Modern Approaches for PFAS LC-MS/MS Analysis in Aqueous and Solid Matrices

SCIEX

phenomenex™

Overview

PFAS Background

Case Studies

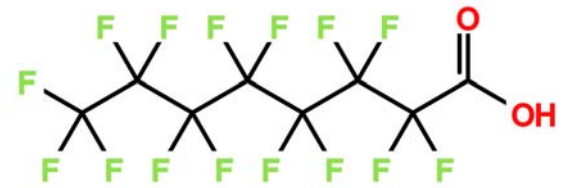
1. SPE + LC-MS/MS
2. Large Volume Direct Inject + LC-MS/MS
3. QuEChERS from Food + UHPLC-MS/MS
4. Online SPE + LC-MS/MS
5. QuEChERS from Sediment + LC-MS/MS

Conclusion

Resources

Acknowledgements

PFAS

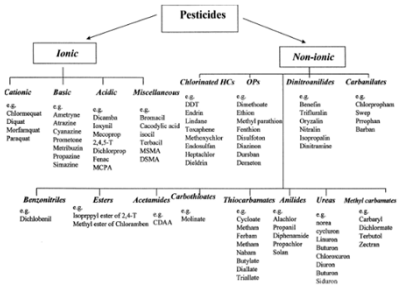


- PFAS – Per and Polyfluorinated Alkyl Substance
- Repellent properties popular for consumer products like surface treatment protection, paper protection, and performance chemicals (i.e. firefighting foams)
- Trace levels found throughout global water sources
- Chemically stable, low reactivity, and resistant to degradation in aqueous environments - Important for use in a wide range of products and industrial applications
- Human exposure to PFAS residues linked to adverse health effects

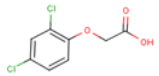
EPA Guidelines for PFOA and PFOS In Drinking Water
=
More Testing

Typical Challenges

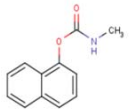
Variety of Compounds



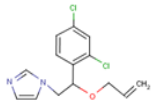
Wide Range of Chemical Properties



Acidic



Neutral



Basic

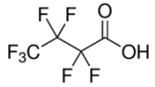
Wide Range of Sample Matrices

PFAS Specific Challenges

System Contamination

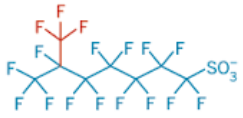
Trace Quantities

Wide Hydrophobic Range



Isobaric

PFOS linear isomer



PFOS branched isomer

The Strata®-X Product Line

Solid Phase Extraction

In SPE, a support particle is modified with different functional groups

- Silica or polymeric particles
- Wide range of functional groups (RP, IEX, NP)

Target analytes bind to the media

- Matrix interferences are washed away using different washing protocols

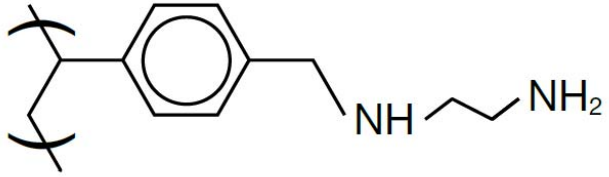
The key distinction is that you optimize your method to target & recover your analytes

- More selective than QuEChERS

Strata®-X-AW and XL-AW

Weak Anion-Exchange

3 Mechanisms of Retention

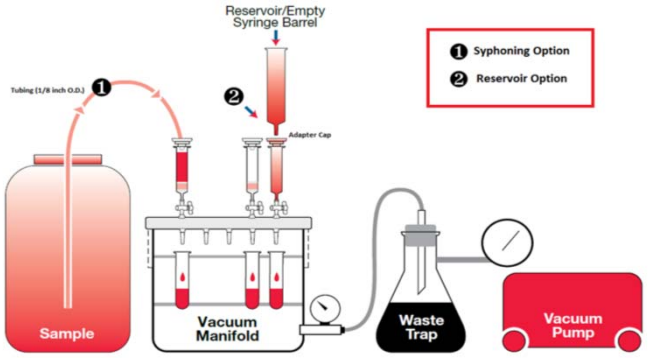


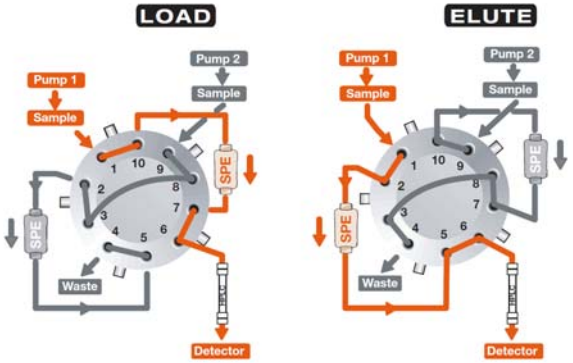
- Weak Anion-Exchange
- π-π Bonding
- Hydrophobic Interaction

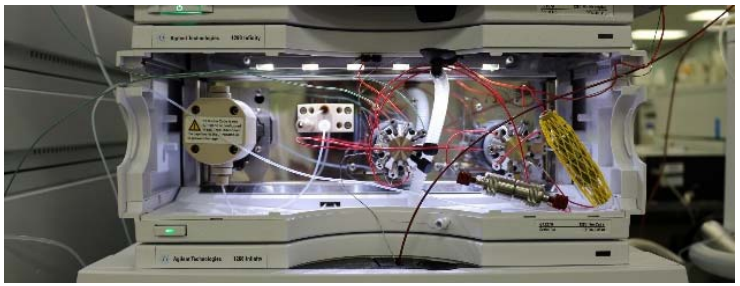
Select Your Particle and Pore Size

	Strata -X-AW, 33 µm, 85 Å	Strata-XL-AW, 100 µm, 300 Å
High Concentration Samples	✓	
Small Target Analytes (< 10 kDa)	✓	
Large Target Analytes (> 10 kDa)		✓
Large Volume Samples		✓
Viscous Samples		✓

Large Volume Processing and Online SPE



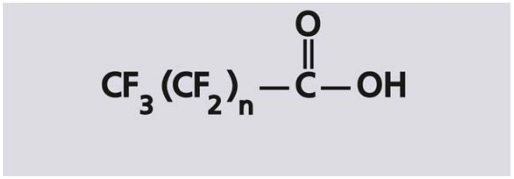




Solid Phase Extraction

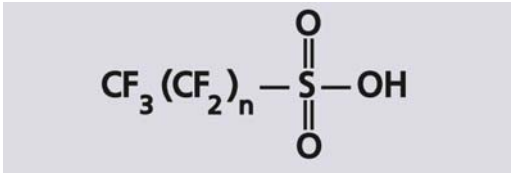
EPA method 537

1. Reversed phase retention (Strata®-X)

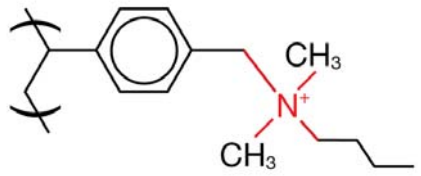


Mixed Mode Anion-Exchange

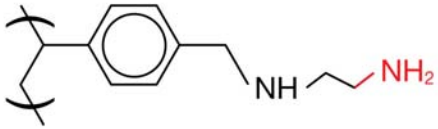
1. Anion-Exchange + Strata-X



Strata-X-A Strong Anion-Exchang



Strata-X-AW Weak Anion-Exchar

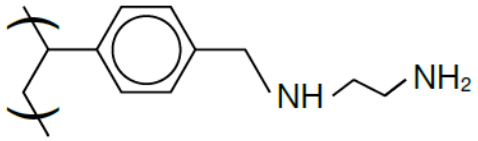


Solid Phase Extraction

Strata®-X-AW Weak Anion-Exchange

Condition: 3 x 5mL methanol
Condition: 2 x 5mL water
Load: 125 mL sample
Wash: 2 x 5 mL water
Bottle rinse: 5 mL 0.5% NH_4OH in methanol Elute: 3 x 5 mL 0.5% NH_4OH in methanol
Evaporate to dryness
Reconstitute with 50/50 methanol/water

3 Mechanisms of Retention

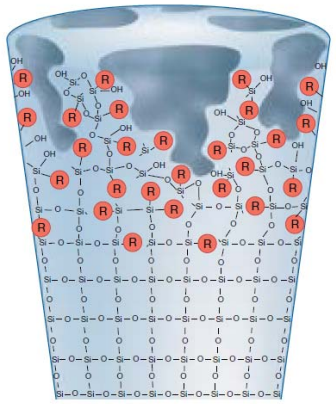


- **Weak Anion-Exchange**
- **π-π Bonding**
- **Hydrophobic Interaction**

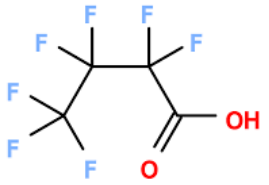
Gemini® TWIN™ Technology

Gemini pH Flexible LC

Gemini columns are rugged reversed phase HPLC columns that offer extended lifetime at extreme pH conditions and excellent stability for reproducible, high efficiency separations.

