Fast and High-Resolution LC-MS Separation of PFAS

P. Lewits[1], J. Simon[1], C. Muraco[3]
1 - Merck KGaA, Frankfurter Str. 250, , Darmstadt, Germany
2 - MilliporeSigma, 585 N Harrison Rd., Bellefonte, PA, 16823, United States

Introduction

PFAS (Per- and poly-fluoroalkyl substances) are persistent, man-made organic compounds, widely found in the environment. Recent awareness has brought attention to the toxicity of these substances. PFAS are associated with health risks such as cancer, infertility, low birth weight, and delayed puberty. Due to their stable chemical structure, PFAS have a high resistance to degradation and also possess a high accumulation potential. In recent years, the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency (EPA) have initiated actions against PFAS. For determination of PFASs, liquid chromatography–mass spectrometry (LC-MS) is a commonly used technique.

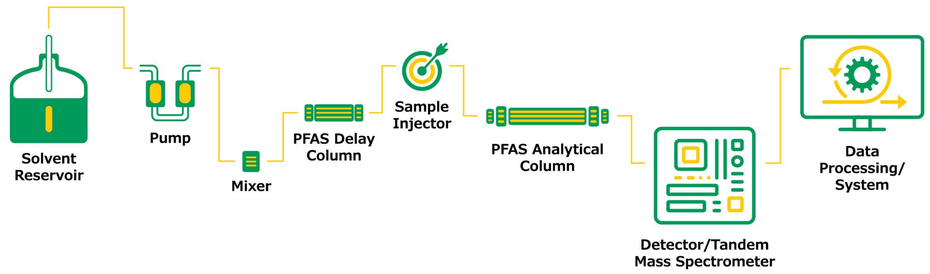

Figure 1: *LC-MS instrumental set-up for PFAS analysis*

Experimental

LC-MS Method

The analysis of the 25 PFAS compounds was performed as detailed in EPA 533 (section 17) using a Shimadzu Nexera X2 system with Shimadzu LCMS-8040. For the chromatographic separation a superficially porous (SPP) stationary phase with 2.7 µm particle size was used.

LC Conditions:

Analytical Column: Ascentis® Express PFAS, 2.7 µm, 10 cm x 2.1 mm, 90A [53559-U]

Delay Column: Ascentis® Express PFAS Delay, 2.7 µm, 5 cm x 3 mm [53572-U]

Mobile Phase A: 10 mM Ammonium Acetete

Mobile Phase B: Methanol

Gradient:

Time	%B
0.0 min	33.0
18.0 min	98.0
18.1 min	100.0
21.0 min	100.0
21.1 min	33.0
26.0 min	End

Flow Rate: 0.4 mL/min
Pressure: 485 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)

MS Conditions:

Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 400 °C

Chromatographic Results

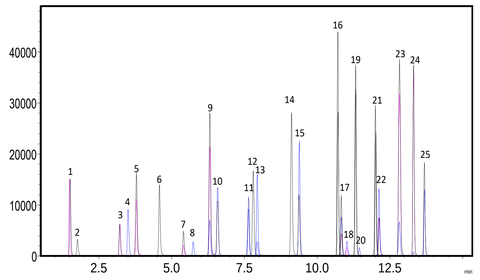

Figure 2: *LC-MS results of the analysis on 25 PFAS compounds from EPA 533*

Peak Number	Compound	Transition	Retention 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	PFMPA	327.0000>307.0000	5.431
8	PFHxA	313.0000>269.0000	5.684
9	PFEESA	349.0000>80.0000	6.099
10	HFPO-DA	285.0000>169.0000	6.335
11	PFHpA	363.0000>319.0000	7.763
12	PFHxS	399.0000>80.0000	7.985
13	ADONA	377.0000>250.9000	8.012
14	PFOA	413.0000>369.0000	9.398
15	PFMBA	449.0000>80.0000	9.512
16	PFNA	463.0000>419.0000	10.751
17	PFOS	499.0000>80.0000	10.793
18	9Cl-PF3ONS	530.9000>351.0000	11.459
19	PFDA	513.0000>469.0000	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>519.0000	12.862
24	11Cl-PF3OUdS	630.7000>451.0000	13.329
25	PFDoA	613.0000>569.0000	13.708

Table 1: *25 PFAS compound from EPA 533*

The HPLC column of choice for PFAS analysis by LC-MS/(MS) is a C18 column based on fully porous silica particles (FPP) or on superficially porous silica particles (SPP). In contrast to ordinary C18 columns Ascentis® Express PFAS columns are tested using a PFAS compound mixture. This ensures the full suitability of the column for PFAS analysis.

