Column Chemistry Considerations for Full Coverage of Sample Matrices and Analyte Ranges in PFAS LC-MS/MS Workflows

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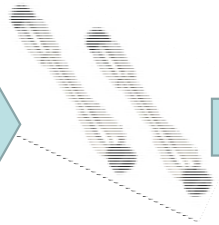
Phenomenex Inc.

Torrance CA

PFAS LC-MS/MS Workflow



Sample/ Analytes

Sample Preparation

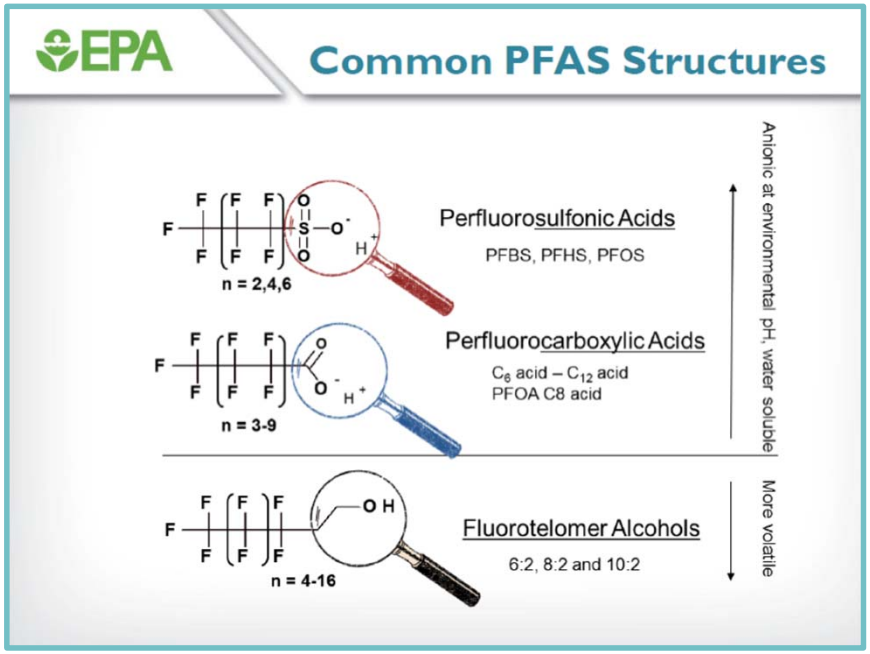
System

Column

Detector

Outline

- PFAS - Brief Introduction
- Samples
- Sample Prep - SPE
- HPLC Instrumentation
- LCMS Detectors
- HPLC Columns
- Summary



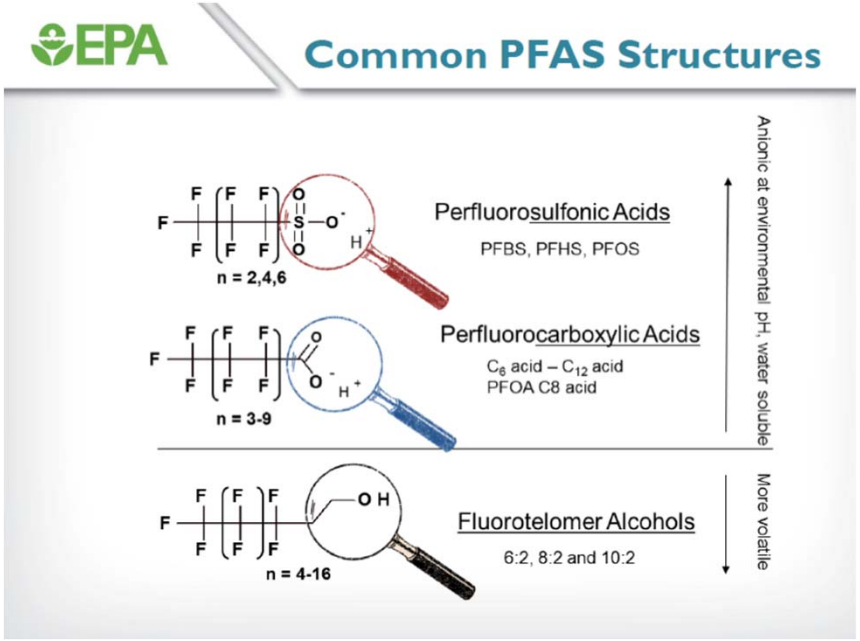
PFAS Introduction

- Per- and polyfluoroalkyl substances (PFAS) are a family of human-made chemicals that are found in a wide range of products used by consumers and industry.
  - https://www.fda.gov/food/chemicals/and-polyfluoroalkyl-substances-pfas
- Per- and polyfluoroalkyl substances (PFAS) are a group of man-made chemicals that includes PFOA, PFOS, GenX, and many other chemicals. PFAS have been manufactured and used in a variety of industries around the globe, including in the United States since the 1940s. PFOA and PFOS have been the most extensively produced and studied of these chemicals.
  - https://www.epa.gov/pfas/basic-information-pfas

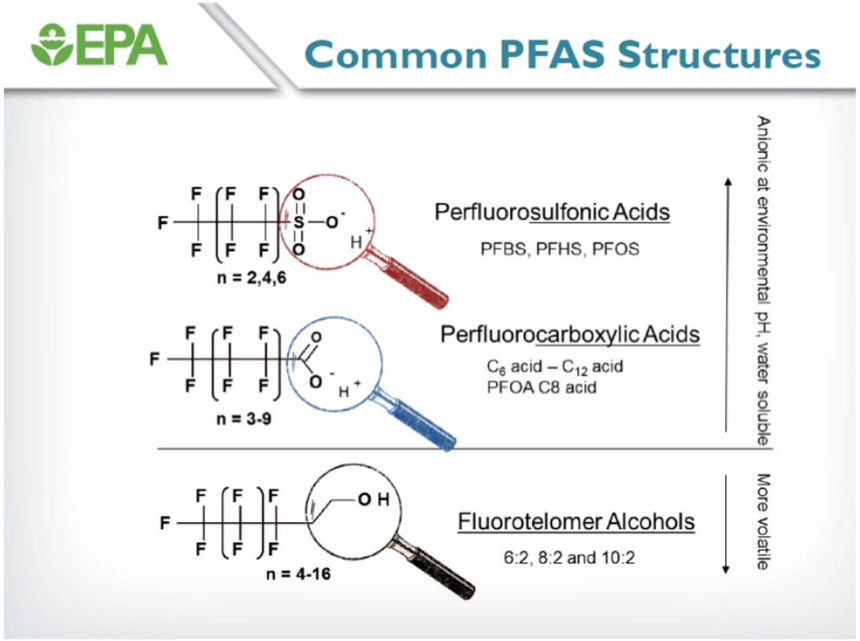
US Regulations for PFAS

- EPA
- DOD
- CERCLA
- Individual States

Common PFAS Structures



Common PFAS Structures



More PFAS

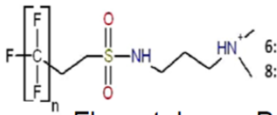
Perfluoroalkyl Sulfonates

PFBS	n = 4	Qn
PFPeS *	n = 5	Ql
PFHxS	n = 6	Qn
PFHpS	n = 7	Qn
PFOS	n = 8	Qn
PFNS *	n = 9	Ql
PFDS	n = 10	Qn

Perfluoroalkyl Carboxylates

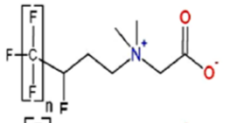
PFBA	n = 4	Qn
PFPeA	n = 5	Qn
PFHxA	n = 6	Qn
PFHpA	n = 7	Qn
PFOA	n = 8	Qn
PFNA	n = 9	Qn
PFDA	n = 10	Qn
PFUdA	n = 11	Qn
PFDoA	n = 12	Qn
PFTrA	n = 13	Qn
PFTeA	n = 14	Qn

