Column Chemistry Considerations affecting PFAS Selectivity for LC-MS/MS Workflows

Richard Jack [1] Sam_Lodge[1] Zara Jalai[1] | 1Phenomenex, Inc., Torrance, CA 90501, USA

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.

Column Chemistry Considerations

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.

Background

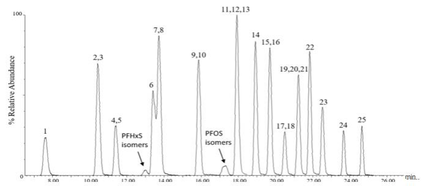


Figure 1. Example chromatogram for reagent water fortified with method 537.1 analytes at 80 ng/L.

Project Scope

A select list of PFAS compounds were chosen 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. Finally, we have chosen to due a further exploration with the complete list of PFAS compounds within Draft EPA 1633 between a C18 and F5 phase

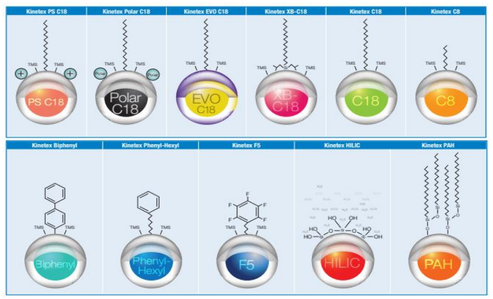
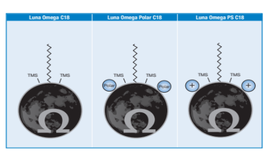


Figure 2. Available column chemistries appropriate for PFAS compound separation. Kinetex are a core shell, Luna Omega are a fully porous phase column



Results

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 ΔRT ≤ 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.

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	5.26	7.37	8.23	9.53	10.02	10.69	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

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

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 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

Results

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	PFDA	9-Cl-PF3ONS	N-MeFOSAA	PFUdA	N-EtFOSAA	PFDoA	11Cl-PF3OUdS	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

Since we began this initial project, Draft EPA Method 1633 was published. We decided to evaluate the most promising phases that showed the most resolution with the expanded PFAS list in EPA 1633. The Figure below shows the PFAS separation between the Luna Omega C18 compared with the Kinetix F5 column. The Luna Omega C18 showed better selectivity for early eluters' while the F5 showed better resolution for later eluting, longer chain, PFAS. This information can be exploited as the list of desired compounds grows and interfering matrices become more problematic.

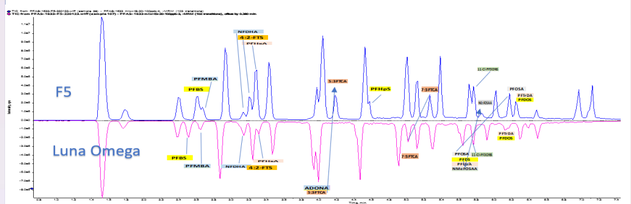


Figure 3. Separation parison between Luna Omega C18 and Kinetix F5 of expanded PFAS panel from EPA 1633.

References

Column Chemistry Considerations for Full Coverage of PFAS Analyte Ranges, TN 1290. 2021
https://phenomenex.blob.core.windows.net/documents/e255f2db-9c9c-4ef6-978d-fb49f5c4a2d5.pdf

Learn more about PFAS analysis, products and solutions at www.phenomenex.com/pfas

Have questions or want more details? We would love to help!
*Visit **www.phenomenex.com/Chat** to get in touch with one of our Technical Specialists*