Pushing PFAS Possibilities: The Hunt for Ultra Sensitivity to Reach ppq EPA Health Advisory Levels

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What are the challenges in meeting the ever-evolving regulations around PFAS?

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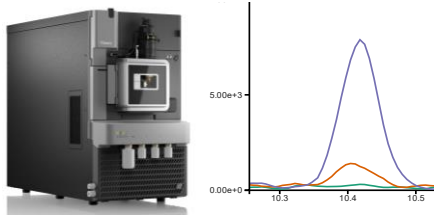
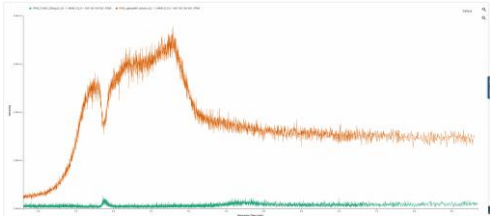
- Sensitivity – Xevo ™ TQ Absolute MS
  - Limits are dropping all the time,
    - EPA HAL levels introduced in 2022, sub ng/L requirement
    - EU Drinking Water Directive introduced in 2022 low ng/L requirement
    - Proposed EPA National Primary Drinking Water Regulation 2023, low ng/L requirement



- Contamination
  - PFAS used in a wide range of products
  - Background contamination an on-going problem



Why Ultra-Trace Detection?

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- In June 2022, the US EPA announced new health advisory levels (HALs) for PFOA, PFOS, PFBS, and HFPO-DA (GenX). The PFOA and PFOS HALs were set at an interim level of 0.004 and 0.002 ng/L (ppt), which raised concern about possibilities of detecting such levels in a reasonable manner.

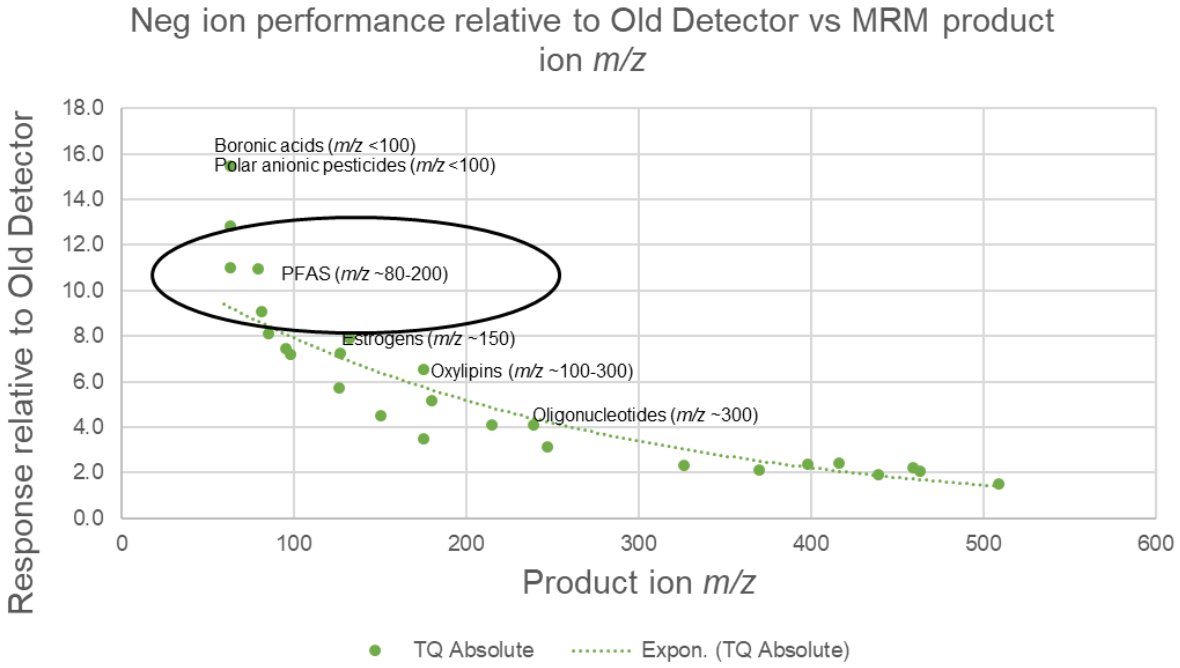
- Solid Phase Extraction (SPE) is required to reach these levels (Oasis™ WAX Cartridge)
- Highly sensitive LC-MS/MS system needed for detection (Xevo TQ Absolute MS)
- **Controlling contamination** most difficult part of this analysis

EPA Announces New Drinking Water Health Advisories for PFAS Chemicals, $1 Billion in Bipartisan Infrastructure Law Funding to Strengthen Health Protections

Compound	US EPA Health Advisory Level (ng/L)	Proposed EPA Maximum Contamination Level (ng/L)
PFOA	0.004 (interim)	4
PFOS	0.02 (interim)	4
PFBS	2000	Hazard Index = 1
GenX	10	Hazard Index = 1

Sensitivity – Enhanced Negative Ion Performance

Comparison of New Detector and Old Detector Technology



Xevo TQ Absolute MS

Sensitivity - Solvent standards – 0.01 ng/mL

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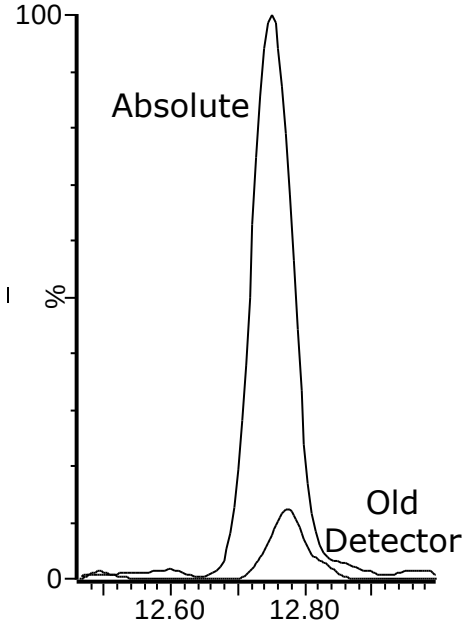
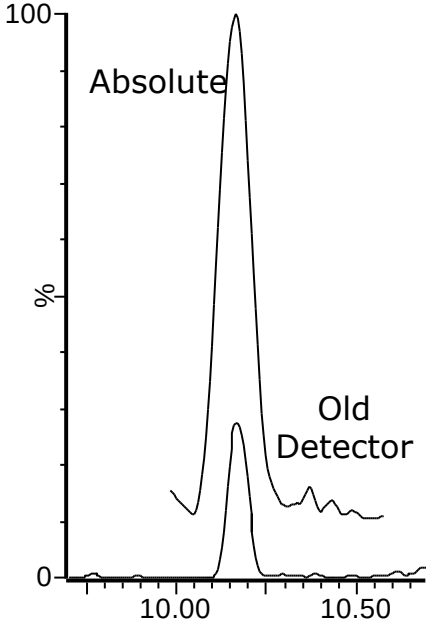
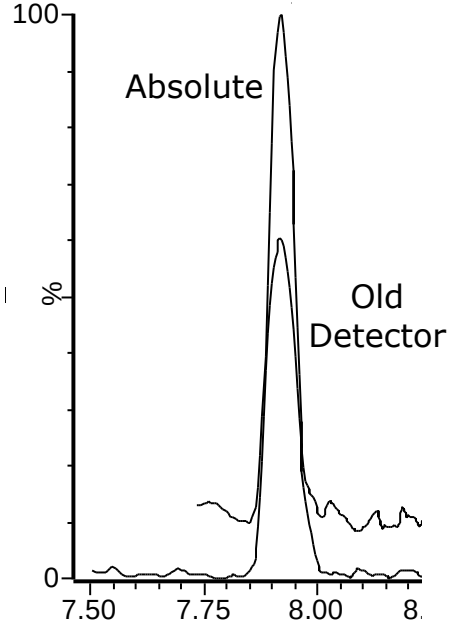
PFOS
498.9 > 80.1