Fluorotelomer Sulfonamide Amines

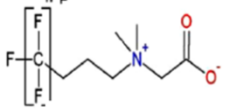


6:2 FtSaAm	n = 6	Sq
8:2 FtSaAm	n = 8	Ql

Fluorotelomer Betaines

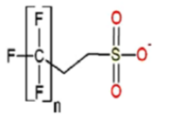


5:1:2 FtB	n = 5	Sq
7:1:2 FtB	n = 7	Sq
9:1:2 FtB	n = 9	Sq



5:3 FtB	n = 5	Sq
7:3 FtB	n = 7	Sq
9:3 FtB	n = 9	Sq

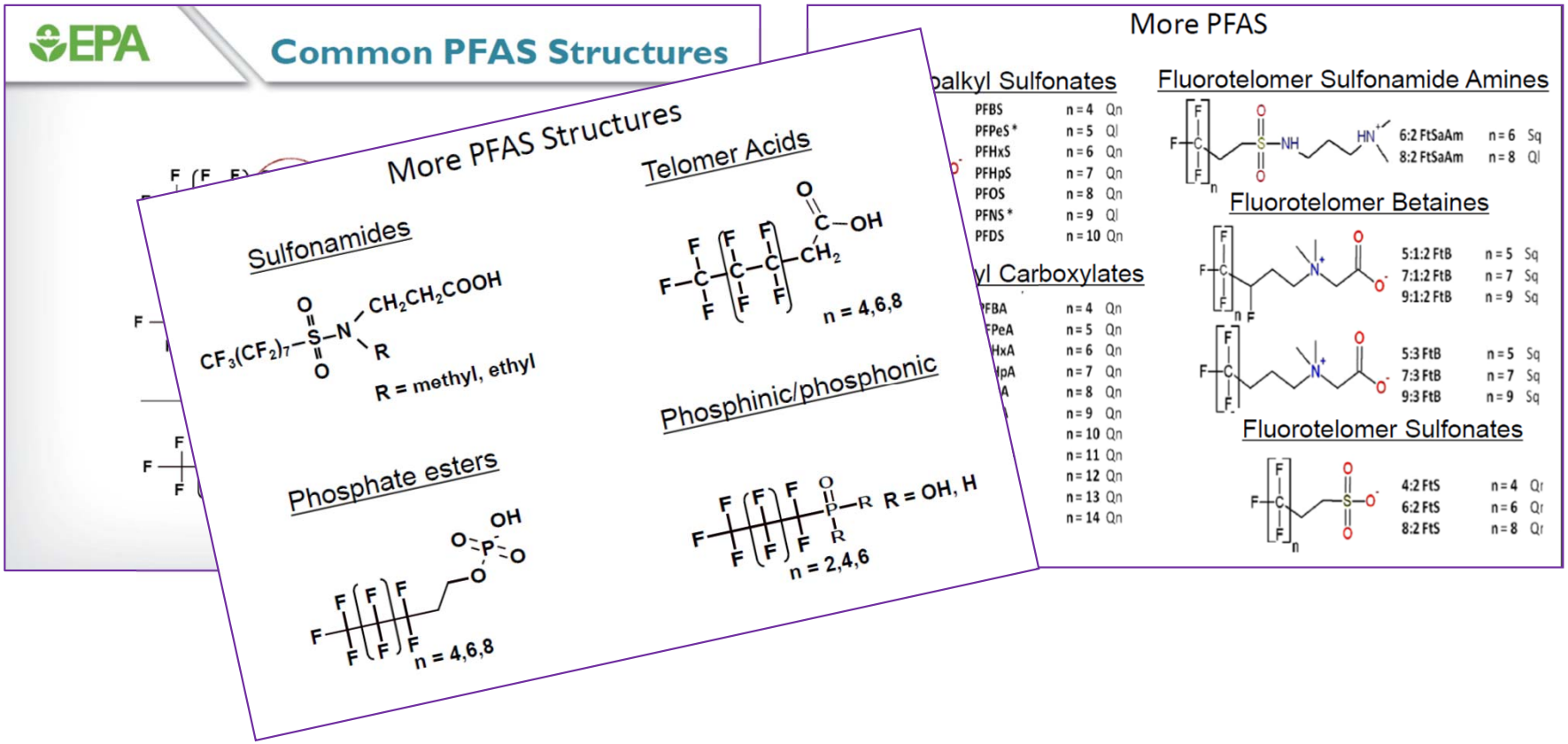
Fluorotelomer Sulfonates



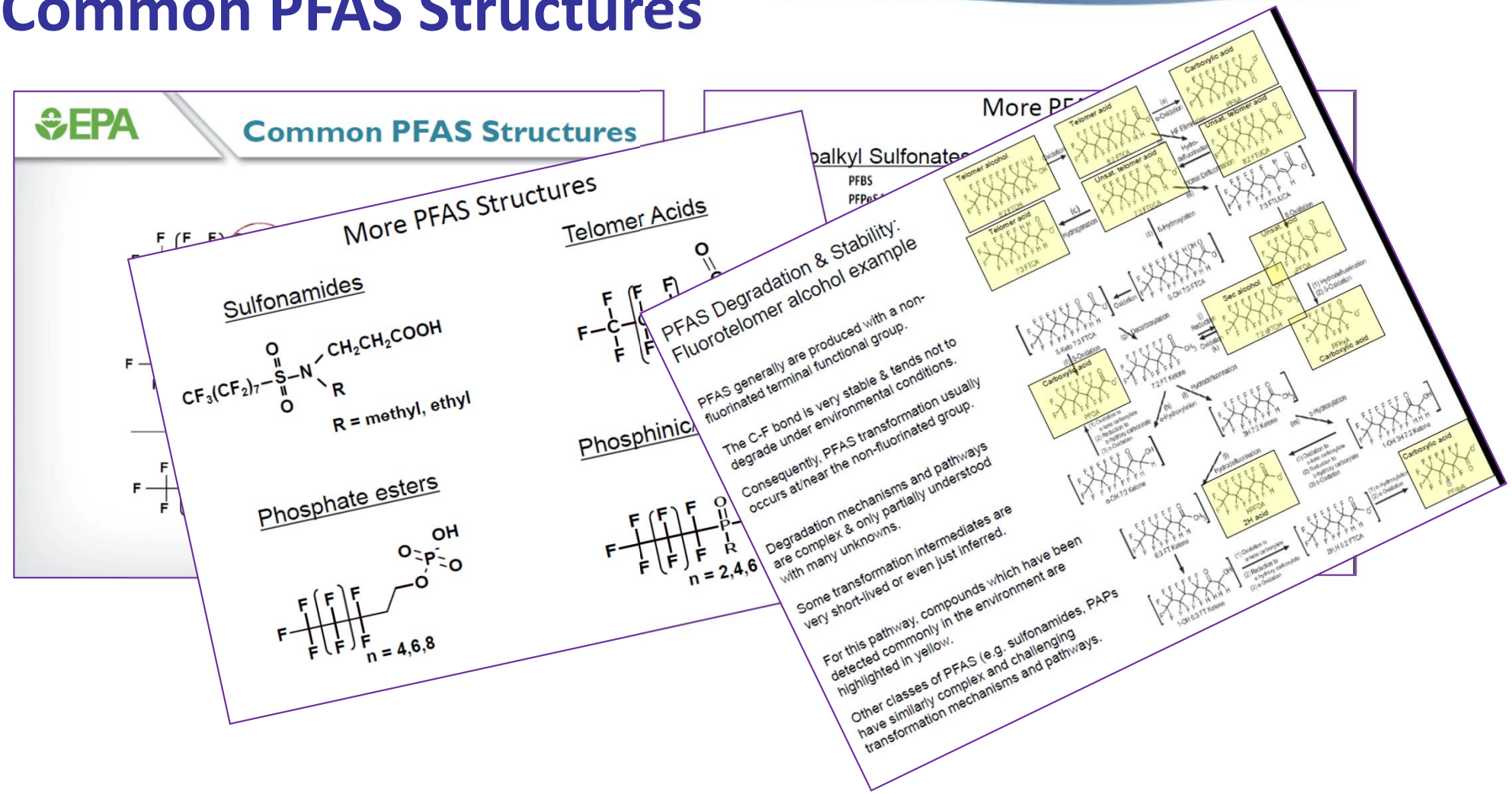
4:2 FtS	n = 4	Qr
6:2 FtS	n = 6	Qr
8:2 FtS	n = 8	Qr

Backe et al. 2013 ES&T

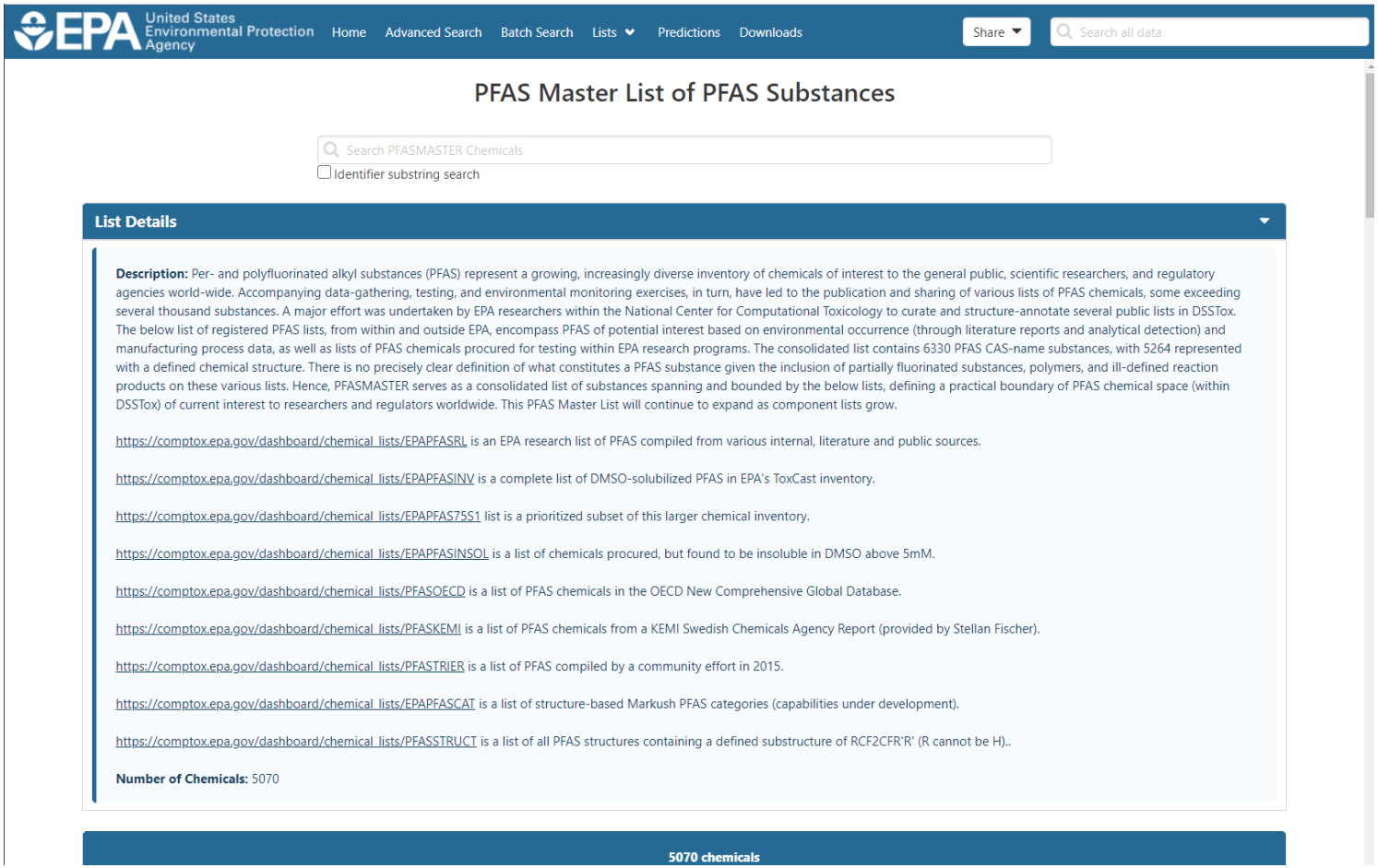
Common PFAS Structures



Common PFAS Structures



EPA Master List of PFAS Substances



EPA United States Environmental Protection Agency

Home | Advanced Search | Batch Search | Lists | Predictions | Downloads | Share | Search all data

PFAS Master List of PFAS Substances

Search PFASMASTER Chemicals

Identifier substring search

List Details

Description: Per- and polyfluorinated alkyl substances (PFAS) represent a growing, increasingly diverse inventory of chemicals of interest to the general public, scientific researchers, and regulatory agencies world-wide. Accompanying data-gathering, testing, and environmental monitoring exercises, in turn, have led to the publication and sharing of various lists of PFAS chemicals, some exceeding several thousand substances. A major effort was undertaken by EPA researchers within the National Center for Computational Toxicology to curate and structure-annotate several public lists in DSSTox. The below list of registered PFAS lists, from within and outside EPA, encompass PFAS of potential interest based on environmental occurrence (through literature reports and analytical detection) and manufacturing process data, as well as lists of PFAS chemicals procured for testing within EPA research programs. The consolidated list contains 6330 PFAS CAS-name substances, with 5264 represented with a defined chemical structure. There is no precisely clear definition of what constitutes a PFAS substance given the inclusion of partially fluorinated substances, polymers, and ill-defined reaction products on these various lists. Hence, PFASMASTER serves as a consolidated list of substances spanning and bounded by the below lists, defining a practical boundary of PFAS chemical space (within DSSTox) of current interest to researchers and regulators worldwide. This PFAS Master List will continue to expand as component lists grow.

https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASRL is an EPA research list of PFAS compiled from various internal, literature and public sources.

https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASINV is a complete list of DMSO-solubilized PFAS in EPA's ToxCast inventory.

https://comptox.epa.gov/dashboard/chemical_lists/EPAPFAS75S1 list is a prioritized subset of this larger chemical inventory.

https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASINSOL is a list of chemicals procured, but found to be insoluble in DMSO above 5mM.

https://comptox.epa.gov/dashboard/chemical_lists/PFASOECD is a list of PFAS chemicals in the OECD New Comprehensive Global Database.

https://comptox.epa.gov/dashboard/chemical_lists/PFASKEMI is a list of PFAS chemicals from a KEMI Swedish Chemicals Agency Report (provided by Stellan Fischer).

https://comptox.epa.gov/dashboard/chemical_lists/PFASTRIER is a list of PFAS compiled by a community effort in 2015.

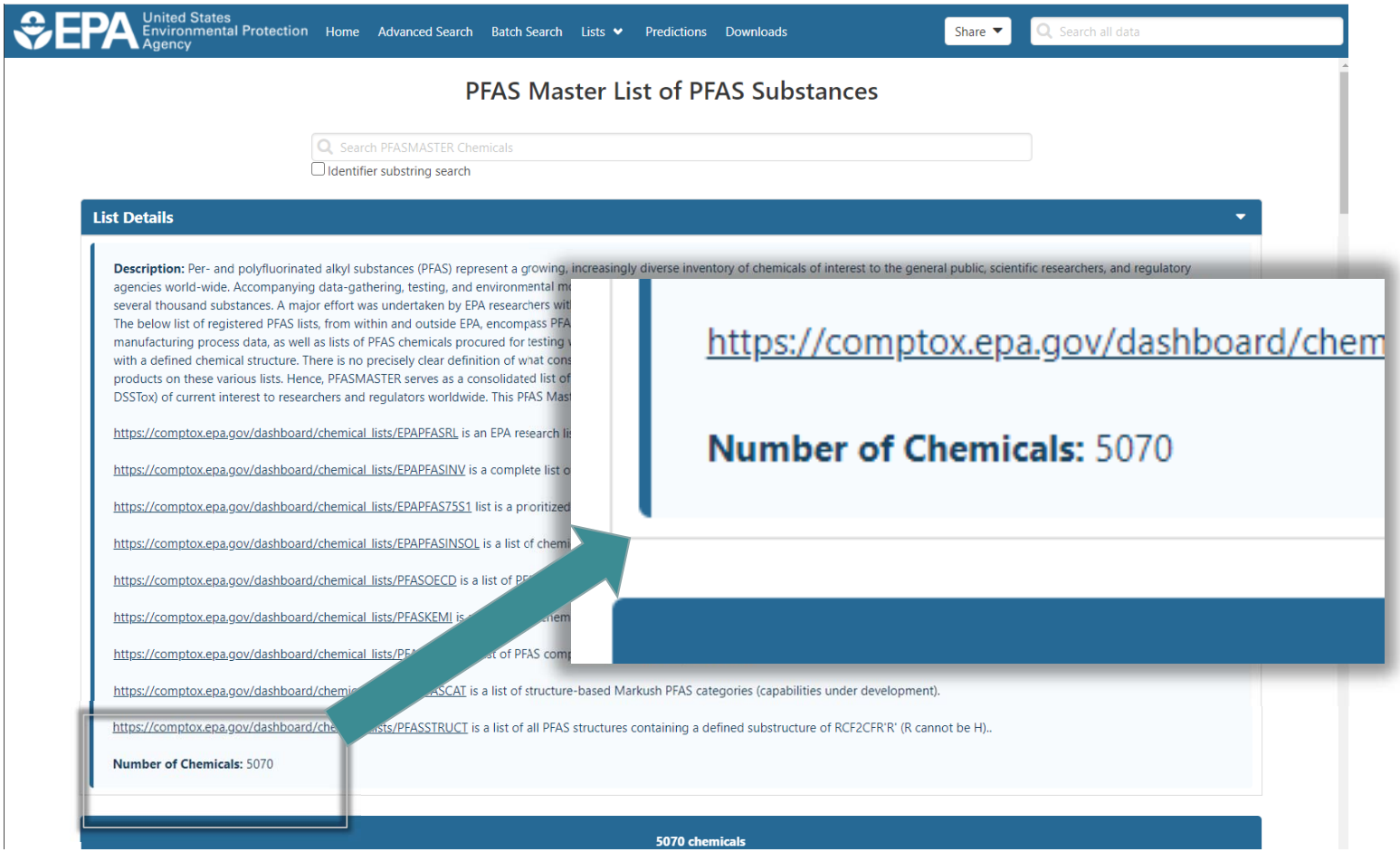
https://comptox.epa.gov/dashboard/chemical_lists/EPAPFASCAT is a list of structure-based Markush PFAS categories (capabilities under development).

