TN-1290

Column Chemistry Considerations for Full Coverage of PFAS Analyte Ranges

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Introduction

Per- and polyfluorinated alkyl substances (PFAS) are man-made chemicals, that have been widely used since the 1940s. They have been employed in a large variety of consumer products, such as nonstick cookware, food containers, stain and water repellent fabrics, polishes, waxes, paints, and cleaning products and are now widely distributed in the global environment. A significant source of PFAS environmental contamination has been the widespread use of PFAS-containing aqueous firefighting foams (AFFF), which are known to migrate into groundwaters at airports and military bases. Further environmental exposure to PFAS comes from industrial production facilities (e.g. chrome plating, electronics, manufacturing, or oil recovery). Living organisms, including plants, animals, and humans, can accumulate PFAS compounds in their tissue, which can build up over time and impact their health.[1-3] A total of 9,252 PFAS are listed in EPA's most recent list of PFAS substances. [4] However, only a handful of these, such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS), have been widely monitored in the environment or have been thoroughly studied for their toxicological effects.

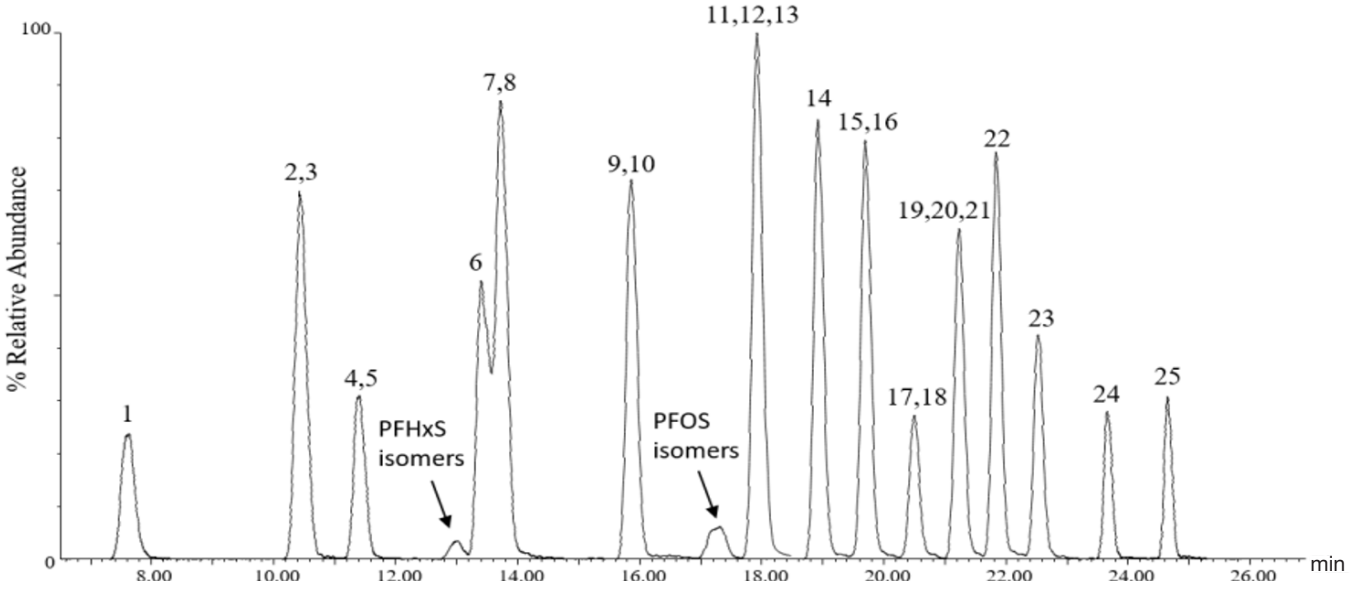
Common Chromatographic Approaches

PFAS compounds are typically determined by LC-MS/MS and LC-HRMS instrumentation. The use of mass spectrometry detection has played a significant role in the quantitation of specific compounds where standards are available. Where standards are not available, the use of time of flight (TOF) and Orbitrap™ MS detectors are used to semi-quantify unknown PFAS compounds. The chromatographic separation of PFAS compounds in currently validated methods typically involves a reversed phase mechanism using a C18 or Phenyl column in an acidic-methanol eluent. For example, EPA method 537.1 uses a C18 column (5 µm, 2.1 x 150 mm C18) and EPA Method 533 was validated using a C18 Phenomenex Gemini® column (3 µm, 2 x 50 mm). Conversely, ASTM D7979 and EPA 8327 were validated using a Phenyl-Hexyl column (1.7 µm, 2.1 × 100 mm), ISO 21675 used a C18 column (5 µm, 2 × 50 mm) and the Department of Agriculture CLG-PFAS 2.01 method used a C8 column, Phenomenex Luna® C8(2) (3 µm, 2 x 50 mm).

