

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, Advanced Materials Technology (AMT) offers HALO® ENVIROCLASS, a product family solution for environmental analysis which consists of Fused-Core® technology columns, packed with particles composed of a solid silica core surrounded by a thin porous silica shell. Researchers can capitalize on the advantage of the superficially porous particle design, which 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

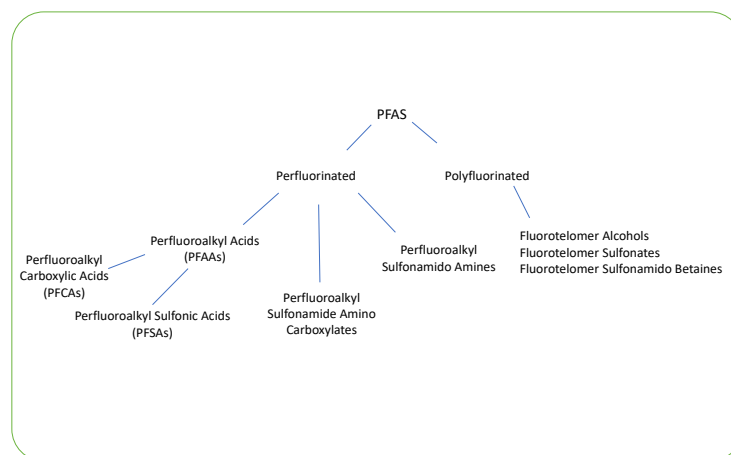
Column	Target Analytes
HALO® PFAS and HALO® PFAS Delay	PFAS
HALO® PAH	PAH
HALO® C18	Pesticides (nonpolar)
HALO® Biphenyl	Pesticides (polar and nonpolar)
HALO® PFP	Mycotoxins

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 perfluorooctanoic 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.

