

Liquid Chromatography

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PAHs in Surface Water by PDA and Fluorescence Detection

Introduction

Heightened awareness of polycyclic aromatic hydrocarbons (PAHs) has become prevalent due to urban background levels found in surface water, soil, air, cosmetics and food. They are

generated by the combustion of fossil fuels and are always found as a mixture of individual compounds that differ in behavior, environmental distribution, and their effect on biological systems. PAHs encompass a wide molecular weight range, differing based on their physical, chemical, and biological characteristics.

Lower molecular weight PAHs, in general, are quite water soluble and have significant acute toxicity to invertebrate aquatic organisms. Naphthalene is structurally the simplest PAH and is especially water soluble. It is the most abundant component of road, driveway and parking lot sealcoating. The U.S. Environmental Protection Agency (EPA) has classified naphthalene as a Group C possible human carcinogen.¹

High molecular weight PAHs are expected to bio-accumulate in aquatic organisms such as oysters, rainbow trout, bluegills and zooplankton.² They have low solubility in water and bind very strongly to soils, sediments and particulate matter. Benzo(a)pyrene, a by-product of incomplete combustion or burning of organic material, such as cigarettes, gasoline and wood, is considered to be one of the most carcinogenic high molecular weight PAHs.³

PAHs in surface water result from a variety of sources including residential, industrial and commercial outlets, streets and parking lots, and atmospheric fallout. In general, most surface waters contain individual PAHs at levels up to 0.05 µg/L (ppb), but highly polluted rivers can have concentrations of up to 6 ppb. The PAH levels in groundwater and drinking water are typically within the range of 0.0002 to 0.0018 ppb. Levels of individual PAHs in rainwater range from 0.01 to 0.2 ppb, whereas, in highly urban areas, levels of up to 1 ppb have been detected in snow and fog.⁴ The EPA has developed ambient water quality criteria to protect human health from the carcinogenic effects of PAH exposure. The current recommendation is a maximum contaminant level (MCL) of no greater than 0.2 ppb in drinking water.⁵

A pre-concentration step, often solid phase extraction, is necessary to concentrate PAHs in aqueous environmental samples to detectable levels by PDA and fluorescence. In this application, via a spiking experiment, we explore the levels at which PAHs in surface water can be monitored by UHPLC with a sub-2 µm particle sized column combined with photo diode array (PDA) and fluorescence (FL) detection.

Experimental

Hardware/Software

A PerkinElmer Altus™ UPLC® system was used, including the A-30 Solvent Module (quaternary pump), Sample Module and Column Module (PerkinElmer, Shelton, CT, USA). A PerkinElmer Brownlee™ HRes DB PAH 1.9 µm 50 x 2.1 mm column (PerkinElmer, Shelton, CT, USA) was used for all separations. Detection was accomplished with PerkinElmer Altus A-30 PDA and FL detectors (PerkinElmer, Shelton, CT, USA). All instrument control, analysis and data processing was performed via Waters® Empower® 3 CDS software.

Method Parameters

The HPLC method parameters are shown in Table 1.

Solvents, Standards and Samples

All solvents and diluents used were HPLC grade.

A stock PAH standard mixture was obtained from PerkinElmer (Part # 00891543; PerkinElmer, Shelton, CT). The stock mixture contained: naphthalene (1000 ppm), acenaphthylene (1000 ppm), 1-methylnaphthalene (1000 ppm), 2-methylnaphthalene (1000 ppm), acenaphthene (1000 ppm), fluorene (500 ppm), phenanthrene (500 ppm), anthracene (1000 ppm), fluoranthene (500 ppm), pyrene (500 ppm), benz(a)anthracene (500 ppm),

Table 1. HPLC Method Parameters.

