

LC/MSMS - a Powerful Analytical Tool, but There are Still Challenges

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Agenda

- PFAS is Everywhere and in Everything
- We Build a Better Mouse Trap with all the Trappings
- Overview and Timeline of US EPA PFAS Methods
- LC/MS/MS – Three Dimensions of Separation
- Regulations From Water to Food Stuffs to Consumer Products
- Matrices, Preparation, and Clean-ups
- Sample Preparation Challenges
- General Qualitative Identification Challenges
- Specific Qualitative Identification and Quantitative Challenges
- Take Away Items

PFAS is Everywhere and in Everything

Fluorochemicals Were Invented in the 1930s

- Dramatically dubbed by the media as “Forever Chemicals.”
- By synthesis design, they repel water and oil and are remarkably thermally stable -They do not biodegrade, but they do bio-transform.
- Little toxicity information is known about ~ 99.8% of PFAS compounds.



We Build Better Mouse Traps with all the Trappings

- First mass spectrometer (MS) -1919
- First gas chromatography (GC) - 1951
- GC data process weighing strip chart paper - 1961
- First Automated GC data processing – 1969
- Use of isotope dilution GC/MS for VOA and SVOAs -1984
- HRGC/HRMS with Selective Ion Monitoring (SIM) -1994
- Solid phase extraction (SPE) -1999
- LC/MS/MS – 2001 (Early 1990s suggested – Centre Analytical)
- PAH and alkylated PAHs by SPE, HRGC/HRMS and SIM – 2007
- PFAS by SPE and LC/MS/MS - 2009

And the Lower We Go...

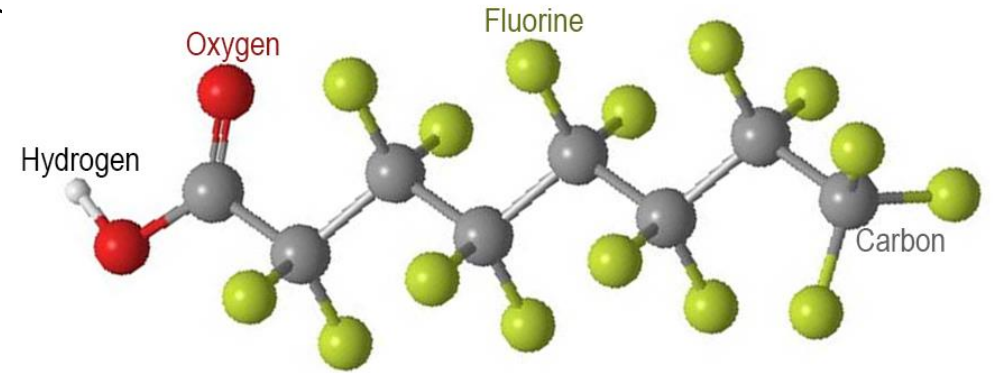
- We have the most sensitive instrument
- We maximize sample extraction mass/volume
- We minimize extract blow-down volume
- We maximum extract injection volume
- We (can even) reduce the signal/noise criteria for positive identifications

