Current LC/MS Approaches for PFAS Analysis with Ultrashort and Long Chain Mixtures

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ASMS 2025: TP- 263

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of chemicals used to make fluoropolymer coatings and products that resist heat, oil, stains, grease, and water. These toxic "forever chemicals" have led to significant public health and environmental concerns and increased needs for diligence in surveillance, production, storage and mitigation.
Current Environmental Protection Agency (EPA) methods will be demonstrated using superficially porous particle technology columns including EPA 533, 537.1, 8327, and 1633. Recently, methods involving significantly larger injection volumes and elimination of solid phase extraction sample preparation have been evaluated in order to speed up analysis times and throughput.
Furthermore, there has been a growing concern over the ultrashort chain PFAS chemicals, including trifluoracetic acid (TFA), and PFPrA which can be challenging to analyze due to low retention and sensitivity via LC/MS/MS. Although mixed mode hydrophilic interaction liquid chromatography (HILIC) has been demonstrated to improve retention, this approach has limitations.

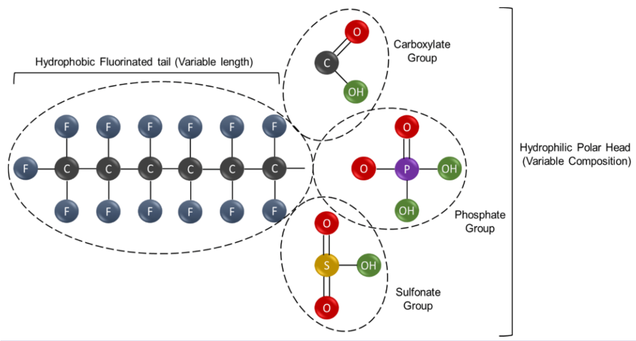


Figure 1: Panieri E, Baralic K, Djukic-Cosic D, Buha Djordjevic A, Saso L. PFAS Molecules: A Major Concern for the Human Health and the Environment. Toxics. 2022; 10(2):44. https://doi.org/10.3390/toxics10020044

Analytical Methods:

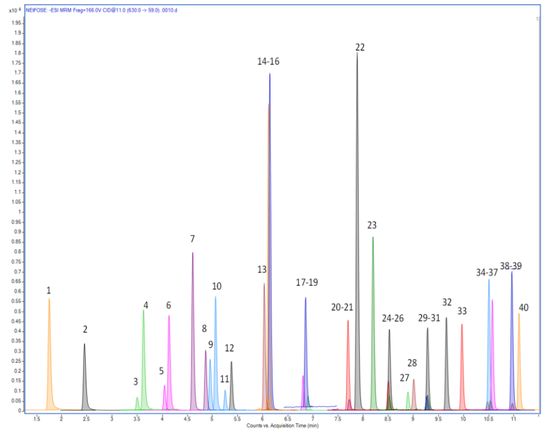
Figure 2: An Agilent 6400 Series Triple Quadrupole (Santa Clara, USA), was coupled to an Agilent 1200 series HPLC system. The Center for PFAS Solutions (Delaware, USA) prepared and supplied standards following the EPA methods. A HALO® PFAS Delay, 3.0x50mm was used as the delay column, and a HALO® PFAS 2.1x100mm column, was used as the analytical column. The delay column was positioned between the mixer and the autosampler.

Figure 3: A Thermo QE-HF was coupled to a Shimadzu Nexera HPLC system. A HALO® PFAS Delay, 3.0x50mm was used as the delay column, and a HALO® PCS Phenyl Hexyl 2.1x100mm column, was used as the analytical column using analytical standards.

Figure 4/5: An Agilent 6400 Series Triple Quadrupole (Santa Clara, USA), was coupled to an Agilent 1200 series HPLC system. A HALO® PFAS Delay, 3.0x50mm was used as the delay column, and a HALO® PCS Phenyl Hexyl 2.1x100mm column

Figure 6/7: An Agilent 6400 Series Triple Quadrupole (Santa Clara, USA), was coupled to an Agilent 1200 series HPLC system. A HALO® PCS Phenyl Hexyl, 3.0x50mm was used as the delay column, and a HALO® PCS Phenyl Hexyl 2.1x100mm column. 0.5mL/min, 40C, 100µL injection, MPA: 2mM NH4OAc, 2mM NH4OAc in MeOH, 2 min hold @ 0%B, 50% B in 3 min, 80%B in 7 min.

