HALO®

TECHNICAL REPORT: AMT-TR092003

TITLE: **RAPID AND HIGH RESOLUTION FUSED-CORE® SOLUTIONS FOR PFAS ANALYSIS**

MARKET SEGMENT: ENVIRONMENTAL

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ABSTRACT

LC-MS/MS methods for the analysis of short-chain and long-chain PFAS are well established. EPA method 537 was introduced in 2009 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. In 2019, EPA method 533 was introduced and focused on "short chain" PFAS, those PFAS with carbon chain lengths between 4 and 12. Method 533 complements EPA Method 537.1 and can be used to test for 11 additional PFAS species, in addition, also 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. All of the EPA methods stipulate 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). 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. Here we introduce the new HALO® PFAS solution consisting of delay and analytical columns with the separation of all PFAS compounds according to EPA methods 537.1, 533 and 8327. In addition, PFAS were detected in well water and a high-speed method was developed.

INTRODUCTION

Per- and polyfluoroalkyl substances (PFAS) are chemicals that have been used in a variety of applications including in stain and water-resistant fabrics and carpeting, cleaning products, paints, and fire-fighting foams. Their chemical structure, which includes both a hydrophobic fluorocarbon section and a hydrophilic carboxylate section, enables PFAS to have such a wide range of applications, but also contributes to their deleterious environmental effects. 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, allowing them to dissolve readily in water and remain largely intact in the environment, resulting in increasing levels of environmental contamination. Accumulation of certain PFAS has also been shown through blood tests to occur in humans and animals. While the science surrounding potential health effects of this bioaccumulation of certain PFAS is developing, evidence suggests it may cause serious health conditions. PFAS analysis, however, is an evolving area of study, and with nearly 5,000 different types of PFAS, undoubtedly more compounds and methods will be developed, refined, and technology improved to enable more accurate analysis of PFAS. As the science on PFAS advances, Advanced Materials Technology introduces our new applicated PFAS delay and analytical columns, to further mitigate the effects of PFAS contamination from instrumentation, and provide a more accurate analysis.

KEY WORDS:

PFAS, EPA 537.1, EPA 533, EPA 8327, MS/MS, HALO®, superficially porous particles, Fused-Core®

EXPERIMENTAL: EPA 537.1, EPA 533, and EPA 8327 using PFAS Standards

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). PFAS standards were purchased from Wellington Laboratories, Inc. (Guelph Ontario, Canada) and were diluted to the desired concentration in 96:4 methanol:water.

Methanol (HPLC grade), water (HPLC grade) and ammonium acetate were purchased from Millipore Sigma (Burlington, MA). A HALO® PFAS Delay, 2.7 µm, 3.0 x 50 mm (Advanced Materials Technology, Wilmington, DE) was used as the delay column, and a HALO® PFAS column, 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.

INSTRUMENT PARAMETERS AND GRADIENT

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 2.7 µm, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 10 mM Ammonium Acetate
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	33
18	98
18.1	100
21.0	100
21.1	33
26.0	End

Flow Rate: 0.4 mL/min
Pressure: 485 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)
Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

MS Source Conditions:
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 ˚C
Heat Block: 400 ˚C

EXPERIMENTAL: Well Water samples

An Agilent 6400 Series Triple Quadrupole (Santa Clara, USA), was coupled to an Agilent 1200 series HPLC system. STRIDE Center for PFAS Solutions (Delaware, USA) prepared and supplied well water samples following the EPA methods. A HALO® PFAS Delay, 2.7 µm, 3.0 x 50 mm (Advanced Materials Technology, Wilmington, DE) was used as the delay column, and a HALO® PFAS column, 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.

TEST CONDITIONS

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, 2.7 µm, 3.0 x 50 mm
Part Number: 92113-415
Mobile Phase A: 20 mM Ammonium Acetate
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	20
15	90
20	90

Flow Rate: 0.4 mL/min
Pressure: 505 bar
Temperature: 44 °C
Detection: -ESI MS/MS
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)
MS System: Agilent 6400 series
LC System: Agilent 1200 series

MS Source Conditions:
Gas Temp: 130 °C
Nebulizer: 25 psi
Gas Flow: 11 L/min
Sheath Gas Heater: 250 °C
Capillary: 3500 V

EXPERIMENTAL: Rapid analysis

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). PFAS standards were purchased from Wellington Laboratories, Inc. (Guelph Ontario, Canada) and were diluted to the desired concentration in 96:4 methanol:water.