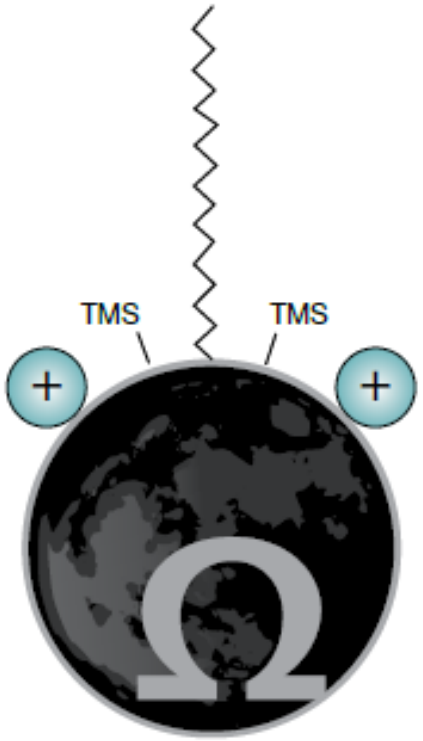


- Take full advantage of high and low pH conditions (pH 1-12) to manipulate selectivity
- Expect longer column lifetime
- For analytical and preparative separations of basic and acidic compounds



PFBA retention and good peak shape

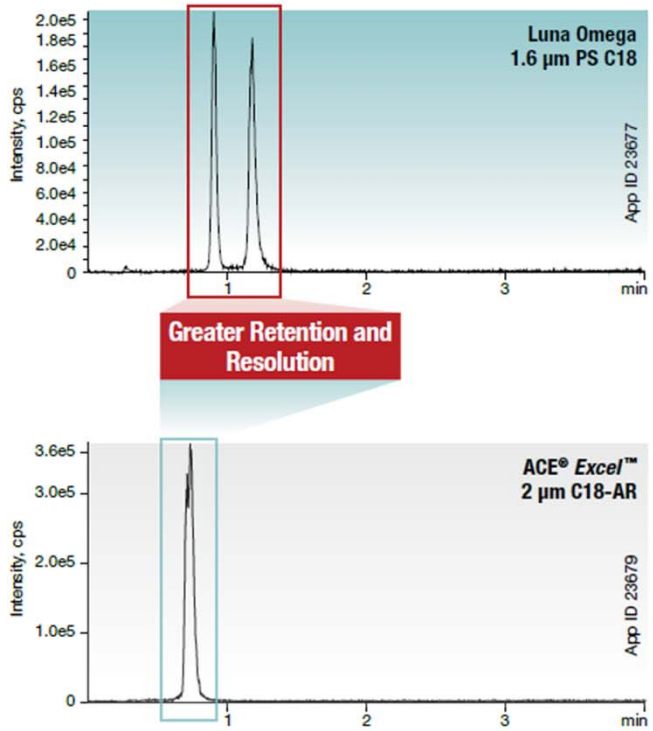
LUNA® Omega PS C18



- **LUNA Omega 1.6 & 5 µm Silica Based**
- **3-step bonding process:**
  - Deposition of **positive charge** on surface
  - C18 bonding
  - TMS endcapping
- **A C18 with:**
  - Enhanced selectivity for **polar acids**
  - Stability in 100% aqueous mobile phases
  - Improved peak shape and loadability for bases

Enhanced Selectivity for Acids

MMA and Succinic Acid



Conditions for all columns:

Columns: Luna Omega 1.6 µm PS C18
ACE Excel 2 µm C18-AR

Dimension: 50 x 2.1 mm

Mobile Phase: A: Water with 0.1 % Formic Acid
B: Acetonitrile with 0.1 % Formic Acid

Gradient:

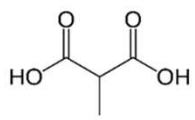
Time (min)	% B
0	0
5	50
5.1	0
7	0

Flow Rate: 0.5 mL/min

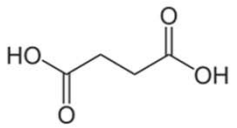
Temperature: 22 °C

Detection: MS/MS (SCIEX API 4000™)

Sample: 1. Succinic acid
2. MMA



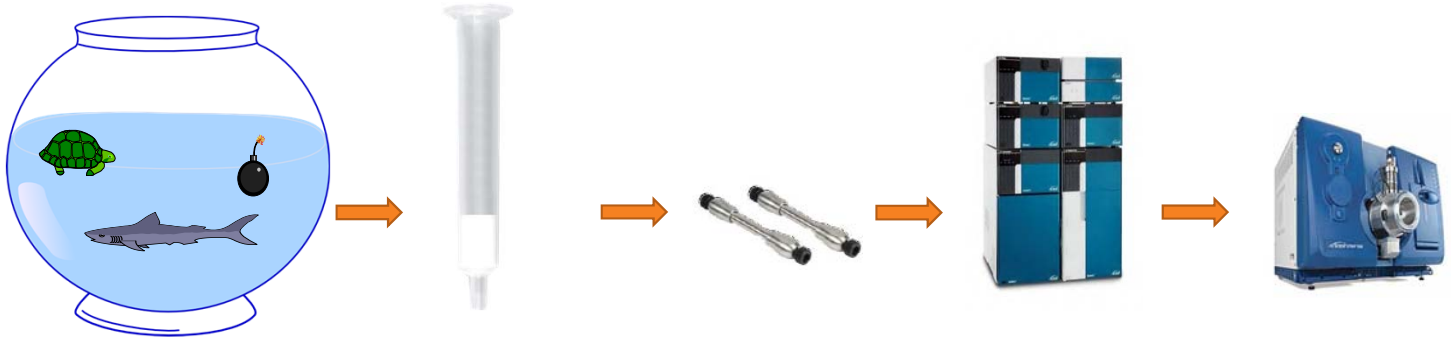
MMA



Succinic Acid

Comparative separations may not be representative of all applications.

So Let's Put These Into Action!



PFAS using SPE and LC/MS/MS

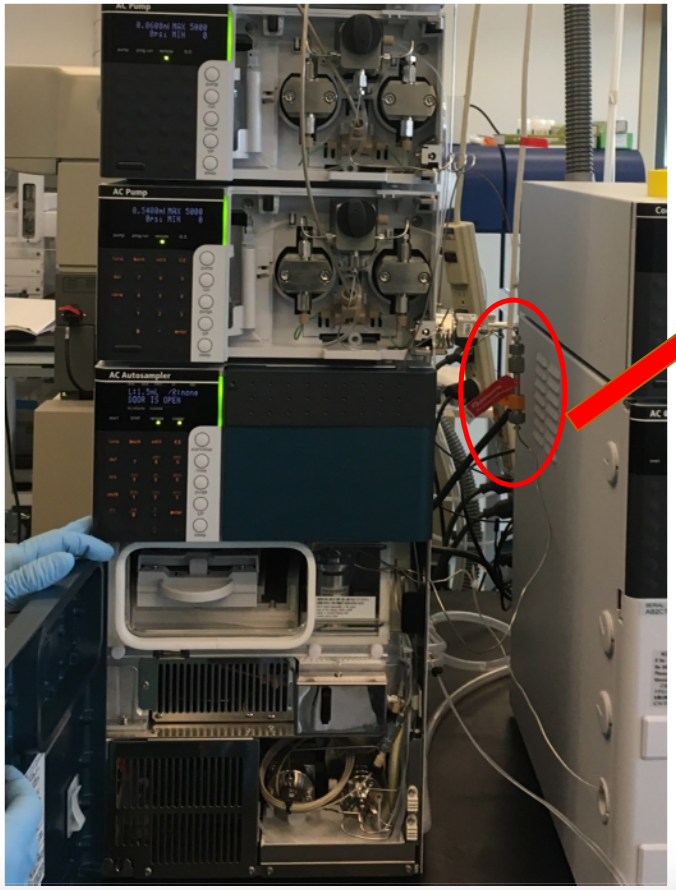
Case Study 1

phenomenex
...breaking with tradition℠

Reducing LC Contamination

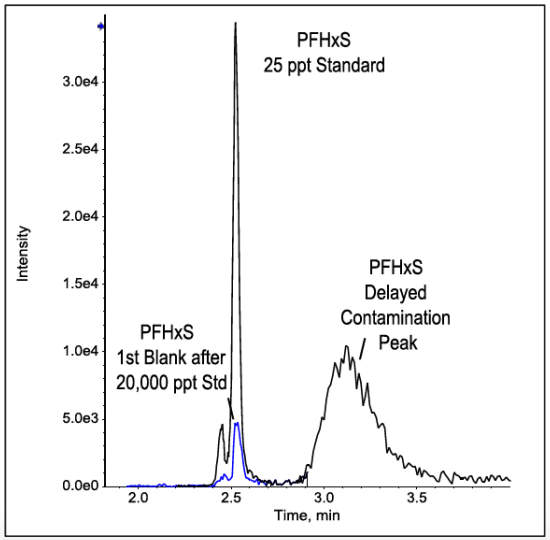
- **Reducing background LC contamination is critical to obtaining low detection limits**
- **Important to use high-purity solvents and modifiers (e.g. ammonium acetate) for mobile phases; test each solvent bottle to verify purity**
- **PFAS may be present in plastic tubing, Teflon® filters**
  - Replace fluorinated tubing with PEEK
  - Remove PFTE solvent filter frits
  - Replace graphite-filled PTFE pump seals with polyethylene seals
- **However, PFAS contamination may still be present …**

LC Contamination: Delay Column



LC Contamination: Reduction Strategies

- Use of “delay” or “trap” column placed between the pumps and autosampler, *upstream* of the analytical column
- PFAS leaching from the LC will be retained on the delay column and separated from the analytical peak
- NOT helpful for contamination from method blanks, contaminated standards



SPE Method Overview

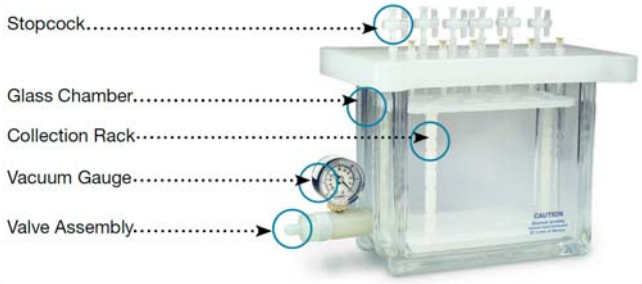
Advantage: Sample Cleanup and Concentration, compatible with DOD QSM 5.1

Overview:

Calibration and mass-labelled surrogate standards (i.e. isotope dilution stds) purchased from Wellington Laboratories (Guelph, ON); *wide coverage for surrogates*

Surrogate standards (25 ng) spiked into 250 mL water samples

PFAS compounds extracted and concentrated on weak anion-exchange SPE column (e.g. Phenomenex Strata® X-AW)



WELLINGTON Reporter

PRODUCT UPDATES FROM WELLINGTON LABORATORIES

November 17, 2016

Catalogue Number	Product (methanol)	Qty	Conc
PFAC-24PAR	Native PFAS Precision and Recovery Standard Solution (24 components)	1.2 ml	2.0 µg/ml ea
MPFAC-24ES	Mass-Labelled PFAS Extraction Standard Solution (19 components)	1.2 ml	1.0 µg/ml ea

These new solution/mixtures complement our existing line of mixed PFAS reference standards.

