Fully Automated, Trace-Level Determination of Parent and Alkylated PAHs in Environmental Waters by Online SPE-LC-APPI-MS/MS

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Key Words

Online SPE, dopant-assisted APPI, seawater, reclaimed water, rainwater runoff, PAH, alkylated PAHs, EQuan, TSQ Quantum Access, mass spectrometry

Goal

To develop a fast, fully automated protocol for determining a large number of parent PAHs and alkylated PAHs in environmental waters, using online solid–phase extraction coupled with liquid chromatography and tandem mass spectrometry.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants produced by both human activities and natural phenomena. PAHs enter surface waters mainly by atmospheric fallout, urban runoff, municipal and industrial effluents, and the spill or leakage of petroleum and its derivates.[1,2] Petroleum-derived mixtures contain large amounts of PAHs[3] and these compounds are often used as markers to determine the source, fate, and potential effects on natural resources after such substances are released to the environment.[4,5] Many PAHs have been found to have toxic, carcinogenic, and mutagenic properties,[6,7] which have prompted the imposition of strict regulations on their releases in industrial and municipal effluents and their concentrations in environmental waters and drinking water supplies. Both the United States Environmental Protection Agency (US EPA) and the European Union have considered at least 16 parent PAHs as priority pollutants for environmental monitoring purposes.[8]

Well-established methodologies are available for the analysis of PAHs in waste and surface waters, usually involving liquid–liquid extraction (LLE) with n-hexane, toluene, benzene, methylene chloride, or cyclohexane,[1] followed by cleanup steps and detection by gas chromatography and mass spectrometry (GC-MS).[9-12] However, LLE requires a high volume of sample, is labor intensive, and time consuming, which severely limits sample throughput. In addition, large amounts of organic solvents are evaporated to concentrate the analytes. Release of the solvent vapor into the atmosphere causes environmental concerns.[1,13,14]

Solid-phase extraction (SPE) provides an alternative to LLE for sample preparation for PAH analysis. The well-established approach of injecting the sample into a large-volume sample loop connected between the SPE pump and the SPE column allows for sample handling by automated autosamplers.[15-17] In this application note, a fast, fully automated protocol for the determination of parent and alkylated PAHs in environmental waters is presented on the basis of work using online SPE coupled with liquid chromatography (LC) and tandem mass spectrometry (MS/MS) detection with the Thermo Scientific™ EQuan™ online SPE LC-MS system.[18] Optimized conditions for SPE extraction, carryover control, LC separation, and APPI-MS/MS detection are also presented. A comparison between the developed method and LLE-GC-MS in terms of chromatographic resolution and sensitivity was performed, and examples of environmental applications are shown.

Experimental

Materials and Reagents

Certified PAH and isotopically labeled PAH standard mixtures, along with additional single PAH standards, were used.[18] Standard reference materials (SRM 2260a and SRM 1491a) were obtained from NIST (Gaithersburg, MD). Stock solutions were stored at –20 °C until needed. Chlorobenzene dopant (extra dry, 99.8% pure) is available from Thermo Fisher Scientific. Artificial seawater (3.5% w/v) was prepared using the commercially available Instant Ocean® salt. Chromatographic studies were performed using Fisher Chemical™ Optima™ LC/MS-grade acetonitrile, methanol, and water.

Sample Collection

All glassware used to collect and store samples was cleaned by heating to 450 °C for at least 6 h before use. Field samples were collected using 60 mL amber glass vials rinsed once with surface water, filled, and capped with PTFE-lined plastic caps being careful to eliminate trapped air. Vials were then placed in plastic bags and transported on ice to the laboratory. A sampling blank, consisting of a 60 mL vial filled with artificial seawater, was placed on ice and transported during sampling. Seawater samples were collected in a single trip during August 2012 around Northern Biscayne Bay, adjacent to the metropolitan area of Miami, Florida. Two reclaimed water samples were collected from the North District Wastewater Treatment Plant in the Miami-Dade County during August and September 2012.

Rainwater runoff samples were collected during a heavy rain event in June 2013 from drainage openings in two parking lots at the Florida International University (FIU) Biscayne Bay campus and at the parking lot of a nearby residential complex. A reference rainwater sample was collected during the same event using a 1 L amber glass bottle and a glass funnel. All samples were stored at 4 °C. Seawater samples were analyzed no more than 14 days after collection. Rainwater, rainwater runoff, and reclaimed waters were analyzed within 24 h of collection.

Sample Preparation

Environmental water

Working solutions of all PAHs were prepared each analysis day in methanol from stock solutions or certified standards. Refrigerated samples were allowed to reach room temperature before preparation. Vials were vigorously shaken for at least 20 s. Then 10 mL aliquots of raw water samples were transferred using disposable glass graduated pipettes directly from the sampling containers into 10 mL LC vials containing 0.55 mL of a methanol solution of isotopically labeled PAHs and 0.45 mL of water. The resulting solutions contained 5% methanol and 95% water with 100 ng/L of each isotopically labeled PAH. Solutions were capped, thoroughly mixed, and loaded into the online SPE system without further treatment.

