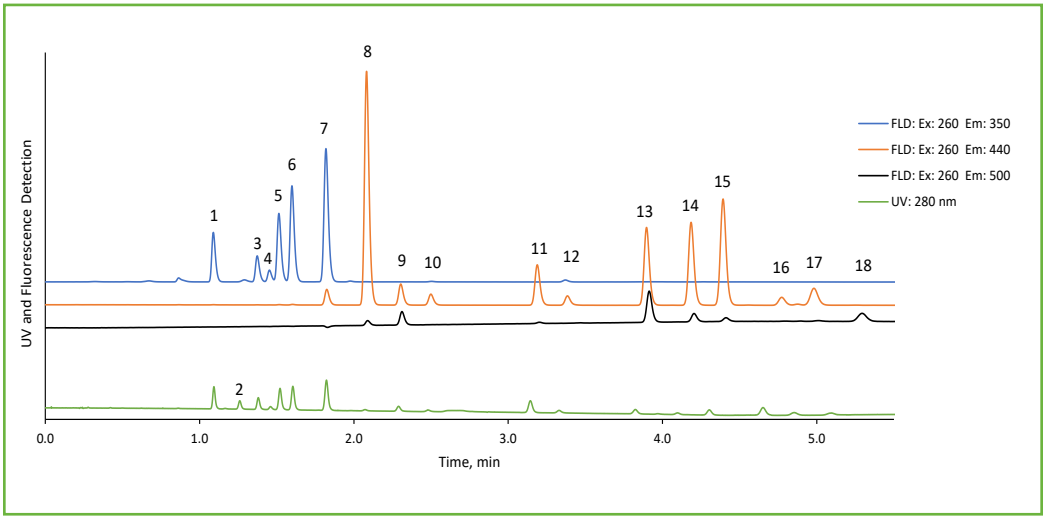
HALO®

ENVIRONMENTAL

Separation of PAH Compounds using UV and Fluorescence Detection

233-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo[a]anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

Time	%B
0.0	50
4.0	100
5.0	100
6.0	100

Flow Rate: 1.8 mL/min
Initial Back Pressure: 256 bar
Temperature: Ambient
Detection: FLD: Ex: 260/ Em: 350/440/500
UV: 280 nm
Injection Volume: 0.3 µL
Sample Solvent: Methanol
LC System: Shimadzu Nexera X2

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. These compounds can be detected several ways including a UV and/or a fluorescence detector (FLD). A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated using a UV and fluorescence detector. The FLD gain in sensitivity compared to the UV is associated to the advantage of no background for FLD and the ability to select both an excitation and emission wavelength; which can be optimized further with systematically testing the S/N as a function of the detector's gain parameter. Slight retention time and peak width increases for the FLD response are due to the greater tubing volume of this detector.

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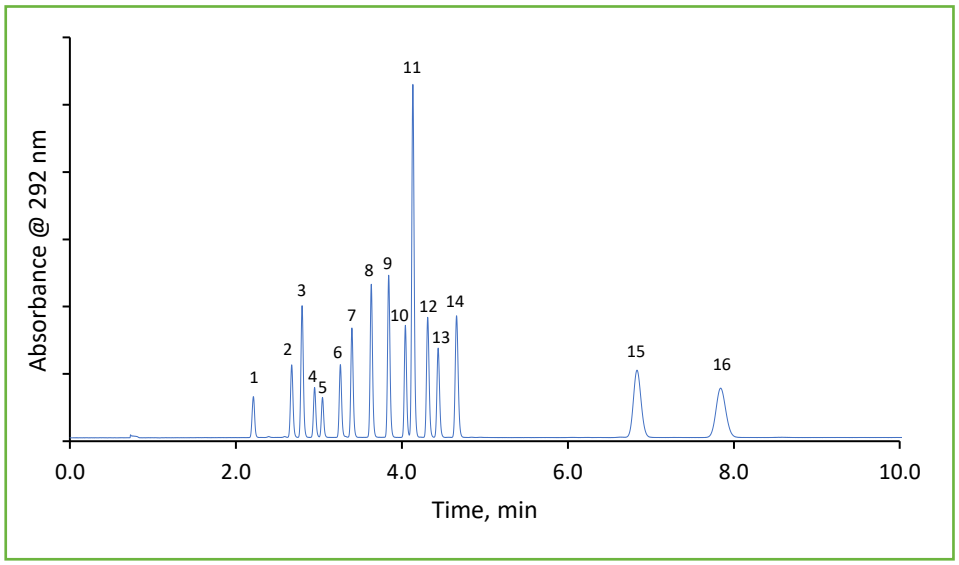
Separation of EU 15 + 1 using HALO® PAH

236-P



PEAK IDENTITIES

1. Benzo[c]fluorene
2. Cyclopenta[cd]pyrene
3. Benzo[a]anthracene
4. Chrysene
5. 5-Methylchrysene
6. Benzo[j]fluoranthene
7. Benzo[b]fluoranthene
8. Benzo[k]fluoranthene
9. Benzo[a]pyrene
10. Dibenzo[a,l]pyrene
11. Dibenz[a,h]anthracene
12. Benzo[ghi]perylene
13. Indeno[1,2,3-cd]pyrene
14. Dibenzo[a,e]pyrene
15. Dibenzo[a,i]pyrene
16. Dibenzo[a,h]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

Time	%B
0.00	50
4.00	100
15.00	100
15.01	50

Flow Rate: 1.8 mL/min
Temperature: 30 °C
Detection: 292 nm
Injection Volume: 10 µL
Data Rate: 100 Hz
LC System: Shimadzu Nexera X2

The EU 15 + 1 list of PAH compounds was established by the European Commission in 2005 specifically for food analysis. The list contains eight of the EPA's priority PAHs along with eight other compounds that are known carcinogens. The separation is completed on a 4.6 x 50 mm HALO® PAH column in less than ten minutes with excellent resolution between the critical pairs 4 and 5 which only differ by the presence of a methyl group.

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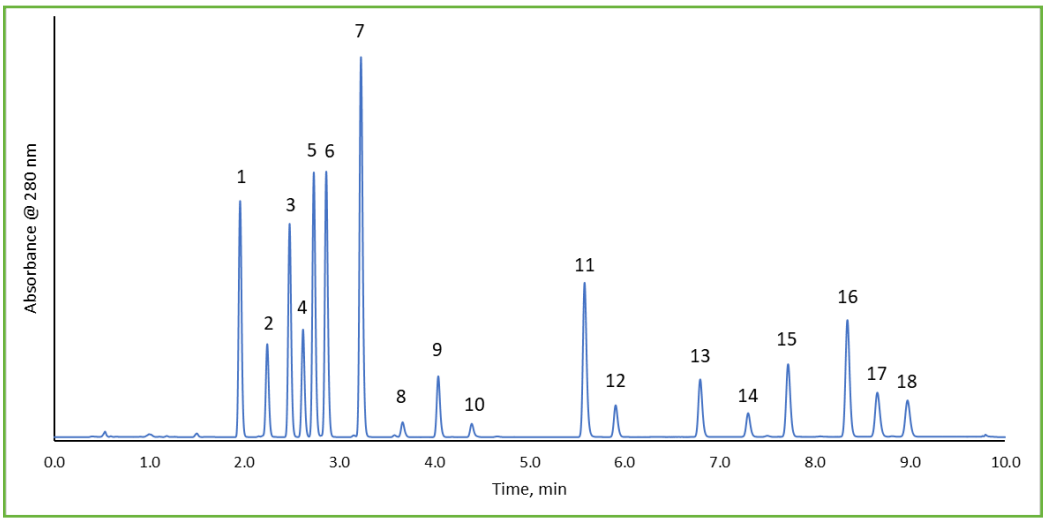
Separation of 18 PAH Compounds using HALO® PAH

231-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 3.0 x 100 mm
Part Number: 92843-612
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

Time	%B
0.0	50
8.0	100
10.0	100

Flow Rate: 0.77 mL/min
Initial Back Pressure: 263 bar
Temperature: 30 °C
Detection: 280 nm
Injection Volume: 2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated on the HALO® PAH column showing excellent speed and resolution.

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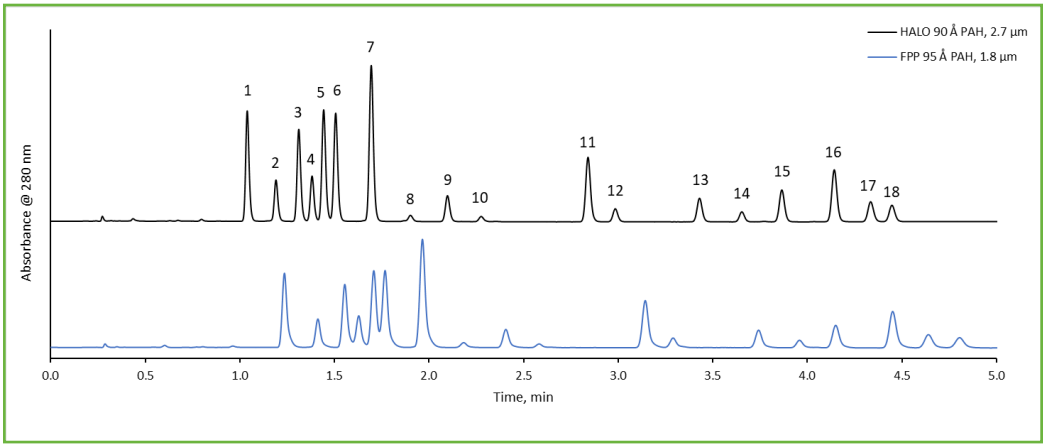
Comparison of HALO® PAH vs. FPP column for 18 PAH Compounds

230-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Competitor Column: FPP 95 Å PAH, 1.8 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:	**Time**	**%B**
	0.0	50
	4.0	100
	5.0	100
	5.01	50

Flow Rate: 1.8 mL/min
HALO® Back Pressure: 256 bar
Competitor Back Pressure: 344 bar
Temperature: 30 °C
Detection: 280 nm
Injection Volume: 2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. A separation of eighteen PAH compounds is performed on a HALO® PAH column and a FPP PAH competitor column. The HALO® column shows excellent peak resolution, along with a lower overall back pressure compared to the competitor's unresolved peaks and peak tailing.

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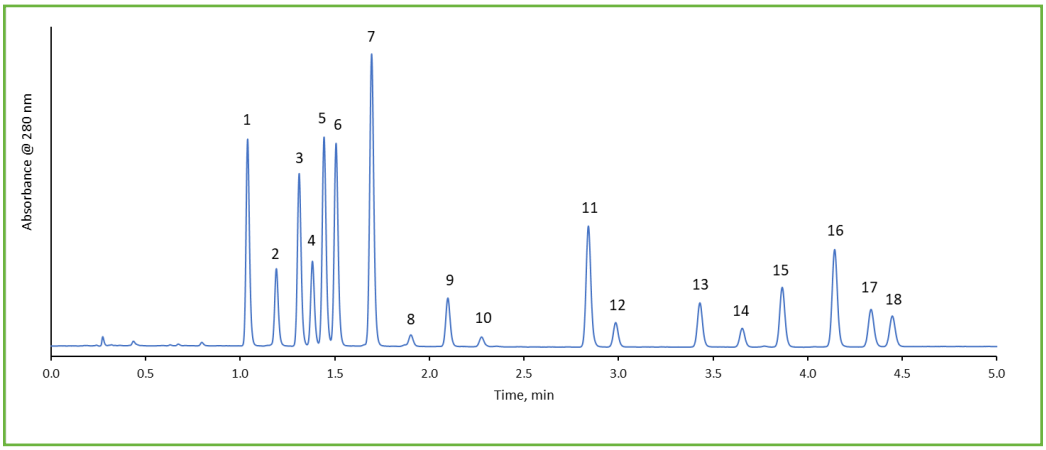
Separation of 16 PAH Compounds Specified in EPA 610 + 2 additional PAH Compounds using HALO® PAH

229-P



PEAK IDENTITIES

1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-cd]pyrene

TEST CONDITIONS:

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50 mm
Part Number: 92844-412
Mobile Phase **A:** Water
B: Acetonitrile

Gradient:

Time	%B
0.0	50
4.0	100
5.0	100
5.01	50

Flow Rate: 1.8 mL/min
Pressure: 256 bar
Temperature: 30 °C
Detection: 280 nm
Injection Volume: 2 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of more than 100 chemicals released from the combustion of coal, oil, gasoline, tobacco, and wood. They can also be found in cooked food. PAHs are persistent chemicals and must be closely regulated for early detection/monitoring to minimize hazardous exposure in the environment and/or use of contaminated raw materials in different industries. A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated on the HALO® PAH column showing excellent speed and resolution.

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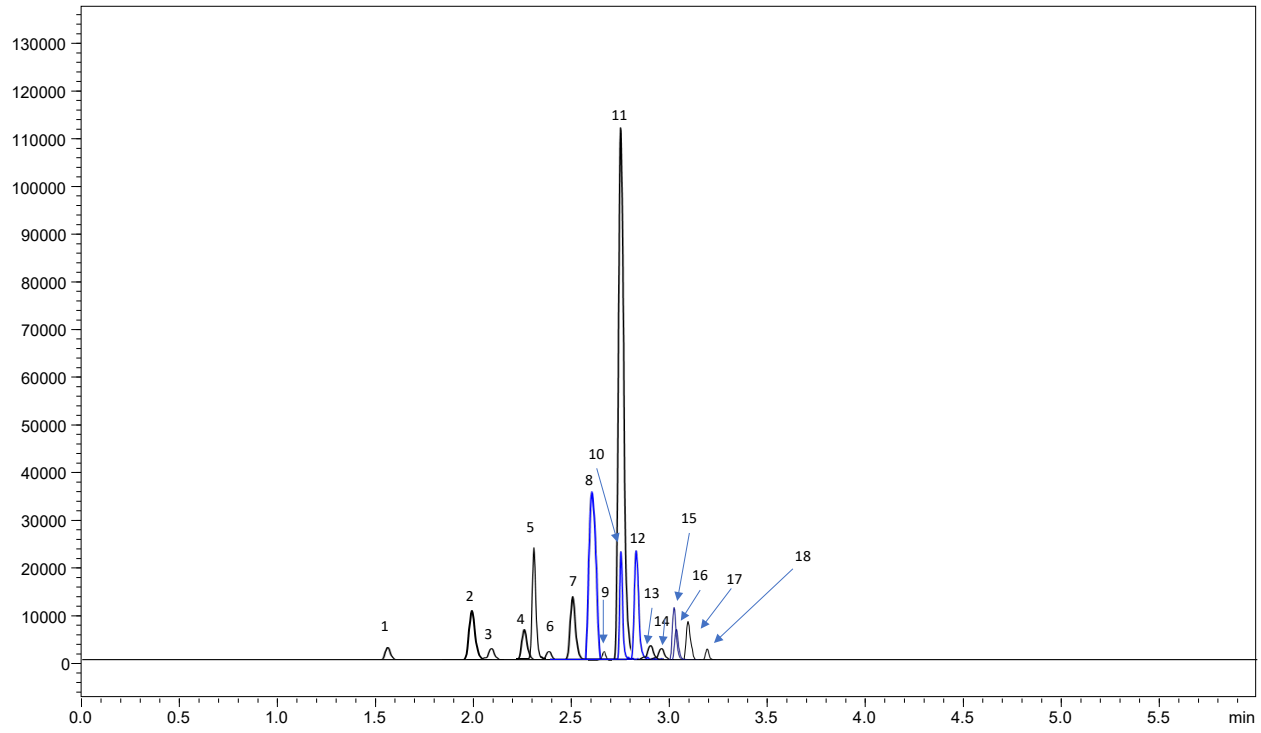
Rapid PFAS Analysis According to EPA 537.1 Using HALO® 90 Å Phenyl-Hexyl, 2.7 µm Peak

219-PF



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 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.

