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Doctor Forum

Optimization of Ultrasonic Extraction and Clean-up Protocol for the Determination of Polycyclic Aromatic Hydrocarbons in Marine Sediments by High-performance Liquid Chromatography Coupled with Fluorescence Detection

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Abstract The procedures of ultrasonic extraction and clean-up were optimized for the determination of polycyclic aromatic hydrocarbons (PAHs) in marine sediments. Samples were ultrasonically extracted, and the extracts were purified with a miniaturized silica gel chromatographic column and analyzed with high performance liquid chromatography (HPLC) with a fluorescence detector. Ultrasonication with methanol-dichloromethane (2:1, v/v) mixture gave higher extraction efficiency than that with dichloromethane. Among the three elution solvents used in clean-up step, dichloromethane-hexane (2:3, v/v) mixture was the most satisfactory. Under the optimized conditions, the recoveries in the range of 54.82% to 94.70% with RSDs of 3.02% to 23.22% for a spiked blank, and in the range of 61.20% to 127.08% with RSDs of 7.61% to 26.93% for a spiked matrix, were obtained for the 15 PAHs studied, while the recoveries for a NIST standard reference SRM 1941b were in the range of 50.79% to 83.78% with RSDs of 5.24% to 21.38%. The detection limits were between 0.75 ng L^{-1} and 10.99 ng L^{-1} for different PAHs. A sample from the Jiaozhou Bay area was examined to test the established methods.

Key words ultrasonic extraction; marine sediment; polycyclic aromatic hydrocarbon; high performance liquid chromatography

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs), which contain at least two fused benzene rings in linear, angular, or cluster arrangements, are ubiquitous environmental contaminants. Because of their high carcinogenic, mutagenic toxicity and their persistence, PAHs are of great concerns and have been extensively studied to understand their fates and distribution in the environment, as well as their toxicity to animals and human (Kahn *et al*., 2008; Neff, 1979; Stegeman *et al*., 1991).The US Environment Protection Agency (US EPA) has included 16 PAHs in its priority pollutants list, and has developed methods for their monitoring in waste-water discharge (Manoli *et al*., 1996). In China, a list including seven PAHs (Naphthalene, fluoranthene, benzo[a]pyrene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[ghi]perylene, indeno [1,2,3-cd]pyrene) was announced in 1989, when these PAHs were firstly among the preferred controlling pollutants to be monitored in drinking water.

PAHs in marine sediments of coastal areas, estuaries and continental shelves are often determined to evaluate their ecological risks. These PAHs are mainly from external inputs including direct aerial fallout and surface water run-off (Lipiatou *et al*., 1994; Witt, 1995). Due to their low water-solubility and high hydrophobicity, PAHs are easily adsorbed onto suspended particulate matters and finally settled down to marine sediments, which make the sediments the major sink of the PAHs. Bottom-dwelling fishes and benthic organisms may be adversely affected by these PAHs and may accumulate the PAHs in the adipose tissues (Harkey *et al*., 1995; Carman *et al*., 1995; Manoli *et al*., 1996). This is the case especially for PAHs with high molecular weight and low water solubility.

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Extraction is essential for determination of PAHs in marine sediments, and it can be achieved with a number of established methods including traditional Soxhlet extraction and modern techniques such as ultrasonication extraction (USE), microwave-assisted extraction (MAE), high-pressurized accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) (Navarro *et al*., 2009; Lopez-Avila *et al*., 1994; Janda *et al*., 1993; Richter *et al*., 1996). Soxhlet extraction is the most conventional technique with high recoveries, which is recommended by the US EPA for extracting semi-volatile and non-volatile organics from solid matrices. However, this technique is very time-consuming with large consumption of hazardous and toxic organic solvents. Ultrasonication is simple and effective for extraction of PAHs in solid samples (Popp *et al*., 1997; Vannoort *et al*., 1990), with equivalent or better recoveries and higher efficiency than those of Soxhlet extraction (Vannoort *et al*., 1990; García *et al*., 1992). Moreover, the equipment for ultrasonication is simple and easy to operate.

In this research, ultrasonication and miniaturized silica gel column chromatography were used for the extraction from marine sediment samples and the purification of the extracts, respectively, prior to the determination of PAHs by HPLC with fluorescence detection. Solvents used for the extraction and purification were optimized for best performance.

