Polycyclic Aromatic Hydrocarbons by Solid Phase Extraction in a Groundwater Matrix with Reduced Sample Volume and Non-Chlorinated Solvent Mixtures

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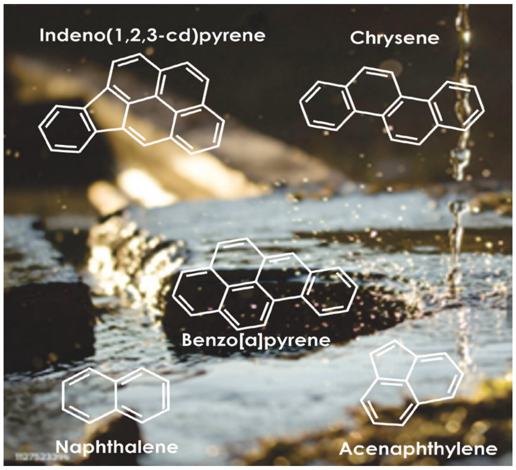

UCT Featured Products

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Enviro-Clean C18 (Octadecyl)
Endcapped 500 mg/6 mL

VMF016GL
Glass Block Vacuum Manifold
System - 16 position

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SPeVAP® 32 Position Multi-Function Solvent Evaporator with Installation Kit

ECHLD(150)6-P
Enviro-Clean® HL DVB
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VOA Vial Tray

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Glass Manifold Polypropylene Collection Rack VOA Vials-12 Position System

Abstract

This study presents a validated method for the quantification of 16 polycyclic aromatic hydrocarbons (PAHs) in groundwater that addresses current analytical challenges by reducing sample volume, employing Solid Phase Extraction (SPE) instead of traditional liquid-liquid extraction methods, and utilizing GC/MS in Selected Ion Monitoring (SIM) mode.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds composed exclusively of carbon and hydrogen atoms, characterized by multiple fused aromatic rings. These rings share carbon–carbon bonds, creating rigid, typically planar structures (with some exceptions). Environmental transport varies depending on the number of ring structures in a particular PAH, with low molecular weight PAHs acting more volatile and high molecular weight PAHs acting less volatile.[1]

PAHs are primarily generated through the incomplete combustion of organic materials such as fossil fuels, wood, and tobacco, as well as from industrial activities and vehicle exhaust. Their stability and resistance to degradation make them environmentally persistent. Because of their hydrophobic nature, PAHs tend to sorb to soil and sediment particles and can enter ground water through leaching from contaminated soil or landfills, leakage from underground storage tanks, or transport via storm water runoff.[2]

High molecular weight PAHs, such as benzo[a]pyrene, are classified by the International Agency for Research on Cancer (IARC) as Group 1 carcinogens, indicating sufficient evidence of carcinogenicity in humans. Chronic exposure to PAHs has been associated with lung, bladder, and skin cancer. The risk is increased for populations exposed to PAHs in occupational settings or in urban areas with heavy traffic and emissions.[3]

Traditional methods for PAH analysis in groundwater typically require large sample volumes and substantial amounts of hazardous organic solvents, such as methylene chloride (DCM). DCM is classified as both a carcinogen and a reproductive toxin, and it can be absorbed through the skin and metabolized into carbon monoxide, which impairs oxygen transport in the body.[4] In contrast, solvents such as hexane and acetone do not carry the same carcinogenic classification, or the severe acute toxicity profile observed with DCM. Aimed at incorporating more environmentally friendly solvents into the sample preparation process, this study analyzes 16 PAH compounds and one methylated PAH from a small sample volume (100 mL). Additionally, this study evaluates the performance of two solid-phase extraction sorbent chemistries: C18 (EEC18) and a highly cross-linked styrene-divinylbenzene (DVB) copolymer (ECHLD).

Experimental

a) Sample Preparation

Sample Pretreatment:

1. Adjust 100 mL sample to pH 2.0 with 6N H_2SO_4.
2. Spike with surrogates and standards.

Tip: *Prepare standards in acetone instead of methanol. Methanol has been shown to cause degradation of the surrogate compound benzo[a]pyrene-d12 when extracted using PE frits.*

SPE Extraction:

1. **Pre-Rinse:** 10 mL hexane:acetone (1:1), followed by 5 mL acetone, dry for 2 minutes.
2. **Condition:** 10 mL methanol, do not let methanol drip below the frit.
3. **Equilibration:** 15 mL of reagent water* (pH 2.0).
4. **Sample Load:** load sample at a flow rate of 3 to 5 mL/minute.
5. **Wash:** 5 mL reagent water into bottle, twice, ensuring to rinse sides of the bottle.
6. **Dry:** for no more than 5 minutes under full pressure or vacuum.

