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Direct Injection Method for LC-MS/MS Detection of Polyfluoroalkyl Substances in Water

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Overview

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- **PFAS Overview**
- **Drinking water analysis**
  - SPE sample prep
- **Other water type analyses**
  - Large volume direct injection (LVI/DI)

Perfluoroalkylated Substances (PFAS)

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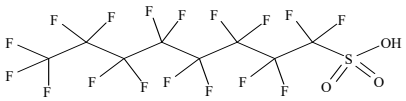
- Widespread applications
  - Surface treatments, surfactants, firefighting foam AFFF's
  - Polymerization aid for polytetrafluoroethylene (PTFE) and other flu
- Stable and persistent in the environment (POP)
  - Bio-accumulative
- Identified in environmental samples worldwide
  - Found in arctic polar bears
  - Many Americans have ~5 ppb of PFOA in their blood!
- Worldwide interest in PFC analysis



PFOS

METHOD 537.	DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS) Version 1.1 September 2009

UCMR3 - PFASs

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Third Unregulated Contaminant Monitoring Rule

Six Perfluorinated Compounds

Contaminant	CAS Registry Number[1]	Minimum Reporting Level	Sampling Points[2]	Analytical Methods
perfluorooctanesulfonic acid (PFOS)	1763-23-1	0.04 µg/L **40 ppt**	EPTDS	EPA 537 Rev 1.1
perfluorooctanoic acid (PFOA)	335-67-1	0.02 µg/L **20 ppt**	EPTDS	EPA 537 Rev 1.1
perfluorononanoic acid (PFNA)	375-95-1	0.02 µg/L 20 ppt	EPTDS	EPA 537 Rev 1.1
perfluorohexanesulfonic acid (PFHxS)	355-46-4	0.03 µg/L 30 ppt	EPTDS	EPA 537 Rev 1.1
perfluoroheptanoic acid (PFHpA)	375-85-9	0.01 µg/L 10 ppt	EPTDS	EPA 537 Rev 1.1
perfluorobutanesulfonic acid (PFBS)	375-73-5	0.09 µg/L 90 ppt	EPTDS	EPA 537 Rev 1.1

Unregulated Contaminant Monitoring Rule

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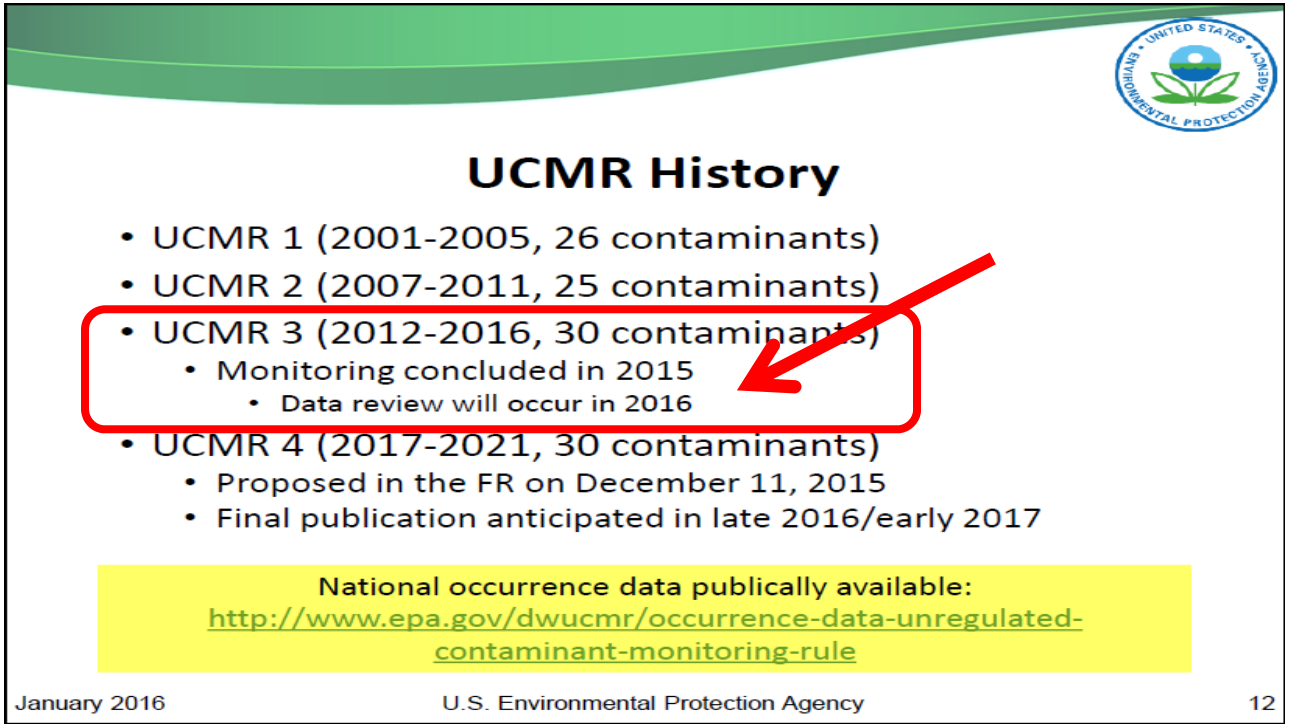
https://www.epa.gov/sites/production/files/2016-02/documents/ucmr4_stakeholderwebinarslides_160113_508.pdf



PFAS's in the Literature

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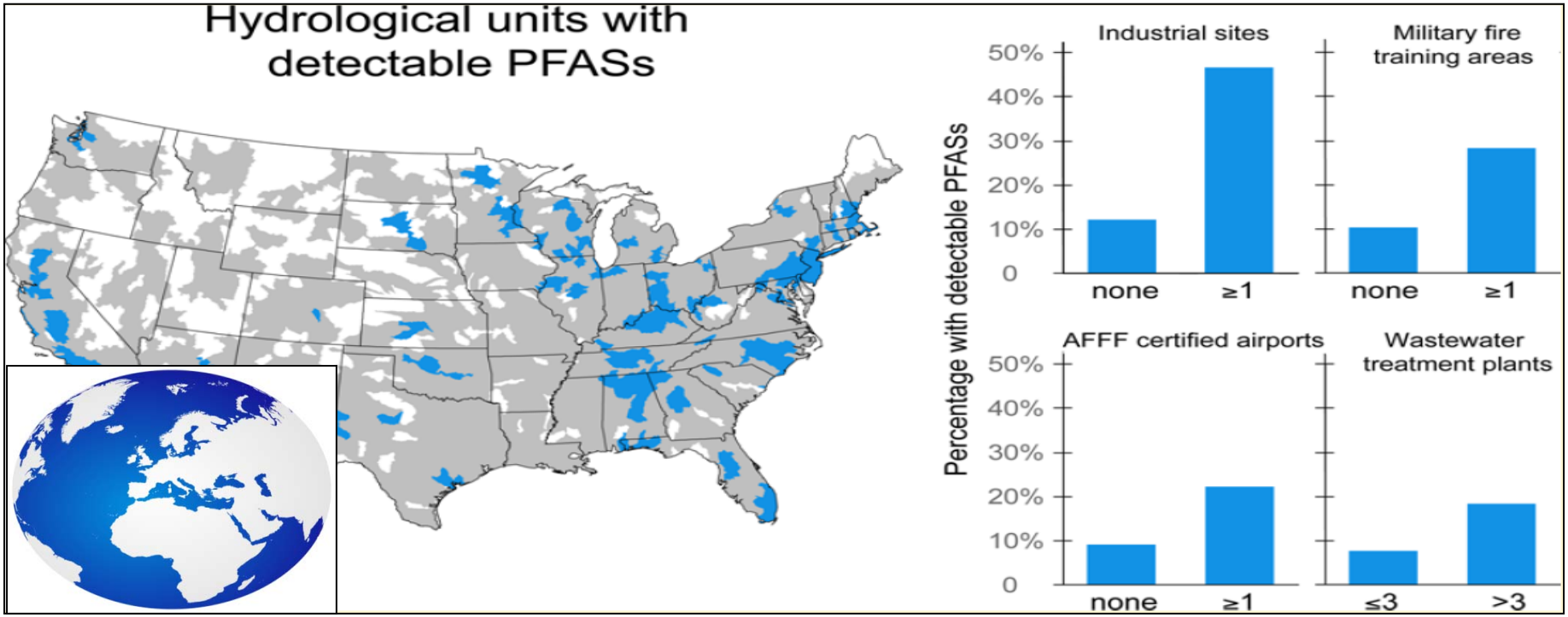
Hu, Xindi C., et al. "Detection of Poly-and Perfluoroalkyl Substances (PFASs) in US Drinking Water Linked to Industrial Sites, Military Fire Training Areas, and Wastewater Treatment Plants." *Environmental Science & Technology Letters* (2016)



