	neutral	2 mM NH ₄ OAc	5.9	poor
10	Luna Omega C18 1.6 μm 50 x 2.1 mm	X	acidic	neutral	2 mM NH ₄ OAc	5.1	poor

* Note: All eluents used a gradient of increasing methanol for elution.

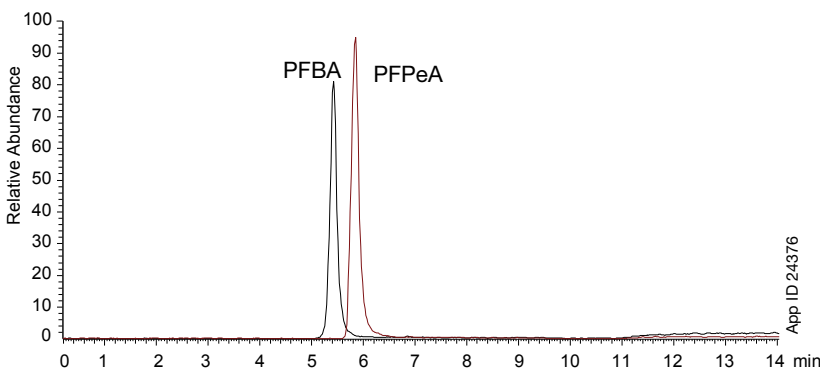
Figure 1.
Online SPE using C18-E sorbent and 2.0 mM ammonium acetate mobile phase modifier on a Luna Omega C18 LC column.



LC-MS/MS Conditions

Column: Luna Omega 1.6 μm C18 100Å
 Dimensions: 50 x 2.1 mm
 Part No.: 00B-4742-AN
 Mobile Phase: A: 2 mM Ammonium Acetate in Water
 B: Methanol
 Gradient: Time (min) % B
 0 40
 4.1 40
 6.1 90
 13 90
 13.01 40
 16 40
 Injection: 5.0 mL
 Flow Rate: 0.20 mL/min
 Temperature: 40 °C
 Detector: Thermo TSQ Quantum Ultra QQQ
 HPLC System: Thermo Accela 1250

Figure 2.
Online SPE using Strata-X-AW sorbent and 0.4-0.8% ammonia mobile phase modifier on a Kinetex C18 EVO LC column (final conditions)



LC-MS/MS Conditions

Column: Kinetex 5 μm EVO C18 100Å
 Dimensions: 100 x 2.1 mm
 Part No.: 00D-4633-AN
 Mobile Phase: A: 0.4% v/v Ammonium Hydroxide in Water
 B: Methanol
 Gradient: Time (min) % B
 0 90
 3.1 20
 4.5 20
 6.1 90
 11 90
 14 90
 Injection: 5.0 mL
 Flow Rate: 0.30 mL/min
 Temperature: Ambient
 Detector: Thermo TSQ Quantum Ultra QQQ
 HPLC System: Thermo Accela 1250

Figure 3. Elution strength of 0.04% NH₃ (left) and 0.24% NH₃ (Right) illustrating more efficient elution of analytes (PFBA and PFPeA) with increased base concentration in the mobile phase.

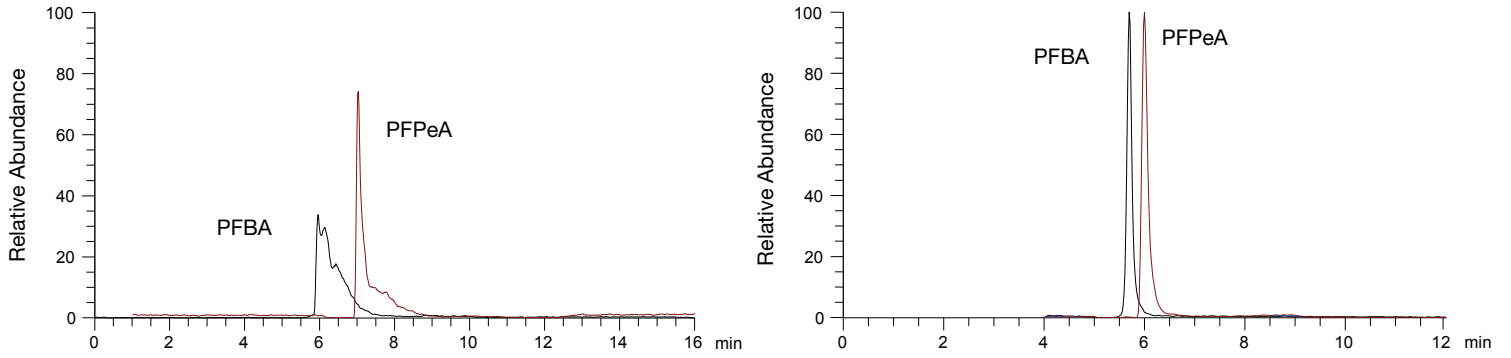


Figure 4. Effect of filtering on recovery of long chain PFCs.