Tip: *Minimize drying time during sample preparation. Extended drying periods can result in the loss of volatile PAHs, potentially compromising quantitation accuracy.*

7. **Elute:**
    a. Add a small layer of Na_2SO_4 to the bottom of the collection vial & top of the SPE cartridge frit
    b. Elute 2x: 8 mL 1:1 hexane:acetone dropwise, then apply full vacuum for 30 seconds.
    c. Elute 2x: 8 mL 9:1 hexane:acetone dropwise, then apply full vacuum for 30 seconds.

Tip: *Rinse the sample bottle thoroughly with the elution solvent and transfer the rinse into the reservoir, making sure to rinse the sides. This step is especially important for increasing the recovery of heavy PAHs.*

8. **Water Removal:**
    a. Swirl eluate to collect water at the bottom of the vial.
    b. Pass organic phase through 20 g of Na_2SO_4 previously rinsed with acetone:hexane (1:1).
    c. Rinse elution vial with 5 mL hexane.
    d. Add 5 g fresh Na_2SO_4 to the top of the reservoir/funnel, then pass the remainder of eluate through it.
    e. Rinse the elution vial with 5 mL hexane and pass through the system.
9. **Evaporate:** at 30°C to 800 µL using a 32 position multi-function solvent evaporator (SPeVAP).
10. **Reconstitute:** to 1 mL with hexane.
11. **Add Internal Standard:** (Cat #31886, Restek Corp.)

** Milli-Q ultrapure water (MilliporeSigma, Burlington, MA, USA) was used as reagent water for all experiments performed in this study.*

b) Analytical Conditions

Table 1. GC-MS Parameters

GC/MS Conditions	
GC/MS	Shimadzu TQ 8050
Column	Rtx-SVOCms, 30 m × 0.25 mm × 0.25 µm
Inlet Temp.	275 °C
Injection	1µL; Split 5:1
Liner	Restek Topaz Splitless 3.5 mm
Oven	40 °C (1.5 min) to 280 °C at 20 °C/min, then to 320 °C at 5 °C/min (hold 0.5 min)
Carrier Gas	Helium (He), constant linear velocity at 39.5 cm/sec
Detector	MSD, SIM mode
MS Parameters	Source at 230 °C; MS transfer line temp. at 300 °C; Electron Energy at 70 eV

Table 2. Monitored SIM Ions and scan times

Analytes	Scan Start & End Time (min)	SIM Ions
1,4-Dichlorobenzene Nitrobenzene-d5	5.2 - 6.31	150, 115, 82, 128
1,3-Dimethyl-2-nitrobenzene Naphthalene Naphthalene-d8	6.31 - 7.21	136, 135, 77, 134
2-Methylnaphthalene 2-Fluorobiphenyl	7.21 - 8.47	142, 141, 172.1, 171.1
Acenaphthylene Acenaphthylene-d8 Acenapthene Acenapthene-d10	8.47 - 9.37	152, 151, 126, 163, 164, 160, 159
Fluorene	9.37 - 10.71	166, 165
Phenanthrene-d10 Phenanthrene Anthracene	10.71 - 11.54	188, 186, 178, 176
Fluoranthene Pyrene p-Terphenyl-d14	11.54 - 13.24	202, 200, 244.2, 240.2
Triphenylphosphate Benzo[a]anthracene Chrysene Chrysene-d12	13.24 - 14.41	169, 326, 228, 226, 240, 238
Benzo[b]fluoranthene Benzo[k]fluoranthene Benzo[a]pyrene Benzo[a]pyrene-d12 Perylene-d12	14.41 - 17.64	252, 250, 264, 132, 260
Indeno[1,2,3-cd]pyrene Dibenzo[a,h]anthracene Benzo[ghi]perylene	17.64 - 19.52	276, 277, 279