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PEAK IDENTITIES

Peak Number	PFAS Species	Observed Transition	Retention Time
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

TEST CONDITIONS:

Delay Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-702
Analytical Column: HALO 90 Å Phenyl-Hexyl, 2.7 µm, 2.1 x 100 mm
Part Number: 92112-730
Mobile Phase A: H_2O 10mM ammonium formate/ 0.1% formic acid
Mobile Phase B: Methanol
Flow Rate: 0.4mL/min
Sample Solvent: (95/5) MeOH/ H_2O

Gradient:

Time	%B
0.00	30
3.00	90
6.00	90
6.01	30
9.00	stop

Initial Pressure: 325 bar
Temperature: 40 °C

MS CONDITIONS:

Detection: -ESI MS
LC System: Shimadzu NexeraX2 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

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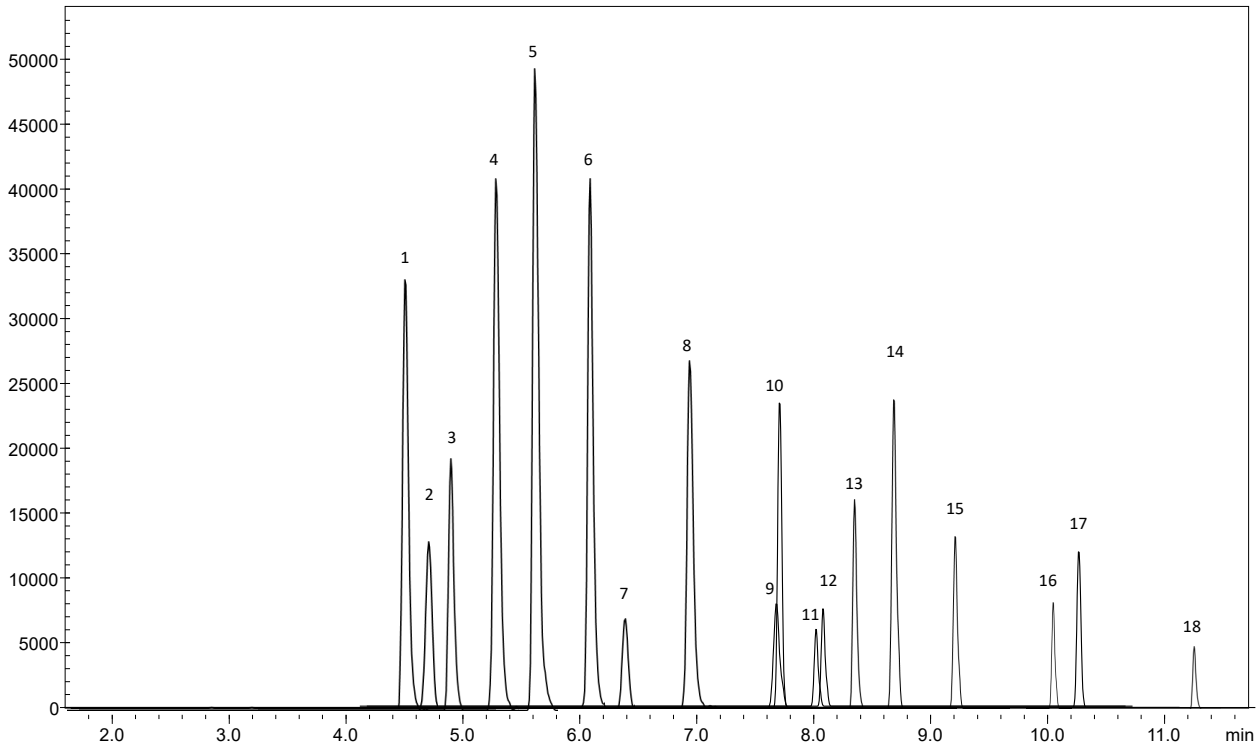
PFAS Analysis According to EPA 537.1 Using HALO® 90 Å C18, 2.0 µm

218-PF



Per-and polyfluoroalkyl substances (PFASs) 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 exposure in humans has been linked to a variety of diseases, including cancer, ulcerative colitis, thyroid disease, and hypercholesterolemia. EPA Method 537.1 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.

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PEAK IDENTITIES

Peak Number	PFAS Species	Observed Transition	Retention Time
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

TEST CONDITIONS:

Delay Column: HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm
Part Number: 92812-402
Analytical Column: HALO 90 Å C18, 2.0 µm, 2.1 x 100 mm
Part Number: 91812-602
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
Flow Rate: 0.3 mL/min
Sample Solvent: (95/5) MeOH/ H_2O

Gradient:

Time	%B
0.0	0
6.0	50
13.0	85
14.0	100
17.0	100
18.0	0
21.0	stop

Initial Pressure: 315 bar
Temperature: 40 °C

MS CONDITIONS:

Detection: -ESI MS
LC System: Shimadzu NexeraX2 ESI
LCMS system: Shimadzu LCMS-8050
Spray Voltage: -2.0 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 400 °C

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TECHNICAL REPORT: AMT-TR042002

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

MARKET SEGMENT: ENVIRONMENTAL

AUTHOR:

Andrew Harron Ph.D., Application Scientist

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

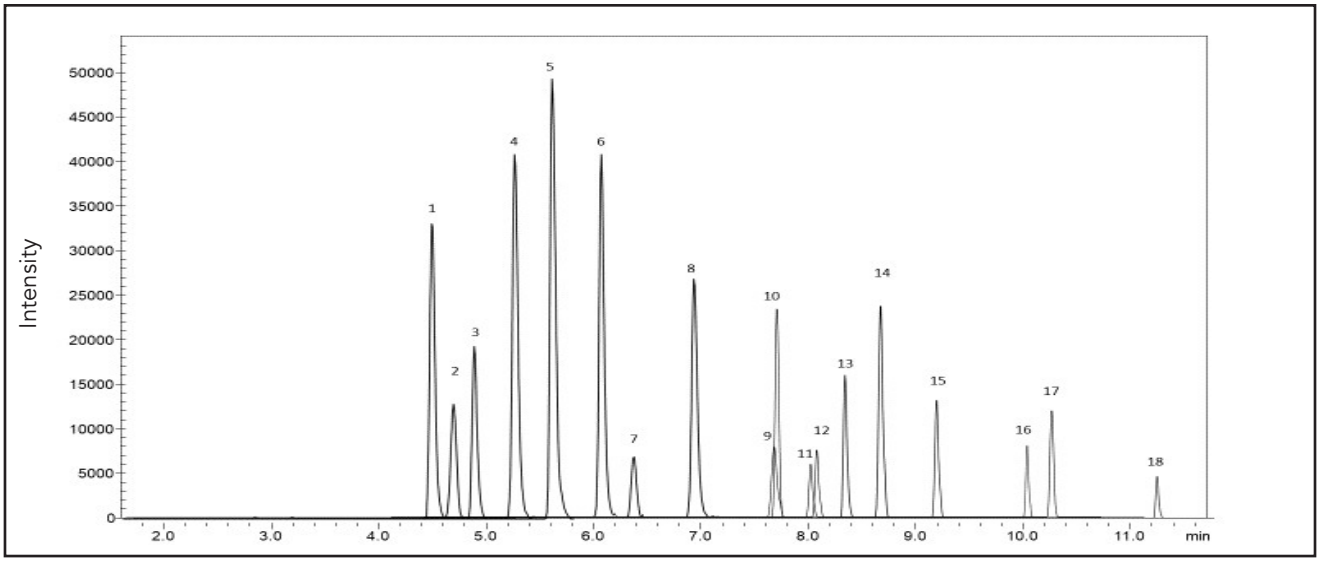


Table 3. Peak identities of 18 PFAS compounds found in EPA 537.1

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

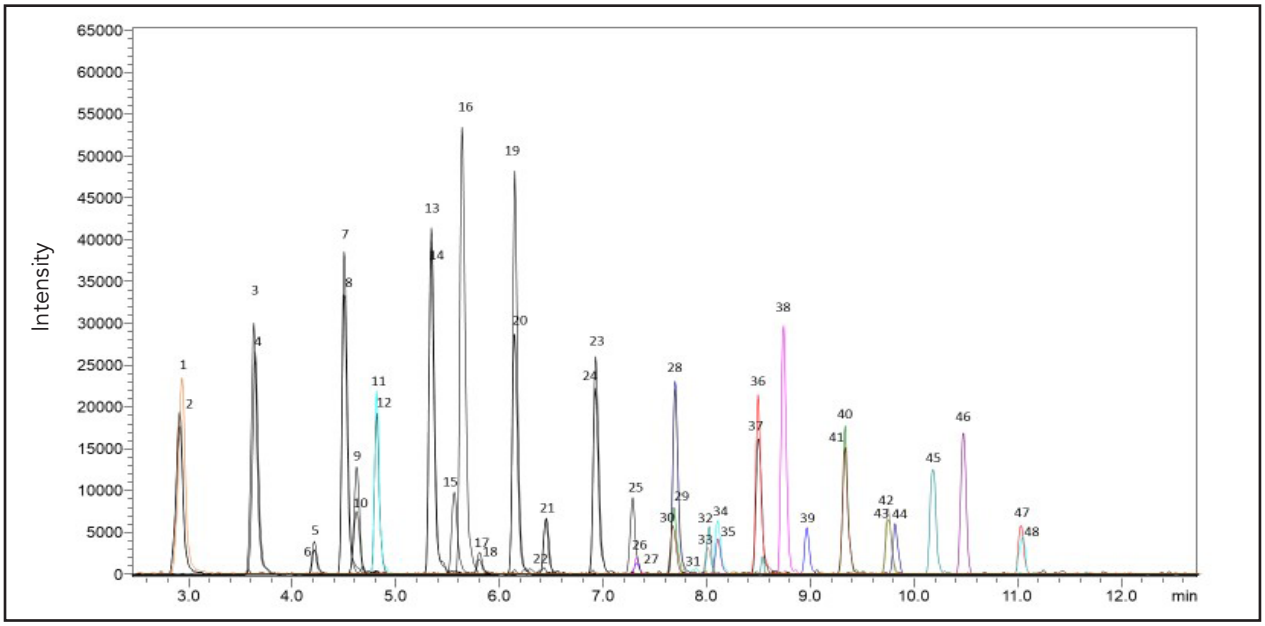


Table 4. Peak identities of PFAS compounds found in EPA 537.1 and EPA 8327

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.

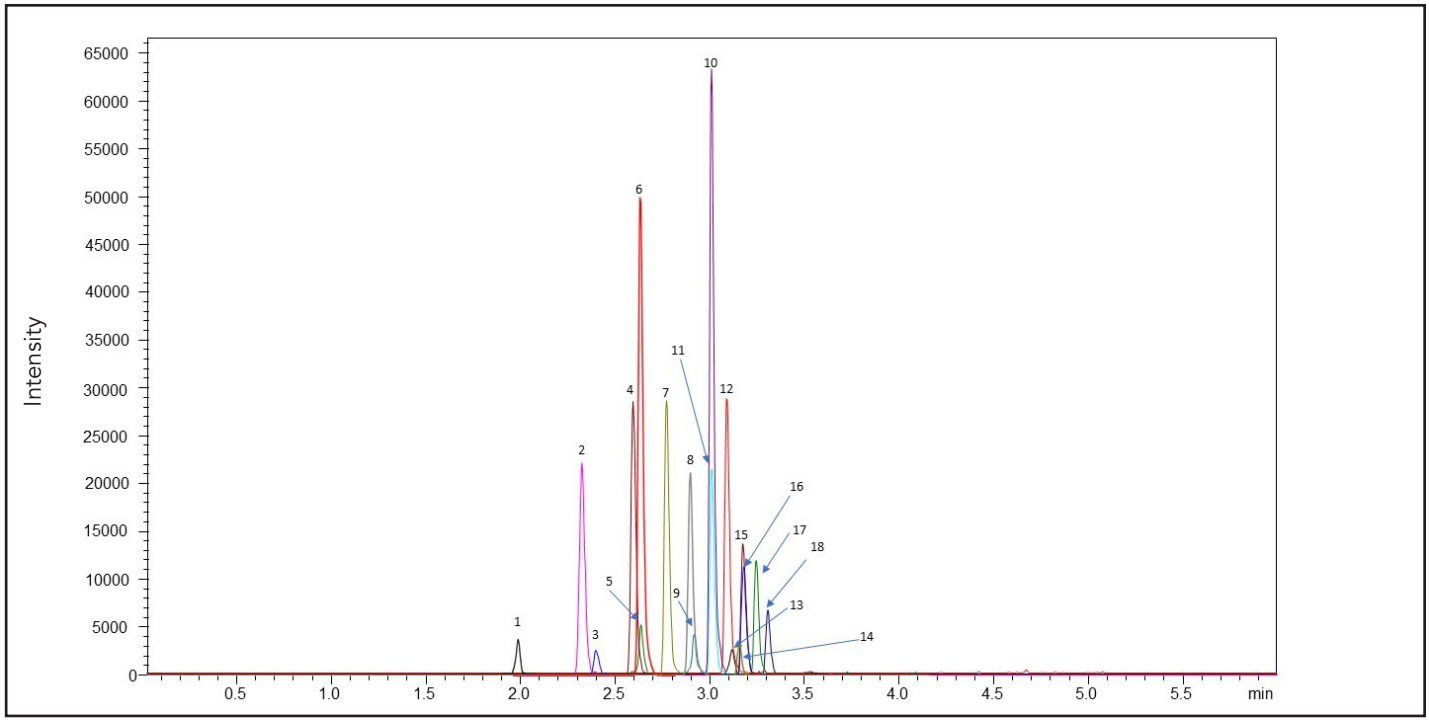


Figure 3. Maximum speed separation of 18 PFAS species according to EPA 537.1 on a HALO® Phenyl-Hexyl column

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.

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.*
2. *Validated Test Method 8327: Per-and Polyfluoroalkyl Substances (PFAS) Using External Standard Calibration and Multiple Reaction Monitoring (MRM) Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).*
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).*

Environmental Analyses of Harmful Chemical and Organic Compounds using Superficially Porous Particle Separations

Introduction

Reports of pollution, unhealthy levels of chemicals in water, soil, and food, and their unknown impact to the environment are becoming increasingly common. For example, in January 2020, per- and polyfluoroalkyl substances (PFAS) were found in the drinking water of several US cities, with some of the highest levels found in Philadelphia, Miami, New Orleans, and the northern New Jersey suburbs of New York City [1]. To aid in the discovery and monitoring of these types of environmentally concerning chemicals, researchers can take advantage of the superficially porous particle design. This offers high throughput without high back pressures in a rugged format less prone to sample clogging therefore making them amenable to challenging environmental sample matrices. Within ENVIROCLASS, AMT offers new specific application assured phases and method solutions to address other environmentally-related applications. See Table 1 for an example of the HALO® ENVIROCLASS column solutions. In this white paper we investigate these offerings and the analysis challenges they address.

Table 1. HALO® ENVIROCLASS column Solutions

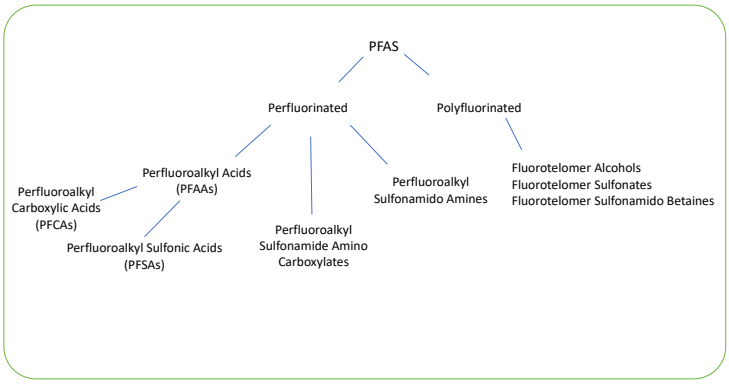
Column	Target Analytes
HALO® PFAS and HALO® PFAS Delay	PFAS
HALO® PAH	PAH
HALO® C18	Pesticides (nonpolar)
HALO® Biphenyl	Pesticides (polar and nonpolar)
HALO® PFP	Mycotoxins

PFAS

There is worldwide concern about the ubiquitous presence of PFAS in the environment. These human-made compounds were designed with carbon-fluorine bonds, which both enhance the stability of the compounds and make it nearly impossible to eliminate them. PFAS have permeated every aspect of life, being present in tap water; food and food packaging; non-stick cookware; plastics; waterproof fabrics for clothing, furniture, and carpets; dust; cleansers; and aqueous film forming foam (AFFF) formulations of fire extinguishers; among others. Contaminated water and soil allow PFAS to enter the food chain. Humans and animals eating contaminated food contribute to the accumulation of PFAS in the tissues of humans and animals. Health risks from exposure to certain PFAS include high cholesterol, liver damage, and cancer.

