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TITLE: **AN EXAMINATION OF PFAS ANALYSIS USING LCMS FOR EPA METHODS 537.1 AND EPA 8327 USING HALO® FUSED-CORE® TECHNOLOGY**

MARKET SEGMENT: ENVIRONMENTAL

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ABSTRACT

EPA Method 537.1 (Shoemaker & Tettenhorst, 2018) can be used for the quantitation of 18 PFAS in drinking water, using solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). The method stipulates two columns be used for chromatography, one to be used as a delay column to mitigate PFAS contamination from the HPLC, and the other to be used as the analytical column and perform the separation (Shoemaker & Tettenhorst, 2018). In 2019 the EPA validated method 8327 for non-potable water testing, which includes the analysis of 24 total PFAS compounds in a variety of aquatic matrices with 14 compounds being common across this method and EPA 537.1.

The EPA allows the analytical testing lab flexibility to improve the separation and detection of PFAS, by changing the LC column, mobile phase composition, LC conditions, and MS and MS/MS conditions. We sat down with environmental chemist Lisa Steinberg, Ph.D. to offer her perspectives on PFAS analysis and herein we present those along with the separation of 18 PFAS compounds according to EPA method 537.1 on the HALO® C18 and the HALO® Phenyl-Hexyl column. In addition, we demonstrate the utility of HALO® Fused-Core® technology for PFAS analysis by simultaneously separating the compounds found in EPA method 8327, including their internal standards. Methods optimized for both high resolution and high speed are presented.

INTRODUCTION

Per and poly fluorinated alkyl substances, collectively PFAS, are a toxic group of chemicals that have found wide ranging application across numerous industries due to their chemical structure, which includes both a hydrophobic fluorocarbon section and a hydrophilic carboxylate section. PFAS are very stable molecules due to the hydrophobic nature of the fluorocarbon section, however they are also highly reactive with polar molecules, due to the hydrophilic nature of the carboxylate section. Environmental chemist Lisa Steinberg, Ph.D. explains that these PFAS compounds are characterized as a long alkyl chain which is fully fluorinated and contains a polar head group. "Due to the polar head group, these chemicals are highly mobile in water so PFAS will quickly leach from soil that is contaminated from rainfall and also groundwater flowing through it. PFAS then ends up in drinking water systems, estuaries and surface water. Their long alkyl chain makes them amenable to accumulating in body fat and tissues."

PFAS exposure in humans has been linked to a variety of diseases, including cancer, ulcerative colitis, thyroid disease, and hypercholesterolemia. PFAS compounds have been used as surfactants, and also in the manufacturing of carpets, upholstery, clothing, food packaging, various types of sealants, firefighting foam, and cookware.

KEY WORDS:

PFAS, EPA 537.1, EPA 8327, MS/MS, HALO® C18, HALO® Phenyl-Hexyl, superficially porous particles, Fused-Core®

According to Steinberg there are over 4,000 possibly upwards of 6,000 PFAS chemicals that have been made and many of which we don't have toxicology data for. This means we don't yet fully realize what the biological effects are on living systems.

The heavy usage of these chemicals throughout the years has led to wide ranging environmental PFAS contamination, as these molecules will readily dissolve in water and are extremely stable. In 2009 the United States Environmental Protection Agency (EPA), introduced EPA method 537 for the detection and quantification of 14 PFAS compounds in drinking water. This method was revised in 2018 to include 4 additional PFAS compounds and labeled EPA 537.1. Recently, the EPA has validated a method for routine analysis and detection of PFAS compounds in non-potable water, method 8327. These two methods contain 28 total compounds between them, and were able to be easily separated by Fused-Core® technology. Methods for both resolution and speed are presented and Steinberg notes "whether you are a state or large private lab, speed is important for routine analysis to save in running costs, solvent usage and waste produced. Speed is a cost and a time saver." Comprehensive methods are necessary because "they are vital for investigative work – is this a new PFAS? Where is it coming from? We keep seeing papers upping the identification numbers and that's where it's going."

EXPERIMENTAL DATA:

Maximum resolution method

A Shimadzu LCMS-8050 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA).

A HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm (Advanced Materials Technology, Wilmington, DE) was used as the delay column, and a HALO 90 Å C18, 2.0 µm, 2.1 x 100 mm (Advanced Materials Technology, Wilmington, DE) was used as the analytical column. The delay column was positioned between the mixer and the autosampler, and a PFAS kit (Shimadzu) was used on the UHPLC. EPA 8327 and 537.1 standards were provided by Shimadzu and obtained from Wellington Laboratories, Inc. (Guelph Ontario, Canada).

Sample prep

Standards were diluted for analysis to a concentration of 0.20 ng/mL 95:5 MEOH:Water.

Instrument Parameters and Gradient

Columns:
HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm (Delay column)
HALO 90 Å C18, 2.0 µm, 2.1 x 100 mm (Analytical column)
Flow Rate: 0.3 mL/min
Initial Pressure: 425 bar
Temperature: 30 ºC
Injection Volume: 10 µL
Sample Solvent: (95/5) MEOH/ H_2O
Mobile Phase A: (95/5) H_2O/ACN/0.1% acetic acid
Mobile Phase B: (95/5) ACN/H_2O 10 mM ammonium formate/0.1% acetic acid

Table 1. Gradient conditions for maximum resolution

Time (min)	%B
0.0	0
6	50
13	85
14	100
17	100
18	0
21.0	stop

Table 2. MS source conditions

MS source conditions	Setting
Spray Voltage	-2.0 kV
Nebulizing gas	2 L/min
Drying gas	15 L/min
DL temp	250 ˚C
Heat Block	400 ˚C

RESULTS:

Maximum resolution method

Column Selection

Due to the freedom given by the EPA for further development of the detection and separation of PFAS compounds, we investigated columns containing superficially porous particles (SPP) as the stationary phase, to act as both the delay column and analytical column. Two different experimental methods were developed: one for maximum resolution and one for maximum speed. The major advantages of SPP, including higher flow rates and lower back pressure, have been well documented, and offer an ideal tool for the chromatographer to employ for high throughput and high-resolution separations (Kirkland et al.).

High Resolution Method

Initial high-resolution experiments included two C18 columns used as both the analytical and the delay column, and ACN as the primary organic component of the mobile phase. Although both columns were composed of the same phase, the separation (Figure 1.) was readily achieved for the 18 components of EPA 537.1 (Table 3) in under 12 minutes.