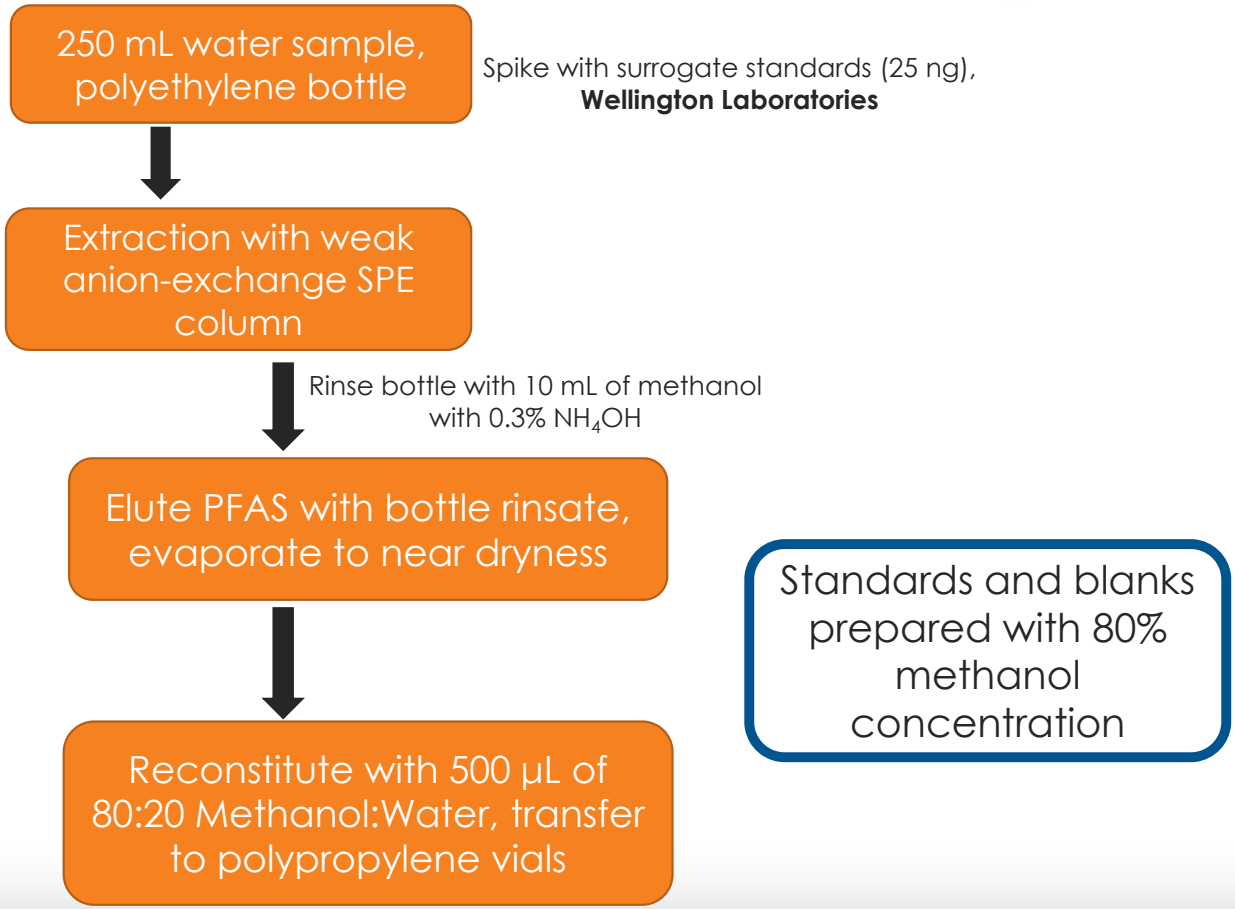
Catalogue Number	Product (methanol)	Qty	Conc
PFC-MXA	Native PFCA Solution/Mixture (C_4-C_{14})	1.2 ml	2.0 µg/ml ea
PFS-MXA	Native PFSA Solution/Mixture (C_4,C_6-C_8,C_{10})	1.2 ml	2.0 µg/ml ea
PFAC-MXA	Native PFCA/PFSA Solution/Mixture (10)	1.2 ml	5.0 µg/ml ea
PFAC-MXB	Native PFCA/PFSA Solution/Mixture (17)	1.2 ml	2.0 µg/ml ea
PFAC-MXC	Native PFCA/PFSA Solution/Mixture (21)	1.2 ml	2.0 µg/ml ea
MPFAC-MXA	Mass-Labelled PFCA/PFSA Solution/Mixture (9)	1.2 ml	2.0 µg/ml ea
MPFAC-C-ES	Mass-Labelled PFCA/PFSA Extraction Standard (13)	1.2 ml	2.0 µg/ml ea
MPFAC-C-IS	Mass-Labelled PFCA/PFSA Injection Standard (4)	1.2 ml	2.0 µg/ml ea
EPA-537IS	U.S. EPA Method 537 Internal Standard PDS (3)	1.2 ml	variable
EPA-537SS	U.S. EPA Method 537 Surrogate PDS (3)	1.2 ml	variable

Please contact your local distributor or info@well-labs.com for pricing and delivery.

Visit our website (www.well-labs.com) for a complete listing of our new products.

Certified System ISO 9001

SPE Method: Flowchart



LC Conditions For: SPE Method

Column: Gemini® 3 µm C18
Dimensions: 50 x 2 mm
Part No.: 00B-4439-B0
Mobile Phase: A: 20 mM Ammonium Acetate in Water
B: Methanol

Gradient:

Time (min)	% B
0.00	10
0.10	55
4.50	99
4.95	99
5.00	10
6.50	00

Injection: 10 µL
Flow Rate: 0.6 mL/min
Temperature: 40 °C
Detection: SCIEX Triple Quad™ 5500 with a Turbo V™ source

Mass Spectrometer & Source Gas Conditions

- SCIEX Triple Quad™5500 system with Turbo V™ source
- ESI probe in negative polarity mode
- Source parameters optimized using Compound Optimization (FIA) function in Analyst® software

Parameter	Value
Curtain Gas (CUR)	35 psi
IonSpray Voltage (IS)	-4500 V
Temperature (TEM)	600 °C
Nebulizer Gas (GS1)	50 psi
Heater Gas (GS2)	50 psi

Mass Spectrometer Parameters: Compound Specific

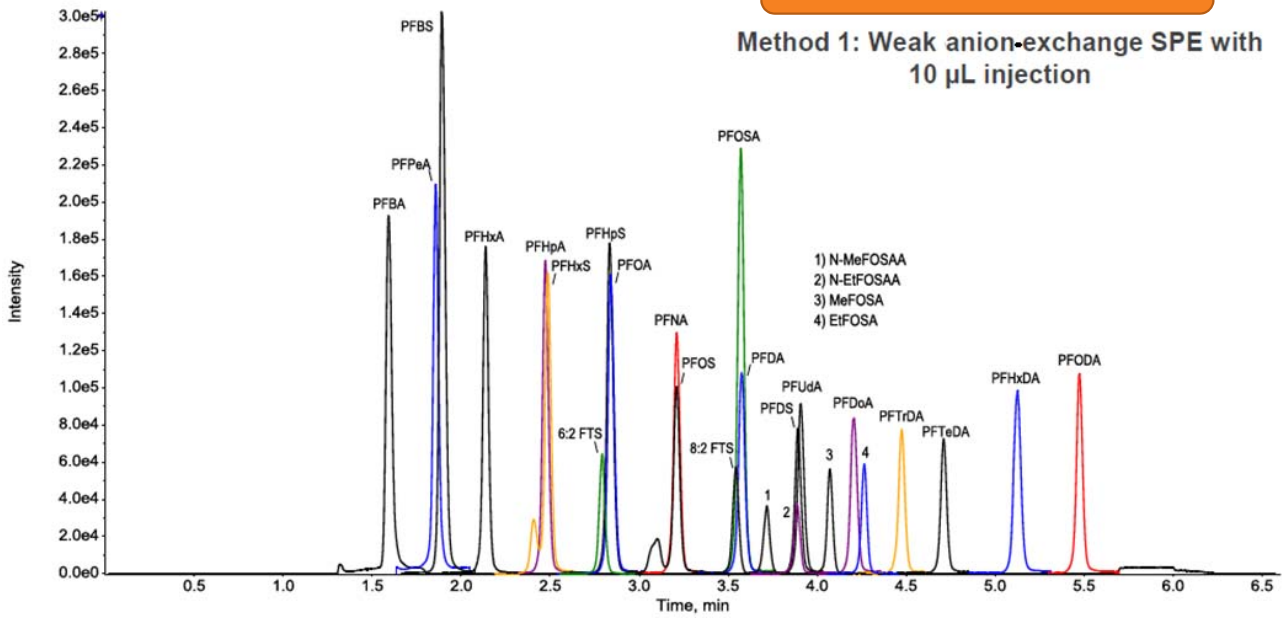
Compound	Q1	Q3	DP	CE
PFCAs				
PFBA	212.9	169	-25	-12
PFPeA	262.9	219	-20	-12
PFHxA	313	269	-25	-12
PFHpA	363	319	-25	-12
PFOA	413	369	-25	-14
PFNA	463	419	-25	-14
PFDA	513	469	-25	-16
PFUdA	563	519	-25	-18
PFDoA	613	569	-25	-18
PFTrDA	663	619	-25	-20
PFTeDA	713	669	-25	-22
PFHxDA	813	769	-25	-24
PFODA	913	869	-25	-26

Compound	Q1	Q3	DP	CE
PFSAs				
PFBS	298.9	80	-55	-58
PFHxS	399	80	-60	-74
PFHpS	449	80	-65	-88
PFOS	499	80	-65	-108
PFDS	599	80	-85	-118
Other PFASs				
6:2 FTS	427	407	-50	-32
8:2 FTS	527	507	-50	-40
PFOSA	498	78	-60	-85
MeFOSA	512	169	-75	-37
EtFOSA	526	169	-75	-37
N-MeFOSAA	570	419	-40	-36
N-EtFOSSA	584	419	-50	-36

- De-clustering Potential (DP) and Collision Energy (CE) optimized for each compound
- One MRM transition monitored each analyte and internal standard
- *Scheduled* MRM™ algorithm used to maximize dwell times and optimize cycle time

Chromatogram: 10 pg on-column injection

6.5 min run time!