https://comptox.epa.gov/dashboard/chemical_lists/PFASSTRUCT is a list of all PFAS structures containing a defined substructure of RCF2CFR'R' (R cannot be H)..

Number of Chemicals: 5070

5070 chemicals

EPA Master List of PFAS Substances



Outline

- PFAS - Brief Introduction
- Samples
- Sample Prep - SPE
- HPLC Instrumentation
- LCMS Detectors
- HPLC Columns
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Published Sample Matrices for PFAS Analysis

- Drinking water
- Wastewater
- Groundwater
- Surface water
- Soil
- Sediment
- Earthworm
- Spinach
- Tomato
- Corn
- Cereal
- Root vegetable
- Melon
- Fish tissue
- Egg
- Milk
- Yogurt
- Cottage cheese
- Sour cream
- Butter
- Meat
- Peppers
- Cabbage
- Cucumber
- Lettuce
- Beans
- Carrot
- Fruits
- Breast milk
- Baby food
- Shellfish

Some Publish Sample Matrices for PFAS Analysis

- Drinking water
- Wastewater
- Groundwater
- Surface water
- Soil
- Sediment
- Earthworm
- Spinach
- Tomato
- Corn
- Cereal
- Root vegetable
- Melon
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- Cottage cheese
- Sour cream
- Butter
- Meat
- Peppers
- Cabbage
- Cucumber
- Lettuce
- Beans
- Carrot
- Fruits
- Breast milk
- Baby food
- Shellfish

What sample matrix doesn't have PFAS

Most Common Matrix

Drinking Water

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- PFAS - Brief Introduction
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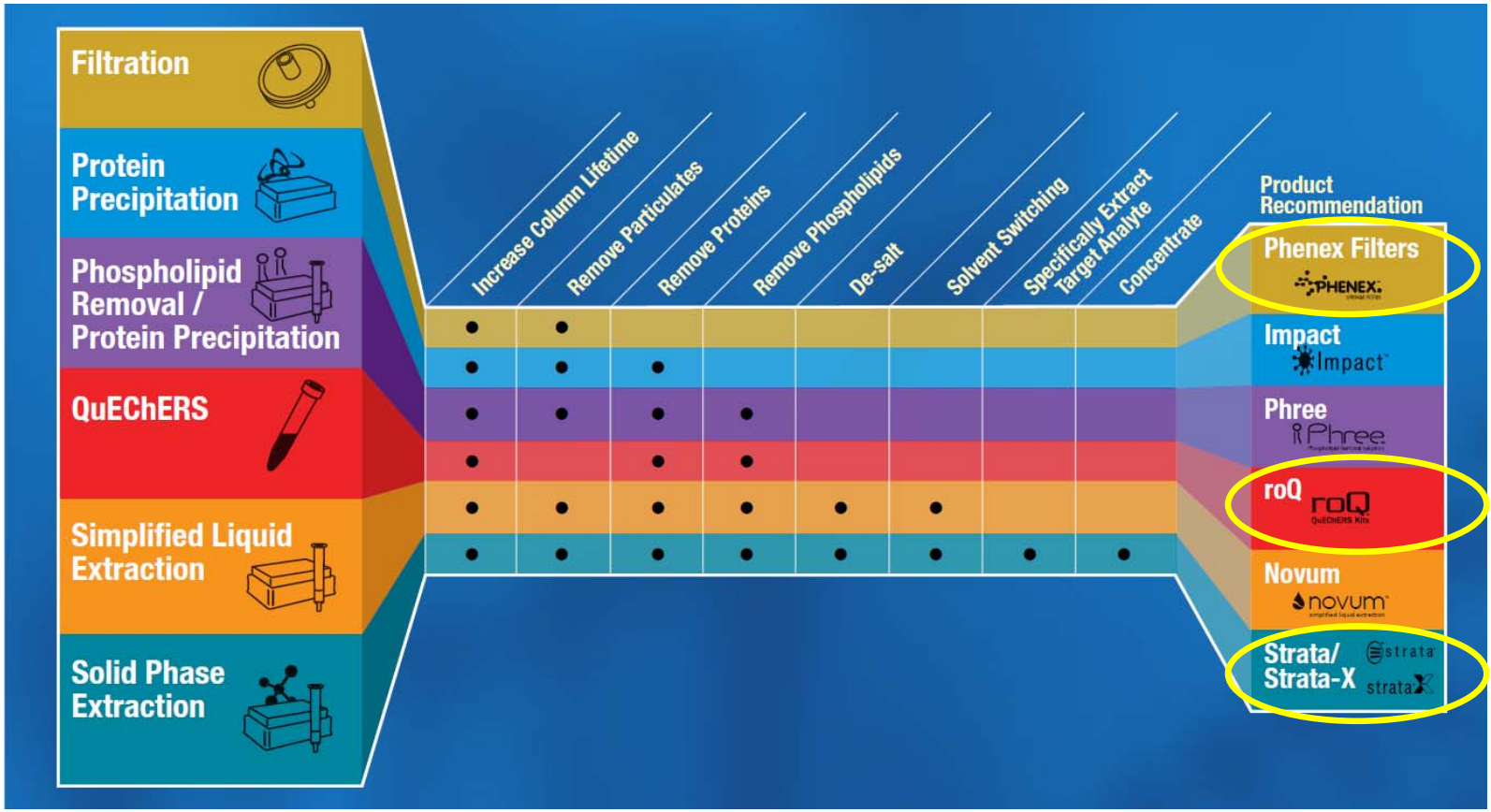
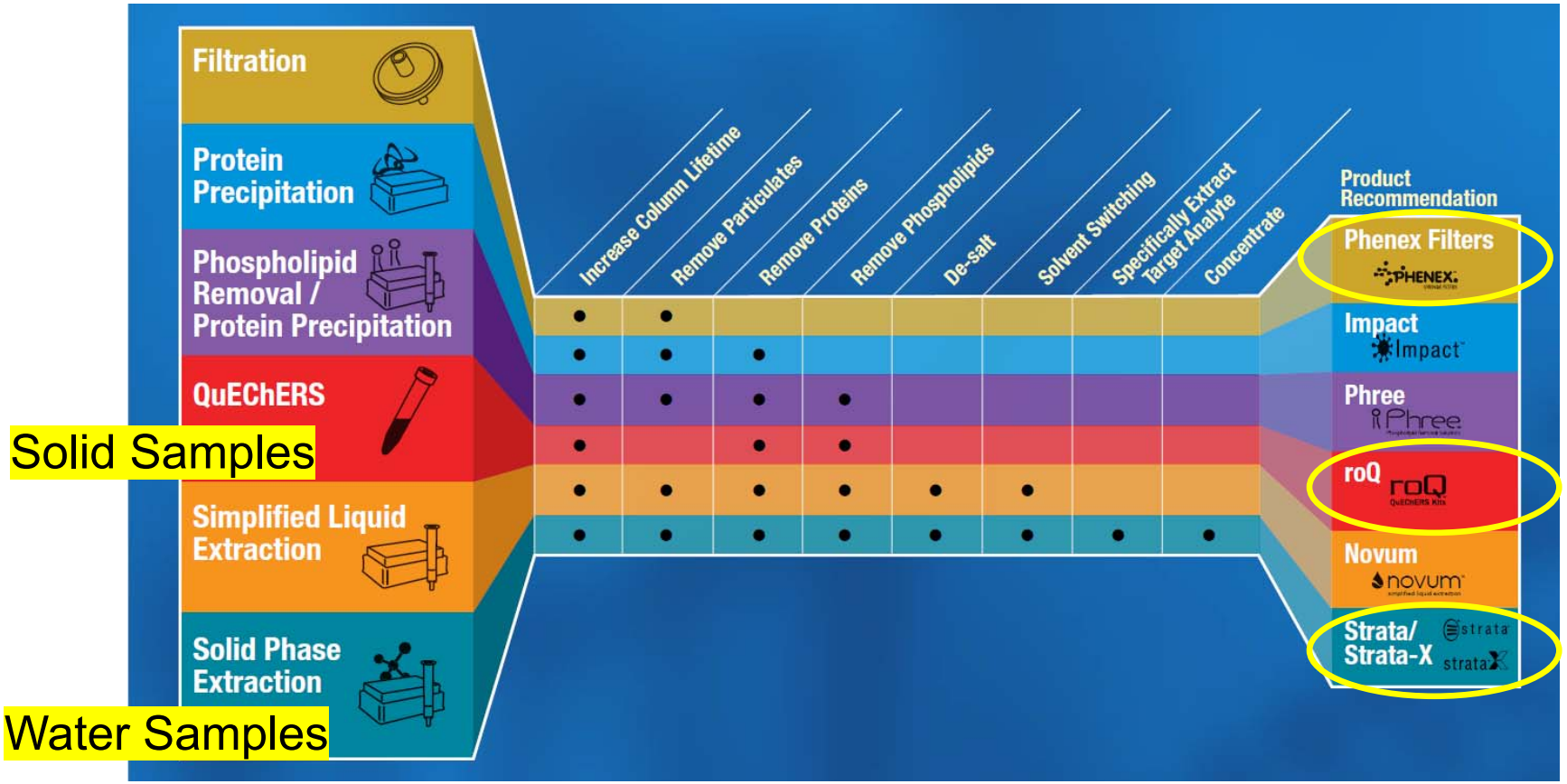
Sample Preparation Techniques / Products



Sample Preparation Techniques / Products



Solid Samples

Water Samples

PFAS Analysis

from Water, Sample Prep/SPE

250 mL water sample, polyethylene bottle

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

↓

Extraction with weak anion-exchange SPE column

→

Rinse bottle with 10 mL of methanol with 0.3% NH_4OH

↓

Elute PFAS with bottle rinsate, evaporate to near dryness

↓

Reconstitute with 500 µL of 80:20 Methanol:Water, transfer to polypropylene vials

strata™X

Strata-XL-AW 100 µm
500 mg / 6 mL

Condition:	4 mL of 0.1% ammonia/methanol 4 mL of methanol, 4 mL of water
Load:	Sample Water
Wash:	4 mL of acetate buffer (pH 4)
Elution:	4 mL of Methanol 4 mL of 0.1% ammonia/methanol fraction.
Rinse:	Rinse Sample bottles with methanol and use for the elution of SPE cartridge.

PFAS Analysis
from Water, On-line SPE

Sample Preparation Procedure

1. Samples are collected in polypropylene bottles and preserved with 0.5 g/L Trizma®.
2. A 10 mL aliquot is spiked with surrogates at a concentration of 50 ng/L.
3. If necessary, filter using a 10 mL syringe fitted to a 1.2 µm glass fiber syringe filter.
4. The filtered sample is spiked with internal standard at 50 ng/L.
5. The filtered sample is loaded and analyzed using a 5.0 mL injection volume.
6. The on-line SPE is completely automated; it includes a sample wash step (2.1 to 4.1 min) to wash Trizma preservative from the media.