Methanol (HPLC grade), water (HPLC grade) and ammonium acetate were purchased from Millipore Sigma (Burlington, MA). A HALO® PFAS Delay, 3.0 x 50 mm (Advanced Materials Technology, Wilmington, DE) was used as the delay column, and a HALO® PFAS column, 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.

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-613
Delay Column: HALO® PFAS Delay, , 2.7 µm, 3.0 x 50 mm
Part Number: 92114-415
Mobile Phase A: 10 mM Ammonium Acetate
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	33
3.0	98
3.1	100
5.1	100
5.2	33
6.5	End

Flow Rate: 0.4 mL/min
Pressure: 479
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)
Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040

MS Source Conditions:

Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 400 °C

RESULTS: EPA 537.1, EPA 533 and EPA 8327

In order to evaluate the performance of the HALO® PFAS columns, we used the target compounds specified in the three different EPA methods. The separation was readily achieved for the 18 components of EPA 537.1 (Figure 1), the 25 components of EPA 533 (Figure 2), and the 24 components of EPA 8327 (Figure 3), with a high degree of resolution.

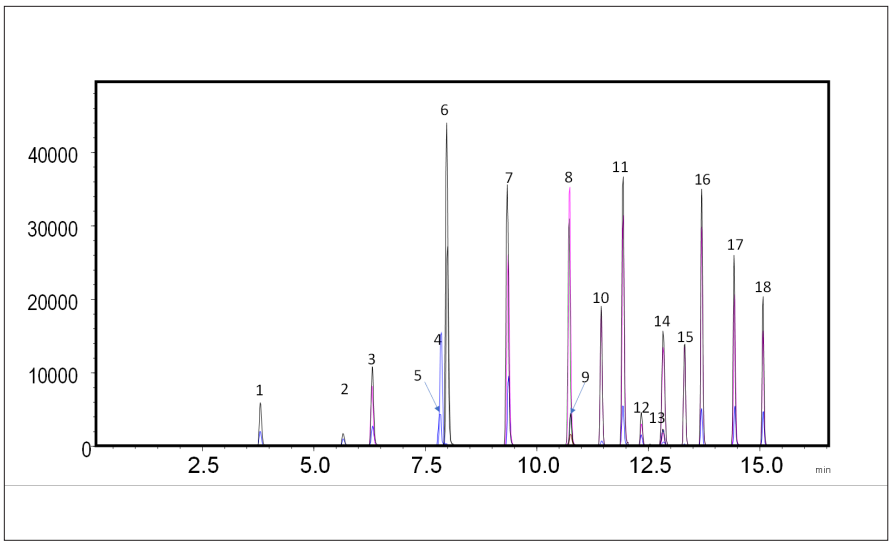


Figure 1. 18 target PFAS compounds specified by EPA 537.1 separated on the HALO® PFAS Delay and HALO® PFAS columns.

Peak #	PFAS Species	Observed Transition	Ret.Time (min)
1	PFBS	299.0000>80.0000	3.789
2	PFHxA	313.0000>269.0000	5.639
3	HFPO-DA	285.0000>169.0000	6.307
4	PFHpA	363.0000>319.0000	7.723
5	PFHxS	399.0000>80.0000	7.936
6	ADONA	377.0000>250.9000	7.978
7	PFOA	413.0000>369.0000	9.368
8	PFNA	463.0000>419.0000	10.715
9	PFOS	499.0000>80.0000	10.762
10	9Cl-PF3ONS	530.9000>351.0000	11.439
11	PFDA	513.0000>469.0000	11.857
12	N-MeFOSAA	570.0000>419.0000	12.336
13	PFUnA	563.0000>519.0000	12.822
14	N-EtFOSAA	584.0000>419.0000	12.827
15	11Cl-PF3OUdS	630.7000>451.0000	13.311
16	PFDoA	613.0000>569.0000	13.690
17	PFTrDA	663.0000>619.0000	14.435
18	PFTeDA	713.0000>669.0000	15.083

