Integration of Ultra-short Chain PFAS Into Routine Analysis Methods: Addressing Retention and Confirmatory Ions

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INTRODUCTION

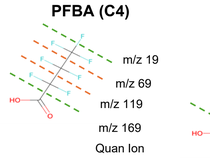
The emerging problem of environmental and health hazards of long chain (≥C8) PFAS has resulted in them being substituted for short (C4 – C7) and ultra-short (<C4) chain PFAS. This has led to the analytical requirement to analyze ultra-short chain PFAS in the same methods developed and applied for longer chain PFAS. This is challenging due to lack of retention of ultra short chain PFAS on commonly used reverse phase columns, but also in terms of ion ratio identification of shorter chain length PFAS. Short and ultra-short chain perfluoroalkyl carboxylates, such as PFPrA and PFBA, generate limited mass fragments in the mass spectrometer's collision cell. Many PFAS methods therefore only monitor for a quantitative ion and lack a confirmatory ion. Current methods are now requiring confirmation of such compounds using either a second injection on a column of different chemistry, or accurate mass determination by high resolution mass spectrometery.

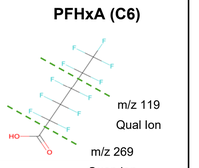
Both challenges are addressed in this work. The ultra-short chain PFAS were chromatographically retained using a mixed mode column that uses both reverse phase and anion exchange retention mechanisms. Additional fragments for the ultra-short chain PFAS were evaluated using the m/z 19 fragment to provide a confirmatory ion for those that historically have not had one easily available by enhancing low mass transmission of the mass spectrometer. By using this combination of a mixed mode separation column and tandem mass spectrometer with automatically applied low mass transmission settings, the challenges of short and ultra-short chain PFAS can be overcome without compromising the performance for other PFAS.

METHODS

Sample Preparation

Water samples were provided by a collaborator and were collected as 5 mL samples from a variety of surface and wastewater sources. Samples were prepared using the ASTM 8421 method. The entire 5 mL water sample was diluted with 5 mL methanol, syringe filtered and then 10 µL of acetic acid was added. For samples that required additional acid to bring pH to 4, acetic acid was added in small increments of 1 µL at a time. Additional acetic acid required ranged from 1-4 µL depending on starting pH.





LC-MS/MS Conditions

LC System: ACQUITY™ Premier System with BSM, FTN and fitted with PFAS Kit

Isolator Column: Atlantis™ Premier BEH™ C18 AX, 2.1 x 50 mm, 5.0 µm Column

Analytical Column: Atlantis Premier BEH C18 AX, 1.7 µm; 2.1mm x 100 mm Column

Column Temp: 35°C

Sample Temp: 10°C

Injection Volume: 30 µl

Flow Rate: 0.3 mL/min

Mobile Phase A: Water + 2 mM ammonium acetate

Mobile Phase B: Methanol + 0.1% Ammonium Hydroxide

Gradient:

Time (min)	%A	%B	Curve
0	99	1	initial
2	99	1	6
3	75	25	6
8	50	50	6
15	15	85	6
16	0	100	6
20	0	100	6
20.1	100	0	6
23.5	100	0	6
24	99	1	6

MS System: Xevo™ TQ Absolute Mass Spectrometer

Software: waters_connect™ for Quantitation

Ionization Mode: ESI-

Capillary Voltage: 0.5 kV

Desolvation Temp: 350°C

Desolvation Gas Flow: 900 L/hr

Cone Gas Flow: 150 L/hr

Source Temperature: 100°C

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RESULTS AND DISCUSSION

Addressing Chromatography for Ultra-short Chain PFAS

Reverse phase (C18) is the most commonly used stationary phase for PFAS analysis, but carboxylic acids ≤C4 are not sufficiently retained on standard reverse phase columns (**Fig. 1A**). In this example, TFA (C2) and PFPrA (C3) elute within the void region (T_0) of the column, distorting peak shape and increasing the chance of matrix interference from the unretained matrix compounds also eluting in this region. Using the mixed mode Atlantis Premier BEH C18 AX Column, containing both reverse phase and anion exchange mechanisms, functional group on each compound also plays a role in retention, allowing for increased retention of the ultra-short chain PFAS like TFA and PFPrA (**Fig. 1B**).

