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# PFAS Analysis by EPA 1633 | Building a Comprehensive Capability

**Waters Corporation**

July 10, 2024

# Today’s presenters

**Stuart Oehrle**
Senior Principal Technical Support Specialist

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Senior Product Marketing Manager

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Field Service Applications Team Leader

# Waters at a glance

- **Liquid chromatography, mass spectrometry, and thermal analysis innovator**
- **Industry-leading service and informatics**
- **Partner with customers around the world to ensure the:**
  - Purity of our water and food
  - Efficacy, safety & quality of medicines and vaccines
  - Durability of products we use everyday



**$2.9BN**

2023 Revenue

**~7,900**

Employees Worldwide

**35+**

Countries with Operations

BARRON'S 100 Most Sustainable Companies 2023

U.S. Green Building Council LEED USGBC

BEST COMPANIES TO WORK FOR U.S.News 2023-2024

MEMBER OF Dow Jones Sustainability Indices Powered by the S&P Global CSA

BIOTECH BREAKTHROUGH AWARDS

EQUALITY 100 2023 2024 Leader in LGBTQ+ Workplace Inclusion

# What we will cover today

- Getting started with PFAS testing: what you need for comprehensive testing capability
  - Spotlight on LC-MS/MS technology
  - Spotlight on sample preparation
- Training and support as foundations for success
- Q&A

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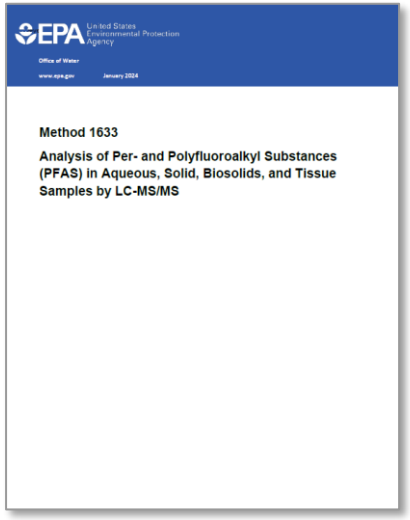
# Reminder of why (and what) we are testing: EPA method 1633




Newly finalized method (January 2024) to test for **40 PFAS compounds** in **wastewater, surface water, groundwater, soil, biosolids, sediment, landfill leachate, and fish tissue**

Created by the **US EPA's Office of Water**, partnership with the **US Department of Defense**'s Strategic Environmental Research and Development Program (SERDP)

Validated through a **multi-laboratory study** to finalize method and add performance criteria

Intended to be used for:

- Clean Water Act Compliance (wastewater discharge permits – NPDES)
- Superfund sites
- Remediation and investigation programs (including for DoD installations)

Source: US Environmental Protection Agency

# Brief intro to liquid chromatography + tandem mass spectrometry (LC-MS/MS): the LC

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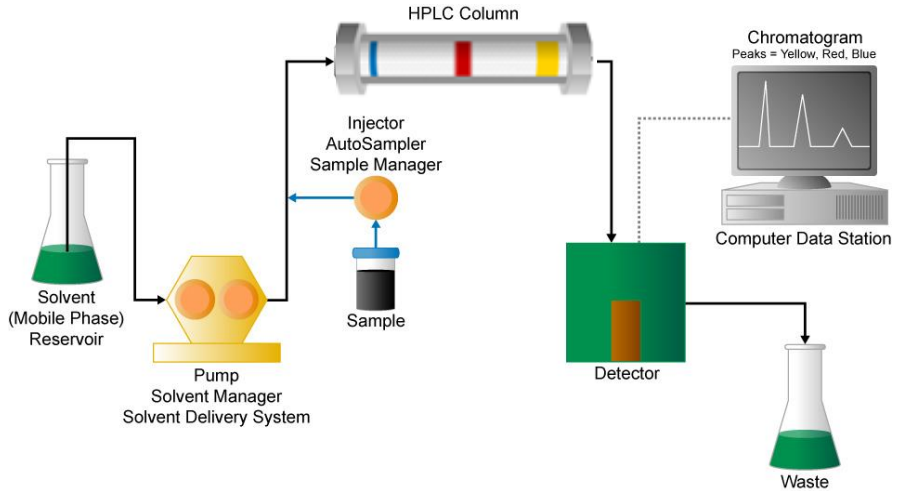
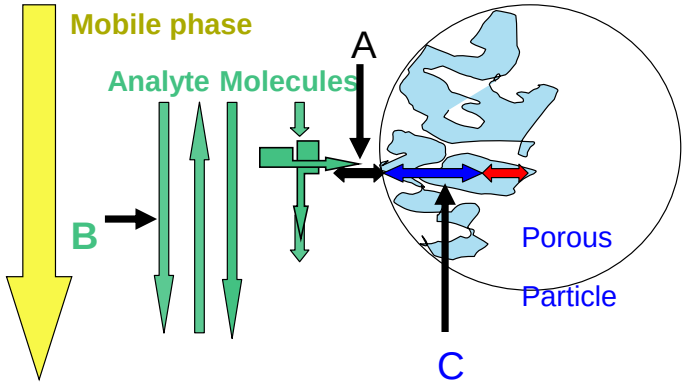
## The inlet (LC)




