Single-Injection Screening and Quantitation of Gen-X and other PFASs in Water and Soil using various Tandem Mass Spectrometry Workflows

Oscar G. Cabrices PhD
Market Development Manager - Americas
Food, Beverage, Environmental, Industrial & Forensics Testing

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Ways SCIEX is working to make environmental testing easier

1 **Simplify the workflows**

- Develop workflows that are rapidly adaptable and compliant

2 **Digging through the noise**

- Enhancing selectivity to quantify and identify with confidence

3 **Taking your sensitivity higher**

- See more or Inject Less

SCIEX and PFAS

INSTRUMENT COMPATIBILITY

- SCIEX instruments excel in negative ion mode

- Although chemically inert, some PFAS congeners are prone to source fragmentation

  - Declustering and transmission by QJet is remarkably soft, prevents fragmentation

  - Only vendor that can prevent source fragmentation for some PFAS analyte classes

    - Extremely important for non-target discovery and quantitation

INDUSTRY-LEADING EXPERTISE

- SCIEX application chemists extensive experience with PFAS applications have authored/co-authored dozens of peer-reviewed publications

  - Combined 30+ years of experience across EFAB team

  - ~2000 total citations

- Extensive experience helping labs get set up for PFAS analysis, building PFAS methods

- Collaborations with PFAS experts

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PFAS
Targeted Quantitation using SCIEX TripleQuad™ Systems

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SCIEX Triple Quad™ 5500+ System - QTRAP Ready

- For labs that need to quantify a large list of targets and demand high quality quantitation results in the 1st injection.

- Only the SCIEX Triple Quad 5500+ system delivers high-end speed and dynamic range, with an upgrade path to include QTRAP® System functionality
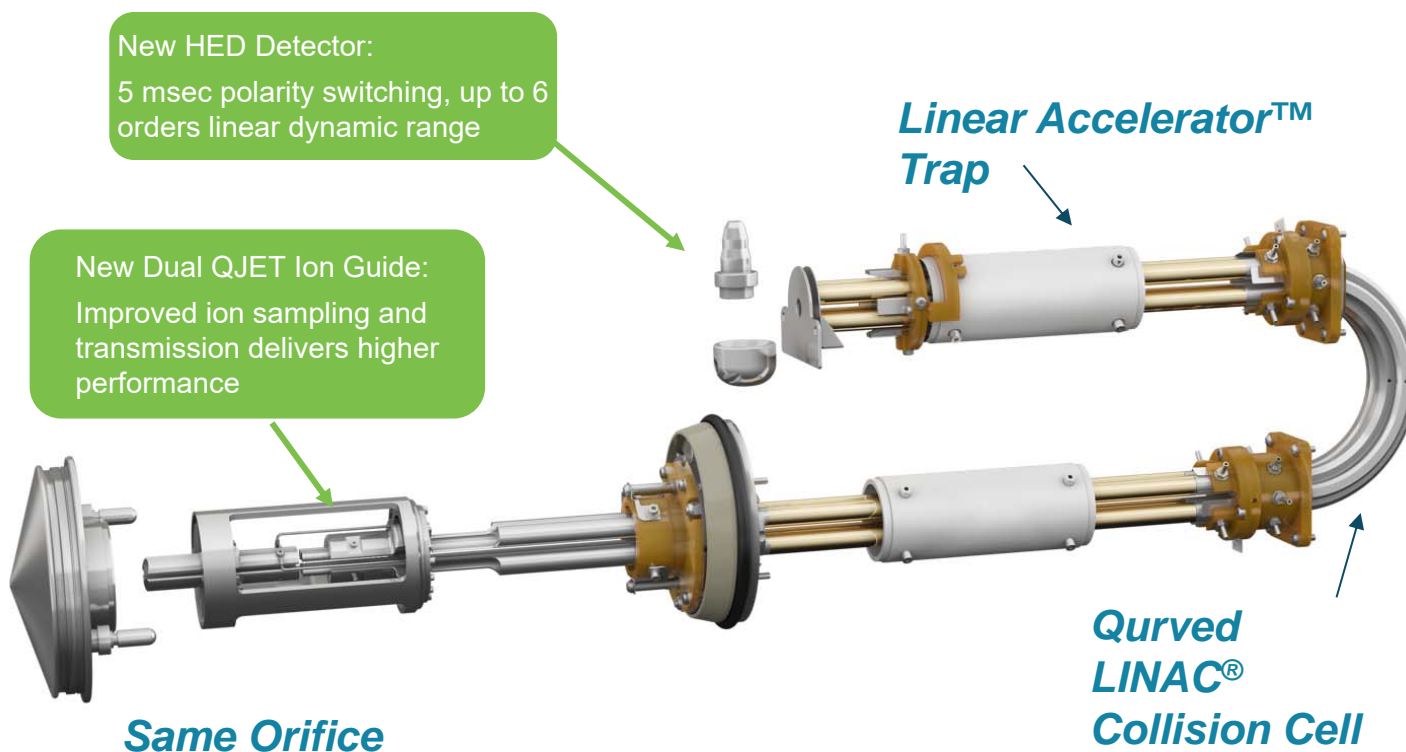
OVERVIEW OF WHAT IS DIFFERENT FROM QTRAP 5500 SYSTEM

- QTRAP System Enabled – field upgradable

- Detector System – HED detector enables

  ✓ Fast polarity switching

  ✓ Expanded Linear Dynamic Range

- Dual QJet® Ion Guide

- Analyst® Software 1.7.1 for Acquisition

- SCIEX OS-Q Software for Data Processing

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Hybrid Triple Quadrupole Linear Ion Trap Technology

NEW ON SCIEX TRIPLE QUAD™ 5500+ SYSTEM – QTRAP® READY

New HED Detector:

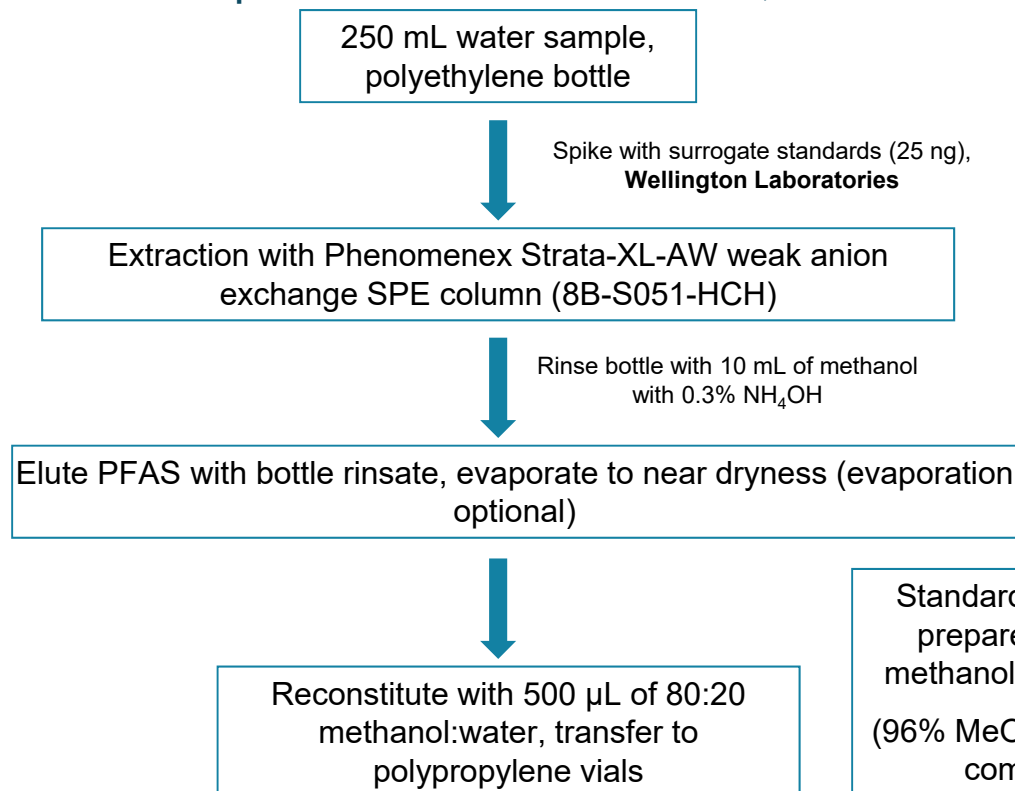5 msec polarity switching, up to 6 orders linear dynamic range

New Dual QJET Ion Guide:

Improved ion sampling and transmission delivers higher performance

Linear Accelerator™ Trap

Qurved LINAC® Collision Cell

Same Orifice

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Turbo V™ Source

- Built for robustness, simple to use

- Orthogonal spray design with V heater configuration focuses heat in the optimal spot to achieve high sensitivity from the ESI droplets