***Besides an amazing
design –
Background &
Matrix REALLY
Start to Matter !!***



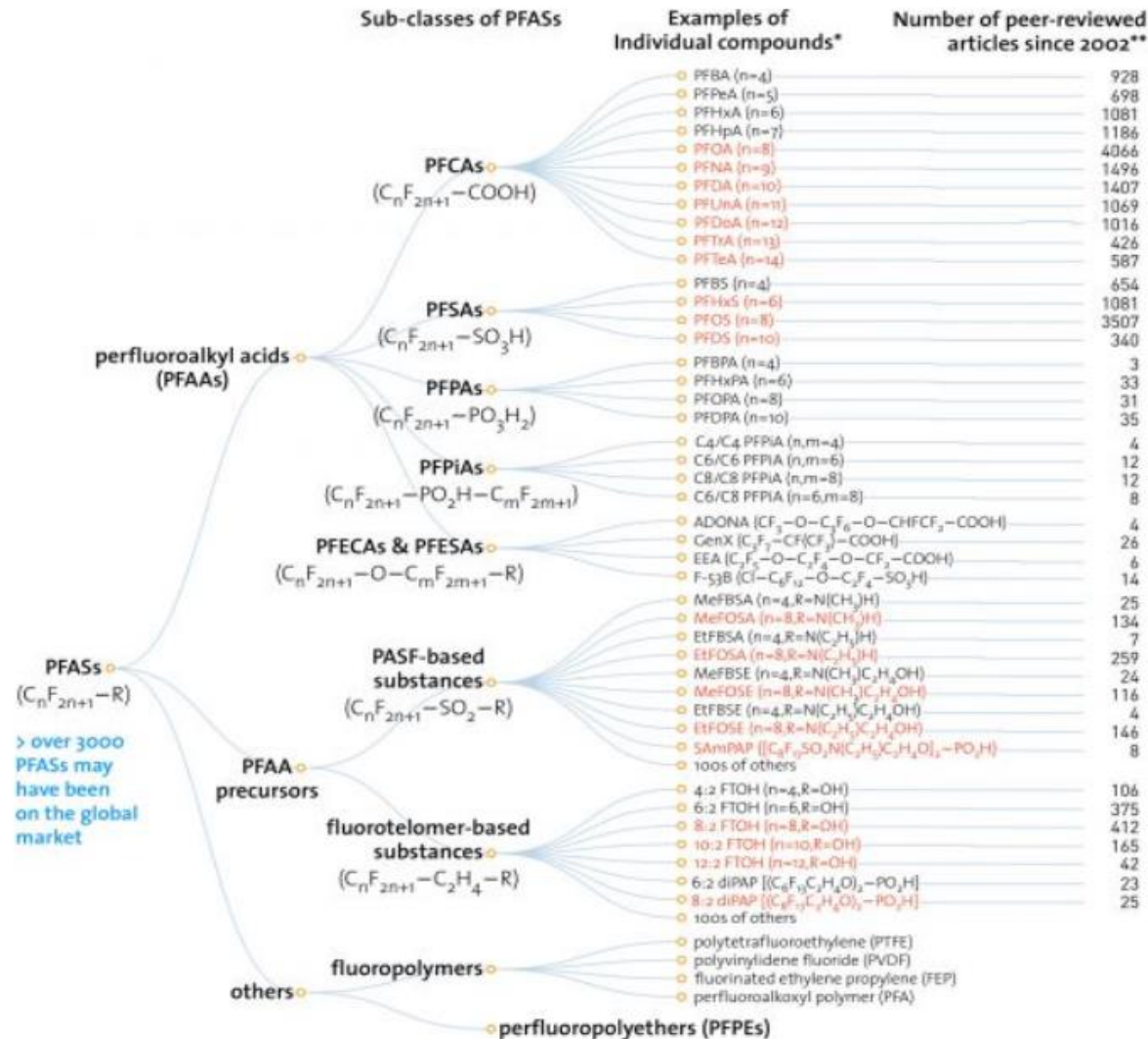
Overview and Timeline of US EPA PFAS Methods

- **US EPA Method 537.0** – Drinking Water – September 2012
(14 PFAS Compounds) UCMR3 May 2012
- **US EPA Method 537.1** – Revised November 2018
(18 PFAS Compounds)
- **US EPA SW-846 Method 8327** – Non-Drinking Water
Aqueous Method June 2019 then September 2021
(24 PFAS Compounds)
- **US EPA Method 533** – Drinking Water **(35 PFAS Compounds)** December 2019
- **US EPA DRAFT Method 1633** - Everything but DW – Draft Rev 4 – July 2023 **(40 PFAS Compounds)**
Still not subjected to formal Rulemaking – Includes every possible complex matrix under the sun !
- **US EPA DRAFT Method 1621** – Adsorbable Organic Fluorine (AOF) – (by Ion Chromatography)
Still not subjected to formal Rulemaking – Does not include fluoropolymers although a Total PFAS Proxy