## Inside the HPLC column (packing)



# Brief intro to liquid chromatography + tandem mass spectrometry (LC-MS/MS): the MS

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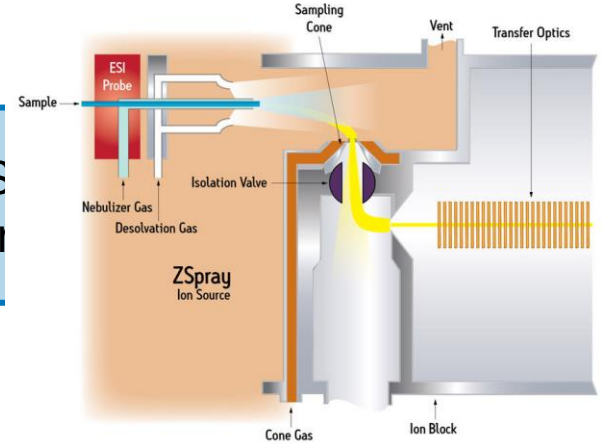


Mass Analyzer

Detector

Mass Spectrometer (MS Detector)

# LC-MS/MS has been selected by regulators for PFAS for the technology's selectivity and sensitivity

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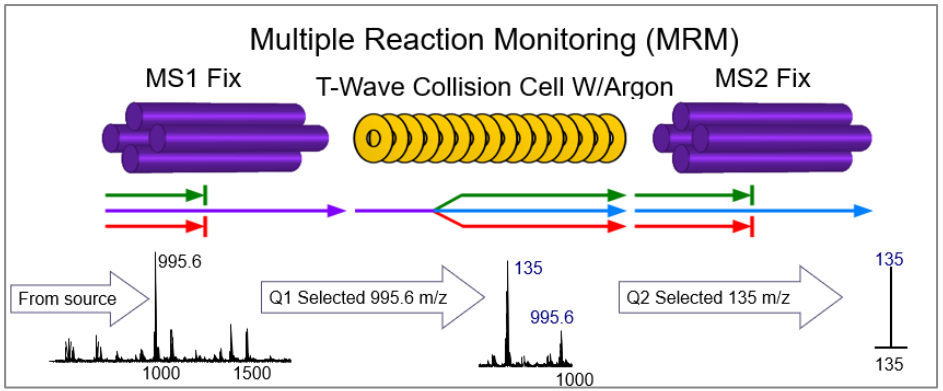
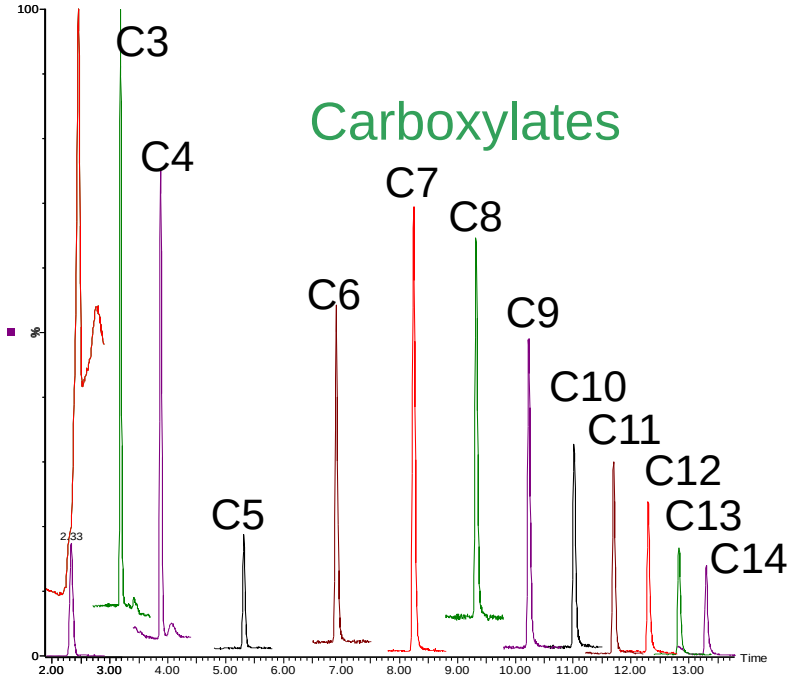
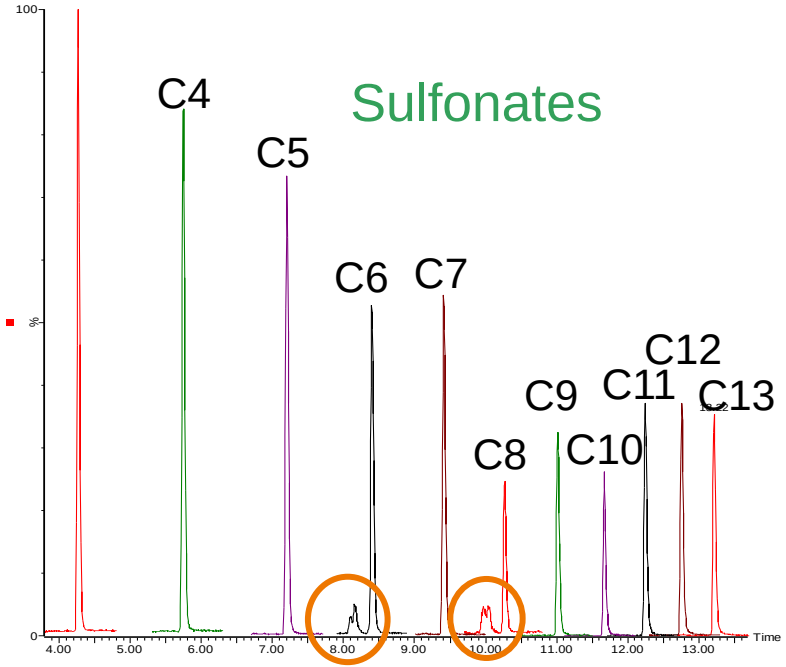
| | |
|---|---|
| High selectivity | Reduce or eliminate matrix interferences |
| High sensitivity | Trace levels - low reporting limits |
| Quantitative accuracy | Reproducibility, stability ,dynamic range and accurate quantitation of targets at low levels in matrix |
| Robustness | Complex sample matrices, reduced sample clean-up |