Calibration solutions

Matrix-matched calibration solutions (5–500 ng/L) were prepared using the same procedure, using artificial seawater and working solutions containing analytes and internal standards in methanol. A seven-point set of calibration solutions was freshly prepared for each analysis batch.

Liquid Chromatography

Online preconcentration was performed using an EQuan online SPE system consisting of an HTC-PAL™ autosampler system (CTC Analytics, Zwingen, Switzerland) equipped with a 5 mL glass syringe, a Thermo Scientific™ Accela™ 1000 LC pump as an analytical HPLC pump, and an Accela 600 LC pump as an SPE loading pump. The online SPE column was a Thermo Scientific™ Hypersil GOLD aQ™ column (20 × 2.1 mm, 12 µm particle size). Analytical separations were carried out using a Hypersil Green PAH column (150 × 2.1 mm, 3 µm particle size), protected by a Hypersil Green PAH guard column (10 × 2.1 mm, 3 µm particle size). Stainless steel tubing was used throughout the SPE-LC-MS/MS system. Dopant to assist photo-ionization was delivered using the combined output of two programmable syringe pumps.

The samples, quality controls, and calibration solutions were loaded onto the 10 mL stainless steel loop (rotary valve A, Figure 1). The SPE column was placed in a second rotary valve (valve B, Figure 1), allowing connection with either the loading pump or the analytical pump. Analysis steps, determined by valve turning events, are graphically presented in Figure 1. Ten milliliters of sample were passed through the SPE column within 5 min, followed by 2 mL of 1% methanol in water to remove inorganic species. Then, 0.5 mL of a short gradient to 60% methanol and 0.5 mL of 60% methanol were passed to prepare the SPE column for connection with the organic-rich analytical stream (Step 1). The SPE column was connected to the analytical column and gradient separation was started, while the sample loop was completely filled with methanol from the SPE LC pump (Step 2). At 15 min, valve A turned and the methanol-filled sample loop was connected with the injection port. The autosampler sequentially injected 5 mL of methanol, 5 mL of water, and two 5 mL portions of the next sample in the queue while the chromatographic separation continued (Step 3). Finally, at 24 min, valve B turned again (Step 4) and connected the SPE LC pump to the SPE column, which was then cleaned with 1 mL of acetonitrile and progressively taken to the highly aqueous initial conditions. These steps added to a total run time of 28 min per sample.