HPLC Conditions								
Column:	PerkinElmer Brownlee HRes 1.9 µm 50 x 2.1 mm DB PAH, Part # N9303995							
Mobile Phase Gradient:	Solvent A: water Solvent B: acetonitrile							
		Time (min)	Flow Rate (mL/min)	%A	%B	%C	%D	Curve
	1	Initial	0.700	50.0	50.0	0.0	0.0	Initial
	2	4.00	0.700	5.0	95.0	0.0	0.0	6
	3	4.50	0.700	5.0	95.0	0.0	0.0	6
4	8.00	0.700	50.0	50.0	0.0	0.0	6	
PDA Detector:	Wavelength: 254 nm							
FL Detector:	Step 1: Ex 280 nm / Em 340 nm Step 2: Ex 280 nm / Em 390 nm at 1.52 mins							
Oven Temp.:	35 °C							
Injection Volume:	1 µL							
Sampling (Data) Rate:	10 pts./sec							

chrysene (500 ppm), benzo(j)fluoranthene (500 ppm), benzo(b)fluoranthene (500 ppm), benzo(k)fluoranthene (500 ppm), benzo(a)pyrene (500 ppm), dibenz(a,h)anthracene (500 ppm), benzo(g,h,i)perylene (500 ppm) and ideno(1,2,3-cd)pyrene (500 ppm) in methylene chloride.

In a 25-mL volumetric flask, a stock PAH standard solution was prepared in methanol to equal the final standard concentration of 1 ppm/2 ppm, depending on the analyte. The solution was further diluted in methanol to 0.5 ppm/1 ppm for PDA detection and to 10 ppb/20 ppb for fluorescence detection. These solutions were used to determine linearity at ppm and ppb levels for the PDA and FL detectors, respectively.

Surface water was collected from: 1) the Housatonic River, downstream of a water treatment plant; 2) a suburban driveway with sealcoating; and 3) the surface surrounding a gas station pump area. Each water sample was spiked 1:1 with the PAH standard solution mix. Recoveries for two PAHs, naphthalene and benzo(a)pyrene, were calculated using both PDA and fluorescence detection.

Prior to injection all samples were filtered through a 0.22-µm filter to remove insoluble particles.

Results and Discussion

Figure 1 shows the peak profile of the 19 PAHs at a concentration of 1 ppm/2 ppm. The upper chromatogram, (a), was collected by PDA, while the lower chromatogram, (b), was collected by fluorescence. Separation for all of the 19 PAHs was achieved in less than 4.5 minutes and most were quantifiable down to ppb levels, as specified in Table 1. For fluorescence detection, fluoranthene and indeno(1,2,3-cd)pyrene are not detected using the excitation and emission wavelengths specified in the method.