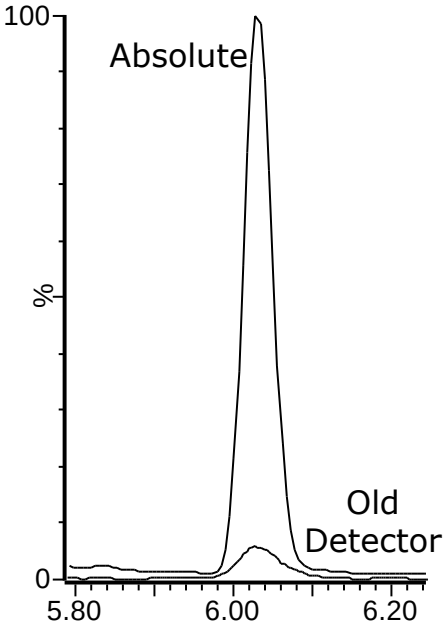
PFOA
412.9 > 169



GenX
285 > 169



PFBS
298.9 > 80.1



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Common Sources of Contamination to Avoid

External Sources	Direct Sources
Clothing/Lab coats treated with waterproofing materials	PTFE (Teflon) containers, lined caps, and tubing
Waterproof papers, notebooks, binders	Aluminum foil
Cosmetics and personal care products (sanitizers, lotions, etc)	Pipette tips branded as being “low retention”
Teflon tape	Permanent markers
Latex gloves	Vacuum grease
Antifog eyewear wipes and sprays	Glass transfer pipettes
Soaps and dishwashing detergents	PTFE filters

Evaluating Background PFAS Levels

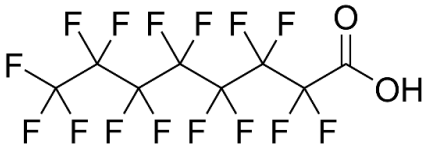
Methanol

Water

Formic or Acetic Acid

Ammonium Hydroxide

Ammonium Acetate



Methanol

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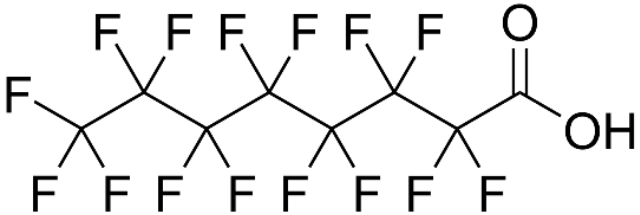
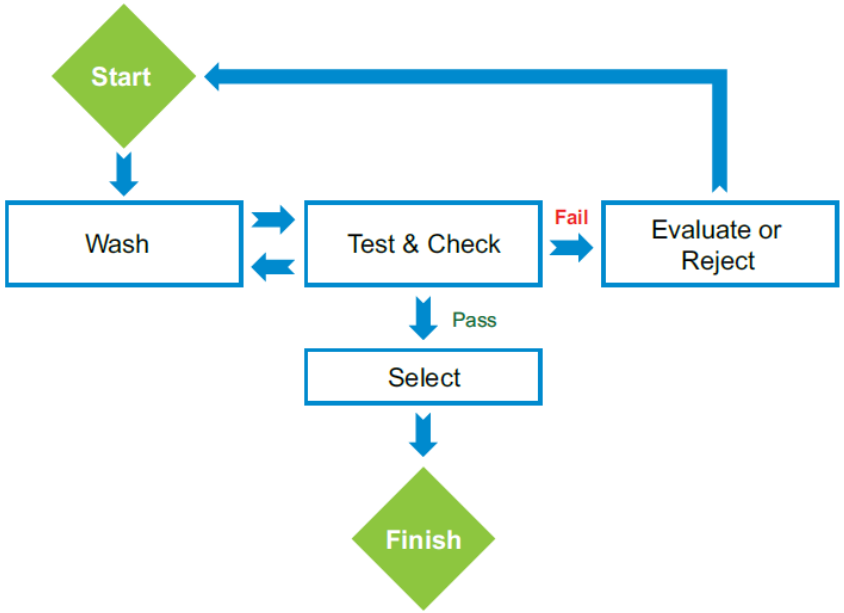


Evaluating Background PFAS Levels

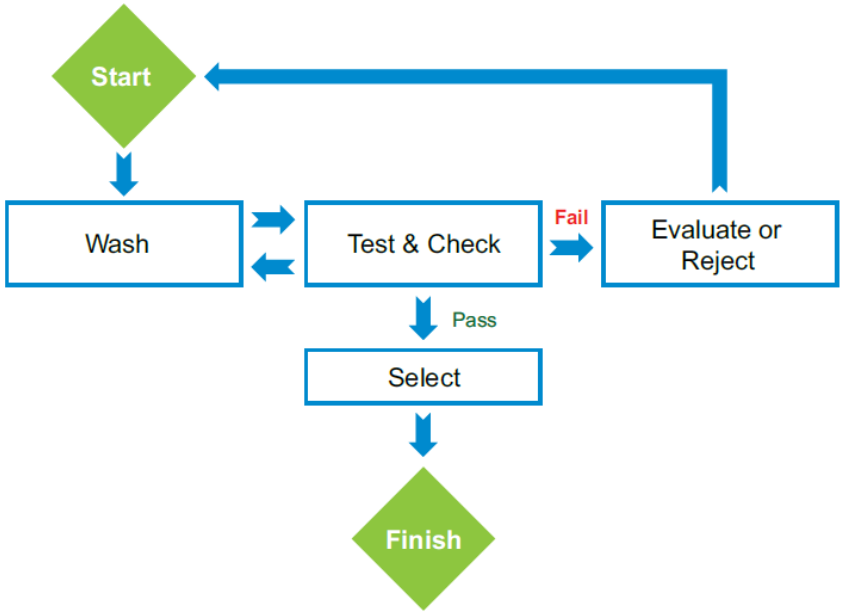


"Best practices for monitoring PFAS contamination in a routine shared-space commercial laboratory"

LINK TO WHITE PAPER

- Consumables
  - Sample Vials and Caps
  - Sample Tubes (15 mL and 50 mL)
  - Sample Collection Bottles
  - Pipette Tips
  - Pipette Filters
  - SPE valves
  - SPE reservoirs
  - Graduated Cylinders
  - Reagent Bottles
- Using "clean" methanol, rinse item, collect rinse
  - Depending on sensitivity of MS and method, can dry rinse and reconstitute in injection solvent OR inject without dry down
- Run using PFAS LC-MS/MS method