PFAS Chromatographic Challenges

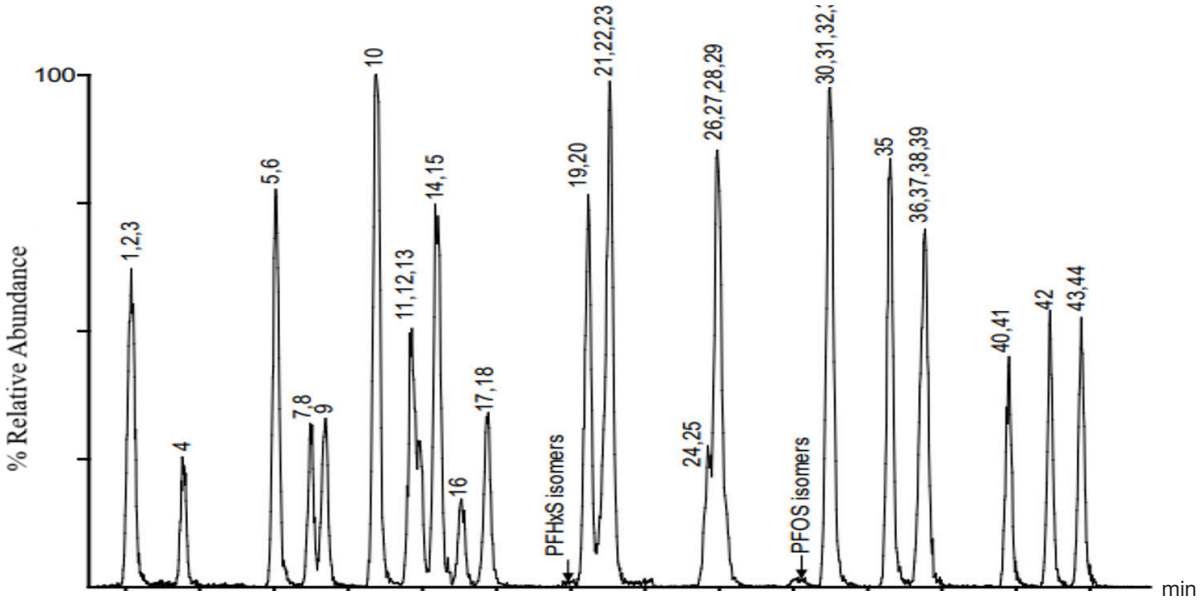
While these methods are generally adequate for a limited list of analytes, the large number of potential PFAS analytes that could potentially be present in a sample will inevitably challenge simple chromatographic separation approaches. This phenomenon was seen early in the development of the EPA drinking water methods. EPA 537.1 when validated, identified several overlapping peaks which can be seen in **Figure 1** as demonstrated by peaks, 2,3; 4,5; 7,8; 9,10; 11,12,13; 15,16; 17, 18; 19, 20, 21.

Figure 1.
Example chromatogram for reagent water fortified with method 537.1 analytes at 80 ng/L.



Likewise, when EPA 533 was developed and validated with an expanded list of PFAS compounds, it also shows several overlapping peaks, as seen in **Figure 2**.

Figure 2.
Example chromatogram for reagent water fortified with method 533 analytes at 80 ng/L.



Whereas many of these overlapping peaks can be successfully resolved by the mass analyzer, the potential presence of isobaric homologues and unresolved matrix interferences point to the continuing need for good chromatographic separation to assure reliable identification and quantitation. Although the problem may be manageable for today's small analyte lists, the challenge will inevitably grow as new PFAS compounds are added for investigational or regulatory purposes.

Looking to the Future

Current PFAS methods primarily rely upon C18 solid phase chemistry and simple methanol-ammonium acetate mobile phase gradients. These methods do not make full use of all the tools in the chromatographer's toolbox, nor need they, given today's limited analyte lists. However, this simple situation will inevitably change and there will be a need to develop more sophisticated chromatographic methods to tease out the subtle chemical and structural differences between closely related PFAS compounds. Chief among these will be the application of novel stationary phases and mobile phases to exploit the different interactions between closely related PFAS molecules. This Tech Note was designed to provide a vision of the potential power of such new chromatographic approaches.