The US Toxic Release Inventory (TRI) reporting now includes 180 PFAS compounds

There are lots of PFAS Compounds

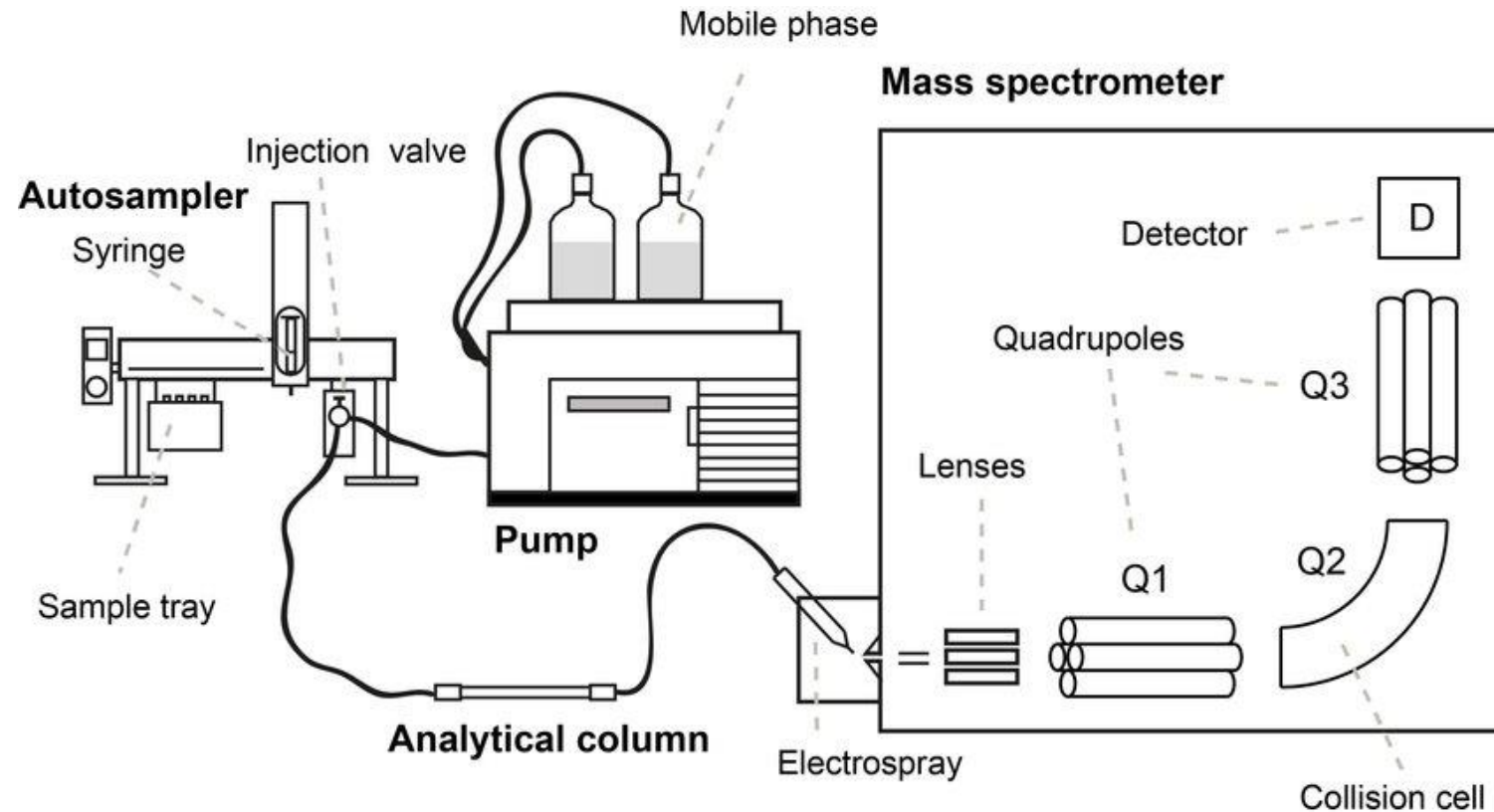


There are 6,330 CAS - named PFAS substances; however, recently proposed PFAS definitions [CF₂, CF₃] put the total over 10,000 compounds

Currently, there are certified native reference standards for about 60 PFAS compounds and far from enough isotopically labeled analogues

* Diagram from centerforenthnography.com

LC/MS/MS – Three Dimensions of Separation



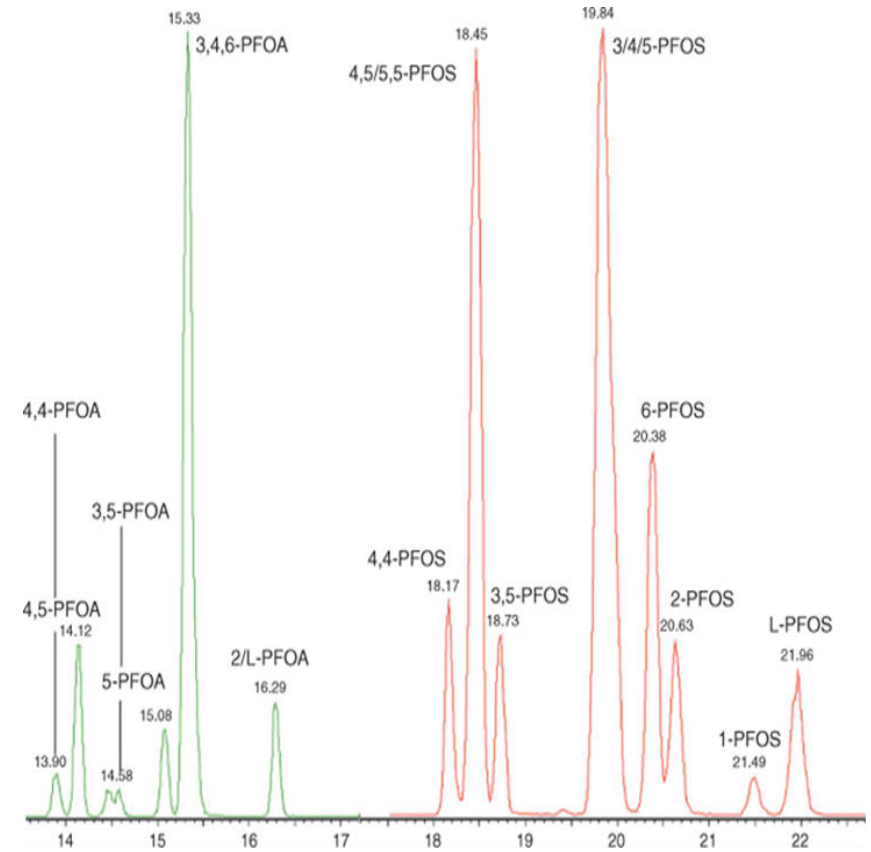
Courtesy of ReseachGate - www.reseachgate.net

LC/MS/MS is a highly sensitive technique that can detect trace levels of PFAS in complex matrices- however it is not immune to false positives/negatives due to a variety on challenges including instrument noise, sample matrix effects and many interferences.