Evaluating Background PFAS Levels



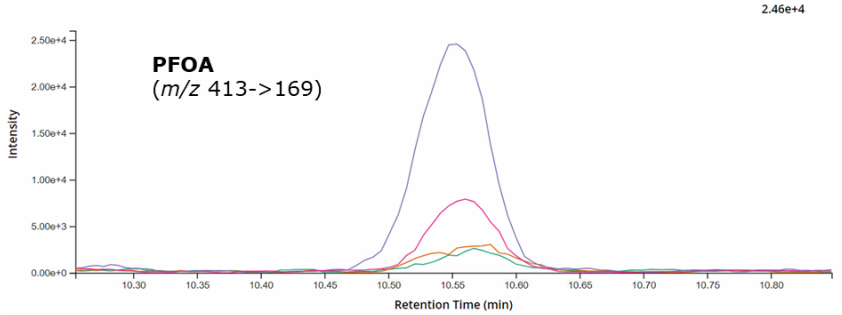
"Best practices for monitoring PFAS contamination in a routine shared-space commercial laboratory"

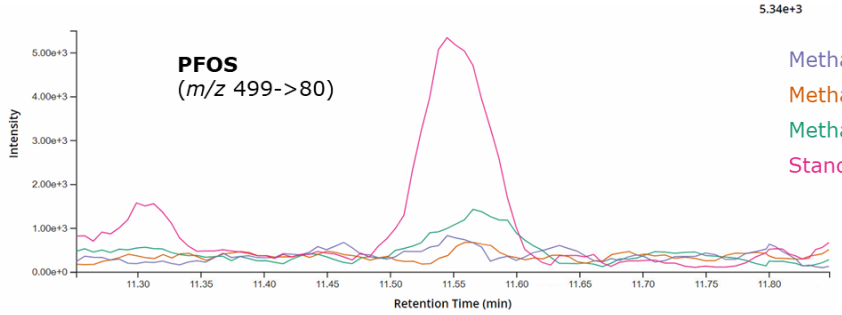
LINK TO WHITE PAPER

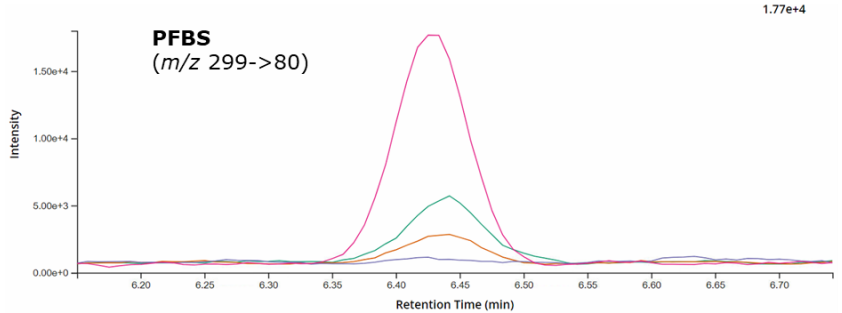
- Solvents and Reagents
  - Methanol
  - Water
  - Formic Acid
  - Acetic Acid
  - Ammonium Hydroxide
  - Ammonium Acetate

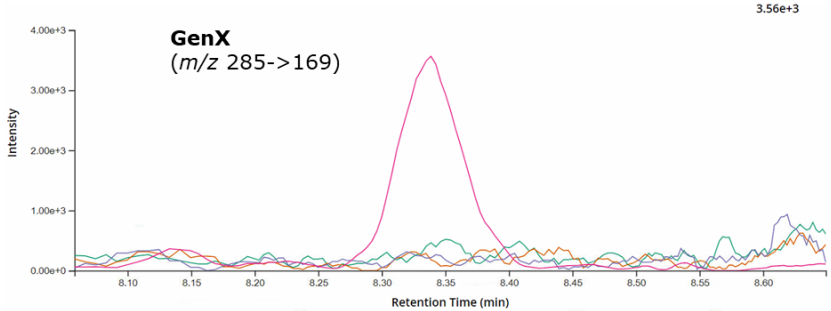
- Aliquot portion (10 mL) of reagent into sample tube and dry under N_2 *(or whatever drying apparatus used in lab for final samples)*
- Dry empty sample tube with reagent aliquots to account for any background from tube
- Reconstitute in injection solvent and run using PFAS LC-MS/MS method