Scope

In this Tech Note we will present data for a select list of PFAS compounds (**Table 1**) that were selected to reflect some of the chemical diversity of the PFAS universe. This grouping will be used to illustrate the differences in chromatographic retention time and elution order between various stationary phases including C8, C18, Phenyl-Hexyl, Biphenyl and F5 which can have significantly different sorptive properties. We will also examine how differing mobile phase polarity (e.g., methanol vs. acetonitrile) influences chromatographic performance for these various phases. Ideally, this information can be used to enhance chromatographic resolution as the list of PFAS compounds continues to increase. The goal is to provide insights that will allow method developers to identify useful separation strategies.

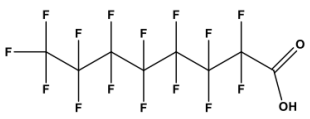
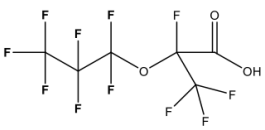
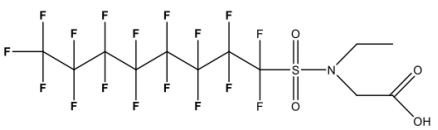
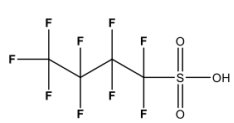
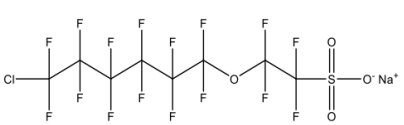
Table 1.

Chemical Name	Abbreviation
Perfluoro alkyl carboxylic acids (PFCAs)	
Perfluorohexanoic acid	PFHxA
Perfluoroheptanoic acid	PFHpA
perfluoro-n-octanoic acid	PFOA
perfluoro-n-nonanoic acid	PFNA
perfluoro-n-decanoic acid	PFDA
perfluoro-n-undecanoic acid	PFUdA
perfluoro-n-dodecanoic acid	PFDoA
perfluoro-n-tridecanoic acid	PFTrDA
perfluoro-n-tetradecanoic acid	PFTeDA
Perfluoroalkyl ether carboxylic acids (PFECA)	
hexafluoropropylene oxide-dimer acid	HFPO-DA
dodecafluoro-3H-4,8-diosanonanoate	NaDONA
Perfluorooctane Sulfonamide and Derivatives	
N-methylperfluoro-1-octanesulfonamidoacetic acid	N-MeFOSAA
N-ethylperfluoro-1-octanesulfonamidoacetic acid	N-EtFOSAA
Perfluorinated sulfonic acids (PFSAs)	
Perfluorobutanesulfonic acid	L-PFBS
perfluoro-1-hexanesulfonate	L-PFHxS
perfluoro-1-octanesulfonate	L-PFOS
Chlorinated Polyfluoroalkyl Ether Sulfonic Acids (Cl-PFESAs)	
9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS
11-chloroeicosalfluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS

Method Variables

PFAS Chemistries

There are established, validated methods set forth by the EPA and ISO for chromatographic separation of PFAS compounds using specific types of columns and packing materials. Unfortunately, not all PFAS compounds can be separated with sufficient accuracy using these methods because of the different types of functional groups that are on different PFAS compounds. In the select list that was used, there are 5 categories of PFAS compounds as shown in **Figure 3**, with an example of each. Owing to the variety of functional groups that can potentially be found on PFAS compounds, there are a variety of HPLC column chemistries that could aid enhanced separation.

Perfluoro alkyl carboxylic acids (PFCAs)	Perfluoro alkyl ether carboxylic acids (PFECAs)	Perfluoro octane Sulfonamide and Derivatives
 Perfluoro-n-octanoic acid	 Hexafluoropropylene oxide-dimcer acid	 N-ethylperfluoro-1-octanesulfonamidoacetic acid
Perfluoro alkyl carboxylic acids (PFCAs)	Perfluoro alkyl ether carboxylic acids (PFECAs)	
 Perfluoro-n-octanoic acid	 Hexafluoropropylene oxide-dimcer acid	