PFAS Analysis Sensitivity Requirements

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SPE enrichment

- Sample prep allows for use of low- to mid-level sensitivity for mass spectrometer

Large Volume Injection

- High sensitivity TQ required

Increasing Sensitivity

Comparison of analysis methods

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	EPA Method 537	ISO 21675	ASTM
Sample Prep	SPE	SPE	Dilute and Filter aka **No Prep**
Injection Volume	10 µL	10 µL	30 µL
Number of compounds	14	27	38

UPLC/TQD Conditions

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- System: ACQUITY UPLC/TQD with PFC Kit
- Analytical column: ACQUITY UPLC BEH C18 2.1x 50 mm
- Mobile Phase A: 2mM ammonium acetate in water/MeOH [95:5]
- Mobile Phase B: MeOH
- Column Temp: 50 °C
- Injection volume: 10 µL (full loop)

Time (min)	Flow (mL/min)	%B	Curve
0.0	**0.40**	**25**	
0.5	**0.40**	**25**	**6**
5.0	**0.40**	**85**	**6**
5.1	**0.40**	**100**	**6**
5.6	**0.40**	**100**	**6**
7.0	**0.55**	**100**	**1**
9.0	**0.40**	**25**	**1**

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PFAS's Interferences/Contamination

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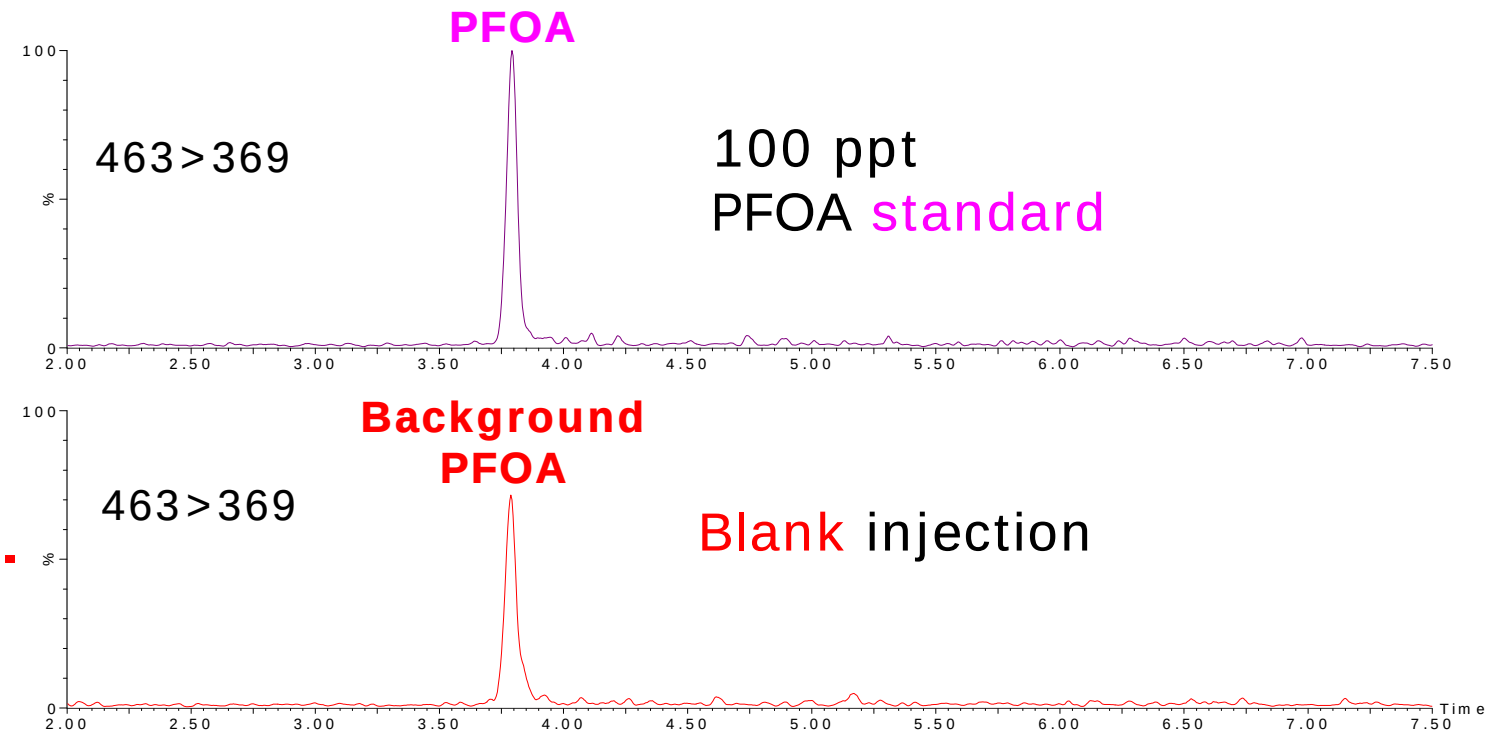

