Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Shrimp

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A simple and rapid method for determining polycyclic aromatic hydrocarbons (PAHs) in shrimp is described. For sample preparation, the quick and simple QuEChERS procedure was used. Reverse-phase chromatography using an octadecyl silica (C_{18}) column and water/acetonitrile gradient elution was used to separate analyte mixtures. After separation, PAHs were detected using liquid chromatography−tandem mass spectrometry (LC−MS/MS) equipped with the atmospheric pressure photoionization (PhotoSpray APPI) source operating in the positive-ion mode. In this methodology, all 16 common PAHs were used and toluene served as a charged dopant to efficiently ionize analyte molecules through secondary reactions. Spikes were performed at 0.2 and 1 μg/g with and without primary and secondary amine (PSA) sorbent cleanup. Recoveries of PAHs were good, with ion ratios that agreed well between the spikes and standards. Without cleanup at 0.2 μg/mL, seven compounds had relatively low recovery (49−69%) and one compound, naphthalene, had a somewhat high recovery of 129%. At 1 μg/mL without cleanup, only three compounds had slightly lower recovery (66−67%). When PSA cleanup was performed, all PAH recoveries were within 75−125% at both spike levels.

KEYWORDS: PAHs; PhotoSpray; LC−MS/MS; shrimp

INTRODUCTION

Oil from the Deepwater Horizon rig has contaminated the Gulf of Mexico; therefore, there is a need to develop methods to analyze shrimp for polycyclic aromatic hydrocarbons (PAHs), which are toxins found in oil. Many PAHs are toxic, mutagenic, and carcinogenic (*1*). Although high-performance liquid chromatography (HPLC) with ultraviolet (UV) or fluorescence detection is often used to analyze environmental samples (*2*), liquid chromatography−tandem mass spectrometry (LC−MS/MS) offers far better selectivity (*3*). Shrimp or any animal tissue contains a myriad of components that are likely to interfere with non-selective analyses only based on UV, fluorescence, or even single-quadrupole MS detection systems. Therefore, LC−MS/MS was chosen as the best method to avoid false positives. Applied Biosystems (also known as AB/Sciex) has an application note (*4*) describing the analysis of atmospheric aerosol samples for PAHs by LC−MS/MS using an atmospheric pressure Photo-Spray source, which "uses photons of light to ionize large quantities of a dopant molecule added along with the vaporized mobile phase". Analyte molecules are efficiently ionized through secondary reactions initiated by the charged dopant (*5*). Atmospheric pressure ionization (API) [electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI)] is usually the choice for ionization for most analytes, but it is inadequate for PAHs. This is because much more energy is needed to ionize the low-polarity C−H bonds in PAHs, while O−H, S−H, and N−H bonds are ionized more easily by the relatively soft ionization techniques of ESI and APCI. Atmospheric pressure photoionization (APPI) is less susceptible to ion suppression than APCI and ESI (*6*). The principal benefit of APPI, as compared to other ionization sources, is in efficiently ionizing broad classes of nonpolar compounds (*6*). Toluene photo-ions react with the solvent, which serves as a reagent for proton-transfer ionization (*7*). Even though APPI is very useful in ionizing nonpolar compounds, polar compounds can be ionized and analyzed by APPI (*6−13*).

MATERIALS AND METHODS

Acetonitrile, acetone, and toluene, HPLC-grade, were from Honeywell, B&J Brand (Morristown, NJ). All 16 individual neat standards were purchased from AccuStandard (New Haven, CT). Enviro Clean tubes prefilled with 6 g of $MgSO_4$ and 1.5 g of NaCl and Enviro Clean prefilled tubes prefilled with 150 mg of $MgSO_4$ and 50 primary and secondary amine (PSA) sorbent (150/50 mg) were from UCT (Bristol, PA). LC was performed with a prominence ultrafast liquid chromatography (UFLC) system, from Shimadzu (Kyoto, Japan). It consisted of two model LC-20AD binary pumps, a model DGU-20A3 online degasser, a model SIL-20AC autosampler set at 15 °C, a model CBM-20A system controller, and a model CTO-20AC oven set at 40 °C. MS was performed with an Applied BioSystems (or AB/Sciex) 4000 QTrap (Foster, CA), operated in triple quad mode. It was equipped with an AB/Sciex PhotoSpray APPI ion source and Analyst software, version 1.5.1. The nitrogen generator was a Source 5000 LC/MS gas generator (Parker Balston, Haverhill, MA). The analytical column for reverse phase was a Waters (Waltham, MA) PAH C18, 150 × 4.6 mm, 5 μm. A gradient elution was used. Solvent A