Figure 1. TIC of 18 PFAS species in EPA 537.1 on a HALO® C18 column

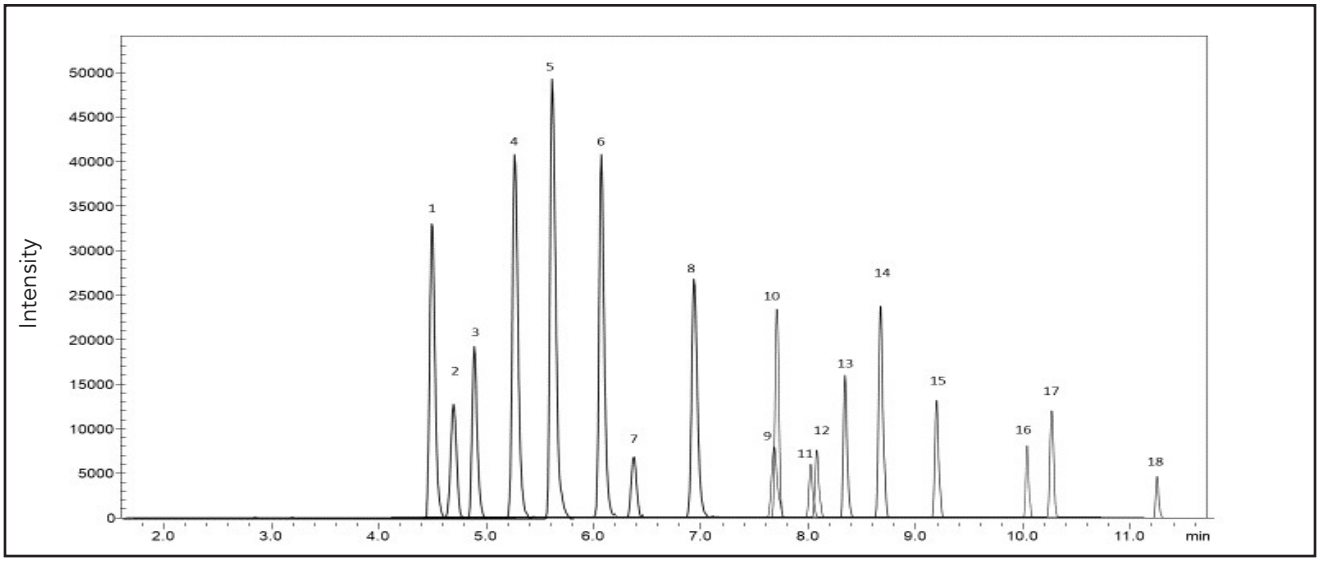

Table 3. Peak identities of 18 PFAS compounds found in EPA 537.1

Peak number	PFAS Species	Observed Transition	Ret. Time (min)
1	PFHxA	313.0000>269.0000	4.502
2	PFBS	299.0000>80.0000	4.618
3	HFPO-DA	285.0000>169.0000	4.812
4	PFHpA	363.0000>319.0000	5.341
5	ADONA	377.0000>250.9000	5.637
6	PFOA	413.0000>369.0000	6.145
7	PFHxS	399.0000>80.0000	6.451
8	PFNA	463.0000>419.0000	6.925
9	N-MeFOSAA	570.0000>419.0000	7.681
10	PFDA	513.0000>469.0000	7.696
11	N-EtFOSAA	584.0000>419.0000	8.022
12	PFOS	499.0000>80.0000	8.102
13	PFUnA	563.0000>519.0000	8.498
14	9Cl-PF3ONS	530.9000>351.0000	8.739
15	PFDoA	613.0000>569.0000	9.333
16	PFTriA	663.0000>619.0000	10.179
17	11Cl-PF3OUdS	630.7000>451.0000	10.475
18	PFTreA	713.0000>669.0000	11.053

We investigated the column's applicability to separate PFAS targets of multiple EPA methods. Figure 2 shows the separation of 28 total PFAS species (Table 4.), including internal standards, and both branched and linear isomers, which are present in a mixture of standards of EPA 537.1 and EPA 8327. The separation was done in under 12 minutes, demonstrating the utility of the column for PFAS analysis by separating multiple PFAS targets of multiple EPA methods.

Figure 2. TIC of PFAS species, including internal standards in EPA 537.1 and EPA 8327 on a HALO® C18 column