Methanol

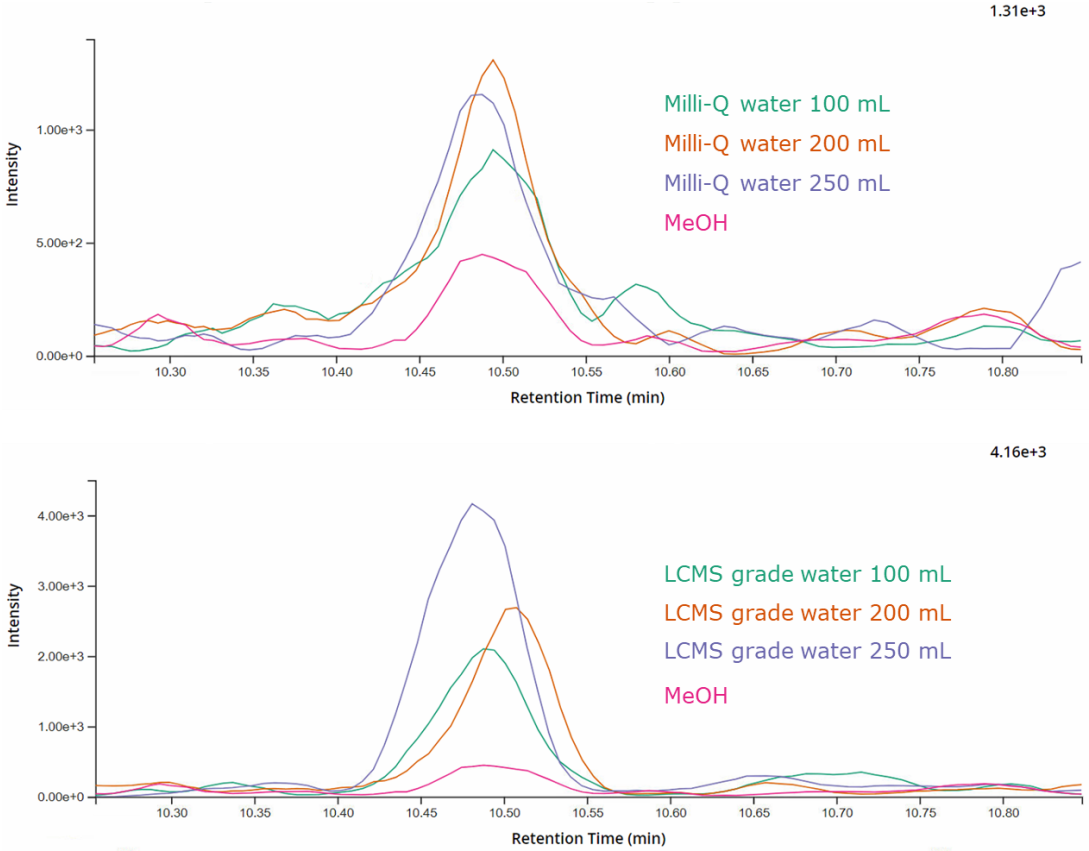








Reagent Water



Ammonium Hydroxide

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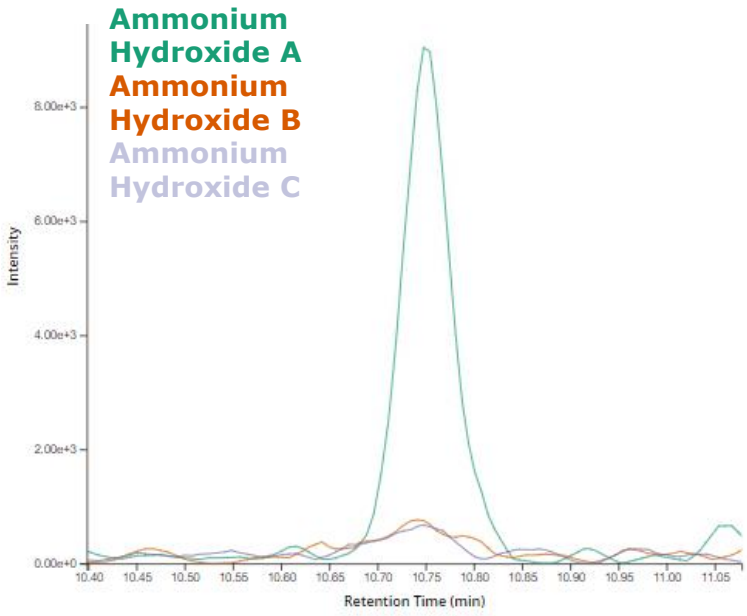
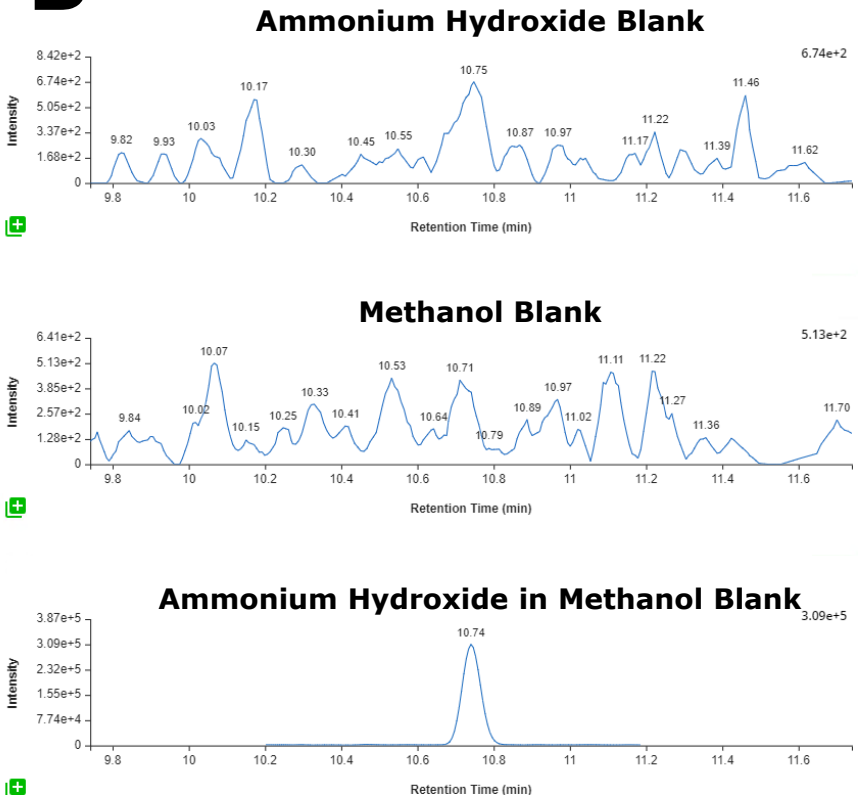
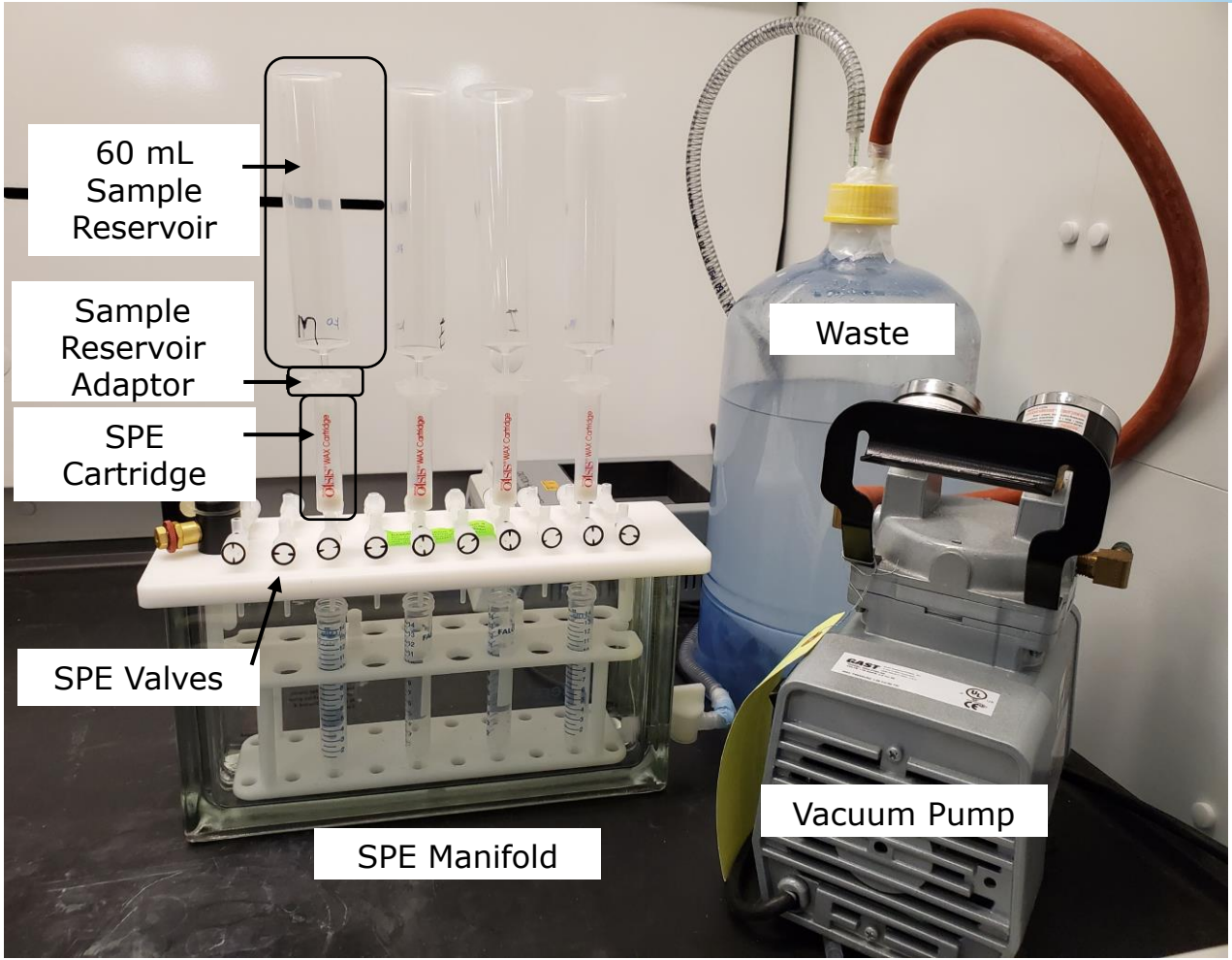
A