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(deionized water) and solvent B (acetonitrile) were used as the mobile phase. Gradient elution (0 min, 50% B; 0−20 min, linear change from 50 to 100% B; 20−25 min, 100% B; 25−26 min, 50% B; 26−30 min, 50% B; 30.10 min, stop; run time, 30 min) was performed with a 0.7 mL/min constant flow rate. The column oven temperature was 40 °C, and the injection volume was 20 μL, for all standards and samples (**Table 1**).

The mass spectrometer was operated in positive mode with the PhotoSpray APPI source. The effluent from the LC column was directly introduced into the source with the addition of toluene pumping at 0.15 mL/min into a mixing "T" connector. Toluene was used as the dopant, instead of chlorobenzene, because toluene is not as toxic as chlorobenzene and has less environmental impact. The only advantage of chlorobenzene over toluene is that it may offer lower sensitivity; however, sufficient sensitivity with toluene was demonstrated to detect PAHs at levels of concern.
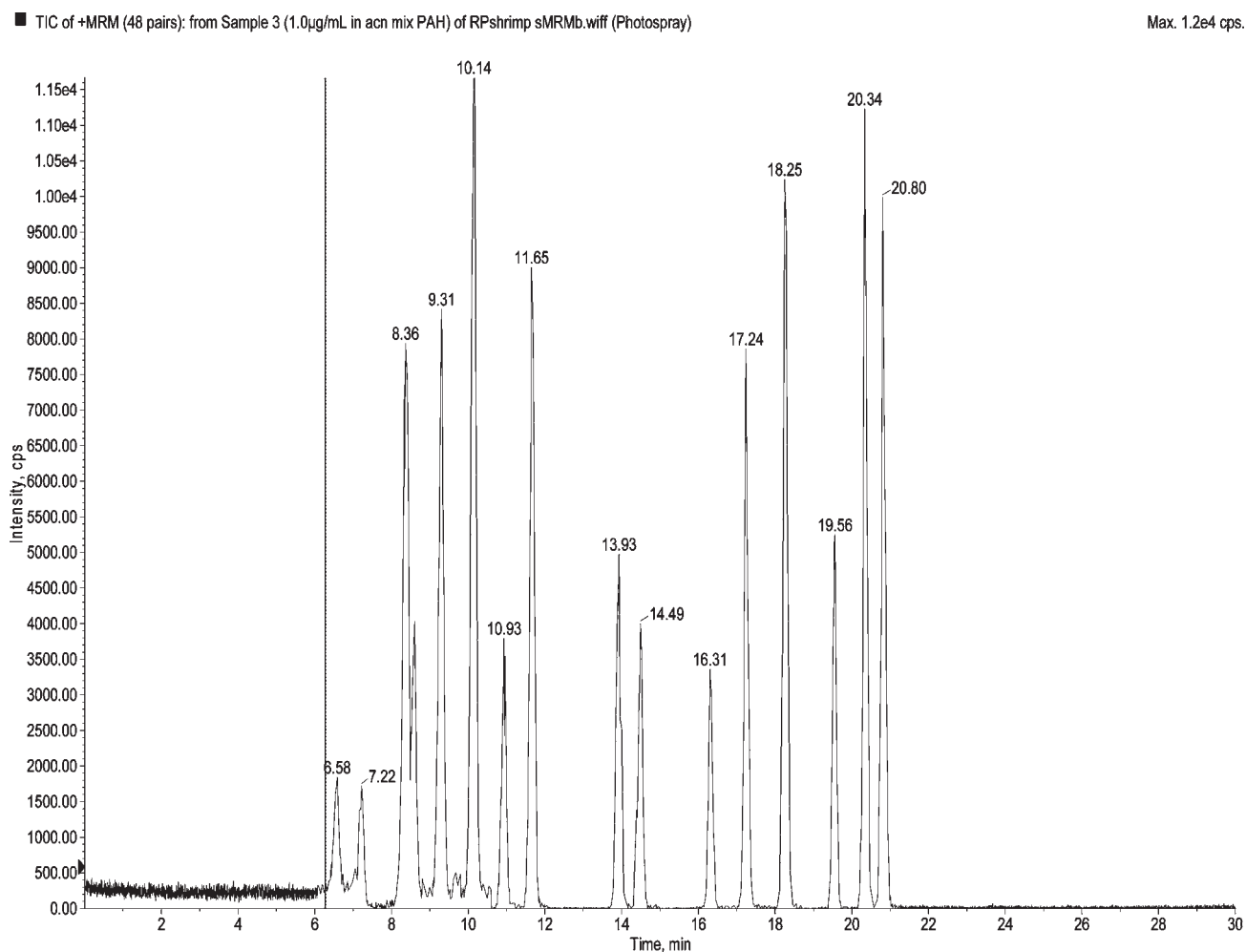
Table 1. Source Settings