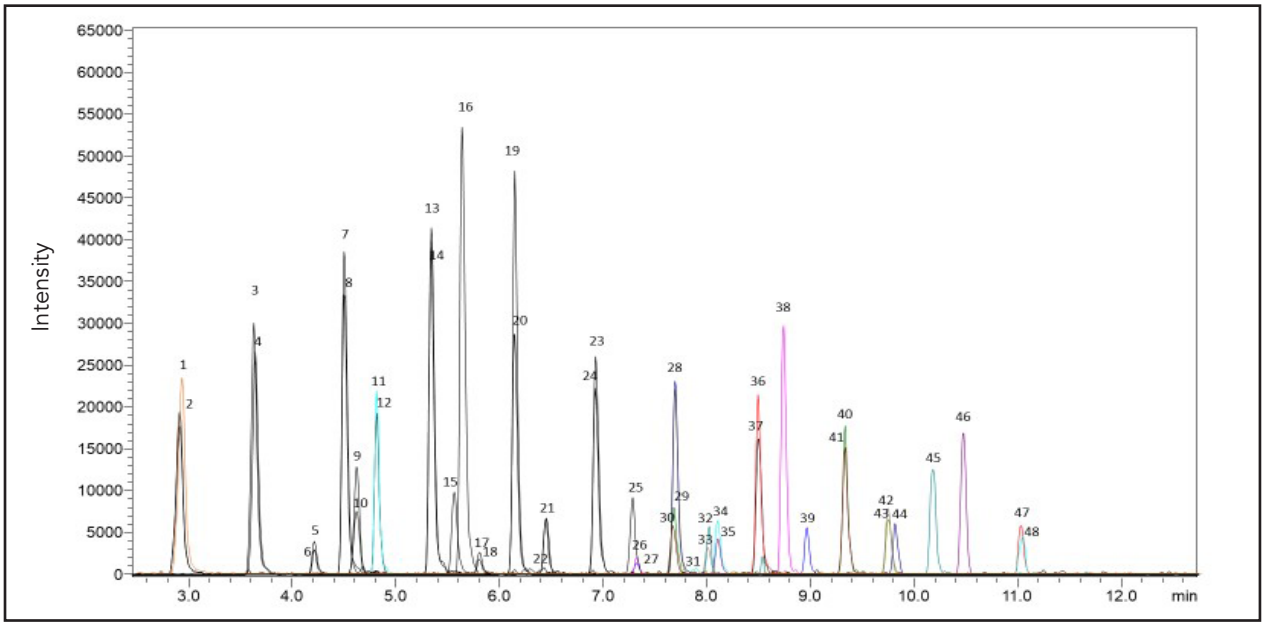

Table 4. Peak identities of PFAS compounds found in EPA 537.1 and EPA 8327

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
1	PFBA	213.0000>169.0000	2.911
2	MPFBA	217.0000>172.0000	2.911
3	M5PFPeA	268.0000>223.0000	3.641
4	PFPeA	263.0000>219.0000	3.646
5	4-2 FTS	327.0000>307.0000	4.209
6	M2-4-2 FTS	329.0000>309.0000	4.213
7	M5PFHxA	318.0000>273.0000	4.499
8	PFHxA	313.0000>269.0000	4.512
9	PFBS	299.0000>80.0000	4.618
10	M3PFBS	302.0000>80.0000	4.618
11	HFPO-DA	285.0000>169.0000	4.812
12	13C-HFPO-DA SURR	287.0000>169.2000	4.812
13	M4PFHpA	367.0000>322.0000	5.337
14	PFHpA	363.0000>319.0000	5.343
15	PFPeS	349.0000>80.0000	5.564
16	ADONA	377.0000>250.9000	5.637
17	6-2 FTS	427.0000>407.0000	5.801
18	M2-6-2 FTS	429.0000>409.0000	5.804
19	M8PFOA	421.0000>376.0000	6.143
20	PFOA	413.0000>369.0000	6.145
21	M3PFHxS	402.0000>80.0000	6.444
22	PFHxS	399.0000>80.0000	6.451
23	M9PFNA	472.0000>427.0000	6.924
24	PFNA	463.0000>419.0000	6.925

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
25	PFHpS	449.0000>80.0000	7.285
26	M2-8-2 FTS	529.0000>509.0000	7.322
27	8-2 FTS	527.0000>507.0000	7.322
28	d3-NMeFOSAA	573.0000>419.0000	7.671
29	N-MeFOSAA	570.0000>419.0000	7.681
30	M6PFDA	519.0000>474.0000	7.695
31	PFDA	513.0000>469.0000	7.696
32	d5-NEtFOSAA	589.0000>419.0000	8.005
33	N-EtFOSAA	584.0000>419.0000	8.022
34	PFOS	499.0000>80.0000	8.102
35	M8PFOS	507.0000>80.0000	8.107
36	M7PFUnA	570.0000>525.0000	8.494
37	PFUnA	563.0000>519.0000	8.498
38	9Cl-PF3ONS	530.9000>351.0000	8.739
39	PFNS	549.0000>80.0000	8.968
40	PFDoA	613.0000>569.0000	9.333
41	M2PFDoA	615.0000>570.0000	9.334
42	FOSA	498.0000>78.0000	9.749
43	M8FOSA	506.0000>78.0000	9.754
44	PFDS	599.0000>80.0000	9.817
45	PFTriA	663.0000>619.0000	10.179
46	11Cl-PF3OUdS	630.7000>451.0000	10.475
47	M2PFTreA	715.0000>670.0000	11.033
48	PFTreA	713.0000>669.0000	11.053

Maximum Speed

As technological advancements continue to progress, mass spectrometers will continue to be improved in regards to the level of sensitivity, mass resolution, and scanning speed. This will undoubtedly change the requirements of EPA 537.1 and EPA 8327, and column performance must be able to handle these advancements. With this in mind, we developed a method for separation at maximum speed to test the suitability of the column for use in these advanced conditions. The higher scanning speed of the MS instruments will lead to faster analysis time and higher flow rates, but a deleterious effect however, is often times an increase in the speed of analysis will lead to a decrease in the resolution therefore causing coelutions. In the case of EPA 537.1 the method stipulates that the PFAS compounds must be sufficiently resolved chromatographically, so the mass spectrometer can dwell on a minimum number of compounds eluting within a retention time window (EPA 537.1).