- Versatile flow ranges - high analytical flow (5 – 3000 µL/min), microflow rates using hybrid electrodes

- Wide compound class coverage with additional APCI probe

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EPA Method 537.1 – Sample Preparation

Method requires SPE concentration, and 10 uL injection on LC-MS/MS System

250 mL water sample, polyethylene bottle

⬇ Spike with surrogate standards (25 ng), **Wellington Laboratories**

Extraction with Phenomenex Strata-XL-AW weak anion exchange SPE column (8B-S051-HCH)

⬇ Rinse bottle with 10 mL of methanol with 0.3% NH_4OH

Elute PFAS with bottle rinsate, evaporate to near dryness (evaporation optional)

⬇

Reconstitute with 500 µL of 80:20 methanol:water, transfer to polypropylene vials

Standards and blanks prepared with 80% methanol concentration

(96% MeOH for EPA 537 compliance)

MUST BE PERFORMED ON TRIPLE QUAD OR QTRAP FOR METHOD COMPLIANCE

phenomenex
...breaking with tradition℠

On-line Extraction Cartridges

Microelution Plates

96- Well Plates

1,3, and 6mL SPE Tubes

Giga SPE Tubes (12 - 150mL tubes)

strata X

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EPA Method 537.1 – Chromatography

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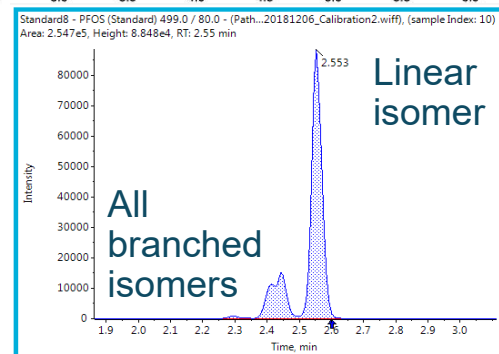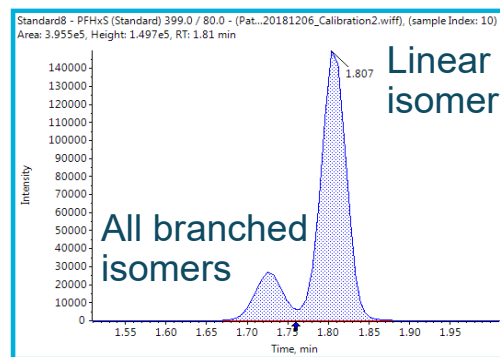**Mobile Phase:** A: 20 mM Ammonium Acetate in Water
B: Methanol

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

PFAS standard mix at 50 ng/L (ppt) containing all 14 compounds in EPA 537.1 eluting within 6 minutes for an 11 minute total run time with a 10 uL injection.

1) N-MeFOSAA
2) N-EtFOSAA
3) MeFOSA
4) EtFOSA

Standard8 - PFHxS (Standard) 399.0 / 80.0 - (Pat...20181206_Calibration2.wiff), (sample Index: 10)
Area: 3.955e5, Height: 1.497e5, RT: 1.81 min

Linear isomer

All branched isomers

Standard8 - PFOS (Standard) 499.0 / 80.0 - (Path...20181206_Calibration2.wiff), (sample Index: 10)
Area: 2.547e5, Height: 8.848e4, RT: 2.55 min

Linear isomer

All branched isomers
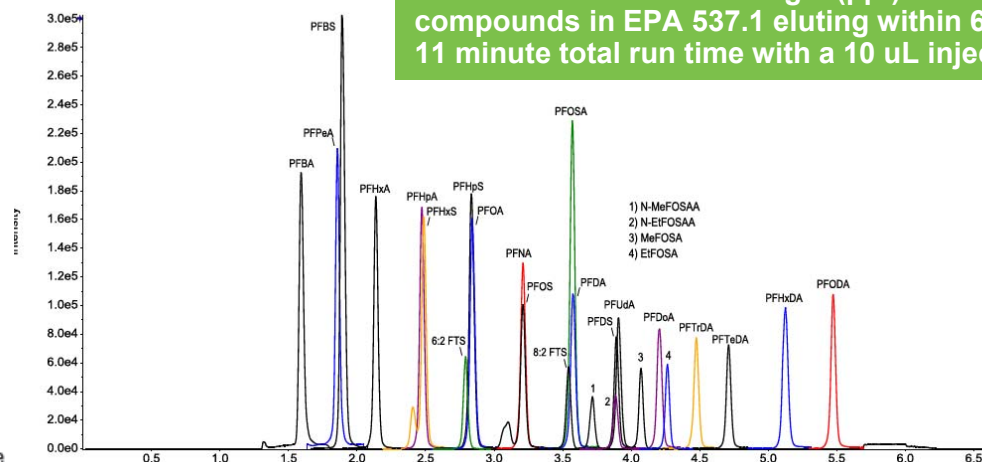
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EPA Method 537.1 – Performance

Calibration for PFBS: y = 748.64042 x (r = 0.99848) (weighting: 1 / x)

PFBS
$r^2 = 0.997$

Calibration for PFHxA: y = 1639.41525 x (r = 0.99759) (weighting: 1 / x)

PFHxA
$r^2 = 0.995$

Calibration for PFOA: y = 1830.80571 x (r = 0.99542) (weighting: 1 / x)

PFOA
$r^2 = 0.991$

Calibration for PFOS: y = 579.44876 x (r = 0.99887) (weighting: 1 / x)

PFOS
$r^2 = 0.998$

Calibration for N-MeFOSAA: y = 179.04309 x (r = 0.99774) (weighting: 1 / x)

n-MeFOSAA
$r^2 = 0.995$

Calibration for PFTeDA: y = 1876.00907 x (r = 0.99690) (weighting: 1 / x)

PFTeDA
$r^2 = 0.994$

✓ Linearity of 6 of the PFASs analyzed out of the suite of 14 showing $R^2 > 0.99$ with a linear fit forced through the origin and 1/x concentration weighting.

✓ The other 8 PFAS compounds also showed $R^2 > 0.99$

✓ Asymmetry factor (AF) calculated for the first 2 eluting peaks, PFBS and PFHxA, at a mid-point standard concentration of 500 ng/L

PFHxA
AF: 1.1

PFBS
AF: 1.0

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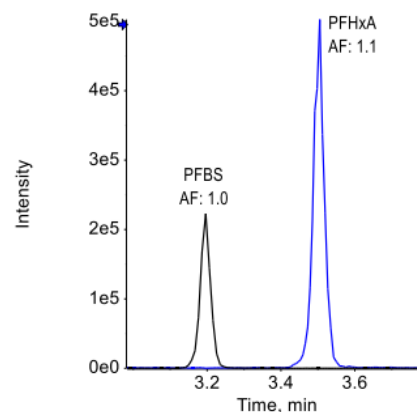
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Mitigating Background PFAS Contamination

FOR ALL PFAS METHODS…

- 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

Delay Column

(Luna C18 30x3mm x 5 µm)