To Complicate Matters Further – Branched/Linear Isomers

As an example, PFOS anion ($C_8F_{17}O_3S$)

- There are 89 possible structural isomers – around 11 isomers in technical-grade standard.
- Reported as PFOS, but actually represents PFOS isomers.
- If branched and linear are included in the PFAS, then all peaks should be integrated.
- Current approaches have been to sum branched and linear isomers together using the linear responses from the to generate the instrument calibration.



Kärman, et al., *Environmental Chemistry* 8(4) 372-380 2011
<http://dx.doi.org/10.1071/EN10145>

Beyond Water We Also Have Food Stuffs – US FDA Findings

Earlier this year, the Food & Drug Administration tested for PFAS in a variety of foods. While the sample sizes were small and may not reflect typical contamination levels, here's what the FDA found.



Regulations From Water to Food Stuffs to Consumer Products

In August of 2023 ASTM F15.81 – PFAS in Consumer Products Released their first DRAFT Standard Guidance

	California	Connecticut	Hawaii	Iowa	Maine	Maryland	Massachusetts	Minnesota	New York	Oregon	Rhode Island	Vermont	Washington
Cosmetics	X					X	P		P		P	P	P
Rugs & Carpets	X				X	X	P		P		P		
Fire-Fighting Foam		X		P		X							X
Food Packaging	X	X	P	P	X	X	P	X	X		P	P	X
Juvenile Products	X						P		X	X	P	X	X
Fabric/Textiles	P				X		P		P		P		
Anti-Fogging Sprays									P				
Ski Wax												X	

X = Regulation Passed

P = Proposed Legislation



Matrices, Preparation, and Clean-ups

Aqueous - Preparation

- Drinking water – SPE or solvent dilution
- Surface water – SPE or solvent dilution
- Groundwater – SPE or solvent dilution
- Beverages – SPE or solvent dilution
- Landfill leachate – SPE
- SPLP/TCLP leachate – SPE

Extract Clean-up

- Carbon powder
- Carbon cartridges



Matrices, Preparation, and Clean-ups (cont.)

Solids - Preparation

- Soils/ Sediment – Solvent extraction
- Biosolids – Solvent extraction
- Tissue and other Foods– Solvent extraction
- Consumer Products:
 - Grinding/Cryomilling
 - Leaching
 - Solvent extraction

Extract Clean-ups

- Carbon powder
- Carbon cartridge



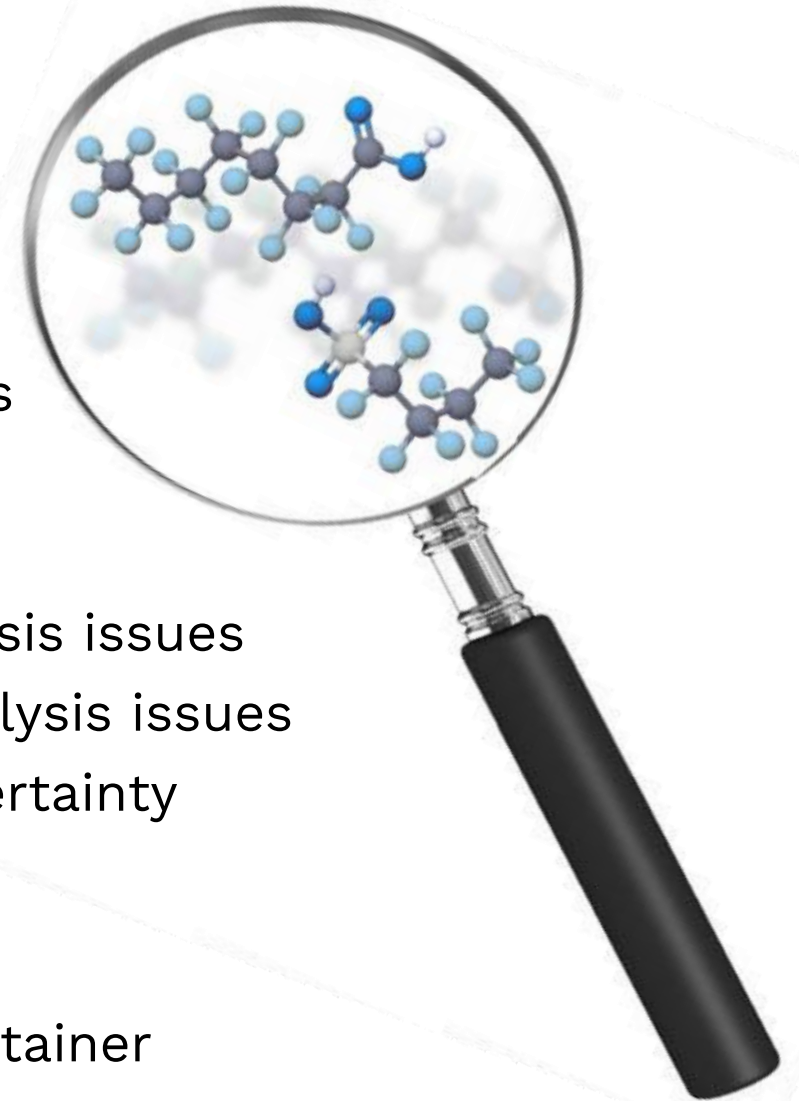
Sample Preparation Challenges

General Challenges

- Equipment contamination – false positive/high bias
- Reagent contamination – false positive/high bias
- Standards impurities – false positive/high bias
- Co-extracted compounds/non-target PFAS – analysis issues
- Inorganic salts (chloride, sulfate, and CaCO_3) – analysis issues
- Sample homogeneity – higher sample/analyte uncertainty

Aqueous – Solid Phase Extraction

- Clogged filters due to particulates
- Decanting due to particulates - but not rinsing container
- Reduced sample volume (and not rinsing container)



General Qualitative Identification Challenges

Chromatographic separation/peak resolution is important.