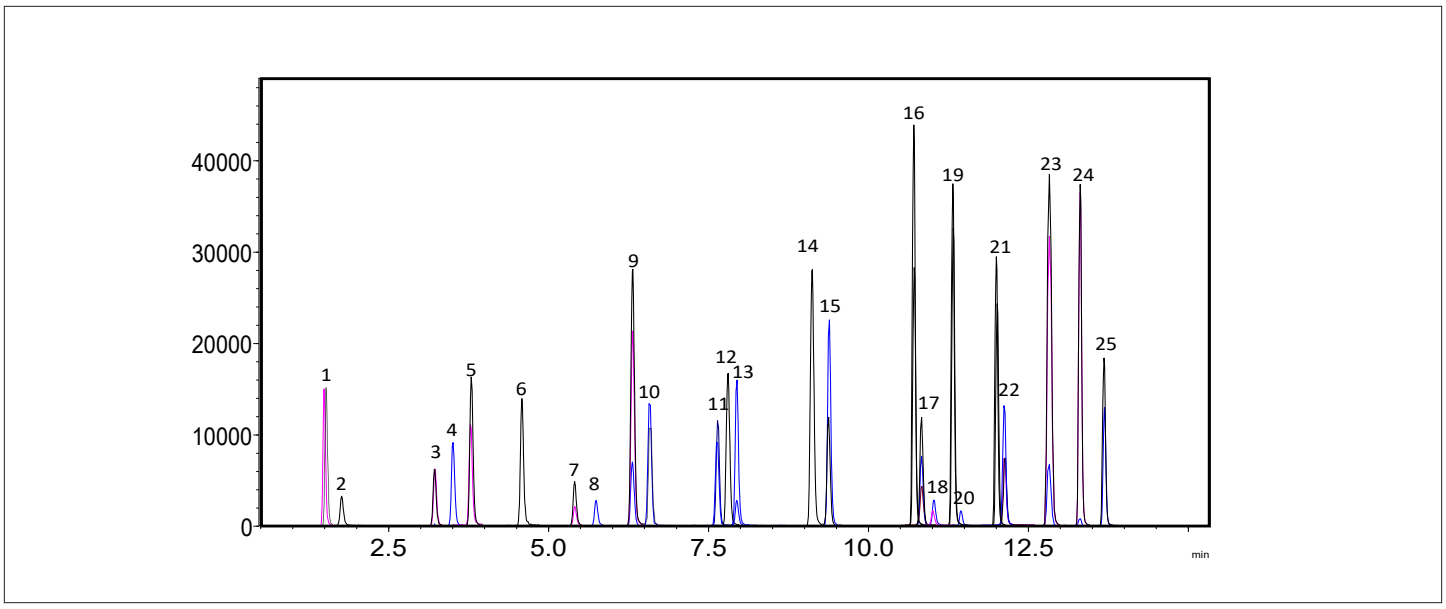


Figure 2. 25 target PFAS compounds specified by EPA 533 separated on the HALO® PFAS Delay and HALO® PFAS columns.

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
1	PFBA	213.0000>169.000	1.358
2	4:2FTS	229.0000>85.0000	1.890
3	PFPeA	263.0000>219.000	3.219
4	PFBS	299.0000>80.0000	3.810
5	PFHpS	279.0000>85.0000	3.967
6	PFPeS	315.0000>135.000	4.791
7	PFMPA	327.0000>307.000	5.431
8	PFHxA	313.0000>269.000	5.684
9	PFEESA	349.0000>80.0000	6.099
10	HFPO-DA	285.0000>169.000	6.335
11	PFHpA	363.0000>319.000	7.763
12	PFHxS	399.0000>80.0000	7.985
13	ADONA	377.0000>250.900	8.012