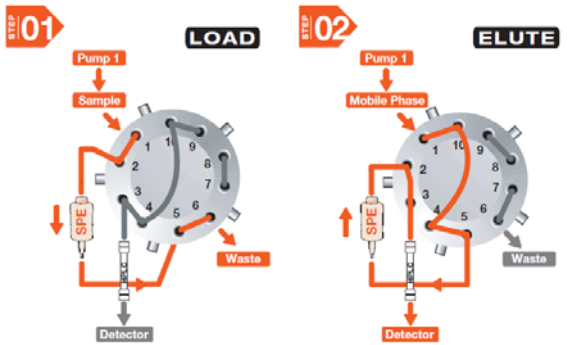
On-line SPE

On-line SPE: Strata-X-AW 33 µm Polymeric Weak Anion-Exchange
Dimensions: 20 x 2.0 mm
Part No.: 00M-S038-B0-CB
On-line SPE Cartridge Holder: 20 mm Cartridge Holder
Part No.: CH0-5845
Sample Filters: Phenex™ Glass Fiber 1.2 µm 28 mm

On-line SPE Program

Time (min)	Water %	MeOH %	ACN %	Flow (mL/min)	Comments
0	100	0	0	2.5	Sample Loading
2	100	0	0	2.5	Sample Loading
2	100	0	0	2.5	SPE Wash
4	100	0	0	2.5	SPE Wash
4.11	30	70	0	0	Idle (Elution into LC)
9	30	70	0	0	Idle (Elution into LC)
9.01	0	0	100	2.0	ACN Wash
9.49	0	0	100	2.0	ACN Wash
9.5	2.0	98	0	3.0	MeOH Wash
11.5	2.0	98	0	3.0	MeOH Wash
11.51	100	0	0	3.0	Cond: Water
14	100	0	0	3.0	Cond: Water

Chemical Abbreviations: Methanol (MeOH); Acetonitrile (ACN)



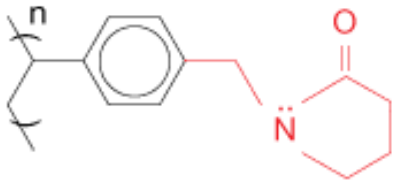
On-line SPE is available in

- Strata C18, C8
- Strata-X, X-C, X-CW, X-A, X-AW

Solid Phase Extraction

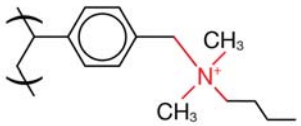
EPA method 537

Reversed phase retention (Strata®-X)

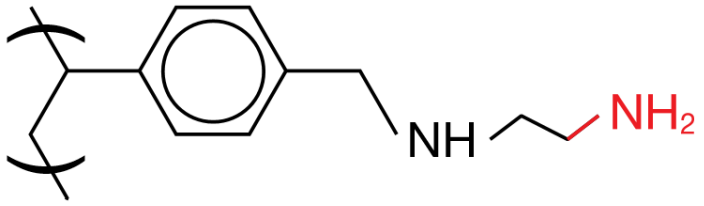


Mixed Mode Anion-Exchange

Anion-Exchange + Strata-X



Strata-X-A Strong Anion-Exchange



Strata-X-AW Weak Anion-Exchange

PFAS Analysis

from Sediment using QuEChERS and LC-MS/MS

Modified roQ™ QuEChERS Protocol

Step 1

Extraction

1. Weigh 2 g of dry sediment into a 50 mL centrifuge tube
2. Spike sample with internal standards
3. Add 10 mL of reagent water and mix
4. Add 10 mL of acidified Acetonitrile (0.1 % Acetic acid) and shake for 10 seconds
5. Add Sodium acetate (1.5 g) and $MgSO_4$ (2.0 g), or weigh out (~) 3.5 g of AOAC 2007.01 roQ extraction packet (AH0-9043)
6. Shake for 10 seconds and vortex for 1 minute
7. Centrifuge at 4000 rpm for 5 minutes
8. Cool sample at – 20 °C for 1.5 hours or until frozen

Step 2

Clean-up

1. Transfer 8-9 mL of the supernatant from the previous step to a 15 mL PSA/C18 roQ dSPE tube (KS0-8926)
2. Shake for 10 seconds and vortex for 1 minute
3. Centrifuge for 10 minutes at 3000 rpm
4. Aspirate 5 mL of supernatant and filter using a Phenex™ 0.2 µm PTFE filter (AF0-2202-12) into a test tube suitable for a dry-down station
5. Evaporate sample using a dry-down station at ≤35 °C to near dryness
6. Reconstitute by first adding 50 µL of Acetone, vortexing, and then adding 950 µL of Methanol/Water (1:1)
7. Transfer the reconstituted sample to an autosampler vial for analysis

Special thanks Syljohn Estil and to the Sanitation Districts of Los Angeles County – San Jose Creek Water Quality Laboratory for contributing this method.

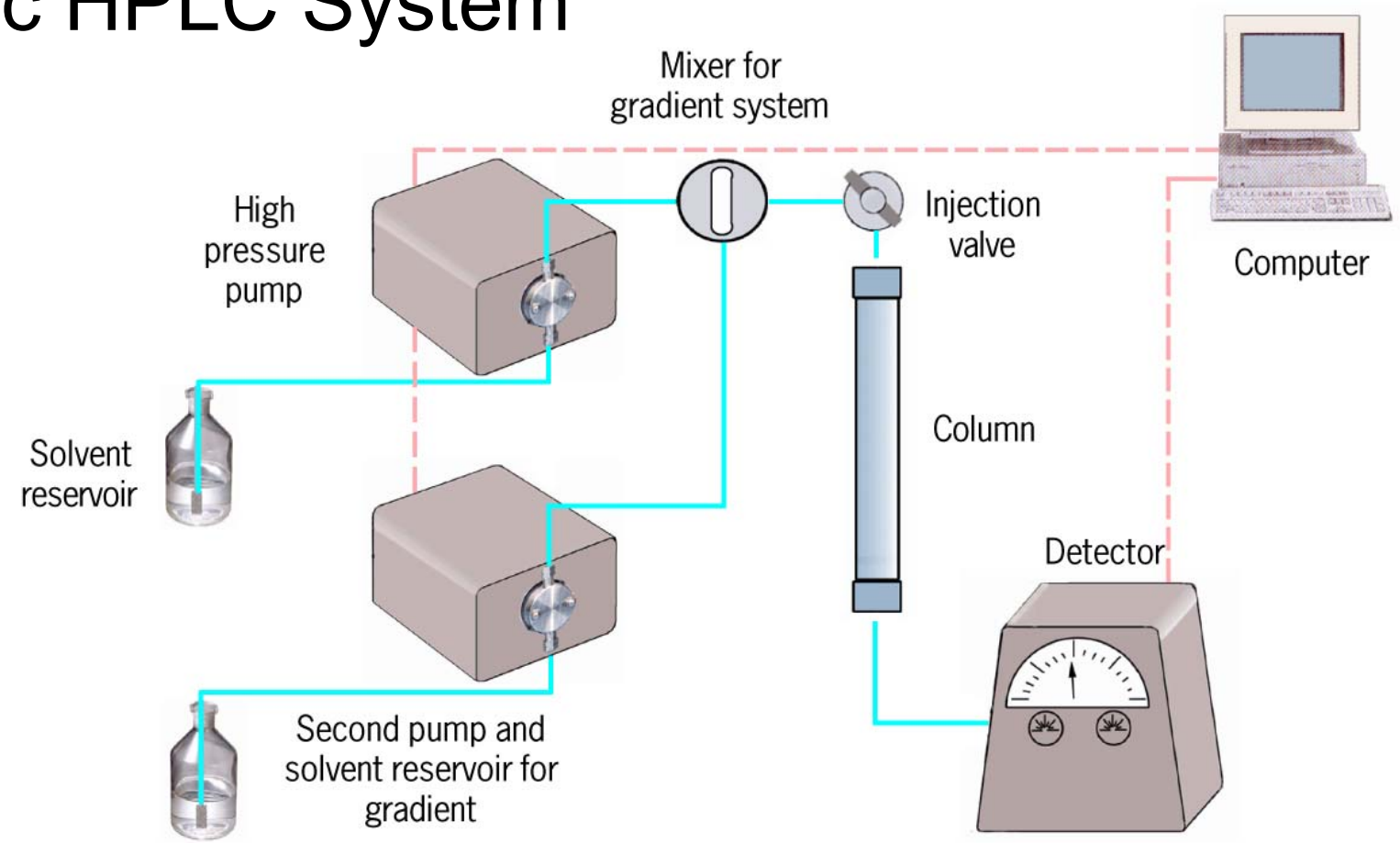
SANITATION DISTRICTS OF LOS ANGELES COUNTY

Converting Waste Into Resources

Outline

- PFAS - Brief Introduction
- Samples
- Sample Prep - SPE
- HPLC Instrumentation
- LCMS Detectors
- HPLC Columns
- Summary

Basic HPLC System



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Mass Spectrometer Parameters: Compound Specific

Compound	Q1	Q3	DP	CE
PFCAs				
PFBA	212.9	169	-25	-12
PFPeA	262.9	219	-20	-12
PFHxA	313	269	-25	-12
PFHpA	363	319	-25	-12
PFOA	413	369	-25	-14
PFNA	463	419	-25	-14
PFDA	513	469	-25	-16
PFUdA	563	519	-25	-18
PFDoA	613	569	-25	-18
PFTrDA	663	619	-25	-20
PFTeDA	713	669	-25	-22
PFHxDA	813	769	-25	-24
PFODA	913	869	-25	-26

Compound	Q1	Q3	DP	CE
PFSAs				
PFBS	298.9	80	-55	-58
PFHxS	399	80	-60	-74
PFHpS	449	80	-65	-88
PFOS	499	80	-65	-108
PFDS	599	80	-85	-118
Other PFASs				
6:2 FTS	427	407	-50	-32
8:2 FTS	527	507	-50	-40
PFOSA	498	78	-60	-85
MeFOSA	512	169	-75	-37
EtFOSA	526	169	-75	-37
N-MeFOSAA	570	419	-40	-36
N-EtFOSSA	584	419	-50	-36

- De-clustering Potential (DP) and Collision Energy (CE) optimized for each compound
- One MRM transition monitored each analyte and internal standard
- *Scheduled* MRM™ algorithm used to maximize dwell times and optimize cycle time

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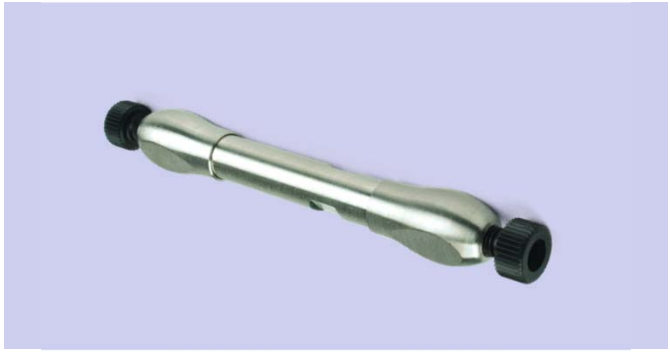


HPLC Column Outline

- Why do we need chromatography
- How does chromatography work
- PFAS examples
- Available column chemistries
- Methodology
- PFAS column screening
  - Sample
  - EPA 533-Similar
  - Epa 533 Acetonitrile Altered