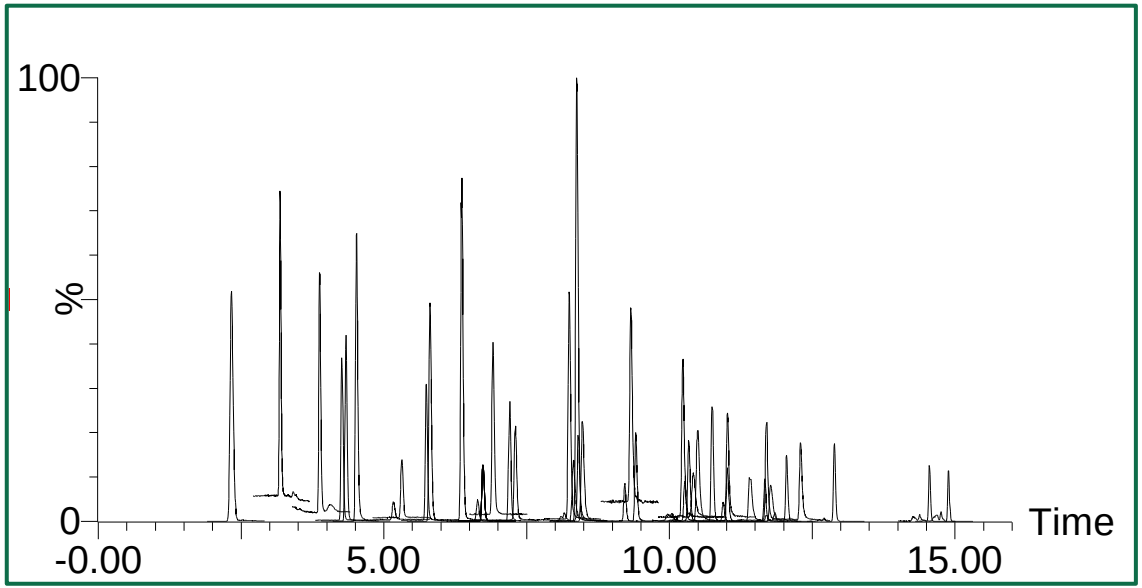

Waters ACQUITY™ Premier
UPLC™ + Xevo™ TQ Absolute MS

# What does that give you?



# The full picture




**46 native PFAS** covering both EPA 1633 and ASTM 8421 lists

# PFAS analysis by EPA 1633 – results from LC-MS/MS

## Certified reference material (CRM) validation

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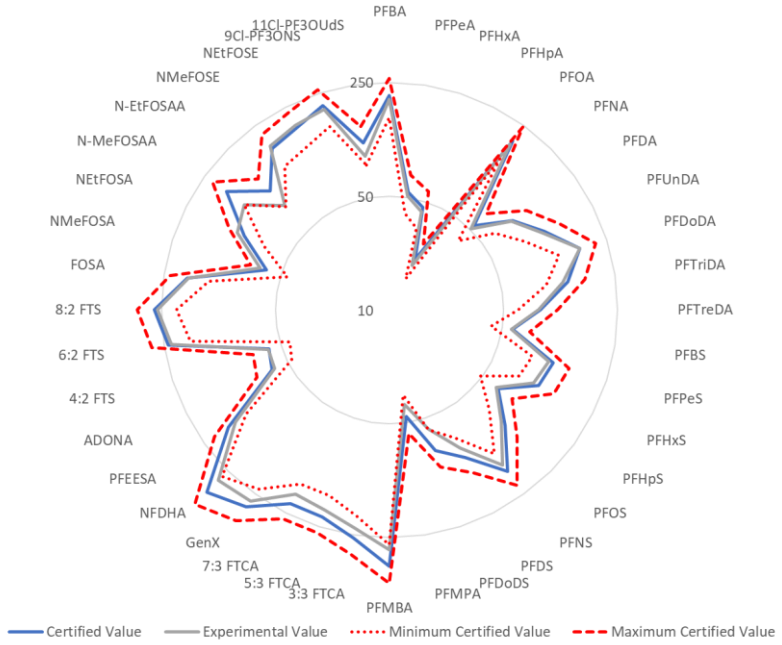
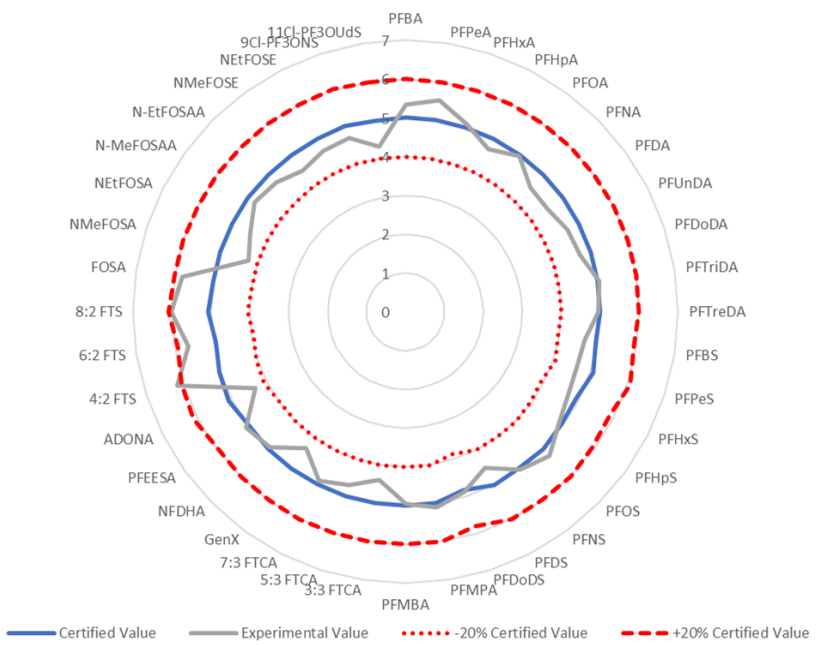
### PFAS in **wastewater** CRM




Mean trueness of 92%
Trueness range of 73 – 112%

### PFAS in **soil** CRM




Mean trueness of 97%