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
14	PFOA	413.0000>369.000	9.398
15	PFMBA	449.0000>80.0000	9.512
16	PFNA	463.0000>419.000	10.751
17	PFOS	499.0000>80.0000	10.793
18	9Cl-PF3ONS	530.9000>351.000	11.459
19	PFDA	513.0000>469.000	11.885
20	8:2FTS	549.0000>80.0000	11.897
21	6:2FTS	498.0000>78.0000	12.680
22	NFDHA	599.0000>80.0000	12.847
23	PFUnA	563.0000>519.000	12.862
24	11Cl-PF3OUdS	630.7000>451.000	13.329
25	PFDoA	613.0000>569.000	13.708

Table 2. Peak identities of 25 PFAS compounds found in EPA 533

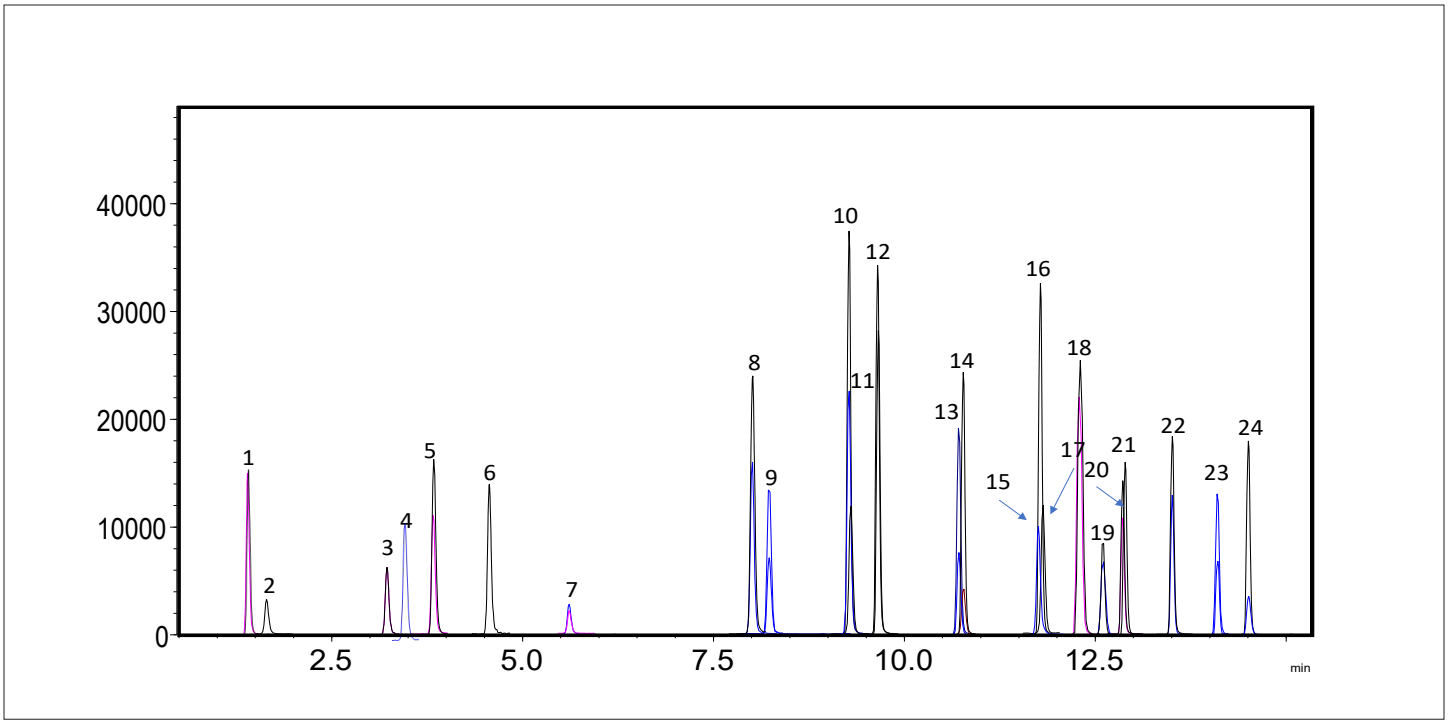


Figure 3. 24 target PFAS compounds specified by EPA 8327 separated on the HALO® PFAS Delay and HALO® PFAS columns.