Pumps

Autosampler
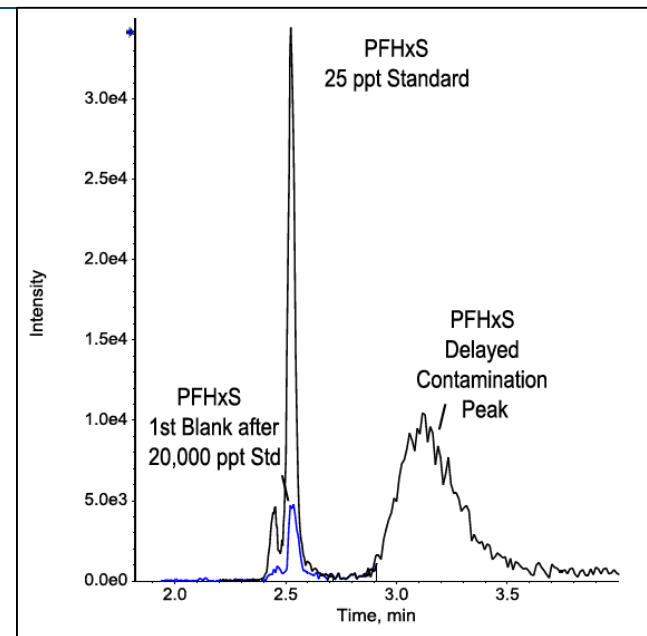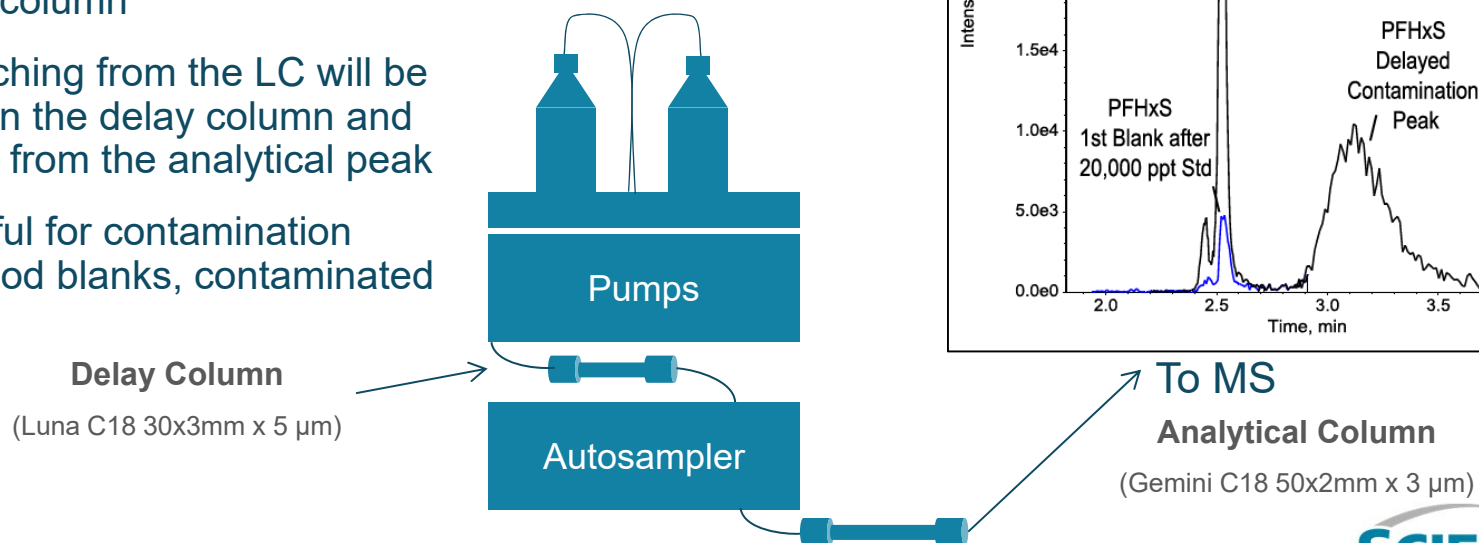
To MS

Analytical Column

(Gemini C18 50x2mm x 3 µm)

PFHxS
25 ppt Standard

PFHxS
1st Blank after
20,000 ppt Std

PFHxS
Delayed
Contamination
Peak

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Something's in the Water: Tackle Your PFAS Analysis

Posted August 15, 2018 at 5:42 AM
By Community Manager

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Exposure to per- and polyfluoroalkyl substances (PFAS) residues could be dangerous. These chemicals have been linked to a variety of adverse health effects including liver damage, thyroid disease, decreased fertility, high cholesterol, obesity, hormone suppression, and cancer. In recognition of these potential risks, these water contaminants are making public headlines as well as becoming core topics for reports, and scientific studies.

What are PFAS'? PFAS are unique chemicals that repel both oil and water; this, among other properties, makes them useful in a variety of applications. Globally, there are more than 3000 products that either once used or still use PFAS[3]. Common examples include food packaging, stain-resistant fabrics and carpets, non-stick kitchenware, paints, adhesives, electronics, personal care products, and firefighting foams[1]. However, there is only a limited amount of information on the extent of PFAS usage as well as how much has accumulated in the environment and made their way into our drinking water systems[2].

These substances are mixtures of humanmade fluoropolymers containing carbon-fluorine monomers[1]. Perfluorinated compounds are those in which all hydrogen atoms have been replaced by fluorine[1,4]. It is the stability of the C-F bonds which prevents these compounds from naturally decomposing in the environment and makes them highly resistant to acids, bases, oxidants, and heat. Whereas, the term 'polyfluorinated' applies to chemicals where not all the hydrogens on the carbons of the molecule are replaced by fluorine[1,4]. Per- and polyfluorinated substances represent a compound class of potentially hundreds of chemicals of varying chemical structures and behaviors.

But how is it getting into our drinking water? Although there is a lack of conclusive data to pinpoint the exact source, there are researchers who suggest that extensive exposure to PFAS in water is partly from firefighting foams and sprays used in training simulations by the military and airport workers.

So, what does this all mean for public health? Health effects are widely debated, though there are many studies that link PFAS exposure to a variety of health problems.[5] For that reason, regulators such as the U.S. Environmental Protection Agency (EPA) set "Health Advisory" levels of PFOA and PFOS.

Food and Environmental   SCIEX

Quantitation of PFASs in Water Samples using LC-MS/MS Large-Volume Direct Injection and Solid Phase Extraction

Simon Roberts[1], KC Hyland[1], Craig Butt[2], Scott Krepich[3], Eric Redman[4], and Christopher Borton[1]
[1] SCIEX (Redwood City, CA), [2] SCIEX (Framingham, MA), [3] Phenomenex (Torrence, CA), [4] TestAmerica Laboratories (Sacramento, CA)

Overview

This application note presents two methods for the quantitation of per- and polyfluorinated alkyl substances (PFASs) in water samples. While the MS/MS detection method using the SCIEX Triple Quad™ 5500 is similar between the two methods, the sample preparation and injection volume differ significantly. The first method presented here utilizes a weak-anion exchange solid phase extraction (SPE) method to concentrate water samples for analysis using a 7.5 minute HPLC gradient. The second method utilizes dilution of a water sample in methanol and direct injection of 950 μL of the diluted sample using a 17.5 minute HPLC gradient. Special modifications to the pumps and autosampler are described to mitigate laboratory-based contamination of PFASs. Both methods achieved accurate quantitation at levels of approximately 1-10 ng/L for more than 17 PFASs.

Introduction

PFASs are unique chemicals whose physicochemical properties make them important for use in a variety of industrial and consumer products including carpets, cookware, food packaging, fire suppressants, and others[1]. Chemically, PFASs are aliphatic structures containing one or more C atoms on which H substituents have been replaced by F atoms. Classification and naming is typically by the particular functional group present, such as carboxylic acids, sulfonates, phosphonic acids, etc., as well as the length of the carbon chain. Desirable in various industrial applications for their chemical stability and low

in water, but these levels have been exceeded in some areas experiencing extreme point source inputs of these chemicals[5].

Given the tremendous persistence of PFASs in the environment and their known presence in human populations exposed via drinking water and other environmental routes, demonstration of the capability for accurate and precise low-level quantitation is paramount for research and testing laboratories. Robust quantitative analytical methods utilize the specificity and sensitivity of LC-MS/MS with MRM monitoring. However, a primary analytical challenge to this assay is the prevention and reduction of background PFASs originating from the LC system and contamination during sample collection and preparation. The two analytical methods described here employ strategies to address PFAS contamination. These include the use of a delay column for separation of a contamination PFAS peak from the

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PFAS
Targeted Quantitation
using High Resolution
Mass Spectrometry
(HRMS)

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Instrumental Methods

- X500R QTOF System

- ExionLC™ system

- Electrospray Ionization with Turbo V™ Ion Source

- Column: Phenomenex Gemini C18, 3 mm x 100 mm, 3 µm particle

- MPA: Water (20 mM ammonium acetate), MPB: Methanol

- Injection Volume = 100 µL

- Column oven = 40 °C

Time (min)	Flow (mL/min)	A%	B%
0.0	0.600	90	10
0.5	0.600	30	70
6.0	0.600	1	99
10.0	0.600	1	99
10.1	0.600	90	10
12.0	0.600	90	10

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14

MRM^{HR}

HIGH RESOLUTION FRAGMENT IONS
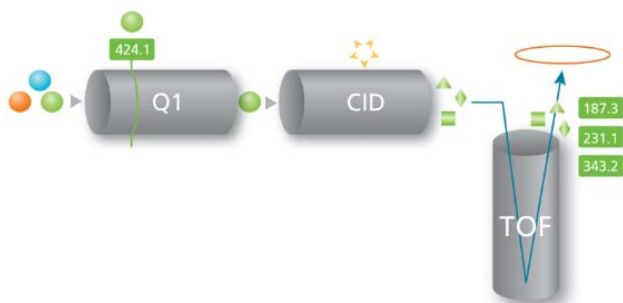
- High resolution fragment ions results in greater compound specificity

- Optimized CE for each MRM

- Scheduled MRM capability to minimize scan time; unique retention time window for each MRM