2 Experimental Reagents and Methods

2.1 Reagents and Apparatus

A standard mixture of the EPA 16 priority PAHs, including naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Anth), fluoranthene (Fla), pyrene (Pyr), benzo[a] anthracene (BaA), chrysene (Chry), benzo[b] fluoranthene (BbF), benzo[k] fluoranthene (BkF), benzo[a] pyrene (BaP), dibenzo [a,h] anthracene (DahA), indeno [1,2,3-cd] pyrene (InP) and benzo[ghi]perylene (BghiP), was purchased from Supelco (Bellefonte, PA, USA). The standard stock solutions were prepared by appropriate dilution of the commercial standard mixture with methanol (MeOH) of HPLC grade and stored in amber volumetric flasks at 4℃. Dichloromethane (DCM), acetone and hexane of analytical grade were redistilled before use. A standard sediment reference (SRM 1941b) was obtained from National Institute of Standards and Technology (NIST), USA. Glassware used was then washed and baked at 450℃ for 4 h, except for volumetric vessels which were washed and rinsed with distilled water, MeOH, DCM and hexane successively before use.

Agilent 1100 series high-performance liquid chromatography equipped with a 20.0 μL loop injector, an Agilent ZORBAX Eclipse® XDB-C18 reversed-phase analytical column (250×4.6 mm, 5 μm) and a fluorescence detector were used to analyze the abundance of the PAHs. Ultrasonic extraction was performed in a benchtop KS-3000 ultrasonic bath (Ningbo, China).

2.2 Sediments Collection

Marine sediments were collected from Hongshiya, Jiaozhou Bay area of Qingdao, China, by using a stainless steel hand shovel on May 22, 2007. Samples were transferred into pre-cleaned amber glass jars and placed in a cooler packed with ice during transportation to laboratory. They were freeze-dried and sieved to a particle size smaller than 0.05 mm to remove gravels, plant roots and other debris. The resulting powder was fully re-mixed to ensure sample homogeneity. The prepared samples were stored in amber glass bottles at 4℃ in a refrigerator before extraction.

2.3 PAHs Extraction

Typically about 5.0 g of sediments was weighed to a glass centrifugation tube with Teflon cap, and mixed with about 0.5 g activated copper powder in the tube to remove sulphide in the sample. The samples were then extracted three times with 10 mL solvent (DCM or MeOH-DCM (2:1, v/v) mixture) at room temperature (25℃) in an ultrasonic bath, for 10 min each time (Sun *et al.*, 1998; Berset *et al*., 1999; Heemken *et al*.,1997). During the extraction, the samples were swirled occasionally to prevent the sediments from sticking to the bottom of the tube. After each extraction, the samples were centrifuged at 3000 rpm for 5 min, and the supernatants were pooled in a pear-shaped flask and concentrated to approximately 0.5 mL with a vacuum rotary evaporator at 30℃ and the solvents were then changed (in 3 times) to 2mL of hexane. The resulted solutions were applied to a silica gel chromatographic column for clean-up.

2.4 Extracts Clean-up

To remove interfering compounds in an extract, clean-up was performed prior to instrumental analysis by introducing the extract onto a silica gel chromatographic column. Silica gels (100–200 mesh) were activated by heating at 180℃ for 12 h and partially deactivated with Milli-Q water (5%, w/w). The glass column (1.0 cm i.d.×18.0 cm in length) was packed with glass wool at its base and filled with 7.0 g of partially deactivated silica gel slurry in hexane by gravity. In order to prevent disturbance by eluting solvent, 1.0 g of anhydrous sodium sulfate was added on the top of the column. The packed column was conditioned prior to sample loading by adding 20.0 mL of hexane to the top of the column and drained. About 2 mL extract was transferred to the top of the conditioned column and eluted with 3 different procedures: (a) the column was pre-eluted with 20.0 mL hexane to remove non-polar aliphatic hydrocarbons followed by the PAHs elution with 20.0 mL DCM-hexane (2:3, v/v) mixture. The PAH fractions were collected to a pear-shaped flask, pre- concentrated to 0.5 mL in a rotary evaporator and transferred into an amber glass vial. The solvent was further evaporated at room temperature under a gentle nitrogen flow, and 1.0 mL hexane was added ex-

actly to re-dissolve the extracted PAHs for instrumental analysis. The procedures (b) and (c) were almost the same as that described in (a), except that there were no pre-elution with hexane and the elution solvents were 20.0 mL DCM-hexane (2:3, v/v) mixture and DCM, respectively.

2.5 PAHs Analysis

After extraction and clean-up, PAHs were analyzed on an Agilent 1100 HPLC by using a gradient elution mode with wavelength-programmed fluorescence detection.