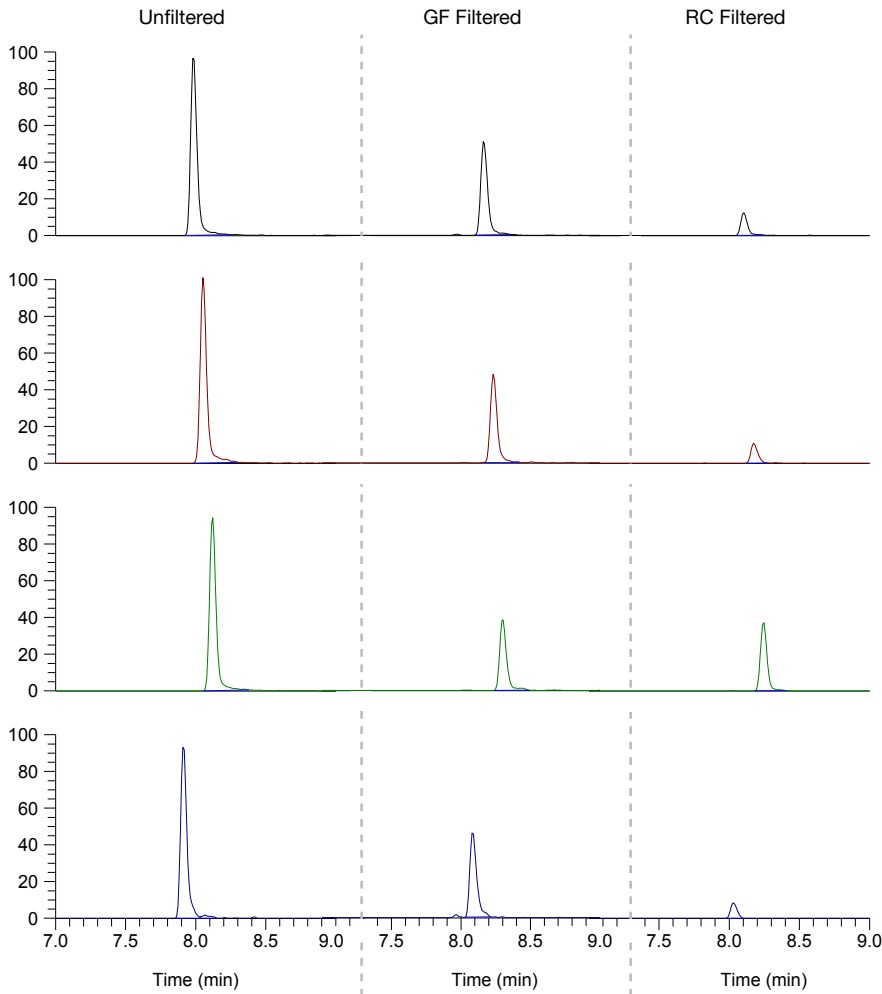
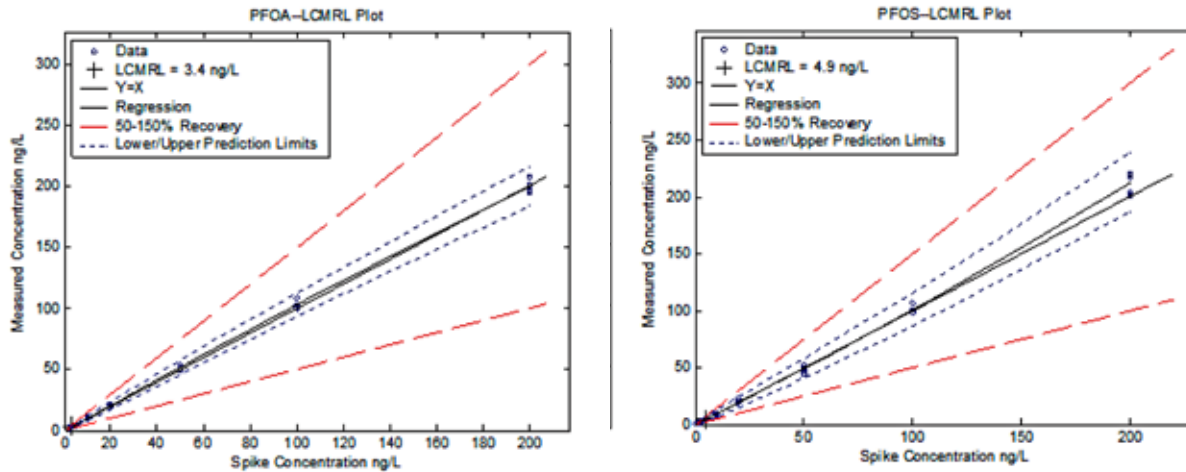