B





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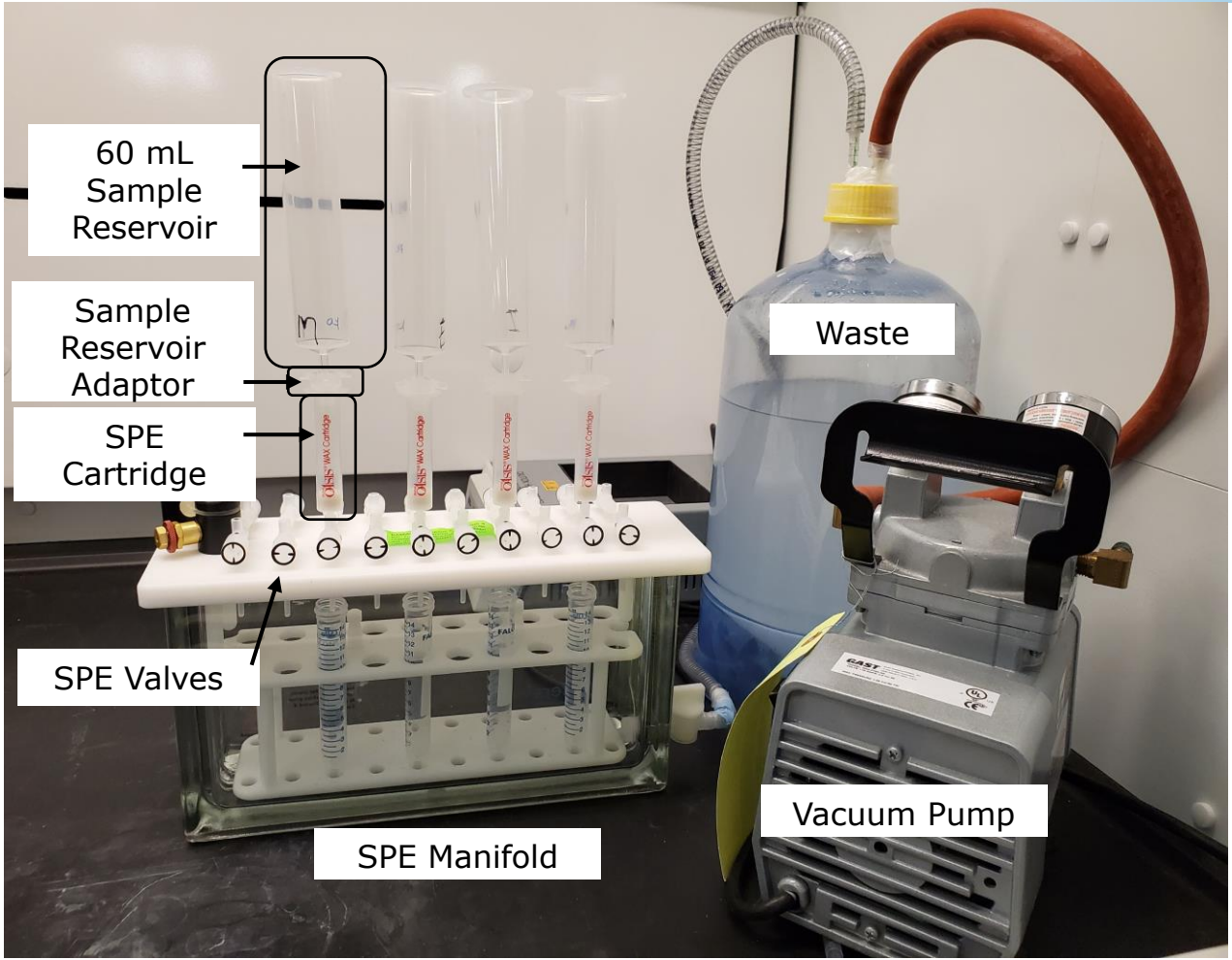
- Cleaning Re-Usable Items:
  - SPE valves
  - SPE adaptors
  - N_2 evaporator needles
    - Sonicate in glass beaker filled with methanol
    - Use dedicated beaker for cleaning purposes only
    - Have SPE valves in “open” position
  - Large volume SPE reservoirs
    - Rinse thoroughly with methanol



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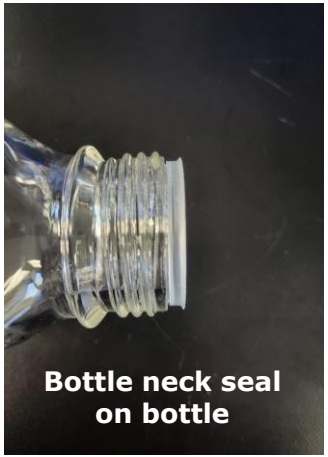
- Cleaning Re-Usable Items:
  - SPE manifold
    - Rinse lid and inside glass chamber thoroughly with methanol between each use
    - If possible, it is advised to have separate “trace” and “high contamination” manifolds
      - Pre-screening samples can determine which manifold each sample is directed to
      - This will prevent high contamination level samples from permanently contaminating trace level analysis

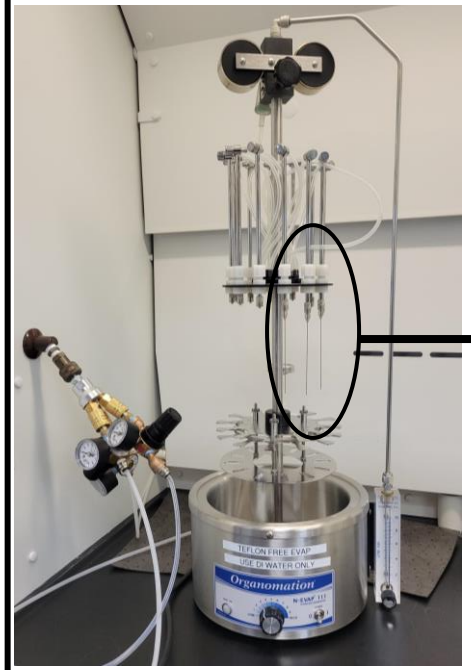
Other Precautions

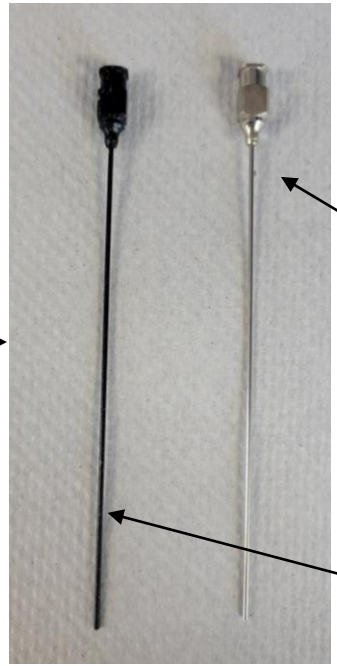


Bottle neck seal on bottle

Bottle neck seal removed from bottle

Reagent and solvent bottle Teflon liner inside cap





Stainless steel needle valve with no coating

Needle valve with Teflon coating

Spike 250 mL water sample with labelled extraction standards (EIS)

↓

Prepare OASIS WAX for PFAS, 6 cc 150 mg by passing the following through cartridge: 4 mL 2% (v/v) ammonium hydroxide in methanol, 4 mL methanol, 4 mL water

↓

Load sample onto cartridge at rate of 2-4 drops per second

↓

Wash cartridge with 4 mL 25 mM ammonium acetate buffer (pH 4)

↓

Dry cartridge briefly to remove any residual water

↓

Wash cartridge with 4 mL of methanol

↓

Elute with 2 x 4 mL 2% (v/v) ammonium hydroxide in methanol into 15 mL collection tubes (pre-washed with methanol)

↓

Dry samples under nitrogen at ~50 °C to near dryness (where only a drop remains at the bottom of the tube)