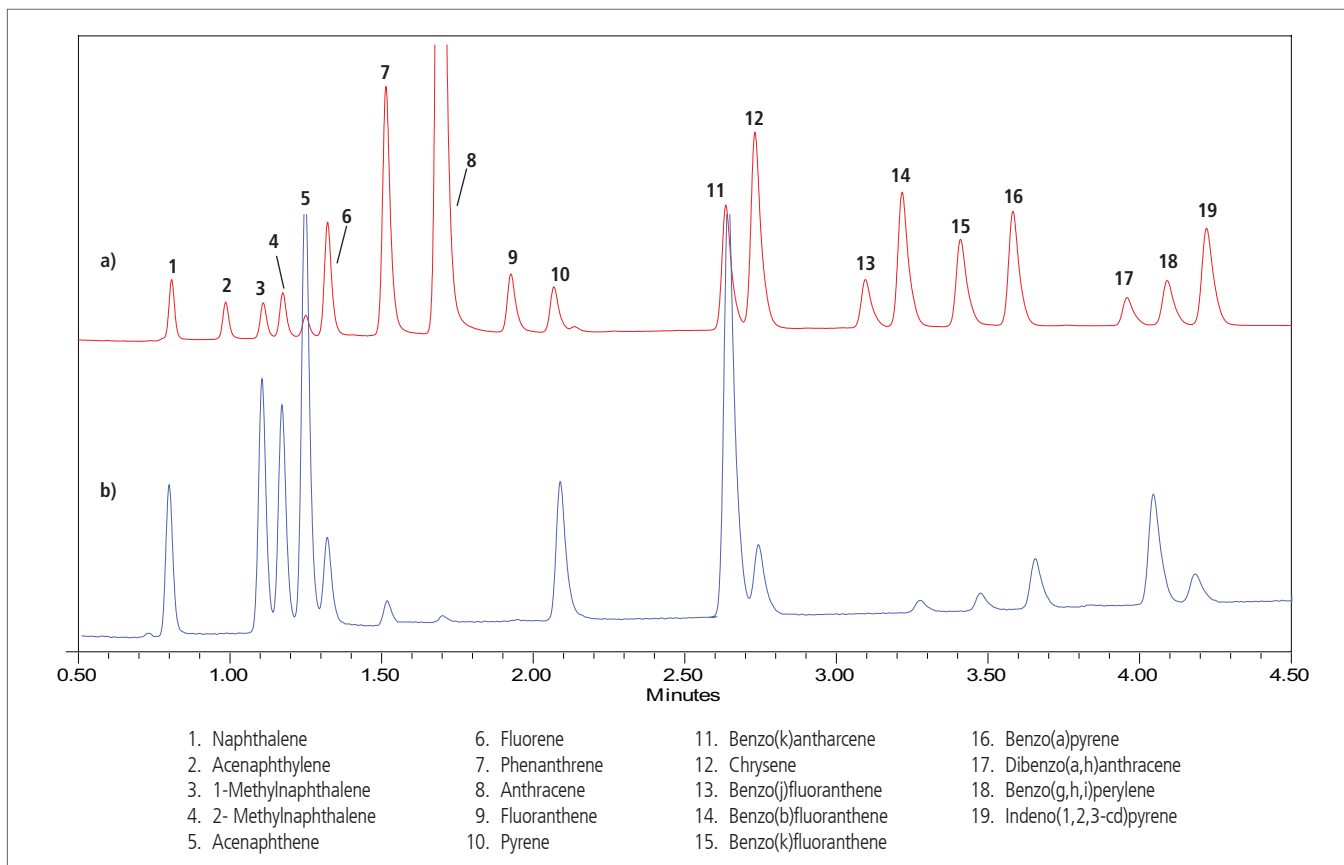


Figure 1. UHPLC chromatogram showing a standard mixture of 19 PAHs at 1 ppm/2 ppm concentrations by a) PDA at 254 nm; b) fluorescence: Ex/Em wavelengths as specified in method.

As shown in Figure 2, chromatographic repeatability was confirmed via ten injections of the 1 ppm/2 ppm PAH standard mixture, (PDA at 254 nm).