Table 3. Peak identities of 24 PFAS compounds found in EPA 8327

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
1	PFBA	213.0000>169.0000	1.358
2	4:2FTS	229.0000>85.0000	1.890
3	PFPeA	263.0000>219.0000	3.219
4	PFBS	299.0000>80.0000	3.810
5	PFHpS	279.0000>85.0000	3.967
6	PFPeS	315.0000>135.0000	4.791
7	PFHxA	313.0000>269.0000	5.684
8	PFHpA	363.0000>319.0000	7.763
9	PFHxS	399.0000>80.0000	7.985
10	FOSA	427.0000>407.0000	9.304
11	PFOA	413.0000>369.0000	9.398
12	PFDS	295.0000>201.0000	9.695
13	PFNA	463.0000>419.0000	10.751

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
14	PFOS	499.0000>80.0000	10.793
15	PFNS	527.0000>507.0000	11.843
16	PFDA	513.0000>469.0000	11.885
17	8:2FTS	549.0000>80.0000	11.897
18	N-MeFOSAA	570.0000>419.0000	12.366
19	6:2FTS	498.0000>78.0000	12.680
20	PFUnA	563.0000>519.0000	12.862
21	N-EtFOSAA	584.0000>419.0000	12.865
22	PFDoA	613.0000>569.0000	13.708
23	PFTrDA	663.0000>619.0000	14.446
24	PFTeDA	713.0000>669.0000	15.103

Delay Column Effectiveness Test

Since PFAS contamination is so prevalent in tubing and other liquid contact points in the HPLC, they can leach into the mobile phase and interfere with sample analysis. This is problematic when trying to reach the low, single digit ppt levels required for drinking water, as PFAS contamination from HPLC components can prevent accurate identification and quantitation of PFAS in samples. In order to prevent, this a delay column must adequately inhibit, or delay, these contaminants from interfering with the analysis.

Figure 4. Delay column effectiveness showing the delay of PFOA contamination by 0.7 minutes

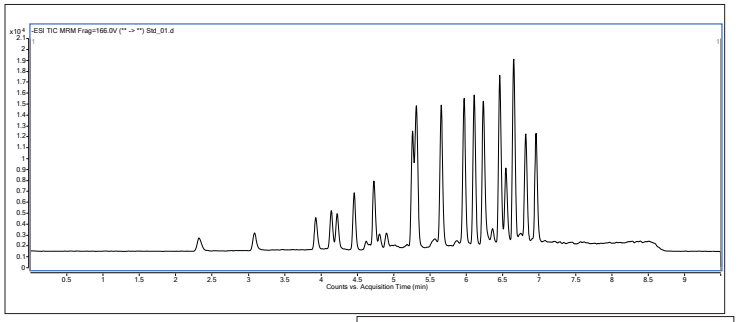
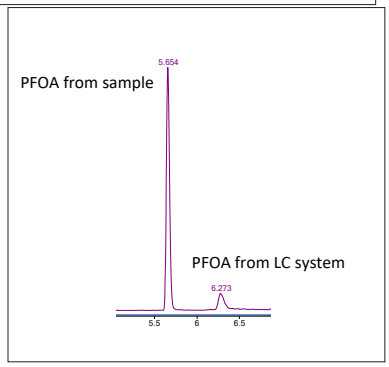


Figure 4 shows the effectiveness of the HALO® PFAS delay column by impeding the instrument PFOA contamination from the sample by 0.7 minutes.



RESULTS: Well Water Samples

The heavy usage of PFAS throughout the years has led to wide ranging environmental contamination, as these molecules will readily dissolve in water and are extremely stable. In the state of Delaware, high levels of PFAS contamination in well water is a serious issue as a large portion of the drinking water is fed by wells. Through a collaboration with Dr. Charles Powley of the Center for PFAS Solutions, part of the Science, Technology & Research Institute of Delaware (STRIDE), spiked well water samples were provided and access to their lab was granted so the columns could be run in an environmental lab on the cutting edge of PFAS analysis.

In addition to the identification of 29 PFAS compounds found in well water, Figure 5 shows a clear separation of the branched and linear isomers of PFAS species PFHxS. Resolution of the branched and linear isomers is important because the presence and ratios of the isomers can be used to aid in identifying the source of the PFAS. Additionally, since the branched isomer are more polar, they are normally found in water samples while linear isomers are found in soil and sediment samples.