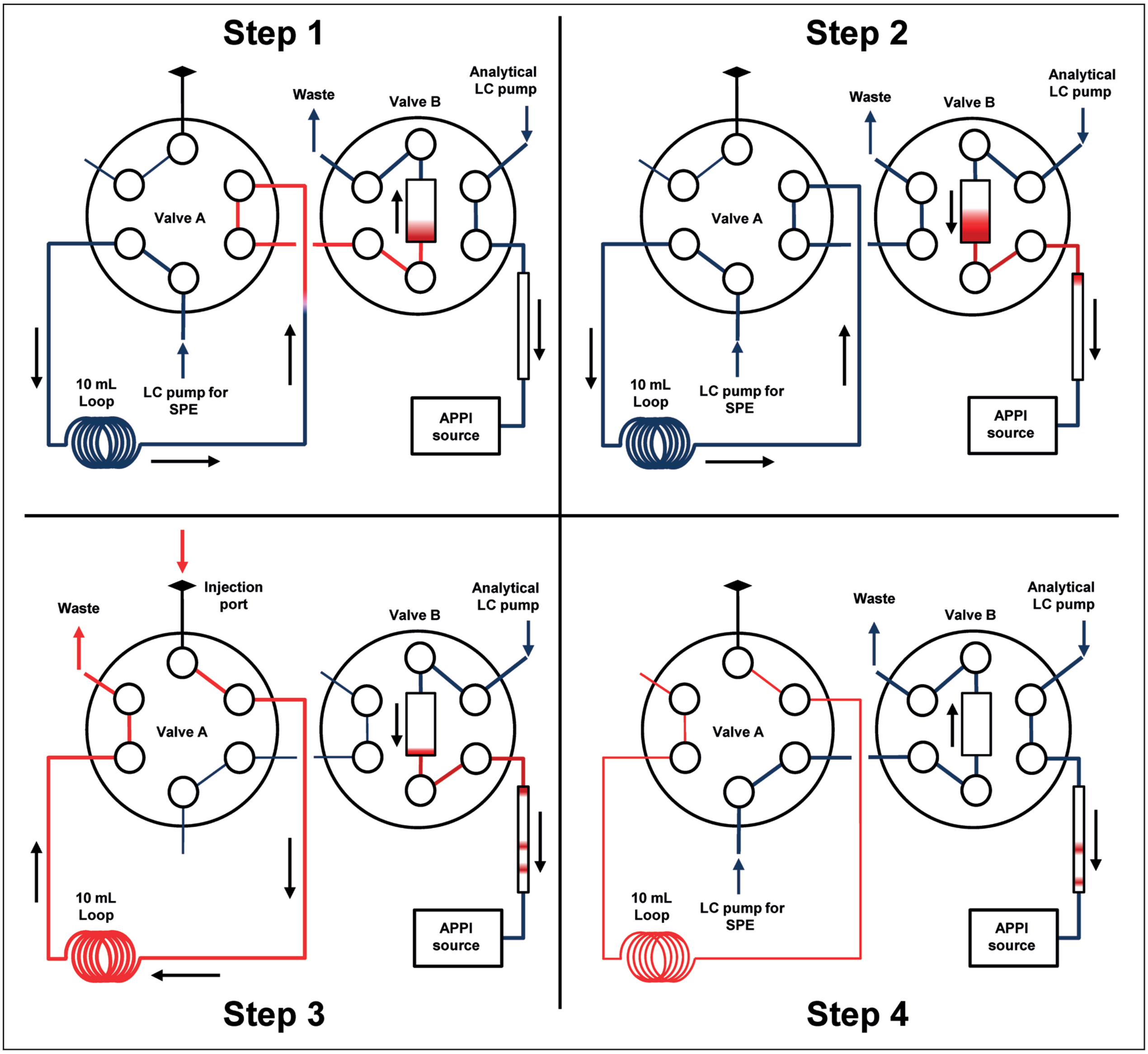

Figure 1. Online SPE system and automated analysis steps. Active flows are shown by arrows and thicker lines. Red: sample and PAHs; blue: mobile phases

Mass Spectrometry

Detection of analytes was performed on a Thermo Scientific™ TSQ Quantum Access™ triple-stage quadrupole mass spectrometer equipped with a Thermo Scientific™ Ion Max™ API source with an APPI probe.

Hydrocarbons (PAHs) are difficult to ionize by conventional LC/MS techniques and yield poor response. Using a dopant-assisted atmospheric pressure photoionization (APPI) interface, an intermediary compound was introduced at high concentrations into the APPI source. This produced large numbers of ions, which in turn underwent a kinetically favored charge transfer with the eluting analytes, provided substantial sensitivity gain relative to dopant-free photoionization.

The following parameters were used for all analytes:

Ion mode	Positive
Skimmer offset	–10 V
Sheath gas (N_2)	40 arbitrary units
Auxiliary gas (N_2)	20 arbitrary units
Capillary temperature	250 °C
Vaporizer temperature	250 °C
Collision gas (Ar) pressure	2.1 mTorr
Scan time	0.020 s
Scan width	0.020 *m/z*

To reduce unnecessary instrument scans, two detection segments were used (segment 1, 8–18 min, and segment 2, 18–28 min). Chlorobenzene dopant was introduced to the APPI source only during the detection period (8–28 min) through the nitrogen auxiliary gas line, delivered by two programmable syringe pumps operating simultaneously for total flow rate of 10% of that of the column eluent. Selected-reaction monitoring (SRM) scan events were obtained by direct infusion of individual PAH solutions and are listed in Table 1.

Table 1. Summary of PAH compounds and their SRM scan events for PAHs tested with this method (**bold: 16 US EPA priority PAHs**, *italic: labeled PAH internal standards*)