C) Chromatography

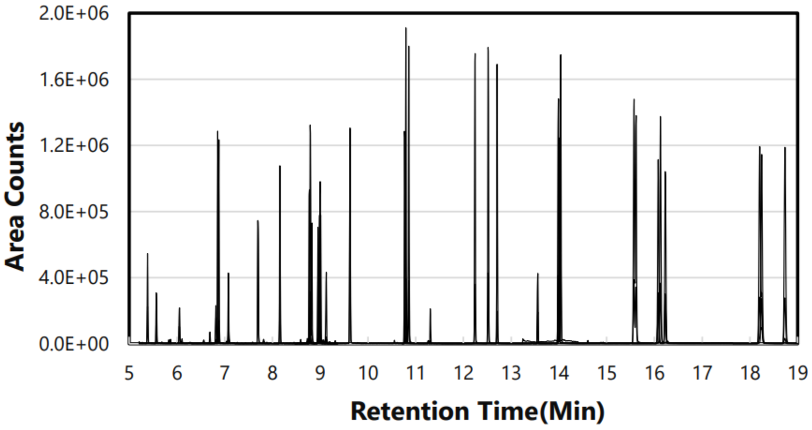

Figure 1. SIM chromatogram of a 100 mL reagent water sample fortified at 10 µg/L, extracted using a C18 sorbent.

Results & Discussion

To prove the robustness of the method, an LOD (Limit of Detection) study (**Table 3**) was conducted over 3 days to determine the lowest concentration of PAHs that could be reliably detected using two different sorbent chemistries (C18 and HLD). In addition to the LOD study, Initial Demonstration of Proficiency (IDP) studies were performed by spiking aliquots of reagent water (Milli-Q ultrapure water) with PAHs at the midpoint (2 µg/L) and high end (10 µg/L) of the calibration curve (**Table 4**). Replicate samples were analyzed to evaluate the method's precision and accuracy. This step ensures that the method consistently produces accurate and reproducible results in a clean matrix. The IDP study at 2 µg/L was performed in reagent water (n = 4) for both sorbents. For C18, PAH recoveries ranged from 91% to 104% with RSDs ≤ 7%, while HLD PAH recoveries ranged from 88% to 107% with RSDs ≤ 6% (**Figures 2** and **4a**).

Following these validation steps, the extraction method was evaluated through a matrix spike study using a simulated groundwater matrix (tap water fortified with mineral salts (NaCl 50mg/L, $CaCl_2$30 mg/L, $MgSO_4$ 20 mg/L, $NaHCO_3$ 50 mg/L to mimic mineral content & oxalic acid 18 mg/L to mimic total organic carbon (TOC) of 5 mg/L). This assessment involved spiking a simulated matrix with known PAH concentrations to evaluate the method's performance under conditions that closely resemble actual environmental samples. The matrix spike study demonstrated that the method effectively detects and quantify PAHs even in complex groundwater matrices, demonstrating robust performance against recovery limits referenced in various EPA methods, such as EPA 8310[5] & EPA 625.1.[6] At the midpoint concentration level, PAH recoveries ranged from 62% to 112% with RSDs ≤ 24%, except for acenaphthylene, which showed a recovery of 41%. In contrast, the HLD sorbent yielded recoveries between 52% and 100% with RSDs ≤ 7% (**Figures 3** and **4b**).

During the development of this study, acenaphthylene initially showed low recovery on C18, an issue not observed on HLD in reagent water. Thus, losses increased with longer drying times after sample loading but improved with optimized cartridge drying time (**Figure 5**). However, in synthetic matrix studies, losses reappeared at the midpoint and high concentration levels on both C18 (62% and 43%) and HLD (57% and 60%). The weak retention of the analyte in the sorbent may be attributed to acenaphthylene's non-planar structure, disrupted aromaticity, and reduced π–π interactions. A deuterated surrogate was added to monitor stability and exhibited low recoveries in line with the native compound, confirming a compound-specific matrix effect. This variability was further demonstrated at the midpoint concentration in matrix on C18, where acenaphthylene exhibited an RSD of 41% (**Figure 4b**).

In synthetic matrix (simulated groundwater), anthracene also showed low recoveries on both sorbents at the concentrations studied, with recoveries between 52% and 65% across both sorbent chemistries. This loss is suspected to result from oxidation of the analyte by hydroxyl radicals, which are generated when trace metals in the tap water react with the oxalic acid used as a TOC source in the synthetic matrix. Both acenaphthylene and anthracene are known to have much higher oxidation potential compared to the other PAHs in their class.[7, 8] Regardless of the observed losses, recoveries for acenaphthylene and anthracene in the synthetic matrix, when extracted with both C18 and HLD sorbents, remained well within the acceptable range in EPA 625.1, which has more stringent QC limits than other EPA PAH methods (33%-145% and 27-123%, respectively).