PFC Kit

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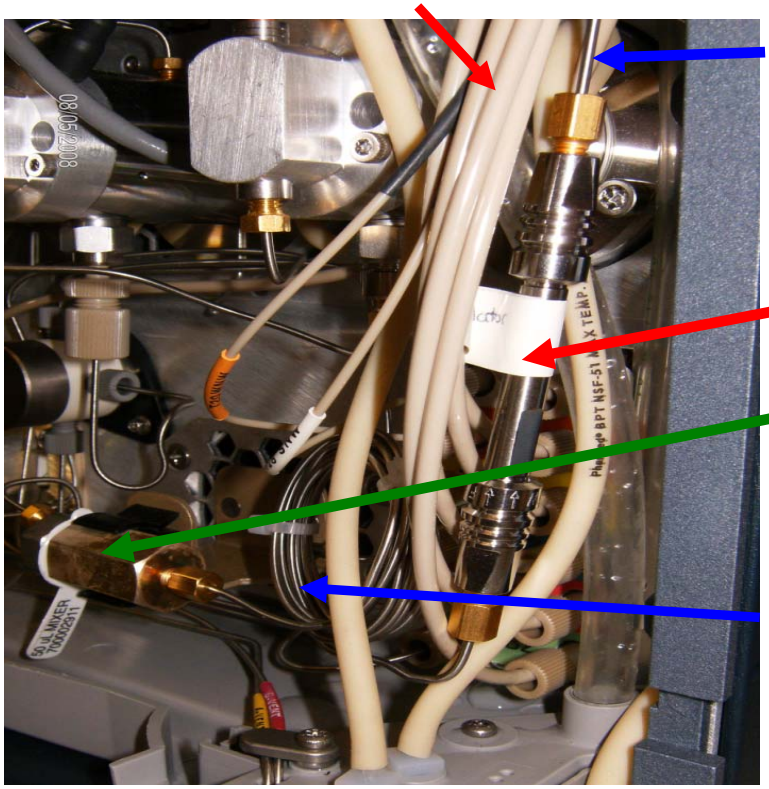
PEEK tubing
for solvent lines



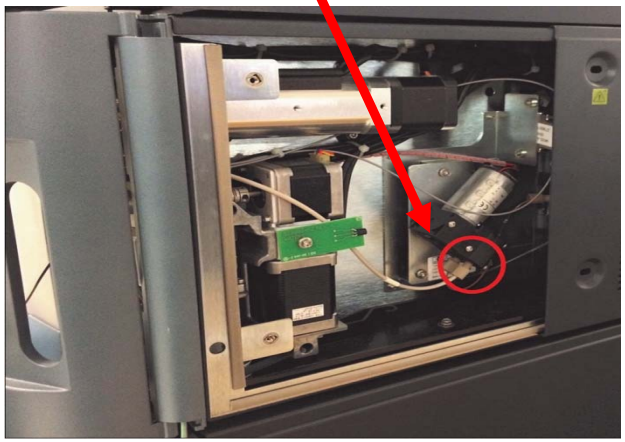
Stainless steel tubing
Isolator column to injector

PFC
Isolator Column

Solvent mixer

Coiled
stainless steel tube
Mixer to
PFC isolator column

PEEK tubing
for autosampler
lines



MRM Chromatograms of PFOA With PFC Kit Installed

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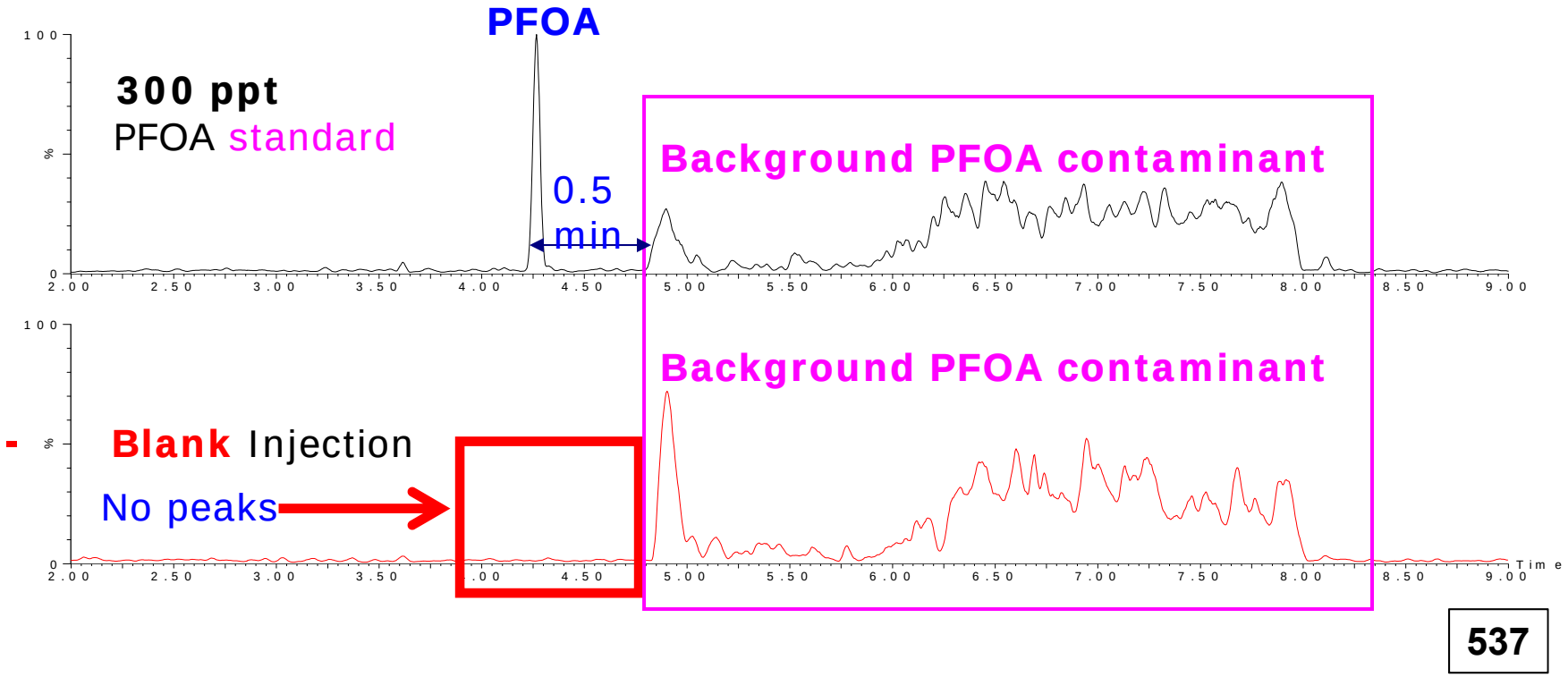