Under mounting pressure from the public, especially those communities whose water became contaminated, the primary US manufacturer of perfluorooctane sulfonate (PFOS) voluntarily phased out its production as of 2002. Similarly, in 2006, eight companies in the PFAS industry voluntarily agreed to phase out production of perfluooctanoic acid (PFOA) and PFOA-related chemicals by 2015. However, PFOS and PFOA are still being used in other countries around the world so products containing them could potentially be imported. Material developers have created replacements including GenX, which is a processing aid technology developed by Chemours and consists of hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt, and short chain PFAS. Short chain PFAS contain 4-7 carbons while long chain PFAS contain 8 or more carbons. Both long and short chain PFAS accumulate in the body, but excretion of long chain takes longer than for short chain. However, recent research is uncovering similar health risks with the short chain versions. Such research has prompted the manufacturers of food packaging that contains 6:2 fluorotelomer alcohol (6:2 FTOH) to voluntarily phase out the sale of these products in the US over a three-year time span beginning in January 2021 [2]. Regulations are being discussed, but there is debate about regulating individual PFAS vs. PFAS as a class. PFAS are divided into two categories: perfluorinated and polyfluorinated. See the flow chart in Figure 1 for the classifications of PFAS.

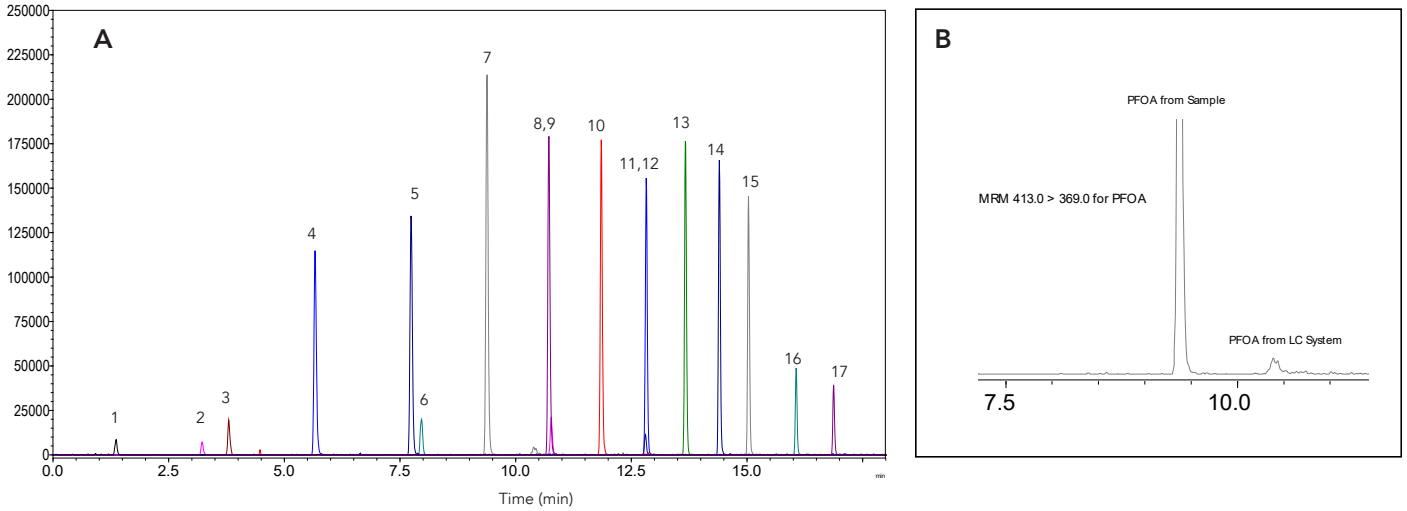
Figure 1. Classifications of PFAS



With all of the health concerns over PFAS, the US Environmental Protection Agency (EPA) has developed, validated, and published methods for PFAS analysis in drinking water: 533 and 537.1. EPA 533 was developed to focus on the short chain PFAS and contains 25 target analytes, while 537.1 has long chain PFAS and contains 18 target analytes. EPA 8327 has been validated for the analysis of groundwater, surface water, and wastewater samples and contains 24 target analytes. There is an appendix method 3512 within 8327, which will eventually become a stand-alone

method after validation and public comment is made. Method 3512 calls for dilution of the samples with organic solvent. With the requirements of EPA methods in mind, HALO® PFAS and HALO® PFAS Delay columns have been developed specifically for the analysis of PFAS and are able to meet the peak asymmetry factor in the range of 0.8 to 1.5 for the first two peaks in a mid-level calibration standard for EPA 537.1. The densely bonded, extensively endcapped ODS stationary phase of HALO® PFAS provides an application assured and method qualified solution for PFAS analysis. The highly retentive endcapped alkyl silane of the HALO® PFAS Delay column provides high retention of PFAS compounds across various mobile phase conditions and is used to delay background instrument PFAS contamination from coeluting with analyzed samples. HALO® PFAS and HALO® PFAS Delay columns are quality assurance tested with a mixture of 17 PFAS compounds that span a range of short chain and long chain structures. An example of this LC-MS/MS is shown in Figure 2A.

Figure 2A. Example of the MRM used to quality test HALO® PFAS columns. **Figure 2B**. Effect of HALO® PFAS Delay column on system PFOA



PEAK IDENTITIES

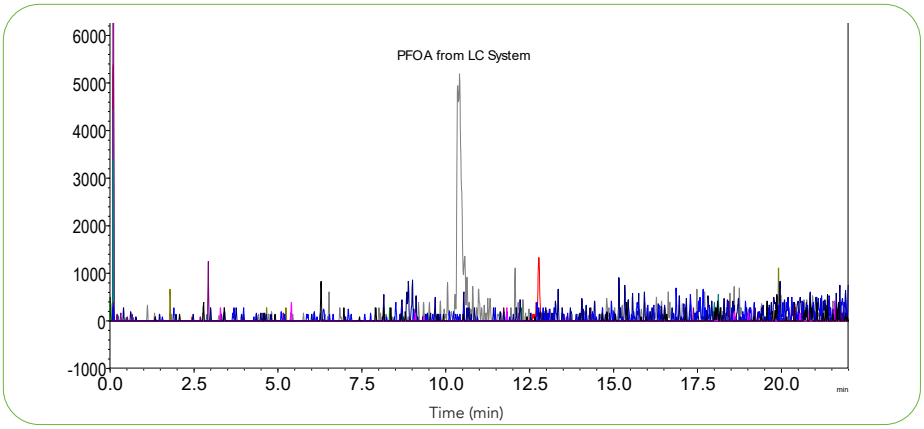
1. PFBA
2. PFPeA
3. PFBS
4. PFHxA
5. PFHpA
6. PFHxS
7. PFOA
8. PFNA
9. PFOS
10. PFDA
11. PFDS
12. PFUnDA
13. PFDoDA
14. PFTrDA
15. PFTeDA
16. PFHxDA
17. PFOcDA

TEST CONDITIONS:

Delay Column: HALO® PFAS Delay, 2.7 µm, 3.0 x 50 mm
Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Mobile Phase A: 10 mM Ammonium Acetate
Mobile Phase B: Methanol
Gradient: 33-98% B in 18 min
Flow Rate: 0.4 mL/min
Initial Back Pressure: 420 bar
Temperature: 35 °C
Detection: Negative ESI, MRM
Injection Volume: 1 µL
Sample: PFAC-MXB (Wellington Laboratories)
Concentration: 100 ppb in 96% Methanol

The short chain compound perfluorobutanoic acid (PFBA) (peak 1) is well retained with symmetrical peak shape. Figure 2B shows the effect of the HALO® PFAS Delay column. The prevalence of PFOA is commonly observed as an instrument materials contaminant. PFOA from the LC system is retained/delayed more relative to the PFOA from the analytical sample. This is crucial for low level quantitation which can be 1 ppt or lower. To illustrate that this later eluting PFOA is originating from the LC system, a null injection (gradient was run while no injection was made) was completed. See Figure 3 for the results of the null injection.

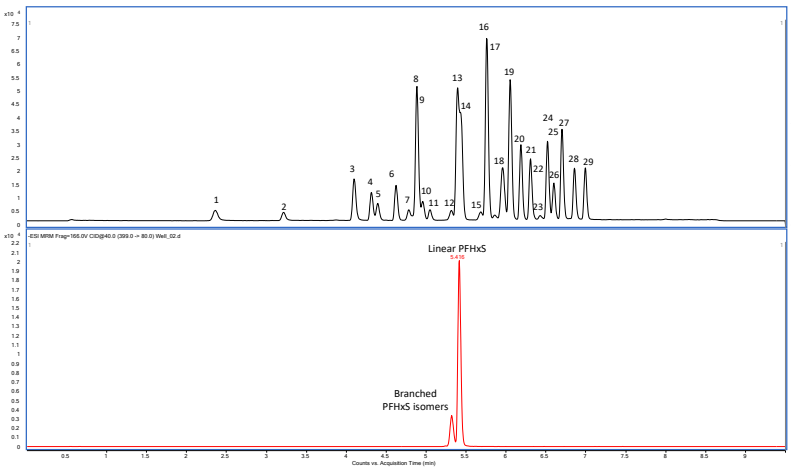
Figure 3. Null injection demonstrating background contamination of PFAS in the LC system. (conditions same as Figure 2)



The cause of the background contamination originates from the LC system itself. The degasser, solvent bottle caps, PTFE tubing, and filters all contribute to the PFAS background of the system. While the degasser can be bypassed and fluoropolymer-free replacements exist for the other components, it is still very difficult to completely remove all of the PFAS contaminants from the system. One group of investigators reported that they rinsed their system for a week to eliminate the background PFAS contamination [3].

The presence of linear and branched isomers contributes to the challenge of PFAS analysis. When electrochemical fluorination (ECF) is used to manufacture PFAS, branched isomers are created as a byproduct of the process. In contrast, when telomerization is used, only linear isomers are formed. Branched PFAS isomers, which are more polar, are less retained compared to the linear PFAS isomers. It has been found that branched isomers are found in water while linear isomers are found in soil and sediment. Furthermore, linear PFOS accumulate in animals while branched PFOS accumulate in people. The health effects of the branched and linear isomers may vary, as well [4]. An example separation demonstrating the resolution of branched and linear isomers of PFHxS in a sample of well water is shown in Figure 4. As more studies are initiated to investigate the effects of branched vs. linear PFAS isomers, it will become more crucial to determine the levels of each.

Figure 4. HALO® PFAS separation of branched and linear PFHxS isomers from a well water sample.



PEAK IDENTITIES

Peak #	Compound	Transition	Retention Time (min)
1	PFBA	213.0 > 169.0	2.36
2	PFMPA	229.0 > 85.0	3.22
3	PFPeA	263.0 > 219.0	4.10
4	PFBS	299.0 > 80.0	4.32
5	PFMBA	279.0 > 85.0	4.40
6	PFEESA	315.0 > 135.0	4.63
7	NFDHA	295.0 > 201.0	4.78
8	4-2FTS	327.0 > 307.0	4.84
9	PFHxA	313.0 > 269.0	4.89
10	PFPeS	349.0 > 80.0	4.96
11	HFPO-DA	285.0 > 169.0	5.05
12	PFHpA	363.0 > 319.0	5.39
13	PFHxS	399.0 > 80.0	5.42
14	ADONA	377.0 > 251.0	5.44
15	6-2FTS	427.0 > 407.0	5.75
16	PFHpS	449.0 > 80.0	5.76
17	PFOA	413.0 > 369.0	5.76
18	PFOS	499.0 > 80.0	6.05
19	PFNA	463.0 > 419.0	6.06
20	9Cl-PF3ONS	531.0 > 351.0	6.19
21	PFDA	513.0 > 469.0	6.31
22	8-2FTS	527.0 > 507.0	6.31
23	NMeFOSAA	570.0 > 419.0	6.43
24	PFUnA	563.0 > 519.0	6.52
25	NEtFOSAA	584.0 > 419.0	6.53
26	11Cl-PF3OUdS	631.0 > 451.0	6.60
27	PFDoA	613.0 > 569.0	6.70
28	PFTrA	663.0 > 619.0	6.86
29	PFTA	713.0 > 669.0	7.00

TEST CONDITIONS

Analytical Column: HALO® PFAS, 2.7 µm, 2.1 x 100 mm
Delay Column: HALO® PFAS Delay, 2.7 µm, 3.0 x 50 mm
Mobile Phase A: 20 mM Ammonium Acetate
Mobile Phase B: Methanol

Gradient:	Time	%B
	0.0	20
	6	90
	8	90
	8.10	20
	10.00	End

Flow Rate: 0.4 mL/min
Pressure: 505 bar
Temperature: 44 °C
Detection: -ESI MRM

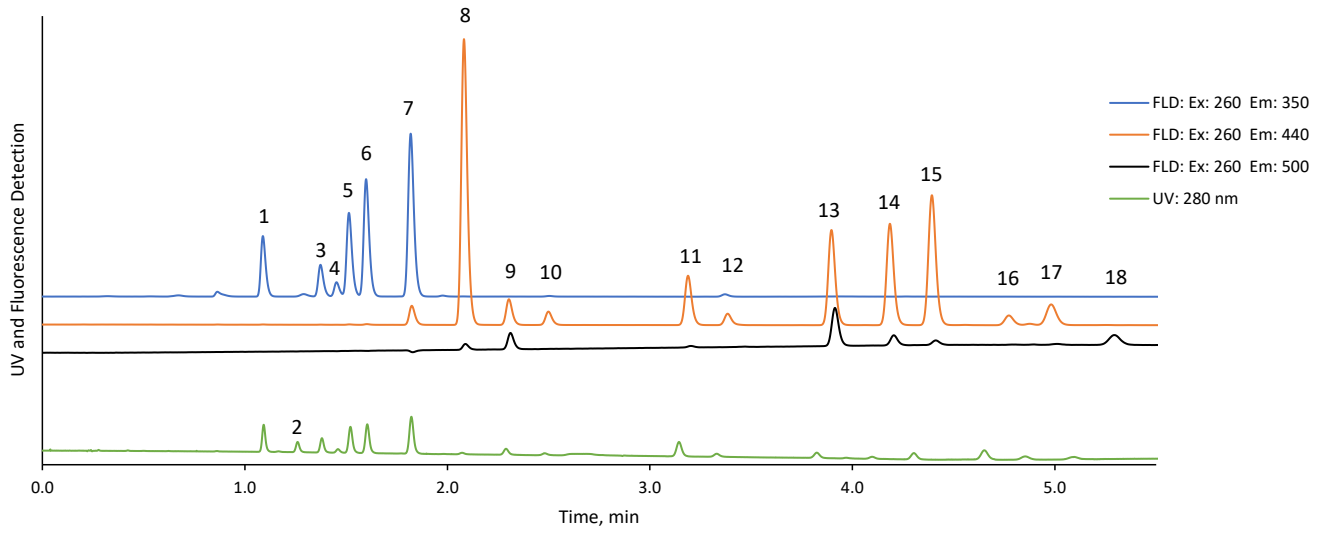
Injection Volume: 2.0 µL
Sample Solvent: Methanol (96%) Water (4%)
LC System: Agilent Triple Quadrupole LC/MS 6400

MS Conditions:
Gas Temp: 130 °C
Nebulizer: 25 psi
Gas Flow: 11 L/min
Sheath Gas Heater: 250 °C
Capillary: 3500 V
Data courtesy of STRIDE Center for PFAS Solutions

PAHs

In addition to methods for PFAS, EPA also has methods for polycyclic aromatic hydrocarbons (PAHs), which are a class of organic compounds that consist of multiple rings containing only carbon and hydrogen. These compounds are produced through natural events (e.g. petroleum seeps, forest fires, volcanic eruptions) or anthropogenically by incomplete combustion or high-pressure processes, such as burning coal, oil, gasoline, trash, tobacco, and wood. Cooking meat over a grill can also form PAHs. These compounds are ubiquitous and exposure to humans can cause irritation, mutation, and cancer. Due to the negative health effects, government agencies have established methods for detection and reporting PAHs, in which they are often screened in panels of 18 compounds or more by environmental laboratories using regulated HPLC methods with either UV or fluorescence detection. There are 100s of PAHs, but regulated methods do not include all of them, specifically alkylated versions, which are more toxic than the parent versions and heterocyclic aromatic compounds. With this is mind, more research and more comprehensive regulations are needed [5]. HALO® PAH columns with trifunctional C18 bonded phase have been specifically designed to provide fast, efficient, selective separations of PAH compounds. A rapid separation of the 16 compounds specified in EPA 610 and an additional 2 PAH compounds that are regularly analyzed is demonstrated in Figure 5.