PAH	CAS Number	$M^{+\bullet}$ Ion (*m/z*)	Quant. Product (*m/z*)	CE (V)	Confirm. Product (*m/z*)	CE (V)	Confirm. Product Int. (%)[a]	Tube Lens (V)	RT (min)	Scan Seg.[b]
Acenaphthene	83-32-9	154	153	16	152	29	72	82	13.1	1
Acenaphthene-D10	15067-26-2	164	162	23	160	35	70	72	12.9	1
Acenaphtylene	208-96-8	152	151	16	150	23	50	69	12.1	1
Anthanthrene	191-26-4	276	274	43	272	69	99	108	24.2	2
Anthracene	120-12-7	178	176	29	152	22	54	82	14.9	1
Benz[a]anthracene	56-55-3	228	226	35	225	52	22	92	17.6	1+2
Benzo[a]fluoranthene	203-33-8	252	250	73	248	41	38	120	18.5	1+2
Benzo[a]pyrene	50-32-8	252	250	73	248	41	42	120	20.3	1+2
Benzo[a]pyrene-D12	63466-71-7	264	260	51	236	47	15	82	20.0	2
Benzo[b]fluoranthene, perylene[c]	205-99-2, 198-55-0	252	250	73	248	41	38	120	19.1	1+2
Benzo[b]fluorene	243-17-4	216	215	14	213	38	50	53	17.4	1+2
Benzo[c]phenanthrene	195-19-7	228	226	35	225	52	24	92	16.3	1+2
Benzo[e]pyrene, benzo[j]fluoranthene[c]	192-97-2, 205-82-3	252	250	73	248	41	37	120	18.8	1+2
Benzo[g,h,i]perylene	191-24-2	276	274	43	272	69	60	108	22.0	2
Benzo[k]fluoranthene	207-08-9	252	250	73	248	41	35	120	19.9	1+2
Biphenyl	92-52-4	154	152	29	153	16	90	82	12.4	1
C1-chrysenes	-	242	239	42	241	22	65	80	18-20	1+2
C1-dibenzothiophenes	-	198	197	10	165	25	49	68	14-16	1+2
C1-fluoranthenes/pyrenes	-	216	215	14	213	38	50	53	15-17	1+2
C1-fluorenes	-	180	165	19	164	35	21	53	14-16	1
C1-naphthalenes	-	142	141	21	115	32	71	57	12.5	1
C1-phenanthrenes/anthracenes	-	192	191	22	189	40	90	67	15-18	1+2
C2-dibenzothiophenes	-	212	211	20	152	39	30	83	15-19	1+2
C2-naphthalenes	-	156	141	19	115	33	60	55	13-15	1
C2-phenanthrenes/anthracenes	-	206	189	39	191	22	58	137	17-19	1+2
C3-naphthalenes	-	170	155	18	153	30	34	75	14-16	1
C4-phenanthrenes/anthracenes	-	234	219	11	204	22	75	10	17-19	1+2
Carbazole	86-74-8	167	166	40	165	40	13	80	10.9	1
Chrysene	218-01-9	228	226	35	225	52	22	92	18.0	1+2
Chrysene-D12	1719-03-5	240	236	37	212	34	14	108	17.8	1+2
Dibenz[a,h]anthracene	53-70-3	278	276	42	274	65	62	105	21.9	2
Dibenzothiophene	132-65-0	184	152	30	139	39	90	85	14.0	1
Fluoranthene	206-44-0	202	200	40	199	57	16	73	15.6	1
Fluorene	86-73-7	166	165	21	164	33	23	101	13.6	1
Fluorene-D10	81103-79-9	176	174	28	172	38	21	65	13.5	1
Indeno[1,2,3-cd]pyrene	193-39-5	276	274	43	272	69	48	108	22.7	2
Naphthalene	91-20-3	128	127	25	102	20	90	48	11.5	1
Naphthalene-D8	1146-65-2	136	134	30	108	30	59	80	11.4	1
Naphthobenzothiophene	239-35-0	234	202	25	189	33	90	100	18.2	1+2
Perylene-D12	1520-96-3	264	260	51	236	47	9	82	18.9	2
Phenanthrene	85-01-8	178	176	29	152	22	75	82	14.2	1
Phenanthrene-D10	1517-22-2	188	184	40	160	32	98	82	14.0	1
Pyrene	129-00-0	202	200	40	199	57	20	73	16.0	1
Triphenylene	217-59-4	228	226	35	225	52	23	92	16.8	1+2

[a] Relative to quantification product ion. [b] Segment 1: 8-18 min, segment 2: 18-28 min. [c] Coelutions observed in Standard Reference Material 2260a.

Data Analysis

Data analysis was performed using Thermo Scientific™ TraceFinder™ EFS software version 3.0.

Results and Discussion

Optimization of Dopant-Assisted APPI Detection

Pure chlorobenzene provides efficient charge transfer ionization for all PAH in the presence of water, methanol, and acetonitrile.[19] Therefore, commercially available high-purity chlorobenzene was used as dopant in this study without any treatment. Under these conditions, a strong positive molecular ion ($M^{+\cdot}$) for each analyte was always observed and isolated as the precursor ion for the SRM scan events, which is consistent with observations by other authors who have used chlorobenzene as dopant for APPI-LC-MS analysis of PAHs.[20]

Two programmable syringe pumps and a spraying device placed in the auxiliary nitrogen gas stream were used. With this system, little or no backpressure was applied to the syringe pumps, which translated into stable dopant delivery. Since analytical signals maximized at a dopant flow rate of approximately 10% of the eluent flow rate, using a programmable dopant system has the advantage of maintaining this optimum ratio as the eluent flow rate changes during the chromatographic separation.

The spraying system was tested with two syringe pumps equipped with four 10 mL syringes (40 mL total), which provided 26 runs (approximately 12 h of continuous operation) before syringe refills were required. This translates into a consumption of about 1.5 mL of chlorobenzene per sample. In comparison, the traditional LLE-GC-MS approach may require up to 150 mL (3 × 50 mL extractions) with organic solvents, such as methylene chloride, to ensure a high recovery. Chlorobenzene has a much shorter atmospheric persistence (half-life of 20–40 h) than methylene chloride and is not considered a carcinogen. Thus, both the lower quantity and the nature of the halogenated waste produced suggest that the online SPE-LC-APPI-MS/MS is a more environmentally friendly methodology than LLE-GC-MS.