3 Results and Discussion

3.1 HPLC Conditions

For HPLC analysis of PAHs, a gradient elution was employed to reduce the analysis time with good resolution of all PAHs peaks. The mobile phase was a binary mixture of MeOH from 70% to 100% and water from 30% to 0. The flow rate was maintained at 0.5 mL min^{-1} for 57 min and finally returned to the original rate (0.3 mL min^{-1}) for 1 min. The total running time was 60 min. The column temperature was kept at 35℃.

In order to obtain best detection of the PAHs, the wavelength of the fluorescence detector was also programmed as shown in Table 1.

HPLC chromatogram for a standard mixture of PAHs using fluorescence detection is given in Fig.l. Since the structures of acenaphthene and fluorine are similar while benzo-(ghi) perylene and indeno-(1,2,3-cd) pyrene are isomeric compounds, the two pairs were not well resolved with the HPLC conditions mentioned above. Among the 16 PAHs, only acenaphthylene did not display any fluorescence, which was therefore not detectable. The other PAHs in the sediment samples could be quantified with the fluorescence abundance of individual PAHs at the optimal wavelength. The retention time of the PAHs are provided in Table 4.

Table 1 Program of gradient elution of mobile phase and the wavelength program of fluorescence detector

Gradient elution			Wavelength programming		
Program time min	Methanol/water %	Flow rate mL min^{-1}	Program time min	Excitation nm	Emission nm
0.0	70/30	0.3	0.00	221	337
2.0	71/29	0.5	13.00	291	365
20.0	80/20	0.5	17.30	250	400
33.0	84/16	0.5	20.50	289	462
40.0	90/10	0.5	23.00	270	390
50.0	100/-	0.5	27.00	254	390
55.0	100/-	0.5	35.00	266	415
60.0	70/30	0.3	41.00	290	430

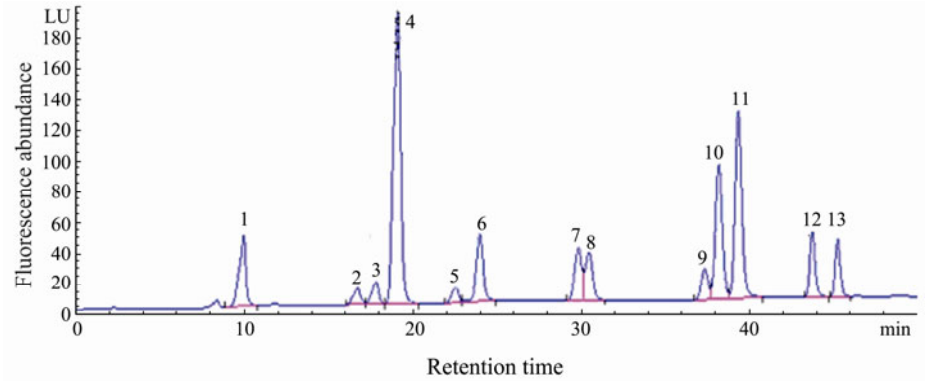

Fig.1 Chromatogram of PAHs obtained under the optimal conditions with HPLC- fluorescence detection. 1. Naph; 2.Ace+Flu; 3. Phe; 4. Anth; 5. Fla; 6. Pyr; 7. Chry; 8. BaA; 9. BbF; 10. BkF; 11. BaP; 12. DahA; 13. BghiP+InP

3.2 Solvents for Ultrasonic Extraction

The solvents used for the extraction of PAHs from solid samples include cyclohexane, MeOH, acetonitrile, DCM, toluene and acetone (Vannoort *et al*., 1990; Popp *et al*., 1997; Codina *et al*., 1994; Blankenhorn *et al*., 1992; Chee *et al*., 1996). The efficiency of different solvents in ultrasonic extraction was reported (Sun *et al*., 1998). Among these solvents, DCM is commonly used since it is relatively inactive in chemical properties and suitable to extract a variety of polar and non-polar compounds. Moreover, its lower boiling point is favorable for reducing the loss of volatile components during pre- concentration. In the present work, the efficiencies of DCM and MeOH-DCM (2:1 v/v) mixture were compared. The extraction efficiencies for the 15 PAHs from the sediments by the two solvents are shown in Table 2. It is obvious that the extraction efficiency of MeOH-DCM mixture was higher than that of DCM for each PAH in the sample. Moreover, the wet sediments need not to be freeze-dried and can be extracted directly using MeOH-DCM mixture because of the increased polarity. In addition, anhydrous sodium sulfate need not to be added to the sediment in the extraction step, which simplified the operation.