Figure 5. LCMRL plot of PFOA and PFOS showing the relationship of the data to the predicted limits. The LCMRL is the highest value of the upper and lower prediction limit intersection points with the 50-150% criteria.



Results & Discussion

One of the first objectives was to find the best SPE sorbent that would cover the target analytes. **Table 1** summarizes the options that were investigated. It quickly became apparent during development that the earliest eluter (lowest molecular weight) analyte was to be the indicator of SPE performance. As such the relative responses of PFBA and PFPeA were tabulated as well as the peak shape in order to meet data quality objectives similar to EPA method 537. The peak shape of the first 2 eluting peaks is addressed in EPA method 537 with a requirement for peak asymmetry factor. It should be noted (lines 1 and 3 in the table) that the Trizma[®] preservative marginally improved the PFBA relative to PFPeA.

It was experimentally determined that the same elution conditions (Methanol and 2.0 mM NH₄OAc) used for C18 or Strata-X online SPE could not work on the Strata-X-AW. Incidentally the mechanism used in weak anion-exchange (high pH elution) is quite different than the one employed using C18. Due to limitations on standard silica C18 analytical columns and online SPE, which typically cannot operate at high pH, a different approach was chosen. The analytical column chosen was a Kinetex[®] EVO C18 50 x 2.1 mm due to its extended pH operability range. This does result in some retention time differences when comparing the analytical systems. For this reason and due to variability associated with electrospray ionization, response was evaluated relative to other analytes to evaluate performance.

The Strata-X-AW provided a significant improvement in trapping smaller chain PFCs. **Figure 1** shows a chromatogram of PFBA (6.71 min) and PFPeA (7.61min) using a C18 online SPE cartridge and 2.0 mM ammonium acetate. Note that the response of PFBA is 11% of the PFPeA response using C18 however this value rises noticeably to 76% when using Strata-X-AW (**Figure 2**). This seems to be a simple limitation of using C18 for small organic acids even if the sample is acidified. The same effect was observed for the sulfonates PFBS and PFHxS, but to a lesser degree.

Another aspect of this analysis that was investigated was the ability to filter aqueous samples. This has a direct effect on whether the method could be adapted to aqueous soil extracts. Two types of filters were evaluated; glass fiber (Phenex[™] GF) and regenerated cellulose (Phenex RC). The biggest impact filtering has on analytes is with longer chain PFCs like PFDoDA, PFTrDA, PFTeDA, and PFDS. **Figure 4** is a comparison between unfiltered, glass fiber filtration, and regenerated cellulose filtration (left to right).

Table 2.

Performance comparison of the online SPE (Strata®-X-AW) method with EPA 537.

Analyte	Online SPE LCMRL	Online SPE DL	EPA537 LCMRL*	EPA537 DL**
PFBA	9.8	1.4	-	-
PFPeA	5.9	0.9	-	-
PFHxA	1.4	0.9	2.9	1.6
PFHpA	5.0	0.5	3.8	0.5
PFOA	3.4	2.0	5.1	1.7
PFNA	3.5	0.8	5.5	0.7
PFDA	11	1.2	3.8	0.7
PFUnDA	14	1.2	6.9	2.8
PFDaA	17	2.5	3.5	1.1
PFTrDA	12	3.3	3.8	2.2
PFTeDA	12	2.1	4.7	1.7
PFBS	6.3	1.6	3.7	3.1
PFHxS	5.5	1.5	8.0	2.0
PFHpS	6.5	1.8	-	-
PFOS	4.9	3.2	6.5	1.4
PFDS	11	4.5	-	-
6:2-FTS	4.1	0.8	-	-
8:2-FTS	5.1	1.7	-	-
N-MeFOSAA	14	2.7	14	6.5
N-EtFOSAA	12	3.2	14	4.2

* LCMRL is the lowest concentration minimum reporting level⁶** DL is the detection limit⁶

Conclusion

Balancing the performance across the wide range of analytes, the Strata-X-AW provides the most robust online SPE, especially if short chain PFCs are of interest. To assess performance as compared to EPA 537 methodology using the Strata-X-AW, a LCMRL (lowest concentration minimum reporting level) study was performed in Trizma® preserved water to determine the lowest concentration at which an accuracy of 50-150% can be achieved with a confidence of 99%. Additionally, a DL (detection limit) study was performed to determine the lowest concentration at which an analyte can be detected with a 99% confidence without an accuracy limit. The results of this study are listed in **Table 2** along with results from EPA method 537.