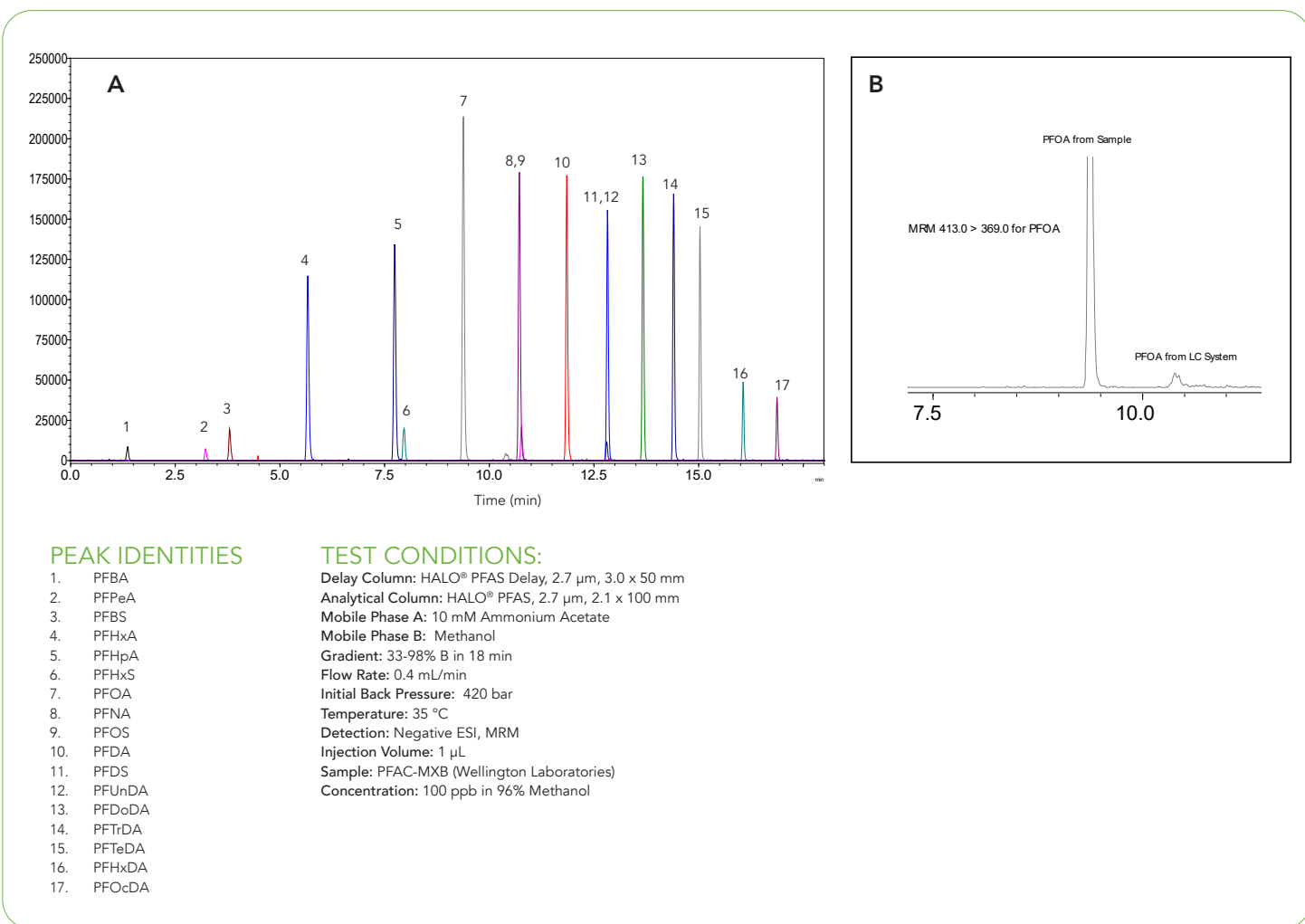
Figure 1. Classifications of PFAS



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

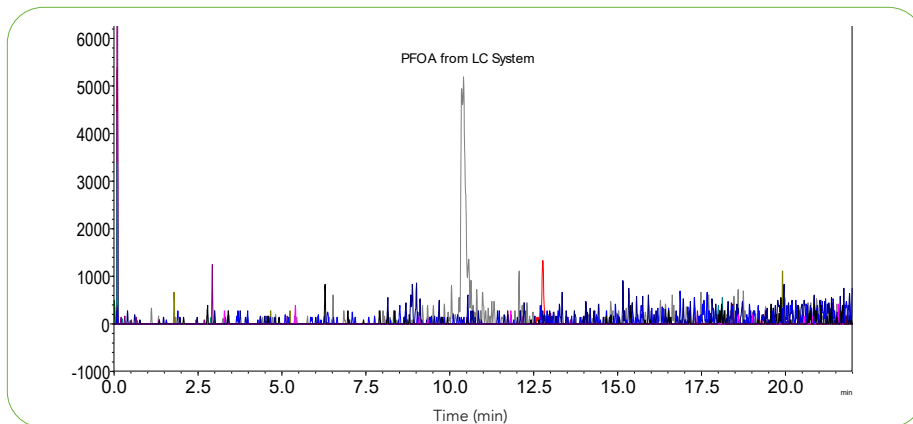
and contains 24 target analytes. There is an appendix method 3512 within 8327, which will eventually become a stand-alone method after validation and public comment is made. Method 3512 calls for dilution of the samples with organic solvent. With the requirements of EPA methods in mind, HALO® PFAS and 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



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.