Table 3. Limit of detection study (µg/L)

Analyte (n=7)	C18	HLD	Analyte (n=7)	C18	HLD
Naphthalene	0.077	0.086	Benzo[a]anthracene	0.072	0.038
2-Methylnaphthalene	0.092	0.189	Chrysene	0.044	0.039
Acenaphthylene	0.061	0.069	Benzo[b]fluoranthene	0.066	0.073
Acenaphthene	0.120	0.080	Benzo[k]fluoranthene	0.058	0.067
Fluorene	0.056	0.046	Benzo[a]pyrene	0.068	0.051
Phenanthrene	0.154	0.071	Indeno[1,2,3-cd]pyrene	0.047	0.056
Anthracene	0.073	0.045	Dibenzo[a,h]anthracene	0.052	0.057
Fluoranthene	0.053	0.125	Benzo[ghi]perylene	0.058	0.056
Pyrene	0.060	0.145	Median LOD	0.061	0.067

Table 4. IDP studies: C18 and HLD recovery and RSD results for a 100 mL sample fortified at 10 µg/L.

Analyte	Reagent Water**				Simulated Groundwater			
	C18 (n=4)		HLD (n=4)		C18 (n=3)		HLD (n=3)	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Nitrobenzene-d5*	105	14	100	1	69	5	100	4
1,3-dimethyl-2-nitrobenzene*	101	10	108	7	82	9	94	7
Naphthalene	88	4	93	3	68	3	77	4
2-Methylnaphthalene	95	4	101	3	70	1	80	6
2-Fluorobiphenyl*	97	7	104	2	74	9	82	13
Acenaphthylene-d8*	109	16	130	1	38	29	61	24
Acenaphthylene	105	7	112	3	43	14	60	21
Acenaphthene	90	4	96	5	66	2	72	8
Fluorene	93	5	101	5	78	2	83	4
Phenanthrene	92	5	100	5	80	2	81	4
Anthracene	98	5	105	5	63	3	59	11
Fluoranthene	97	7	107	5	84	4	84	6
Pyrene	96	8	105	5	77	2	75	8
p-Terphenyl-d14*	105	9	119	4	95	3	92	10
Triphenylphosphate*	113	14	135	8	113	3	101	5
Benzo[a]anthracene	96	9	107	5	83	6	80	8
Chrysene	91	9	101	5	84	3	83	5
Benzo[b]fluoranthene	96	8	107	5	88	7	90	6
Benzo[k]fluoranthene	89	13	97	4	82	3	82	11
Benzo[a]pyrene-d12*	105	10	118	4	88	1	89	11
Benzo[a]pyrene	93	10	102	4	82	5	82	6
Indeno[1,2,3-cd]pyrene	89	12	102	4	87	4	85	2
Dibenzo[a,h]anthracene	86	11	97	4	84	4	82	4
Benzo[ghi]perylene	84	12	94	4	82	3	80	4

Surrogates (*); Milli-Q Water (**)