- Increased method flexibility

Best MS/MS workflow for sensitivity

MS/MS only acquired for targeted compounds

	Compound ID	Group name	Precursor ion (Da)	Fragment ion (Da)	Accumul...	Declusteri...	Collision energy (V)	Retention time (min)	Retention ti...
1	A5760 1	A5760	205.97	122.0255	0.1500	-100	-20	3.40	30
2	A5760 2	A5760	205.97	106.0305	0.1500	-100	-20	3.40	30
3	A5760 3	A5760	205.97	78.0354	0.1500	-100	-24	3.40	30
4	F4106 1	F4106	219.94	156.0239	0.1500	-60	-18	6.24	30
5	F4106 2	F4106	219.94	77.9664	0.1500	-60	-32	6.24	30
6	F4106 3	F4106	219.94	141.0004	0.1500	-60	-24	6.24	30
7	UJV12 1	UJV12	293.00	205.9705	0.1500	-60	-24	2.49	30
8	UJV12 2	UJV12	293.00	77.9662	0.1500	-60	-72	2.49	30
9	UJV12 3	UJV12	293.00	142.0080	0.1500	-60	-36	2.49	30
10	UNS90 1	UNS90	293.99	205.9688	0.1500	-60	-26	3.61	30
11	UNS90 2	UNS90	293.99	77.9657	0.1500	-60	-62	3.61	30
12	UNS90 3	UNS90	293.99	142.0073	0.1500	-60	-32	3.61	30
13	QZY47 1	QZY47	307.02	219.9870	0.1500	-60	-20	5.10	30
14	QZY47 2	QZY47	307.02	77.9668	0.1500	-60	-48	5.10	30
15	QZY47 3	QZY47	307.02	156.0244	0.1500	-60	-32	5.10	30

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MRM^{HR} "MAX": Quantitation and Compound ID

WE SIMULTANEOUSLY PERFORM SENSITIVE QUANTITATION AND COMPOUND IDENTIFICATION USING AN MRM^{HR} APPROACH

- First MRM transition for Compound Sensitivity:
    - Use optimized CE for fragment ion
    - Data processing extracts high resolution fragment ion
- Second MRM transition for Compound ID:
    - Use CE spread (-35 ±30V) to capture full range of fragmentation
    - TOF scans entire fragment ion mass range
    - MS/MS spectrum searched against novel, expanded PFAS library

TOF MSMS

Enhance dynamic range ☐

Mass Table   ◯ Apply fragment ion mass   ⦿ Apply TOF start/stop mass   ☐ Apply scan schedule   Import and autofill...   Sort by precursor ion

	Compound ID	Group name	Precursor ion (Da)	TOF start mass (Da)	TOF stop mass (Da)	Accumulati...	Declusteri...	Collision energy (V)	CE spread (V)
1	PFBA	PFBA	212.90	50.00000	1200.00000	0.0500	-25	-35	30
2	PFBA_2	PFBA	212.90	50.00000	1200.00000	0.0500	-25	-12	0
3	13C4_PFBA	PFBA	217.00	162.00000	182.00000	0.0500	-25	-12	0
4	PFHxA	PFHxA	312.90	50.00000	1200.00000	0.0500	-55	-35	30
5	PFHxA_2	PFHxA	312.90	50.00000	1200.00000	0.0500	-55	-12	0
6	13C2_PFHxA	PFHxA	312.90	260.00000	280.00000	0.0500	-55	-12	0
*									

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SCIEX Fluorochemical HR-MS/MS Library 2.0

HIGH RESOLUTION MS/MS SPECTRAL LIBRARY RECENTLY UPDATED TO INCLUDE ADDITIONAL COMPOUNDS DETECTED IN AFFF AND AFFF-IMPACTED WATER

- 252 PFAS compounds covering negative, positive and zwitterionic compound classes

- Built specifically for the X500R QTOF system but also compatible with SCIEX TripleTOF® and QTRAP® systems

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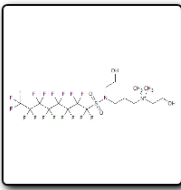
MRM^HR "MAX": Ground Water Sample #1 - PFBS

MRM^{HR} "MAX": Ground Water Sample #TMP6 GW15 – PFBS

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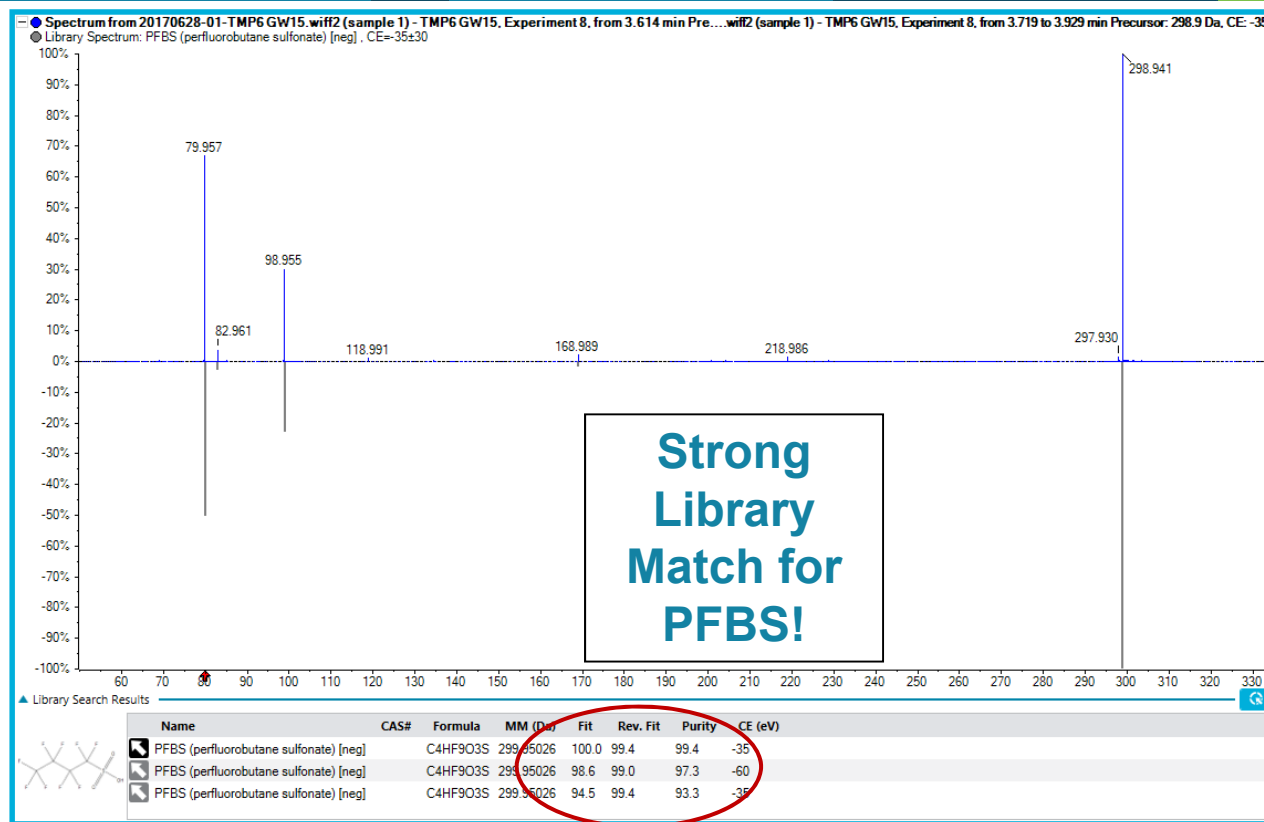
MRM^HR "MAX": Ground Water #TMP8 GW17 – 6:2 FTS
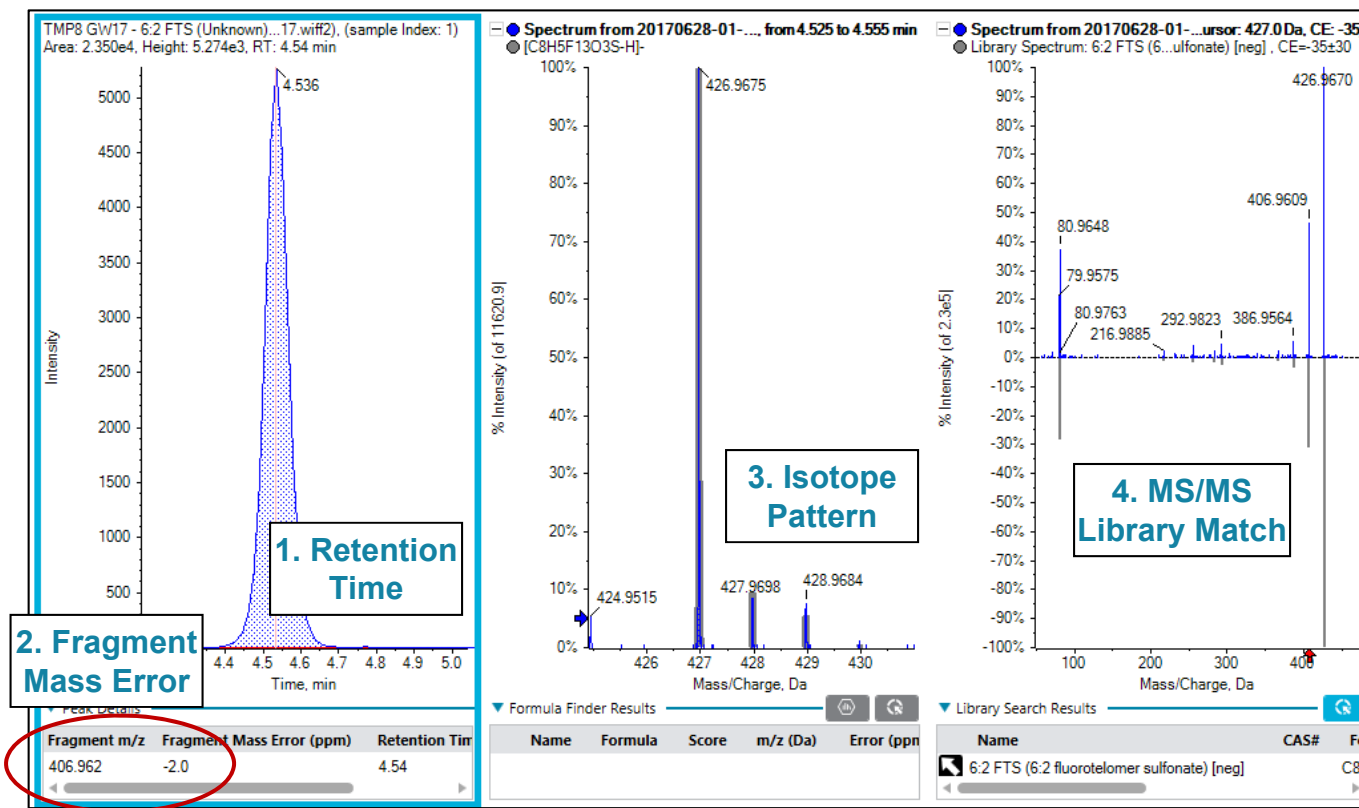
MRM^{HR} "MAX": Ground Water Sample #EISW-001 – PFOS