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Quanpedia

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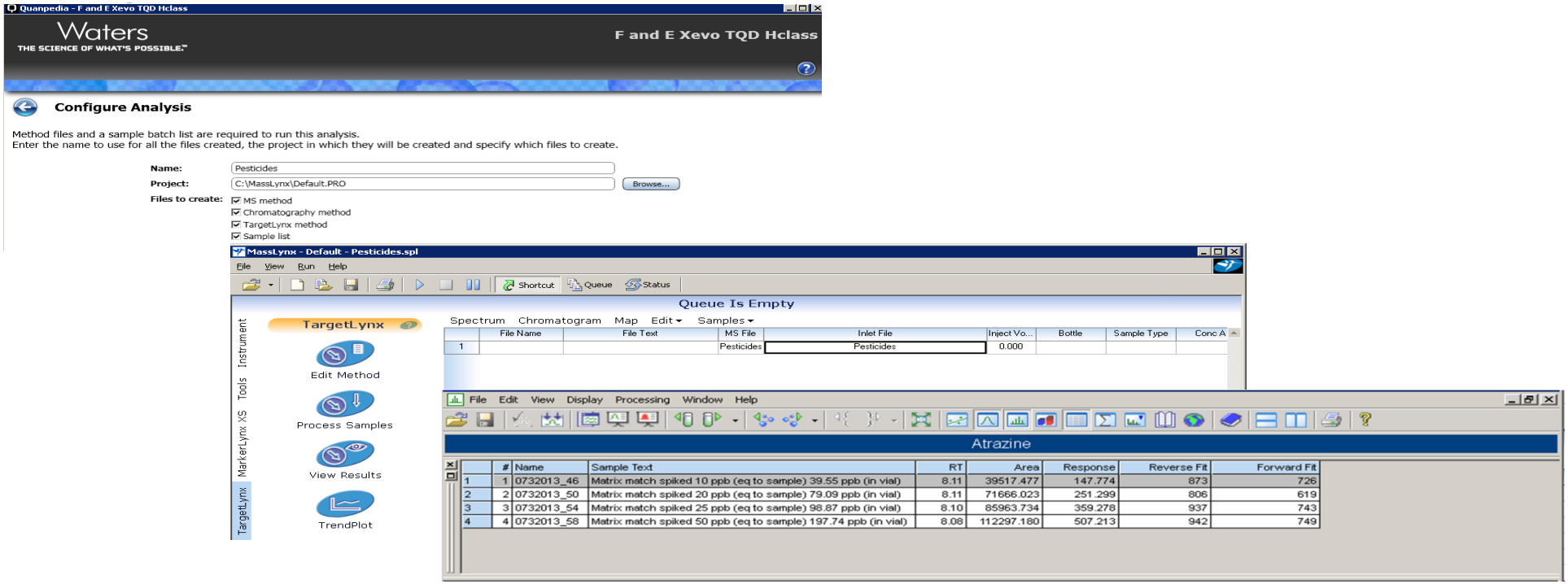
- **Generates all the necessary methods for routine analysis**
  - Methods -> Sample List -> Data Processing



Typical Calibration Curve

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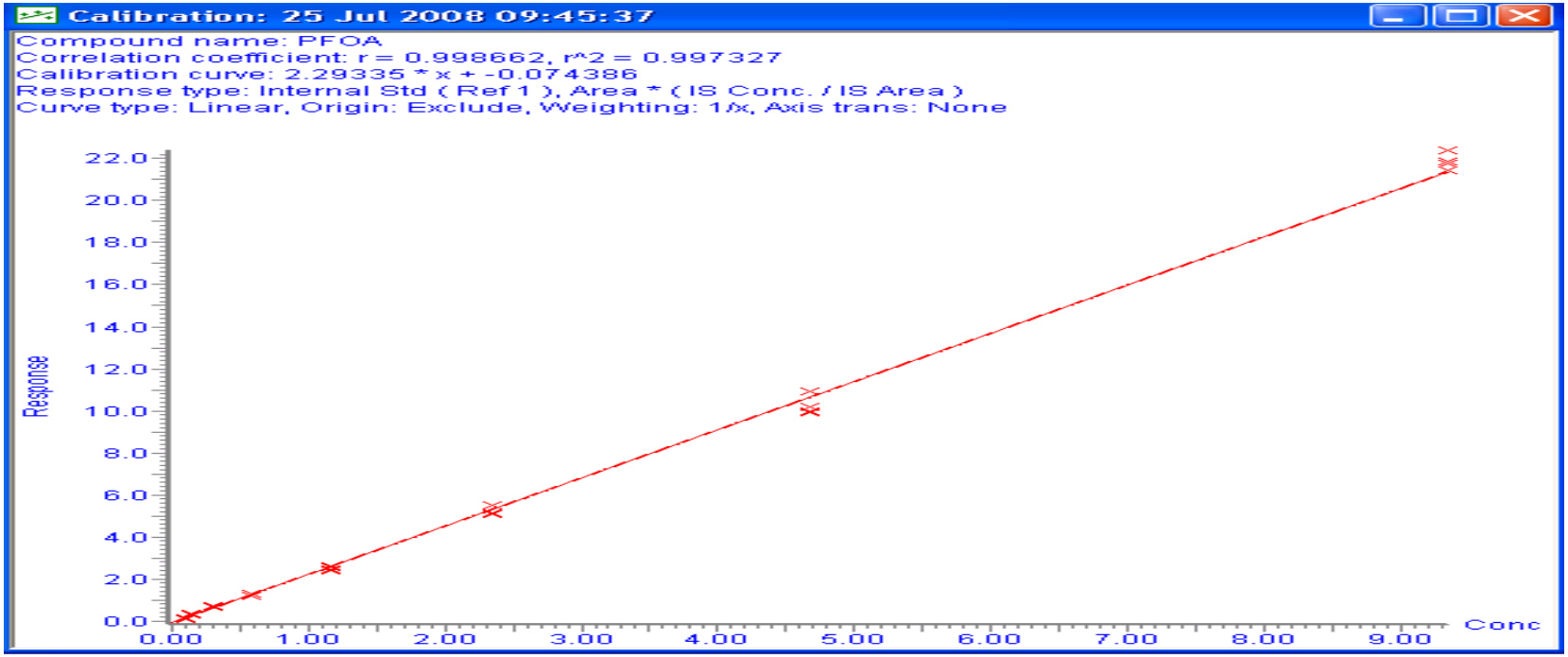
PFOA calibration curve (0.09 ppb to 9.4 ppb) r^2 > 0.997



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Recovery Study

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Target 10 PFCs	**1 ppt** spiked water (n=6)	
	Recovery (%)	RSD (%)
PFBuS	105	6
PFHxA	112	11
PFHpA	124	15
PFHxS	103	5
PFOA	**122**	**17**
PFNA	107	9
PFOS	**104**	**6**
PFDA	109	8
PFUnA	100	9
PFDoA	105	12

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PFOS in Bottled Water

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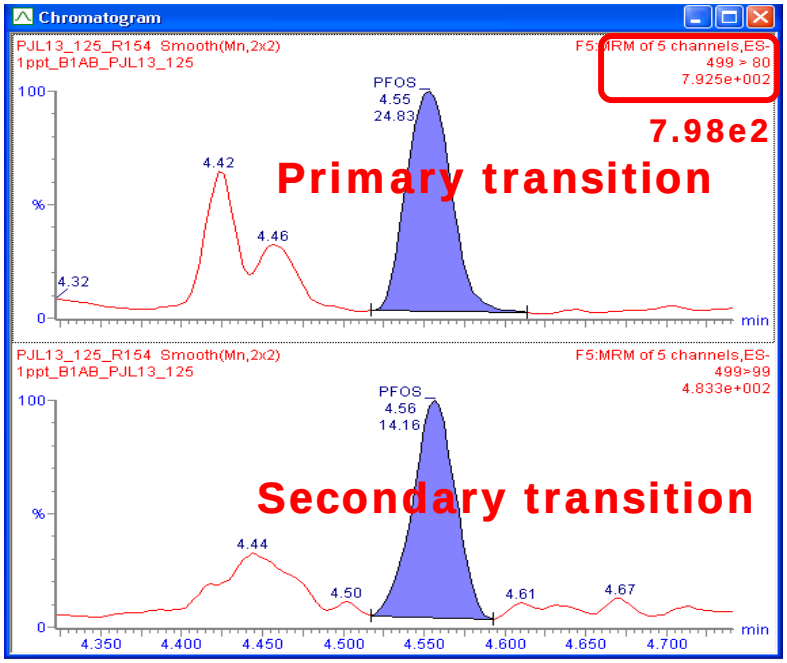