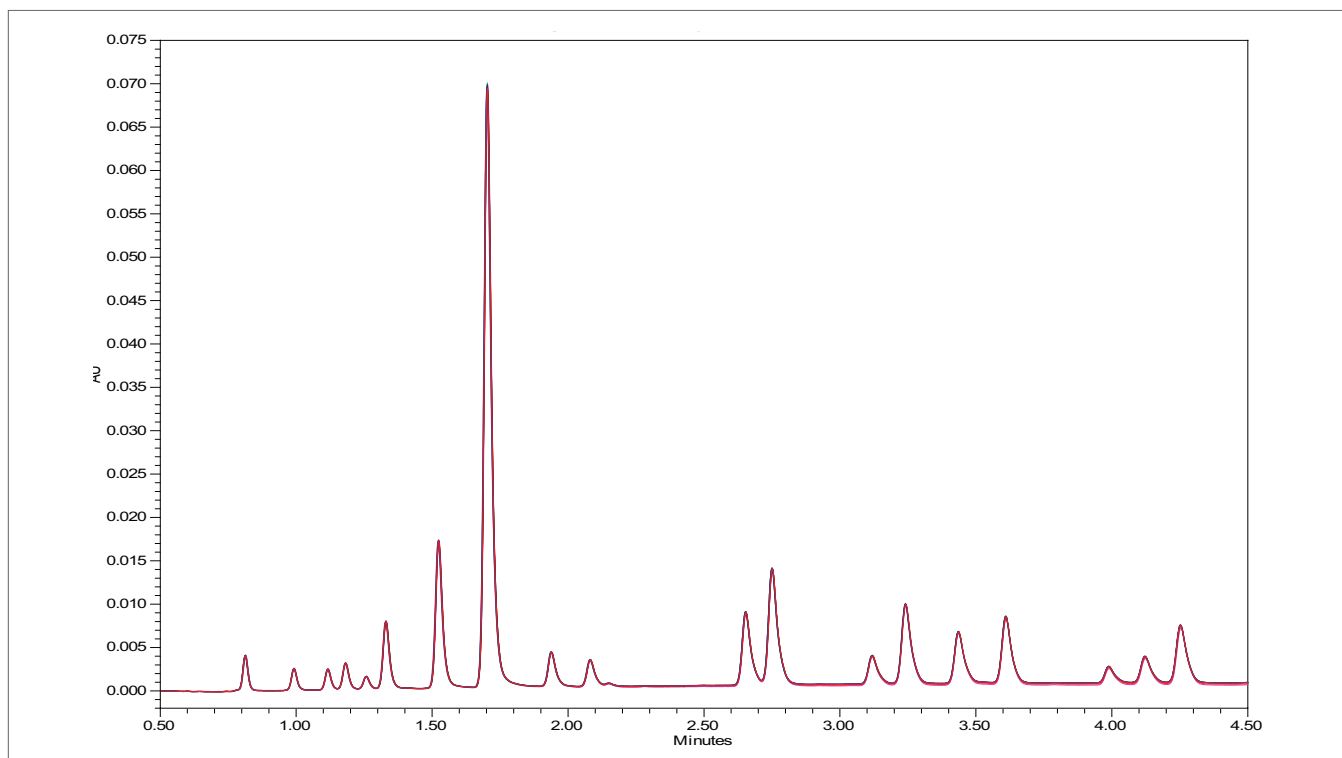


Figure 2. Chromatographic repeatability of the 19 PAH standard mixture by PDA at 254 nm.

Linearity, at ppm levels by PDA detection and ppb levels by fluorescence, was determined for each of the 19 PAHs using a 1:1 water-diluted stock solution. Representative of the 19 PAHs, the linearity plots for both naphthalene and benzo(a)pyrene are shown below.

In Figure 3, we demonstrate linearity for naphthalene between 0.5-5 ppm by PDA detection at 254 nm. Similarly, linearity is demonstrated from 20-100 ppb by fluorescence detection (Ex/Em wavelengths as specified in method).

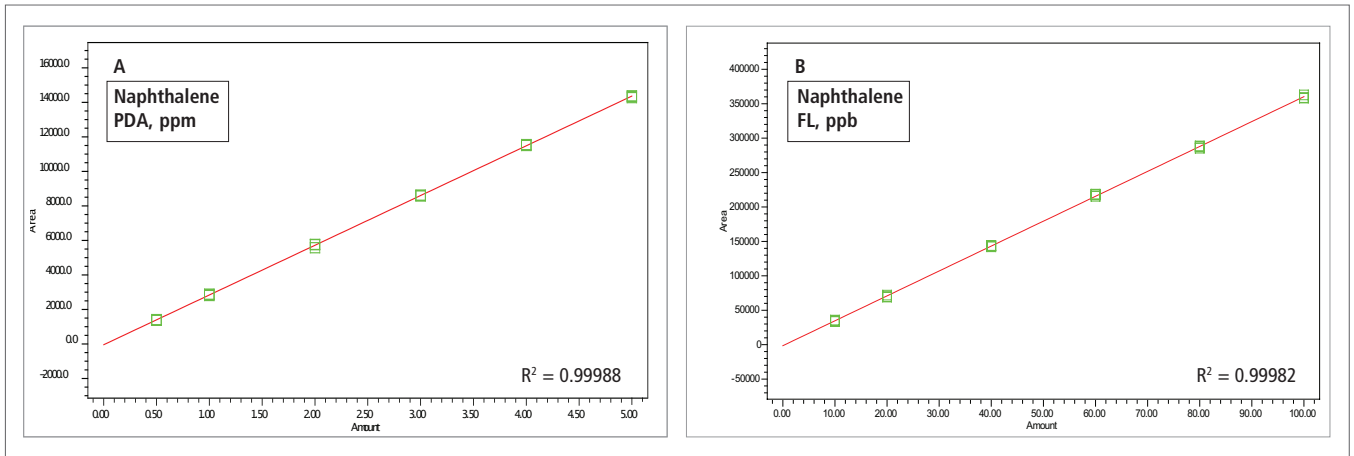


Figure 3. Linearity plots of naphthalene a) 0.5-5 ppm by PDA; b) 20-100 ppb by fluorescence.