MRM 1: Optimized CE

- CE = -108

- Library fit = **15.9**

- Too much fragmentation for library match ✘

MRM 2: CE Spread

- CE = -35±30 V

- Library fit = **100**

- ID confirmation ✓
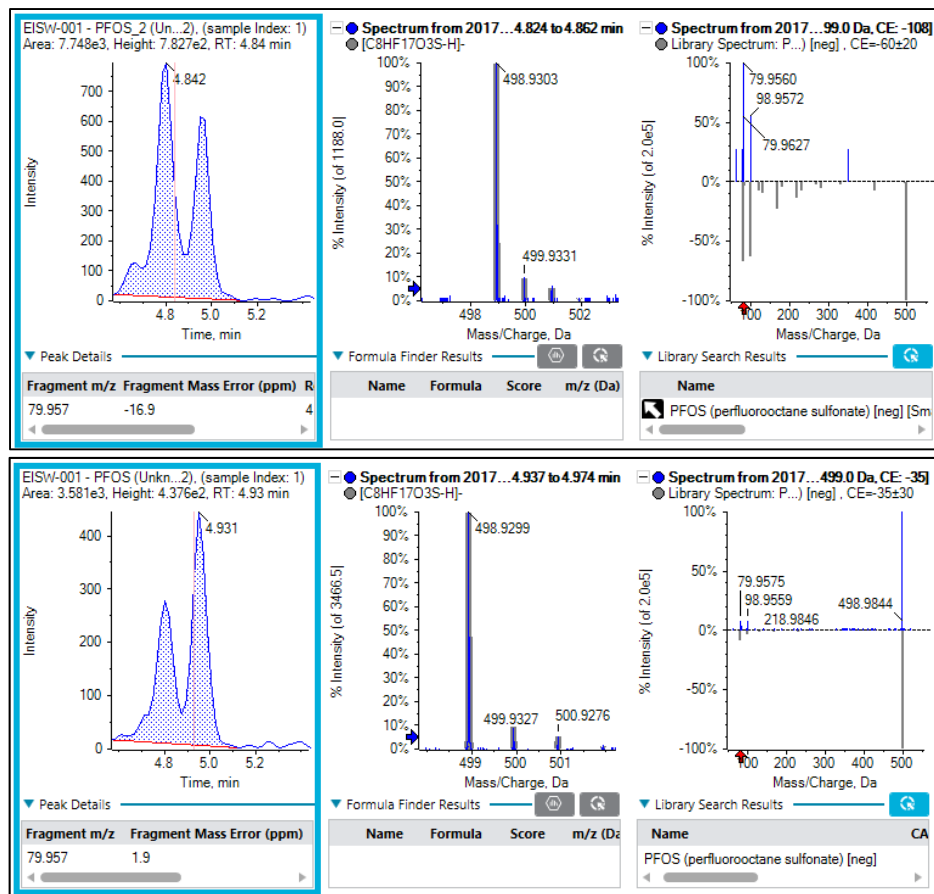
Ion Ratios Calculated From MRMHR "MAX" Spectrum: PFBS

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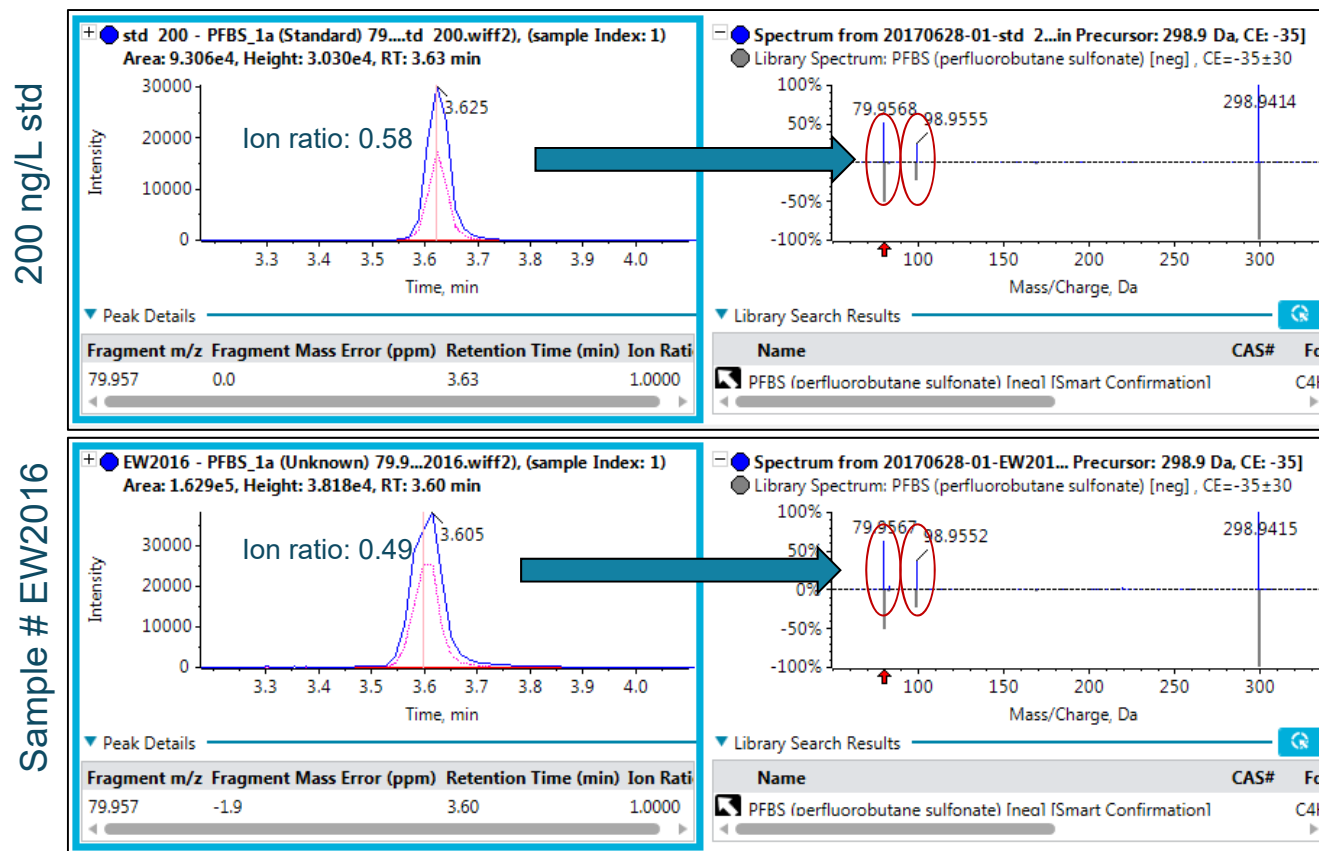
MRM^{HR} "MAX": Quantitation using Optimized CE