Peak retention time can be impacted by non-target peaks and bile salts.

Signal-to-noise can be impacted by the following:

- Non-target peaks
- Electro-spray suppression (inorganic salts)

Quantitation to confirmation ion ratio issues:

- Non-target peak interferences
- Mass transition interferences
- Branched/linear isomer composition for select PFAS

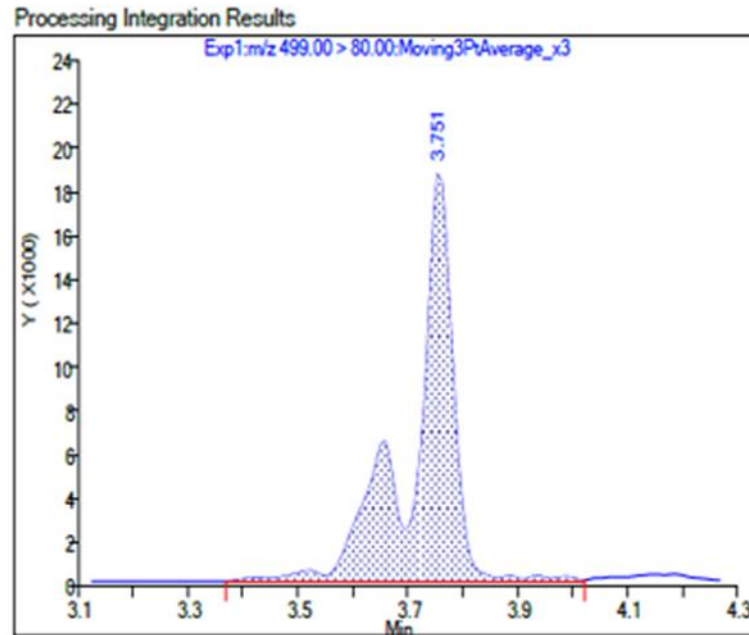


Qualitative Challenges – Separation/Resolution

Chromatographic separation of target compound isomers is important.

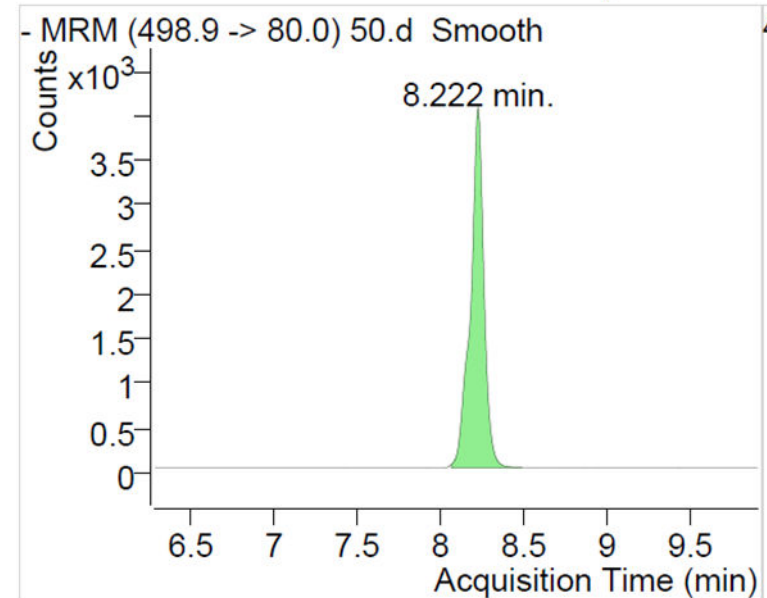
28 Perfluorooctanesulfonic acid, CAS: 1763-23-1
Signal: 1