PFOS in the bottled water				
Injection	sample 1	Sample 2	sample 3	sample 4
1	0.42	0.39	0.44	0.29
2	0.37	0.44	0.43	0.35
3	0.48	0.52	0.39	0.35
4	0.48	0.46	0.39	0.38
5	0.44	0.52	0.42	0.35
6	0.36	0.43	0.36	0.36
Average (ppt)	**0.42**	**0.46**	**0.40**	**0.35**
RSD (%)	**12.7**	**11.1**	**7.7**	**8.1**
Mean = **0.41 ppt**				**537**

Instrument LOQ <25ppt; SPE enrichment factor =250; Method LOQ <0.1ppt

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Direct (large volume) Injection Approach

ASTM 7979-17

ASTM 7979-17 Direct Injection Method
Sample Prep

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1. 5 mL MeOH + 5 mL sample in 15mL tube, vortex
2. Filter: Glass fiber + GHP (polypropylene)
3. Add 10 ul acetic acid
4. 500 ul to LC vial

- Reagent Water
- Surface (River) Water
- Ground Water
- Influent Waste Water
- Effluent Waste Water

June 2018 Application Note: **720006329EN**

Instrument Methods

Source Parameters

- Instrument: **Xevo TQ-XS**
- Ion Mode: ESI-
- Capillary Voltage: 1.0 kV
- Desolvation Temperature: 500°C
- Desolvation Flow: 1100 L/hr
- Cone Flow: 150 L/hr

MS Method

- Developed using **QuanOptimize**
  - MRMs, CV, CE
- Divert flow to waste from 15 – 21 mins

LC Method

- Acquity I-Class with PFC Kit
- CSH Phenyl Hexyl 2.1mm x 100 mm, 1.7 um
- A: 95:5 H2O:MeOH + 2 mM am ac
- B: MeOH + 2 mM am ac
- **Inj Vol: 30 ul**
- Gradient:

Time (min)	Flow (mL/min)	%A	%B
0	0.3	100	0
1	0.3	80	20
6	0.3	55	45
13	0.3	20	80
14	0.4	5	95
17	0.4	5	95
18	0.3	100	0
22	0.3	100	0

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Compounds Included in Method

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- 39 Native Compounds
- 27 Isotope Labels – Recovery only!

Abbreviation	Molecular Formula	CAS Number	PFAS Class
PFBA	$C_4HF_7O_2$	375-22-4	carboxylate
PFPeA	$C_5HF_9O_2$	2706-90-3	carboxylate
PFHxA	$C_6HF_{11}O_2$	307-24-4	carboxylate
PFHpA	$C_7HF_{13}O_2$	375-85-9	carboxylate
PFOA	$C_8HF_{15}O_2$	335-67-1	carboxylate
PFNA	$C_9HF_{17}O_2$	375-95-1	carboxylate
PFDA	$C_{10}HF_{19}O_2$	335-76-2	carboxylate
PFUnDA	$C_{11}HF_{21}O_2$	2058-94-8	carboxylate
PFDoDA	$C_{12}HF_{23}O_2$	307-55-1	carboxylate
PFTriDA	$C_{13}HF_{25}O_2$	72629-94-8	carboxylate
PFTreDA	$C_{14}HF_{27}O_2$	376-06-7	carboxylate
PFHxDA	$C_{16}HF_{31}O_2$	67905-19-5	carboxylate
PFOcDA	$C_{18}HF_{35}O_2$	16517-11-6	carboxylate
PFBS	$C_4HF_9SO_3$	29420-49-3	sulfonate
PFPeS	$C_5HF_{11}SO_3$	2706-91-4	sulfonate
PFHxS	$C_6HF_{13}SO_3$	3871-99-6	sulfonate
PFHpS	$C_7HF_{15}SO_3$	375-92-8	sulfonate
PFOS	$C_8HF_{17}SO_3$	1763-23-1	sulfonate
PFNS	$C_9HF_{19}SO_3$		sulfonate
PFDS	$C_{10}HF_{21}SO_3$	335-77-3	sulfonate
FOSA	$C_8H_2F_{17}NO_2S$	754-91-6	sulfonamide
N-EtFOSAA	$C_{12}H_8F_{17}NO_4S$	2991-50-6	sulfonamidoacetic acid
N-MeFOSAA	$C_{11}H_6F_{17}NO_4S$	2355-31-9	sulfonamidoacetic acid
N-MeFOSA	$C_9H_4F_{17}NO_2S$	31506-32-8	sulfonamide
N-EtFOSA	$C_{10}H_6F_{17}NO_2S$	4151-50-2	sulfonamide
FHUEA	$C_8H_2F_{12}O_2$	70887-88-6	unsaturated telomer acid
FOUEA	$C_{10}H_2F_{16}O_2$	70887-84-2	unsaturated telomer acid
8:2 diPAP	$C_{20}H_9F_{34}PO_4$	678-41-1	phosphate ester
4:2 FTS	$C_6H_5F_9SO_3$	757124-72-4	telomer sulfonate
6:2 FTS	$C_8H_5F_{13}SO_3$	29420-49-3	telomer sulfonate
8:2 FTS	$C_{10}H_5F_{17}SO_3$	39108-34-4	telomer sulfonate
PFecHS	$C_8HF_{15}SO_3$	67584-42-3	cyclic
FHEA	$C_8H_3F_{13}O_2$	53826-12-3	telomer acid
FOEA	$C_{10}H_3F_{17}O_2$	27854-31-5	telomer acid
FDEA	$C_{12}H_3F_{21}O_2$	N/A	telomer acid
FHpPA	$C_{10}H_5F_{15}O_2$	812-70-4	other
ADONA	$C_7H_2F_{12}O_4$	958445-44-8	other
9Cl-PF3ONS	$C_8HF_{16}ClSO_4$	73606-19-6	other
11Cl-PF3OUdS	$C_{10}HF_{20}ClSO_4$	73606-19-6	other