↓

Reconstitute samples with 0.25 mL of methanol and 0.25 mL of 4 mM ammonium acetate solution containing injection standard (IIS)

↓

Transfer sample to polypropylene vial and cap

SPE method used for drinking water analysis using Oasis WAX

- Oasis WAX SPE Cartridge using 150 mg, 6 cc cartridges for drinking water samples
- Isotope labelled internal standards spiked before extraction (EIS) and after extraction (IIS)
- Enrichment factor of 500x
  - 250 mL drinking water sample → 0.5 mL extract volume

PFAS Instrument Methods

Source Parameters

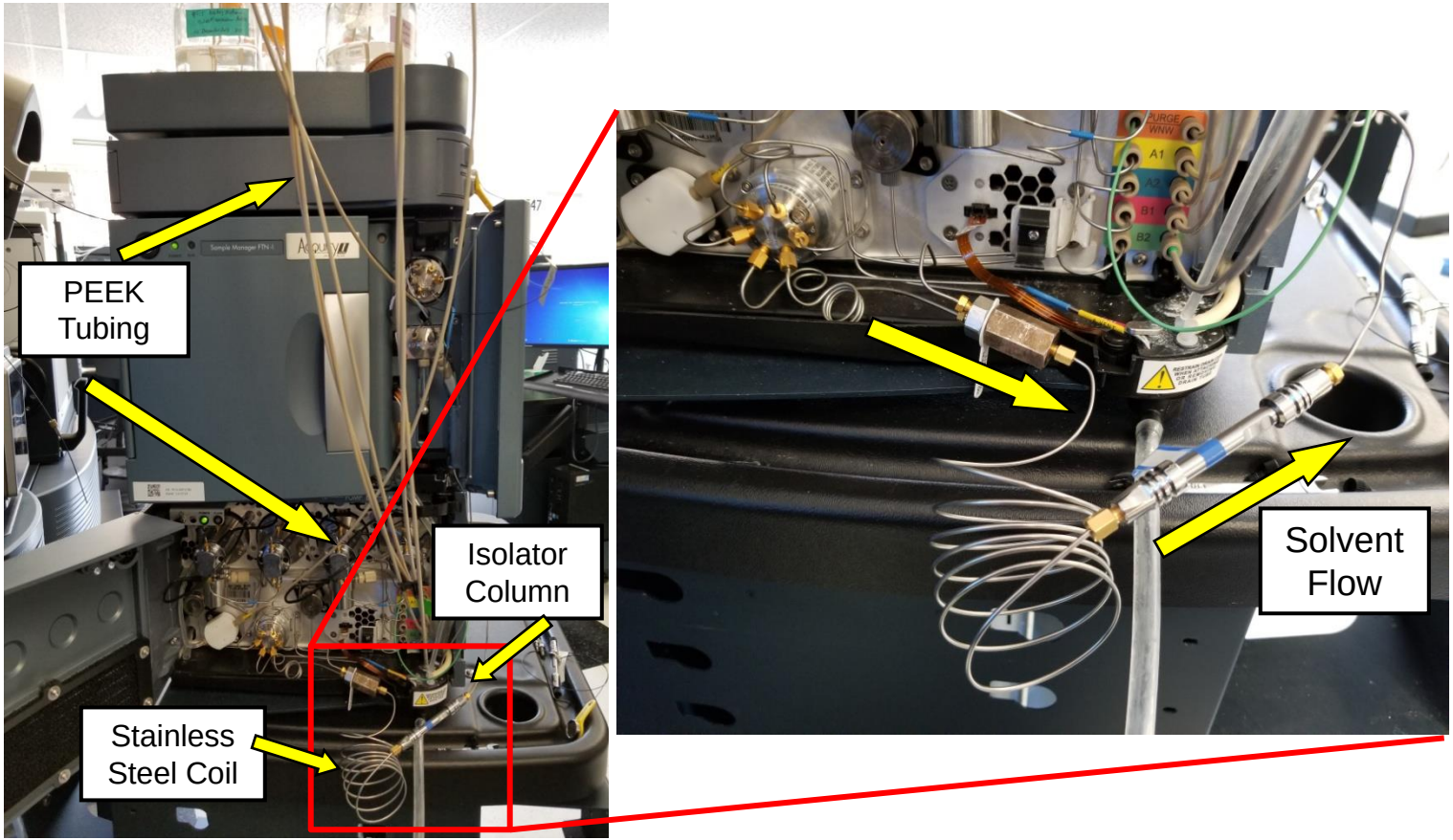
- Instrument: Xevo TQ Absolute MS
- Ion Mode: ESI-
- Capillary Voltage: 0.5 kV
- Desolvation Temperature: 350° C
- Desolvation Flow: 900 L/hr
- Cone Flow: 150 L/hr

LC Method

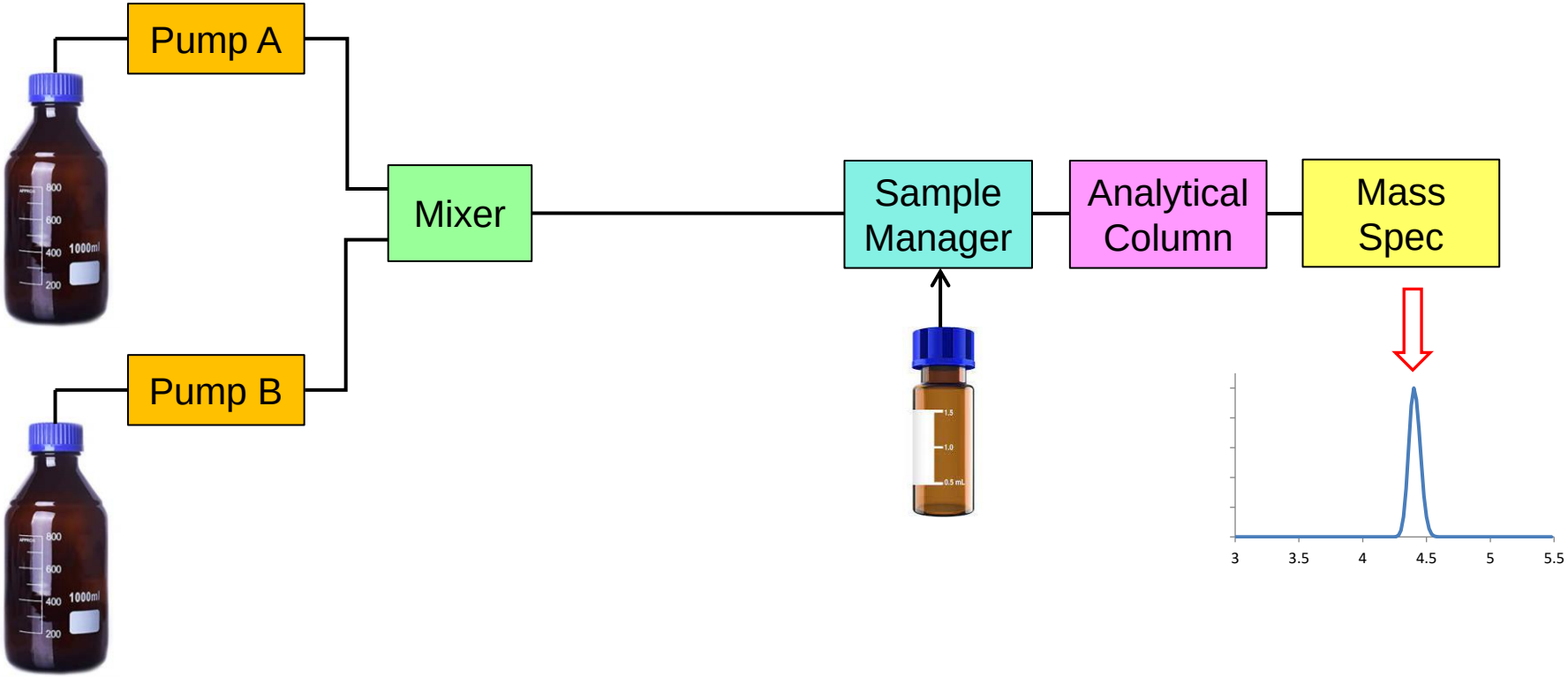
- Instrument: ACQUITY™ Premier BSM FTN System with **PFAS Kit**
- Column: ACQUITY Premier BEH™ C18 Column 2.1mm x 100 mm, 1.7 µm
- Isolator Column: Atlantis™ Premier BEH C18 AX Column 2.1mm x 50 mm, 5.0 µm
- Mobile Phase A: Water + 2 mM ammonium acetate
- Mobile Phase B: Methanol + 2 mM ammonium acetate
- Injection Volume: 10 uL
- Gradient:

Time (min)	Flow (mL/min)	%A	%B
0	0.3	95	5
1	0.3	75	25
6	0.3	50	50
13	0.3	15	85
14	0.3	5	95
17	0.3	5	95
18	0.3	95	5
22	0.3	95	5

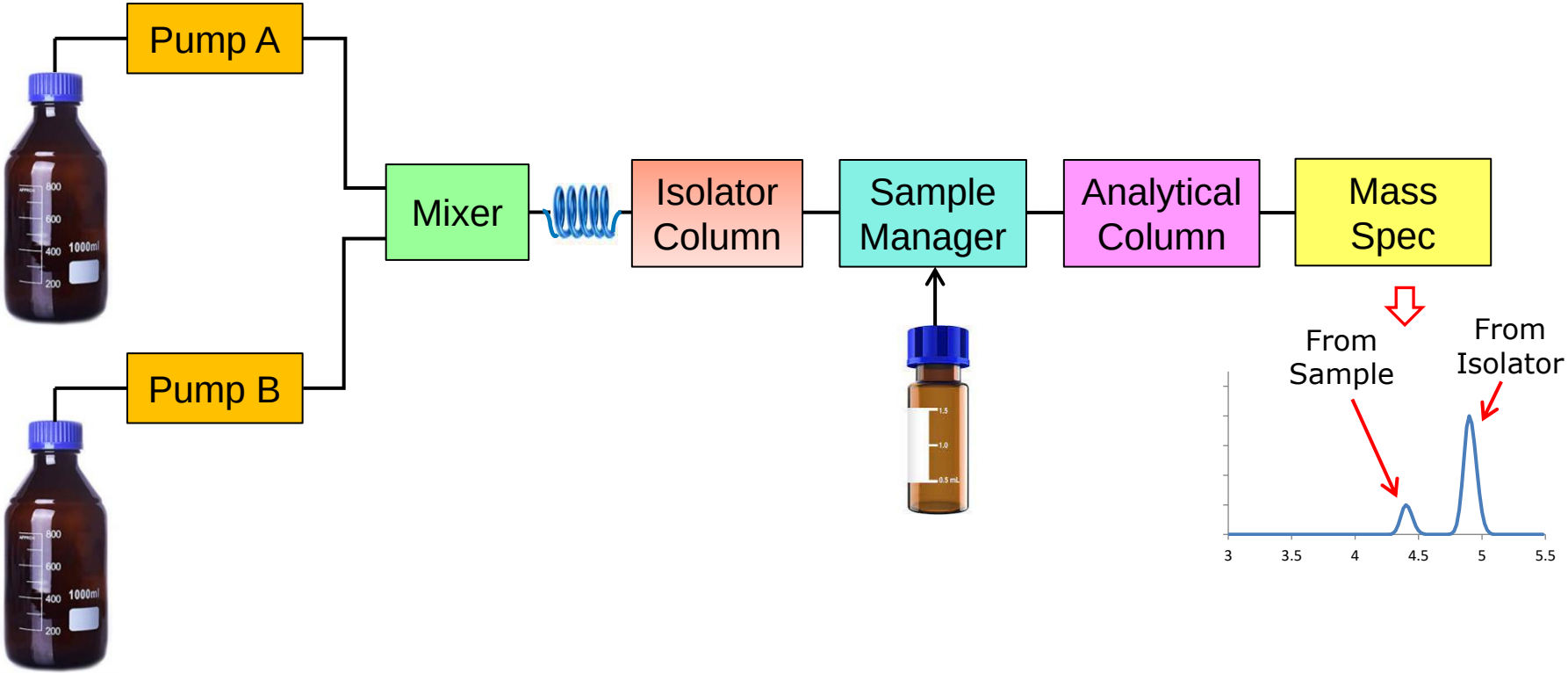
The Waters PFAS Kit



Standard LC Configuration



LC Configuration with Isolator Column



Sensitivity - Limit of quantitation (LOQ) and signal:noise (S:N) from two laboratories using two different TQ Absolute systems

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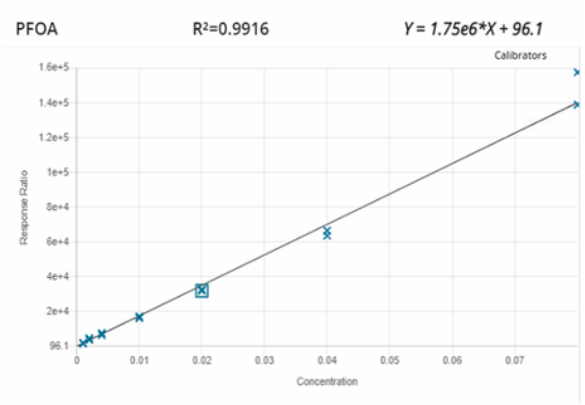
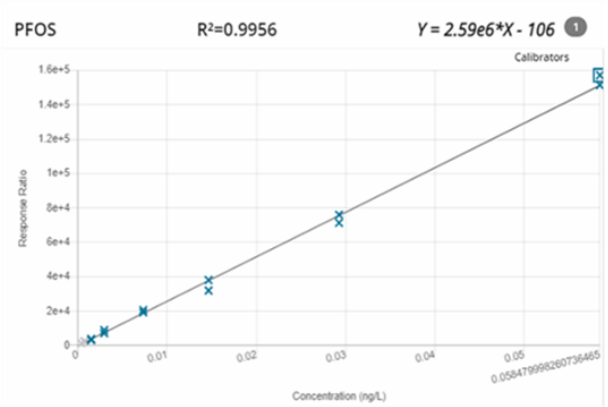
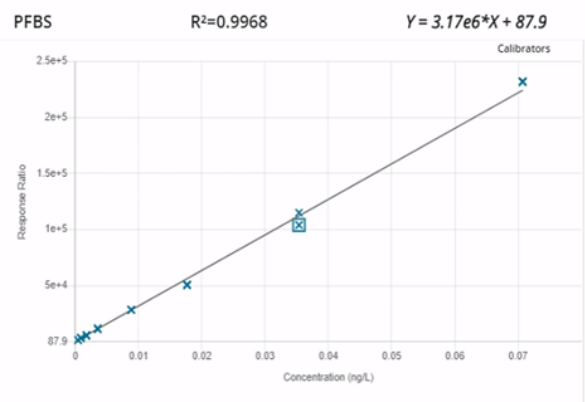
- LOQ was determined on two different Xevo TQ Absolute MS systems in two different laboratories to show consistent ability to reach HALs
- LOQs are all below the EPA HALs, even the interim levels for PFOA and PFOS
- LOQs are well below the interim minimum reporting levels