RT: 3.75
Area: 92339
Amount: 0.053136
Amount Units: ng/ml



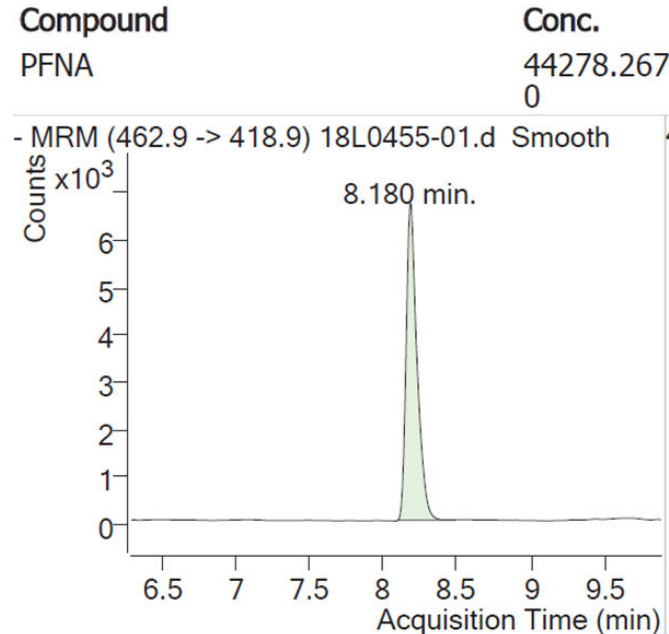
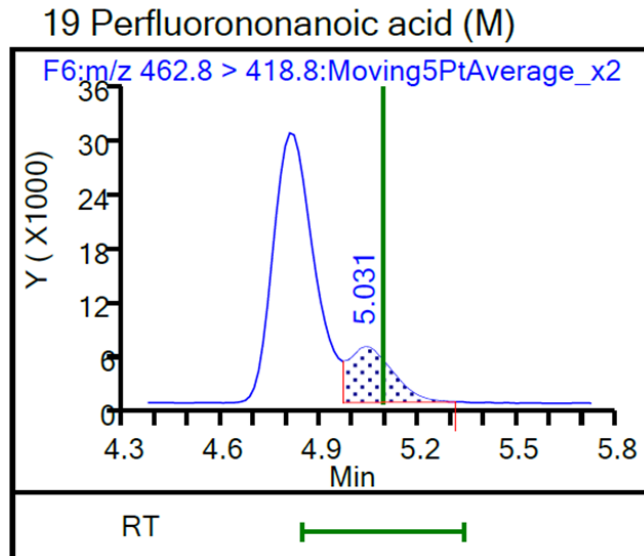
Compound
PFOS-Total

Conc.
45106.007
7



These are two chromatograms with PFOA from split samples, two different labs. The lab on the left would report only the linear as the total. The lab on the right reports a higher concentration total PFOA.

Qualitative Challenges – Separation/Resolution (cont.)

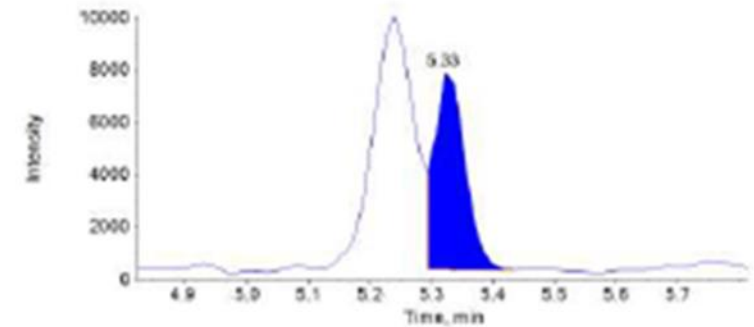


- Aqueous split sample for PFNA (5.031 min is the correct target).
- Concentration difference for PFNA between these split samples, two different labs report 10-fold difference.
- Method 537.1 – No secondary ion monitoring is required.

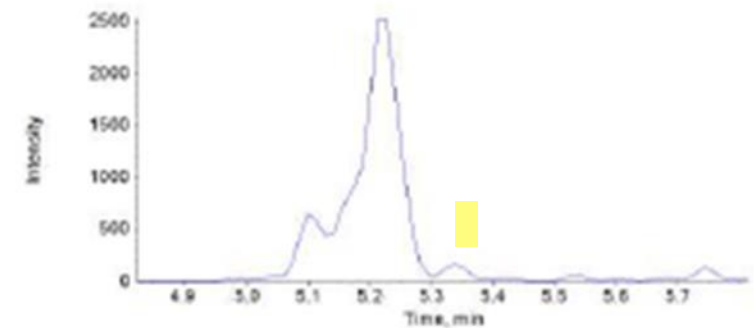
Qualitative Challenges – Secondary Ion

- PFNA peak observed for the quantitation ion.
- Peak prior to quantitation ion (blue) is an interference.
- Confirmation ion for PFNA was not observed (> s/n) (yellow).

Component:
PFNA
Mass Transition: 463.0 / 419.0
Retention Time: 5.33
S/N: 52.3



Ratio Ion:
PFNA_2
Mass Transition: 463.0 / 169.0
Retention Time: N/A
S/N: N/A



Some laboratories would report PFNA as a positive detection with the ion ratio out, while other laboratories would report this PFNA as “not-detected.”