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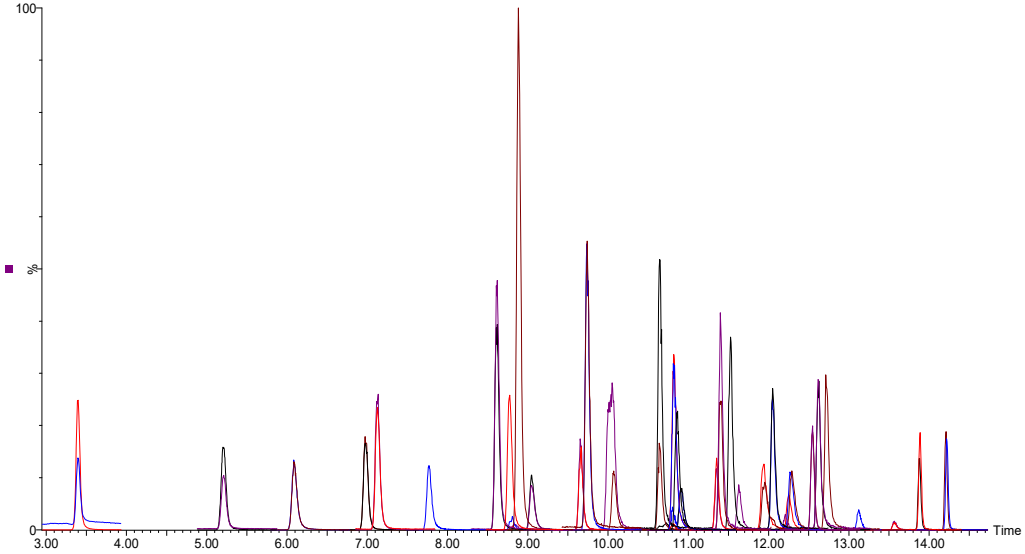
Overall Method Summary



Reagent Blanks	50:50 Water:Methanol + 0.1% acetic acid	Concentration must be < half the reporting limit	✓
Method Blank	Reagent water + surrogates. Taken through sample prep	Concentration must be < half the reporting limit	✓
Reporting Limit Check	Reagent water + targets at RL + surrogates. Taken through sample prep	Targets detectable; Recovery 35 – 150%	✓
Lab Control	Reagent water + targets + surrogates. Taken through sample prep	Must fall within 99.7% confidence interval	✓

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Spiked Surface Water

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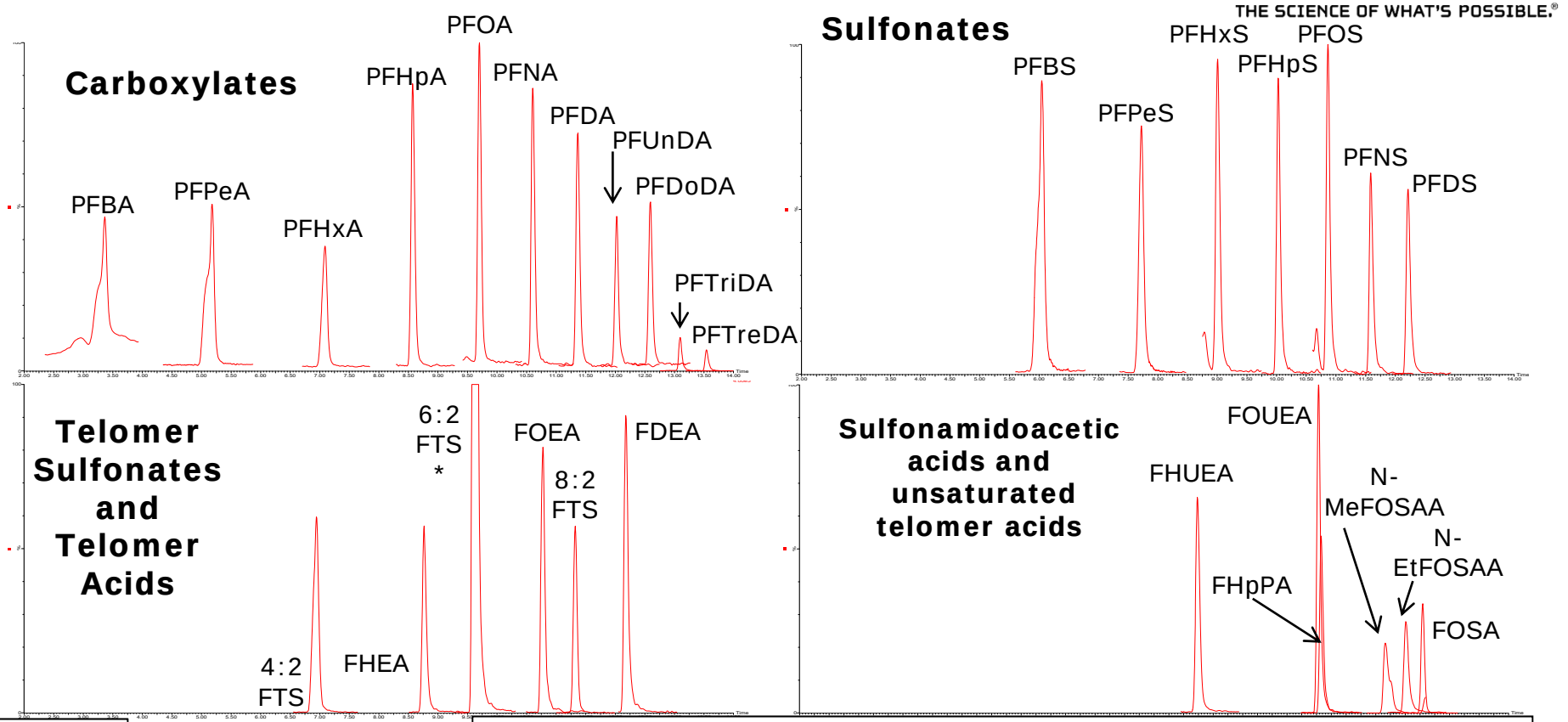

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PFBA and PFPeA at 300 ng/L; 4:2, 6:2, and 8:2 FTS at 1200 ng/L; all other compounds at 60 ng/L.
(*) this compound shown off scale.

Data Independent Acquisition (DIA)
Concurrent MRM + Full Scan (aka RADAR)

