INTEGRATED EXTRACTION AND CLEANUP METHOD BASED ON PRESSURIZED LIQUID EXTRACTION FOR 33 POLYCYCLIC AROMATIC HYDROCARBONS IN MARINE SEDIMENTS

Choi M[1]*, Kim YJ[1], Lee IS[1], Choi HG[1]

[1] Marine Environment Research Division, National Fisheries Research and Development Institute, Busan, Republic of Korea

Introduction

Polycyclic aromatic hydrocarbons (PAHs) and PAH alkylated homologs are widespread environmental contaminants that result from natural and anthropogenic activities including petrogenic or pyrogenic sources. PAHs are present in exhaust as incomplete fuel combustion, and in crude oil in significant amounts. Because of their mutagenic and carcinogenic potential, they have been determined in several matrices, particularly, waters, sediments and seafood. In the marine environment, PAHs are bioavailable to marine species via the food chain, as waterborne compounds, and contaminated sediments. PAHs have been monitored in marine environments, in order to protect and control habitats of marine species.

Sample preparation prior to the determination of PAHs in marine sediments usually consists of many steps because of the complexity of the matrix. Extractions and cleanups for PAHs are the most critical steps in the analytical procedure for their good recovery. Methods based on classical Soxhlet or sonication techniques for extraction of PAHs have been performed in marine sediments. Alternatively, faster and more effective, automated extraction techniques such as pressurized liquid extraction (PLE) have been developed and applied.[1] After the extraction step, cleanup step is needed to remove many interfering components such as lipids, sulfur, pigment, or cholesterols. Most common post-cleanup approaches include adsorption columns using alumina, florisil, and silicagel. In addition, copper is often used for sulfur removal, especially from marine sediments. Frequently, more than two steps are required prior to GC analysis. If the extraction and cleanup procedure could be combined in one-step on the automated extraction device, it could shorten sample preparation time and solvent cost. In the present study, integrated PLE and cleanup procedure is proposed as a simple, rapid, and reliable alternative for 33 PAHs (parent PAHs and alkylated homologs) in marine sediments.

Materials and methods

All reagents were analytical or pesticide grade. Hexane, dichloromethane, and activated silica gel were purchased from Merck (Darmstadt, Germany). Copper was purchased from Sigma-Aldrich (St. Louis, MO, USA), and sea sand washed was from Fisher Scientific (Phillipsburg, NJ). Diatomaceous earth tube was obtained from Thermo scientific (Sunnyvale, CA). Standard stock solutions were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and Chiron NPD cocktail. 17 surrogate standards were obtained from Dr. Ehrenstorfer.

Marine sediment samples were sampled at 17 locations around Busan Harbor, the biggest harbor in Korea. Surface sediment samples (0-4 cm depth) were collected using a box core sampler deployed from a research vessel. Collected samples were individually wrapped in aluminum foil and immediately frozen in a refrigerator on the vessel. Marine sediment samples were transported to the laboratory and were stored at -20 °C and freeze-dried later. An unpolluted marine sediment sample was obtained to develop an analytical procedure. Extractions were performed on an ASE 350 system (Thermo, Co., Sunyvale, CA, USA) equipped with stainless-steel extraction cells. Five grams of activated silica gel was placed on top of the filter, and 5 g of activated copper, 5 g of sample, and tube were placed on top of silica gel. The samples were extracted as follows: extraction solvent, 2 × 20mL of hexane and dichloromethane (9:1 v/v); temperature, 100 °C; pressure, 1700 psi; extraction time, 2 × 5 min . The clean extract was concentrated to about 1 mL by Turbo Vap. The residue was further reduced in volume to 0.5 mL by blowing with a nitrogen stream after adding an internal standard solution. Analyses were performed with a gas chromatograph (Agilent 6890; Wilmington, DE) coupled with a mass spectrometer (Agilent 5973 N). Detailed descriptions of instrumental analyses have been presented elsewhere[2,3]. The capillary column used was a DB5-MS column (30-m length, 0.25-mm inner diameter, 0.25-µm film thickness; J & W Scientific, Palo Alto, CA). The mass spectrometer was operated in the selective ion monitoring mode using molecular ions of individual PAHs.

For PAH spike and recovery experiments, 5 g of marine sediment was weighed into a extraction cell, and spike recovery samples were fortified with 20 and 100 µL of 0.25−2 ng/ µL composite of PAHs directly onto the sediment matrix, and allowed to acclimate for 1 hour at room temperature. Marix blanks were prepared similarly but were not fortified. Samples were then extracted (see Figure 1).

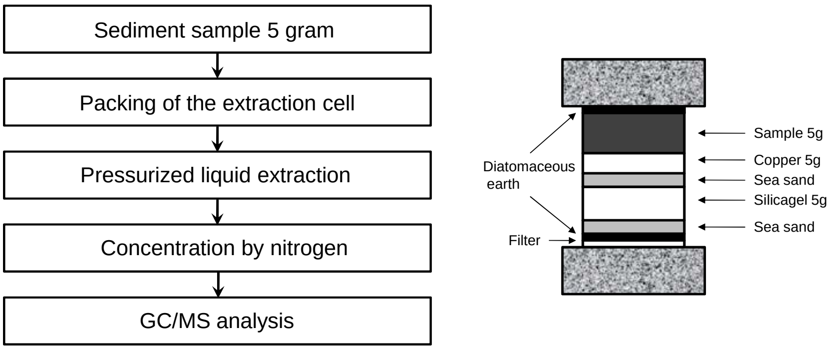

Figure 1. Analytical method for PAHs (left) and packing of the extraction cell (right).