Qualitative Challenges – Chromatographic Interference

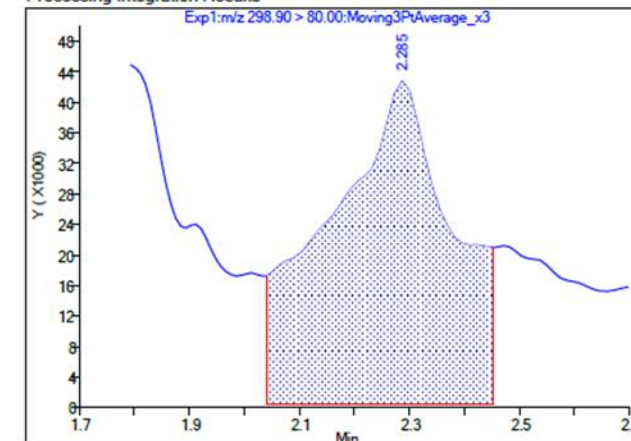
- PFBS does not have branched isomer components in the calibration.
- Non-target peak is co-eluting with quantitation peak for PFBS with manual integration (quantitation ion to right).
- Second transition for PFBS (below) has no interference, but never used for quantitation

5 Perfluorobutanesulfonic acid, CAS: 375-73-5

Signal: 1

RT: 2.28
Area: 653394
Amount: 1.276607
Amount Units: ng/ml

Processing Integration Results

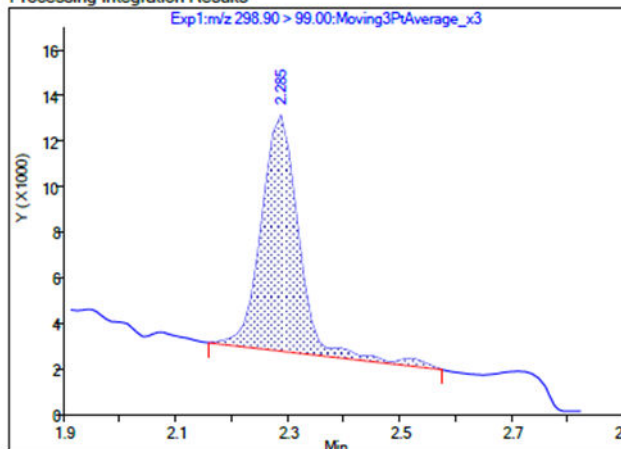


5 Perfluorobutanesulfonic acid, CAS: 375-73-5

Signal: 2

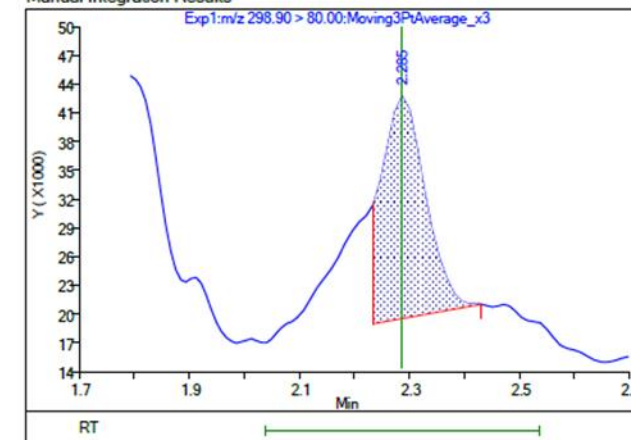
RT: 2.28
Area: 46321
Amount: 1.276607
Amount Units: ng/ml

Processing Integration Results



RT: 2.28
Area: 124786
Amount: 0.243808
Amount Units: ng/ml

Manual Integration Results



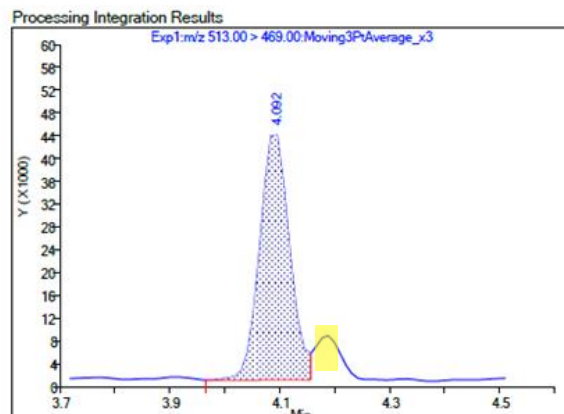
The differences in automated versus (proper) manually integrated PFBS peak to the right yields substantially different (potentially reported) concentrations

Qualitative Challenges – Chromatographic Interference

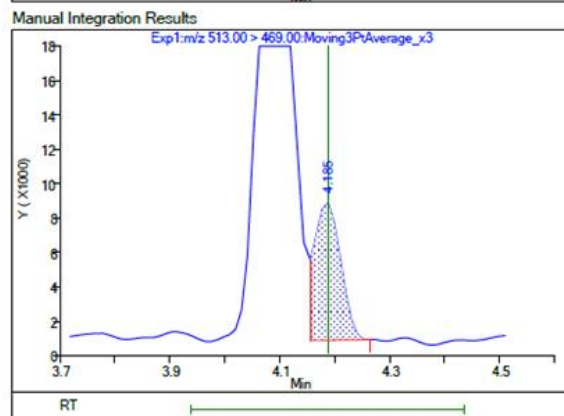
24 Perfluorodecanoic acid, CAS: 335-76-2