Trueness range of 85 – 120%

# LC-MS/MS is also used for EPA PFAS drinking water methods

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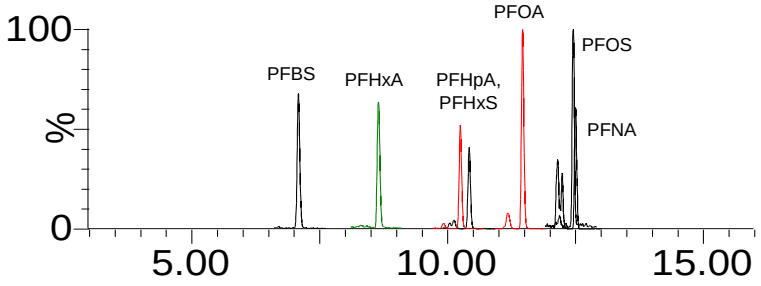
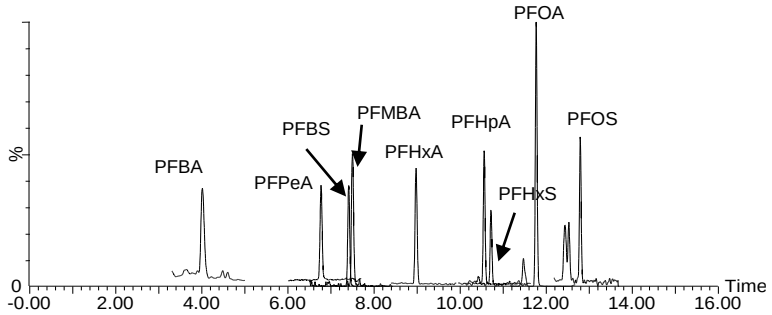
## EPA 537.1




## EPA 533




Identification and amounts (typically in ng/L)

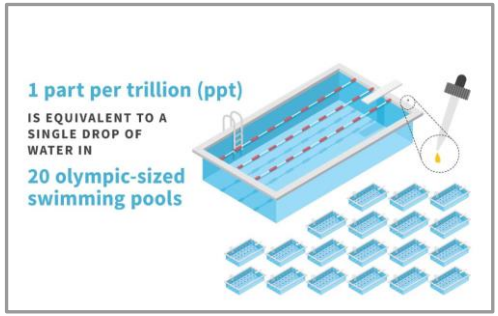
# Putting **ng/L** into perspective

Nanograms of compound per liter of solution (aka part-per-trillion (ppt))

- **One** part per 1,000,000,000,000 ($10^{12}$) parts

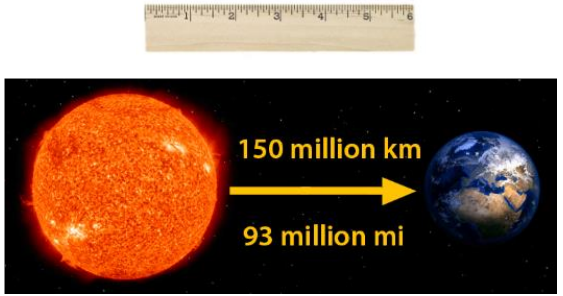
## Some ways to visualize one ng/L ….