Optimization of Chromatographic Separation

During compound optimization for SRM detection, it was observed that PAHs with the same parent masses have similar behavior upon collision-induced dissociation (same product ions, same collision energy, see Table 1), eliminating the possibility of selective detection of isobaric PAHs. Because comprehensive PAH analysis requires quantitation beyond the 16 priority PAHs, a carefully controlled LC separation is required to solve most of these isobaric interferences. In addition, since PAH molecules have fixed planar conformations, chromatographic selectivity is governed solely by their molecular dimensions.[21]

Furthermore, complete chromatographic resolution of the 16 PAHs listed as priority by the EPA using the Hypersil Green PAH stationary phase has been previously reported.[22,23] This stationary phase was selected to explore the possibility of a liquid chromatography separation of most alkylated PAHs as these compounds are often used as markers to identify pollution sources and environmental transformations.[4, 24] Light PAHs (i.e., alkylnaphthalenes) could be only efficiently separated using a methanol/water gradient system, as the use of acetonitrile/water caused fast elution with no resolution control. On the other hand, methanol proved to be a weak solvent for PAHs *m/z* 228 and above, causing excessively high retention times and peak shape broadening even at 100% methanol isocratic elution. A second gradient between methanol and acetonitrile was then used after the water/methanol system. Still, retention times for PAHs *m/z* 252 and above were also very high even at 100% acetonitrile conditions. To perform an efficient, wide mass range separation, a flow rate gradient was also used in combination with solvent strength control, taking advantage of the steep backpressure drop observed as water is removed from the analytical column during the gradient.

Figure 2 compares the obtained resolution of alkylated PAHs contained in the Standard Reference Material 1491a to that obtained by traditional GC-MS analysis. Although resolution for C1-naphthalenes was lower than GC, two marginally resolved peaks are observed in the SPE-LC-MS/MS separation of these compounds that differ only in the position of a single methyl group between adjacent carbon atoms. Since C1-naphthalenes are detected as a group, the limited resolution does not affect quantitation. As analyte mass increased, the observed resolution behavior tended to be similar to that obtained by GC-MS. Both techniques had the same difficulty in separating C1-fluoranthenes and C1-pyrenes (four peaks should be observed in the *m/z* 216 chromatogram), while complete resolution was observed for 3-methylchrysene and 6-methylchrysene in both methods. All four methylphenanthrenes are visible and well separated from the 2-methylanthracene signal, in contrast to the GC-MS separation where a coelution of the two groups is observed. These results indicate that isobaric-alkylated PAHs can be partially resolved using single-column liquid chromatography.

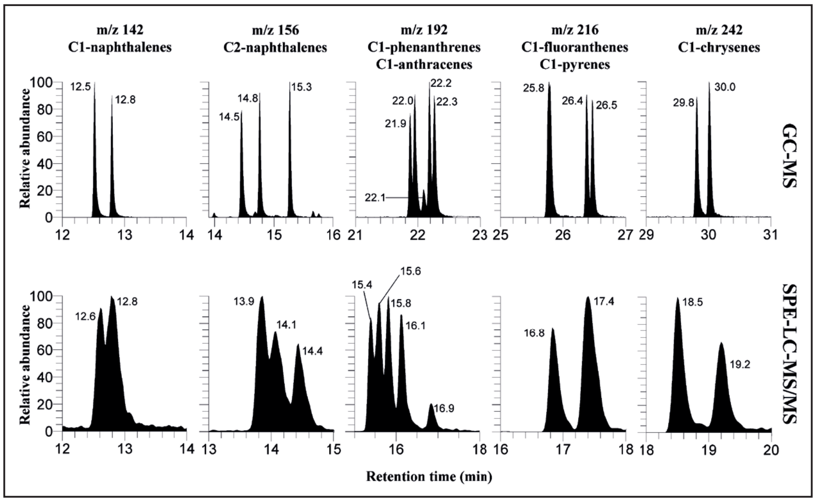

Figure 2. Comparison of peaks of PAHs contained in the Standard Reference Material 1491a, obtained by GC-MS analysis (1/10 dilution in hexane, top) and by SPE-LC-MS/MS analysis (1/27,500 serial dilution in seawater, bottom). Reference material listed compounds: C1-naphthalenes (1-methyl, 2-methyl); C2-naphthalenes (1,2-dimethyl, 1,6-dimethyl, 2,6-dimethyl); C1-phenanthrenes (1-methyl, 2-methyl, 3-methyl, 9-methyl); C1-anthracenes (2-methyl); C1-fluoranthenes (1-methyl, 3-methyl); C1-pyrenes (1-methyl, 4-methyl); C1-chrysenes (3-methyl, 6 methyl). Standard Reference Material 1491a also contains one C2- phenanthrene (1,7-dimethyl, not shown)

Although the observed resolution of alkylated PAHs may not be enough to replace capillary GC-MS for PAH fingerprinting applications, the resolution obtained by SPE-LC-MS/MS could be enough to be used as a screening tool to decide if a given sample should be analyzed using those time-consuming techniques, taking advantage of the low sample consumption and the speed of this methodology. Additionally, the absence of sample preparation could provide the ability to track in almost real time the extent of a contamination by monitoring for the alkylated PAH-specific concentration patterns observed at the pollution source. With the gradient separation used, baseline resolution of the 16 priority PAHs from their isobaric interferences present in Standard Reference Material 2260a was obtained except for benzo[*b*]fluoranthene, which coeluted with perylene. Attempts to separate these compounds without a significant increase in run time were unsuccessful, and since method speed was a priority, these compounds were quantified as a group.