Overlaid chromatogram of 1 µg/L standard

Method Performance: Calibration Range, Sensitivity and Accuracy

Compound	Calibration Range (ng/L)	Linear Correlation (r^2)	S:N of 25 ng/L standard	Accuracy of 25 ng/L standard
PFCAs				
PFBA	25-20,000	0.997	108	104%
PFPeA	25-20,000	0.998	88	103%
PFHxA	25-20,000	0.998	104	93%
PFHpA	25-20,000	0.999	116	101%
PFOA	25-20,000	0.999	117	106%
PFNA	25-20,000	0.990	91	109%
PFDA	25-20,000	0.998	103	105%
PFUdA	25-20,000	0.995	84	101%
PFDoA	25-20,000	0.998	60	101%
PFTrDA	25-20,000	0.998	32	104%
PFTeDA	25-20,000	0.994	15	107%
PFHxDA	25-20,000	0.999	21	103%
PFODA	25-20,000	0.999	33	102%

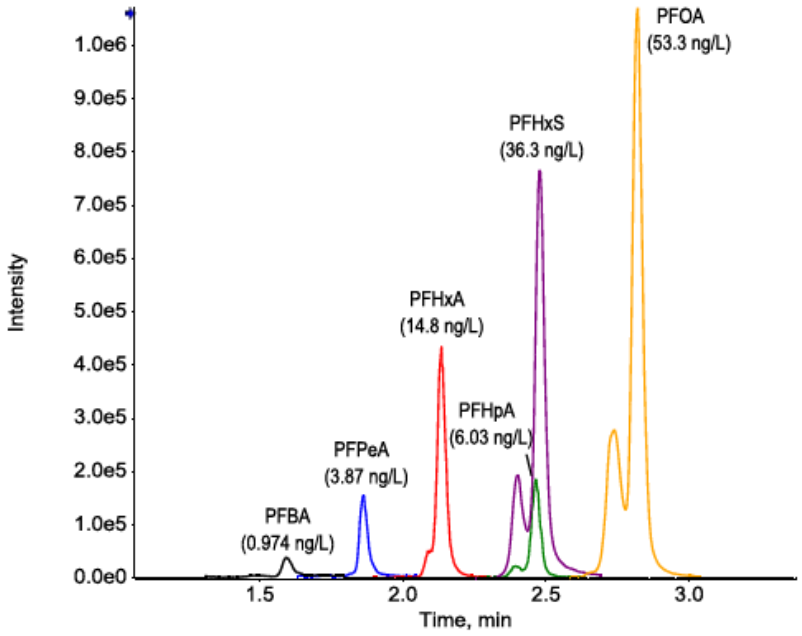
- 3 orders of linear dynamic range
- Excellent signal-to-noise and accuracy of lowest calibrator

Method Performance: Calibration Range, Sensitivity and Accuracy

Compound	Calibration Range (ng/L)	Linear Correlation (r^2)	S:N of 25 ng/L standard	Accuracy of 25 ng/L standard
PFSAs				
PFBS	25-20,000	0.995	31	92%
PFHxS	25-20,000	0.999	604	103%
PFHpS	25-20,000	0.997	103	105%
PFOS	25-20,000	0.995	312	105%
PFDS	25-20,000	0.998	88	102%
Other PFASs				
6:2 FTS	25-20,000	0.991	100	98%
8:2 FTS	25-20,000	0.992	113	97%
PFOSA	25-20,000	0.997	118	104%
MeFOSA	25-20,000	0.996	96	103%
EtFOSA	25-20,000	0.994	90	101%
N-MeFOSAA	25-20,000	0.996	109	100%
N-EtFOSSA	25-20,000	0.994	61	103%

500x concentration factor:
25 ng/L injection std -> 0.05 ng/L in sample; method flexibility

SPE Method Application: Real-World Water Sample



Overlaid chromatogram of PFAS in water sample

* Chromatographic separation of isomers

SPE and LC/MS/MS - Method Summary

- **Sample cleanup and concentration, compatible with DOD QSM 5.1**
- **Short 6.5 min LC method; separation of PFAS compounds and isomers *with HLPC***
  - Good peak shape for lower chain-length PFAS
- **Use of delay column to separate contamination from LC**
- **Method performance:**
  - 3 orders of linear dynamic range, +/- 10% accuracy and excellent S/N for lowest calibrator (25 ng/L), r^2 >0.990
- **Applied to real-world water sample; PFAS detected at 0.97- 53.3 ng/L**
- **Can detect levels well below new EPA drinking water guidelines for PFOA and PFOS**

PFAS using HPLC and Direct Inject LC/MS/MS

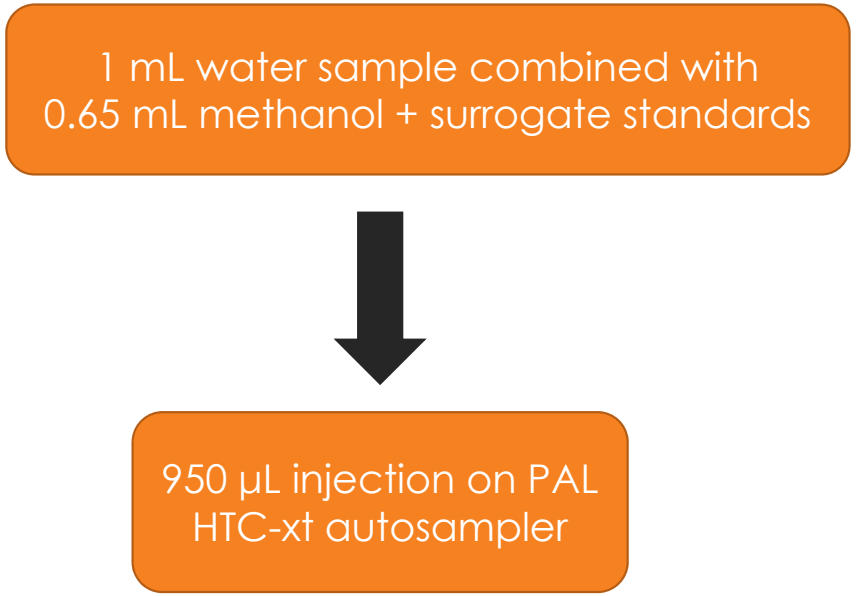
Case Study 2

Craig Butt, Ph.D.
Product Application Specialist

Large Volume Injection Method Overview

- **Advantage: Minimal sample preparation, reduced contamination potential**
- **Overview:**
  - Water samples diluted with methanol + surrogate standards
  - Direct injection of 950 µL onto analytical column
  - Longer and larger diameter column used to improve retention; resulting in longer runtime (17.5 min)

Large Volume Injection Method: Flowchart



Method Overview

- Method not optimized for PFHxDA (C16) and PFODA (C18)
- Presence of 5 g/L Trizma is not compatible with large volume injection method due to ionization suppression (but is compatible with SPE method)
- Mass Spectrometer:
  - SCIEX Triple Quad™5500 system with Turbo V™ source; ESI negative mode
  - Identical source gas and compound-specific parameters as the SPE method

LC Conditions For: Large Volume Injection

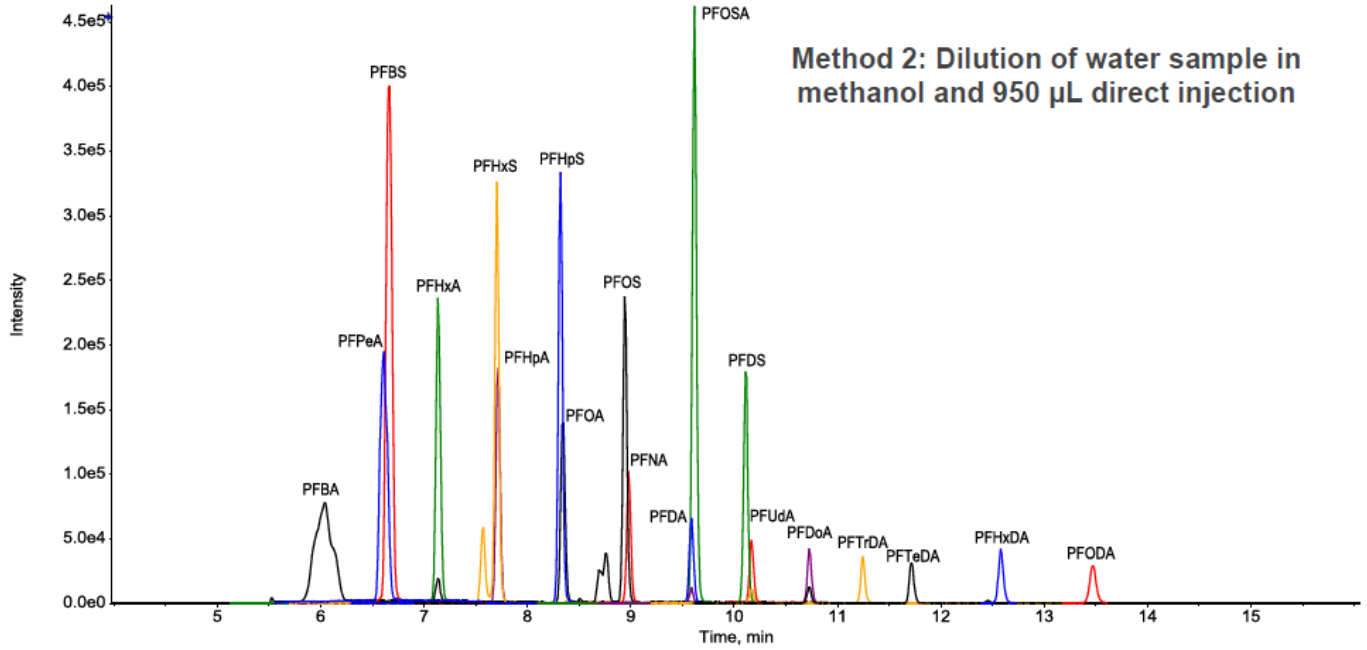
Column: Gemini® 3 µm C18
Dimensions: 100 x 3.0 mm
Part No.: 00D-4439-Y0
Mobile Phase: A: 20 mM Ammonium Acetate in Water
B: Methanol