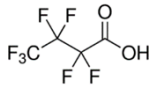
Why Do We Need Chromatography

PFAS Specific Challenges

System Contamination

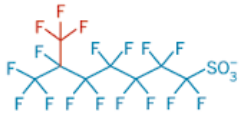
Trace Quantities

Wide Hydrophobic Range



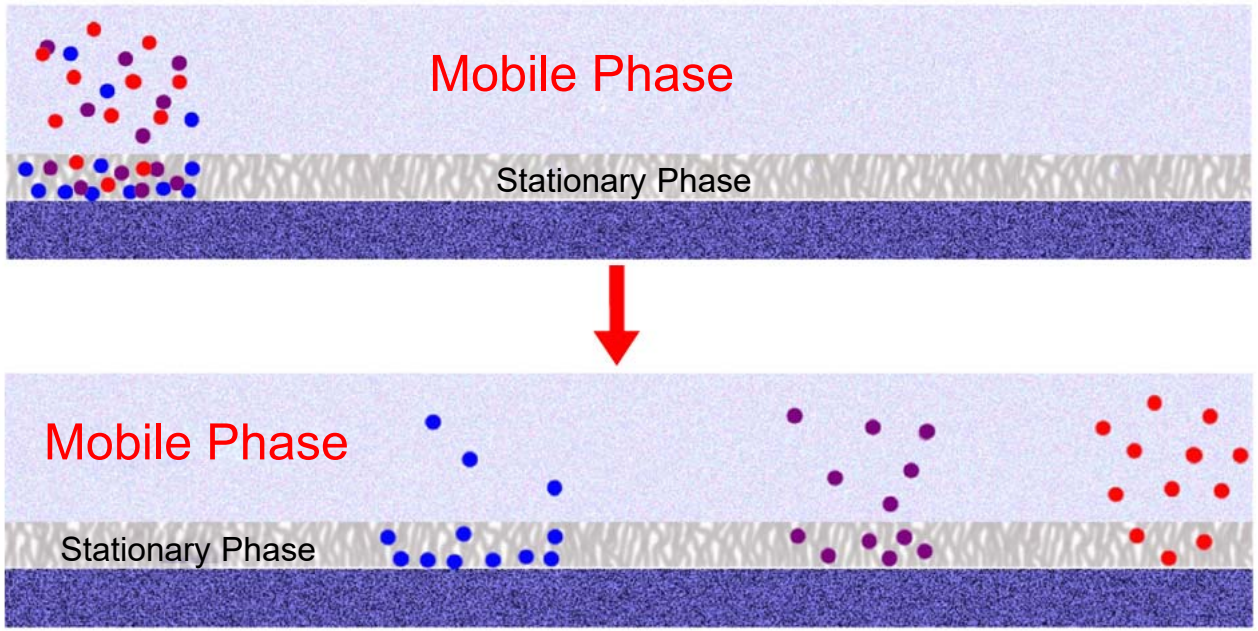
Isobaric

PFOS linear isomer



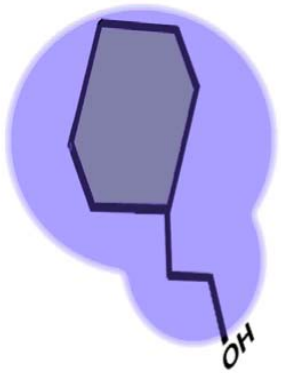
PFOS branched isomer

How Does Chromatography Work

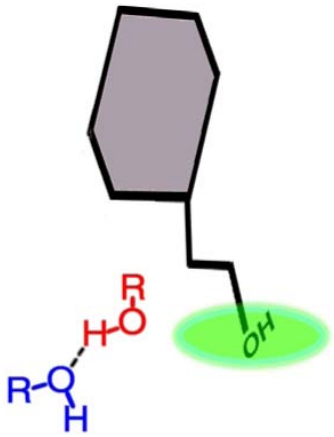


Interactions of the Analytes with the Stationary Phase

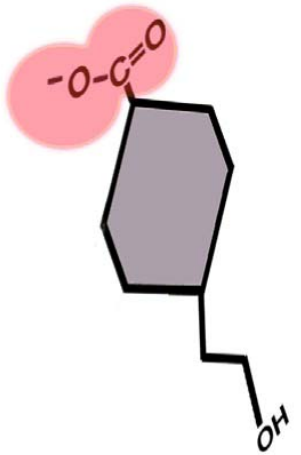
Hydrophobic



Polar

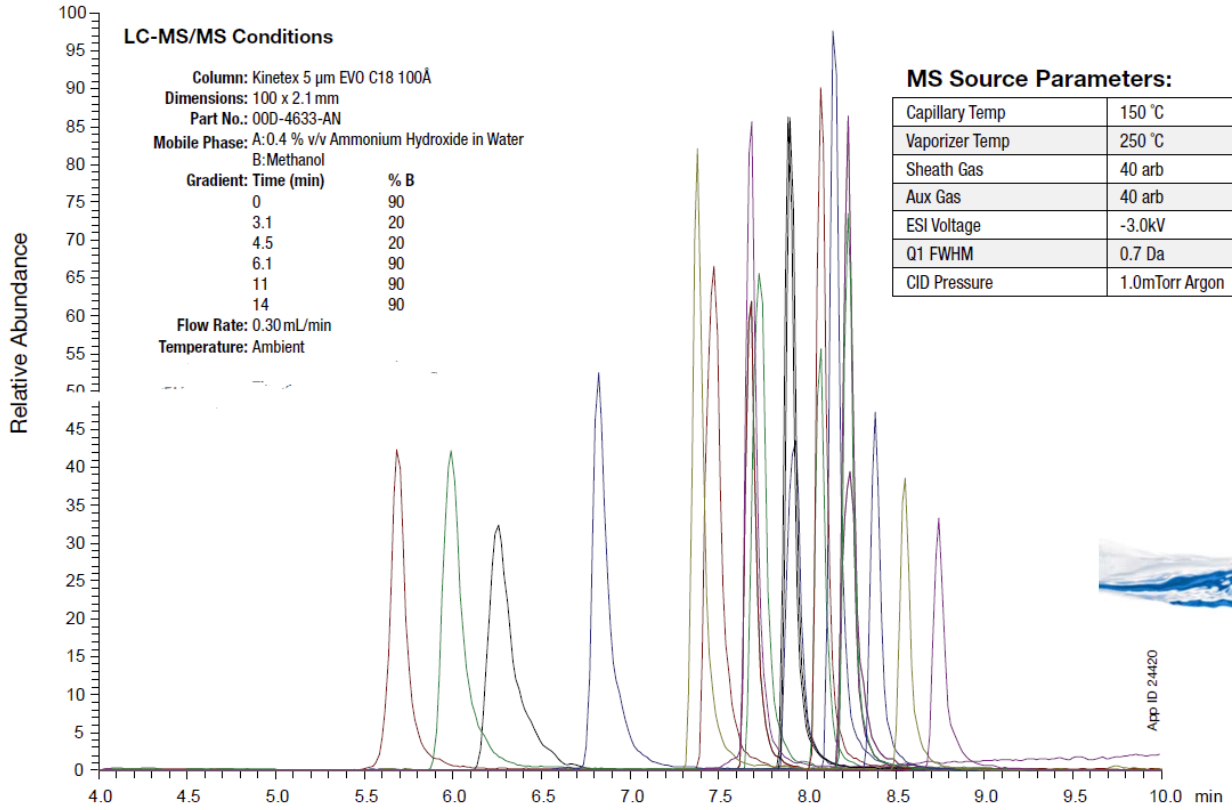


Ionic



Specific PFAS Example 1

PFAS Analysis from Water, LC-MS/MS



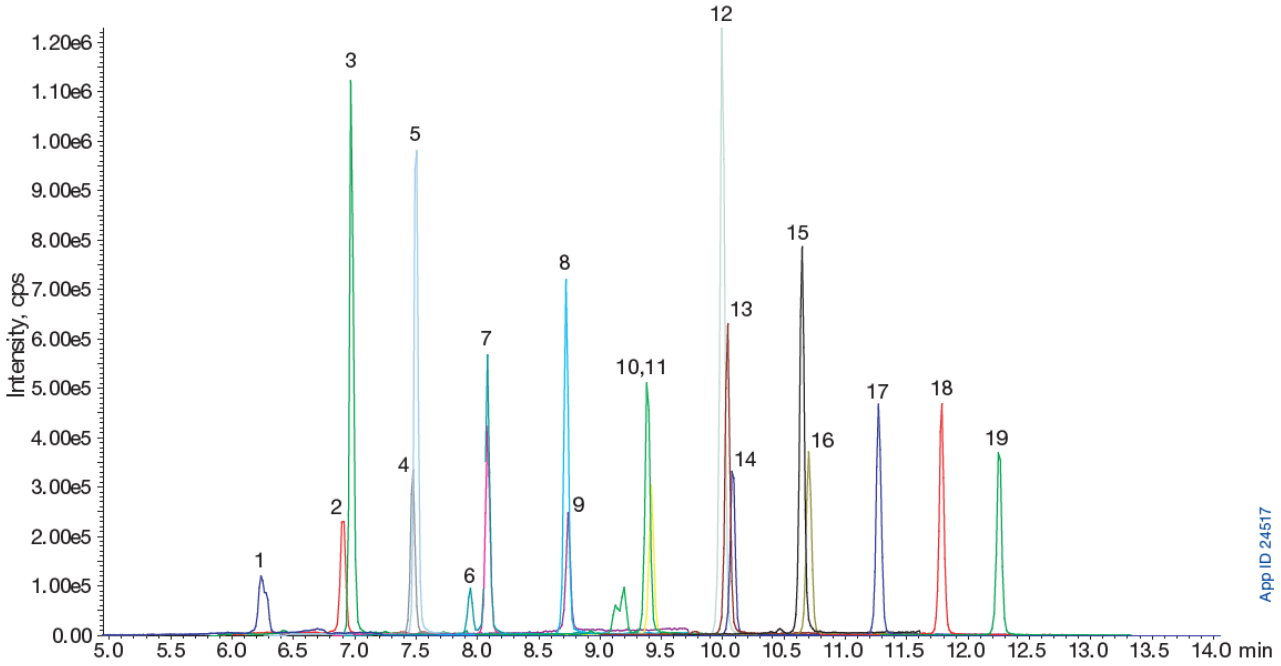
MS Source Parameters:

Capillary Temp	150 °C
Vaporizer Temp	250 °C
Sheath Gas	40 arb
Aux Gas	40 arb
ESI Voltage	-3.0kV
Q1 FWHM	0.7 Da
CID Pressure	1.0mTorr Argon

Specific PFAS Example 2

PFAS Analysis from Sediment using QuEChERS and LC-MS/MS

Sediment Spiked with 1ng/g



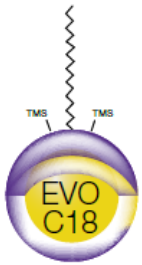
LC-MS/MS Conditions

Column:	Gemini® 3 µm C18
Dimensions:	100 x 3.0 mm
Part No.:	00D-4439-Y0
Inline Filter:	Phenomenex KrudKatcher™ Ultra (AF0-8497)
Delay Column:	Luna® 5 µm C18(2) 30 x 2.0 mm
Part No.:	00A-4252-B0
Mobile Phase:	A: 20 mM Ammonium acetate in Water B: Methanol
Gradient:	Time (min) % B 0 10 1.5 65 8 95 8.1 99 12 99 12.5 10
Flow Rate:	0.6 mL/min
Injection:	90 µL
Temperature:	40 °C
Detector:	SCIEX 5500 QTRAP®
Detection:	MS/MS ESI Negative (sMRM)
Analytes:	1. PFBA 2. PFPeA 3. PFBS 4. PFHxA 5. PFPS 6. PFHxS 7. PFHpA 8. PFHpS 9. PFOA 10. PFOS 11. PFNA 12. PFOSA 13. PFNS 14. PFDA 15. PFDS 16. PFUdA 17. PFDoA 18. PFTrDA 19. PFTeDA