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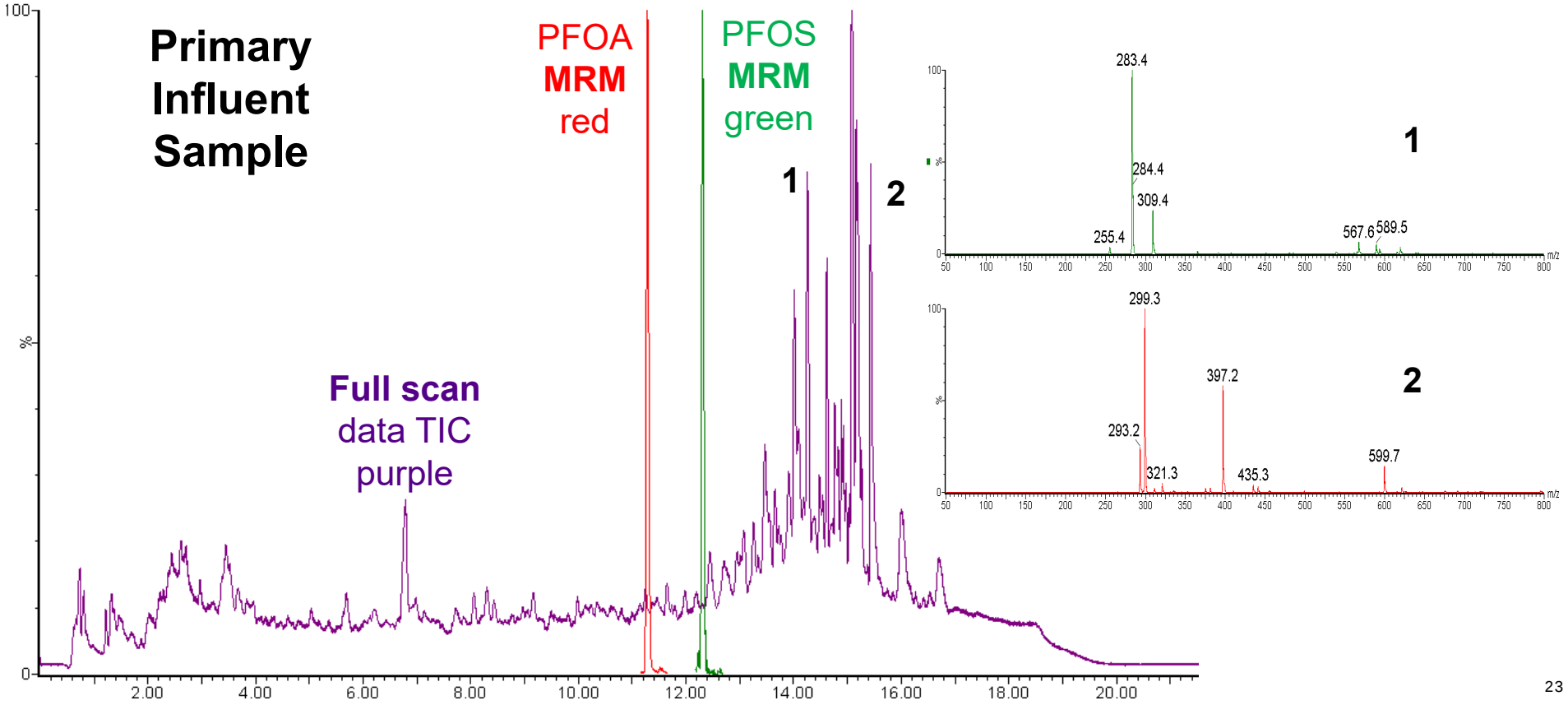

Method Detection Limits (MDL)

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Below reporting limits described by ASTM 7979

Compound	Sample spike (ng/L)	MDL (ng/L)	Reporting range (ng/L)*	R^2
PFBA	100	25.20	50–2000	0.993
PFPeA	10	1.04	50–2000	0.999
PFHxA	10	1.33	10–400	0.999
PFHpA	10	0.91	10–400	0.999
PFOA	10	1.42	10–400	0.999
PFNA	10	1.33	10–400	0.999
PFDA	10	0.84	10–400	0.998
PFUnDA	10	2.52	10–400	0.996
PFDoDA	10	1.76	10–400	0.993
PFTriDA	10	2.34	10–400	0.991
PFTreDA	10	1.99	10–400	0.993
PFHxDA	200	25.41	–	0.984
PFOcDA	400	41.99	–	0.983
PFBS	10	1.21	10–400	0.999
PFPeS	10	1.07	10–400	0.999
PFHxS	10	1.41	10–400	0.999
PFHpS	10	1.57	10–400	0.999
PFOS	10	1.61	10–400	0.999
PFNS	10	1.37	10–400	0.999
PFDS	10	1.44	10–400	0.997

Compound	Sample spike (ng/L)	MDL (ng/L)	Reporting range (ng/L)*	R^2
FOSA	10	1.29	10–400	0.999
N-Et-FOSAA	10	1.90	10–400	0.997
N-Me-FOSAA	10	1.59	10–400	0.999
N-Et-FOSA	10	1.45	–	0.997
N-Me-FOSA	10	1.19	–	0.999
FHUEA	10	1.53	10–400	0.999
FOUEA	10	1.36	–	0.999
8:2 diPAP	300	50.16	–	0.988
4:2 FTS	10	1.50	10–400	0.999
6:2 FTS	10	N/A	10–400	0.999
8:2 FTS	10	2.62	10–400	0.997
PFecHS	10	1.17	10–400	0.998
FHEA	200	42.19	300–8000	0.994
FOEA	200	50.38	200–8000	0.997
FDEA	200	79.48	200–8000	0.993
FHpPA	10	1.47	10–400	0.999
ADONA	10	0.82	–	0.999
9Cl-PF3ONS	10	1.06	–	0.999
11Cl-PF3OUdS	10	1.45	–	0.998

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Linearity and Sensitivity

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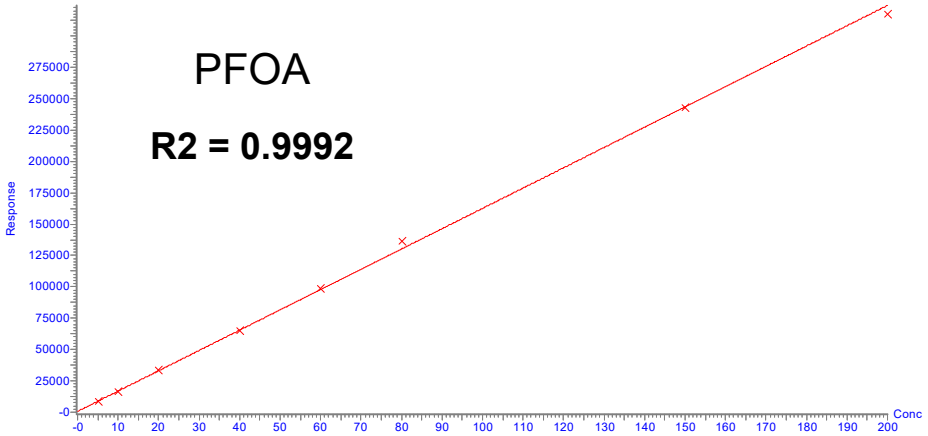
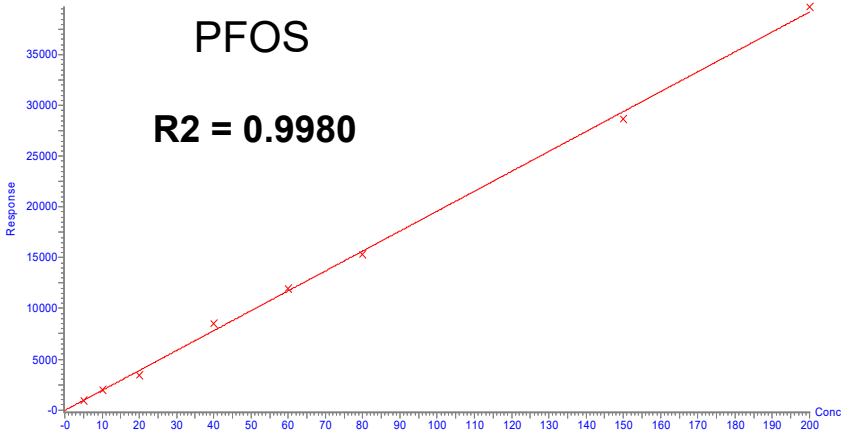
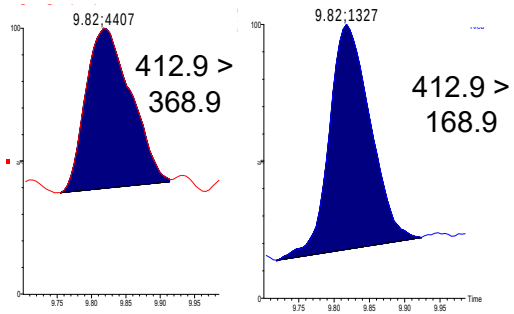
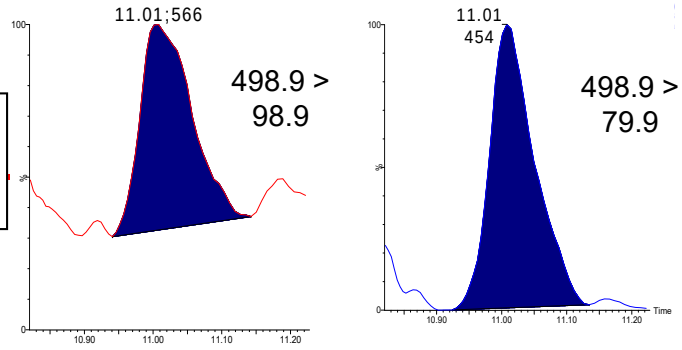