EXPERIMENTAL: Maximum speed method

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). EPA 537.1 standards were purchased from Wellington Laboratories, Inc. (Guelph Ontario, Canada) and were diluted to the desired concentration in 95:5 methanol:water. Methanol (HPLC grade), water (HPLC grade) and ammonium formate were purchased from Millipore Sigma (Burlington, MA). A HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm (Advanced Materials Technology, Wilmington, DE) was used as the delay column, and a HALO 90 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm (Advanced Materials Technology, Wilmington, DE) was used as the analytical column. The delay column was positioned between the mixer and the autosampler, and a PFAS kit (Shimadzu) was used on the HPLC.

Sample Preparation

Standards were diluted for analysis to a concentration of 0.20 ng/mL 95:5 MEOH:Water.

Instrument Parameters and Gradient

Columns:
HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm (Delay column)
HALO 90 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm (Analytical column)
Flow Rate: 0.4 mL/min
Initial Pressure: 350 bar
Temperature: 30 ºC
Injection Volume: 10 µL
Sample Solvent: (95/5) MEOH/ H_2O
Mobile Phase A: H_2O 10mM ammonium formate/0.1% acetic acid
Mobile Phase B: MEOH/0.1% acetic acid

Table 5. Gradient conditions for maximum resolution

Time (min)	%B
0.00	30
3.00	90
6.00	90
6.01	30
9.00	stop

MS source conditions	Setting
Spray Voltage	-2.0 kV
Nebulizing gas	2 L/min
Drying gas	15 L/min
DL temp	250 ˚C
Heat Block	400 ˚C

Table 6. MS conditions for maximum speed method

RESULTS: Maximum speed method

Column Selection

As speed was the primary goal of this analysis, the analytical column was changed to a Phenyl-Hexyl and the delay column remained a C18. The reason for the change was that the retentive nature of identical phases, in this case C18, would limit the effectiveness of the separation. By changing to Phenyl-Hexyl, and also changing the mobile phase conditions, the delay column, as C18 phase, was more retentive than the analytical column Phenyl Hexyl phase, mitigating any interference from instrument contamination. This gives the increased speed needed and enables the mass spectrometer to have sufficient dwell times for all the components. Figure 3 shows the 18 PFAS compounds (Table 7) of EPA method 537.1 separated in under 3.5 minutes with no coelutions of isobaric compounds.