3 Orders of Linear Dynamic Range

LOQ: 1 ng/L

Blank

Calibration for 4:2 FTS_2: y = 0.00806 x + 0.00914 (r = 0.99150, r² = 0.98307) (weighting: 1 / x)

4:2 FTS

Standards: 1-1000 ng/L

Calibration for PFHxA_2: y = 0.01003 x + 0.03466 (r = 0.99785, r² = 0.99571) (weighting: 1 / x)

PFHxA

Standards: 1-1000 ng/L

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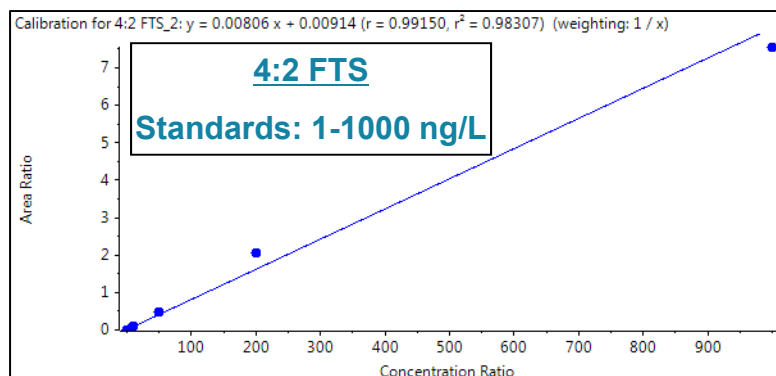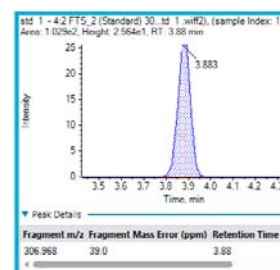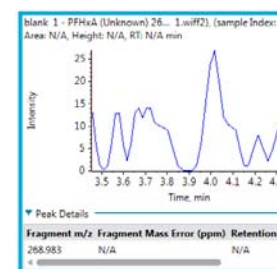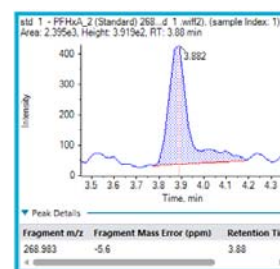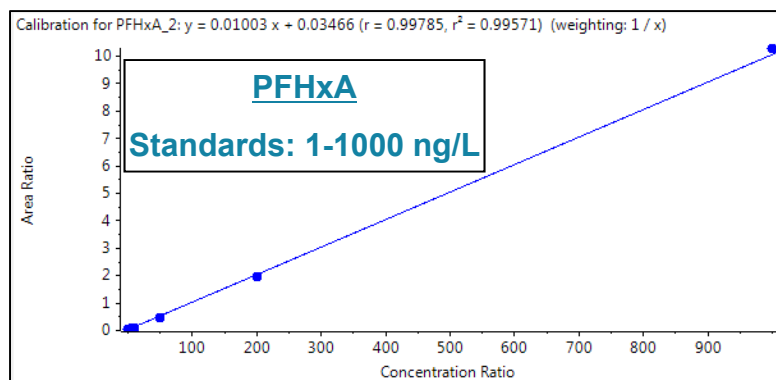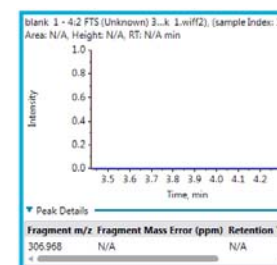
MRM^{HR} "MAX": PFHxA in Solvent Standard
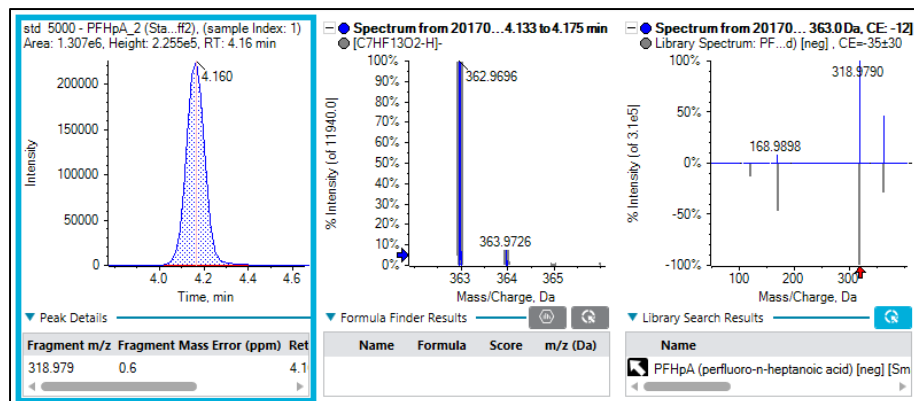
MRM 1: Optimized CE

- **Higher area count (sensitivity)**

- **Simple MS/MS spectrum**
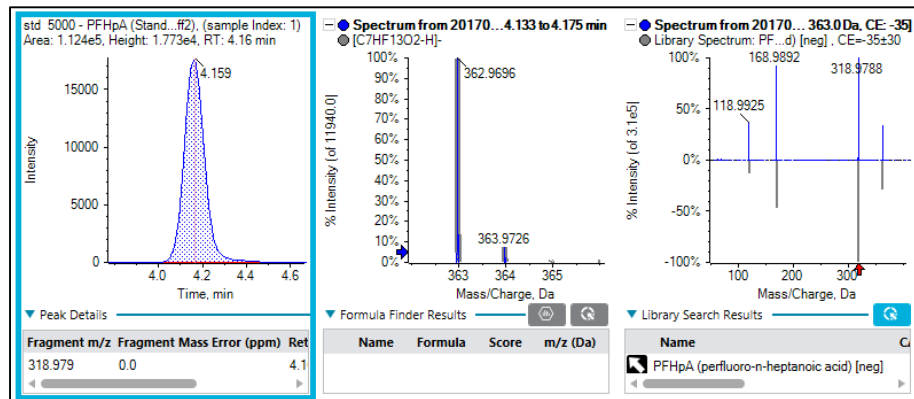
MRM 2: CE Spread

- **Lower area count**

- **Complex MS/MS spectrum (library searching)**

What about GenX?
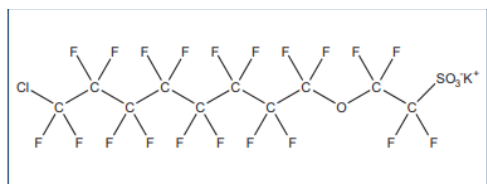
What's Next? GenX, PFOA replacement compound

- Cape Fear River basin near Wilmington, NC is contaminated with GenX and other PFOA replacement compounds

- What else is there? How do these chemicals behave in the environment? Are there degradation compounds?

- SWATH allows for sample re-interrogation
    - Digital sample archival

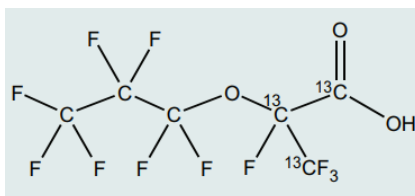- Wellington is already making native and mass-labelled standards for some of these:

Hydro-EVE acid

Nafion by-product 2

PFO4DA

PFO5DoDA
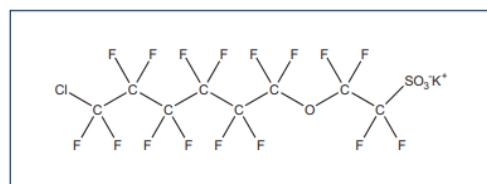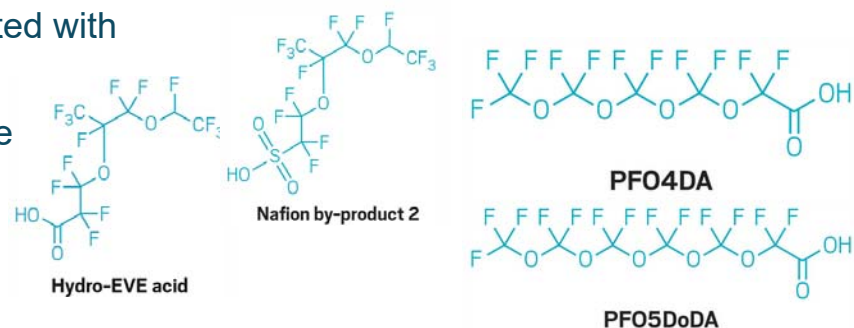
9Cl-PF3ONS (main component of F-53B)