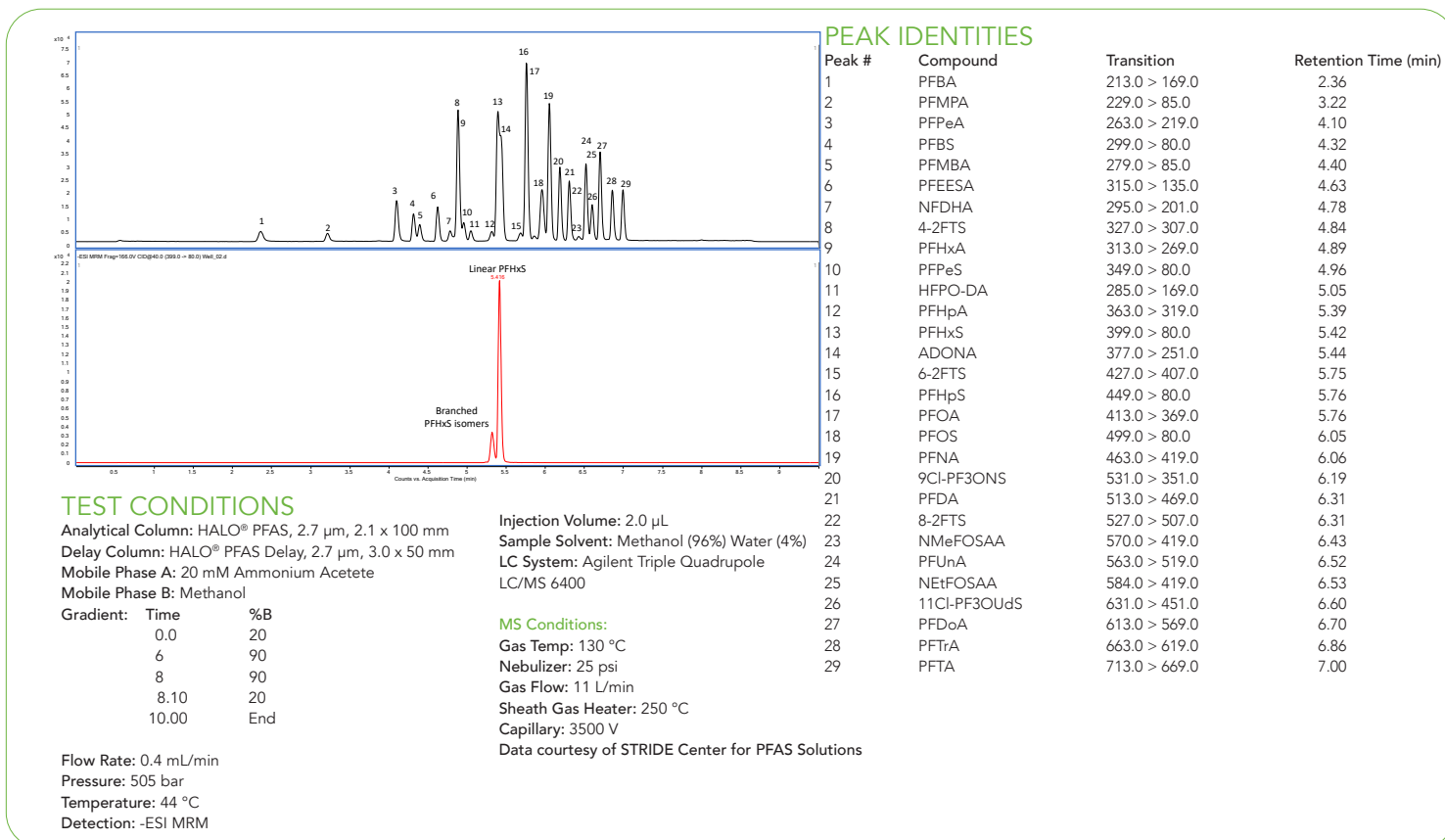
Figure 3. Null injection demonstrating background contamination of PFAS in the LC system. (conditions same as Figure 2)



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.