Gradient:

Time (min)	% B
0	10
1.5	65
8	95
8.1	99
12	99
12.5	10

Injection: 950 µL
Flow Rate: 0.6 mL/min
Temperature: 40 °C
Detection: SCIEX Triple Quad™ 5500 with a Turbo V™ source

Chromatogram: Large Volume Injection Method

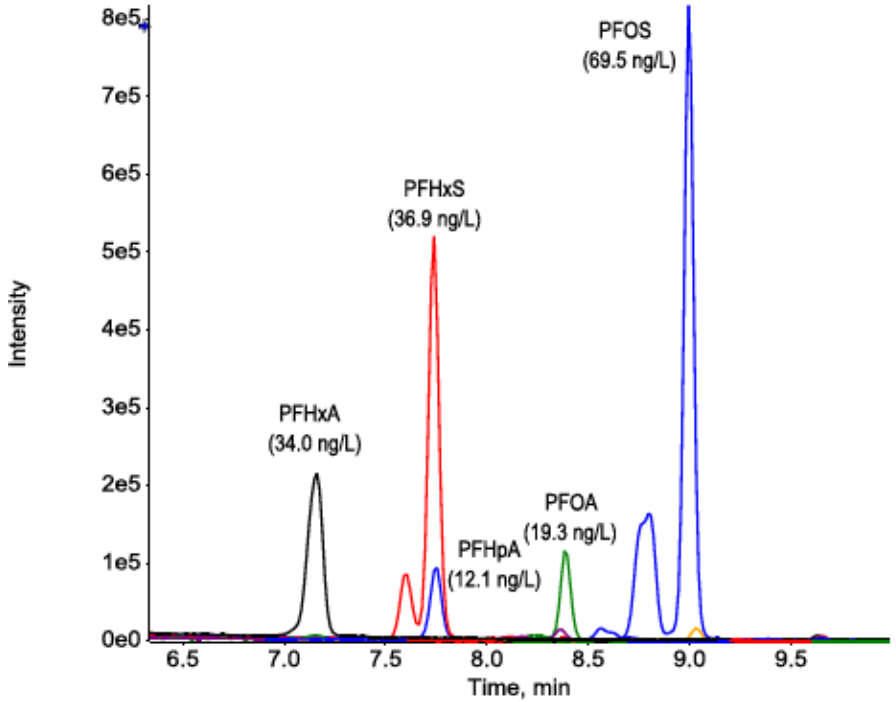


Overlaid chromatogram of 10 ng/L spike into groundwater

Method Performance: Calibration Range, Sensitivity and Accuracy

Compound	Calibration Range (ng/L)	Linear Correlation (r^2)	S:N of 1 ng/L standard	Accuracy of 1 ng/L standard
PFCAs				
PFBA	1-200	0.997	328	97%
PFPeA	1-200	0.999	137	101%
PFHxA	1-200	0.999	284	101%
PFHpA	1-200	0.993	267	96%
PFOA	1-200	0.999	113	99%
PFNA	1-200	0.999	137	101%
PFDA	1-200	0.997	176	96%
PFUdA	1-200	0.998	168	99%
PFDoA	1-200	0.994	127	94%
PFTrDA	1-200	0.995	125	95%
PFTeDA	1-200	0.998	56	98%
PFSAs				
PFBS	2-200	0.994	1178	100%
PFHxS	1-200	0.998	229	96%
PFHpS	1-200	0.999	327	99%
PFOS	1-200	0.999	251	99%
PFDS	1-200	0.999	516	98%
PFOSA	1-100	0.997	1012	96%

Large Volume Injection Method Application



Overlaid chromatogram of PFAS in groundwater sample as analyzed by large volume injection

*Chromatographic separation of isomers

Large Volume Injection Method Summary

- **Minimal sample preparation; dilute with methanol + surrogate standard and inject ("dilute & shoot")**
- **Method performance:**
  - 2 orders of linear dynamic range, +/- 10% accuracy and excellent S/N for lowest calibrator (25 ng/L), r^2 >0.990
- **Excellent method robustness; precision (% CV) was <5% for 9 replicates (20 ng/L) over 1 week analysis**
- **Applied to real-world groundwater sample; PFAS detected at 12.1-69.5 ng/L**
- **Can detect levels below EPA drinking water guidelines (70 ng/L for PFOS and PFOA)**

Environmental Testing

PFAS using SPE or Direct Inject by UHPLC-MS/MS

Case Study 3

LC-MS/MS

Agustin Pierri, Ph.D.
Technical Director

WECK LABORATORIES, INC.

UHPLC Conditions For:

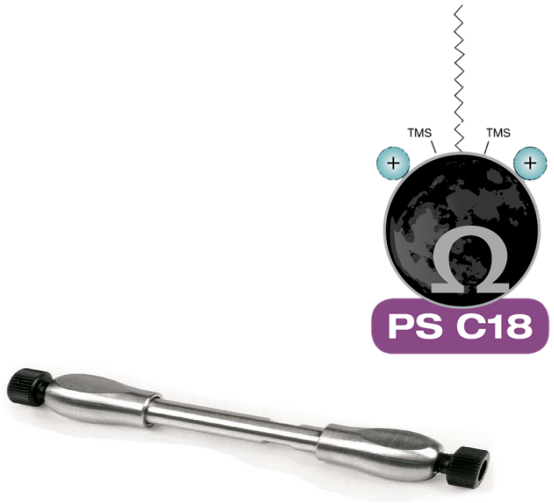
Large Volume Injection and SPE

Column:	Luna Omega 1.6 µm PS C18
Dimensions:	50 x 2.1 mm
Part No.:	00B-4752-AN
Mobile Phase:	A: 5 mM Ammonium Acetate in Water B: Acetonitrile

Gradient:

Time (min)	B (%)
0.0	40
0.5	40
3.0	90
3.1	100
4.0	100

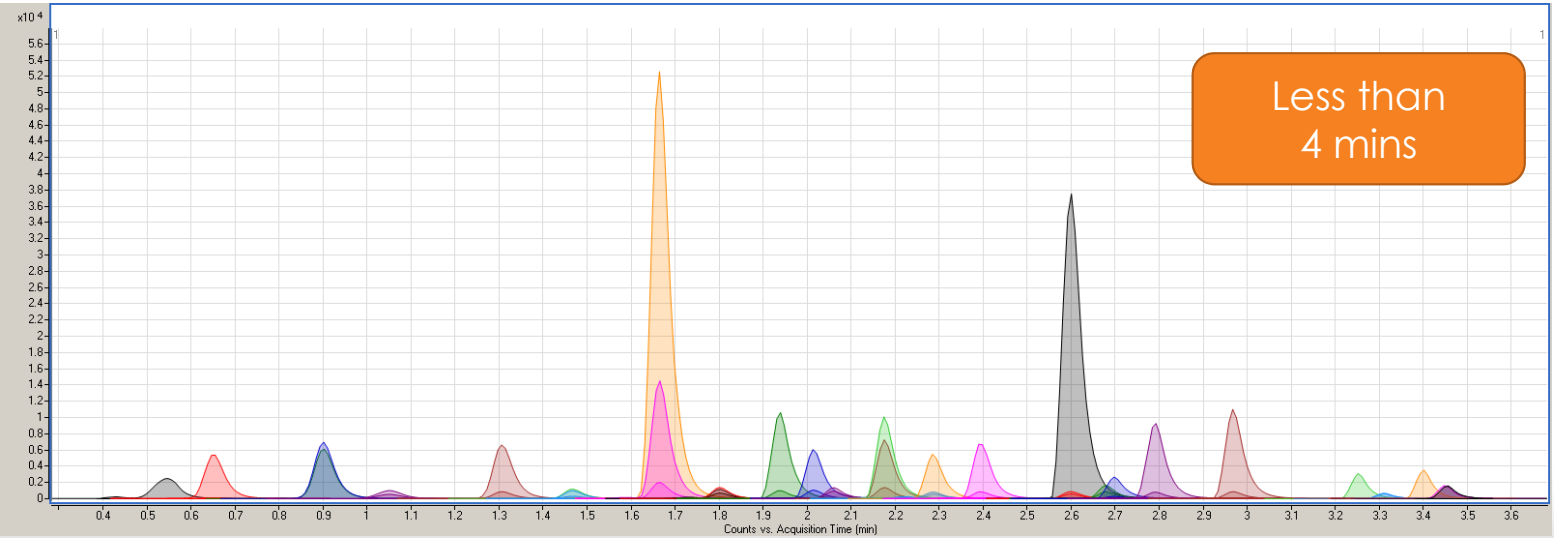
Injection:	1 µL
Flow Rate:	0.55 mL/min
Temperature:	40 °C



Direct Injection Chromatogram

23 PFAS Analytes

6:2 FTS	EtFOSE	PFDS	PFNA	PFTeDA
8:2 FTS	MeFOSE	PFHpA	PFOA	PFTrDA
EtFOSA	PFBA	PFHpS	PFOS	PFUdA
MeFOSA	PFBS	PFHxA	PFPeA	
	PFDA	PFHxS	PFDoA	



PFAS in Butter, Egg, and Milk QuEChERS, SPE and LC-MS/MS

Case Study 3

Dr. Agustin Pierri

WECK LABORATORIES, INC.