The contamination of PFAS compounds from the HPLC system and materials used in analytics is a concern. Therefore, it is recommended to use a delay column, which is placed before injection in the system set-up *(Figure 1)*.

The highly retentive endcapped silane of the Ascentis® Express PFAS Delay column provides high retention of PFAS compounds across various mobile phase conditions and is used to delay background instrument PFAS contamination from interference with analyzed samples.

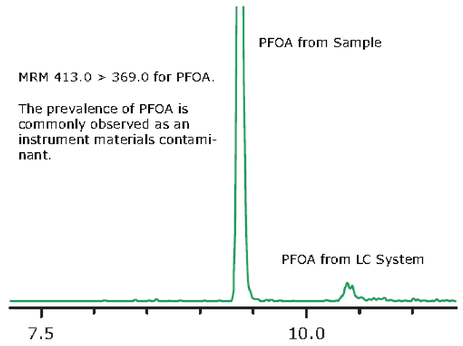

Figure 3: *LC-MS results of PFOA using a delay column.*

Typically, the delay column is used with a larger ID than the analytical column:

Analytical column	->	Delay Column
2.1 mm ID	->	3 mm ID
3 mm ID	->	4.6 mm ID

Analysis of 33 PFAS Compounds in 5 Minutes

LC Conditions:

Analytical Column: Ascentis® Express PFAS, 2.7 µm, 10 cm x 2.1 mm, 90A [53559-U]
Delay Column: Ascentis® Express PFAS Delay, 2.7 µm, 5 cm x 3 mm [53572-U]
Mobile Phase A: 10 mM Ammonium Acetete
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0 min	33.0
4.0 min	98.0
4.1 min	100.0
6.0 min	100.0
6.1 min	33.0
7.5 min	End

Flow Rate: 0.4 mL/min
Pressure: 479 bar
Temperature: 35 °C
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)

MS Conditions:

Detection: -ESI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 400 °C

Sample Compounds:

1	PFBA	17	PFHpA
2	4:2FTS	18	PFOS
3	PFPeA	19	9Cl-PF3ONS
4	PFBS	20	8:2FTS
5	PFHpS	21	PFNS
6	PFPeS	22	PFDA
7	PFMPA	23	N-MeFOSAA
8	PFHxA	24	PFNA
9	PFEESA	25	NFDHA
10	HFPO-DA	26	PFUnA
11	PFHxS	27	N-EtFOSAA
12	NaDONA	28	6:2FTS
13	ADONA		11Cl-
14	FOSA	29	PF3OUdS
15	PFOA	30	PFTrDA
16	PFMBA	31	PFDoA
		32	PFTeDA
		33	PFDS

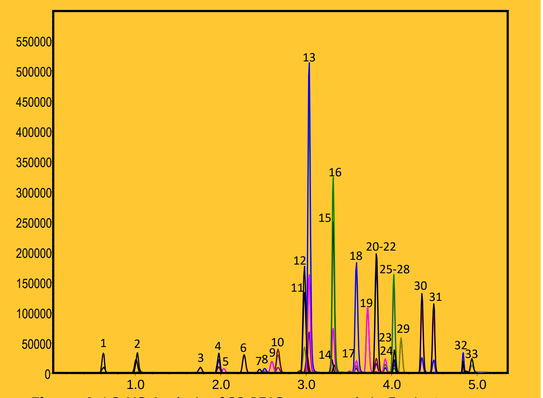

Figure 4: *LC-MS Analysis of 33 PFAS compounds in 5 minutes.*

Conclusion

The new Ascentis® Express PFAS HPLC column is designed for the separation of novel and legacy short chain and long chain PFAS compounds containing branched and linear isomers, whilst adhering to EPA methodology requirements. Furthermore, a specific PFAS delay column prevents background PFAS contamination from interfering with the sample results in quantitative LC-MS methods. The Ascentis® Express PFAS HPLC column, with its Fused-Core® technology and a particle size of 2.7 µm, delivers fast and high-resolution separations with excellent selectivity, peak shape, and necessary retention to perform in EPA methods 537.1, 533 and 8327. These advantages are demonstrated, in one particular example, by the separation of all PFAS analytes from EPA methods 537.1, 533, and 8327 in under five minutes.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada

SigmaAldrich.com

© 2021 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved. MilliporeSigma, Supelco, and the vibrant M are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.