Optimization of the Online SPE Procedure

SPE column loading, washing, and reconditioning parameters were optimized for extraction recovery, seawater salt elimination, and prevention of carryover using isotopically labeled PAHs as testing compounds. Same-day 10 mL injections of 100 ng/L (online SPE) and 100 µL direct injections of 10,000 ng/L solutions in 70% methanol/water were made, accounting for 1.0 ng on column for each compound (the 5 mL injection mode was tested against 50 µL direct-injection, 0.5 ng on column). Percent recoveries were obtained using averaged peak areas, using at least three direct-injection runs and two online SPE runs. The direct-injection method had the same analytical gradient as the online SPE method. The observed retention times were in agreement with an 8 min offset due to the online SPE time, ensuring similar APPI source conditions at elution in both injection modes thus enabling the direct comparison of peak areas. Passing at least 2 mL of aqueous mobile phase through the loading column after the SPE step was enough to prevent the transfer of salt residues to the APPI source.

Method Validation

Calibration and quality control

Calibration curves were obtained by plotting the peak area ratio of each PAH to an isotopically labeled PAH internal standard against concentration in nanograms per liter. Linearity was observed for all analytes in the range used (R^2>0.99; 5 to 500 ng/L). Calibration stability was evaluated every 10 runs by injecting seawater fortified at 100 ng/L. Calibration and method accuracy was verified by injecting artificial seawater fortified with serially diluted standard reference materials 1491a and 2260a. With every analysis batch, a negative (reagent and sampling) and a positive (fortified at 100 ng/L) blank were also used. Additionally, one sample duplicate and one fortified matrix experiment were always analyzed per every five samples. The system was continuously tested for carryover by injecting a reagent blank after the highest calibration standard and after every calibration verification standard. Compound identification was considered positive when signals with a S/N ratio above 3 were present in both the quantification and confirmation SRM transitions, with a maximum retention time difference of 0.2 min relative to calibration standards or standard reference materials. Calculated concentrations below method detection limits (MDLs) were considered non-detections. A reporting limit (RL) of three times the MDLs was set in order to reduce the risk of false positives and ensure data quality.

Determination of method detection limits

MDLs were calculated by multiplying the standard deviation from seven measurements by the Student *t* value ($t_{(7-1,\ 99)}$=3.143), according to procedures outlined by the US EPA,[9] using natural seawater (from FIU Campus Beach, see Table 2), fortified at 50 ng/L. For sensitivity comparison, MDLs for the traditional LLE+GC-MS methodology were determined using 1,000 mL of the same seawater sample also fortified to 50 ng/L and extracted three times with 50 mL portions of methylene chloride. The extract was obtained, evaporated, and cleaned according to established methods (EPA 3510C and 3630C)[10,11] and analyzed by a GC-MS method available elsewhere.[12] The average MDLs corrected for sample size obtained by LLE-GC-MS analysis are an order of magnitude higher than those obtained by SPE-LC-MS/MS.[18] Although in practice lower MDL values can be obtained with LLE due to the possibility of using larger sample volumes, the higher per volume sensitivity of the online SPE approach is more useful when limited amounts of sample are available. Also, the low sample volume required and high sample throughput of this method facilitate the analysis of multiple quality controls such as duplicates and fortified matrix experiments.

Table 2. Method performance upon analysis of surface seawater of US EPA priority PAHs.

Location			Haulover Boat Ramp	Haulover Marina	Dinner Key Marina			Bayfront Park	Pelican Harbor Park	FIU Campus Beach	Miami Beach Marina			
Latitude N			25.91684	25.90613	25.7272			25.77274	25.84713	25.90994	25.77194			
Longitude W			80.12383	80.12396	80.23767			80.18491	80.16782	80.1364	80.14027			
PAH	**MDL (ng/L)**	**RL (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Duplicate (ng/L)**	**Ave±σ (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc in Fortified Matrix Experiment (ng/L)**	**Fortification Level (ng/L)**	**% Rec**
Acenaphthene	15	45	-	-	-	-		-	-	-	-	184	176	104
Acenaphthylene	16	49	-	-	-	-		-	-	-	-	179	176	102
Antracene	29	86	-	-	-	-		-	-	-	-	179	176	101
Benz[*a*] anthracene	12	36	-	-	-	-		-	-	-	-	160	176	91
Benzo[*b*] fluoranthene, perylene	34	102	-	-	-	-		-	-	-	-	370	373	99
Benzo[*g,h,i*] perylene	19	57	-	-	-	-		-	-	-	-	185	176	105
Benzo[*k*] fluoranthene	21	63	-	-	-	-		-	-	-	-	189	176	108
Crysene	11	33	-	-	-	-		-	-	-	-	173	176	98
Dibenz[*a,h*] anthracene	16	48	-	-	-	-		-	-	-	-	188	176	107
Fluoranthene	12	36	-	-	-	-		-	-	-	-	180	176	101
Fluorene	7.9	24	-	-	-	-		-	-	-	-	187	176	106
Indeno[1,2,3-*cd*]pyrene	26	78	-	-	-	-		-	-	-	-	197	176	112
Naphthalene	20	60	101	-	104	100	102±2	-	-	-	-	189	176	107
C1-naphthalenes	13	40	129	-	74	76	75±1	-	-	-	-	419	353	119
C2-naphthalenes	15	44	<RL	-	47	45	46±1	-	-	-	-	177	176	101
Phenanathrene	19	57	-	-	-	-		-	-	-	-	167	176	94
Pyrene	17	50	-	-	-	-		-	-	-	-	166	176	94
Total PAH			230	0	225	221	**223±2**	0	0	0	0			
% Recovery Average														**103±7**