Figure 5. Comparison of PAH Separations Detected by UV and Fluorescence Using a HALO® PAH column



TEST CONDITIONS

Column: HALO 90 Å PAH, 2.7 µm, 4.6 x 50mm
Mobile Phase A: Water
Mobile Phase B: Acetonitrile

Gradient:	Time	%B
	0.0	50
	4.0	100
	5.0	100
	6.0	100

Flow Rate: 1.8 mL/min
Pressure: 256 bar
Temperature: Ambient
Detection: FLD: Ex: 260/ Em: 350/440/500
UV: 280 nm
Injection Volume: 0.3 µL
Sample Solvent: Methanol
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

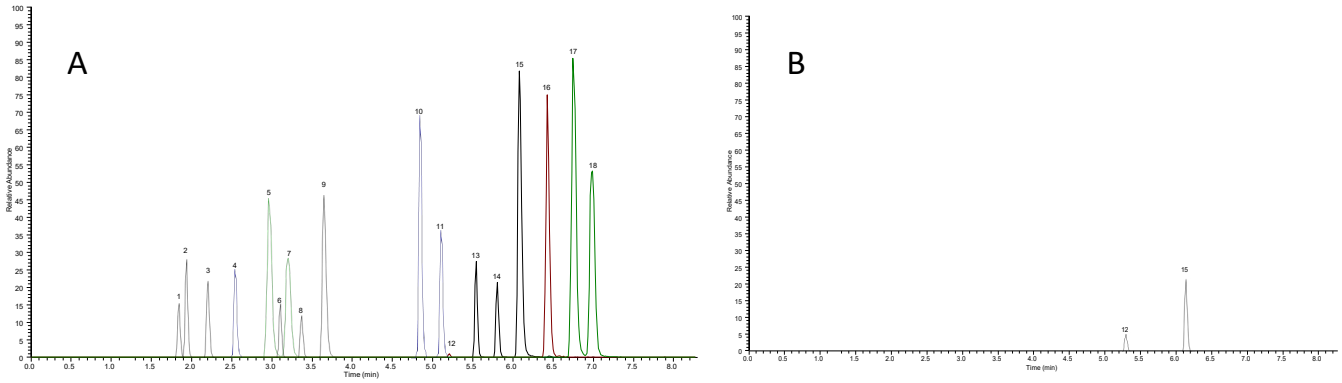
1. Naphthalene
2. Acenaphthylene
3. 1-methylnaphthalene
4. 2-methylnaphthalene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo[a]anthracene
12. Chrysene
13. Benzo[b]fluoranthene
14. Benzo[k]fluoranthene
15. Benzo[a]pyrene
16. Dibenzo[a,h]anthracene
17. Benzo[g,h,i]perylene
18. Indeno[1,2,3-c,d]pyrene

The advantage of using a fluorescence detector is its increased sensitivity, provided the compounds fluoresce. Notice that acenaphthylene (peak 2) does not fluoresce and is only visible in the green trace from the UV signal. The fluorescence detector's larger extra column volume (contributed from the flow cell) reduces the resolution between peaks 4 and 5 (2-methylnaphthalene and acenaphthene) compared to the UV separation, which has smaller extra column volume. This brings attention to LC system optimization and how an optimized system, including low volume flow cells, if available, can greatly assist with increased resolution.

Mass spectrometry can also be used for PAH analysis when compound identities are difficult to determine via retention times of standards or when standards are not available. Figure 6A shows the LCMS analysis of 18 PAHs from a solution of standards while Figure 3B shows the results of an extracted grilled steak sample.

Figure 6A. LCMS of 18 PAHs using a HALO® PAH column.

Figure 6B. LCMS of an extracted grilled steak sample.



PEAK IDENTITIES

Peak #	Compound	Precursor Ion	Fragment 1	Fragment 2
1	Naphthalene	128	78	102
2	Acenaphthylene	152	126	151
3	1-Methylnaphthalene	142	89	115
4	2-Methylnaphthalene	142	115	141
5	Acenaphthene	154	126	153
6	Fluorene	166	115	165
7	Phenanthrene	178	151	176
8	Anthracene	178	152	176
9	Fluoranthene	202	150	200
10	Pyrene	202	150	200
11	Benzo[a]anthracene	228	150	226
12	Chrysene	228	200	226
13	Benzo[b]fluoranthene	252	224	250
14	Benzo[k]fluoranthene	252	224	250
15	Benzo[a]pyrene	252	224	250
16	Dibenzo[a,h]anthracene	278	248	276
17	Benzo[ghi]perylene	276	248	274
18	Indeno[1,2,3-cd]pyrene	276	246	274

TEST CONDITIONS

Column: HALO 90 Å PAH, 2.7 µm, 2.1 x 100 mm
Mobile Phase A: Water/0.1% formic acid
Mobile Phase B: Acetonitrile/0.1% formic acid

Gradient:	Time	%B
	0.0	40
	5.0	100
	8.0	100
	8.01	40

Flow Rate: 0.4 mL/min
Pressure: 289 bar
Column Temperature: 30 °C
Injection Volume: 1 µL
Sample Solvent: Methanol
LC System: Shimadzu Nexera

MASS SPECTROMETRY CONDITIONS:

MS System: Thermo Scientific™ Q Exactive™ HF
ESI voltage: 5.5 kV
Heater Temp: 400 °C
Sheath gas: 35 (arbitrary units)
Aux gas: 8 (arbitrary units)
Tube lens voltage: 40 V

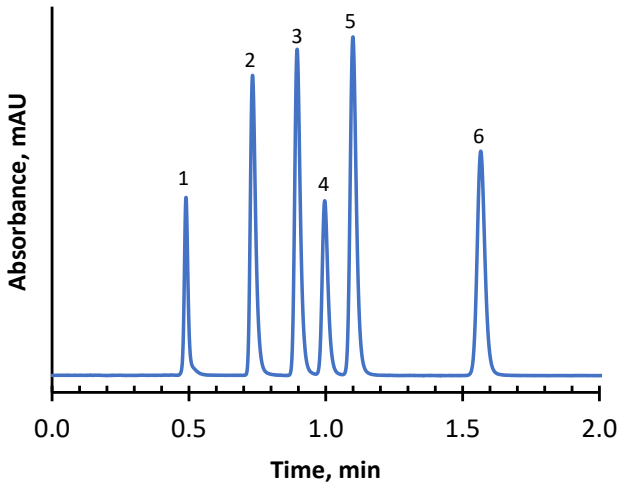
Calibration curves were run from 1 ppb – 100 ppb in order to quantitate the levels of PAHs detected in the extracted steak sample. The level of chrysene was determined to be 2.55 ppb and the level of benzo[a]pyrene was determined to be 1.98 ppb. Although these two PAHs were detected in the steak sample, the levels of both (individual and combined) were below the 5 ppb limit for benzo[a]pyrene by itself and less than the 30 ppb limit for the sum of benzo[a]pyrene, benzo[a] anthracene, benzo[b]fluoranthene, and chrysene set by the EU Commission Regulation (EC) No 1881/2006 for PAHs in key foodstuffs [6]. Up to this point, the U.S. Food and Drug Administration has not set any maximum limits for PAHs in food. Water, however, may not contain more than 0.2 ppb of benzo[a]pyrene according to the US EPA.

Pesticides

A never-ending source of environmental impact to humans, animals, and insects, particularly the honey bee population, is the prevalent use of pesticides, which are any substance or mixture whose purpose is to prevent, destroy, repel, or mitigate any pest. Some of the more well-known pesticides are insecticides, herbicides, rodenticides, and fungicides. Currently there are over 1000 pesticides used across the world. The US EPA is responsible for regulating pesticides and setting tolerances or Maximum Residue Limits (MRLs) which are the highest amount of a pesticide allowed to stay in or on a food. Different pesticides pose different health risks, which is why it is important to monitor the levels of these compounds in food for both animals and people. AMT offers several options for pesticide analysis. For nonpolar pesticides, HALO® C18 is recommended since it is a universal phase with excellent retention. For mixtures of polar and nonpolar pesticides, HALO® Biphenyl is recommended since it has increased retention over alkyl phases for polar compounds and is 100% aqueous compatible.

Neonicotinoids are systemic insect neurotoxins that are applied to seeds and protect seedlings from aphids and chewing insects [7]. These pesticides permeate the plant and are present in the nectar and pollen. The EU has banned outdoor use of four neonicotinoids (clothianidin, imidacloprid, thiamethoxam, and thiacloprid) because of their negative health risks to bees and reproductive toxicity to humans. Studies have shown that bees are not killed immediately, but die sooner than normal, are less healthy, have difficulty finding their way back to flowers and the hive, and lose their sense of smell, among other effects [8]. The US EPA has proposed continuing to allow the use of neonicotinoids with additional precautionary measures, which include requiring workers to wear additional personal protective equipment, regulations on when to apply the pesticides, advising homeowners against using neonicotinoids, and proposing to ban imidacloprid's use on residential lawns and turf because of health effects such as rashes and skin irritation, nausea, facial numbness and swelling, lethargy, and numbing and tingling on fingers and lips. HALO® C18 is successfully implemented as a solution for the fast analysis of neonicotinoids (Figure 7), which were detected using UV. The method conditions are also amenable to detection using a mass spectrometer.

Figure 7. Under 2-minute separation of six neonicotinoids on a HALO® C18 column.



PEAK IDENTITIES

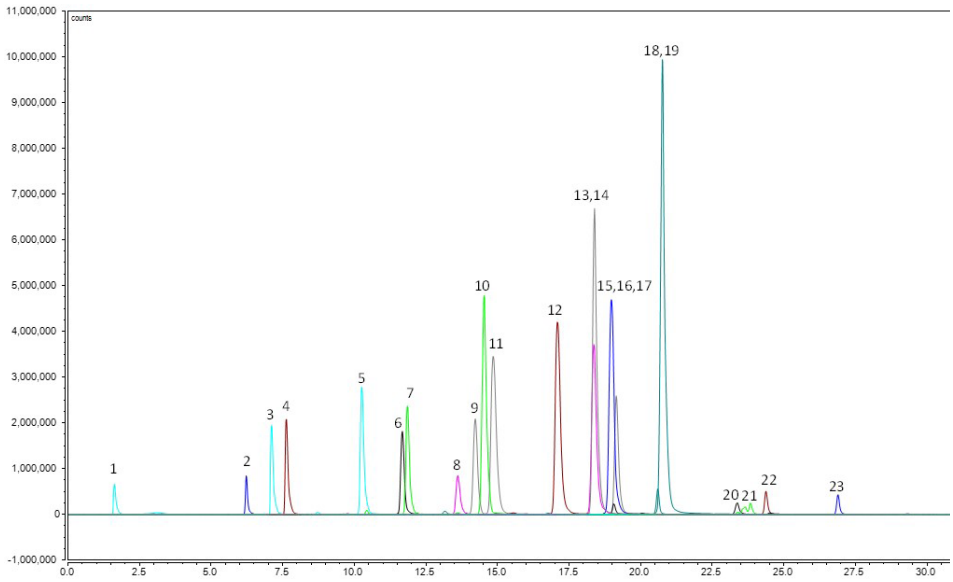
1. Nitenpyram
2. Thiamethoxam
3. Clothianidin4. Imidacloprid
5. Acetamiprid
6. Thiacloprid

TEST CONDITIONS

Column: HALO 90 Å C18, 2.7 µm, 3.0 x 100 mm
Mobile Phase: 70/30: A/B
Mobile Phase A: 0.1% Formic acid in water
Mobile Phase B: Acetonitrile
Flow Rate: 0.8 mL/min
Pressure: 252 Bar
Temperature: 35 °C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 50/50: Water/acetonitrile

Another option for pesticide analysis is the HALO® Biphenyl column. Being 100% aqueous compatible, it is an ideal choice for polar pesticides that require low or no organic initial gradient conditions. Figure 8 shows a mix of pesticides with a broad range of polarities separated on a HALO® Biphenyl column. These pesticides are typical of the ones screened in medical marijuana samples.

Figure 8. Separation of 23 pesticides ranging in polarities on a HALO® Biphenyl column.



TEST CONDITIONS

Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm
Mobile Phase A: Water/0.1% formic acid/4 mM ammonium formate
Mobile Phase B: Acetonitrile/0.1% formic acid/4 mM ammonium formate

Gradient:	Time (min.)	%B
	0.00	0
	1.01	15
	4.00	35
	5.00	62
	30.00	100
	34.00	100

Flow Rate: 0.2 mL/min
Pressure: 89 bar (initial)
Temperature: 40 °C
Injection Volume: 1 µL
Sample Solvent: Acetonitrile
Detection: + ESI

PEAK IDENTITIES

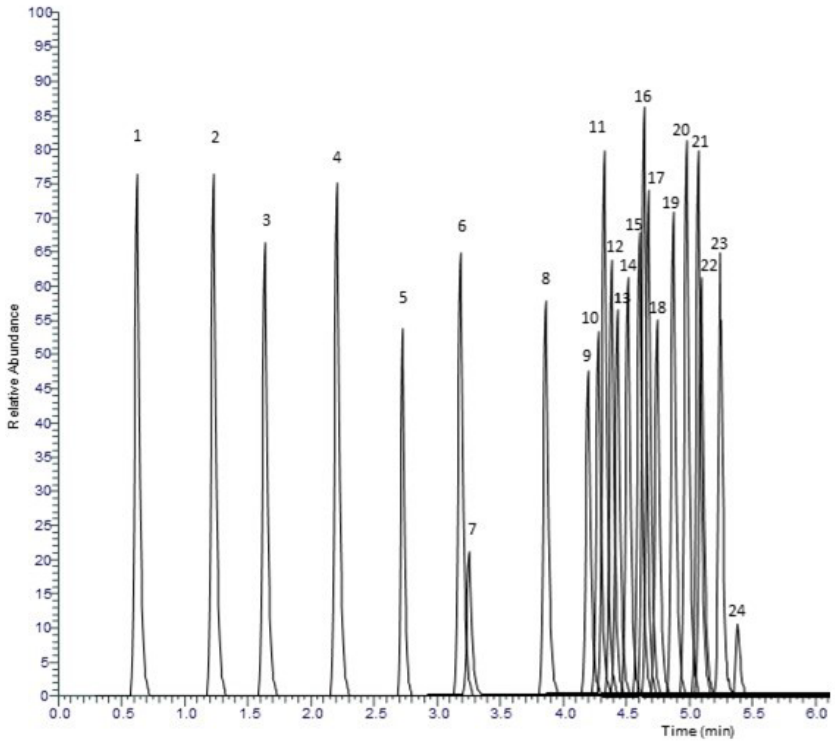
Peak #	Compound	m/z	Retention (min)
1	Daminozide	161.096	1.616
2	Flonicamid	230.000	6.224
3	Thiamethoxam	292.000	7.109
4	Imidacloprid	256.050	7.631
5	Paclobutrazol	294.130	10.256
6	Fenhexamid	302.079	11.678
7	Myclobutanil	289.129	11.849
8	Bifenazate	301.150	13.610
9	Dimethomorph Isomer 1	388.130	14.226
10	Spirotetramat	374.190	14.535
11	Dimethomorph Isomer 2	388.130	14.846
12	Spinosad A	732.480	17.089
13	Spinosad D	746.490	18.363
14	Trifloxystrobin	409.100	18.391
15	Spinetoram	748.520	18.970
16	Pyrethrin II	373.200	19.068
17	Piperonyl butoxide	356.240	19.151
18	Pyrethrin I	329.210	20.594
19	Etoxazole	360.180	20.759
20	Abamectin A	895.500	23.370
21	Cypermethrin	433.110	23.610
22	Bifenthrin	440.160	24.370
23	Acequinocyl	407.230	26.890
observed in negative ion mode	Fludioxonil	247.048	9.763

Mycotoxins

Mycotoxins gained much attention when 100,000 turkey chicks died near London after eating contaminated peanut meal in 1962. This is when the term was first coined even though mycotoxins had existed for ages. As secondary metabolites of fungi, mycotoxins are low molecular weight, toxic in low concentrations, and chemically and thermally stable during food processing. The major mycotoxins are aflatoxins, citrinin, ergot alkaloids, fumonisins, ochratoxins, patulin, trichothecenes, and zearalenone and are found in crops and food. Depending on the mycotoxin, they can be carcinogenic, teratogenic, hepatotoxic, mutagenic or have immunosuppresive effects [9]. Challenges to mycotoxin analysis include the presence of many isomeric compounds and the fact that the different mycotoxins have various chemistries [10].