NaDONA

PERSISTENT POLLUTANTS

Chemours to clean up GenX and pay $12 million fine

Legal deal would require company to test fluoroethers for health and environmental effects

by *Cheryl Hogue*
NOVEMBER 27, 2018
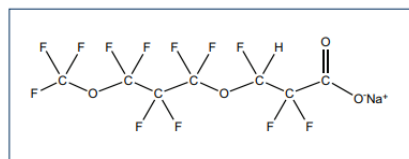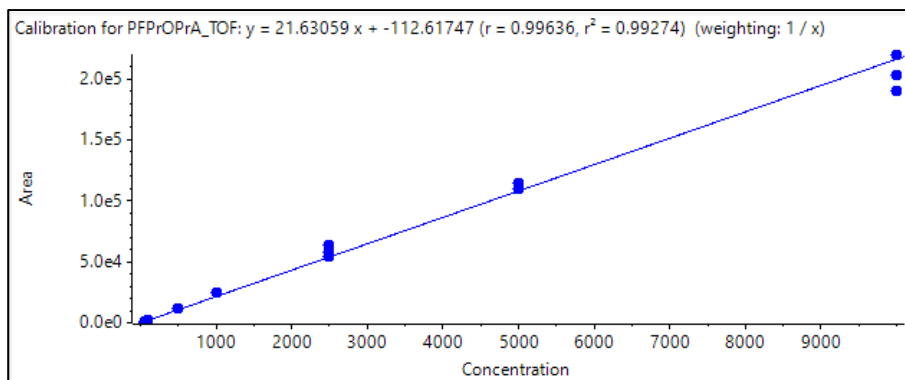
11Cl-PF3OUdS (minor component of F-53B)

HFPO-DA

PERSISTENT POLLUTANTS

The hunt is on for GenX chemicals in people

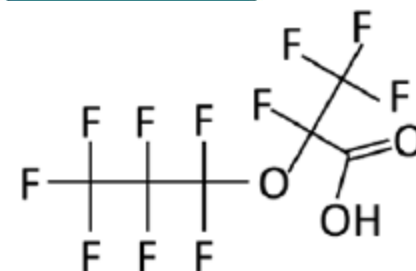Analysis of North Carolina residents' blood for Chemours PFAS yields surprises

by *Cheryl Hogue*
APRIL 7, 2019 | APPEARED IN **VOLUME 97, ISSUE 14**

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PFPrOPrA (GenX), TOFMS

- LOQ = 50 ppt

Data courtesy of:

PFPrOPrA ("GenX")

$C_6HF_{11}O_3$

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PFPrOPrA (GenX), MRM^{HR} (m/z 168.987 fragment)

- LOQ = 10 ppt

Data courtesy of:

Detection of GenX Compounds in Soil Sediment Samples

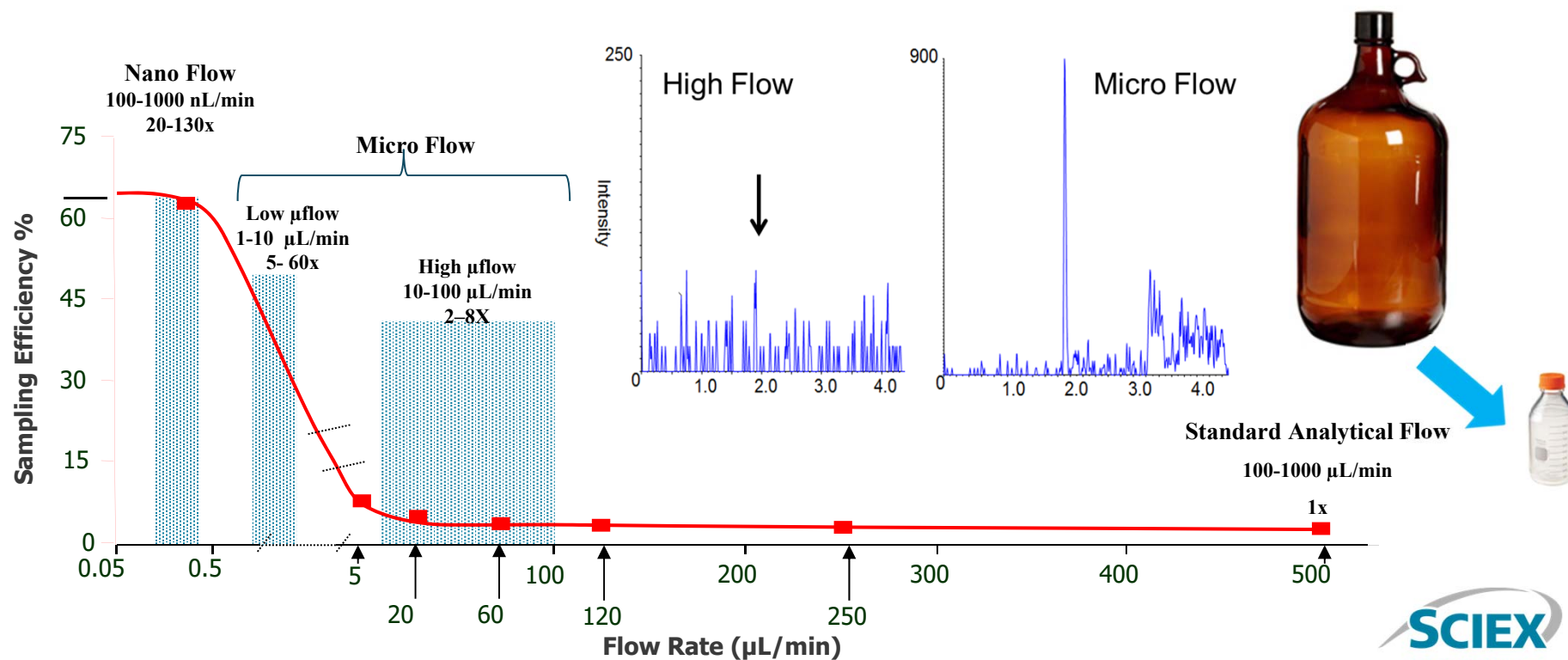## PFPrOPrA found in 4 different unknown samples within calibration range

Data courtesy of: UNCW

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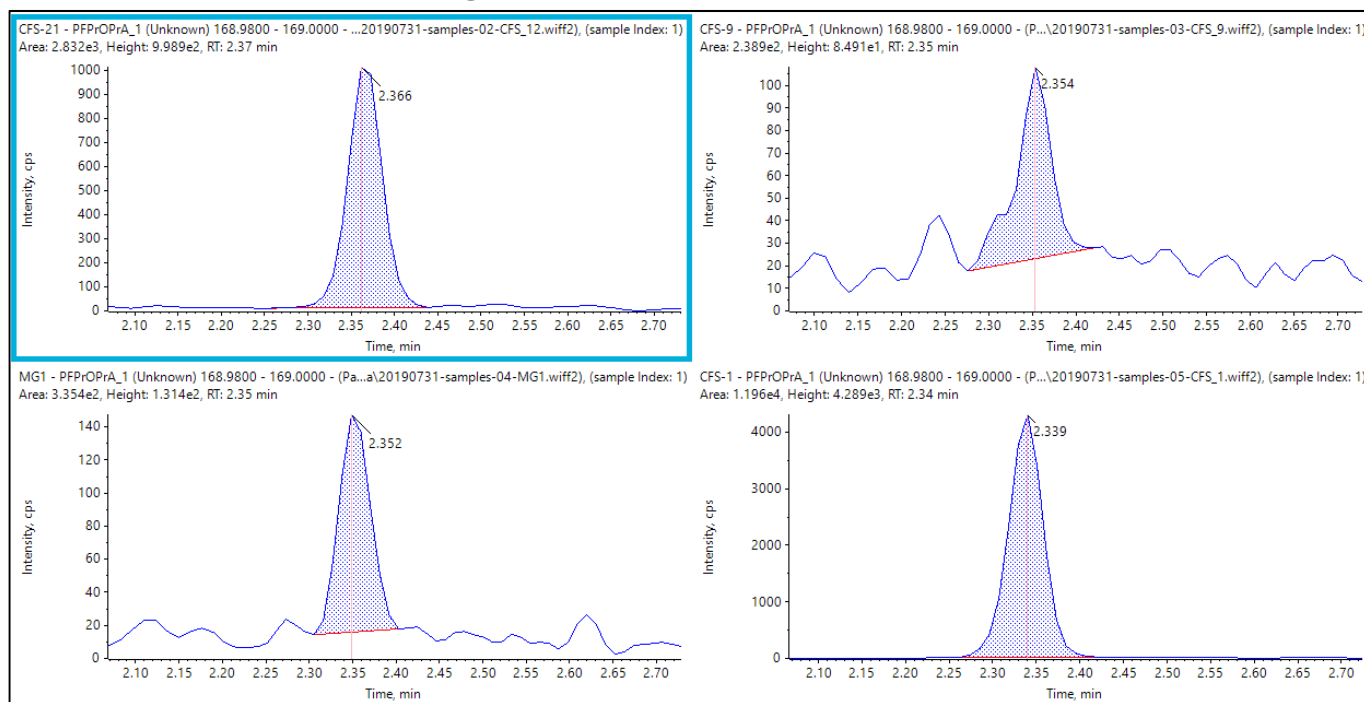
Increasing Sensitivity