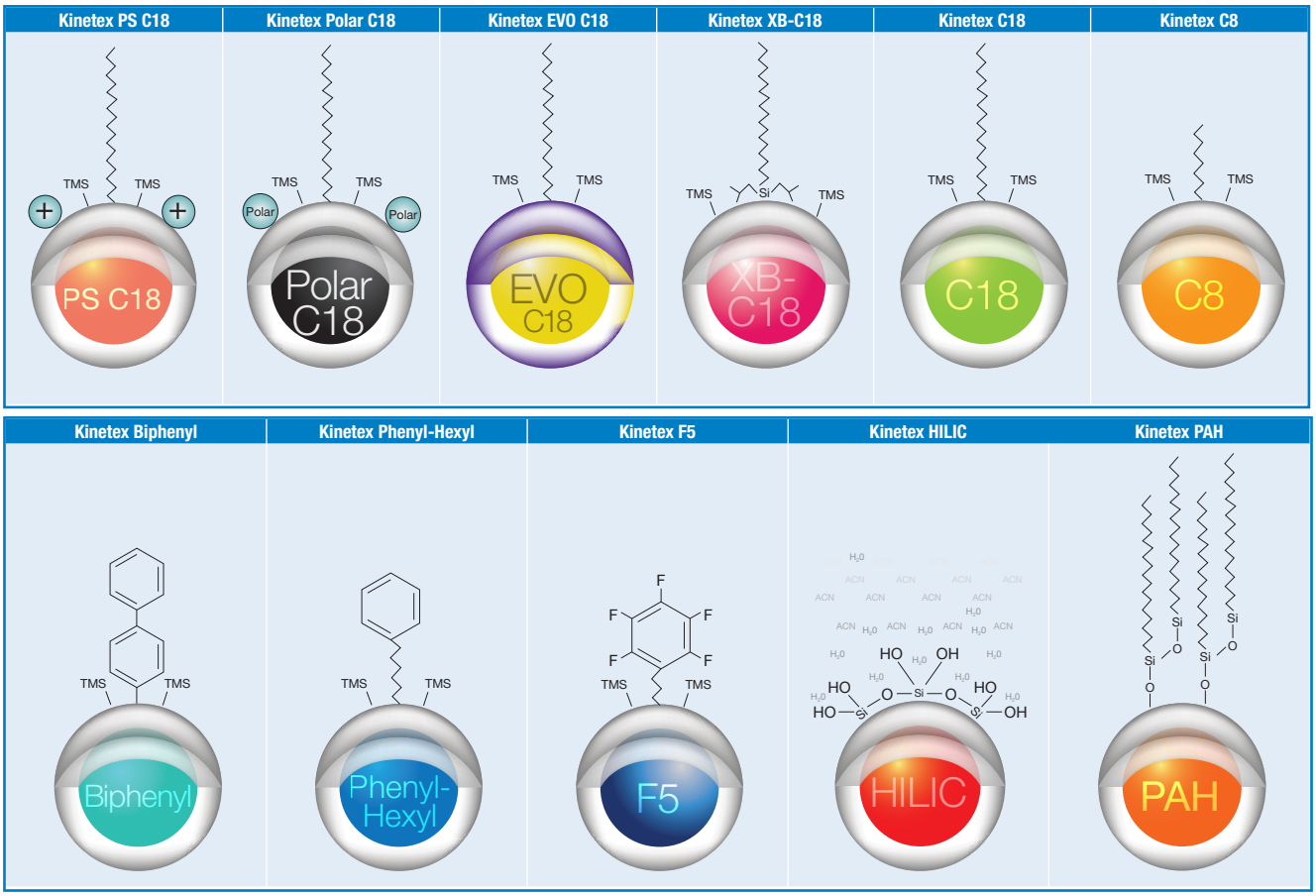
Solid Phase Chemistries

A representation of the different solid phase chemistries that are available in Phenomenex HPLC columns that could be used in PFAS separations is presented in **Figure 4**. This wide variety of ligand chemistries – combined with differences in porosity and other morphological variations – was developed to offer a wide range of variables for method development.

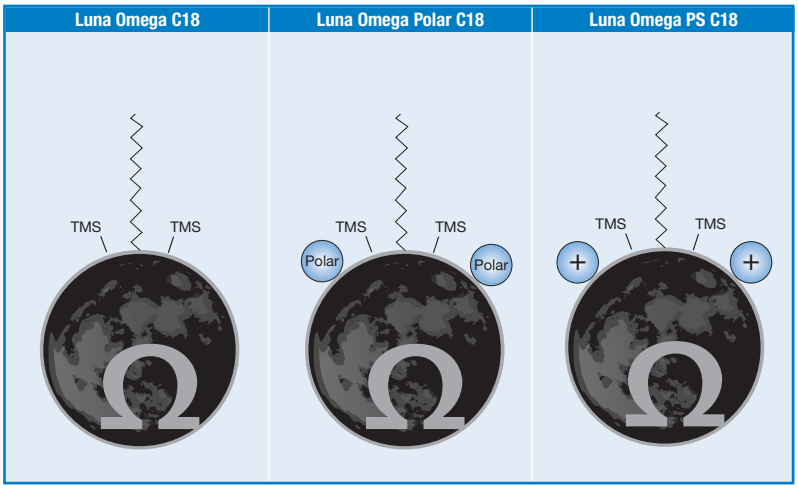
Different combinations of these variables serve to enhance the separation of polar compounds, increase surface areas, add pH stability, decrease system backpressures, etc. These, and additional column properties, provide chromatographers with a high degree of flexibility with which to tackle challenging separations.