It was reported that a second extraction improved the recovery of some PAHs (Barco-Bonilla *et al.*, 2009). To further explore the extent of extraction, the residues extracted by DCM were extracted again using the two solvents (Fig.2). It is clearly shown that the 15 PAHs were still detected and the intensity of each peak was much greater when the residues were extracted using the solvent mixture (Fig.2b). In contrast, the 15 PAHs were almost undetectable when the residues were extracted using DCM only (Fig.2a). For the same PAH component, the sum of the content of the first extraction by DCM and that of a second extraction by mixture solvent agreed well with that extracted directly using MeOH-DCM (2:1 v/v) mixture. Furthermore, if the residues extracted firstly by MeOH-DCM (2:1 v/v) mixture was extracted additionally by mixture solvent again, no distinct PAH peaks were seen (data not shown). Therefore, MeOH-DCM (2:1 v/v) mixture was selected as the one-step extraction solvent.

Table 2 Comparison of extraction efficiency with different solvents

PAHs	Extraction content ng g^{-1}, dry *wt* *		a/b
	a	b	%
Naph	5.02	5.58	89.97
Ace+Flu	2.63	3.67	71.52
Phe	18.63	27.75	67.15
Anth	6.68	8.95	74.70
Fla	48.81	67.26	72.56
Pyr	75.26	100.88	74.60
Chry	30.49	39.63	76.95
BaA	24.69	33.21	74.35
BbF	49.98	72.39	69.04
BkF	12.02	16.28	73.84
BaP	15.17	18.85	80.48
DahA	11.88	18.49	64.23
BghiP+InP	17.08	23.69	72.08

Note: * extracted by DCM (a) and MeOH-DCM (2:1 v/v) mixture (b), respectively.

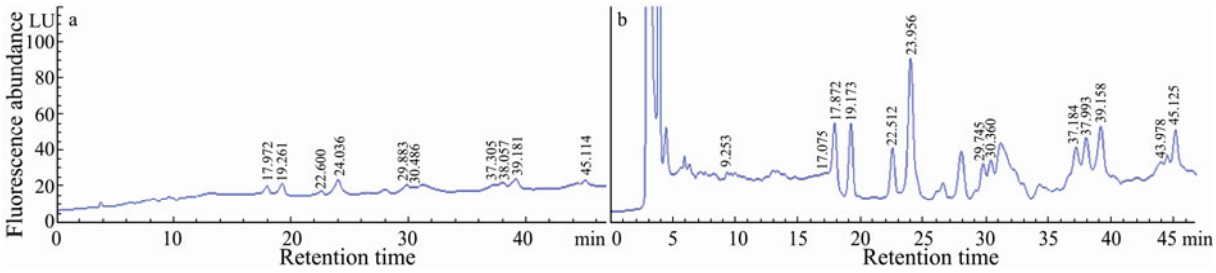

Fig.2 Chromatograms of the extracts for an additional extraction with DCM (a) and MeOH-DCM (2:1 v/v) mixture (b), respectively, for DCM-extracted residues.

3.3 Clean-up of the Extracts

To eliminate the interference of co-extracted compounds from marine sediments for the determination of PAHs, a clean-up process for concentrated extracts is indispensable before the analysis by HPLC. A simple and effective approach is silica gel column chromatography, which was adopted in this study. Three elution procedures as described in section 1.4 were compared for their purification effects. They were sequential elution with hexane and DCM-hexane mixture (2:3, v/v) (a), DCM-hexane mixture only (b) and DCM only (c).HPLC chromatograms for different procedures are shown in Fig.3. No much difference was observed between procedure (a) and (b) (see Figs.3a and 3b). It is natural since only compounds with fluorescence can be detected with a fluorescent detector and aliphatic hydrocarbons usually do not show fluorescence. Moreover, we noticed that a certain portion of PAHs were eluted along with aliphatic hydrocarbons by hexane pre-elution (data not shown), resulting in under-estimation of PAHs in the samples. On the other hand, co-elution of substances with higher polarity (such as pigments) is inevitable while eluted with DCM, causing enhanced baseline and possible interference (Fig.3c). It is therefore preferable to elute silica gel column directly with DCM- hexane mixture (2:3 v/v) without hexane pre-elution.