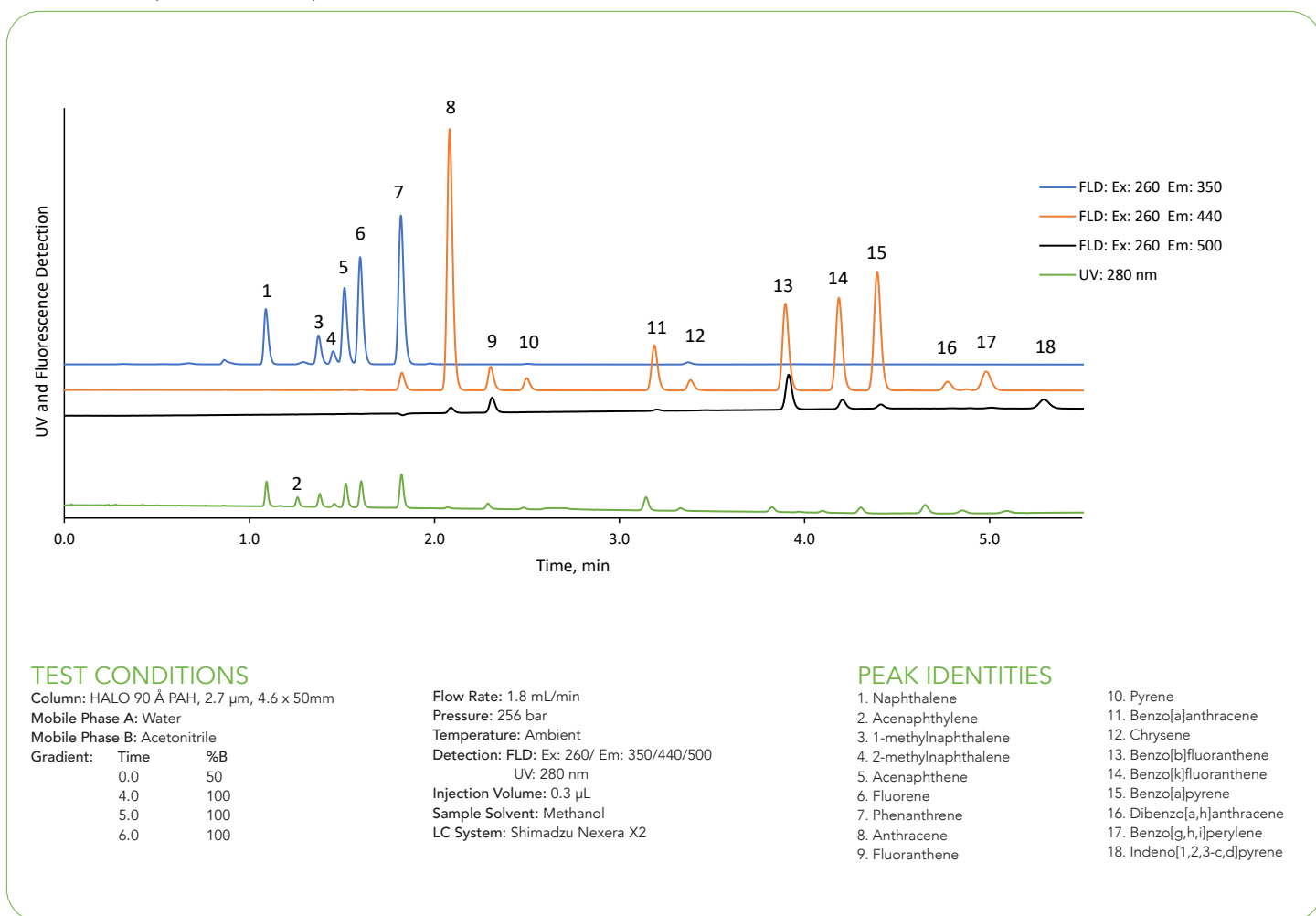
Figure 4. HALO® PFAS separation of branched and linear PFHxS isomers from a well water sample.



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 in 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

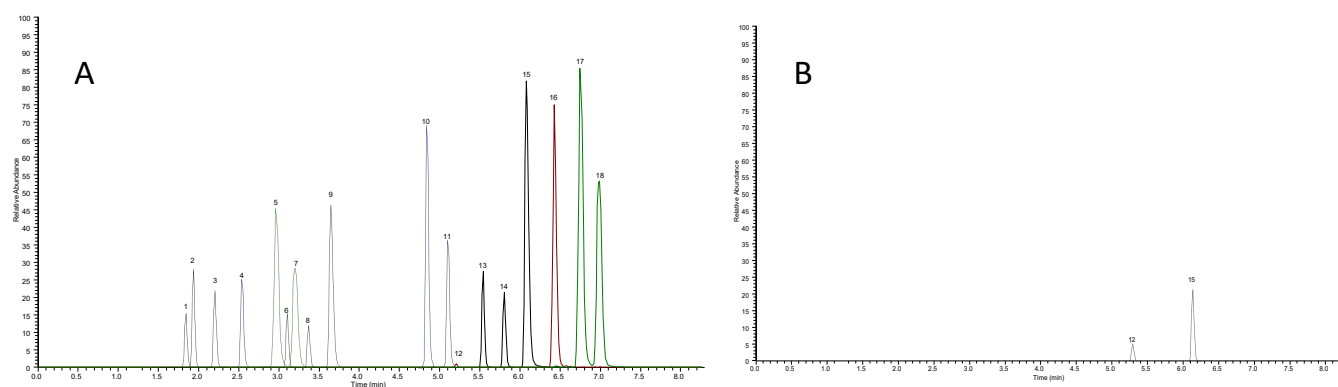


The advantage of using a fluorescence detector is its increased sensitivity, provided the compounds fluoresce. Notice that acenaphthylene (peak 2) does not fluoresce and is only visible in the green trace from the UV signal. The fluorescence detector's larger extra column volume (contributed from the 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