Since the anion exchange selectivity of the mixed mode column is pH dependent, the LC method increased pH over the gradient using ammonium hydroxide to achieve the best resolving power. Even though an initial 10 µL acetic acid was added to each sample after dilution and filtration some samples still had elevated pH values causing retention time shifting of the most pH sensitive early eluting compounds (**Fig. 2A**). To resolve this, additional acetic acid was added to each sample to adjust the pH to 4 making the retention time of the early eluting compounds more stable (**Fig. 2B**).

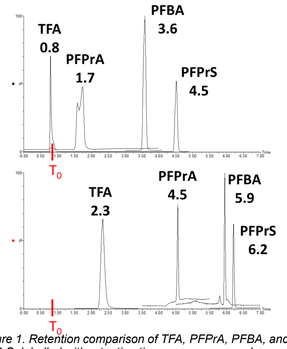

Figure 1. Retention comparison of TFA, PFPrA, PFBA, and PFPrS, labelled with retention times, on a reverse phase only column (A) and the mixed mode column (B).

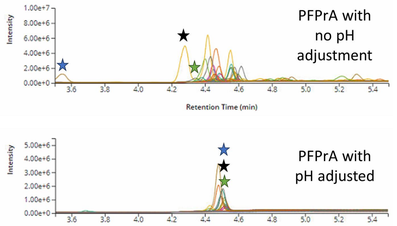

Figure 2. Overlay of PFPrA retention in different types of samples without (A) and with (B) additional pH adjustment. Stars indicate peaks from same sample.

TFA, PFPrA, and PFPrS were identified in the landfill leachate sample that previous analysis could not chromatograph. Routinely targeted C4-C14 PFAS could also be analyzed on the same column using a single injection. **Table 2** lists the PFAS identified in the landfill leachate sample and the calculated percent difference. The percent difference was within 15% for all PFAS identified except for PFBA, which is assumed to be an overestimation on the reverse phase column due to co-eluting matrix peaks that were resolved using the C18 AX column (**Fig. 3**).

Compound	C18 AX (ng/L)	Reverse Phase (ng/L)	% Difference
TFA	7790	-	NA
PFPrA	1063.6	1204	11.7
PFBA	1904.8	2853.2	33.2
PFPeA	3150.8	3351.4	6.0
PFHxA	5004.4	5002.4	0.0
PFHpA	743.2	682	9.0
PFOA	1431	1379	3.8
PFNA	133	129.2	2.9
PFDA	153.4	147.4	4.1
PFUnDA	ND	ND	ND
PFDoDA	ND	ND	ND
PFTrDA	ND	ND	ND
PFTeDA	ND	ND	ND
PFPrS	552	-	NA
PFBS	4055.8	4293.4	5.5
PFPeS	348	361	3.6
PFHxS	1133.8	1158.4	2.1
PFHpS	29.4	32.2	8.7
PFOS	452.8	454	0.3
PFNS	ND	ND	ND
PFDS	ND	ND	ND
PFDoDS	ND	ND	ND
HQ115	689.6	639	7.9

Compound	C18 AX (ng/L)	Reverse Phase (ng/L)	% Difference
PFMPA	10.6	9.8	8.2
PFMBA	3	2.8	7.1
3:3 FTCA	183.8	210	12.5
5:3 FTCA	6343.8	6176.2	2.7
7:3 FTCA	151.4	147.4	2.7
GenX	4.2	4	5.0
NFDHA	ND	ND	ND
PFEESA	ND	ND	ND
FHUEA	48.8	48.4	0.8
FOUEA	ND	ND	ND
ADONA	ND	ND	ND
4:2 FTS	42.2	40.4	4.5
6:2 FTS	6829.2	7012.2	2.6
8:2 FTS	69.2	69.4	0.3
FOSA	11	10	10.0
NMeFOSA	ND	ND	ND
NEtFOSA	ND	ND	ND
N-MeFOSAA	254.6	222.4	14.5
N-EtFOSAA	76.4	71.4	7.0
NMeFOSE	ND	ND	ND
NEtFOSE	ND	ND	ND
9Cl-PF3ONS	ND	ND	ND
11Cl-PF3OUdS	ND	ND	ND

Table 1. Quantitation of 46 PFAS compounds in a landfill leachate sample using both the mixed mode column and reverse phase column, indicating the percent difference between both sets of data. (ND) not detected, (NA) not applicable.