1 drop of water in 20 Olympic-sized swimming pools




One second of time in approximately 31,700 years

Traveling 6 inches out of a 93-million-mile journey to the sun[1]




1. The earth is 93 million miles (avg.) from the sun

# Testing for PFAS requires a comprehensive analysis solution

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1



## Sample prep

- SPE cartridges
- Sample preparation apparatus (manual vacuum manifold or an automated system)
- Nitrogen sample evaporator

2

## Instruments

- Ultra-Performance Liquid Chromatography (UPLC)
- Tandem quadrupole mass spectrometer (MS/MS)
- PFAS hardware kit
- Analytical columns

3

## Informatics

- Instrument control and acquisition software
- Data processing and reporting
- Output to LIMS system or similar database
- Local quality system

4



## CRM and PT

- Certified reference materials (CRM)
- Proficiency testing (PT) program for analysts

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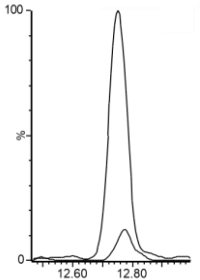
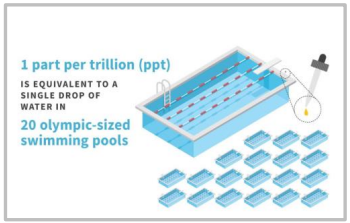
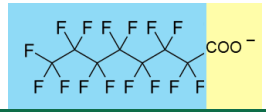
# Solid-phase extraction (SPE) - why we use it for PFAS analysis

- Regulatory compliance
  - US EPA 533, 537.1, and 1633 require solid phase extraction clean up steps
  - Reproducibility across laboratories running same method
- Clean up from complex matrices
  - Environmental waters, solids, biosolids, and other samples contain interferences
  - Reduce ion suppression and background
- Concentration of PFAS
  - Measuring at low levels (ng/L)
  - Increase limits of detection
- Selectivity in extraction
  - Use of WAX sorbent more specific to PFAS



# Sample preparation for EPA Method 1633

EPA Method 1633 requires cleanup with both a weak-anion exchange (WAX) SPE cartridge AND carbon

Depending on the sample matrix, the order of clean up is determined

EPA 1633 is a **performance-based** method, allowing for modifications as long as recoveries and RSDs% meet the method criteria


WAX SPE cartridges and loose graphitized carbon black (GCB)

**Ground, surface, and wastewaters**

↓

WAX cleanup first, then GCB

**Soils, sediments, biosolids, tissue**

↓

GCB cleanup first, then WAX


WAX/GCB and GCB/WAX dual-phase, or bilayer cartridges combine these two cleanup steps into one single cartridge for faster and easier use in EPA 1633

# Why WAX and why GCB?

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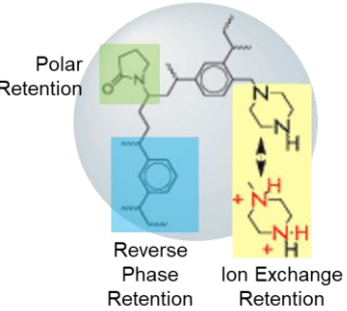
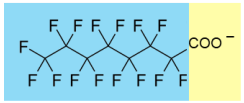
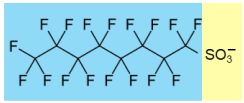
**Oasis™ WAX**



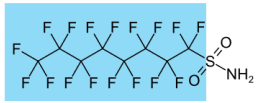

**PFOA**



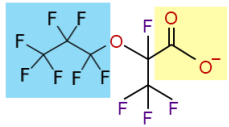
**PFOS**



**FOSA**



**Gen X**



**Prep Samples (250 mL)**
*pH adjust to < 3*

Activates ion exchange sites in WAX sorbent when loaded

**Condition Cartridge**

**Load Sample**

**Rinse/Wash Cartridge**

Remove neutrals

**Elute**
*Eluent with pH > 8*
*Dry and dilute*

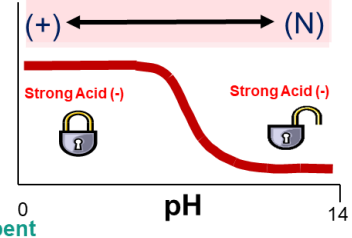
"Turns off" ion exchange sites in WAX sorbent to release PFAS