Two-step extraction procedure- QuEChERS and Solid Phased Extraction (SPE)-

QuEChERS Protocol

- Add 1.0 gram homogenized sample to 50 mL tube
- Add 10 mL H2O and 10 mL MeCN
- Add 4 g MgSO4 and 1 g NaCl
- Vortex 3 minutes, centrifuge 5 minutes
- Transfer 1 mL aliquot to tube with 150 mg MgSO4 and 50 mg PSA
- Transfer Aliquot for LC-MS/MS analysis
- Analysis was done using on Luna Omega PS C18 50 x 2.1mm

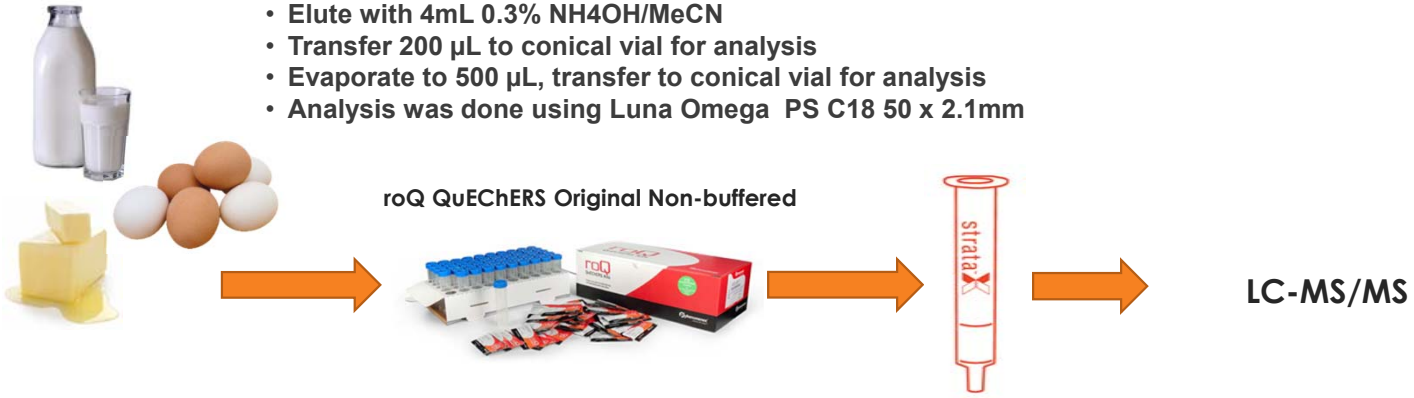
roQ QuEChERS Original Non-buffered

LC-MS/MS

QuEChERS and SPE Protocol

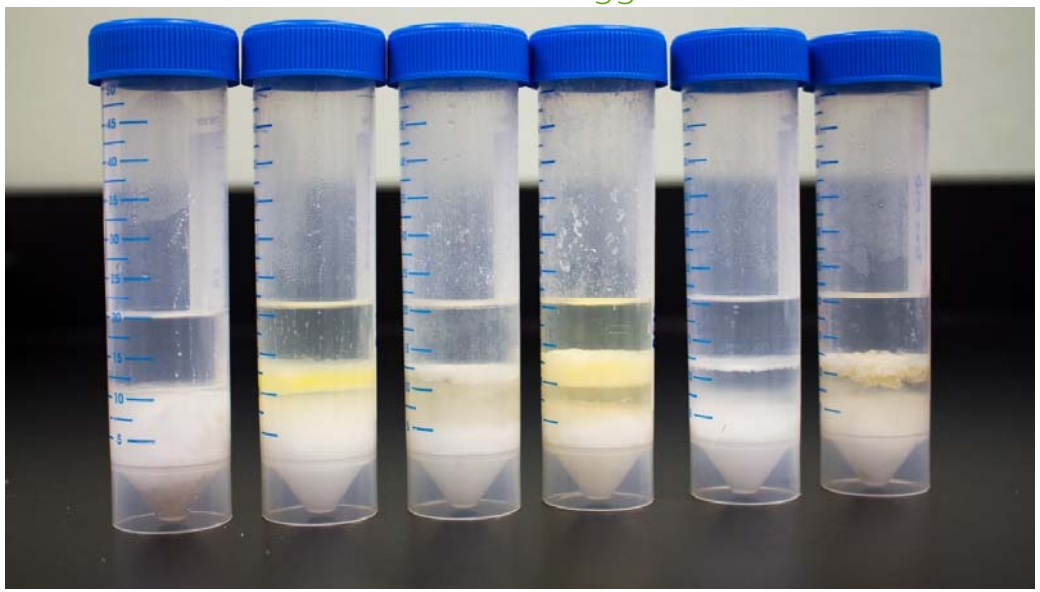
QuEChERS
- Add 1.0 grams homogenized sample to 50 mL tube
- Add 10 mL H2O and 10 mL MeCN
- Add 4 g MgSO4 and 1 g NaCl
- Vortex 3 minutes, centrifuge 5 minutes
- Transfer 1 mL aliquot to tube with 150 mg MgSO4 and 50 mg PSA
- Transfer 500 µL, dilute to ~15 mL with H2O for SPE

SPE
- Condition Strata-XAW 200 mg/3 mL SPE cartridge with 0.3% NH4OH/MeCN
- Load diluted QuEChERS extract, wash with 5 mL H2O
- Elute with 4mL 0.3% NH4OH/MeCN
- Transfer 200 µL to conical vial for analysis
- Evaporate to 500 µL, transfer to conical vial for analysis
- Analysis was done using Luna Omega PS C18 50 x 2.1mm

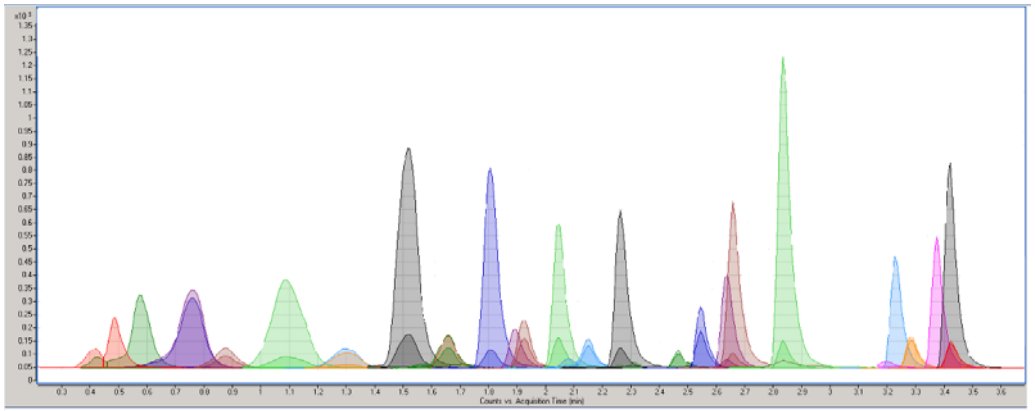


Extracts

Blank Butter Cheese Egg Milk Fish



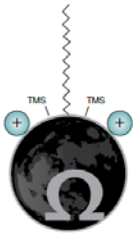
PFAS in Butter using SPE and LC-MS/MS



We would like to provide special thanks to Agustin Pierre from Weck Laboratories for contributing this application.

WECK LABORATORIES, INC.

LUNA OMEGA PS C18



Column:	Luna Omega 1.6 µm PS C18
Dimensions:	100 x 2.1 mm
Part No.:	00D-4752-AN
Mobile Phase:	A: 5 mM Ammonium Acetate in Water B: Acetonitrile

Gradient:

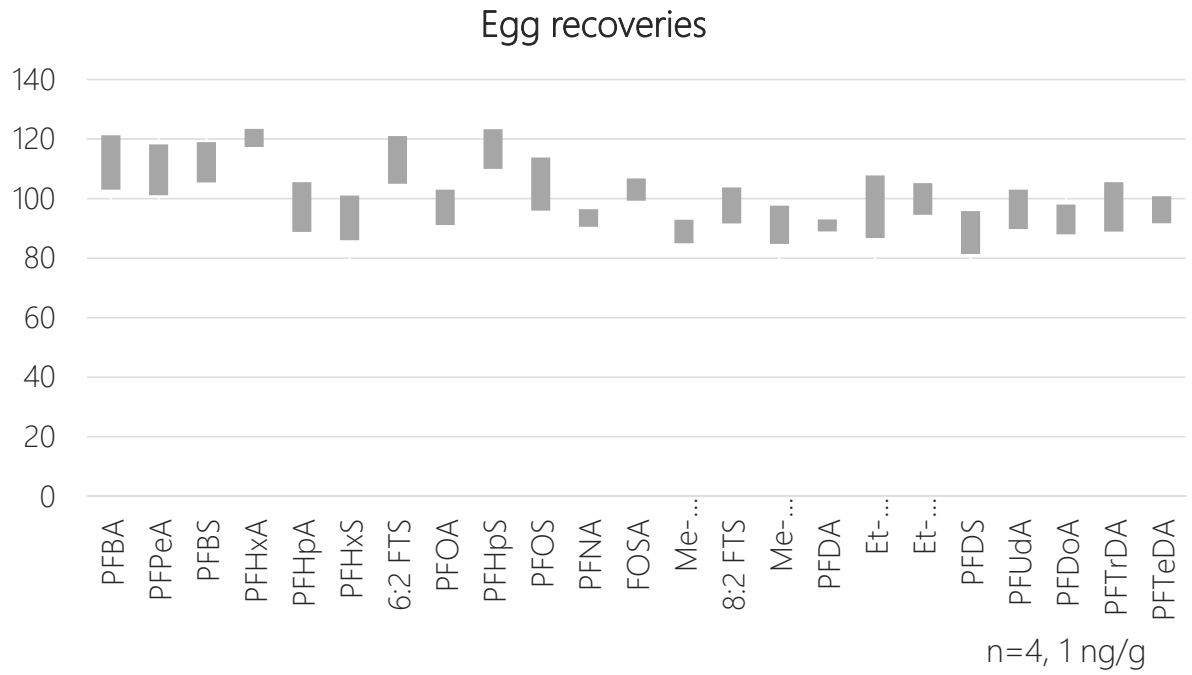
Time (min)	B (%)
0.0	40
0.5	40
3.0	90
3.1	100
4.0	100