Figure 4.
Available column chemistries appropriate for PFAS compound separation.

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



Luna® Omega Fully Porous 1.6, 3, and 5 µm



Mobile Phase Chemistries

However, in addition to column selection, chromatographers can also make changes in mobile phase polarity to further enhance selectivity. For example, EPA method 533 was altered in several ways to enhance separation of the selected PFAS compounds used in the present study. In the first elution regime, the percentage of methanol was increased at run initiation and then further increased to a higher percentage than had been previously used in the published method. This decreased the overall run time but kept the percentage increase of methanol roughly the same. This elution regime will be referred to later in this Tech Note as "533 Similar" (**Figure 5**).

In the second elution regime, acetonitrile was added to the mobile phase at a ratio of 80:20 methanol:acetonitrile to increase mobile phase polarity (but with all other factors remaining the same as in the "533 Similar" elution regime). This second elution regime will be referred to as "533 Acetonitrile Altered" (**Figure 6**). The results from these two elution regimes will be addressed separately. Clearly, there are many other potential mobile phase variations that could be investigated. However, the two variations presented here will suffice to demonstrate the power of mobile phase polarity combined with solid phase chemistry variation to effect PFAS chromatographic behavior.

Figure 5.

EPA 533 - As Published		
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		
Time (min)	% 20 mM Ammonium Acetate	% Methanol
Initial	55	45
15	10	90
21	10	90
21.5	55	45
40 → 90 in 15 min 3.0 % per min		

Figure 6.

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

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

Results and Discussion

For ease of comparison, all chromatographic data will be presented in tabular format with the chromatography columns on the left, the PFAS compounds across the top, and the specific analyte retention times under the PFAS compounds. The highlighted boxes identify two compounds that have overlapping retention times (generally $\Delta RT \leq 0.1$ min) and the arrows at the bottom indicate when two compounds have changed elution order. The different PFAS compound classes are represented by the colors referenced in **Table 1**. This representation is a more insightful way to present the data because overlaying or stacking individual chromatograms makes it very difficult to compare results across columns. The two mobile phase chemistry regimes identified above will now be discussed separately.

1. EPA 533 Similar

In order to determine how the selected PFAS compounds would elute and separate, seven different chromatography columns with different solid phase chemistries were examined. **Figure 7** displays columns that have C18-functionality or PAH-functionality. The PFAS elution order was generally consistent for most of the C18 phases, although specific elution times varied. The Kinetex® PAH column demonstrated two compound functional pairs with a reverse elution order: NaDONA (a perfluoroether carboxylic acid) vis-á-vis L-PFHxS (a perfluronated sulfonic acid) and PFUdA (a perfluoroalkyl carboxylic acid) vis-á-vis N-EtFOSSA (a perfluorooctane sulfonamide). In addition, there were slight differences in overlapping peaks amongst the various C18 phases, whereas the Kinetex PAH phase had only one overlapping pair. When compared to two C8 phases (**Figure 8**), the elution order was similar to the C18 phases, and the retention times were similar, but there were fewer overlapping peak pairs (one pair vs. 3 pairs).

However, the C8 phases also demonstrated two compound functionality pairs with a reverse order elution from the C18 phases: L-PFOS (a perfluoronated sulfonic acid) vis-á-vis PFNA (a perfluroalkyl carboxylic acid) and (again) PFUdA vis-á-vis N-EtFOSSA, presumably is response to the lower hydrophobicity of the C8 phase functionality. Interestingly, both C8 phases and the PAH phase had fewer overlapping peaks compared to the C18 phases, but in different parts of the elution order spectrum. This likely represents the greater contribution of pi-electron interaction with the PAH phase in contrast with more consistent hydrophobic interaction characteristic of the C18 phases. These variations are subtle rather than dramatic, but they offer insights into interactions between solid phase chemistry and PFAS compound class that could be useful for better separating adjacent compound pairs or shifting analytes away from mass spectral interferences.