Strong acid are always ionized

Sorbent is a weak base that uses pH to selectively retain and release strong acid when gaining a (+) charge

pKa ~6

Oasis WAX SORBENT CHARGE




WAX uses reversed-phase and ion exchange retention that is selective to PFAS

But EPA 1633 also requires a cleanup with GCB


GCB removes **even more** interferences than WAX alone

Useful in **complex matrices** beyond drinking water

Benefit to the retention of **neutral** PFAS

# SPE protocol for EPA 1633 using dual-phase cartridges

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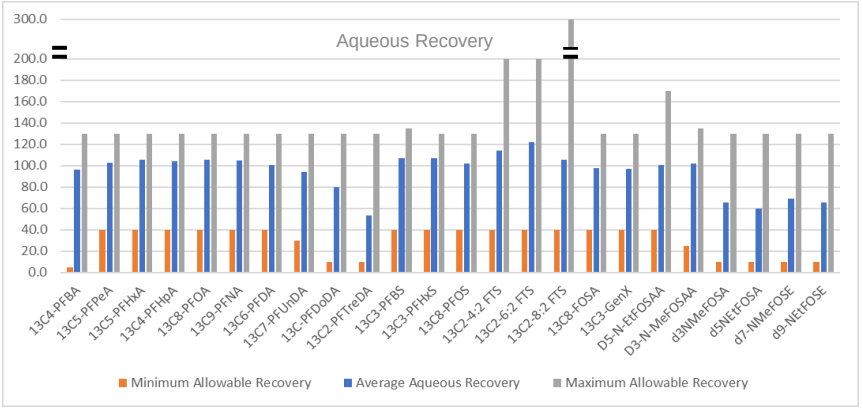
| | | |
|---|---|---|
| 1 | Prepare sample | ▪ Some Pre-SPE steps dependent on sample matrix type<br>▪ Check pH and adjust to 6.5 ± 0.5% using formic acid or ammonium hydroxide as needed<br>▪ Check cartridge orientation, pack glass wool to half height of the SPE barrel |
| 2 | Condition and equilibrate SPE cartridge | ▪ 15 mL of 1% (v/v) ammonium hydroxide methanol<br>▪ 5 mL of 0.3 M formic acid |
| 3 | Load sample onto SPE cartridge and washes | ▪ Load sample at 5 mL/min<br>▪ Wash cartridge with 10 mL of reagent water, ensuring to rinse reservoir with this solution<br>▪ Wash with 5 mL of 1:1 0.1M formic acid:methanol, ensuring to rinse the reservoir with this solution<br>▪ Dry cartridge for 15 seconds |
| 4 | Elute sample from SPE cartridge | ▪ Place collection tubes in manifold<br>▪ Rinse bottle with 5 mL 1% (v/v) ammonium hydroxide in methanol. Transfer to cartridge and elute<br>▪ Add 25 µL acetic acid to each sample<br>▪ Spike each sample with non-extracted internal standard |

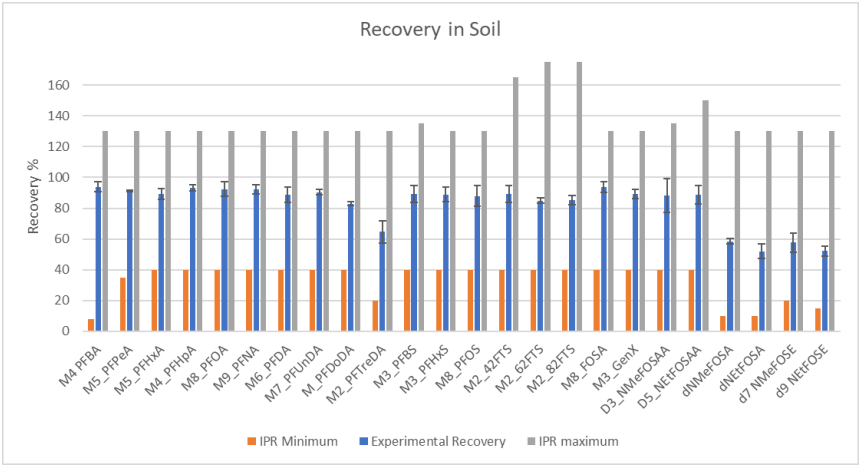
Full workflow with WAX/GCB and GCB/WAX cartridges video

# Sample prep that meets EPA Method 1633 requirements

- Proof of equivalence *must* be demonstrated when using a dual-phase cartridge.
- Dual-phase cartridges remove 4 steps in the protocol, and are faster, easier, and cleaner to use than loose GCB.
- **Recoveries and repeatabilities** for GCB/WAX for soil and fish tissue and WAX/GCB for ground, surface, influent and effluent waters **were measured and are within acceptance criteria and are repeatable.**

- For soil (n=3):
  - Mean recovery of all EIS was 81%
  - Mean %RSD was 2.8%
- For tissue (n=3):
  - Mean recovery of all EIS was 85%
  - Mean %RSD was 9.2%
- For environmental waters (n=5):
  - Mean recovery of all EIS was 91.2%
  - Mean %RSD was 9.2%