– Below MDL

Examples of Environmental Applications

The developed methodology was tested by analyzing a group of multi-origin environmentally relevant water samples. Seawater collections were made from seven sites in northern Biscayne Bay. Naphthalene and alkylnaphthalenes were detected in two of the sites in which activity of small vessels was observed (Table 2). The elevated water solubility of naphthalenes relative to other PAHs[1] may increase their permanence in the water long enough to be detected by the grab sampling performed. Although the method sensitivity was not enough to detect background concentrations in samples where no active boating was observed, a capability of fast detection of focalized emission of petroleum-derived products was demonstrated.

Suspended particles in rainwater runoff resulting from the erosion of impervious surfaces have been documented as an important source of PAHs in the environment.[25,26] To assess the performance of the developed methodology for this type of monitoring, rainwater and runoff samples from the drainage openings at three parking lots were collected during a heavy rain event in June 2013. As can be seen in Table 3, PAHs were detected in runoff from only the partially flooded parking lot located in a residential complex. Chromatograms for the priority PAHs detected in that sample are compared with reference rainwater in Figure 3, showing that interference-free detection and positive identification were obtained for these analytes except for benzo[*b*]fluoranthene, which is not resolved from perylene by this methodology as discussed before. Also, since no PAHs were observed in the reference rainwater, this data strongly suggests that the parking lot was the source of the contamination. The high number of parent PAHs detected, the predominance of heavy PAHs such as fluoranthene and pyrene, and their relative concentrations are in agreement with previous reports of PAHs in rainwater runoff from coated parking lots,[27] suggesting that the presented methodology is applicable for this type of study.

Table 3. Method performance upon analysis of rainwater and rainwater runoff of US EPA priority PAHs.

Sample Source			**Direct Collection**	**Apartment Complex**			**FIU Biscayne Bay Campus**				
Sample Description			Reference Rainwater	Partially Flooded Parking Lot			Parking Lot A	Parking Lot B			
PAH	**MDL (ng/L)**	**RL (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Duplicate (ng/L)**	**Ave±σ (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc (ng/L)**	**Measured Conc in Fortified Matrix Experiment (ng/L)**	**Fortification Level (ng/L)**	**% Rec**
Acenaphthene	15	45	-	-	-		-	-	105	110	95
Acenaphthylene	16	49	-	-	-		-	-	119	110	109
Antracene	29	86	-	-	-		-	-	118	110	107
Benz[*a*]anthracene	12	36	-	190	202	196±6	-	-	105	110	96
Benzo[*b*] fluoranthene, perylene	34	102	-	112	108	110±2	-	-	216	220	98
Benzo[*g,h,i*] perylene	19	57	-	60	61	60.4±0.7	-	-	124	110	113
Benzo[*k*] fluoranthene	21	63	-	<RL	<RL		-	-	105	110	96
Crysene	11	33	-	153	169	161±8	-	-	112	110	101
Dibenz[*a,h*] anthracene	16	48	-	-	-		-	-	137	110	124
Fluoranthene	12	36	-	410	387	399±12	-	-	104	110	95
Fluorene	7.9	24	-	<RL	<RL		-	-	100	110	91
Indeno[1,2,3-*cd*] pyrene	26	78	-	<RL	<RL		-	-	130	110	118
Naphthalene	20	60	-	-	-		-	-	91	110	83
C1-naphthalenes	13	40	-	-	-		-	-	209	220	95
C2-naphthalenes	15	44	-	-	-		-	-	111	110	101
Phenanathrene	19	57	-	183	186	184±1	-	-	116	110	105
Pyrene	17	50	-	293	315	304±11	-	-	117	110	107
Total PAH			0	1401	1428	**1415±14**	0	0			
% Recovery Average											**102±10**

– Below MDL, <RL Detection below reporting limit (RL=3×MDL)