Results and discussion

Table 1 summarizes PAH spike recoveries from marine sediment fortified with composite PAHs at two different levels. Recoveries for level 1 (5−40 ng/g-dry) were reasonable, on average 87%, with individiul PAH recoveries typically ranging from 53 to 118%. Recoveries for level 2 (25−200 ng/g-dry) were equally reasonable, with average PAH recoveries being 91% and individual PAH recoveries ranging from 54 to 114%. Both recoveries for two levels were less than 70% at naphthalene (53−54%), acenaphthylene (68%), and 1-methyldibenzothiophene (66−71%).

In order to evaluate the effectiveness and utility of simultaneous extraction and cleanup (one step) method, a comparison to an existing PLE method is presented in Figure 2. The existing PLE method includes three steps; PLE extraction using hexane:acetone 1:1(v/v) (US EPA method 3545A for analysis of semivolatiles), sulfur removal using activated copper, and cleanup using activated silicagel column chromatography for analysis of16 prior PAHs.[2,3] It was found that recoveries of PAHs were very close between one step and three steps except for dibenzothiophene and 1-, 2-, 4-methyldibenzothiophenes. The one step method substantially improved recoveries of four compounds (71−84%), compared to three steps (0−1%). Their low recoveries in the three steps are probably associated with the activated silicagel column chromatography, which is suitable for 16 prior PAHs. Compared to the three step method, the one step method has advantages to save time (50% for nitrogen concentration, and 100% for cleanup duration), and to save money (to use less than 30% of solvent volume and 50% of glassware consumption).

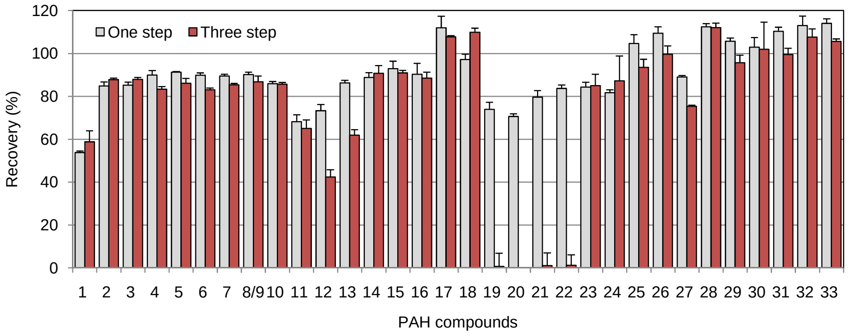

Figure 2. The developed method (one step) performance compared to traditional PLE method (three steps; extraction + sulfur removal + silicagel cleanup) for the fortified marine sediment sample. The number of PAH compounds are shown in Table 1.

Method accuracy was also evaluated by analyzing a certified reference material (NIST SRM 1944) containing 23 target compounds. Table 2 shows the estimated concentrations and the recovery values for PAHs. The mean accuracy of the developed method was 79 ± 25 %. The PAHs with accuracy higher than 70% were phenanthrene, 1-, 2-, 3-, & 9-methylphenanthrenes, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, Indeo(1,2,3-c,d)pyrene, and benzo(ghi)perylene. However, naphthalene, acenaphthene and fluorene showed the value less than 50%, suggesting relatively poor in lower molecular weight PAHs.

The proposed method was applied to the determination of PAHs in 17 sediment samples collected from Busan Harbor, which is the biggest commercial harbor of Korea and the world's fifth largest container port. PAHs were detected in all samples, and were determined without additional sample preparations. Σ_{33}PAH ranged from 122 to 3024 ng/g-dry, and Σ_{16}PAH (the sum of No. 1, 11−14, 23−33; Table 1) ranged from 81 to 2441 ng/g-dry.

A rapid and accurate integrated PLE and cleanup analysis method was developed for the determination of 33 PAHs in marine sediment samples. The analytes extraction and cleanup were accomplished in a single step via an on-line and concurrent column chromatographic cleanup by PLE. Results illustrated that this developed method was equal to or better than the traditional methods. With the developed one step method, the most relevant criteria required for an extraction procedure, such as low solvent consumption, short process times, and possibility of automation, were fulfilled.

Acknowledgements

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References

1. Fernadez-Alvarez M, Llompart M, Lama P, Lores M, Garcia-Jares C, Garcia-Chao M, Dagnac T. (2009) ; *J .Agric. Food Chem.* 57: 3963-73.
2. Moon HB , Kannan K, Lee SJ, Ok G. (2006) ; *Arch. Environ. Contam .Toxicol.* 51: 494-502.
3. Moon HB, An YR, Choi SG, Choi M, Choi HG. (2012) : *Environ. Toxicol. Contam .* 31: 477-85.