The HALO® PFP column, known for its ability to resolve isomeric and isobaric compounds, is able to address the challenging mycotoxin separations and is suited for their fast analysis. See Figure 9. Two isobaric pairs of compounds were resolved: 15-acetyldeoxynivalenol and 3-acetyldeoxynivalenol (peaks 6 & 7), which are Type B trichothecenes; and aflatoxin M1 and aflatoxin G1 (peaks 10 & 11). With new mycotoxins being identified, the HALO® PFP column can be used for discovery experiments, as well as routine quantitation.

Figure 9. Rapid separation of 24 mycotoxins in less than 5.5 min using a HALO® PFP column.



TEST CONDITIONS

Column: HALO 90 Å PFP, 2 µm, 2 x 50 mm
Mobile Phase A: Water/2mM ammonium formate/0.1% Formic acid
Mobile Phase B: Methanol/2mM ammonium formate/0.1% Formic acid
Gradient:

Time	% B
0.01	15
1.0	25
2.0	40
2.50	41
4.50	100
5.50	100
5.51	15
6.50	Finished

Flow Rate: 0.4 mL/min
Initial Pressure: 485 bar
Temperature: 40 °C
Injection Volume: 1 µL
Sample Solvent: 95/5 water/methanol
Detection: +ESI MS/MS

PEAK IDENTITIES

Peak #	Compound	Retention Time	Precursor Ion	Product Ion
1	Nivalenol	0.71	313.1235	175.10
2	Deoxynivalenol	1.38	297.1335	249.09
3	Deoxynivalenol-3-glucoside	1.70	459.1850	193.10
4	Fusarenon X	2.37	355.1387	247.10
5	Neosolaniol	2.87	383.1702	365.16
6	15-Acetyldeoxynivalenol	3.33	339.1378	321.15
7	3-Acetyldeoxynivalenol	3.36	339.1378	231.15
8	Gliotoxin	3.97	327.0436	196.08
9	Aflatoxin G2	4.27	331.0759	312.97
10	Aflatoxin M1	4.39	329.0604	273.12
11	Aflatoxin G1	4.40	329.0601	242.90
12	Aflatoxin B2	4.44	315.0820	284.87
13	HT-2 + Na	4.47	447.1934	345.10
14	Diacetoxyscirpenol	4.49	367.2637	307.15
15	Aflatoxin B1	4.52	313.0662	286.99
16	Ochratoxin A	4.67	404.0855	238.99
17	T-2 +Na	4.72	489.2049	245.09
18	Ochratoxin B	4.88	370.1321	324.15
19	Citrinin	4.96	251.0860	233.09
20	Zearalenone	5.11	319.1491	283.08
21	Patulin +MEOH	5.11	187.0723	98.95
22	Fumonisin B1	5.24	722.3868	334.25
23	Fumonisin B3	5.41	706.3901	336.25
24	Fumonisin B2	5.44	704.3901	336.25

Conclusion

AMT is committed to providing solutions using Fused-Core® technology, which is well suited for environmental analysis. HALO® ENVIROCLASS provides a portfolio of selectivities and particle sizes designed for analysis of small molecules of interest to environmental scientists. In particular, HALO® PFAS and HALO® PAH are specifically quality assured using the relevant samples and methods for which they are designed. This ensures that every column will have reproducible retention profiles and peak widths critical for environmental analysis. Environmental chemists can rely on the rugged performance, rapid separations, and high throughput of HALO® ENVIROCLASS columns for solutions to challenging samples, including PFAS, PAHs, pesticides, mycotoxins, and other ecologically-related investigations.

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HALO®

TECHNICAL REPORT

TITLE: **HALO® ENVIROCLASS APPLICATIONS: THE ANALYSIS OF VETERINARY DRUGS, PESTICIDES AND ENVIRONMENTAL CONTAMINANTS**

MARKET SEGMENT: ENVIRONMENTAL

AUTHOR:

Andrew Harron Ph.D., Application Scientist

ABSTRACT

Many challenges exist in environmental and food safety analysis, including the number of potentially carcinogenic compounds that have been reported in our food and water. In the last 20 years, this number has grown significantly, and the nature of these compounds is becoming increasingly more complex. Pharmaceuticals and hormones used in veterinary medicine and animal husbandry and pesticides, are examples of compounds that are commonly found in the environment and food supply, and although regulated, these regulations are subject to revision on a constant basis. In addition, maximum allowable limits are decreasing and emerging environmental contaminants are now leaching into the food supply as well. This will require highly sensitive methods for detection and separation of these compounds for analysis. Here we present how using Fuse-Core® technology enhances this analysis for pesticides, emerging environmental contaminants and veterinary drugs, and can provide detection at limits below regulation.

INTRODUCTION

The scale of agriculture and food production in the United States of America (USA), is truly massive. In the USA, over 37% of the total land mass (or more than 900 million acres) is used for agricultural and livestock farming, and with over 2 million active farms, contributed $1.109 trillion to the U.S. gross domestic product (GDP) in 2019. On average, one farmer can produce enough food to feed over 150 people for the year.[1]

Veterinary drugs and hormones, are a complex group of substances that can be differentiated into various chemical classes and therapeutic areas, such as antivirals, antifungals, and antibiotics. The pharmaceuticals can further be differentiated based on their classifications, such as macrolides, quinolones, sulfonamides, benzimidazoles, tricyclines, and NSAIDs.[2-3] The primary use of the therapeutics is to promote animal growth and maintain good animal health, which lowers the potential of a transmission of a disease from animal to human. However, meat can be contaminated with large quantities of therapeutics as well as steroids, which can lead to many negative health effects for humans. In addition to carcinogenic risks of consuming high levels of hormone infused beef, the use of antibiotics can breed antibiotic-resistant microorganisms in animals, transferable to humans.[2-3]

The use of pesticides is critical for successful crop production, and in turn, the overall economic health of the country. Pesticides are used not only at various stages

KEY WORDS:

Environmental contaminants, veterinary drugs, HALO® Enviroclass

during the crop production cycle, but also during storage, and transportation as well. This creates numerous exposure points for toxic exposure of the residues remaining on vegetables, fruits, grain and cereals, and meats, resulting in negative health and environmental effects.[4-6] Another area of pesticide exposure is bodies of water, usually rivers, resulting from ground water contamination. Upon application to the crops, the pesticides leach into the aquifer and penetrate underground streams. These streams then drain into the rivers, thus providing exposure risks to both humans and animals.[6]

The continued use of the veterinary drugs and pesticides in farming has also led to an increase in the number of environmental contaminants that have proliferated through the food supply as well. Environmental contaminants often result from human waste and contamination, or from naturally occurring sources. One such example of contamination is PFAS. PFAS, a known environmental and water contaminant, has grown to such high levels in animals, plastics, and food packaging, that it can be considered as an emerging food contaminant. In addition, large scale contamination of waterways by pharmaceuticals, is also being detected at alarming levels.

Governmental agencies, including the World Health Organization (WHO), the U.S. Environmental Protection Agency (EPA), and the European Union (EU) have all issued statements to guide on pesticide usage and limits, while the Codex Alimentarius issues statements on the use of veterinary drugs on food-producing animals.[4-8] Maximum residue levels (MRLs), have been established as the highest level that is allowable on foodstuffs, when pesticides or veterinary drugs have been applied. In the United States, these levels are in the PPM range for agricultural products and meat, for tolerated pesticides. In the EU these levels are lower, with the average being 10 ppb for most pesticides except for explicitly prohibited compounds. In the case of veterinary drugs, the EU mandate is in the ppb range as well as the USA.[4-8]

These low levels require analysis that is capable of achieving high sensitivity, to ensure high quality data for meeting MRL requirements in complex food matrices. In addition, it is critical in an evolving situation such as food safety analysis to expect new pesticides, veterinary drugs and environmental contaminants, to be added to the regulations, and imposed limits to be decreased. Therefore, technology must not only meet the demands and regulations of today, but also be able to address the future regulations that will be imposed. Here we present the HALO® Biphenyl and HALO® C18 for the analysis of pesticides and Veterinary drugs, achieving lower levels of detection than stipulated by the EU and US EPA.

EXPERIMENTAL DATA:
Maximum resolution method

Experimental: Pesticides and Environmental contaminants

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Acetonitrile (HPLC grade), Methanol (HPLC grade), water (HPLC grade), formic acid, and ammonium formate were purchased from Millipore Sigma (Burlington, MA).

Samples: Pesticides and Environmental contaminants

Two experiments were performed, the first was a screening of a mixture 191 pesticides, containing both polar and non-polar pesticides to determine the suitability of the column for the analysis. The pesticide mixture was acquired from Millipore Sigma, at a concentration of 5ppm. The second experiment was the analysis of a mixture of environmental contaminants and pesticides that were spiked into egg samples. The pesticides and environmental contaminants were spiked into egg samples, at a concentration of 0.045 ng/mL, and then extracted via QuEChERSER method, and provided by the USDA for the analysis. Briefly, 2 g of sample, using liquid nitrogen, is extracted with 10 mL of 4/1 (v/v) acetonitrile/water for 10 min by shaking, followed by centrifugation for 3 min. A 200 µL portion was transferred to a mini-centrifuge tube and quickly evaporated under nitrogen flow, followed by addition of initial LC mobile phase solvent and ultracentrifuged for 5 min before analysis by LC- (MS/MS).

Column and Gradient: Pesticides and Environmental Contaminants:

Standard mix of 191 pesticides

Analytical Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm, **Part Number:** 92812-611, **Mobile Phase A**: 5mM ammonium formate, 0.1 % Formic Acid, **Mobile Phase B**: MEOH, 0.1% Formic Acid, 0.4 mL/min. 0-100 %B in 12min. 100 %B 12-16 min, 0 %B 16.10min, 20 End

Spiked Pesticides and Environmental contaminants

Analytical Column: HALO 90 Å Biphenyl, 2.7 µm, 2.1 x 100 mm, Part Number: 92812-611, Mobile Phase A: 5mM ammonium formate, 0.1 % Formic Acid, Mobile Phase B: ACN, 0.1% Formic Acid, 0.4 mL/min. 0-100 %B in 20min. 100 %B 20-22 min, 0 %B 22.10min, 25 End

MS source conditions: Pesticides and Environmental contaminants

Spray Voltage: 3.5 kV Nebulizing gas: 2 L/min Drying gas: 15 L/min DL temp: 250 ˚C Heat Block: 400 ˚C

Experimental: Veterinary Drugs

A Shimadzu LCMS-8040 triple quadrupole mass spectrometer was coupled to a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Acetonitrile (HPLC grade), water (HPLC grade), formic acid, and ammonium formate were purchased from Millipore Sigma (Burlington, MA).

Samples: Veterinary drugs

The veterinary drugs were spiked into egg samples, at a concentration of 0.045 ng/mL, and then extracted via QuEChERSER method, and provided by the USDA for the analysis. Briefly, 2 g of sample, using liquid nitrogen, is extracted with 10 mL of 4/1 (v/v) acetonitrile/water for 10 min by shaking, followed by centrifugation for 3 min. A 200 µL portion was transferred to a mini-centrifuge tube and quickly evaporated under nitrogen flow, followed by addition of initial LC mobile phase solvent and ultracentrifuged for 5 min before analysis by LC- (MS/MS).

Column and Gradient: Veterinary drugs:

Analytical Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm, **Part Number:** 92812-602, **Mobile Phase A:** Water, 0.1 % Formic Acid, **Mobile Phase B:** ACN, 0.1% Formic Acid, 0.4 mL/min. 10-100% B in 14 minutes. 100-100% B 14-16min, 10% B 16.10 min, 19.0 End

MS source conditions: Veterinary drugs:

Spray Voltage: 3.5 kV Nebulizing gas: 2 L/min Drying gas: 15 L/min DL temp: 275 ˚C Heat Block: 375 ˚C

Results Pesticides and Environmental Contaminants

Previous experimentation had shown the Biphenyl phase's combination of hydrophobic, aromatic, and polar selectivity, provided a fast separation of a pesticide mix consisting of 191 pesticides, showing good retention of both polar and non-polar pesticides. **Figure 1, Table 1** shows the screening of 191 pesticides in less than 16 minutes, and demonstrates the HALO® Biphenyl column to be an excellent choice for high throughput screening for environmental applications.

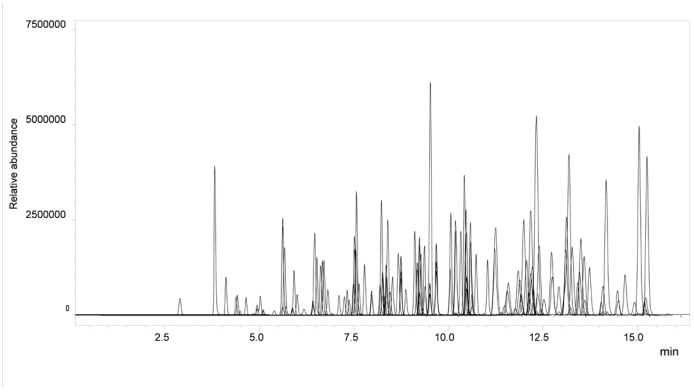

Figure 1. 191 pesticides detected by HALO® Biphenyl
See Table 1 on Page 6

Spiked Samples

Modifications to the gradient, including extending the time, were made to account for the mixture of environmental contaminants that were included in the analysis, and also for any possible unknown matrix effects that could potentially arise. The resulting chromatogram, **Figure 2**, shows the separation of 160 pesticides and environmental contaminants (**Table 2**) in less than 20 minutes.

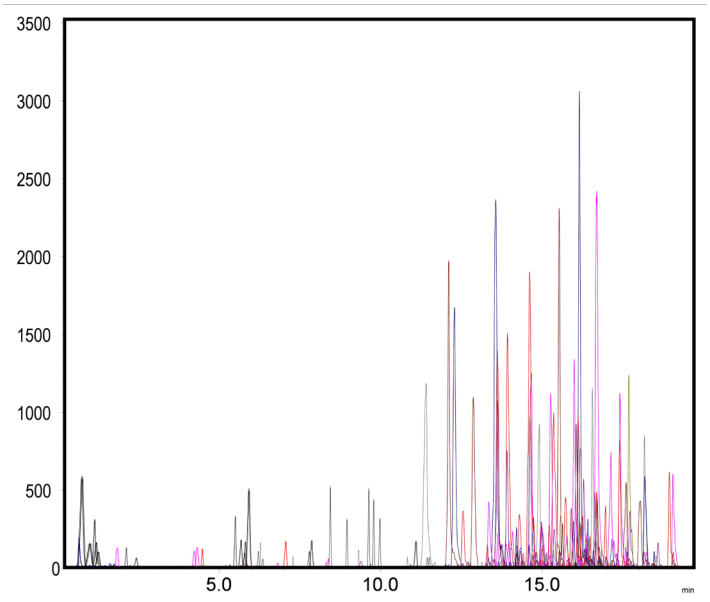

Figure 2. 160 pesticides and environmental contaminants in spiked egg samples

See Table 2 on Page 9

The concentration of the compounds was 0.045 ng/mL, which is significant as it is below the MRL levels established by both the EPA and the EU, demonstrating the sensitivity of the separation, with no matrix effects contributing to ion suppression evident.

Results: Veterinary Drugs

45 veterinary drugs were spiked into egg samples and subjected to QuEChERSER extraction and were obtained from the USDA. A C18 column was chosen for the analysis as it is a universal phase for acids, bases and neutral solutes and has excellent stability at low to mid mobile phase pH, which would provide ample retention for all compounds in the sample. **Figure 3**, shows 45 veterinary drugs detected and classified (**Table 3**) in just over 13 minutes on the HALO® C18. The concentration of the drugs was 0.045 ng/mL, which like the pesticides, is below the MRL recommended by the Codex Alimentarius.

The HALO® C18 enabled the separation and identification of a complex mix of veterinary drugs, including macrolides, quinolones, sulfonamides, benzimidazoles, tetracyclines, NSAIDs and 4 dye species which have also been used for therapeutic purposes in veterinary medicine. The high-speed separation is easily accomplished and can definitely find application in high throughput environments.