EPA Method 1633: HALO® PFAS C18



Compound	Retention Time
PFBA	1.75135
PFMPA	2.45145
3-3 FTCA	3.50342
PFPeA	3.62722
PFBS	4.04783
PFMBA	4.13398
PFEESA	4.60998
NFDHA	4.86550
4-2FTS	4.95623
PFHxA	5.06188
PFPeS	5.25643
HFPO-DA	5.37952
PFHpA	6.03142
PFHxS	6.11412
5-3 FTCA	6.11698
ADONA	6.13967
6-2FTS	6.80270
PFOA	6.85485
PFHpS	6.90540
PFNA	7.69722
PFOS	7.72580
7-3 FTCA	7.88195
9Cl-PF3ONS	8.19443

Compound	Retention Time
8-2FTS	8.49818
PFDA	8.51965
PFNS	8.52130
NMeFOS-AA	8.89147
PFOSA	9.00733
PFDS	9.26428
PFUnA	9.28205
NEtFOSAA	9.28502
11Cl-PF3OUdS	9.65908
PFDoA	9.96475
NMeFOSA	10.47117
NMeFOSE	10.50327
PFDoS	10.52672
PFTrA	10.57155
NEtFOSE	10.96262
NEtFOSA	10.96952
PFTeDA	11.09977

Figure 2: Calibration standards for EPA method 1633

The latest EPA 1633 method includes 40 PFAS compounds across nine different compound classes (including linear and branched isomers) using a reversed phase HALO® PFAS column along with a HALO® PFAS delay column.

Ultrashort and Long Chain Mixtures: HALO® PCS Phenyl-Hexyl

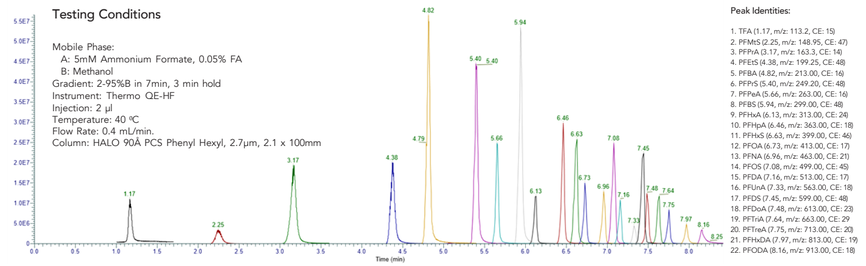
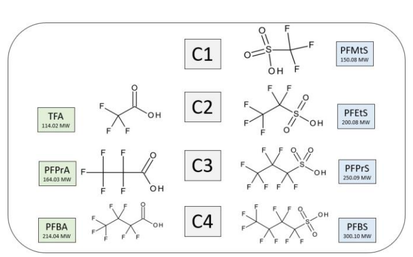
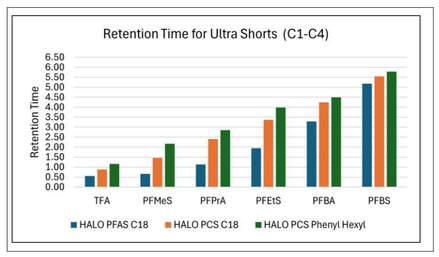