Injection:	2 µL
Flow Rate:	0.55 mL/min
Temperature:	40 °C
Detection:	MS/MS

Analytes

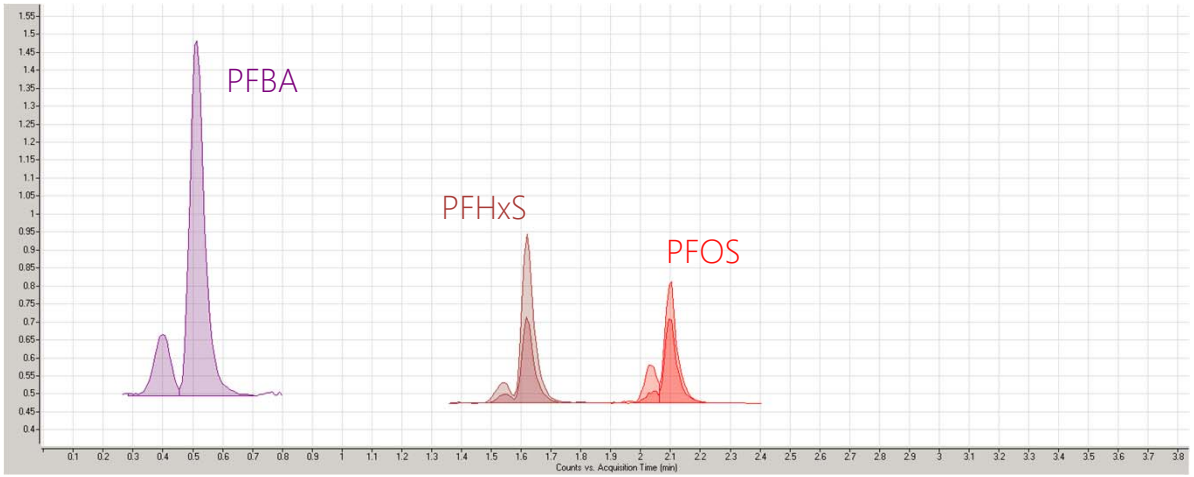
1. 6:2 FTS
2. 8:2 FTS
3. EtFOSA
4. EtFOSE
5. FOSA
6. MeFOSA
7. MeFOSE
8. PFBA
9. PFBS
10. PFDA
11. PFDoA
12. PFDS
13. PFHpA
14. PFHpS
15. PFHxA
16. PFHxS
17. PFNA
18. PFOA
19. PFOS
20. PFPeA
21. PFTeDA
22. PFTrDA
23. PFUdA

QuEChERS Recoveries, Egg



Egg recoveries: QuEChERS + SPE

Branched vs. linear



PFAS using Online SPE by HPLC-MS/MS

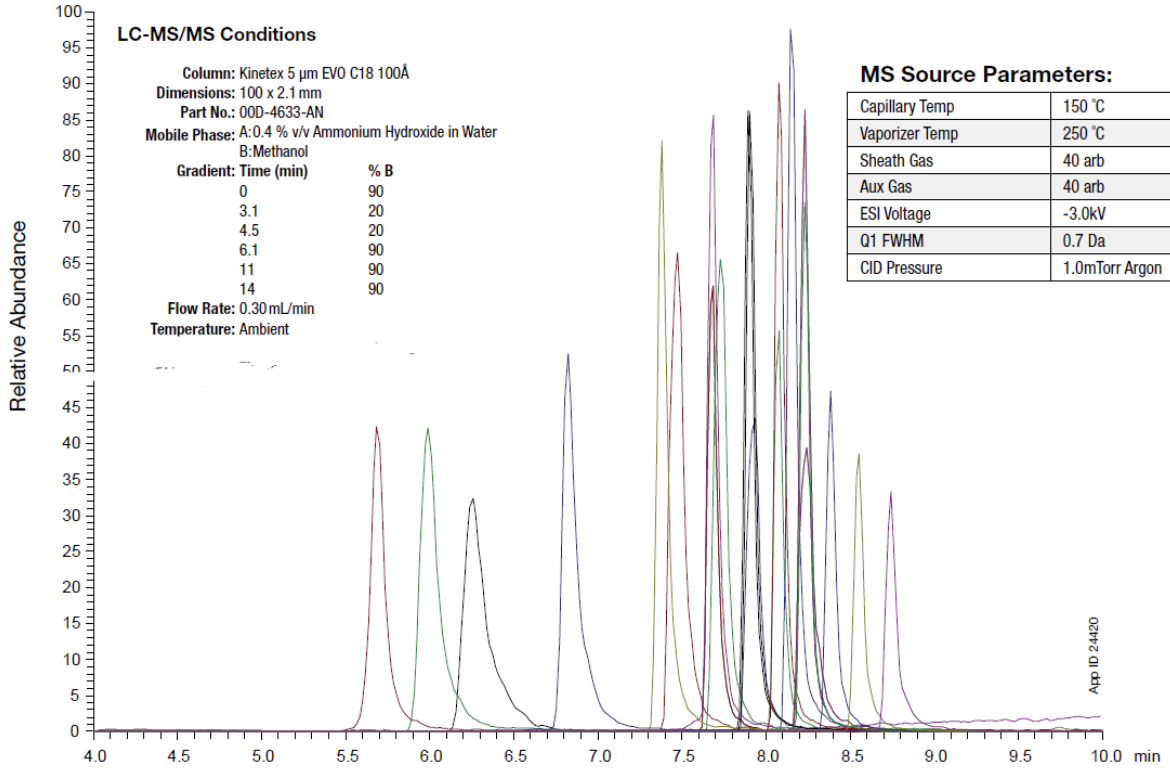
Case Study 4

LC-MS/MS

David Schiessel

BABCOCK Laboratories, Inc.
The Standard of Excellence for Over 100 Years

Evaluation of Online SPE Sorbents for PFAS



Sample Prep 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 µm 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.

LC Gradient (pump 1)

Time (min)	Water (%)	MeOH (%)	0.4% NH3 (%)
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.

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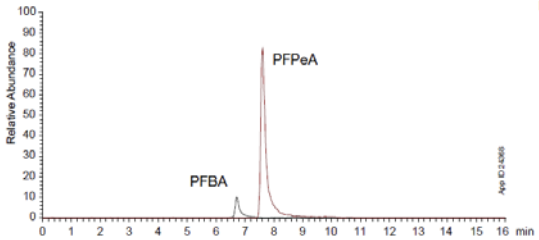
Online SPE Program (pump 2)

Time (min)	Water (%)	MeOH (%)	AcN (%)	Flow (mL/min)	Step
0.00	100	0	0	2.5	Load
2.00	100	0	0	2.5	Load
2.10	100	0	0	2.5	Wash
4.10	100	0	0	2.5	Wash
4.11	30	70	0	0	Idle
9.00	30	70	0	0	Idle
9.01	0	0	100	2.0	Wash
9.49	0	0	100	2.0	Wash
9.50	2.0	98	0	3.0	Wash
11.50	2..0	98	0	3.0	Wash
11.51	100	0	0	3.0	Equil
14.00	100	0	0	3.0	Equil

Evaluation of Online SPE Sorbents for PFAS

Online SPE using C18-E sorbent and 2.0 mM ammonium acetate mobile phase modifier on a Luna Omega C18 50 mm column.



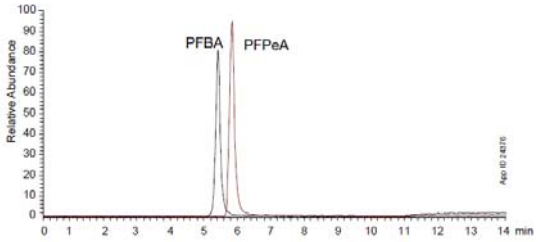
LC-MS/MS Conditions

Column: Luna® Omega 1.6 µm C18 100Å
Dimensions: 50 x 2.1 mm
Part No.: 008-4742-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

Online SPE using Strata-X-AW sorbent and 0.4-0.8% ammonia mobile phase modifier on a Kinetex C18 EVO 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

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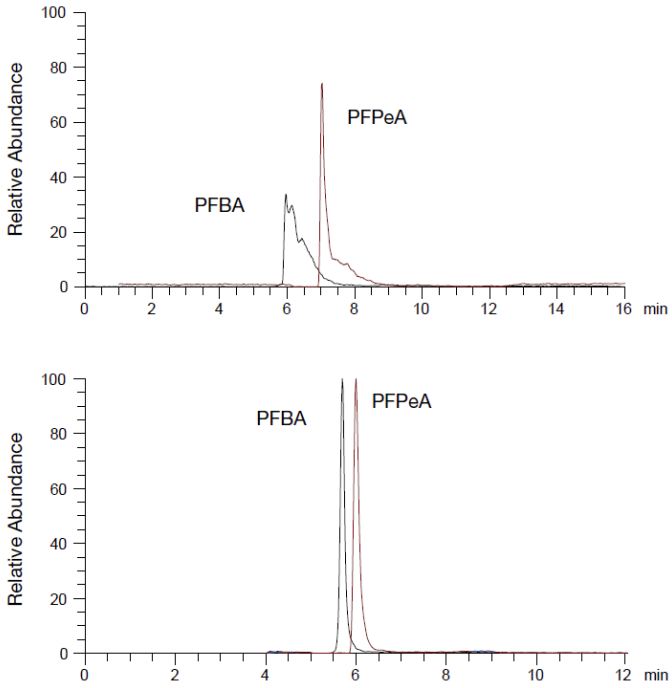
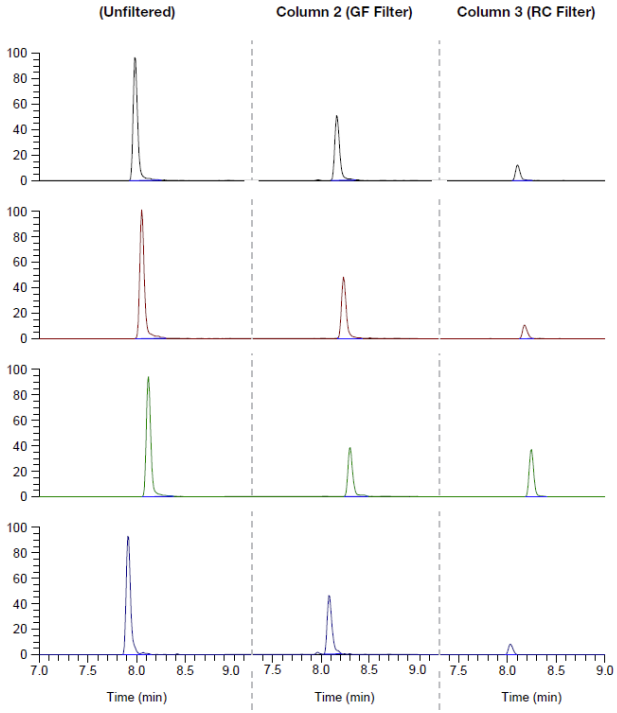
Environmental Testing

Elution strength of 0.04% NH_3 (top) and 0.24% NH_3 (bottom) illustrating more efficient elution of analytes (PFBA and PFPeA) with increased base concentration in the mobile phase.