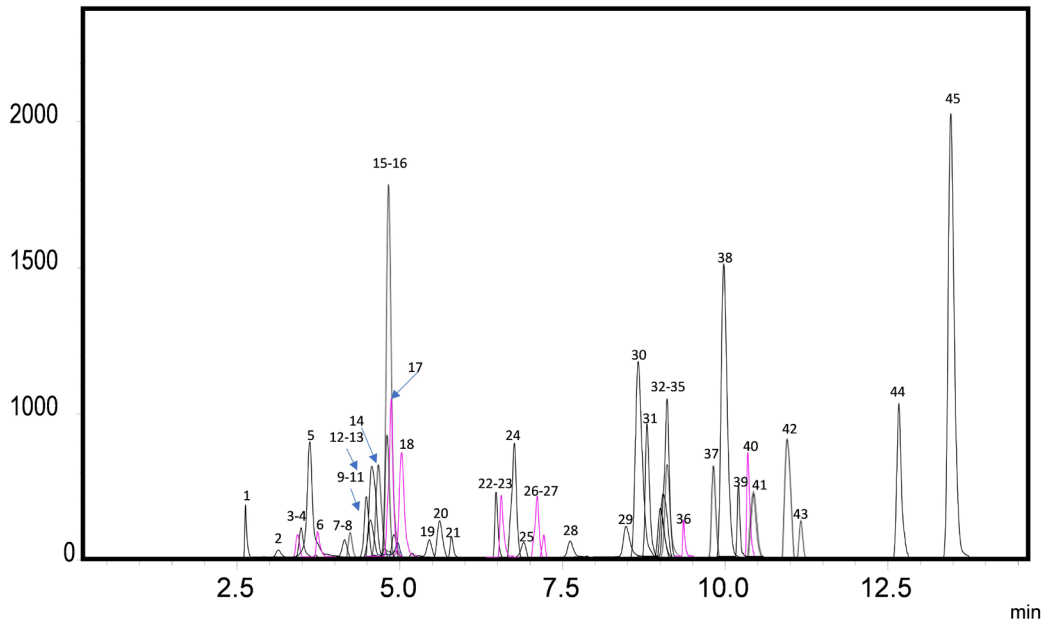

Figure 3. 45 veterinary drugs on HALO® C18
See Table 3 on Page 12

CONCLUSION:

Environmental and food safety analysis can be challenging for a number of reasons, including matrix effects which could lead to ion suppression, incomplete extraction from the various matrices, and difficult separations due to the chemical nature of the compounds. In addition to the current list of challenges, emerging contaminants are sure to add to these challenges and the technology will need to answer those challenges. Coupled with new sample prep technology such as QuEChERSER, HALO® Fused-Core® columns play a critical role in new environmental and food safety analysis workflows, enabling the detection of pesticides and veterinary drugs in food matrices below MRLs established by both the EU and EPA. HALO® columns enable fast, efficient separations covering a full range of compounds for food safety testing, and have the capability to exceed current testing limits, and are well equipped to handle future levels as they arise.

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TABLE 1 Pesticides transitions and retention times

Peak number	Name	m/z transition	Ret. Time
1	Cyromazine	167.0000>85.1000	2.894
2	Terbufos sulfone	321.1000>171.0000	3.427
3	Metoxuron	229.1000>72.0500	4.135
4	Propamocarb	189.0000>102.1000	4.530
5	Omethoate	214.0000>125.1000	4.840
6	Monolinuron	215.1000>126.1000	4.841
7	Simetryn	214.0000>124.0000	4.841
8	Butocarboxim sulfoxide	207.1000>75.1000	5.117
9	Butocarboxim	208.1000>75.1000	5.118
10	Aldicarb sulfoxide	207.1000>89.2000	5.119
11	Dinotefuran	203.2500>129.0500	5.157
12	Butoxycarboxim	223.0000>106.2000	5.700
13	Aldoxycarb	240.2000>86.1000	5.787
14	Flonicamid	230.1000>203.0000	5.867
15	Sebuthylazine	229.9000>174.0500	5.868
16	Atrazine-desisopropyl	173.8000>68.1000	6.174
17	Carbendazim	192.1000>160.1000	6.408
18	Pymetrozine	218.1000>105.1000	6.459
19	Oxamyl	237.1000>72.1000	6.721
20	Nitenpyram	271.0000>56.1500	6.747
21	Methomyl	162.8000>106.0000	6.807
22	Oxydemeton-methyl	247.0000>169.0000	6.920
23	Clothianidin	250.1000>132.0000	7.243
24	Demeton-s-methyl sulfone	262.8000>169.0000	7.352
25	Fuberidazole	185.0000>157.0500	7.458
26	Fenuron	164.9000>72.0500	7.505
27	Thiabendazole	202.1000>175.0000	7.547
28	Cyproconazole Isomer	292.0000>70.0000	7.708
29	3-hydroxycarbofuran -21	255.1000>163.1000	7.963
30	Ethidimuron	264.9000>114.0000	8.188
31	Chloridazon	222.1000>104.1000	8.241
32	Ethirimol	210.1000>98.0500	8.363
33	Dioxacarb	224.0000>123.1000	8.392
34	Methiocarb	226.1000>169.1000	8.395
35	Vamidothion	288.0000>146.0500	8.447
36	Cymoxanil	199.1000>128.0000	8.517
37	Ametryn	242.1000>122.1000	8.669
38	Mesurol sulfoxide	242.1000>122.1000	8.669
39	Terbutryn	242.1000>186.1000	8.671
40	Imidacloprid	256.1000>175.0000	8.871
41	Oxycarboxin	268.1000>175.0000	9.137
42	Monuron	199.1000>72.0500	9.170
43	Cycluron	199.1000>72.0500	9.171
44	Methiocarb-sulfone	258.1000>122.1000	9.230
45	Metolcarb	166.1000>109.0000	9.267
46	Thidiazuron	221.1000>102.1000	9.378
47	Diuron	232.8000>72.1000	9.675
48	Fluometuron	233.1000>72.1000	9.677
49	Propoxur	210.1000>111.0000	10.059
50	Fenthion sulfoxide	295.1000>280.0000	10.169
51	Imazamethabenz-methyl	289.1000>144.0000	10.186
52	Myclobutanil	289.0000>70.0000	10.194
53	Bendiocarb	224.1000>109.1000	10.194
54	Chlorotoluron	212.9000>72.0000	10.325
55	Terbumeton	226.1000>170.1000	10.355
56	Propargite	368.3000>231.2000	10.358
57	Pyracarbolid	218.1000>125.1000	10.415
58	Thiacloprid	253.0000>126.0500	10.462
59	Forchlorfenuron	248.1000>129.0000	10.473
60	Methabenzthiazuron	222.1000>165.2000	10.488
61	Carbofuran	222.2000>165.1000	10.489

Peak number	Name	m/z transition	Ret. Time
62	Quinoclamine	208.0000>89.0000	10.515
63	Isoprocarb	194.1000>95.0000	10.576
64	Carbaryl	202.1000>145.1000	10.589
65	Metobromuron	259.0000>148.0500	10.623
66	Benoxacor	260.0000>149.1000	10.630
67	Buturon	237.1000>84.1000	10.731
68	Isoproturon	207.0000>72.1500	10.747
69	Sulfentrazone	387.0000>309.0000	10.789
70	Ethiofencarb	226.0000>107.1000	10.831
71	Naptalam	292.1000>144.1000	10.875
72	Thiobencarb	258.0000>125.0000	10.894
73	Tepraloxydim	342.2000>250.1500	10.963
74	Spiroxamine	298.0000>144.1500	11.083
75	Carboxin	236.0000>143.0500	11.087
76	Tebuthiuron	229.1000>172.4000	11.090
77	Fenpropimorph	304.2000>147.1000	11.266
78	Linuron	249.0000>159.9500	11.276
79	Fenobucarb	208.0000>95.1000	11.304
80	Siduron	233.3000>94.0000	11.377
81	Penconazole	284.1000>70.0000	11.393
82	Ethiprole	396.9500>350.8500	11.402
83	Ethoxyquin	218.0000>174.0500	11.452
84	Desmedipham	318.0000>182.5000	11.496
85	1-Dodecylguanidine	228.1000>71.1000	11.517
86	Phenmedipham	318.1000>168.0000	11.602
87	Disulfoton sulfoxide	291.0000>213.0000	11.612
88	Halofenozide	331.1000>105.0000	11.636
89	Azamethiphos	325.0000>183.0000	11.636
90	Promecarb	208.1000>109.0000	11.753
91	Thifensulfuron-methyl	388.1000>167.1000	11.798
92	Diethofencarb	268.2000>226.1000	11.802
93	Tridemorph	298.1000>130.1000	11.814
94	Flurtamone	334.1000>247.0500	11.950
95	Tebufenpyrad	334.0000>145.0000	11.950
96	Fenthion sulfone	311.0000>109.0000	11.956
97	Cyprodinil	226.0000>93.0000	11.960
98	Pencycuron	329.2000>125.1000	11.961
99	Fomesafen	456.1000>344.0000	12.044
100	Iprovalicarb	321.2000>119.2000	12.131
101	Flutolanil	324.1000>242.0000	12.154
102	Chlorantriniliprole	484.1000>452.9000	12.251
103	Trinexapac-ethyl	253.2000>69.0000	12.252
104	Neburon	275.1000>88.1000	12.257
105	Isoxaflutole	360.1000>251.1000	12.308
106	Benalaxyl	326.2000>294.1000	12.316
107	Chloroxuron	291.1000>72.1000	12.407
108	Dimethametryn	256.1000>186.0500	12.409
109	Fenazaquin	307.1000>161.0000	12.439
110	Terbufos-sulfoxide	305.1000>186.8000	12.444
111	Ethofumesate	287.1000>258.9000	12.449
112	Fenamidone	312.1000>92.1000	12.493
113	Clethodim	360.0000>164.0500	12.528
114	Piperonyl butoxide	356.2000>177.2000	12.554
115	Boscalid	343.0000>307.0000	12.568
116	Methoxyfenozide	369.2000>149.0000	12.585
117	Bioresmethrin	339.2000>171.0500	12.619
118	Hydramethylnon	495.2000>323.2000	12.632
119	Rimsulfuron	432.1000>182.0000	12.698
120	Fenchlorphos oxon	304.9000>109.0000	12.699
121	Tralkoxydim	330.2000>284.1500	12.720
122	Epoxiconazole	330.1000>121.1000	12.721
123	Ipconazole Isomer	334.2000>70.0000	12.827
124	Thiofanox	219.2000>57.2000	12.834
125	Fenbuconazole	337.0000>124.9000	12.909
126	Zoxamide	336.1000>187.0000	12.910
127	Benthiazole	239.0000>179.9500	12.922
128	Isoxaben	333.2000>165.0000	13.019
129	Metconazole	320.2000>70.0500	13.032
130	Triflumuron	359.1000>156.0000	13.057
131	Mandipropamid	412.2000>328.1500	13.071

Peak number	Name	m/z transition	Ret. Time
132	Isoprothiolane	291.0000>230.9500	13.084
133	Acibenzolar-s-methyl	210.9000>136.0000	13.166
134	Cyflufenamid	413.2000>295.1000	13.247
135	Dimethomorph	388.2000>301.1000	13.266
136	Flutriafol	302.1000>70.1000	13.278
137	Fenoxycarb	302.2000>116.0000	13.284
138	Spirotetramat	374.3000>302.1500	13.301
139	Novaluron	491.1000>471.1000	13.308
140	Fluquinconazole	376.1000>349.0500	13.393
141	Spinosad (Spinosyn A)	732.5000>142.2000	13.430
142	Bensulfuron-methyl	411.2000>149.1000	13.439
143	Cyazofamid	325.1000>108.0000	13.485
144	Carfentrazone-ethyl	412.1000>346.0000	13.515
145	Pinoxaden	401.2000>317.2000	13.527
146	Picoxystrobin	368.1000>145.0000	13.570
147	Pyraflufen-ethyl	413.1000>339.0000	13.610
148	Phoxim	299.0000>77.1000	13.632
149	Fenothiocarb	254.1000>72.1000	13.634
150	Mefenacet	298.9000>148.0500	13.636
151	Triflusulfuron-methyl	493.2000>264.1000	13.659
152	Azoxystrobin	404.2000>372.1000	13.724
153	Hexaflumuron	462.8000>158.1000	13.726
154	Chlorimuron-ethyl	415.1000>186.0000	13.746
155	Haloxyfop-methyl	376.0500>316.0000	13.769
156	Lufenuron	509.0000>339.0000	13.782
157	Metaflumizone	507.2000>178.0000	13.788
158	Kresoxim-methyl	313.9500>267.3000	13.844
159	Anilofos	368.2000>125.0000	13.963
160	Tetraconazole	372.1000>159.0000	13.964
161	Sethoxydim	328.1000>296.3000	14.062
162	Famoxadone	392.0000>331.1000	14.078
163	Teflubenzuron	381.1000>141.2000	14.082
164	Clofentezine	303.0000>138.0000	14.122
165	Haloxyfop-etotyl	434.0500>315.9000	14.186
166	Trifloxystrobin	409.1000>186.1000	14.197
167	Pretilachlor	312.0000>252.1000	14.237
168	Diflubenzuron	328.0000>141.0000	14.265
169	Diclobutrazol	328.2000>70.2000	14.266
170	Fluoxastrobin	459.1000>427.1000	14.287
171	Flufenoxuron	489.1000>158.1000	14.374
172	Metrafenone	409.1000>209.0500	14.403
173	Piperophos	354.1000>170.9000	14.425
174	Fenoxaprop-ethyl	362.1000>288.1000	14.475
175	Pyraclostrobin	390.1000>194.1000	14.483
176	Benzoximate	364.0000>199.0000	14.560
177	Diniconazole	326.2000>70.2000	14.633
178	Isocarbophos	307.0000>121.1000	14.721
179	Spiromesifen	371.3000>273.2000	14.724
180	Chlorfluazuron	540.1000>383.0000	14.744
181	Chlorthiophos	360.7500>304.9000	14.761
182	Furathiocarb	383.2000>195.1000	14.772
183	Pyriproxyfen	322.0000>96.0000	14.821
184	Chinomethionate	235.0000>207.0500	14.833
185	Spirodiclofen	411.2000>71.1000	15.001
186	Propaquizafop	444.2000>100.1000	15.117
187	Avermectin B1a.	890.5000>567.5000	15.265
188	Rotenone	395.2000>213.1000	15.267
189	Fenpyroximate	422.3000>366.2000	15.339
190	Cyphenothrin	376.2000>181.0000	15.351
191	Phenothrin	351.2000>183.0000	15.423

TABLE 2 Pesticides and Environmental Contaminants Trasnitions and Retention Times

ID#	Name	m/z	Ret. Time
1	Mercapto -Methylimidazole	114.8800>57.1000	0.449
2	Dimetridazole hydroxy	158.0000>140.1000	0.544
3	Diuron	232.9400>72.0000	0.557
4	Daminozide	161.0100>143.0000	0.669
5	Ketoprofen	255.1000>77.0000	0.729
6	Propanil	218.0000>162.1000	0.921
7	Nalidixic Acid	233.1000>215.1000	1.095
8	Methamidophos	141.9000>94.0000	1.739
9	Methomyl	163.0200>106.0000	2.163
10	Niflumic Acid	283.0000>265.0000	2.441
11	Acephate	184.0000>143.1000	2.515
12	Aldicarb sulfoxide	207.0200>132.1000	4.269
13	Dinotefuran	203.0600>129.1000	4.376
14	Omethoate	214.0000>182.9000	4.552
15	Quinclorac	241.9000>224.0000	5.051
16	Flonicamid	230.0200>203.1000	5.758
17	Aldicarb sulfone	223.0200>86.1000	5.812
18	Salbutamol	240.2000>148.1000	5.842
19	Ipronidazole hydroxy	186.0000>168.0000	6.089
20	Pymetrozine	217.9900>104.9000	6.104
21	Carbendazim	192.0000>160.1000	6.219
22	Flunixin	297.0000>279.0000	6.641
23	Nitenpyram	271.0000>126.0000	6.969
24	OxamylNH4	237.0100>72.0000	7.051
25	Oxydemeton Methyl	246.9300>169.1000	7.348
26	Clothianidin	250.0000>169.2000	7.705
27	AldicarbNH4	208.1000>116.1000	7.821
28	Ciprofloxacin	332.1000>314.1000	8.318
29	Dicrotophos	238.0000>112.0000	8.371
30	Thiamethoxam	292.0100>211.1000	8.426
31	Dimethoate	229.9500>199.1000	8.843
32	Cymoxanil	199.0000>128.1000	9.295
33	SulfoxaflorNH4	294.9700>174.1000	9.396
34	Atrazine	216.0300>174.1000	9.398
35	Meclofenamic Acid	296.0000>278.0000	9.681
36	Imidacloprid	255.9400>209.0000	9.987
37	Xylazine	221.0000>164.0000	10.047
38	Mercapto benzimidazole	150.9600>93.0000	10.399
39	Dichlorvos	220.9000>109.0000	10.762
40	Acetamiprid	223.0100>126.0000	10.948
41	Cyprodinil	226.0500>93.1000	11.113
42	Tebuthiuron	229.0000>172.3000	11.389
43	Morantel	221.1000>123.0000	11.395
44	Imazethapyr	290.0200>245.1000	11.445
45	Trimethoprim	291.1000>230.0000	11.467
46	Diflufenzopyr	335.0000>206.2000	11.574
47	Metalaxyl	280.0100>220.2000	11.796
48	Carbofuran	222.0000>123.0000	12.106
49	Thiacloprid	252.9800>126.2000	12.282
50	Imazalil	296.9700>159.1000	12.551
51	Albendazole Sulfone	298.0000>159.0000	12.561
52	Fenbufen	255.1000>181.1000	12.675
53	Flunixin-d3	300.0000>282.0000	12.785
54	Thiophanate Methyl	343.0200>151.0000	12.871
55	C-lencyclohexerol	319.1000>301.0000	12.986
56	Propyphenazone	231.1000>189.1000	13.135
57	Linuron	248.9000>160.1000	13.304
58	2-Aminoflubendazole	256.0000>123.0000	13.347
59	Fenobucarb	208.0500>95.0000	13.355
60	Fosthiazate	283.9800>228.0000	13.513
61	Dodemorph	282.2000>116.1000	13.556
62	Azamethiphos	324.9000>183.0000	13.613
63	Ethiprole	398.9000>352.9000	13.626
64	EthiproleNH4	413.9000>351.0000	13.641
65	Pronamide	256.0000>190.0000	13.716