Figure 7.
C18 and PAH summary

		L-PFBS	PFHxA	HFPO-DA	PFHpA	L-PFHxS	NaDONA	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Gemini®	C18	5.28	7.37	8.23	9.53	10.02	10.09	11.41	13.12	13.12	13.58	14.28	15.03	15.35	15.36	16.05	16.34	17.23	18.11
Luna®Omega	Polar C18	4.15	6.18	7.10	8.31	8.38	8.47	10.16	11.43	11.43	12.34	12.58	13.30	14.04	14.05	14.38	14.59	15.47	16.29
Luna Omega	Ps C18	4.29	6.34	7.21	8.46	8.55	9.05	10.35	12.06	12.03	12.53	13.24	14.01	14.31	14.36	14.58	15.23	16.08	16.44
Kinetex	C18	3.36	6.38	7.38	9.44	10.04	10.07	12.03	13.48	13.52	14.4	15.1	15.41	16.17	16.15	16.5	17.15	18.06	18.48
Kinetex	XB-C18	3.27	5.30	6.18	7.56	8.10	8.13	9.54	11.31	11.34	12.24	12.51	13.28	13.58	14.02	14.31	14.56	15.47	16.31
Kinetex	Polar C18	3.05	4.59	5.49	7.18	7.33	7.37	9.16	10.54	10.57	11.52	12.17	12.51	13.25	13.27	14.03	14.25	15.18	16.05

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	PAH	1.24	2.07	2.60	3.99	4.27	4.31	5.81	7.59	7.76	8.61	9.16	9.73	10.32	10.52	11.14	11.69	12.75	13.71

Elution Order Shifts from C18

Elution Order Shifts from C18
Overlaps are Eliminated

Figure 8.
C8 Summary

		L-PFBS	PFHxA	HFPO-DA	PFHpA	L-PFHxS	NaDONA	PFOA	L-PFOS	PFNA	9-Cl-PF3ONS	PFDA	N-MeFOSAA	N-EtFOSAA	PFUdA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Luna	C8	5.41	7.51	8.38	9.51	10.04	10.15	11.33	12.47	12.49	13.28	13.53	14.12	14.40	14.46	15.13	15.33	16.14	16.48
Kinetex	C8	5.30	7.54	8.47	10.14	10.26	10.33	11.57	13.18	13.19	14.03	14.26	14.45	15.15	15.24	15.56	16.14	16.56	17.33

Elution Order Shifts from C18

Finally, additional differences are seen when comparing Kinetex® Biphenyl, Phenyl-Hexyl, and F5 columns. These phases were designed with different chemistries having varying polarities to provide better selectivity for aromatic compounds. However, these polarity differences and greater pi-electron interactability also come into play with the different PFAS chemistries, as evidenced by the various reverse order elution pairs from the C18 phases.

The elution order in the Kinetex Biphenyl and Phenyl-Hexyl columns are consistent, but markedly different from the Kinetex F5 column. The Biphenyl and F5 phases showed only one set of overlapping peaks, but the Phenyl-Hexyl column had 3 sets of overlapping peaks. Interestingly, the compound classes that overlapped were different between the Phenyl-Hexyl and Biphenyl columns (**Figure 9**).

Figure 9.
Phenyl Stationary Phase Summary

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	PFDA	9-Cl-PF3ONS	N-MeFOSAA	PFUdA	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	Biphenyl	1.47	2.06	2.19	3.67	4.00	4.43	5.53	7.02	7.49	8.64	8.21	9.23	9.24	9.88	10.16	10.56	10.96	11.67
Kinetex	Phenyl-Hexyl	2.53	3.89	4.57	5.97	6.39	6.42	7.69	9.10	9.35	10.30	10.37	11.13	11.33	11.69	12.21	12.31	13.01	13.67

Elution Order Shifts from C18

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFOA	PFNA	L-PFOS	9-Cl-PF3ONS	PFDA	N-MeFOSAA	PFUdA	N-EtFOSAA	11Cl-PF3OUdS	PFDoA	PFTrDA	PFTeDA
Kinetex	F5	3.37	5.12	5.84	7.49	7.85	7.98	9.45	11.10	11.35	11.88	12.47	13.37	13.63	13.84	14.09	14.61	15.47	16.18

Elution Order Shifts from C18

2. EPA 533 Acetonitrile Altered

Acetonitrile is a highly polar molecule and is often added to the mobile phase to alter how analytes interact with the solid phase. The previously discussed experimental sequence was repeated using a 80:20 methanol:acetonitrile mobile phase with the same PFAS compounds and HPLC columns. The C18 columns all still had a consistent elution order as compared to 533 Similar but displayed earlier retention times (**Figure 10**). However, compared to 533 Similar, the conditions of 533 Acetonitrile Altered resulted in a much larger number of retention time elution order shifts.