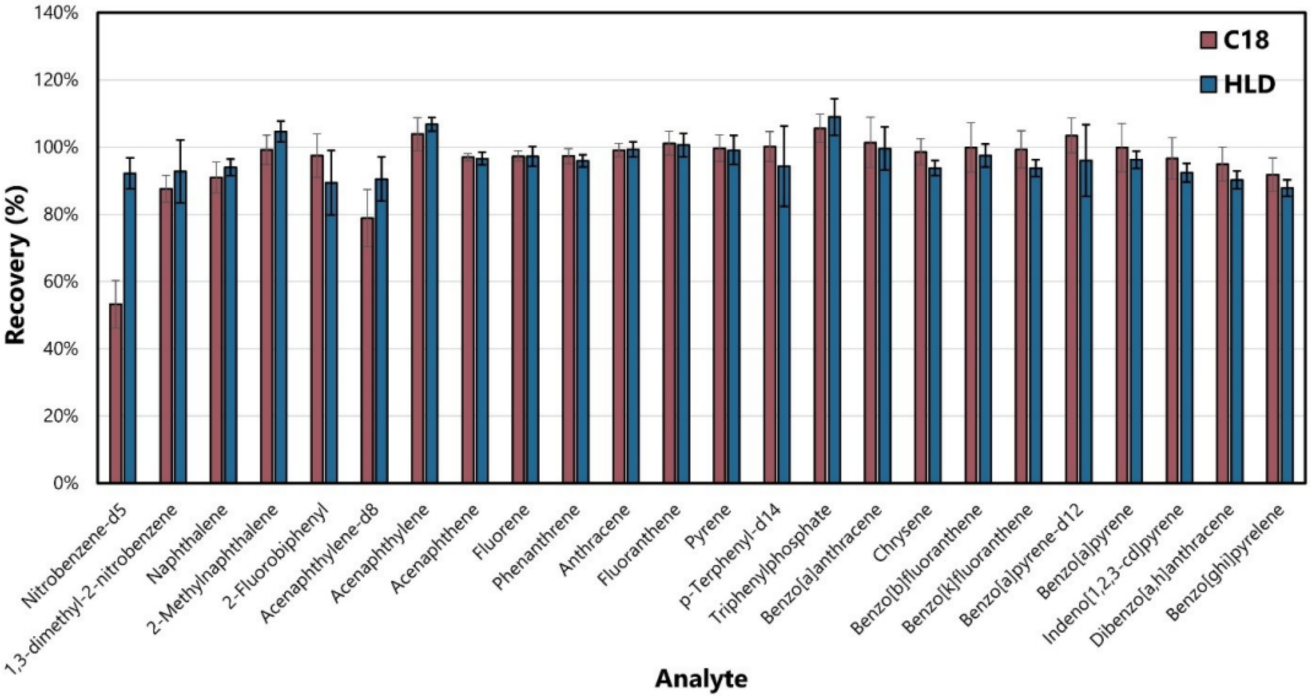

Figure 2. Average recoveries for 100 mL reagent water fortified at 2 µg/L (10 µg/L for surrogate compounds) using C18 (n=4) and HLD (n=4) as SPE sorbents.