instrument parameter	value
curtain gas	30
collision gas	high
ion spray voltage (V)	800
temperature (°C)	550
ion source gas 1	60
ion source gas 2	20
interface heater	on
entrance potential (EP)	10
collision cell exit potential (CXP)	15
horizontal probe position	5
vertical probe position	5

For sample preparation, the QuEChERS procedure was used (*14*). To each of three centrifuge tubes (control, spike 1, and spike 2) were added 10 g of blended head-off, unpeeled shrimp. The spike 1 tube was fortified with 100 μL of a 20 μg/mL spike solution in acetonitrile. The spike 2 tube was fortified with 500 μL of a 20 μg/mL spike solution in acetonitrile. Then, 10 mL of acetonitrile, 6.0 g of anhydrous $MgSO_4$, and 1.5 g of NaCl were added to each tube. Samples were then shaken in Geno/Grinder with a $^3/_8 \times ^7/_8$ in. ceramic grinding cylinder at 1000 strokes/min for 1 min. Next, they were centrifuged for 5 min at 4000 rpm. Supernatants were filtered using a 0.45 μm nylon syringe filter into an autosampler vial for analysis. Also, a 1.5 mL aliquot of the extract was treated with 50/150 mg of $PSA/MgSO_4$ prior to filtration into an autosampler vial.

Instrument linearity was evaluated using a calibration range, including 0.2, 0.5, 1, 2, and 10 μg/mL standard mixtures prepared in acetonitrile. Spike quantitation was performed against a single-point standard with matching concentrations in acetonitrile.

RESULTS AND DISCUSSION

Figure 1 is an example chromatogram showing separation of mixtures of standards at 1.0 μg/mL in acetonitrile. The precursor and fragment ions as well as the collision energies and declustering potentials are shown in **Table 2**. These values were optimized by infusion of standards with the addition of HPLC mobile phase and dopant. **Table 3** summarizes linearity results. Two compounds had r^2 values of 0.996; all others were 0.998 or higher, indicating excellent linearity between the calibration range of 0.2 and 10 μg/mL. Once linearity was demonstrated, single-point quantitation was preferred because of analysis time savings. Spike recoveries showed little ion suppression without PSA cleanup.

■ TIC of +MRM (48 pairs): from Sample 3 (1.0μg/mL in acn mix PAH) of RPshrimp sMRMb.wiff (Photospray)   Max. 1.2e4 cps.

Figure 1. Reverse-phase LC−MS/MS of PAH standards. Total ion current (TIC) of the mixture of 1.0 μg/mL standards in acetonitrile.