Table 1. Recovery of 33 PAHs (Mean ± RSD; n=3) from marine sediment fortified with composite PAHs

		Recovery (%)			
		Level 1 (5−40 ng/g-dry)		Level 2 (25−200 ng/g-dry)	
No.	Compound	Mean	RSD	Mean	RSD
1	Naphthalene	53.1	9.8	53.8	0.7
2	1-methylnaphthalene	76.5	9.3	84.8	1.9
3	2-methylnaphthalene	76.6	6.8	85.2	1.4
4	1,3- & 1,7-dimethylnaphthalene	82.5	10.1	89.9	2.0
5	1,4-dimethylnaphthalene	82.4	9.6	91.3	0.3
6	1,5-dimethylnaphthalene	79.4	9.8	89.9	1.0
7	1,6-dimethylnaphthalene	81.5	9.6	89.4	0.8
8/9	2,6- & 2,7-dimethylnaphthalene	80.6	10.5	90.1	1.2
10	Biphenyl	73.6	10.1	85.9	1.0
11	Acenaphthylene	68.1	3.7	68.1	3.3
12	Acenaphthene	71.3	5.2	73.3	2.9
13	Fluorene	85.3	2.6	86.2	1.2
14	Phenanthrene	86.3	1.0	88.8	2.2
15	1-methylphenanthrene	85.9	13.4	92.9	3.4
16	2-methylphenanthrene	81.6	7.8	90.3	5.0
17	3-methylphenanthrene	114.5	9.5	111.9	5.3
18	9-methylphenanthrene	99.3	12.3	97.1	2.4

18	Dibenzothiophene	70.2	13.0	73.9	3.3
20	1-methyldibenzothiophene	66.1	13.1	70.6	1.2
21	2-methyldibenzothiophene	76.5	15.1	79.6	3.0
22	4-methyldibenzothiophene	78.2	14.0	83.7	1.6
23	Anthracene	85.2	4.4	84.3	2.3
24	Fluoranthene	80.4	1.9	81.7	1.3
25	Pyrene	101.4	4.1	104.6	4.1
26	Benz(a)anthracene	106.9	3.3	109.4	3.0
27	Chrysene	86.8	2.6	89.1	0.6
28	Benzo(b)fluoranthene	108.4	2.9	112.3	1.5
29	Benzo(k)fluoranthene	102.2	3.5	105.7	1.5
30	Benzo(a)pyrene	103.7	8.0	102.9	4.5
31	Indeo(1,2,3-c,d)pyrene	117.8	7.5	110.3	1.9
32	Dibenz(a,h)anthracene	115.0	7.4	113.0	4.4
33	Benzo(ghi)perylene	116.5	7.3	114.0	2.1

Table 2. PAH certified and determined amounts (μg/g) for NIST SRM 1944 marine sediment

	Certified value [a]		Control limit range [b]		Determined amount	
Compound	Mean	SD	Lower	Upper	Mean	SD
Naphthalene	1.65	0.31	0.94	2.55	0.75	0.02
1-methylnaphthalene	0.52	0.03	0.34	0.72	0.32	0.01
2-methylnaphthalene	0.95	0.05	0.63	1.30	0.48	0.01
Biphenyl	0.32	0.07	0.18	0.51	0.17	0.01
Acenaphthene	0.57	0.03	0.38	0.78	0.25	0.03
Fluorene	0.85	0.03	0.57	1.14	0.29	0.01
Phenanthrene	5.27	0.22	3.54	7.14	4.34	0.39
1-methylphenanthrene	1.7	0.1	1.12	2.34	1.19	0.09
2-methylphenanthrene	1.9	0.06	1.29	2.55	1.76	0.11
3-methylphenanthrene	2.1	0.1	1.40	2.86	2.33	0.11
4+9-methylphenanthrene	1.6	0.2	0.98	2.34	1.43	0.09
Dibenzothiophene	0.62	0.01	0.43	0.82	0.40	0.04
Anthracene	1.77	0.33	1.01	2.73	1.00	0.42
Fluoranthene	8.92	0.32	6.02	12.01	8.50	0.56
Pyrene	9.7	0.42	6.50	13.16	7.84	0.31
Benz(a)anthracene	4.72	0.11	3.23	6.28	3.30	0.49
Chrysene	4.86	0.1	3.33	6.45	4.12	0.94
Benzo(b)fluoranthene	3.87	0.42	2.42	5.58	3.20	0.35
Benzo(k)fluoranthene	2.3	0.2	1.47	3.25	1.69	0.31
Benzo(a)pyrene	4.3	0.13	2.92	5.76	4.43	3.01
Indeo(1,2,3-c,d)pyrene	2.78	0.1	1.88	3.74	2.54	0.50
Dibenz(a,h)anthracene	0.424	0.069	0.249	0.641	0.615	0.181
Benzo(ghi)perylene	2.84	0.1	1.92	3.82	2.88	0.80

[a] Dry mass basis as given in NIST certificate.

[b] NOAA defines the lower control limit as 0.7 × (certified value – uncertainty value), and the upper control limit as 1.3 × (certified value + uncertainty value).