In Figure 4, linearity for benzo(a)pyrene between 0.25-2.5 ppm is shown for PDA at 254 nm. Linearity is also demonstrated from 5-50 ppb by fluorescence detection (Ex/Em wavelengths as specified in method).

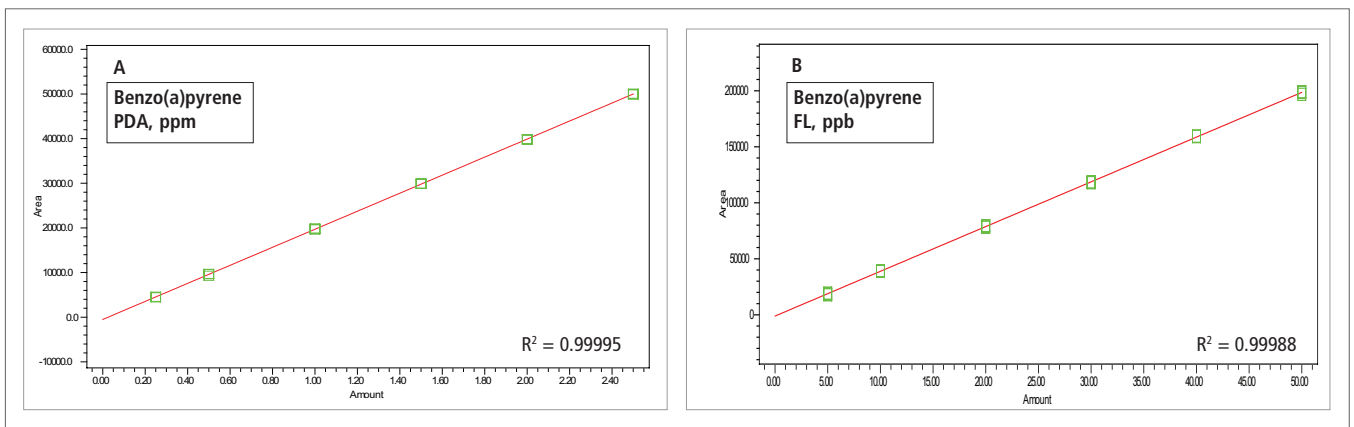


Figure 4. Linearity plots of benz(a)pyrene a) 0.25-2.5 ppm by PDA; b) 5-50 ppb by fluorescence.

As listed in Table 2, LOQ and LOD levels were established for each of the PAHs, based upon a s/n of > 10/1 for LOQ and > 3/1 for LOD. It should be noted that for all PAHs, the LOQs established by PDA were below 1 ppm.

Table 2. PAHs and their calculated LOQs and LODs, by PDA and FL detection.

Polyaromatic Hydrocarbon	RT (min)	PDA LOQ (ppb)	PDA LOD (ppb)	FL LOQ (ppb)	FL LOD (ppb)
Naphthalene	0.81	207	62	17	5
Acenaphthylene	0.99	343	103	ND	ND
1-Methylnaphthalene	1.12	376	113	8	2
2-Methylnaphthalene	1.18	286	86	9	3
Acenaphthene	1.26	598	179	4	1
Fluorene	1.33	56	17	10	3
Phenanthrene	1.52	26	8	38	11
Anthracene	1.70	13	4	227	68
Fluoranthene	1.94	110	33	ND	ND
Pyrene	2.08	146	44	6	2
Benzo(a)anthracene	2.65	52	16	2	1
Chrysene	2.75	33	10	11	3
Benzo(j)fluoranthene	3.12	134	40	11	3
Benzo(b)fluoranthene	3.24	48	14	64	19
Benzo(k)fluoranthene	3.44	74	22	45	13
Benzo(a)pyrene	3.61	57	17	15	5
Dibenzo(a,h)anthracene	3.99	232	69	7	2
Benzo(g,h,i)perylene	4.12	146	44	26	8
Indeno(1,2,3-cd)pyrene	4.25	67	20	ND	ND

ND = Not Detected

As can be observed in Figure 5, no quantifiable PAHs were found in any of the three unspiked, undiluted surface waters analyzed. In order to visualize the amount of PAHs recoverable by PDA and FL detection, surface water samples were spiked with the PAH standard mixture, as shown in Figure 6.