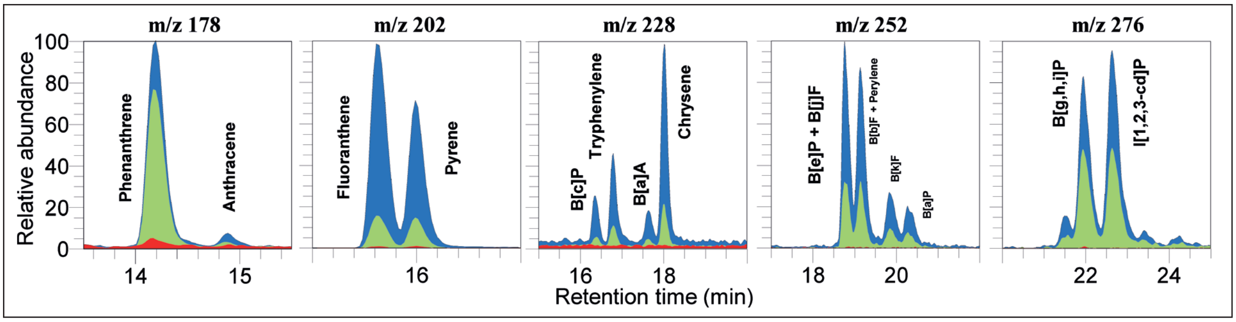

Figure 3. Chromatograms obtained upon analysis of a rainwater runoff sample from a residential parking lot and its comparison with reference rainwater. (Blue trace: main (quantitative) SRM transition in runoff sample; green trace: secondary (confirmation) SRM transition in runoff sample; red trace: main SRM transition from injection of reference rainwater)

Although not enough data is available to explain the non-occurrence of PAHs in runoff from the campus parking lots, the residential parking lot had a much slower drainage capability, and thus, the sample was collected under moderate flooding conditions. The lower drainage rate may have enhanced the possibility of detections as PAH-containing suspended particles could not be washed out by the rain as fast as in the campus parking lots. It is also possible that the nature of the coatings is different, as it has been shown that asphalt-based coatings contain many fewer PAHs than coal-based coatings.[25]

Samples of reclaimed water used for irrigation at FIU Biscayne Bay campus were collected in two different dates and analyzed in order to assess the performance of the developed methodology to detect PAHs discharged with WWTP effluents. Alkylnaphthalenes were detected in one on the samples (Table 4), but concentrations were lower than the reporting limit. Good recoveries were obtained in the fortified matrix experiment for reclaimed water, suggesting that method sensitivity rather than a severe matrix effect prevented positive quantification in these samples. Excellent recoveries were also obtained in fortified matrix experiments with the other two types of environmental waters tested with this method, which may suggest that the use of a wide range of molecular sizes of isotopically labeled PAHs normalizes analyte behavior during the automated preconcentration and analysis, keeping matrix effects under control in spite of the lack of any other sample preparation steps such as filtration. In addition, method reproducibility was also good upon analysis of duplicates of PAH-containing seawater and runoff samples.

Table 4. Method performance upon analysis of reclaimed water obtained from the Miami-Dade North District Wastewater Treatment Plant for US EPA priority PAHs.

PAH	MDL (ng/L)	RL (ng/L)	Measured Conc (ng/L)	Measured Conc (ng/L)	Measured Conc in Fortified Matrix Experiment (ng/L)	Fortification Level (ng/L)	% Recovery
Acenaphthene	15	45	-	-	203	176	115
Acenaphthylene	16	49	-	-	162	176	92
Antracene	29	86	-	-	185	176	105
Benz[*a*]anthracene	12	36	-	-	164	176	117
Benzo[*b*] fluoranthene, perylene	34	102	-	-	363	373	97
Benzo[*g,h,i*] perylene	19	57	-	-	150	176	85
Benzo[*k*] fluoranthene	21	63	-	-	219	176	124
Crysene	11	33	-	-	210	176	119
Dibenz[*a,h*] anthracene	16	48	-	-	156	176	88
Fluoranthene	12	36	-	-	209	176	116
Fluorene	7.9	24	-	-	168	176	95
Indeno[1,2,3-*cd*] pyrene	26	78	-	-	174	176	99
Naphthalene	20	60	-	-	161	176	91
C1-naphthalenes	13	40	-	-	364	353	103
C2-naphthalenes	15	44	-	<RL	228	176	118
Phenanathrene	19	57	-	-	175	176	99
Pyrene	17	50	-	-	200	176	111
Total PAH			0	0			
% Recovery Average							**104±12**

Conclusion

An automated protocol for the comprehensive analysis of 28 parent PAHs and their extended alkylated homologues by online SPE-LC-MS/MS was successfully developed with optimized parameters for extraction, separation, and detection using dopant-assisted APPI. Method performance and the control of matrix effects were demonstrated by obtaining good recoveries upon analysis of seawater, reclaimed water, and rainwater runoff fortified with certified standards, showing the utility of this method to survey the occurrence of PAHs in waters at the urban environment. A survey of PAH concentration in a seawater environment influenced by a large urban area was conducted, and although background concentrations were below MDLs, localized PAH input events from boating activities were detected above reporting limits. With lower run times, very simple sample preparation, lower generation of toxic solvent waste, and higher sensitivity per volume of sample used, this method could represent a viable alternative to LLE-GC-MS for routine PAH monitoring, providing laboratories with a much higher sample throughput while reducing overall operation costs and the environmental impact of PAH analysis.

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