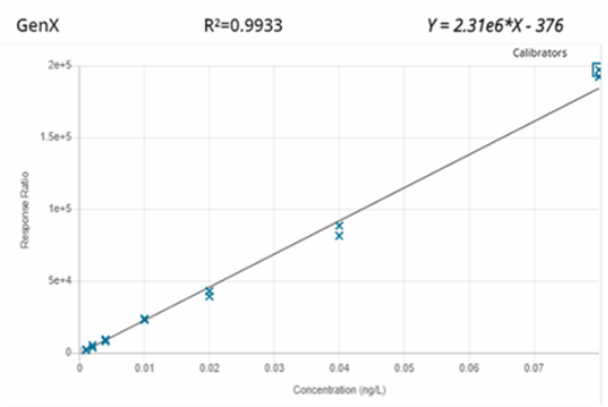
Compound	Laboratory 1 LOQ (ng/L)	Laboratory 2 LOQ (ng/L)	Laboratory 1 S:N of LOQ	Laboratory 1 S:N of LOQ	EPA HAL (ng/L)	Proposed Maximum Contamination Level (ng/L)
PFOA	0.001	0.001	10	10	0.004 (interim)	4
PFOS	0.001	0.001	12	27	0.02 (interim)	4
PFBS	0.0009	0.002	24	27	2,000	Hazard Index 1
HFPO-DA (GenX)	0.004	0.004	21	10	10	Hazard Index 1

Sensitivity - Calibration curves on the Xevo TQ Absolute MS









- Calibration curves were acquired in the range of 0.0005 to 0.08 ng/L (0.25 to 40 ng/L in vial equivalent).
- All curves were linear over this range
- All curves had a correlation coefficient of ≥ 0.991, with residuals within 30%
- Data acquisition and processing using waters_connect™ for Quantitation

Sensitivity - EPA HALs Testing

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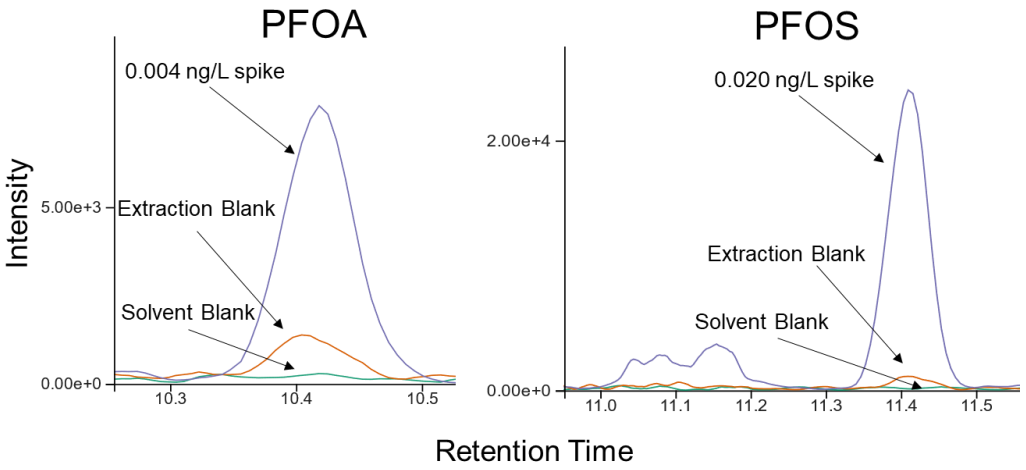
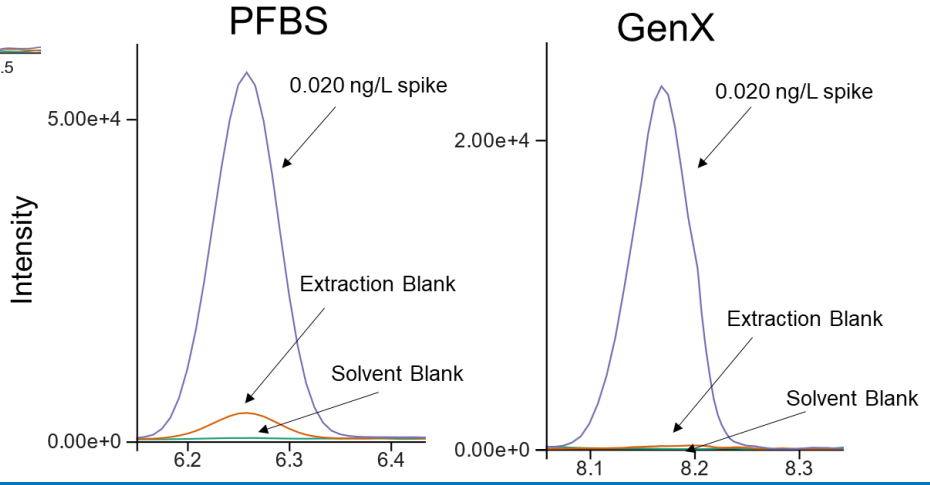


- Critical assessment of the background of the laboratory vs the response from the spiked samples
- Blanks response <30% of the method LOQ

- It may be impossible to completely eliminate contamination during sample preparation but <u>it can be controlled to an acceptable amount</u>.



Accuracy and repeatability of drinking water extractions and analysis

- This table demonstrates sample preparation method performance showing calculated concentrations and recovery values
- The average recovery was in the range of 90 – 111%
- %RSD was within the range of 2 to 13%
- The average recovery and %RSD values demonstrate excellent method accuracy and repeatability.

0.004 ng/L spike				
	Expected Concentration (ng/L)	Average Calculated Concentration (ng/L)	% RSD	Average % Recovery
PFOA	0.0040	0.0042	5	105
PFOS	0.0029	0.0030	5	103
PFBS	0.0035	0.0032	2	90
GenX	0.0040	0.0037	13	91
0.02 ng/L spike				
	Expected Concentration (ng/L)	Average Calculated Concentration (ng/L)	% RSD	Average % Recovery
PFOA	0.020	0.022	9	111
PFOS	0.015	0.014	6	94
PFBS	0.018	0.017	3	96
GenX	0.020	0.021	10	107

Conclusions

- Xevo TQ Absolute MS along with sample enrichment gained by sample extraction using Oasis WAX SPE Cartridges, this work has demonstrated that a typical laboratory can reach the HAL requirements from the EPA.
- The interim sub-ppt levels for PFOA and PFOS were reached without additional or special resources outside the normal range of an analytical prep shared-space laboratory.
- The challenging factor in this analysis was demonstrated to be cleanliness during sample preparation to limit the amount of PFAS contamination during this step. With focus on laboratory practices and sample handling, contamination can be kept to a minimum during sample preparation.
- The SPE method using Oasis WAX Cartridges was shown to be accurate and repeatable with excellent recoveries and % RSD of replicates.
- The full workflow presented in this study demonstrates **it is possible to reliably detect challenging trace levels of PFAS, in the ppq range, in drinking water samples**.

Acknowledgements - The Waters PFAS Team

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