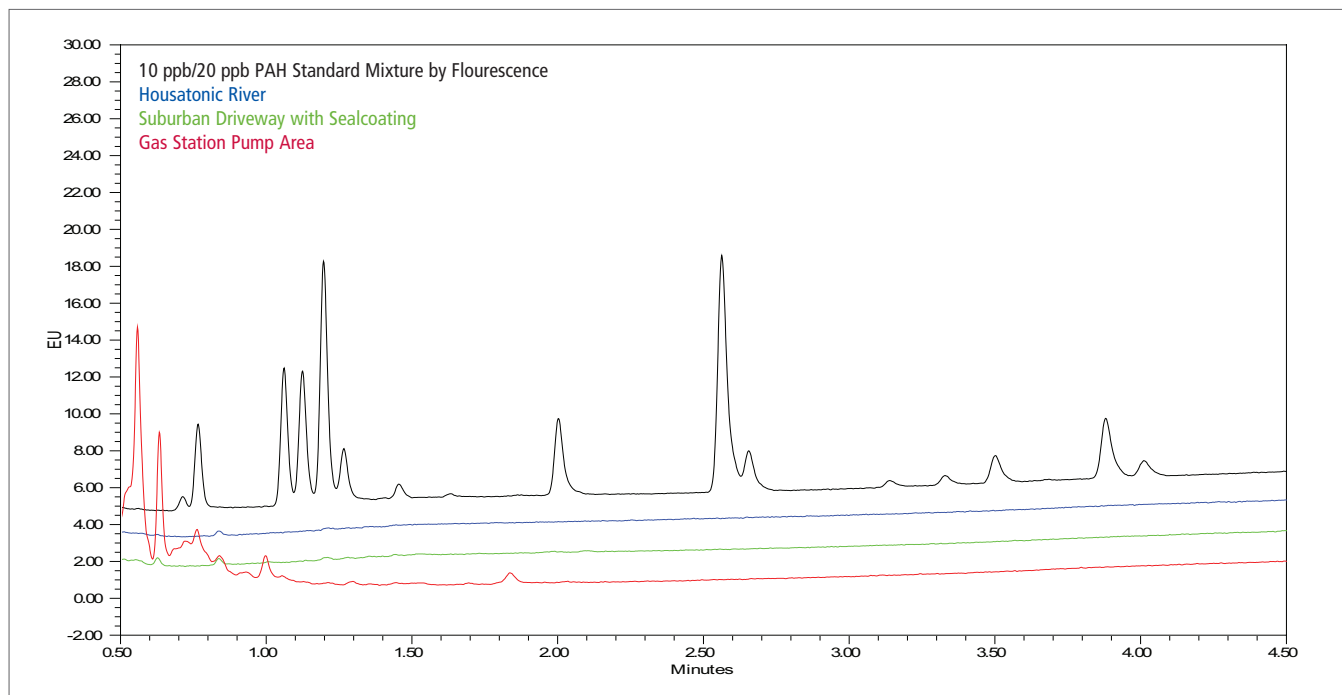


Figure 5. Fluorescence overlay of 10 ppb/20-ppb PAH standard mixture (black) with undiluted surface water from the Housatonic River (blue), a suburban driveway with sealcoating (green), and a gas station pump area (red).