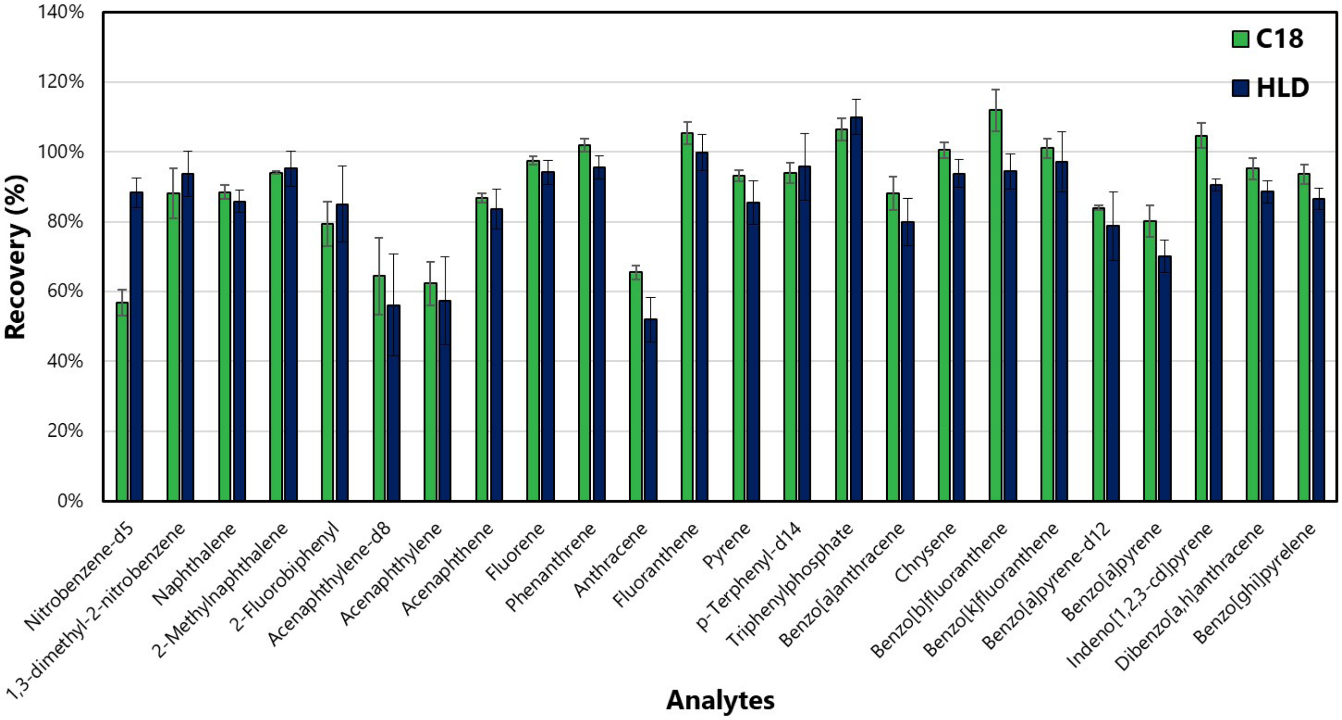

Figure 3. Matrix spike study showing average recoveries for 100 mL simulated groundwater fortified at 2 µg/L (10 µg/L for surrogate compounds) using C18 (n=3) and HLD (n=3) as SPE sorbents.

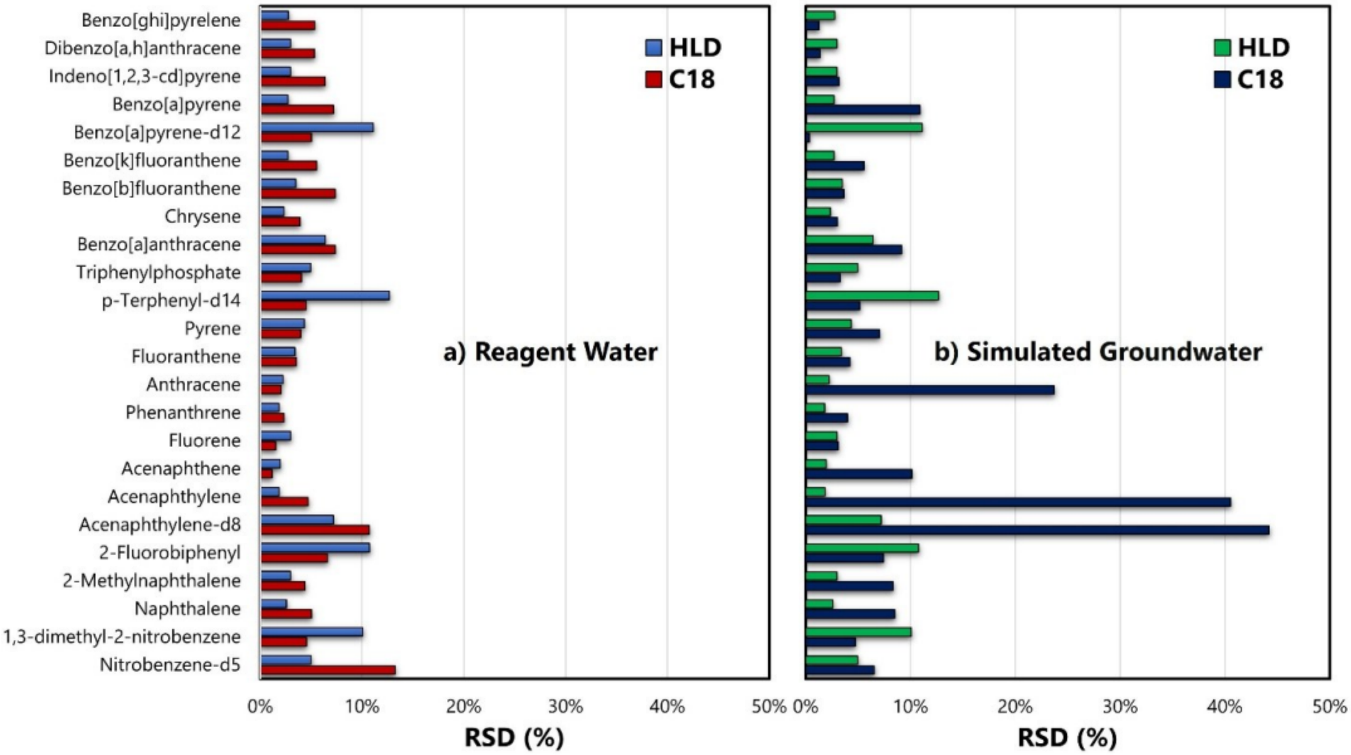

Figure 4. Relative standard deviations for 100 mL samples fortified at 2 µg/L (10 µg/L for surrogate compounds) in (a) reagent water and (b) simulated groundwater.