# Looking ahead - Making analysis by EPA 1633 ‘easier’

## Research underway

Fully automate SPE using Oasis WAX/GCB using PromoChrom SPE-03

Introduction of dual-phase cartridges with WAX and GCB to reduce manual carbon steps

Utilize the high sensitivity of the Xevo TQ Absolute to reduce aqueous sample sizes

PromoChrom SPE-03


Oasis WAX/GCB and GCB/WAX

ACQUITY Premier with Xevo TQ Absolute

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# Decoding PFAS literature


LFSM

NMeFOSAA

SUR

$^{13}$C-PFHxA

EIS

NIS

CCC

ADONA

# What do the methods *not* give us?

- Instrument-specific information
  - Methods
  - Parameters
- Software-specific information
  - Data processing
  - Calculations
- A partner

# Education and training are fundamental to successfully establishing a PFAS analysis capability

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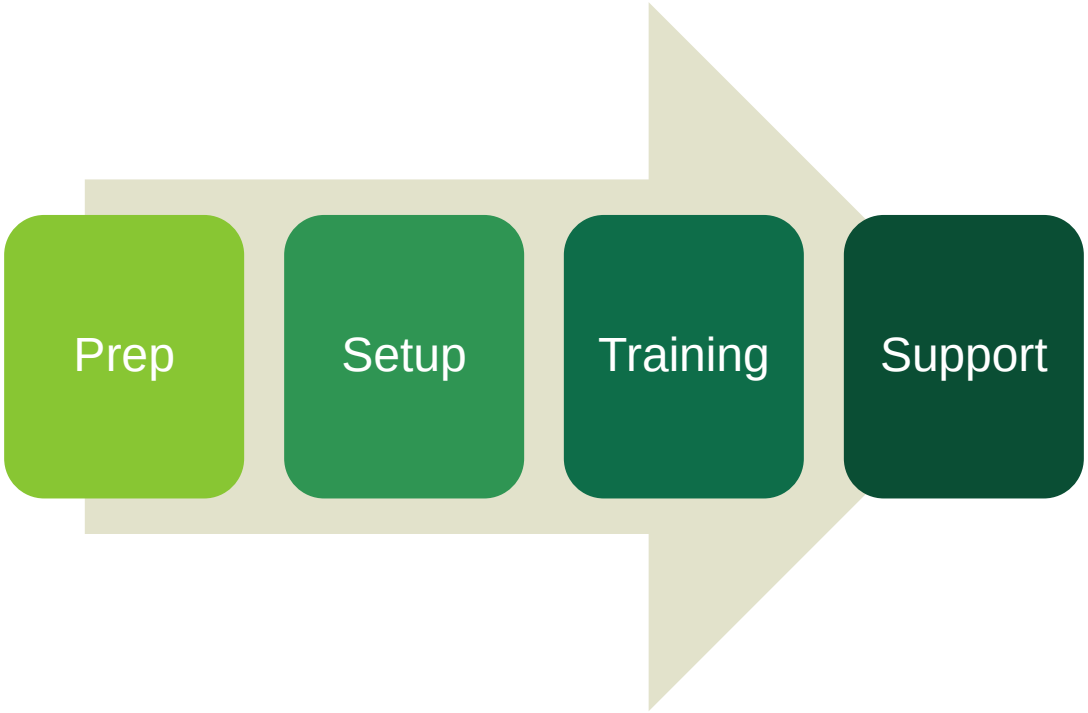
## Example suite of training options

- Custom onsite training
- Virtual training
- Advanced method consultation
- Education subscriptions
- Certified in-house courses
- E-learning courses


**No matter what you choose, we support you.**

The Waters™ Customer Education Team is here for you with expert instructors whether you have just purchased a new Waters instrument, need help learning a new software or application, or simply require refresher training on any of our platforms.

# Support for PFAS analysis should span from preparing your lab through the ability to run the analysis independently




We are with you every step of the way

# Example training collateral: detailed analysis guidance

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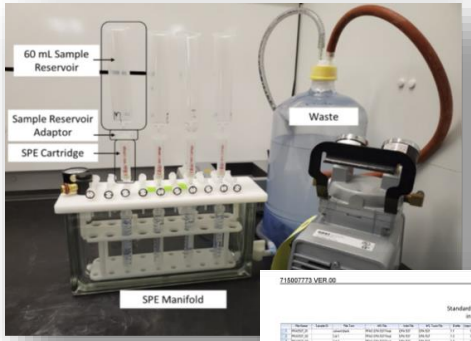
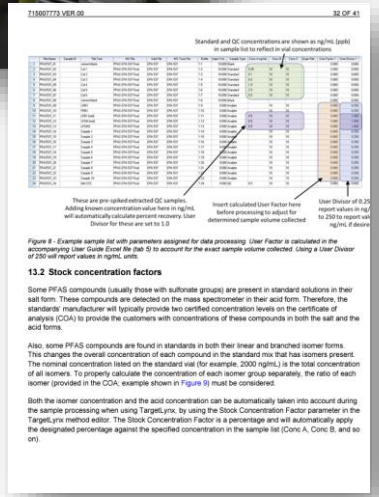
## PFAS Solution Setup Guide