Figure 3: Ultrashort/ Long chain PFAS analysis using HALO® PCS Phenyl Hexyl

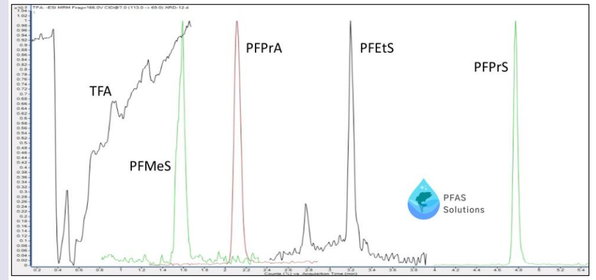


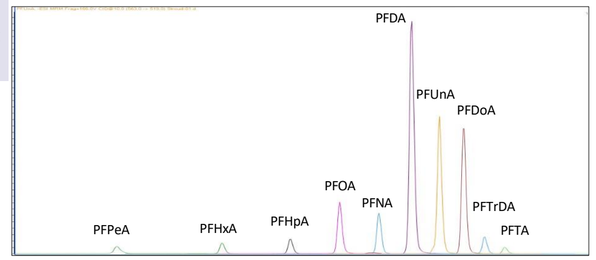


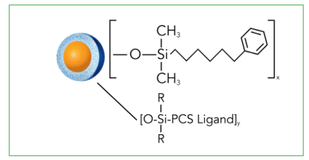
A broad reverse phase gradient ranging from 2-95% organic was used in order to retain the polar and non-polar analytes. Overall best retention was achieved using PCS Phenyl-Hexyl. A separation of the ultra-short and long chain PFAS can be seen in figure 3, showing excellent retention for the ultra-short chain PFAS using a HALO 90Å, 2.7µm PCS Phenyl Hexyl column with the combination of a HALO® PFAS delay column. A combination of ammonium formate and methanol was determined to give the overall best results.

Direct Injection Sample Analysis: Well Water and Soil Extract: HALO® PCS Phenyl-Hexyl

PFAS HPLC methods are continuing to improve including newer methods involving no solid phase extraction steps, reducing time and money in laboratory workflows. This is achieved by injecting a large amount of sample on column. Figure 4 is an example of an ultrashort chain PFAS analysis (10-100 ng/L) in a well water sample, avoiding solid phase extraction and injecting a large injection volume (20µL) for analysis.
Figure 5 shows PFAS in soil extract (0.1-1 ng/g







A new reversed phase, 90Å superficially porous particle (SPP) silica with a positive charge surface chemistry has shown advantages for short and long chain PFAS HPLC analyses compatible with several mass spectrometry platforms.

Direct Injection Sample Analysis: 100µL/ Standards

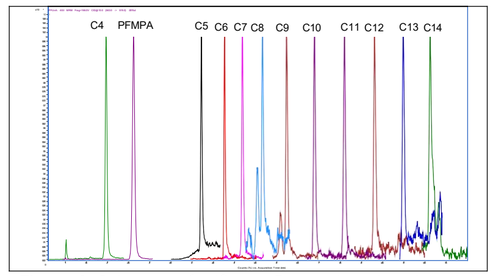


Figure 6: Soil extract fortified at 5ppb using direct injection (100µL) using a combination of a HALO® PCS Phenyl Hexyl analytical and delay column. Carboxylates shown above

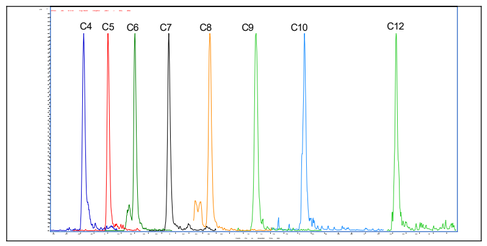


Figure 7: Soil extract fortified at 5ppb using direct injection (100µL) using a combination of a HALO® PCS Phenyl Hexyl analytical and delay column. Sulfonates shown above.

Conclusions

Reversed phase HPLC with the combination of LC/MS/MS is the most common approach to analyze PFAS compounds. As HPLC column technologies along with improvements to LC/MS sensitivity continue to grow, more and more of these analytes are being discovered.

One of the problems analyzing short chain PFAS is low retention under reversed phase conditions like EPA 1633. Even under high aqueous conditions retention for short chain PFAS becomes a challenge for standard C18 column chemistries. Having analytes too close to the column void can also cause issues with any unwanted interferences or ionization suppressing species, making it more difficult to accurately quantitate and measure the peak of interest.

HALO® PCS Phenyl-Hexyl has shown advantages for short chain PFAS HPLC analyses. With the addition of the charged surface ligand, retention is increased for the ultrashort PFAS compounds allowing reversed phase HPLC to be a viable option. The HALO® PCS columns incorporate a 90Å, 2.7µm SPP particle including C18 and Phenyl-Hexyl ligand options.