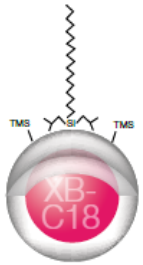
Some Available Column Chemistries

Kinetex® Core-Shell 1.3, 1.7, 2.6, 5 µm

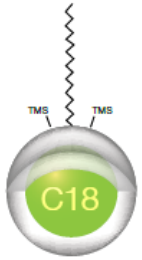
Kinetex EVO C18



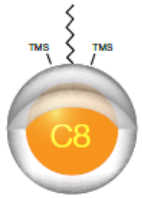
Kinetex XB-C18



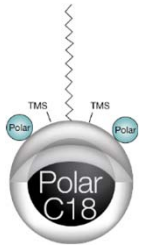
Kinetex C18



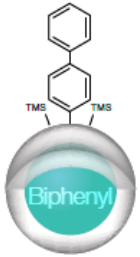
Kinetex C8



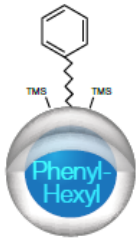
Kinetex Polar C18



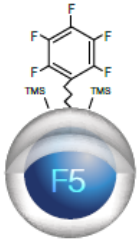
Kinetex Biphenyl



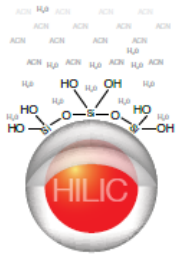
Kinetex Phenyl-Hexyl



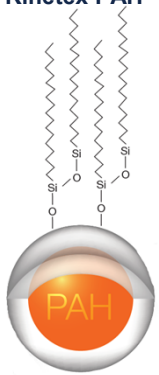
Kinetex F5



Kinetex HILIC

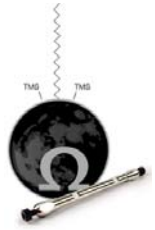


Kinetex PAH

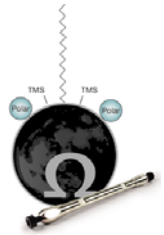


Luna® Omega Fully Porous 1.6, 3, 5 µm

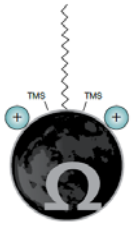
LUNA® OMEGA C18



LUNA OMEGA Polar C18



LUNA OMEGA PS C18



Methodology

METHOD 533: DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY ISOTOPE DILUTION ANION EXCHANGE SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

Chromatographic Method Conditions

EPA 533

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	95	5
0.5	95	5
3	60	40
16	20	80
18	20	80
20	5	95
22	5	95
25	95	5
35	95	5

Chromatographic Method Conditions

EPA 533

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	95	5
0.5	95	5
3	60	40
16	20	80
18	20	80
20	5	95
22	5	95
25	95	5
35	95	5

533 Similar

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45

Chromatographic Method Conditions

EPA 533

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	95	5
0.5	95	5
3	60	40
16	20	80
18	20	80
20	5	95
22	5	95
25	95	5
35	95	5

40 → 80
in 13 min
3.08% per min

533 Similar

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45

45 → 90
in 15 min
3.0% per min

Column Screening: Sample

Wellington Laboratories: EPA-537PDSL-R1

Contains all of the linear isomers of native PFAS analytes required by Method 537

PFHxA	HFPO-DA
PFHpA	N-MeFOSAA
PFOA	N-EtFOSAA
PFNA	L-PFBS
PFDA	L-PFHxS
PFUdA	L-PFOS
PFDoA	NaDONA
PFTrDA	9Cl-PF3ONS
PFTeDA	11Cl-PF3OUdS

EPA 537 vs EPA 533

EPA 537

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	60	40
1	60	40
25	10	90
32	10	90
32.1	60	40
37	60	40

40 → 90
in 24 min
2.08% per min

EPA 533

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	95	5
0.5	95	5
3	60	40
16	20	80
18	20	80
20	5	95
22	5	95
25	95	5
35	95	5

40 → 80
in 13 min
3.08% per min

EPA 537 vs EPA 533

EPA 537

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	60	40
1	60	40
25	10	90
32	10	90
32.1	60	40
37	60	40

40 → 90
in 24 min
2.08% per min

533 Similar

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45

45 → 90
in 15 min
3.0% per min

Column Screening: Sample

Wellington Laboratories: EPA-537PDSL-R1

Contains all of the linear isomers of native PFAS analytes required by Method 537

Abbreviations for Experts

PFHxA	HFPO-DA
PFHpA	N-MeFOSAA
PFOA	N-EtFOSAA
PFNA	L-PFBS
PFDA	L-PFHxS
PFUdA	L-PFOS
PFDoA	NaDONA
PFTrDA	9Cl-PF3ONS
PFTeDA	11Cl-PF3OUdS

Column Screening: Sample

Wellington Laboratories: EPA-537PDSL-R1

Contains all of the linear isomers of native PFAS analytes required by Method 537

Chemical Names for Chemists

- Perfluorobutanesulfonic acid
- Perfluorohexanoic acid
- hexafluoropropylene oxide-dimer acid
- Perfluoroheptanoic acid
- dodecafluoro-3H-4,8-diosanonanoate
- perfluoro-1-hexanesulfonate
- perfluoro-n-octanoic acid
- perfluoro-n-nonanoic acid
- perfluoro-1-octanesulfonate
- 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate
- perfluoro-n-decanoic acid
- N-methylperfluoro-1-octanesulfonamidoacetic acid
- N-ethylperfluoro-1-octanesulfonamidoacetic acid
- perfluoro-n-undecanoic acid
- 11-chloroeicosalfluoro-3-oxaundecane-1-sulfonate
- perfluoro-n-deodecanoic acid
- perfluoro-n-tridecanoic acid
- perfluoro-n-tetradecanoic acid

Column Screening: Sample

Wellington Laboratories: EPA-537PDSL-R1

Contains all of the linear isomers of native PFAS analytes required by Method 537

Chemical Names Sorted by Functional Groups

Perfluorobutanesulfonic acid
perfluoro-1-hexanesulfonic acid
perfluoro-1-octanesulfonic acid

Perfluorohexanoic acid
Perfluoroheptanoic acid
perfluoro-n-octanoic acid
perfluoro-n-nonanoic acid
perfluoro-n-decanoic acid
perfluoro-n-undecanoic acid
perfluoro-n-deodecanoic acid
perfluoro-n-tridecanoic acid
perfluoro-n-tetradecanoic acid

hexafluoropropylene oxide-dimer acid

dodecafluoro-3H-4,8-dioxanonanoate

9-chlorohexadecafluoro-3-oxanonane-1-sulfonate
11-chloroeicosafluoro-3-oxaundecane-1-sulfonate

N-methylperfluoro-1-octanesulfonamidoacetic acid
N-ethylperfluoro-1-octanesulfonamidoacetic acid

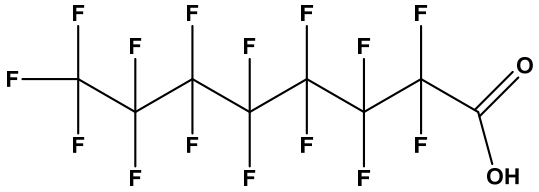
Wellington Laboratories: EPA-537PDSL-R1

Contains all of the linear isomers for native PFAS analytes required by Method 537

Functional Groups in This Set of Compounds

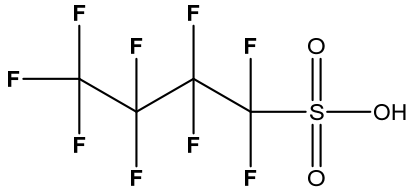
- Perfluoro alkyl carboxylic acids
- Perfluoro alkyl sulfonic acids
- Perfluoro octane sulfon amido acetic acid
- Perfluoro oxide-dimer acid
- Polyfluoro dioxa nonanoate
- Chloro Perfluoro oxa sulfonate

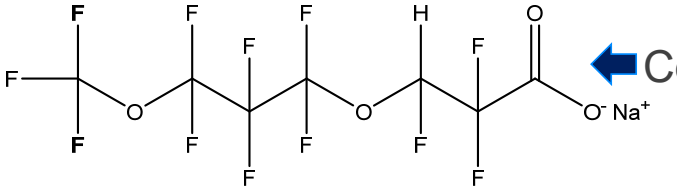
Functional Groups Applied to Chromatography