- Consultant-led guidance
- Ensures necessary preparation for PFAS analysis

## PFAS Application Success Guide

- Detailed reference document
- Instructions for entire end-to-end workflow





715007773 VER 00
32 OF 41
Standard and QC concentrations are shown as ng/mL (ppb) in sample list to reflect in vial concentrations
These are pre-spiked extracted QC samples. Adding known concentration value here in ng/mL will automatically calculate percent recovery. User Divisor for these are set to 1.0
Insert calculated User Factor here before processing to adjust for determined sample volume collected
User Divisor of 0.25 report values in ng/mL to 250 to report values in ng/mL if desired
Figure 8 - Example sample list with parameters assigned for data processing. User Factor is calculated in the accompanying User Guide Excel file (tab 5) to account for the exact sample volume collected. Using a User Divisor of 250 will report values in ng/mL units.
13.2 Stock concentration factors
Some PFAS compounds (usually those with sulfonate groups) are present in standard solutions in their salt form. These compounds are detected on the mass spectrometer in their acid form. Therefore, the standards' manufacturer will typically provide two certified concentration levels on the certificate of analysis (COA) to provide the customers with concentrations of these compounds in both the salt and the acid forms.
Also, some PFAS compounds are found in standards in both their linear and branched isomer forms. This changes the overall concentration of each compound in the standard mix that has isomers present. The nominal concentration listed on the standard vial (for example, 2000 ng/mL) is the total concentration of all isomers. To properly calculate the concentration of each isomer group separately, the ratio of each isomer (provided in the COA; example shown in Figure 9) must be considered.
Both the isomer concentration and the acid concentration can be automatically taken into account during the sample processing when using TargetLynx, by using the Stock Concentration Factor parameter in the TargetLynx method editor. The Stock Concentration Factor is a percentage and will automatically apply the designated percentage against the specified concentration in the sample list (Conc A, Conc B, and so on).

# PFAS application user training: recap

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- Solutions setup and success guides
- Acquisition and processing methods ready to go
- Expert guidance – onsite and virtual
- Certified LCMS method implementation success
- Time and effort saved

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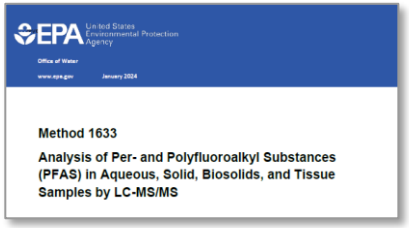
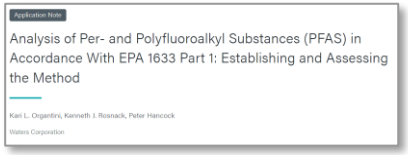
# What questions do you have?

# Want to learn more? Additional resources

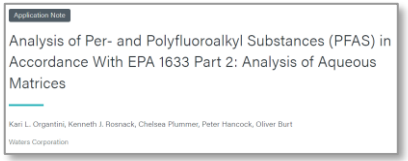
## Regulatory method and application notes




**EPA Method 1633**



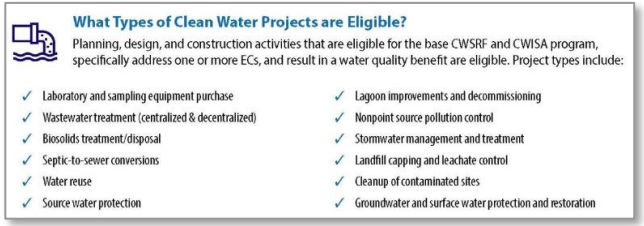

**Waters EPA 1633 Application Note Part 1**



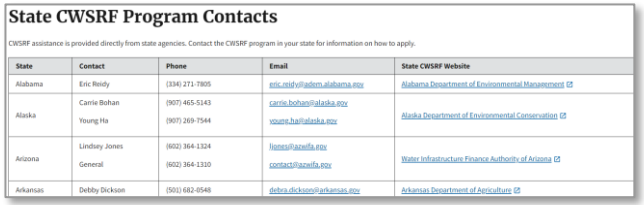

**Waters EPA 1633 Application Note Part 2**


**Best practices for monitoring PFAS contamination**

## Clean Water State Revolving Fund (CWSRF) resources




**CWSRF Emerging Contaminants Home Page**



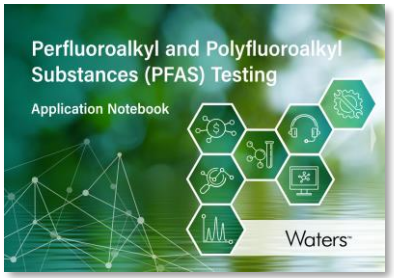

**State CWSRF websites and contacts**


Source: US Environmental Protection Agency, Waters Corporation

# Conquer your analytical PFAS challenges today

- Visit our PFAS Resource Hub for a complete guide to PFAS analysis

PFAS Application Notebook

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