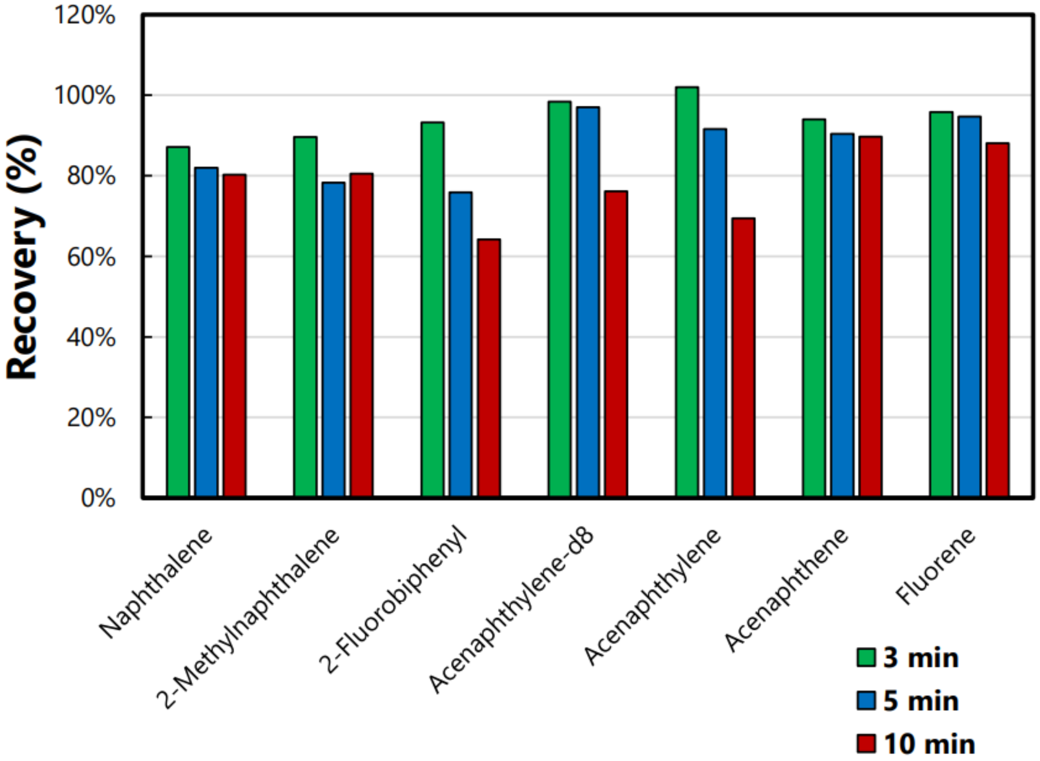

Figure 5. Impact of cartridge drying time on the recovery of low molecular weight PAHs using C18 sorbent.

Conclusion

Both sorbents demonstrated excellent analyte retention in reagent water, with HLD (**ECHLD(150)6-P**) having more consistent recovery of volatile surrogate compounds. In synthetic groundwater, both sorbents exhibited some recovery loss and higher variability for the nonplanar, volatile PAH acenaphthylene, as well as recovery loss for anthracene. These compounds are known to be highly susceptible to oxidation from matrix components. Nonetheless, all recoveries across all matrices remained within the quality control limits specified by current EPA methodologies. It was demonstrated that both sorbents can retain both low and high molecular weight PAHs, as well as alkylated PAHs. By using hexane and acetone as alternative solvents, the method eliminates the need for DCM while maintaining the high sensitivity required to detect PAHs at concentrations that meet or exceed groundwater quality standard (GWQS) limits. This application note presents an efficient and fast SPE method that highlights the versatility of two sorbent chemistries for extracting PAHs from ground and surface water. SPE requires less solvent and less hands-on extraction time than traditional methods such as liquid-liquid extraction (LLE). The method combines a low sample volume approach with the use of environmentally friendly solvents, effectively avoiding the need to handle chlorinated solvents, allowing laboratories to incorporate greener chemistry practices into their workflow.

Acknowledgement

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