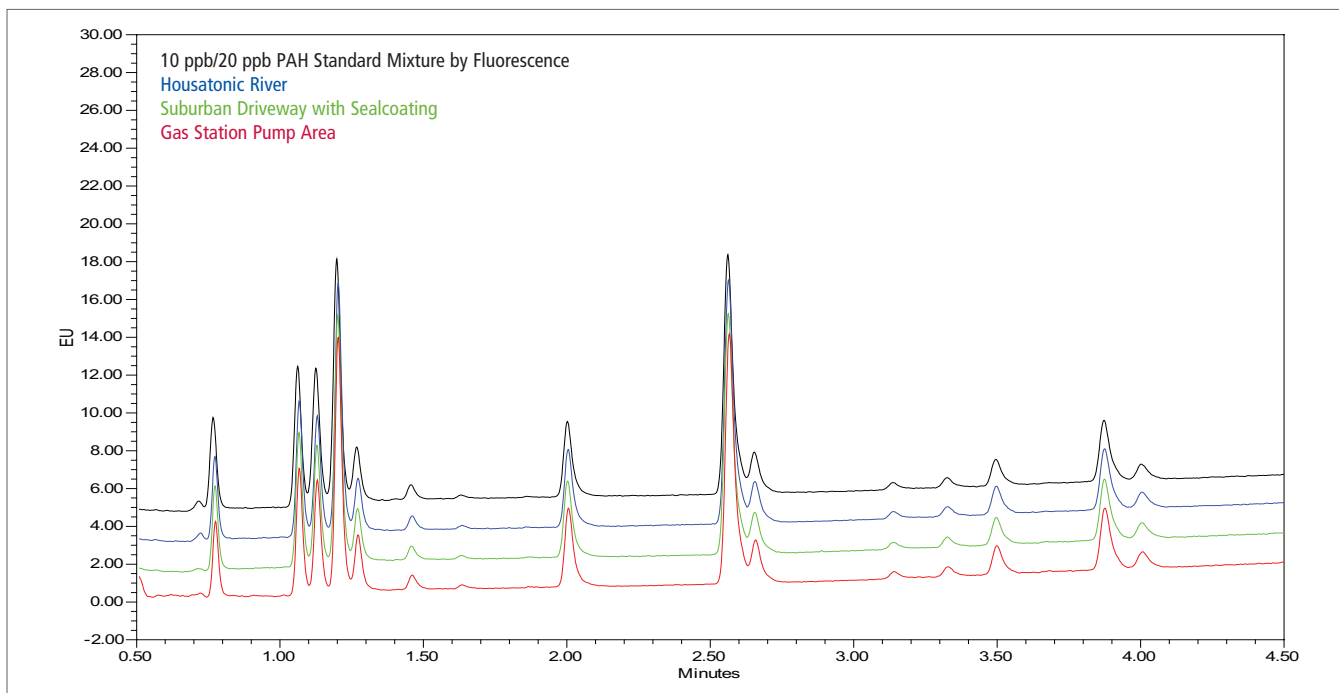


Figure 6. Fluorescence overlay of surface water from the Housatonic River (blue), a suburban driveway with sealcoating (green), and a gas station pump area (red), each spiked 1:1 with the 10 ppb/20 ppb PAH standard mixture (black).

Recoveries for naphthalene and benzo(a)pyrene, in each spiked sample, are shown in Table 3. Recovery was determined at both ppm and ppb levels, using both PDA and fluorescence detection.

Naphthalene and benzo(a)pyrene are reported for each water sample, using the PDA and fluorescence detectors at ppm and ppb levels. Recoveries were between 89-107 %.

Table 3. Recoveries of naphthalene and benzo(a)pyrene in spiked surface water collected from the Housatonic River, a suburban driveway with sealcoating, and gas station pump area, each diluted 1:1 with methanol.

PAH of Interest	Housatonic River	% Recovery from Surface Water Suburban Driveway with Sealcoating	Gas Station Pump Area
PDA Detection			
1-ppm benzo(a)pyrene	107	104	107
2-ppm naphthalene	102	101	98
FL Detection			
10-ppb benzo(a)pyrene	102	100	102
20-ppb naphthalene	89	92	89

Conclusion

This work has demonstrated the fast, effective chromatographic separation of 19 PAHs using a PerkinElmer Altus UPLC® system with A-30 PDA and FL detectors. The results exhibited exceptional linearity for each PAH over the tested concentration ranges.

Though none of the analyzed surface waters showed any detectable amount of PAHs, the naphthalene and benzo(a)pyrene spike recovery analysis demonstrated the ability of the A-30 PDA and A-30 FL detectors to detect PAHs at ppb levels.

References

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