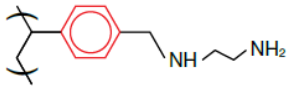
Effect of filtering on recovery of long chain PFCs.



Evaluation of Online SPE Sorbents for PFAS

Options	Column	Strata SPE Sorbent	Sample pH	SPE Conditioning pH	Eluent*	PFBA / PFPeA %	Shape
1	Kinetex EVO C18 5 µm 100 x 2.1mm	X-AW	Trizma (pH=7)	neutral	0.24-0.04 % NH_3	100	excellent
2	Kinetex EVO C18 5 µm 50 x 2.1mm	X-AW	neutral	neutral	0.04 % NH_3	106	very poor
3	Kinetex EVO C18 5 µm 50 x 2.1mm	X-AW	neutral	neutral	0.24-0.04 % NH_3	76	OK
4	Kinetex EVO C18 5 µm 50 x 2.1mm	X-AW	acidic	neutral	0.02 % Formic Acid	13	OK
5	Luna Omega C18 1.6 µm 50 x 2.1mm	C18	neutral	neutral	2 mM NH_4OAc	<1	—
6	Luna Omega C18 1.6 µm 50 x 2.1mm	C18	acidic (pH=2)	acidic (pH=2)	0.02 % Formic Acid	22	very poor
7	Luna Omega C18 1.6 µm 50 x 2.1mm	C18	acidic (pH=2)	acidic (pH=2)	2 mM NH_4OAc	11	OK
8	Luna Omega C18 1.6 µm 50 x 2.1mm	C18	neutral	acidic (pH=2)	2 mM NH_4OAc	11	OK
9	Luna Omega C18 1.6 µm 50 x 2.1mm	X	neutral	neutral	2 mM NH_4OAc	5.9	poor
10	Luna Omega C18 1.6 µm 50 x 2.1mm	X	acidic	neutral	2 mM NH_4OAc	5.1	poor

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



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PFAS in Sediment using QuEChERS

Case Study 5

Syljohn Estil and Eric Nelson

SANITATION DISTRICTS OF LOS ANGELES COUNTY

PFAS in Sediment using QuEChERS

Sample Preparation

QuEChERS Extraction Protocol

Step 1

Extraction

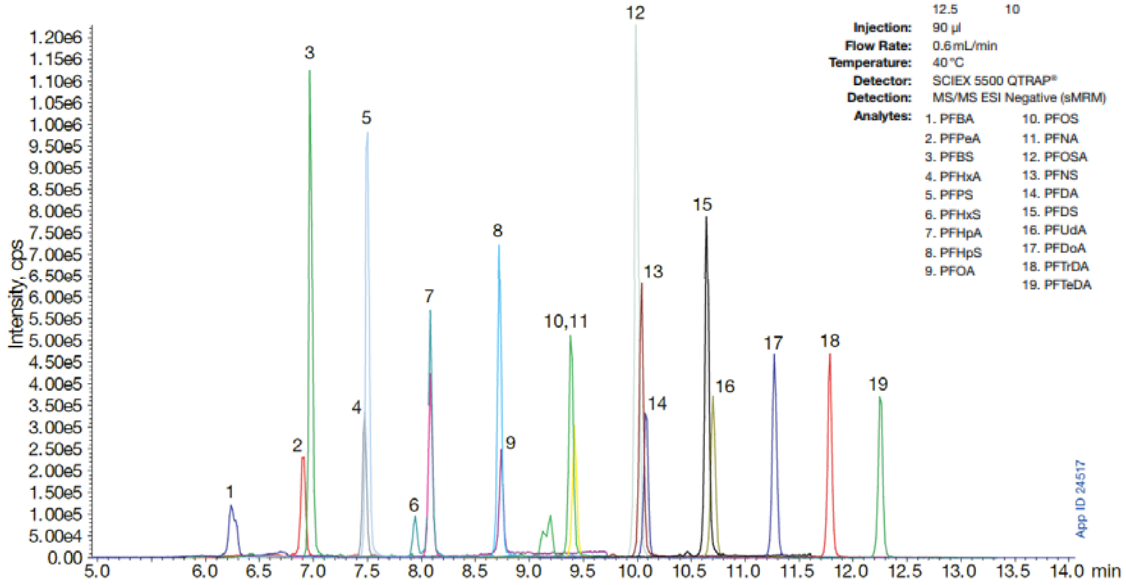
1. Weigh 2.0 g of dried sediment into a polypropylene container and spike with isotopically-labeled internal standards
2. Add 10mL deionized water and vortex. Add 10mL acidified acetonitrile (1% acetic acid) to the slurry and vortex
3. Add the extraction salts (1.5 g Sodium Acetate and 2 g $MgSO_4$) to the sample and vortex for 1 minute
4. Centrifuge the samples for 5 minutes at 4000 rpm
5. Place the samples in a rack and freeze at -20° for 30-60 minutes. This freezing step allows for easier extraction of the supernatant

Step 2

Clean-up

1. Transfer 8-9mL of the acetonitrile supernatant into a roQ QuEChERS PSA/C18 dSPE clean-up tube (Part no. KS0-8926) and vortex for one minute
2. Centrifuge the dSPE tubes for 10 minutes at 3000 rpm
3. Place an aliquot of the extract in a HPLC vial and dilute 1:1 with deionized water. The sample is now ready for analysis

Extracted ion chromatogram of sediments spiked with 1.0 ng/g of the target analytes



HPLC-MS/MS Conditions

Column:	Gemini® 3 µm C18
Dimensions:	100 x 3 mm
Part No.:	00D-4439-Y0
Inline Filter:	Phenomenex Krudkatcher™ Ultra
Delay Column:	Luna® 5 µm C18 (2) 30 x 2.0 mm
Part No.:	00A-4252-B0
Mobile Phase:	A: 20 mM Ammonium acetate in water
	B: Methanol
Gradient:	**Time (min) % B**
	0.0 10
	1.5 65
	8.0 95
	8.1 99
	12.0 99
	12.5 10
Injection:	90 µl
Flow Rate:	0.6 mL/min
Temperature:	40 °C
Detector:	SCIEX 5500 QTRAP®
Detection:	MS/MS ESI Negative (sMRM)

Analytes:

1. PFBA
2. PFPeA
3. PFBS
4. PFHxA
5. PFPS
6. PFHxS
7. PFHpA
8. PFHpS
9. PFOA
10. PFOS
11. PFNA
12. PFOSA
13. PFNS
14. PFDA
15. PFDS
16. PFUdA
17. PFDoA
18. PFTrDA
19. PFTeDA

Conclusion

Solid Phase Extraction or Large-Volume Injection are both suitable sample preparation techniques

Delay column to distinguish system related PFAS interferences

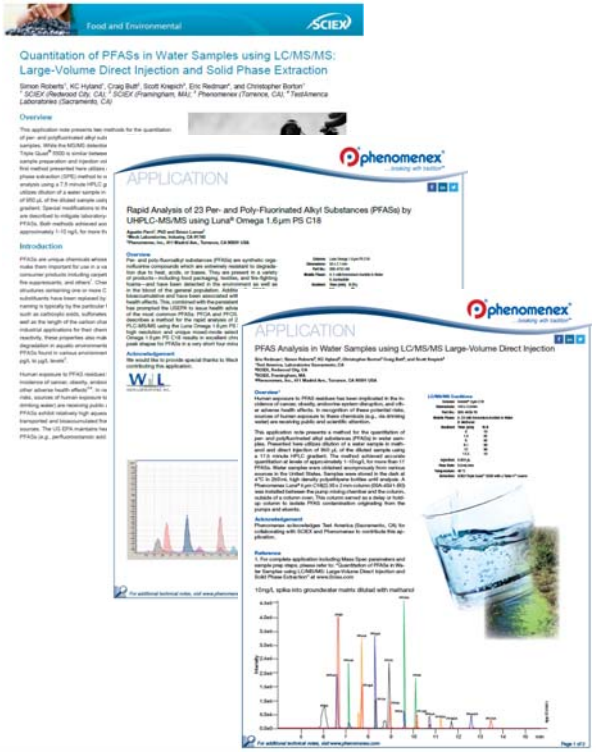
HPLC and UHPLC column chemistries suitable for the chromatographic range of polar acids through non-polar acids, esters, amides, and sulfonamides with selectivity of branched vs. linear isomers

SCIEXTriple Quad™ 5500 with Turbo V™ source

Detection limits at low ppt levels with the ability to detect below drinking water guidelines

Environmental Support and Resources

- SPE Method Development Tool
- 1,000s of Applications
- On-Site Lab Demos
- Environmental Edge Newsletter
- Technical Notes
- Digital Learning Tutorials



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Thank You
Acknowledgements

We would like to provide special thanks to Dr. Craig Butt and Dr. Simon Roberts at SCIEX, Dr. Agustin Pierri from Weck Laboratories, Syljohn Estil at Sanitation Districts of LA, and David Schiessel at BABCOCK Laboratories, for their contributions.