Perfluoro-n-octanoic acid

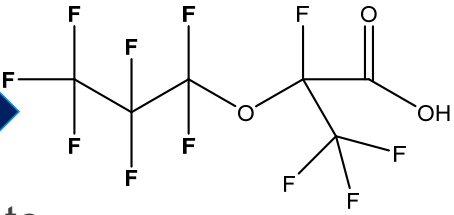
Perfluorobutanesulfonic acid

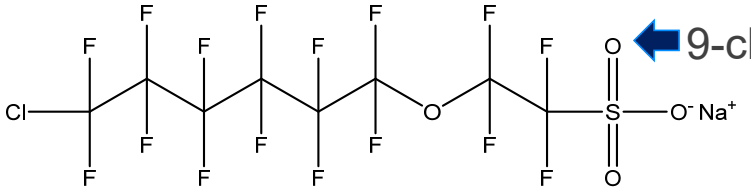




Codecafluoro-3H-4,8-dioxanonanoate

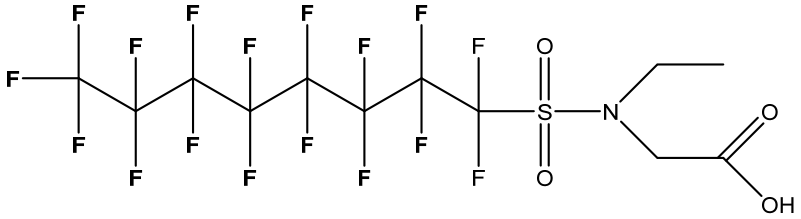
Hexafluoropropylene oxide-dimer acid



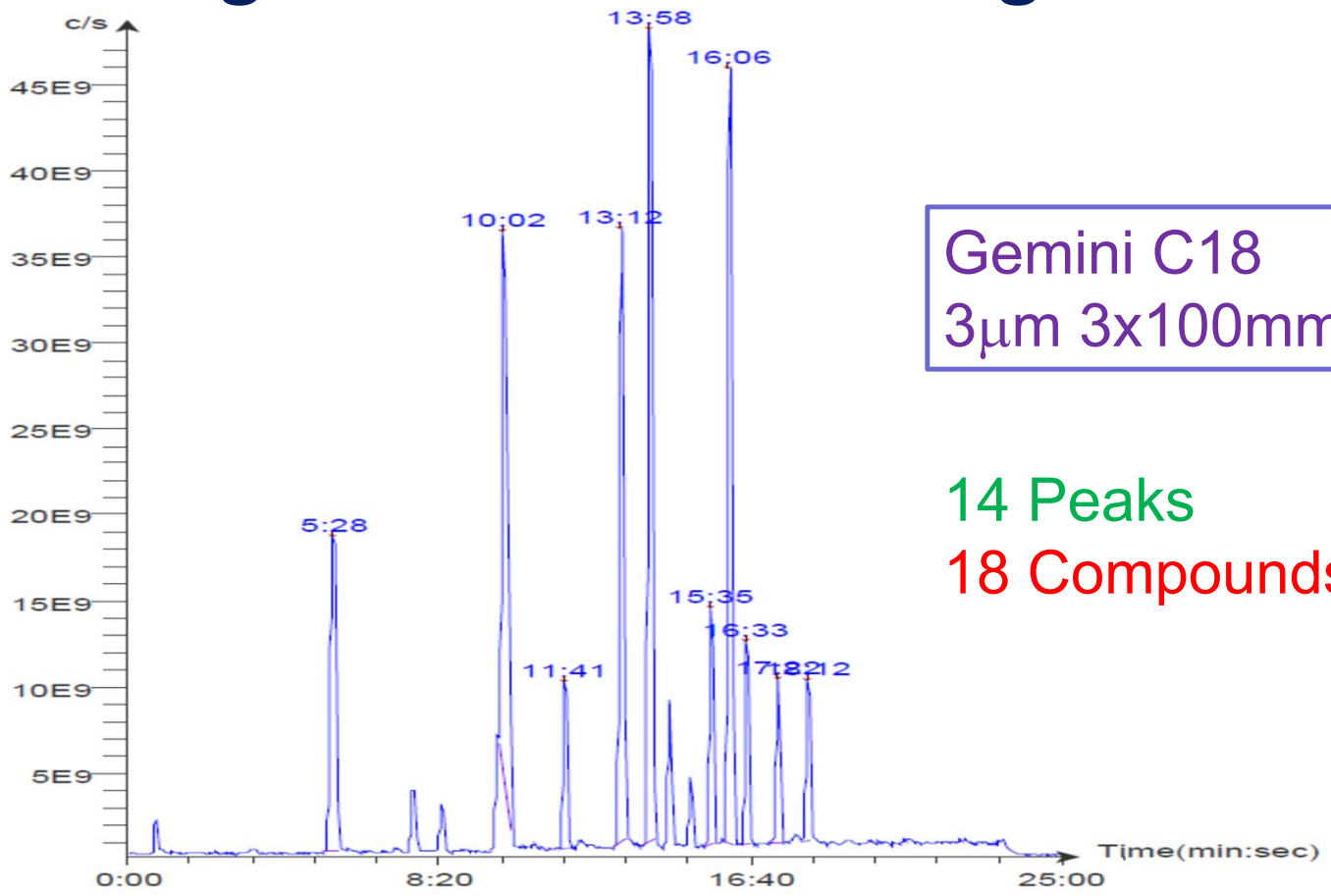


9-chlorohexadecafluoro-3-oxanonane-1-sulfonate

N-ethylperfluoro-1-octanesulfonamidoacetic acid



Screening Data: TIC Chromatogram

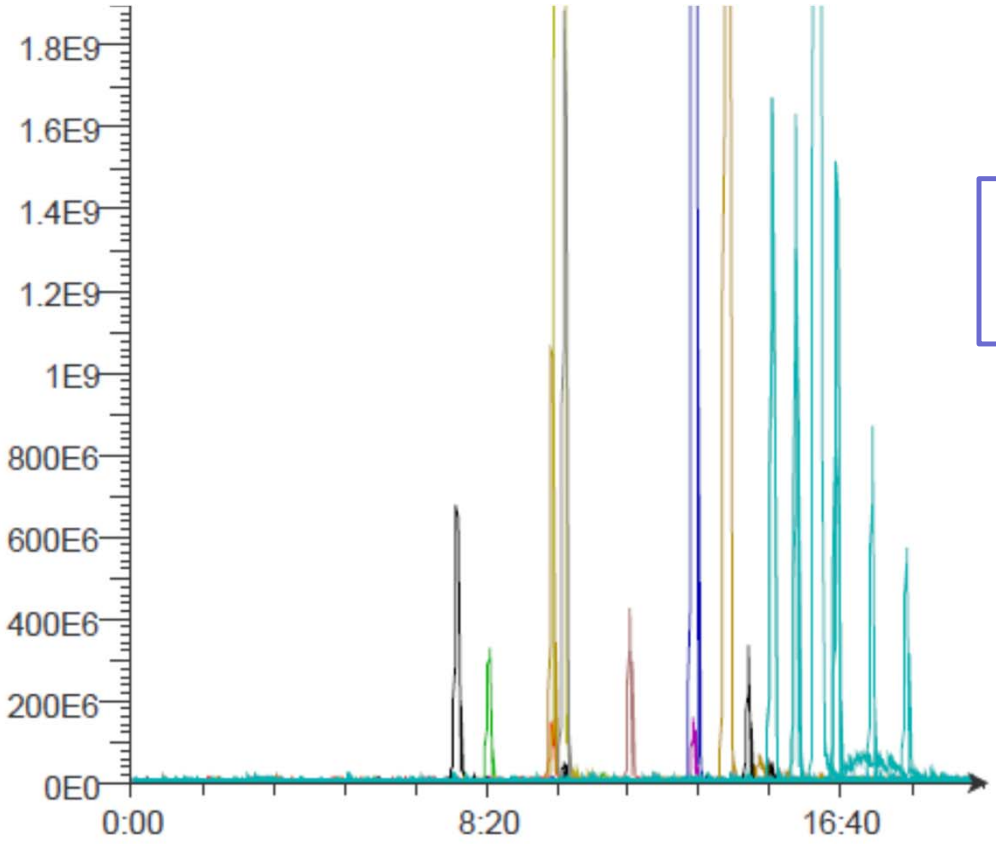


Gemini C18
3µm 3x100mm

14 Peaks
18 Compounds

Screening Data: Extracted Ion Chromatograms

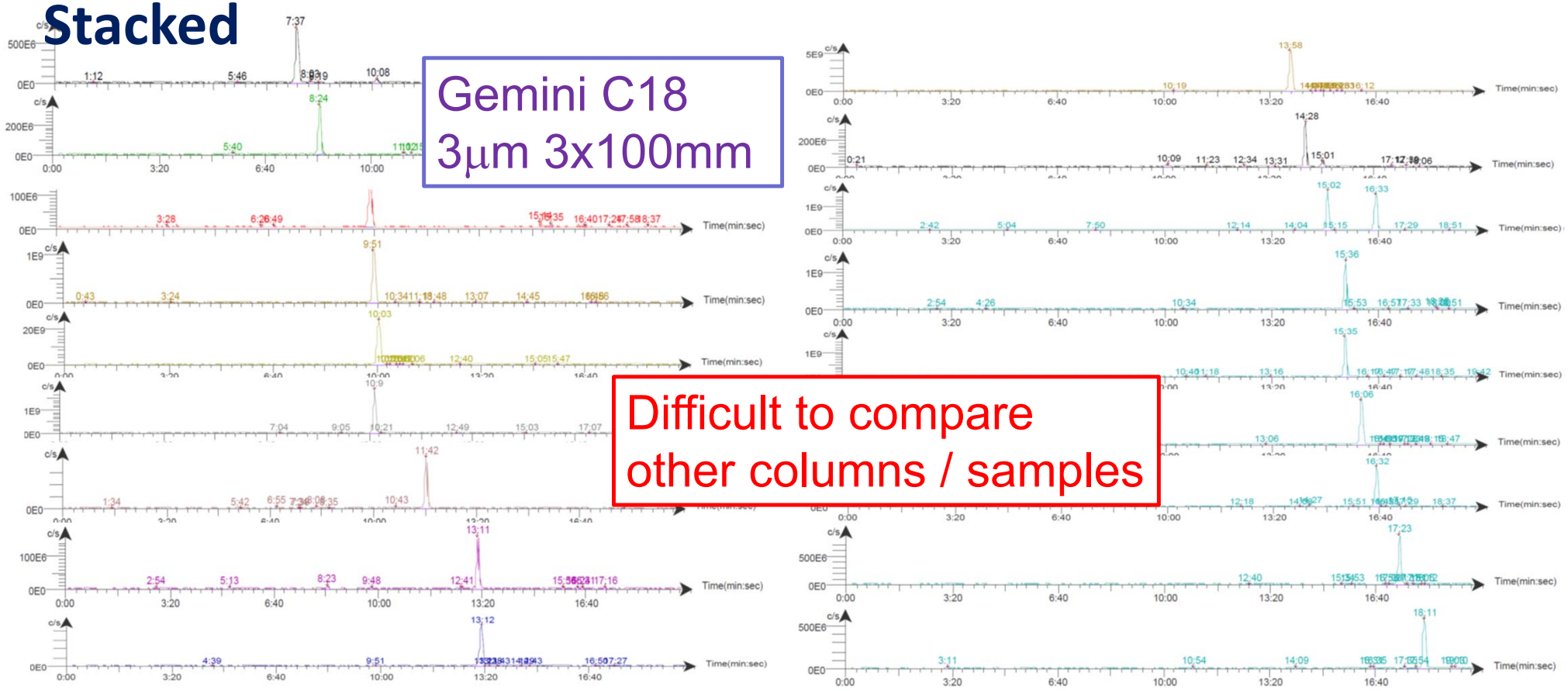
Overlayed



Gemini C18
3µm 3x100mm

18 Peaks
18 Compounds
Difficult to visualize

Screening Data: Extracted Ion Chromatograms Stacked



Screening Data: Tabulate Compounds and C18 Retention Times

EPA 533-similar: Ammonium Acetate / Methanol

Carboxylic Acid	Ether + Carboxylic Acid	Carboxylic Acid	Sulfonic Acid	Sulfonic Acid	Ether + Carboxylic Acid	Carboxylic Acid	Carboxylic Acid	Sulfonic Acid	Ether + Sulfonic Acid	Carboxylic Acid	Sulfon Amido Acid	Carboxylic Acid	Sulfon Amido Acid	Ether + Sulfonic Acid	Carboxylic Acid	Carboxylic Acid	Carboxylic Acid
PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11

Retention Time (min.sec)

Overlapping Peaks

Gemini C18
3µm 3x100mm

Compare Gemini C18 + Kinetex C18

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48

Compare Kinetex XB-C18 + Gemini C18 + Kinetex C18

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.03	14.31	14.55	15.47	16.30

Compare Kinetex Polar C18 + Other C18 Columns

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.03	14.31	14.55	15.47	16.30

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	Polar C18	4.58	5.48	7.19	7.19	7.33	7.37	9.17	10.54	10.57	11.53	12.17	12.51	13.26	13.27	14.03	14.25	15.19	16.05

Compare Luna Omega Polar C18 + Other C18 Columns

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.03	14.31	14.55	15.47	16.30
Kinetex	Polar C18	4.58	5.48	7.19	7.19	7.33	7.37	9.17	10.54	10.57	11.53	12.17	12.51	13.26	13.27	14.03	14.25	15.19	16.05

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Luna Omega	Polar C18	6.19	7.11	8.29	8.31	8.39	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.03	14.05	14.38	14.59	15.47	16.29

Compare Luna Omega PS C18 + Other C18 Columns

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.03	14.31	14.55	15.47	16.30
Kinetex	Polar C18	4.58	5.48	7.19	7.19	7.33	7.37	9.17	10.54	10.57	11.53	12.17	12.51	13.26	13.27	14.03	14.25	15.19	16.05
Luna Omega	Polar C18	6.19	7.11	8.29	8.31	8.39	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.03	14.05	14.38	14.59	15.47	16.29

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Luna Omega	PS C18	6.34	7.21	8.46	8.46	8.55	9.05	10.35	12.03	12.06	12.53	13.24	14.01	14.31	14.35	14.58	15.23	16.08	16.45

Compare Kinetex PAH + Other C18 Columns

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.03	14.31	14.55	15.47	16.30
Kinetex	Polar C18	4.58	5.48	7.19	7.19	7.33	7.37	9.17	10.54	10.57	11.53	12.17	12.51	13.26	13.27	14.03	14.25	15.19	16.05
Luna Omega	Polar C18	6.19	7.11	8.29	8.31	8.39	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.03	14.05	14.38	14.59	15.47	16.29
Luna Omega	Ps C18	6.34	7.21	8.46	8.46	8.55	9.05	10.35	12.03	12.06	12.53	13.24	14.01	14.31	14.35	14.58	15.23	16.08	16.45

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	PAH	2.04	2.36	3.57	3.59	4.17	4.18	5.49	7.37	7.46	8.37	9.10	9.44	10.19	10.30	11.09	11.42	12.45	13.43

Elution Order Changed

C18 Summary

EPA 533-similar: Ammonium Acetate / Methanol

The elution order was consistent for most of the C18 columns

The Kinetex PAH column had 2 compounds reverse elution order

There were slight differences in overlapping peaks

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11	16.13	16.47	17.11	18.02	18.48
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.03	14.31	14.55	15.47	16.30
Kinetex	Polar C18	4.58	5.48	7.19	7.19	7.33	7.37	9.17	10.54	10.57	11.53	12.17	12.51	13.26	13.27	14.03	14.25	15.19	16.05
Luna Omega	Polar C18	6.19	7.11	8.29	8.31	8.39	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.03	14.05	14.38	14.59	15.47	16.29
Luna Omega	PS C18	6.34	7.21	8.46	8.46	8.55	9.05	10.35	12.03	12.06	12.53	13.24	14.01	14.31	14.35	14.58	15.23	16.08	16.45

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	PAH	2.04	2.36	3.57	3.59	4.17	4.18	5.49	7.37	7.46	8.37	9.10	9.44	10.19	10.30	11.09	11.42	12.45	13.43

Elution Order Changes

Compare Kinetex Phenyl-Hexyl + Gemini C18

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	PFDA	9-Cl-PF3ONS	N-MeFOSAA	PFUdA	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	Phenyl-Hexyl	3.54	4.34	5.58	5.58	6.23	6.25	7.41	9.06	9.21	10.18	10.23	11.08	11.20	11.42	12.13	12.18	13.01	13.41

Elution Order Changes

Compare Kinetex Biphenyl + Kinetex Phenyl-Hexyl + Gemini C18

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	Phenyl-Hexyl	3.54	4.34	5.58	5.58	6.23	6.25	7.41	9.06	9.21	10.18	10.23	11.08	11.20	11.42	12.13	12.18	13.01	13.41

		PFHxA	HFPO-DA	PFHpA	L-PFBS	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA	9-Cl-PF3ONS	N-EtFOSAA	PFUdA	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	Biphenyl	2.06	2.14	3.49	3.49	4.07	4.32	5.35	7.02	7.28	8.11	8.34	9.44	9.90	9.90	10.00	10.21	10.45	11.23