While all 3 SPE sorbents evaluated could be used for PFC analysis, the Strata-X-AW shows markedly improved recoveries for the widest analyte class. This is not surprising considering offline SPE using weak anion-exchange is fairly robust in other matrices^{2,3}. However, if only certain priority PFCs are required such as PFOA and PFOS, C18 would provide adequate peak shape and recovery. One caveat with this system is that the sample pH should be adjusted to 2 using formic acid and the sorbent should be conditioned using 0.2% formic acid. This provides sufficient strength to protonate and trap analytes.

With the advent of analytical columns like the Kinetex® EVO C18 core-shell that can support a higher pH, it's possible to couple weak anion-exchange online SPE to a suitable analytical column. Additionally, the use of aqueous ammonia in the eluent has no limiting factors when coupled to MS due to its volatility and preliminary data show that ammonia provides better ionization than ammo-

niacum acetate. Glass fiber filters can allow samples to be filtered without detrimentally affecting performance. This makes it possible to further expand the applicability of the method. For example, one method of extracting PFCs from soil uses methanol followed by SPE cleanup⁵. It's quite possible that a large volume (0.5-1.0 mL) of methanol could be filtered using glass fiber, diluted with water, and pre-concentrated using a Strata-X-AW as described above. Preliminary tests show that up to 10% methanol can be injected onto Strata-X-AW before the high organic starts affecting the smallest acids (i.e.: PFBA).

Acknowledgements

We would especially like to thank David Schiessel and BABCOCK Laboratories for developing and contributing this application.



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References

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Ordering Information

Kinetex® EVO C18 Core-Shell LC Columns

5 µm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
00A-4633-AN	00B-4633-AN	00D-4633-AN	00F-4633-AN	AJO-9298
for 2,1 mm ID				

5 µm MidBore™ Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
50 x 3.0	100 x 3.0	150 x 3.0	3/pk
00B-4633-YO	00D-4633-YO	00F-4633-YO	AJO-9297
for 3,0 mm ID			

5 µm Analytical Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
00B-4633-EO	00D-4633-EO	00F-4633-EO	00G-4633-EO	AJO-9296
for 4,6 mm ID				

2.6 µm Minibore Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
00A-4725-AN	00B-4725-AN	00D-4725-AN	00F-4725-AN	AJO-9298
for 2,1 mm ID				

2.6 µm MidBore Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
50 x 3.0	100 x 3.0	150 x 3.0	3/pk
00B-4725-YO	00D-4725-YO	00F-4725-YO	AJO-9297
for 3,0 mm ID			

2.6 µm Analytical Columns (mm)				SecurityGuard™ ULTRA Cartridges‡
50 x 4.6	100 x 4.6	150 x 4.6	3/pk	
00B-4725-EO	00D-4725-EO	00F-4725-EO	AJO-9296	
for 4,6 mm ID				

1.7 µm Minibore Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
50 x 2.1	100 x 2.1	150 x 2.1	3/pk
00B-4726-AN	00D-4726-AN	00F-4726-AN	AJO-9298
for 2,1 mm ID			

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJO-9000

Luna® Omega LC Columns

1.6 µm Microbore Columns (mm)			
Phases	50 x 1.0	100 x 1.0	150 x 1.0
C18	00B-4742-AO	00D-4742-AO	00F-4742-AO

1.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges‡
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
C18	00A-4742-AN	00B-4742-AN	00D-4742-AN	00F-4742-AN	AJO-9502
for 2.1 mm ID					

3 µm Minibore Columns (mm)					SecurityGuard™ Cartridges (mm)
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	4 x 2.0*
PS C18	00A-4758-AN	00B-4758-AN	00D-4758-AN	00F-4758-AN	AJO-7605
for ID: 2.0 - 3.0 mm					

Strata® SPE Ordering Information

On-line Extraction Cartridge

Description	Part Number	Unit/Box
Strata-X on-line extraction cartridge, 20 x 2.0 mm	00M-S033-B0-CB	ea
Strata-X-AW online extraction cartridge, 20 x 2.0 mm	00M-S038-B0-CB	ea
Strata C18 online extraction cartridge, 20 x 2.0 mm	00M-S039-CB	ea
Cartridge holder, 20 mm	CH0-5845	ea



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