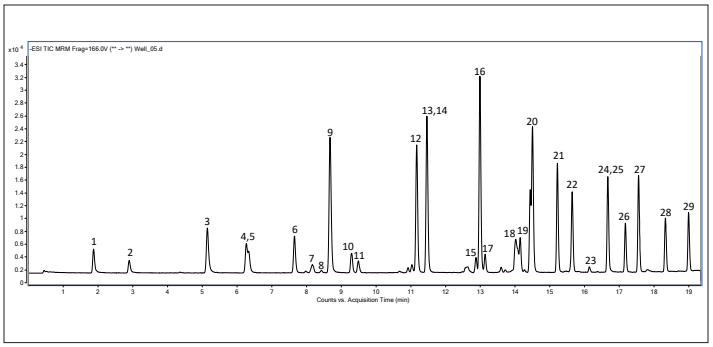


Figure 5. EIC of a branched and linear isomer resolution of PFHxS.

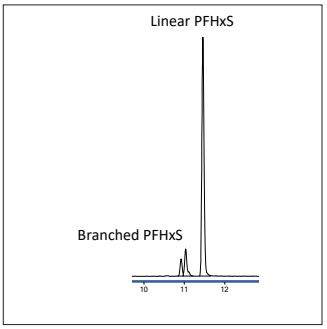


Table 4. Peak identities of 29 PFAS compounds found in spiked well water

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
1	PFBA	213.0 > 169.0	1.88
2	PFMPA	229.0 > 85.0	2.90
3	PFPeA	263.0 > 219.0	5.15
4	PFBS	299.0 > 80.0	6.27
5	PFMBA	279.0 > 85.0	6.34
6	PFEESA	315.0 > 135.0	7.66
7	NFDHA	295.0 > 201.0	8.18
8	4:2FTS	327.0 > 307.0	8.43
9	PFHxA	313.0 > 269.0	8.67
10	PFPeS	349.0 > 80.0	9.29
11	HFPO-DA	285.0 > 169.0	9.49
12	PFHpA	363.0 > 319.0	11.17
13	PFHxS	399.0 > 80.0	11.46
14	ADONA	377.0 > 251.0	11.47
15	6:2FTS	427.0 > 407.0	12.87
16	PFOA	413.0 > 369.0	12.99
17	PFHpS	449.0 > 80.0	13.14
18	PFNA	463.0 > 419.0	14.43
19	PFOS	499.0 > 80.0	14.50
20	9Cl-PF3ONS	531.0 > 351.0	15.22
21	8:2FTS	527.0 > 507.0	15.59
22	PFDA	513.0 > 469.0	15.64
23	NMeFOSAA	570.0 > 419.0	16.13
24	NEtFOSAA	584.0 > 419.0	16.66
25	PFUnA	563.0 > 519.0	16.67
26	11Cl-PF3OUdS	631.0 > 451.0	17.17
27	PFDoA	613.0 > 569.0	17.55
28	PFTrA	663.0 > 619.0	18.32
29	PFTeDA	713.0 > 669.0	18.99

RESULTS: Rapid Analysis

As technological advancements continue to progress, mass spectrometers will continue to be improved in regards to their level of sensitivity, mass resolution, and scanning speed. This will undoubtedly impact future developments in PFAS analysis, 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 columns 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. Figure 6 shows the separation of 33 PFAS species found in EPA 537.1, EPA 533, and EPA 8327, completed in under 5 minutes.