ID#	Name	m/z	Ret. Time
66	Pyrimethanil	200.1000>107.2000	13.739
67	Paclobutrazol	294.0300>70.0000	13.914
68	Norflurazon	303.9000>284.0000	13.936
69	Cyantraniliprole	475.1000>286.0000	13.956
70	Triadimenol	296.0000>70.1000	14.074
71	Methiocarb	226.0100>169.0000	14.209
72	Etoxazole	360.1700>141.0000	14.222
73	Chlorsulfuron	357.9000>167.1000	14.234
74	Triasulfuron	401.9800>167.0000	14.299
75	Fenthion Sulfone	311.0000>125.0000	14.328
76	Mabuterol	311.1000>237.0000	14.576
77	Fluxapyroxad	382.0000>362.1000	14.591
78	Iprovalicarb	321.1000>119.0000	14.611
79	Fluopyram	396.9800>208.0000	14.619
80	Flutolanil	324.0000>242.1000	14.652
81	Chlorantraniliprole	484.0000>452.9000	14.715
82	Fenhexamid	302.0600>96.9000	14.732
83	Myclobuthanil	289.0100>70.0000	14.903
84	Penthiopyrad	360.0400>276.1000	14.961
85	Tetraconazole	372.0000>159.1000	14.987
86	Fenamidone	312.0000>236.2000	15.003
87	Saflufenacil	501.0000>349.0000	15.014
88	Boscalid	343.0000>307.1000	15.092
89	Clethodim	360.0000>164.0000	15.123
90	Ethoprophos	243.0600>172.9000	15.222
91	Methidathion	302.8800>144.9000	15.233
92	Methoxyfenozide	369.1000>149.1000	15.266
93	Fenarimol	330.9000>268.1000	15.309
94	Hexaconazole	315.9900>69.9000	15.353
95	Thiodicarb	354.9600>88.0000	15.361
96	Tebuconazole	308.0200>70.0000	15.375
97	Fenoterol	304.1000>107.2000	15.491
98	Fenamiphos	304.0000>217.1000	15.524
99	Diflubenzuron	310.9600>158.0000	15.536
100	Penconazole	285.9500>70.0000	15.568
101	Flufenacet	363.9500>194.1000	15.582
102	Bifenazate	301.1000>198.1000	15.726
103	Penoxsulam	484.0000>195.2000	15.758
104	Benzovindiflupyr	398.0500>342.0000	15.897
105	Flusilazole	315.9900>247.0000	15.965
106	Epoxiconazole	330.0000>121.1000	15.991
107	Dimethomorph	388.0600>301.0000	16.048
108	Phosmet	318.0100>160.0000	16.114
109	Fenoxycarb	302.0000>116.0000	16.145
110	Triazophos	313.9200>162.0000	16.152
111	Spirotetramat	374.1200>302.1000	16.161
112	Diazinon	305.0000>169.1000	16.176
113	Spiromesifen	388.1100>273.2000	16.237
114	Fenbuconazole	337.0200>125.0000	16.238
115	Bitertanol	338.1100>269.1000	16.367
116	Cyazofamid	324.9000>107.9000	16.372
117	Tolylfluanid	347.0000>137.0000	16.377
118	Novaluron	493.0100>158.2000	16.396
119	Tetrachlorvinphos	364.9000>127.0000	16.414
120	Triflumizole	346.0500>277.9000	16.473
121	Chlorfenvinphos	358.9000>155.1000	16.476
122	Isofenphos	346.0100>245.1000	16.531
123	Phorate	260.9300>74.9000	16.554
124	Picoxystrobin	368.0000>145.1000	16.556
125	Propiconazole	342.0700>159.1000	16.564
126	Pyraflufen Ethyl	412.9000>339.0000	16.633
127	Pirimiphos Methyl	305.9000>164.2000	16.686
128	Azoxystrobin	404.0400>372.1000	16.686
129	Chlorimuron Ethyl	414.9680>186.0000	16.699
130	Disulfoton	274.9500>88.9000	16.834
131	Fenthion	279.0000>247.0000	16.838
132	Tebufenpyrad	334.0900>145.1000	16.954
133	Prallethrin	301.0500>123.1000	16.975

ID#	Name	m/z	Ret. Time
134	Spinetoram	748.4000>142.2000	17.118
135	Prochloraz	375.9000>308.2000	17.16
136	Profenofos	372.9000>302.8000	17.162
137	Chlorpyriphos Methyl	321.9000>125.0000	17.206
138	Clofentezine	303.0000>138.0000	17.322
139	Fluoxastrobin	459.0000>427.0000	17.396
140	Trifloxystrobin	409.1000>186.1000	17.411
141	Malachite Green leuco	331.2000>239.1000	17.577
142	Difenoconazole	406.0000>250.9000	17.584
143	Phosalone	367.9000>182.1000	17.631
144	Piperonyl Butoxide	356.1100>177.2000	17.687
145	Pyraclostrobin	388.1000>194.2000	17.699
146	Fenoxaprop Ethyl	361.9800>288.0000	17.716
147	Indoxacarb	527.9000>248.8000	17.751
148	Quizalofop Ethyl	373.0000>299.1000	18.024
149	Crystal Violet leuco	374.2000>238.2000	18.168
150	Pyriproxyfen	322.0600>95.9000	18.172
151	Pyrazophos	374.0100>222.0000	18.186
152	Coumaphos	362.8000>227.0000	18.217
153	PropargiteNH4	368.1000>231.2000	18.245
154	Hexythiazox	353.0100>228.1000	18.431
155	Spirodiclofen	411.1000>313.0000	18.463
156	Acequinocyl	357.2000>329.3000	18.521
157	Fenpropathrin NH4	367.1100>125.0000	18.586
158	Fenpyroximate	422.2000>366.1000	18.934
159	Phenothrin	351.0800>183.0000	19.048
160	Pyridaben	365.0500>309.0000	19.054

TABLE 3 Veterinary Drugs Transition Times and Retention Times

Peak id	Drug	Transition	Retention Time	Classification
1	Ciprofloxacin	332.1000>314.1000	2.515	Quinolones
2	Sulfathiazole	256.0000>92.0000	3.021	Benzimidazoles
3	Lincomycin	407.2000>126.1000	3.334	Quinolones
4	Sulfa pyridine	250.1000>184.0000	3.341	Sulfonamide
5	Albendazole-2-amino	240.0000>133.1000	3.582	Benzimidazoles
6	Trimethoprim	291.1000>230.0000	3.641	Quinolones
7	Ormetoprim	275.1000>123.1000	4.228	Quinolones
8	Tetracycline	445.1000>410.1000	4.234	Tetracycline
9	Enrofloxacin	360.1000>342.1000	4.524	Quinolones
10	Danofloxacin	358.1000>340.0000	4.532	Quinolones
11	Sulfaclozine	285.0000>156.0000	4.534	Sulfonamide
12	Sulfachloropyridazine	285.0100>92.0000	4.548	Sulfonamide
13	Sulfamerazine	265.0000>108.0000	4.591	Sulfonamide
14	Diclofenac	296.0000>214.0000	4.625	NSAID
15	Difloxacin	400.1000>382.1000	4.941	Quinolones
16	Amoxicillin	366.0000>113.9000	5.015	Macrolide
17	Chlortetracycline	479.1000>444.0000	5.027	Tetracyline
18	Sulfadoxine	311.0000>92.0000	5.283	Sulfonamide
19	Sulfaethoxypyridazine	295.0000>140.1000	5.542	Sulfonamide
20	Penicillin G	335.0000>159.9000	5.626	Macrolide
21	Neospiramycin 2H	350.2000>174.2000	5.858	Macrolide
22	Spiramycin	422.4000>174.2000	6.521	Macrolide
23	Sulfadimethoxine	311.1000>108.0000	6.527	Sulfonamide
24	Albendozole Sulfoxide	282.1000>208.0000	6.638	Benzimidazoles
25	Albendazole Sulfone	298.0000>159.0000	6.669	Benzimidazoles
26	Sulfaquinoxaline	301.1000>156.0000	7.027	Sulfonamide
27	Phenylbutazone	309.1000>120.1000	7.106	NSAID
28	Tilmicosin	435.4000>174.1000	7.527	Macrolide
29	Flumequin	262.0000>244.1000	8.508	Quinolones
30	Nalidixic Acid	233.1000>215.1000	8.542	Quinolones
31	Oxolinic Acid	261.9000>244.0000	8.646	Quinolones
32	Kitasamycin	772.3000>174.2000	9.015	Macrolide
33	Tylosin	916.5000>174.1000	9.018	Macrolide
34	Florfenicol Amine	248.0000>230.1000	9.051	Sulfonamide
35	Erythromycin A	734.4000>576.4000	9.122	Macrolide
36	Malachite Green	329.2000>313.2000	9.389	Dye
37	Albendazole	266.0000>234.0000	9.829	Benzimidazoles
38	Cloxacillin	436.0000>277.0000	10.031	Macrolide
39	Dicloxacillin	470.0000>160.0000	10.081	Macrolide
40	Crystal Violet leuco	374.2000>238.2000	10.363	Dye
41	Crystal violet	372.2000>356.2000	10.452	Dye
42	Brilliant Green	385.2000>341.1000	11.001	Dye
43	Dapsone	249.0000>156.0000	11.110	Sulfonamide
44	Carprofen	274.0000>228.1000	12.602	NSAID
45	Ivermectin	897.6000>240.1000	13.142	Macrolide

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TECHNICAL REPORT

TITLE: **LC-MS METHOD DEVELOPMENT AND COLUMN SCREENING FOR PHARMACEUTICAL AND PERSONAL CARE PRODUCTS (PPCPS) IN THE ENVIRONMENT**

MARKET SEGMENT: ENVIRONMENTAL

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ABSTRACT:

Pharmaceutical and personal care products (PPCPs) have been a growing concern to our environment which include prescription and over-the-counter medications, veterinary drugs, soaps, lotions, and even insect repellents. These products have entered the environment through various sources which permeate the water table, contaminating wastewater, ground water, and even drinking water. Validated LC-MS methods have been completed in order to screen for these wide range of chemical compounds which can further be optimized in order to achieve better resolution and selectivity. LC-MS method development is performed based on the EPA 542 PPCP method in order to achieve an improved chromatographic resolution and selectivity for environmental applications.

INTRODUCTION:

Pharmaceutical and personal care products that are a concern to the environment range from a wide variety of compounds and come from a variety of different sources. PPCPs include prescription and non-prescription human drugs, illegal drugs, and veterinary drugs, as well as their subsequent metabolites and conjugates, including antibiotics, hormones, anticonvulsants, antidepressants, lipid regulators, antihypertensives, and nonsteroidal anti-inflammatory drugs. PPCPs also include sunscreen, soaps, moisturizers, lipsticks, fragrances, insect repellent, and shampoo.[1] There are many different ways that these chemicals can enter the environment. Whether through a manufacturing process, aquaculture treatments, inappropriate disposal of unused medicine, treatment of animals (pets), and livestock treatments these chemicals eventually enter the soil or wastewater treatment plants which then leads to receiving water. Figure 1 represents the variety of sources where these chemicals can come from.

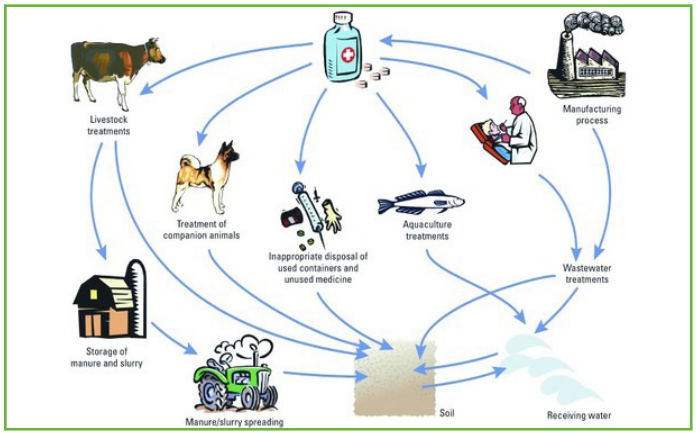


Figure 1: Common sources of PPCP in the environment (nih.gov)

KEY WORDS:

pharmaceutical, personal care products, superficially porous particles, HPLC

These broad range of chemical compounds lead to a wide variety of chemical structures and can make it challenging to analyze, especially at very low levels around the parts per trillion (ng/L) range. Because of this, LC-MS detection is needed. Choosing the right method conditions such as mobile phases, acidic modifiers, gradient, and column selection can all lead to an overall better separation. Column screening was performed in order to choose the best stationary phase along with the use of method optimization software to further improve the method.

EXPERIMENTAL DATA:

Method development is based on the EPA method 542: Determination of Pharmaceuticals and Personal Care Products in Drinking Water by Solid Phase Extraction and Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). This method screens twelve common PPCP compounds that are listed in Figure 2.

Analyte	Drug Category	Use
Carbamazepine	Anticonvulsant	can treat seizures, nerve pain, and bipolar disorder
Diazepam	Anxiolytic and Sedative	can treat anxiety, muscle spasms, and seizures
Diclofenac (sodium salt)	NSAID	can treat pain, migraines, and arthritis
Enalapril (maleate salt)	ACE inhibitor medication	can treat high blood pressure, diabetic kidney disease, and heart failure
Erythromycin	Antibiotics and Gut motility stimulator	can treat infections/ acne
Fluoxetine (HCl)	Selective Serotonin Reuptake Inhibitor (SSRI)	can treat depression, OCD, bulimia nervosa, and panic disorder
Gemfibrozil	Cholesterol Medication	can lower high cholesterol and triglyceride levels in the blood
Naproxen	NSAID	can treat fever and pain
Phenytoin	Anticonvulsant	can treat and prevent seizures
Sulfamethoxazole	Antibiotics	can treat or prevent infections
Triclosan (Irgasan)	Antibacterial and Antifungal Agent	antibacterial/ antifungal agent present in some consumer products (toothpaste, soap, detergents)
Trimethoprim	Antibiotics and Folate synthesis inhibitor	can treat infections, including urinary tract and ear infections

Figure 2: EPA 542 analyte list

EPA 542 recommends using a C18 column with two separate gradients, one for positive ion electrospray, and the other for negative due to protonation or deprotonation of an analyte. Water and methanol mobile phases using ammonium acetate are used for the analysis. These methods can be seen in Tables 1 and 2 followed by the MS method conditions in Tables 3 and 4.