Elution Order Changes

Compare Kinetex F5 + Biphenyl, Phenyl-Hexyl + Gemini C18

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	Phenyl-Hexyl	3.54	4.34	5.58	5.58	6.23	6.25	7.41	9.06	9.21	10.18	10.23	11.08	11.20	11.42	12.13	12.18	13.01	13.41
Kinetex	Biphenyl	2.06	2.14	3.49	3.49	4.07	4.32	5.35	7.02	7.28	8.11	8.34	9.44	9.90	9.90	10.00	10.21	10.45	11.23

		PFHxA	HFPO-DA	PFHpA	L-PFBS	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	F5	5.07	5.50	7.29	7.30	7.51	7.59	9.27	11.06	11.21	11.53	12.28	13.22	13.38	13.50	14.05	14.37	15.28	16.12

Elution Order Change

Phenyl Stationary Phase Summary

EPA 533-similar: Ammonium Acetate / Methanol

The elution order was different from the C18 columns and between phenyl columns

The Kinetex F5 column had similar order to the C18 columns and only 1 overlap

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11
Kinetex	Phenyl-Hexyl	3.54	4.34	5.58	5.58	6.23	6.25	7.41	9.06	9.21	10.18	10.23	11.08	11.20	11.42	12.13	12.18	13.01	13.41
Kinetex	Biphenyl	2.06	2.14	3.49	3.49	4.07	4.32	5.35	7.02	7.28	8.11	8.34	9.44	9.90	9.90	10.00	10.21	10.45	11.23

		PFHxA	HFPO-DA	PFHpA	L-PFBS	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	F5	5.07	5.50	7.29	7.30	7.51	7.59	9.27	11.06	11.21	11.53	12.28	13.22	13.38	13.50	14.05	14.37	15.28	16.12

Elution Order Change

Altered Chromatographic Method Conditions

533 Similar (previous data)

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45

533 Acetonitrile Altered

- HPLC Method Conditions

Time (min)	% 20 mM Ammonium Acetate	% 80-20 Methanol Acetonitrile
Initial	55	45
15	10	90
21	10	90
21.5	55	45

Screening Data: Tabulate Compounds and C18 Retention Times

EPA 533-similar

Gemini C18
3µm 3x100mm

Methanol

Previously Shown

PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-[illegible]A	11Cl-PF3[illegible]	PFDoA	PFTrDA	PFTeDA
7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11

Screening Data: Compare Methanol with Methanol - Acetonitrile

EPA 533-similar

533 Acetonitrile Altered

Gemini C18
3µm 3x100mm

Methanol

PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.35	15.36	16.06	16.32	17.23	18.11

80-20 Methanol - Acetonitrile

PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	PFOA	NaDONA	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
2.25	2.58	3.52	3.53	4.27	5.33	5.45	7.14	8.54	9.05	9.05	9.49	10.15	10.26	11.51	12.58	13.10	14.22

Compare Kinetex PAH + Other C18 Columns

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	[illegible]FUdA	[illegible]A	[illegible]	PF[illegible]	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.[illegible]		16.32	17.23	18.11
Kinetex	C18	6.41	7.38	9.39	9.40	9.59	10.02	11.57	13.40	13.44	14.33	15.03	15.36	16.11		.47	17.11	18.02	18.48
Kinetex	XB-C18	5.31	6.18	7.55	7.56	8.10	8.14	9.54	11.31	11.34	12.24	12.51	13.28	13.58	1[illegible]	14.31	14.55	15.47	16.30
Kinetex	Polar C18	4.58	5.48	7.19	7.19	7.33	7.37	9.17	10.54	10.57	11.53	12.17	12.51	13.26	13.27	14.03	14.25	15.19	16.05
Luna Omega	Polar C18	6.19	7.11	8.29	8.31	8.39	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.03	14.05	14.38	14.59	15.47	16.29
Luna Omega	Ps C18	6.34	7.21	8.46	8.46	8.55	9.05	10.35	12.03	12.06	12.53	13.24	14.01	14.31	14.35	14.58	15.23	16.08	16.45

Previously Shown
Note the similarity for
all except PAH

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	PAH	2.04	2.36	3.57	3.59	4.17	4.18	5.49	7.37	7.46	8.37	9.10	9.44	10.19	10.30	11.09	11.42	12.45	13.43

Elution Order Changed

Compare Gemini C18 + Kinetex C18, XB-C18 and Polar C18

533 Acetonitrile Altered

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	PFOA	NaDONA	PFNA	PFDA	L-PFOS	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini	C18	2.25	2.58	3.52	3.53	4.27	5.33	5.45	7.14	8.54	9.05	9.05	9.49	10.15	10.26	11.51	12.58	13.1	14.22
Kinetex	XB-C18	1.22	1.38	2.14	2.14	2.42	3.41	3.45	5.16	6.48	6.52	7.16	7.56	7.57	8.13	9.31	10.34	10.5	12.05
Kinetex	Polar C18	1.5	2.15	2.55	2.56	3.26	4.18	4.31	5.44	7.08	7.2	7.22	7.59	8.26	8.27	9.41	10.51	10.52	12
Luna Omega	Polar C18	1.19	1.35	1.58	2	2.23	3.08	3.18	4.27	5.54	6.05	6.1	6.49	7.13	7.16	8.31	9.37	9.4	10.45
Luna Omega	PS C18	1.52	2.21	3.06	3.07	3.4	4.41	4.52	6.17	7.45	7.54	8	8.43	8.56	9.06	10.23	11.27	11.42	12.55
Kinetex	PAH	0.46	0.5	0.56	0.57	1.03	1.2	1.21	2.12	3.27	3.3	3.48	4.19	4.2	4.46	6.05	6.54	7.28	8.49

		HFPO-DA	PFHxA	PFHpA	L-PFBS	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA	9-Cl-PF3ONS	N-MeFOSAA	N-EtFOSAA	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	C18	1.25	1.41	2.18	2.2	2.48	4.08	4.18	6.24	8.17	8.27	8.32	9.17	9.37	9.54	11.21	11.21	12.31	13.6

Elution Order Changes

C18 Summary

533 Acetonitrile Altered

There were more overlapping pairs than with Methanol

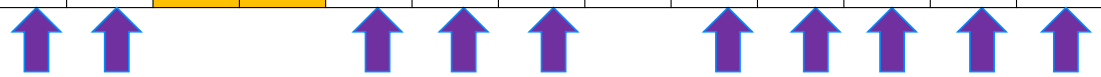
The elution order was consistent for most of the C18 columns

The Kinetex C18 column had several compounds change elution order

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	PFOA	NaDONA	PFNA	PFDA	L-PFOS	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini	C18	2.25	2.58	3.52	3.53	4.27	5.33	5.45	7.14	8.54	9.05	9.05	9.49	10.15	10.26	11.51	12.58	13.1	14.22
Kinetex	XB-C18	1.22	1.38	2.14	2.14	2.42	3.41	3.45	5.16	6.48	6.52	7.16	7.56	7.57	8.13	9.31	10.34	10.5	12.05
Kinetex	Polar C18	1.5	2.15	2.55	2.56	3.26	4.18	4.31	5.44	7.08	7.2	7.22	7.59	8.26	8.27	9.41	10.51	10.52	12
Luna Omega	Polar C18	1.19	1.35	1.58	2	2.23	3.08	3.18	4.27	5.54	6.05	6.1	6.49	7.13	7.16	8.31	9.37	9.4	10.45
Luna Omega	PS C18	1.52	2.21	3.06	3.07	3.4	4.41	4.52	6.17	7.45	7.54	8	8.43	8.56	9.06	10.23	11.27	11.42	12.55
Kinetex	PAH	0.46	0.5	0.56	0.57	1.03	1.2	1.21	2.12	3.27	3.3	3.48	4.19	4.2	4.46	6.05	6.54	7.28	8.49

		HFPO-DA	PFHxA	PFHpA	L-PFBS	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA	9-Cl-PF3ONS	N-MeFOSAA	N-EtFOSAA	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	C18	1.25	1.41	2.18	2.2	2.48	4.08	4.18	6.24	8.17	8.27	8.32	9.17	9.37	9.54	11.21	11.21	12.31	13.6

Elution Order Changes



Compare Kinetex F5 + Biphenyl, Phenyl-Hexyl + Gemini C18

EPA 533-similar: Ammonium Acetate / Methanol

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini	C18	7.37	8.24	9.51	9.52	10.03	10.90	11.42	13.11	13.12	13.58	14.28	15.02	15.3		16.06	16.32	17.23	18.11
Kinetex	Phenyl-Hexyl	3.54	4.34	5.58	5.58	6.23	6.25	7.41	9.06	9.21	10.18	10.23	11.08	11.	.42	12.13	12.18	13.01	13.41
Kinetex	Biphenyl	2.06	2.14	3.49	3.49	4.07	4.32	5.35	7.02	7.28	8.11	8.34	9.44	9.90	9.90	10.00	10.21	10.45	11.23

Previously Shown

		PFHxA	HFPO-DA	PFHpA	L-PFBS	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	F5	5.07	5.50	7.29	7.30	7.51	7.59	9.27	11.06	11.21	11.53	12.28	13.22	13.38	13.50	14.05	14.37	15.28	16.12

Elution Order Change

Compare Kinetex F5 + Biphenyl, Phenyl-Hexyl + Gemini C18

533 Acetonitrile Altered

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	PFOA	NaDONA	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini	C18	2.25	2.58	3.52	3.53	4.27	5.33	5.45	7.14	8.54	9.05	9.05	9.49	10.15	10.26	11.51	12.58	13.1	14.22
Kinetex	Phenyl-Hexyl	1.03	1.11	1.23	1.24	1.36	1.59	2.16	2.57	4.06	4.2	4.28	4.49	5.1	5.28	6.1	7.05	7.27	7.56
Kinetex	Biphenyl	0.38	0.51	0.59	0.59	1.04	1.12	1.2	1.34	2.06	2.2	2.22	2.41	2.49	3.14	3.36	4.19	4.49	5.02
Kinetex	F5	1.16	1.27	1.56	1.56	2.17	3.08	3.15	4.33	5.56	6.03	6.32	6.5	7.02	7.13	8.26	9.14	9.38	10.45

Elution Order Change

Phenyl Stationary Phase Summary

533 Acetonitrile Altered

The elution order was different from the C18 columns and between phenyl columns

The Kinetex F5 column was less similar to the C18 columns with acetonitrile in the eluent

The Kinetes F5 still only had 1 overlap

		PFHxA	HFPO-DA	PFHpA	L-PFBS	L-PFHxS	PFOA	NaDONA	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini	C18	2.25	2.58	3.52	3.53	4.27	5.33	5.45	7.14	8.54	9.05	9.05	9.49	10.15	10.26	11.51	12.58	13.1	14.22
Kinetex	Phenyl-Hexyl	1.03	1.11	1.23	1.24	1.36	1.59	2.16	2.57	4.06	4.2	4.28	4.49	5.1	5.28	6.1	7.05	7.27	7.56
Kinetex	Biphenyl	0.38	0.51	0.59	0.59	1.04	1.12	1.2	1.34	2.06	2.2	2.22	2.41	2.49	3.14	3.36	4.19	4.49	5.02
Kinetex	F5	1.16	1.27	1.56	1.56	2.17	3.08	3.15	4.33	5.56	6.03	6.32	6.5	7.02	7.13	8.26	9.14	9.38	10.45

Elution Order Change

Outline

- PFAS - Brief Introduction
- Samples
- Sample Prep - SPE
- HPLC Instrumentation
- LCMS Detectors
- HPLC Columns
- Summary

Summary

- PFAS analysis is a long list of complicated topics
  - Sampling, Sample Prep, HPLC instrumentation, Methodology, Columns, Detectors
- Specific methods are designed around a particular set of compounds from a specific sample matrix
- The HPLC methodology in EPA 533 and EPA 537, uses methanol and ammonium acetate as eluent components.
  - Various C18 stationary phases, show subtle differences between the different “flavors” of C18
  - Phenyl based phases show wider differences in selectivity
- When a method can be adjusted or is being developed, using a mixture of acetonitrile and methanol can provide different selectivity than using methanol alone
- A variety of HPLC column chemistries are needed for the wide range of PFAS related compounds such as: polar acids, non-polar acids, esters, amides, and sulfonamides. All of which can be complicated with branched vs. linear isomers

Thank You