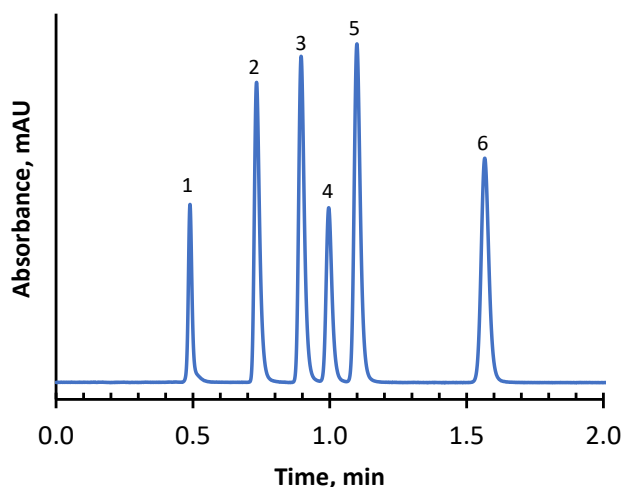
Calibration curves were run from 1 ppb – 100 ppb in order to quantitate the levels of PAHs detected in the extracted steak sample. The level of chrysene was determined to be 2.55 ppb and the level of benzo[a]pyrene was determined to be 1.98 ppb. Although these two PAHs were detected in the steak sample, 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

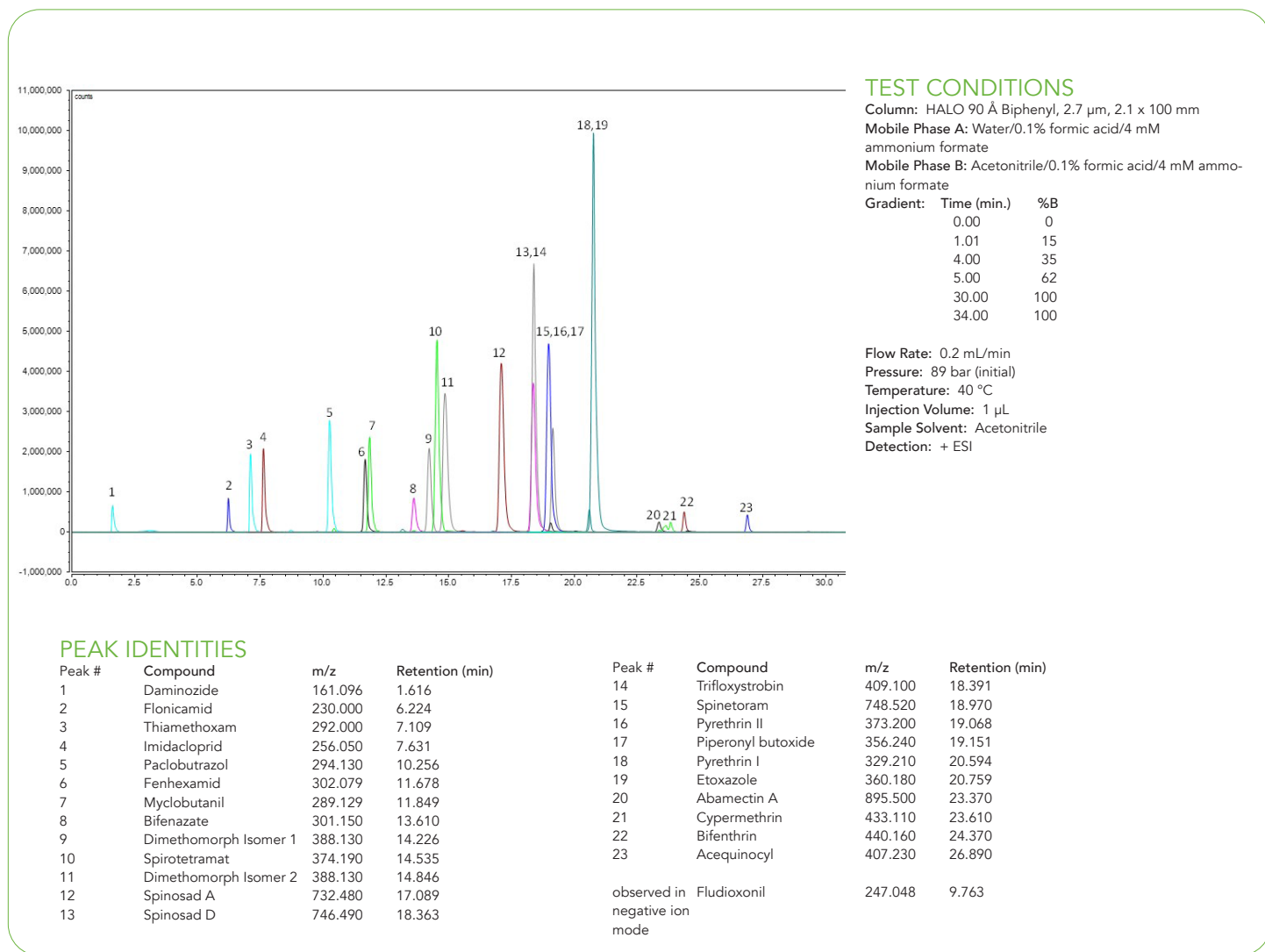
1. Nitenpyram
2. Thiamethoxam
3. Clothianidin
4. Imidacloprid
5. Acetamiprid
6. Thiacloprid