The addition of acetonitrile to the mobile phase increased the number of overlapping peaks for the Gemini® C18, Luna Omega Polar C18, and Kinetex Polar C18 columns, but it conversely decreased the number of overlapping peaks for the Luna Omega PS-C18 and Kinetex C18 columns. In the Kinetex PAH column, the methanol:acetonitrile mobile phase also significantly changed the elution order as compared to methanol-only mobile phase, but with some differences in the effected compounds (**Figure 10**). However, with Kinetex PAH there were also more overlapping peaks, resulting in compromised separation for early eluters.

Figure 10.
C18 and PAH Summary

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini	C18	2.42	2.55	2.97	3.88	4.45	5.55	5.47	7.25	8.89	9.08	9.10	9.82	10.24	10.43	11.85	12.96	13.15	14.40
Luna Omega	Polar C18	1.83	1.97	2.25	2.92	3.43	4.31	4.51	5.75	7.14	7.35	7.35	7.98	8.44	8.45	9.69	10.84	10.88	12.01
Luna omega	Ps C18	1.87	1.99	2.34	3.12	3.66	4.69	4.87	6.29	7.74	7.90	8.02	8.71	8.91	9.10	10.38	11.46	11.71	12.91
Kinetex	C18	1.42	1.47	1.69	2.30	2.79	4.12	4.31	6.40	8.28	8.45	8.54	9.28	9.62	9.90	11.36	12.51	12.72	14.00
Kinetex	Polar C18	1.32	1.39	1.58	1.99	2.38	3.13	3.29	4.45	5.90	6.09	6.16	6.80	7.21	7.29	8.53	9.62	9.65	10.76

Elution Order Shifts from "EPA 533 Similar"

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	PFUdA	9-Cl-PF3ONS	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	XB-C18	1.37	1.42	1.63	2.23	2.69	3.68	3.76	5.27	6.80	6.87	7.26	7.94	7.96	8.20	9.52	10.57	10.82	12.08

Elution Order Shifts from "EPA 533 Similar"

		L-PFBS	PFHxA	HFPO-DA	PFHpA	NaDONA	L-PFHxS	PFNA	PFNA	L-PFOS	PFDA	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex®	PAH	0.76	0.76	0.83	0.95	1.05	1.33	1.34	2.19	3.44	3.49	3.78	4.30	4.31	4.78	6.10	6.90	7.47	8.82

Elution Order Shifts from "EPA 533 Similar"

The methanol:acetonitrile mobile phase also resulted in more overlapping pairs and changes in elution order with the C8 columns (**Figure 11**). The elution order was consistent between the two C8 columns using this method, but there were many shifts in elution order compared to the methanol-only eluent. Finally, the methanol:acetonitrile method and the methanol-only method showed similar but not identical elution orders in the Kinetex Biphenyl and Phenyl-Hexyl Columns. The elution order with Kinetex F5 was less comparable with Kinetex Biphenyl and Phenyl-Hexyl columns with the acetonitrile altered eluent than previously seen with the methanol-only eluent. However, with the acetonitrile altered eluent, Kinetex F5 was more similar in elution order to the C18 columns than to the phenyl stationary phases.

The 533 Acetonitrile Altered method also showed increased overlapping peaks in all phenyl and F5 stationary phases (**Figure 12**), although the shorter run times may have contributed significantly to these increases. All things considered, the methanol:acetonitrile data demonstrate that mobile phase polarity (in conjunction with stationary phase chemistry) has a great deal of influence over the sorption behavior of the different classes of PFAS compounds and could be a powerful tool with which to influence chromatographic behavior.

Figure 11.
C8 Summary

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Luna®	C8	2.05	2.15	2.34	3.22	3.55	4.51	5.07	6.22	7.48	7.53	8.04	8.28	9.05	9.09	10.25	11.33	11.36	12.42
Kinetex	C8	2.14	2.22	2.41	3.30	4.10	5.19	5.34	7.01	8.32	8.35	8.47	9.11	9.50	9.53	11.06	12.17	12.14	13.17

Elution Order Shifts from "EPA 533 Similar"

Figure 12.
Phenyl Stationary Phase Summary

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	N-EtFOSAA	9-Cl-PF3ONS	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Gemini®	C18	2.42	2.55	2.97	3.88	4.45	5.55	5.47	7.25	8.89	9.08	9.10	9.82	10.24	10.43	11.85	12.96	13.15	14.40