EVERYONE WANTS MORE

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Moving to Microflow LC

IMPROVE SAMPLING AND IONIZATION EFFICIENCY

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M5 Micro LC System

- Delay column installed to retain PFAS contamination from tubing

- Initial tests shown here were performed without replacing any FEP (fluoroethylene polymer) tubing or PTFE parts

- Eluent A: Water + 10 mM ammonium acetate

- Eluent B: Methanol + 10 mM ammonium acetate

- Run time: 10 min

- Injection volume: 1 uL

- 15 uL/min LC flow

- Phenomenex Gemini C18 50 mm x 0.3 mm x 3 um
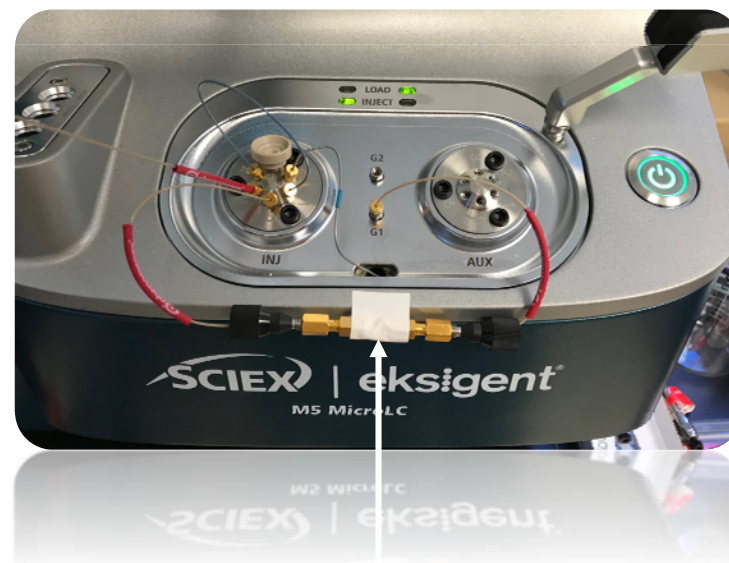
Delay Column
(*Phenomenex Luna C18(2) 30 mm x 0.3 mm*)

32

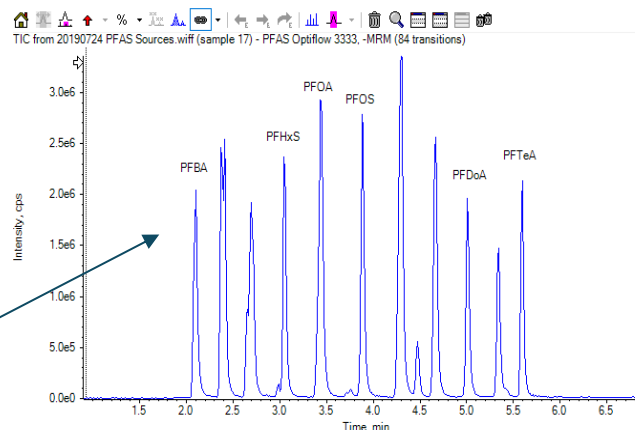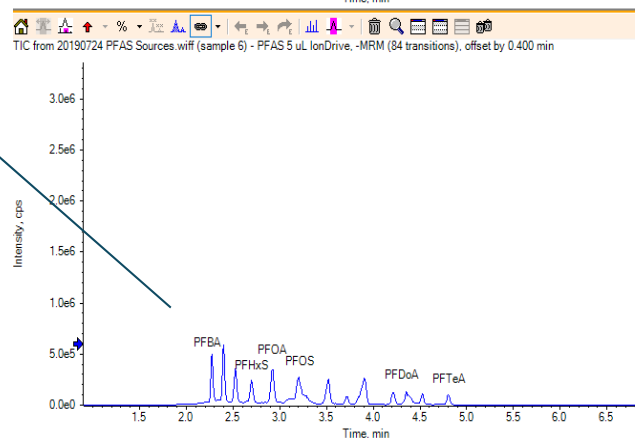SCIEX
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Micro vs Standard Flow: Sensitivity Improvements

M5 Micro
15 uL/min
1 uL injection

15-20x sensitivity improvement

Analytical HPLC
800 uL/min
2 uL injection

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M5 Micro LC: PFOA Calibration (15 µL/min; 1 µL injection)
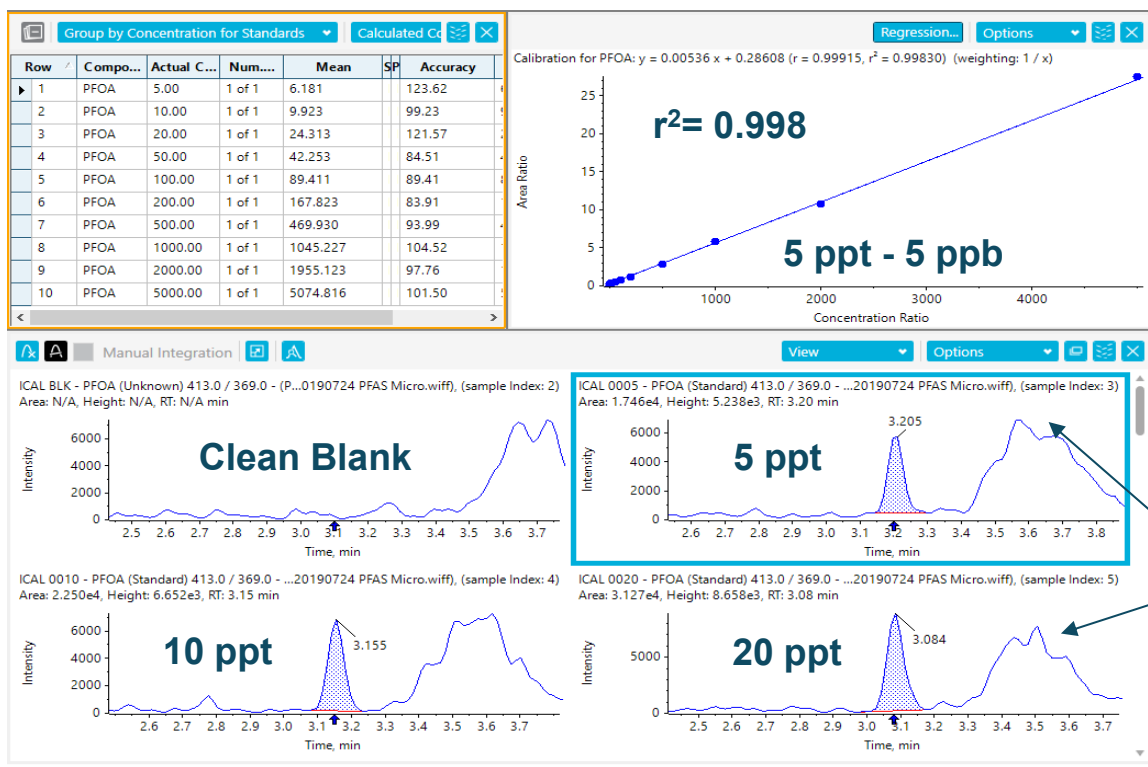
Preliminary Results Findings:
- ✓ 3 Orders Magnitude Good for PFOA. Potential improvements on LDR

- ✓ Potential for overcoming In source saturation

- ✓ Results obtained without modifying tubing in the LC

Background PFOA

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Using a Trap n Elute Workflow

- M5 system can use Ion Exchange online SPE
  - Phenomenex Luna NH2 30 mm x 0.3 mm
- Samples are loaded with 10 mM ammonium acetate (pH ~ 7)
- PFAS are eluted from the ion exchange column with 0.1% ammonium hydroxide (pH ~ 10)

Contamination Trap Column

Ion Exchange Column

Outlet to Analytical Column and MS

To be continued…

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