Table 1. HPLC Conditions (Positive ion electrospray)

HPLC
Column: Waters Xterra® MS C18, 2.1 x 150 mm, 3.5 µm
Column Temperature: 30 °C
Column Flow Rate: 0.200 mL/min
Autosampler Temperature: 10 °C
Injection Volume: 10 µL
Gradient:

Time (min)	%5 mM ammonium acetate in 10% MeOH/90% reagent water[a]	%MeOH
0.00	90	10
0.50	90	10
0.51	50	50
8.00	25	75
8.01	0	100
10.00	0	100
14.00	90	10
24.00	90	10

[a]Preparation of 5 mM ammonium acetate in 10% MeOH/90% reagent water: Combine 385 mg ammonium acetate and reagent water in 1 L volumetric flask. Add 100 mL MeOH and dilute to volume.

Table 2. HPLC Conditions (Negative ion electrospray)

HPLC
Column: Waters Xterra® MS C18, 2.1 x 150 mm, 3.5 µm
Column Temperature: 30 °C
Column Flow Rate: 0.200 mL/min
Autosampler Temperature: 10 °C
Injection Volume: 50 µL
Gradient:

Time (min)	%5 mM ammonium acetate in 10% MeOH/90% reagent water[a]	%MeOH
0.00	90	10
0.50	90	10
0.51	40	60
8.00	0	100
11.00	0	100
15.00	90	10
25.00	90	10

[a]Preparation of 5 mM ammonium acetate in 10% MeOH/90% reagent water: Combine 385 mg ammonium acetate and reagent water in a 1 L volumetric flask. Add 100 mL MeOH and dilute to volume.

Table 3. Positive Mode ESI-MS/MS Method Conditions

MS Parameter	HPLC-MS/MS
Polarity	Positive ion electrospray
Capillary Voltage, kV	2.50
Source Temperature, °C	120
N2 Desolvation Temperature, °C	400
N2 Desolvation Gas Flow, L/hr	900
Cone Gas Flow, L/hr	50
Extractor Lens, V	2.00
RF Lens, V	0.2

Table 4. Negative Mode ESI-MS/MS Method Conditions

MS Parameter	HPLC-MS/MS
Polarity	Negative ion electrospray
Capillary Voltage, kV	2.50
Source Temperature, °C	120
N2 Desolvation Temperature, °C	400
N2 Desolvation Gas Flow, L/hr	900
Cone Gas Flow, L/hr	50
Extractor Lens, V	1.00
RF Lens, V	0.1

A HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm column from Advanced Materials Technology, Inc. (Wilmington, DE) was used for the initial analysis. In order to further increase peak resolution, a column screening approach was performed using a scouting gradient and nine different stationary phases (2.7 µm, 2.1 x 100 mm) from Advanced Materials Technology, Inc. The best performing stationary phase as determined by overall best selectivity and resolution was then used for DryLab® optimization to further improve the separation. Scouting gradient conditions used for the column screening experiment are shown in Table 5 followed by DryLab® optimization conditions in Table 6.

Table 5. Scouting Gradient

TEST CONDITIONS:
Column: HALO® 2.7 µm, 2.1 x 100mm
Mobile Phase A: Water, 0.1% FA
Mobile Phase B: Acetonitrile, 0.1% FA
Gradient:

Time	%B
0.0	10
0.5	10
10	100
11	100

Flow Rate: 0.3 mL/min
Temperature: 30°C
Detection: 220 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: 50/50 Water/MeOH
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL

Table 6. DryLab® optimization method conditions

TEST CONDITIONS:
Column: HALO 90 Å RPA, 2.7 µm, 2.1 x 100mm
Mobile Phase A: Water, 0.1% FA
Mobile Phase B: Acetonitrile, 0.1% FA
Gradient:

Time	%B
0.0	10
18.0	67.5

Flow Rate: 0.3 mL/min
Temperature: 34 °C
Detection: LC-MS/MS
Injection Volume: 1.0 µL
Sample Solvent: 50/50 Water/MeOH

All experiments were conducted on a Shimadzu Nexera HPLC instrument using LabSolutions software (Shimadzu Scientific Instruments, Columbia, MD). A UV diode array detector (1 µL flow cell) was used for the scouting gradient experiments. Initial LC/MS runs were performed on a Shimadzu 8040 LC-MS/MS and finalized using a Thermo Q-Exactive (Waltham, MA). Standards were obtained from Millipore Sigma (St. Louis, MO). Methanol (MS grade), Acetonitrile (MS grade), water (HPLC grade), formic acid, and ammonium acetate were purchased from Millipore Sigma (Burlington, MA).

Analytical standards were prepared at 1000 µg/mL in 50/50 methanol/ water and used as stocks. LC-MS analysis required dilution of standards to 8.33 µg/mL with water for column screening and method development to better serve MS analysis.

RESULTS:

The original EPA 542 method was performed on a HALO 90 Å C18, 2.7 µm 2.1 x 150 mm column. These results can be seen in Figure 3. This method requires two separate multi-step gradients using ammonium acetate and methanol as the mobile phases.

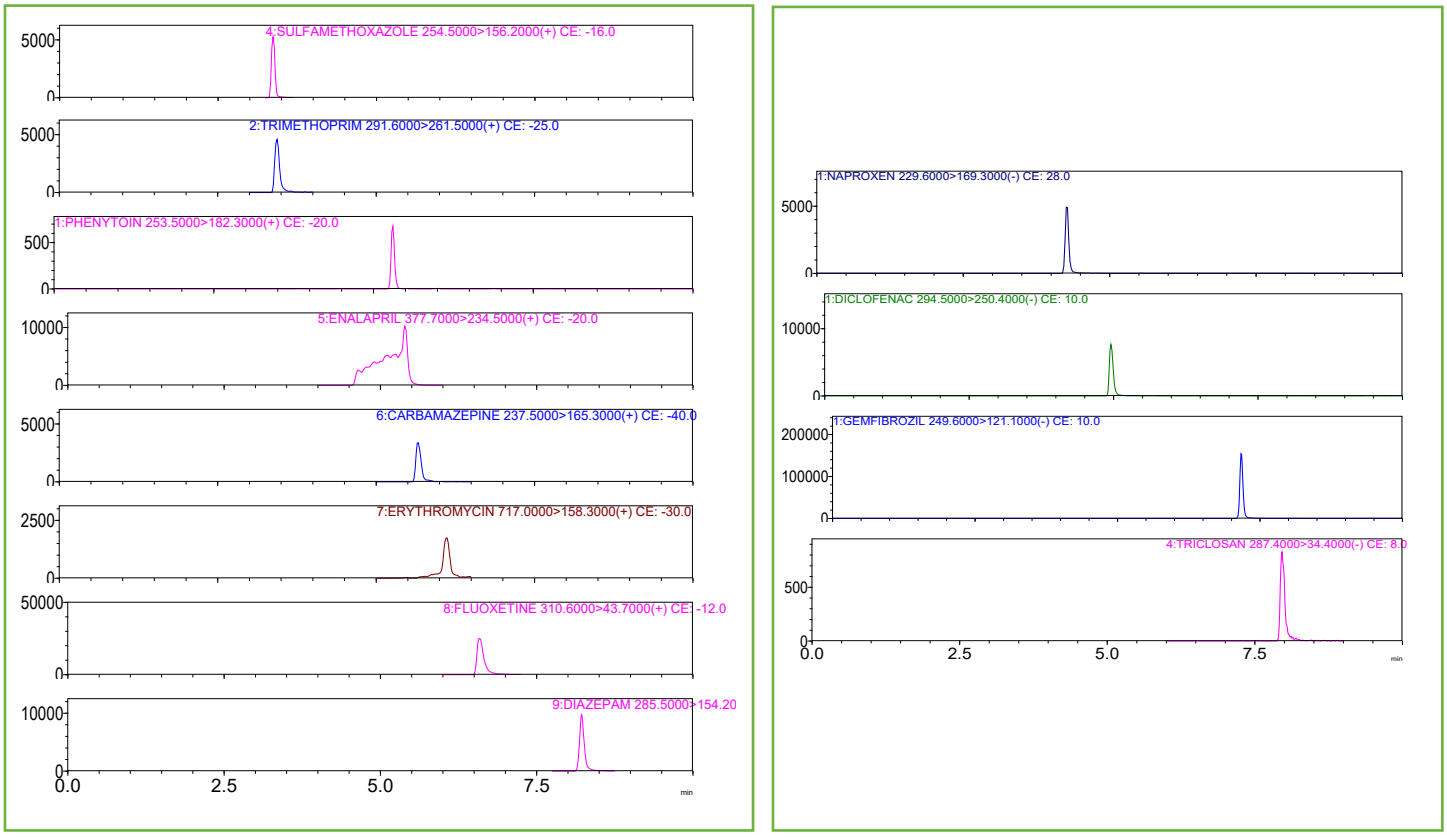


Figure 3: PPCP separation on HALO® C18 following EPA 542

The C18 stationary phase is known for being a very universal phase, ideal for many different types of compounds. However, C18 is not always the best column of choice. Many different phases exist to help improve peak shape and resolution and, in some cases, show advantages compared to C18. During method development, different stationary phases should be screened in order to make sure maximum resolution is achieved. Figure 4 shows a PPCP panel screened on nine different stationary phases using the scouting gradient (Table 5).

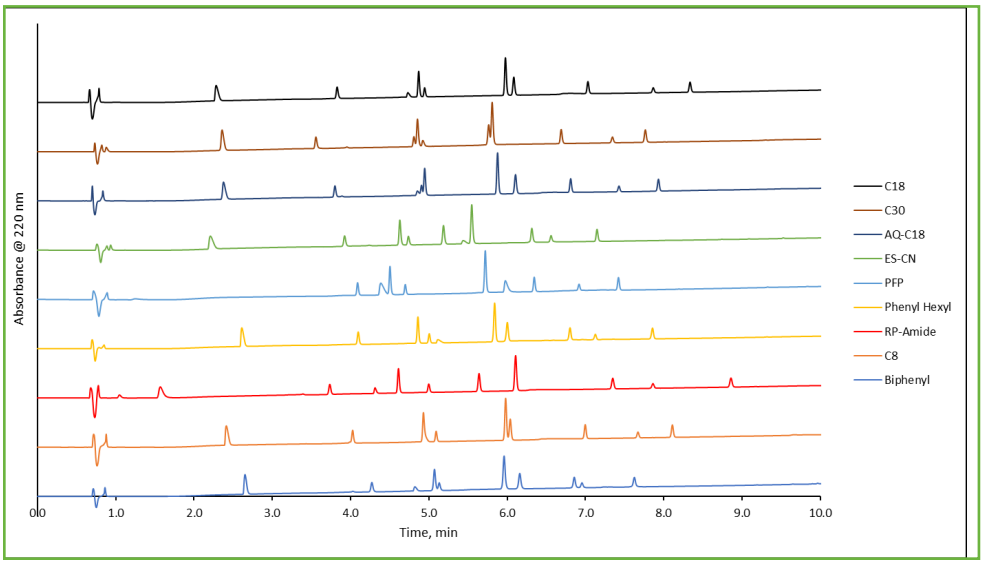


Figure 4: PPCP phase screening using a scouting gradient

The dimethylpalmitamideopropylsilane, better known as the RP-Amide stationary phase showed the overall best selectivity and resolution compared to other available phases. (red trace in Figure 4). This phase is ideal for reversed-phase separations of basic compounds as well as alcohols, acids, phenols, and catechins. The functionality of polar embedded phases can be attributed to the proximity of the polar group to the silica surface, allowing hydrogen bonding to occur with unreacted silanols, deactivating them making the surface base-friendly. Additionally, the presence of the polar group near the surface allows more water in the mobile phase to get near the silica surface making the column less hydrophobic and friendlier to separations of polar solutes. The RP-Amide stationary phase can be seen in Figure 5.

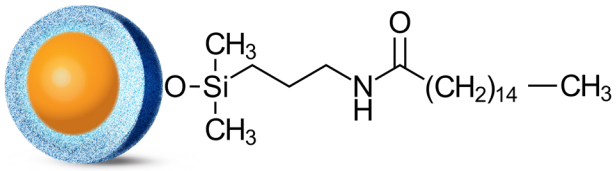
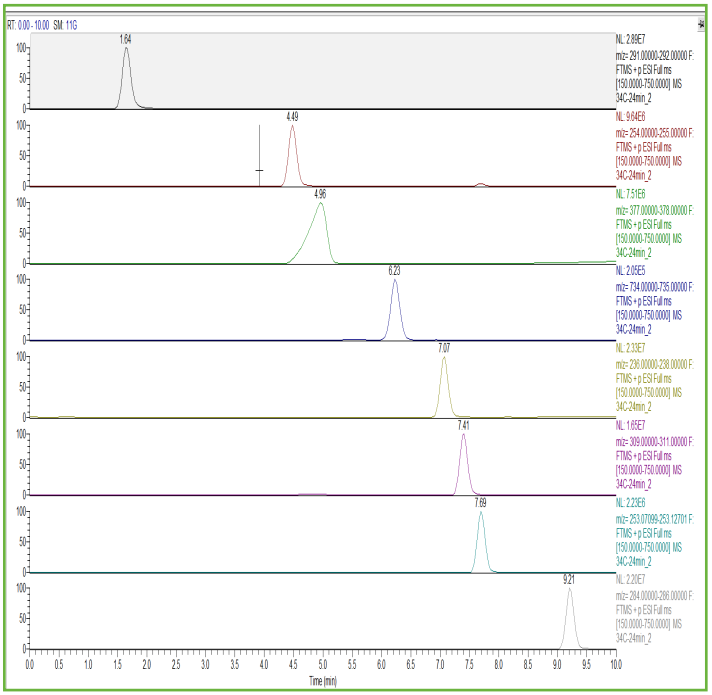


Figure 5: HALO® RP-Amide stationary phase

Method optimization software such as DryLab® can be used in order to further increase method performance. This software helps predict chromatograms under a wide range of experimental conditions and allows for quicker method development for complex samples while further improving method validation. Figure 6 shows the PPCP panel under DryLab® recommended conditions.



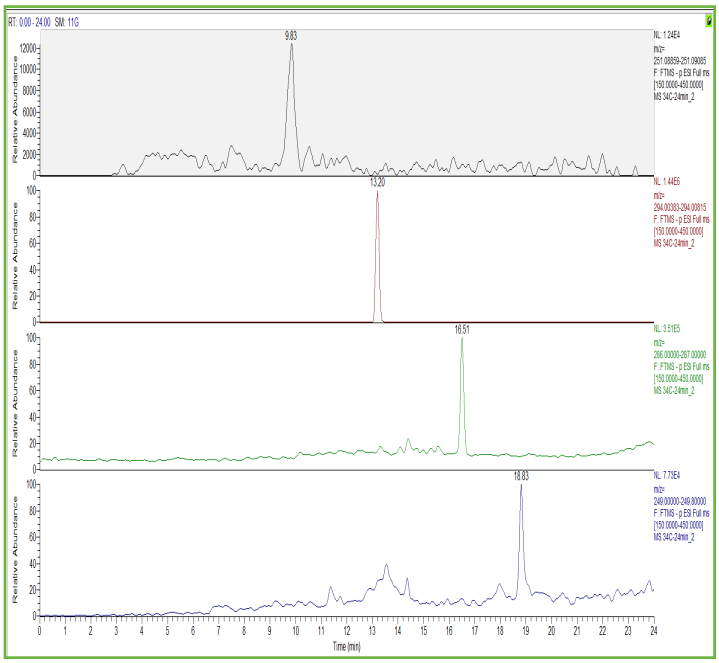


Figure 6: DryLab® optimization of separation with RP-Amide stationary phase

CONCLUSION:

Over the next century, the combination of increasing global population size and potential droughts may result in reduced water availability, increased need for water reuse, and increasing concentrations of PPCPs in water systems. The current wastewater treatment methods do not remove all PPCPs effectively. This, coupled with the possibility that antibiotics may promote the development of antibiotic-resistant bacteria and antibiotic-resistant genes, leads to concerns about the sustainability of global water supplies.[1] This work serves to show how screening columns and conducting method development with available software tools for optimal method conditions can lead to improved and faster separations.

REFERENCES:

1. Cizmas L, Sharma VK, Gray CM, McDonald TJ. Pharmaceuticals and personal care products in waters: occurrence, toxicity, and risk. Environ Chem Lett. 2015 Dec;13(4):381-394. doi: 10.1007/s10311-015-0524-4. Epub 2015 Aug 26. PMID: 28592954; PMCID: PMC5459316.