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Table 2. MRM Table[a]

name	transition type	Q1 mass	Q3 mass	DP[b]	CE[b]
naphthalene	quant	128	102	80	40
	confirm	128	78	80	40
acenaphthylene	quant	152	126	80	45
	confirm	152	102	80	50
acenaphthene	quant	154	152	60	55
	confirm	154	127	60	55
fluorene	quant	166	115	60	60
	confirm	166	139	60	60
anthracene	quant	178	152	80	45
	confirm	178	176	80	45
phenanthrene	quant	178	152	80	40
	confirm	178	176	80	40
fluoranthene	quant	202	200	80	80
	confirm	202	150	80	80
pyrene	quant	202	200	80	65
	confirm	202	151	80	70
benzo[a]anthracene	quant	228	226	80	80
	confirm	228	200	80	80
chrysene	quant	228	226	80	80
	confirm	228	200	80	80
benzo[a]pyrene	quant	252	250	60	60
	confirm	252	226	60	60
benzo[b]fluoranthene	quant	252	224	60	85
	confirm	252	250	60	85
benzo[k]fluoranthene	quant	252	250	60	55
	confirm	252	226	60	55
benzo[g,h,i]perylene	quant	276	274	80	70
	confirm	276	250	80	70
indeno[1,2,3-c,d]pyrene	quant	276	274	80	70
	confirm	276	250	80	70
dibenzo[a,h]anthracene	quant	278	276	55	80
	confirm	278	250	55	80

[a] Dwell times were determined by the timed MRM algorithm of Analyst software.
[b] DP is the declustering potential, and CE is the collision energy (in eV).

Table 3. Correlation Coefficient (r^2) and Retention Times for Reverse-Phase LC−MS/MS

compound name	quantitation ion (r^2)	retention time (min)
naphthalene	0.998	6.6
acenaphthylene	0.998	7.2
acenaphthene	0.998	8.4
fluorene	0.996	8.6
phenanthrene	0.998	9.3
anthracene	1.000	10.1
fluoranthene	0.999	10.9
pyrene	0.996	11.6
benzo[a]anthracene	1.000	13.9
chrysene	1.000	14.5
benzo[b]fluoranthene	0.999	16.3
benzo[k]fluoranthene	0.998	17.2
benzo[a]pyrene	0.999	18.2
dibenzo[a,h]anthracene	1.000	19.5
benzo[g,h,i]perylene	1.000	20.3
indeno[1,2,3-c,d]pyrene	0.999	20.8

Once PSA cleanup was applied, recoveries were even better, indicating the absence of ion suppression or enhancement.

Note that the precursor ion is not formed by the addition of a proton or other charged species and the mass is the same as the molecular weight of the native analyte molecule. An unexplained phenomenon occurred with benzo[g,h,i]perylene and indeno-[1,2,3-c,d]pyrene. These isobaric compounds had unusually high ion ratios (area of quantitation MRM versus confirmation). Even with the high ion ratios, quantitation and confirmation were acceptable at the levels needed.

Table 4. Comparison of Estimated LODs (ppm in Shrimp)

compound name	level of concern in shrimp (ppm)	reverse-phase QuEChERS extraction, (1 g of shrimp/mL of acetonitrile)	
		with no cleanup	with PSA cleanup
acenaphthene	a	0.08	0.08
acenaphthylene	a	0.10	0.11
anthracene	233	0.10	0.06
benzo[a]anthracene	3.80	0.06	0.07
benzo[a]pyrene	0.05	0.02	0.02
benzo[b]fluoranthene	a	0.04	0.04
benzo[g,h,i]perylene	a	0.42	0.51
benzo[k]fluoranthene	a	0.03	0.02
chrysene	4.1	0.04	0.04
dibenzo[a,h]anthracene	a	0.06	0.05
fluoranthene	0.26	0.12	0.06
fluorene	31	0.06	0.07
indeno[1,2,3-c,d]pyrene	a	0.32	0.30
naphthalene	31	0.29	0.42
phenanthrene	a	0.07	0.07
pyrene	0.41	0.08	0.08

[a] The limit of concern for some compounds was not known at the time of publication, and values that are listed are subject to change.