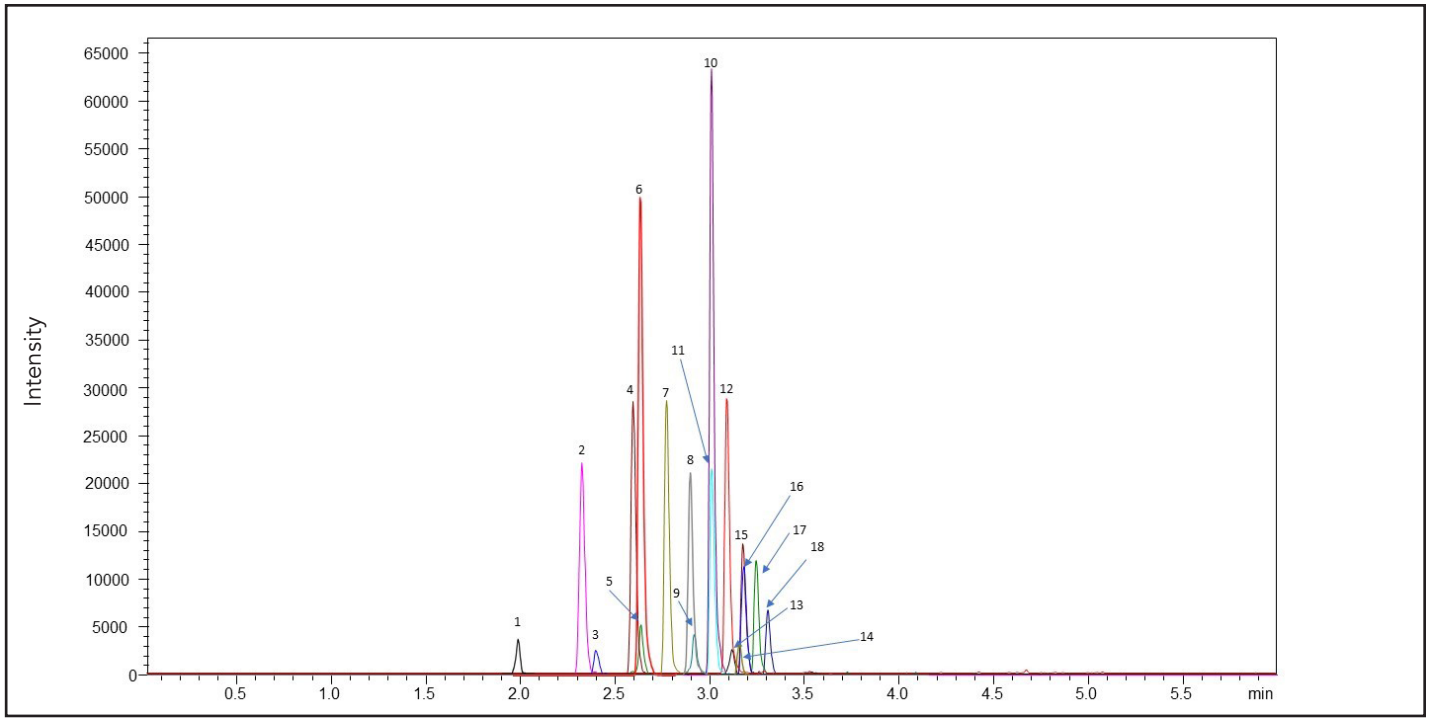

Figure 3. Maximum speed separation of 18 PFAS species according to EPA 537.1 on a HALO® Phenyl-Hexyl column

Table 7. PFAS species separated at maximum speed according to EPA method 537.1

Peak number	PFAS Species	Observed Transition	Ret. Time (min)
1	PFBS	299.0000>80.0000	2.008
2	PFHxA	313.0000>269.0000	2.325
3	HFPO-DA	285.0000>169.0000	2.339
4	PFHpA	363.0000>319.0000	2.595
5	PFHxS	399.0000>80.0000	2.630
6	ADONA	377.0000>250.9000	2.631
7	PFOA	413.0000>369.0000	2.771
8	PFNA	463.0000>419.0000	2.901
9	PFOS	499.0000>80.0000	2.917
10	9Cl-PF3ONS	530.9000>351.0000	3.009
11	PFDA	513.0000>469.0000	3.011
12	PFUnA	563.0000>519.0000	3.099
13	N-MeFOSAA	570.0000>419.0000	3.106
14	N-EtFOSAA	584.0000>419.0000	3.166
15	11Cl-PF3OUdS	630.7000>451.0000	3.176
16	PFDoA	613.0000>569.0000	3.177
17	PFTriA	663.0000>619.0000	3.244
18	PFTreA	713.0000>669.0000	3.311

CONCLUSION:

Due to the high levels of environmental contamination, PFAS analysis of water, both potable and non-potable, is of critical importance. As PFAS analysis continues to evolve and technology improves, the ability to separate multiple PFAS species quickly and efficiently will become paramount. The HALO® C18 and Phenyl-Hexyl have been shown to be highly efficient at separating PFAS species, and equally adept as both delay and analytical columns. The ability of the HALO® C18 to separate the 48 unique PFAS species found in EPA 537.1 and EPA 8327, as well as the HALO® Phenyl-Hexyl separating the PFAS species of EPA 537.1 in under 3.5 minutes, demonstrate that superficially porous particle (Fused-Core®) technology benefits PFAS analysis.

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3. *Kirkland, J.J., Schuster, S.A., Johnson, W.L., Boyes, B.E., (2013) Fused-core particle technology in high-performance liquid chromatography: An overview. Journal of Pharmaceutical Analysis. Volume 3, Issue 5, (303-312).*