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, WWD
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.

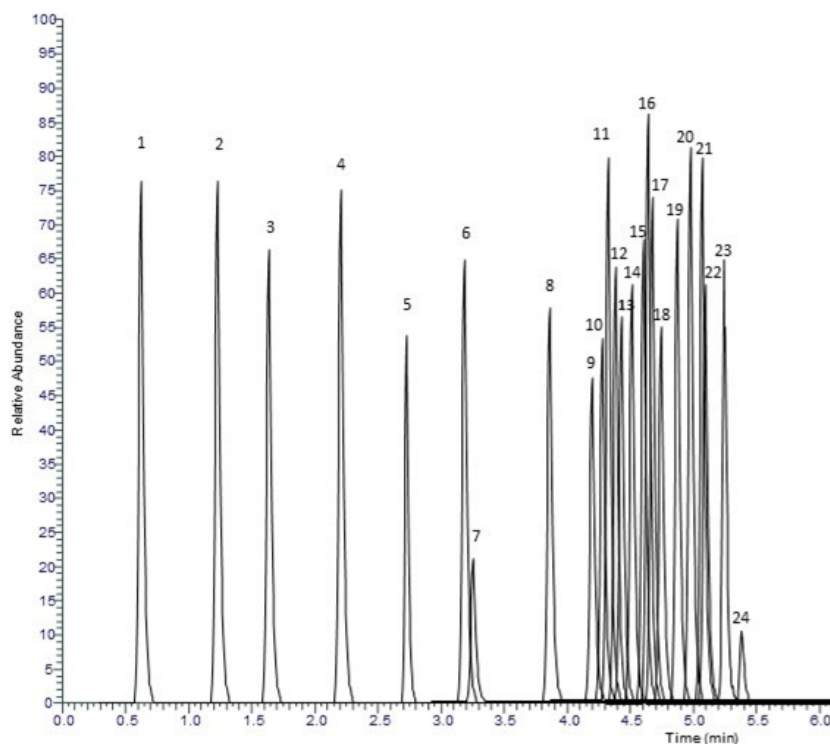


Mycotoxins

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 immunosuppressive 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	Neosolanol	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.

References

1. Environmental Work Group: <https://www.ewg.org/release/pfas-contamination-tap-water-far-more-prevalent-previously-reported/>; accessed August 21, 2020.
2. <https://www.fda.gov/news-events/press-announcements/fda-announces-voluntary-agreement-manufacturers-phase-out-certain-short-chain-pfas-used-food>, accessed August 21, 2020.
3. Journal of Chromatography B, 1049–1050 (2017) 24.
4. Science of The Total Environment, 733 (2020) 139186.
5. Polycyclic Aromatic Compounds, 35 (2015) 330.
6. Commission Regulation (EC) No 1881/2006.
7. <https://www.chemistryworld.com/news/what-you-need-to-know-about-neonicotinoids/3008816.article?adredir=1#/> accessed August 21, 2020.
8. Environmental Science and Pollution Research International, 26 (2019) 34723.
9. Clinical Microbiology Reviews, 16 (2003) 497.
10. Toxins, 8 (2016) 361.

HALO® and Fused-Core® are registered trademarks of Advanced Materials Technology, Inc.