Estimated limits of detection (LODs) are in **Table 4**. The LODs were estimated by calculating the concentration equivalent for the confirmation ion at a signal-to-noise ratio of 3. The signal-to-noise ratio was measured using the signal-to-noise script of Analyst software based on 3 times the standard deviation of the manually selected noise region. They are compared to the levels of concern (*15*). Chromatograms are shown in the Supporting Information.

Table 5 summarizes spike recovery results and ion ratios at 0.2 and 1 μg/g in shrimp present at 1 g/mL in acetonitrile, with and without PSA cleanup. All mobile-phase blanks and shrimp control samples had zero positive findings (see the Supporting Information). Recoveries were good, with ion ratios that agreed well between the spikes and standards. The best recoveries were observed after PSA cleanup. Without cleanup (at the 0.2 μg/mL spike level), seven compounds had lower recoveries (49−69%) and one compound, naphthalene, had a slightly high recovery of 129%. At 1 μg/mL (without cleanup), only three compounds had slightly lower recoveries (66−67%). When PSA cleanup was performed, all PAH recoveries were within 75−125% at both 0.2 and 1 μg/g spike levels. With no PSA cleanup at the 0.2 μg/g spike level, some of the more toxic compounds, such as dibenzo[a,h]anthracene and indeno-[1,2,3-c,d]pyrene, had lower than average recoveries. Also, at the 0.2 μg/g level without cleanup, ion ratios did not closely match those of the standard for naphthalene and indeno-[1,2,3-c,d]pyrene. Chromatograms of recoveries of spiked shrimp samples are in the Supporting Information.

With PSA cleanup, all recoveries were within the generally accepted range of 75−125% and ion ratios of the matrix spikes matched very well with those of the standards. The only exception was indeno[1,2,3-c,d]pyrene at the 0.2 μg/g level, which had an ion ratio 169% of the standard. However, the ion ratio for this compound was > 44, because of the poor secondary transition. Further optimization would likely fix this issue.

The results of this study indicate acetonitrile QuEChERS extraction with PSA cleanup, combined with LCMS/MS and APPI, is a quick, highly selective, and efficient method for determination of PAHs in shrimp matrix.

Table 5. Reverse-Phase Spike Recovery and Ion Ratios

compound name	0.2 μg/g shrimp, with no PSA		0.2 μg/g shrimp, with PSA[a]		1 μg/g shrimp, with no PSA		1 μg/g shrimp, with PSA	
	spike recovery (%)	ion ratio vs standard	spike recovery (%)	ion ratio vs standard	spike recovery (%)	ion ratio vs standard	spike recovery (%)	ion ratio vs standard
acenaphthene	85	88	98	113	99	99	100	101
acenaphthylene	89	133	109	118	105	93	94	93
anthracene	103	99	102	96	83	103	95	98
benzo[a]anthracene	78	92	88	113	82	88	86	89
benzo[a]pyrene	62	100	77	101	76	99	87	100
benzo[b]fluoranthene	69	106	78	112	79	95	89	95
benzo[g,h,i]perylene	49	107	74	142	67	110	84	117
benzo[k]fluoranthene	64	111	77	115	81	104	90	105
chrysene	82	89	88	115	80	96	89	104
dibenzo[a,h]anthracene	59	99	77	99	66	98	85	95
fluoranthene	67	79	100	113	85	99	101	101
fluorene	98	117	118	120	92	100	89	100
indeno[1,2,3-c,d]pyrene	58	147	87	169	67	103	84	102
naphthalene	129	149	113	99	83	91	96	89
phenanthrene	102	114	122	109	94	101	102	103
pyrene	94	102	88	106	107	103	105	107

[a] PSA is a cleanup sorbent that contains primary and secondary amines, used here in dispersive mode.

Supporting Information Available: Reverse-phase LC−MS/MS (Figures S1−S14). This material is available free of charge via the Internet at http://pubs.acs.org.

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