Benchmark: C 18 "EPA 533 Acetonitrile Altered"

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	L-PFOS	N-MeFOSAA	9-Cl-PF3ONS	N-EtFOSAA	PFUdA	PFDoA	11Cl-PF3OUdS	PFTrDA	PFTeDA
Kinetex	F5	1.27	1.32	1.45	1.93	2.29	3.12	3.26	4.55	5.92	6.05	6.54	6.84	7.03	7.22	8.44	9.24	9.63	10.76

Elution Order Shifts from C18 Acetonitrile Altered

		PFHxA	L-PFBS	HFPO-DA	PFHpA	NaDONA	PFOA	L-PFHxS	PFNA	PFDA	N-MeFOSAA	L-PFOS	N-EtFOSAA	PFUdA	9-Cl-PF3ONS	PFDoA	PFTrDA	11Cl-PF3OUdS	PFTeDA
Kinetex	Phenyl-Hexyl	1.05	1.14	1.18	1.39	1.60	1.99	2.25	2.96	4.09	4.33	4.47	4.82	5.16	5.46	6.17	7.09	7.45	7.95
Kinetex	Biphenyl	0.85	0.90	0.90	0.99	1.07	1.20	1.32	1.55	2.11	2.32	2.37	2.68	2.81	3.23	3.59	4.31	4.83	5.05

Elution Order Shifts from C18 Acetonitrile Altered

Conclusions

The HPLC methodology in EPA methods 537, 537.1 and 533 are all based upon a C18 stationary phase and a methanol-water mobile phase. In this study we have shown that the use of alternative stationary phases of varying surface chemistry and eluents of varying polarity can significantly alter the sorption-elution characteristics of different classes of PFAS compounds. This orthogonal approach to PFAS HPLC chromatography should serve as a fruitful approach to future method development. As analyte lists increase in size and complexity, a variety of HPLC column chemistries and eluent compositions will be needed to accommodate the wide range of PFAS related compounds that might be encountered such as polar acids, non-polar acids, esters, amides, sulfonamides, and telomere length, all of which can be complicated with branched vs. linear isomers.

The work presented here is merely illustrative and should be considered a starting point for column chemistry and mobile phase considerations for PFAS HPLC methodology. Even though the demonstration sample contained a nice mix of PFAS compounds with varied functional groups, there are certainly many more compounds in the 9000-strong (and growing) PFAS inventory that will challenge LC-MS methodology. National and state PFAS analyte panels are constantly being updated and expanded. There is increasing emphasis on identifying and quantifying PFAS related isomers, unique functional groups and degradation products across a wide range of sample matrices. With regulated detection and quantitation limits being driven lower and lower, sensitivity is a significant issue. The choice of HPLC column chemistry will play a significant role in successfully meeting all these future challenges.

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4. PFAS List of PFAS Substances (Version 2). CompTox Chemicals Dashboard | PFASMASTER Chemicals (epa.gov) Accessed April 7, June 9, 2001.

Ordering Information

PFAS CRM Native Standards. All analytes at the same concentration in acid form for easy calculation and dilution.

Product	Part Number	Qty.	Conc.
EPA 533 mix	AL0-101838	1 mL	2 µg / mL in methanol
EPA 537.1 mix	AL0-101839	1 mL	2 µg / mL in methanol
EPA 533 + 537.1 mix	AL0-101840	1 mL	2 µg / mL in methanol

More PFAS Products for Your PFAS Methods

Description	Part Number
Luna™ Omega Column 3 µm PS C18 50 x 3 mm	00B-4758-Y0
Kinetex™ EVO Column 5 µm C18 100 x 2.1 mm	00D-4633- AN
Strata™ PFAS (WAX/GCB) SPE 200 mg, /50 mg, /6 mL tubes, 30/pk	CS0-9207
Strata SDB-L SPE 500 mg/6 mL tubes, 30/pk	8B-S014-HCH
Verex™ Vial, 9 mm Screw, PP, 1.7 mL, 1000/pk	AR0-39P0-13
Verex Vial, 9 mm Screw, PP, 300 µL, 1000/pk	AR0-39P2-13
Verex Vial, 9 mm Screw, PP, 700 µL, 1000/pk	AR0-39P1-13
Vial Cap Verex™ Cert+ Cap (one-piece), 9 mm, PE w/ Starburst pre-Slit, 2 mL, 1000/pk	AR0-89P6-13-C

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