2.5 ng/L injection (0.5x Reporting Limit)



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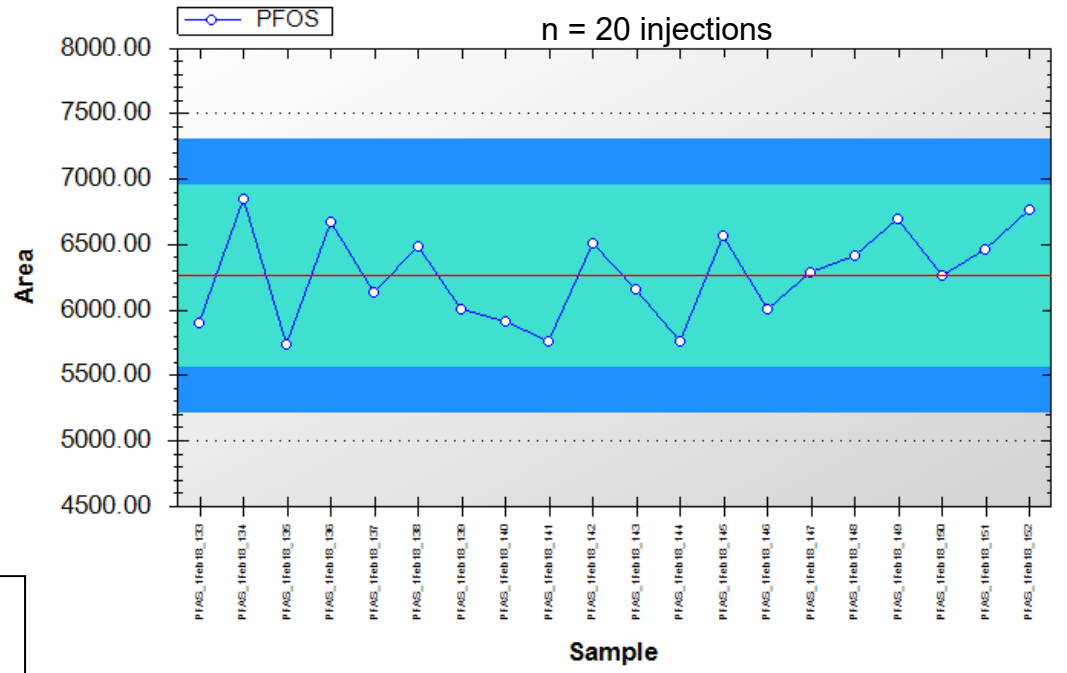
Recovery and Repeatability

Matrix	% Recovery PFOS
Reagent Water	92.8
Ground Water	90.6
Surface Water	94.6
Influent Water	94.1
Effluent Water	93.5

Overall Recoveries: 80 – 125%

*PFTreDA, PFTriDA ~ 200% - matrix effects



Overall RSD: < 10 %

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Water Sample Types and Recoveries

- Reagent Water
- Surface (River) Water
- Ground Water
- Influent Waste Water
- Effluent Waste Water

	Average Percent Recovery in Matrix					
	Reagent Water	Ground Water	Surface Water	Influent Water	Effluent Water	Recovery Compound
PFBA	82.7	82.1	80.9	80.8	85.5	^{13}C-PFBA
PFPeA	89.1	87.7	90.2	88.1	91.4	$^{13}C_5$-PFPeA
PFHxA	89.7	90.1	91.7	91.3	93.3	$^{13}C_5$-PFHxA
PFHpA	90.6	89.8	92.6	91.3	91.9	$^{13}C_4$-PFHpA
PFOA	**92.5**	**92**	**94.2**	**94.7**	**94.3**	**$^{13}C_8$-PFOA**
PFNA	93	92.2	94.3	94.8	95.2	$^{13}C_9$-PFNA
PFBS	94.6	92.1	96.8	93.8	96.1	$^{13}C_3$-PFBS
PFPeS	94.6	92.1	96.8	93.8	96.1	$^{13}C_3$-PFBS
PFHxS	89.8	88.1	91.3	91.9	93.5	$^{13}C_3$-PFHxS
PFHpS	92.8	90.6	94.6	94.1	93.5	$^{13}C_8$-PFOS
PFOS	**92.8**	**90.6**	**94.6**	**94.1**	**93.5**	**$^{13}C_8$-PFOS**
PFNS	92.8	90.6	94.6	94.1	93.5	$^{13}C_8$-PFOS

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Water Sample Reproducibility

- Reagent Water
- Surface (River) Water
- Ground Water
- Influent Waste Water
- Effluent Waste Water

	% RSD in Matrix			
	Ground Water		Surface Water	
	Low spike (n=3)	High spike (n=3)	Low spike (n=3)	High spike (n=3)
PFBA	15.6	2.7	2.1	2.8
PFPeA	7.4	1.6	2.2	0.4
PFHxA	6.6	1.7	1.6	1.9
PFHpA	4.4	2.4	8.9	2.1
PFOA	**5.9**	**1.5**	**9.5**	**2.5**
PFNA	6.3	2.5	8.2	1.0
PFDA	8.7	1.3	5.2	1.9
PFBS	6.2	1.2	1.5	1.5
PFPeS	2.5	0.3	1.4	0.1
PFHxS	2.6	1.7	2.5	4.1
PFHpS	6.2	3.8	2.6	1.6
PFOS	**7.9**	**3.1**	**7.2**	**1.5**
PFNS	4.0	2.8	5.6	1.3

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PFAS's Summary

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- Occurrence studies have led to increased PFAS's testing needs
- Drinking water analysis is increasing steadily due to increased public awareness
- Other water types (non-drinking water) of increasing interest as well
- SPE enrichment allows entry level TQ to achieve ppt detection. HP TQ required for LVI/DI water analysis
- MRM database, analyte optimization tool and isolator column facilitate implementation

Thank you for your attention!

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Questions???