Signal: 1

RT: 4.09
Area: 159811
Amount: 0.285155
Amount Units: ng/ml



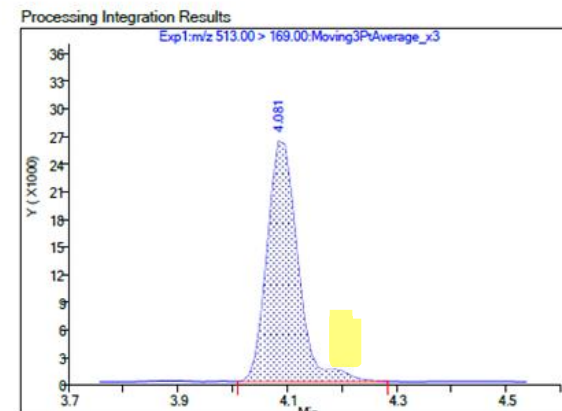
RT: 4.19
Area: 24699
Amount: 0.044071
Amount Units: ng/ml



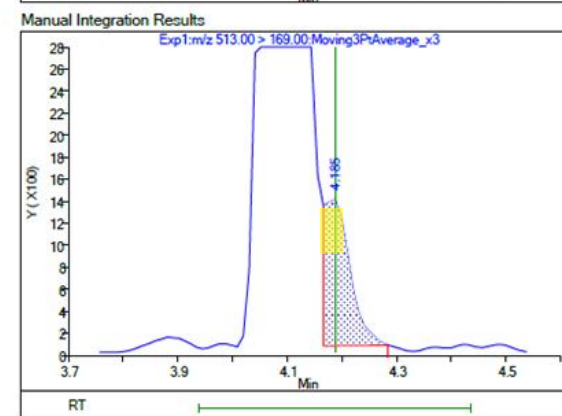
24 Perfluorodecanoic acid, CAS: 335-76-2

Signal: 2

RT: 4.08
Area: 101364
Amount: 0.285155
Amount Units: ng/ml

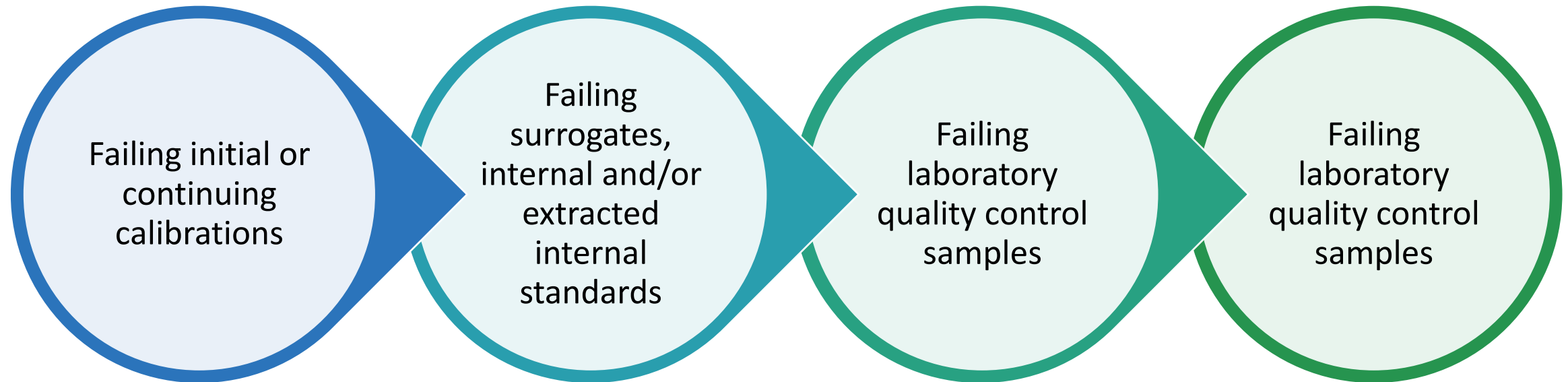


RT: 4.19
Area: 4092
Amount: 0.044071
Amount Units: ng/ml



The PFDA quantitation peak retention time is 4.186 (yellow) not 4.092 (blue) and in this case both transitions have a large non-target interference. Some laboratories might report PFDA as a positive detection while other laboratories would report this PFDA as “not-detected.”

Quantitative Challenges – Assessing the Bias



- Method and reagents blanks
- Laboratory control samples/fortified blanks
- Matrix spikes (not required in some methods)

- Variety of blanks prepared in the field
- Field duplicates /replication



Take Away Items

- 1 PFAS is everywhere – in environmental media, food/beverages, and as of late, in every and all consumer products
- 2 LC/MS/MS is a highly sensitive technique that can detect trace levels of PFAS in complex matrices and the qualifications of the Analyst behind the instrument is mission critical
- 3 We need more readily available native and isotopic PFAS reference materials
- 4 Sample matrices and preparation techniques can substantially impact data quality – non-target PFAS and other co-extracted materials will play havoc with analysis
- 5 Out-of-Criteria calibrations and/or laboratory and field quality control samples need careful interpretation

BE SKEPTICAL OF USING PFAS DATA UNTIL IT IS VALIDATED BY QUALIFIED PROFESSIONALS

The Future of Environmental Solutions





Thank you – Questions?



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