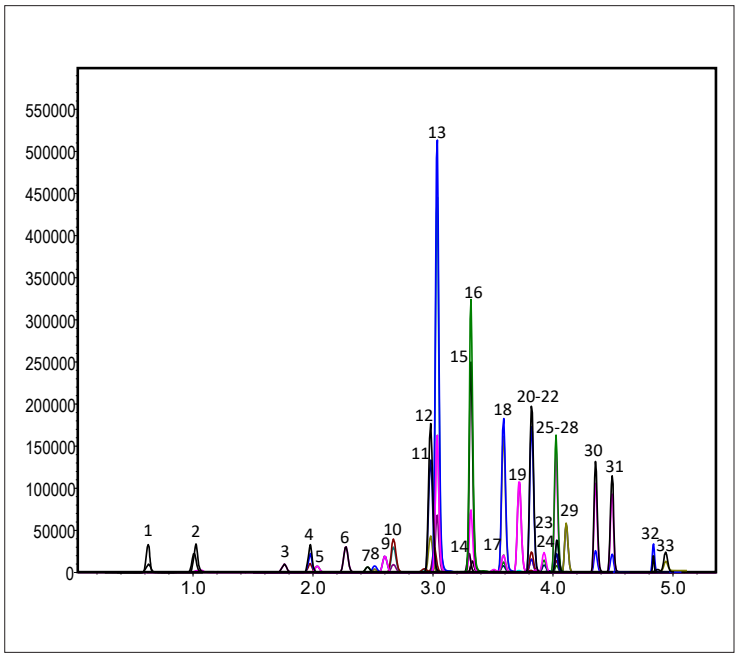


Figure 6. 33 target PFAS compounds specified by EPA methods 537.1, 533, and 8327 separated on the HALO® PFAS Delay and HALO® PFAS columns.

Table 5. Peak identities of 33 PFAS compounds from EPA methods 537.1, 533, and 8327 separated in less than 5 minutes on the HALO® PFAS DELAY and HALO® PFAS columns.

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
1	PFBA	213.0000>169.0000	0.755
2	4:2FTS	229.0000>85.0000	1.031
3	PFPeA	263.0000>219.0000	1.762
4	PFBS	299.0000>80.0000	1.979
5	PFHpS	279.0000>85.0000	2.035
6	PFPeS	315.0000>135.0000	2.273
7	PFMPA	327.0000>307.0000	2.454
8	PFHxA	313.0000>269.0000	2.514
9	PFEESA	349.0000>80.0000	2.599
10	HFPO-DA	285.0000>169.0000	2.670
11	PFHxS	399.0000>80.0000	3.013
12	NaDONA	377.0000>251.0000	3.033
13	ADONA	377.0000>250.9000	3.034
14	FOSA	427.0000>407.0000	3.299
15	PFOA	413.0000>369.0000	3.316
16	PFMBA	449.0000>80.0000	3.328
17	PFHpA	363.0000>319.0000	3.388
18	PFOS	499.0000>80.0000	3.588

Peak #	PFAS Species	Observed Transition	Ret. Time (min)
19	9Cl-PF3ONS	530.9000>351.0000	3.719
20	8:2FTS	549.0000>80.0000	3.816
21	PFNS	527.0000>507.0000	3.820
22	PFDA	513.0000>469.0000	3.822
23	N-MeFOSAA	570.0000>419.0000	3.925
24	PFNA	463.0000>419.0000	3.942
25	NFDHA	599.0000>80.0000	4.015
26	PFUnA	563.0000>519.0000	4.025
27	N-EtFOSAA	584.0000>419.0000	4.029
28	6:2FTS	498.0000>78.0000	4.033
29	11Cl-PF3OUdS	630.7000>451.0000	4.110
30	PFTrDA	663.0000>619.0000	4.355
31	PFDoA	613.0000>569.0000	4.496
32	PFTeDA	713.0000>669.0000	4.745
33	PFDS	295.0000>201.0000	4.921

CONCLUSION:

Due to the high levels of environmental contamination, PFAS analysis of potable and non-potable water is of critical importance. The HALO® PFAS column solutions have been shown to be highly efficient at separating PFAS species as fast as 5 minutes, and equally adept at delaying PFAS contamination originating from the instrument. The examples highlighted in this report using the target analytes of EPA Methods 537.1, 533, and 8327 demonstrate that Fused-Core® technology benefits PFAS analysis for fast, efficient, and rugged separations which are paramount to environmental analysis.

ACKNOWLEDGEMENT:

We gratefully acknowledge Dr. Charles Powley from the STRIDE Center for PFAS Solutions for his collaboration, and for sharing his knowledge with many beneficial discussions and advice on PFAS.

REFERENCES:

1. *Shoemaker, J.; Tettenhorst, D. Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC, 2018.*