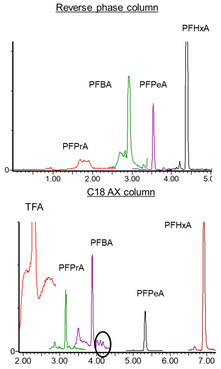

Figure 3. Zoomed in chromatogram of the landfill leachate sample showing the co-eluting peaks that cause the higher PFBA quantitation on the reverse phase column (A) and are resolved on the C18 AX column (B). TFA and PFHxA peaks are cut off due to the zoom.

Additional Confirmation Ions

On a standard mass spectrometer, the m/z 19 fragment transition can be detected for PFAS, but with very low response. On Xevo TQ Mass Spectrometers operating waters_connect Software, low mass transmission settings are automatically enabled when a fragment mass of ≤ 50 is used in the MS method. The response of the m/z 19 fragment is significantly enhanced on the modified system as seen in **Figure 4**, demonstrating the peak area improvement over the calibration range of the m/z 19 fragment transition for PFBA. Additionally, the overlay of both the peaks for the quantitative transition (262.9 > 219) and new confirmatory transition (262.9 > 19) for PFPeA at all points of the calibration curve is shown in **Figure 5** demonstrating this transition is sensitive enough to be used over a large concentration range. This is also demonstrated in **Figure 6** showing the stable ion ratios (confirm/quan) at the low end (1-20 ng/L) of the calibration curve for PFBA. The ion ratios easily fall within the ±30% range most methods require for ion ratios. The use of the m/z 19 fragment was applied to authentic water samples to confirm the identification of PFBA in surface water samples at 2.9 and 20 ng/L (**Fig. 7**). In this example, the 212.9 > 19 transition was easily detected at both levels on the modified system, compared to the standard system where the peak was not detectable at the 2.9 ng/L concentration. This indicates the m/z 19 fragment transition can be utilized in conjunction with the quantitative ion, at even trace concentrations in real samples. To both quantitate and confirm the identification of PFAS previously lacking a confirmatory ion in a single injection.

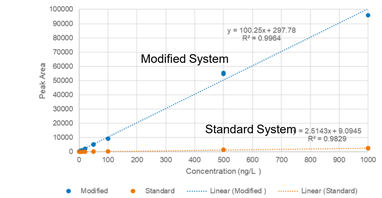

Figure 4. Peak area of m/z 212.9>19 peaks for PFBA on modified (blue) and standard (orange) Xevo TQ Absolute MS over 1 to 1,000 ng/L range.

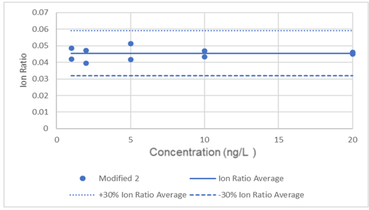

Figure 6. Ion ratios for PFBA from 1 to 20 ng/L. Ion ratio calculated by dividing the confirmation ion (m/z 212.9>19) response by the quantitative ion (m/z 212.9>169) response.

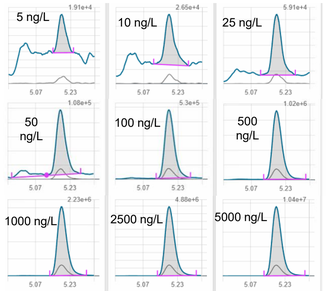

Figure 5. Overlay of peaks for the quantitative (262.9 > 219) and confirmation (262.9 > 19) transitions for PFPeA across the calibration curve.

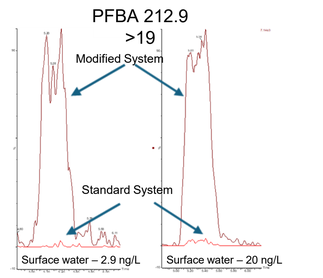

Figure 7. Overlay of PFBA peak 212.9>19 in a surface water sample at 2.9 ng/L (left) and 20 ng/L (right) comparing the response of the modified system with better low mass transmission than the standard system.

CONCLUSIONS

- **The Atlantis Premier BEH C18 AX Column successfully retains ultra-short chain PFAS, like TFA and PFPrA.**
- **Ultra-short through long chain PFAS can be analyzed on the same column in a single injection.**
- **Using the m/z 19 fragment transition is a promising option for confirmation of PFAS that previously only known to have one transition.**
- **This approach makes it possible to both quantitate and confirm the identification of common compounds like (but not limited to) PFBA and PFPeA in a single injection without the need for injecting on a second column or additional LC-HRMS analysis.**