The recoveries of PAHs for purification procedure (b) (*i.e.* elution with DCM-hexane mixture only) showed that 2 or 3-ring PAHs (such as naphthalene and phenanthrene *etc.*) were vulnerable to loss with lower recoveries (30.68% to 63.74%) and higher RSDs (Table 3), while higher recoveries (82.59% to 97.32%) and lower RSDs were observed for high-molecular-weight PAHs. Low recovery with high RSDs is usually caused by co-evaporation of targeted compounds with solvents in a vacuum rotary evaporator or under a gentle N_2 stream (Wenclawiak *et al.*, 1992). However, our results are still comparable to those reported earlier in literatures (Miège *et al.*, 2003; Filipkowska *et al.*, 2005), in which the recoveries were less than 30% and 20% to 70% for naphthalene and 3-ring PAHs, respectively.

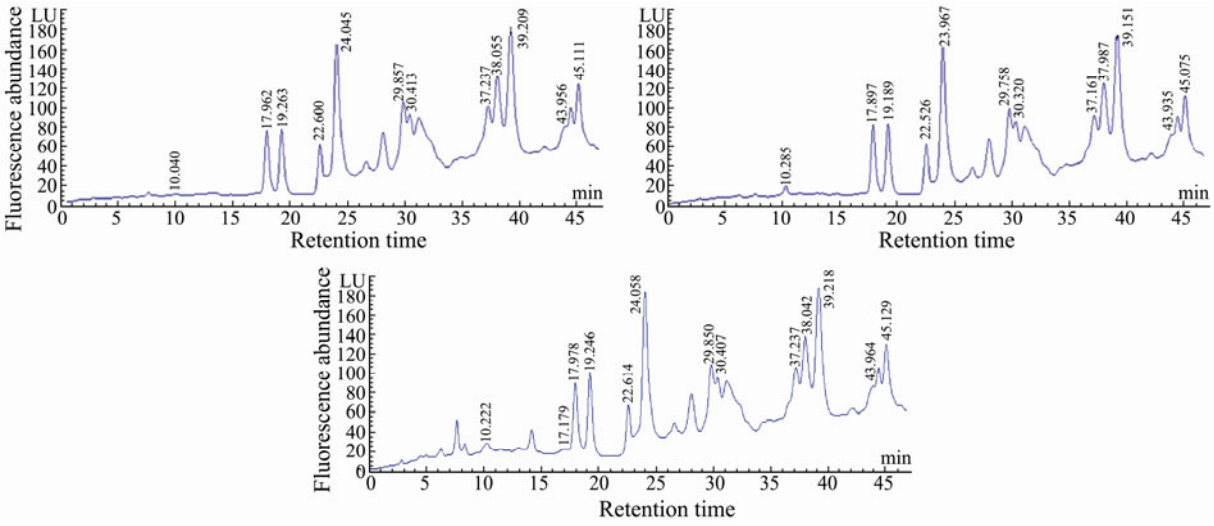

Fig.3 HPLC chromatograms of extracts purified by sequential elution with hexane and DCM-hexane mixture (2:3, v/v) (a), DCM-hexane mixture (2:3, v/v) only (b) and DCM only (c).

Table 3 Recoveries and RSDs of PAHs for clean-up procedure (n=4)

PAHs	Spiking amount ng	Recovering amount ng				Average recovery %	RSD %
Naph	50	16.92	14.07	11.44	18.94	30.68	21.38
Ace+Flu	10	4.99	4.31	5.81	3.84	47.39	18.02
Phe	5	2.91	3.47	3.75	2.62	63.74	16.06
Anth	5	3.20	2.67	3.41	3.44	63.65	11.17
Fla	10	9.16	7.68	8.51	7.69	82.59	8.63
Pyr	5	3.91	4.70	4.89	4.52	90.13	9.40
Chry	5	4.35	4.88	4.72	4.14	90.50	7.48
BaA	5	4.38	4.52	4.19	3.71	84.00	8.40
BbF	10	9.14	9.87	8.66	9.59	93.18	5.68
BkF	5	4.69	4.70	4.93	4.81	95.67	2.33
BaP	5	4.77	4.93	4.99	4.78	97.32	2.25
DahA	10	9.30	9.66	9.32	9.69	94.93	2.20
BghiP+InP	5	9.30	9.66	9.32	9.69	94.70	3.34

3.4 Method Linearity, Detection Limits, Precision and Accuracy

Under the chromatographic conditions described in section 2.1, a series of standard mixtures of PAHs at different concentrations were analyzed to determine the method linearity and detection limits. An external reference method was used for quantification of PAHs, in which a linear regression of the peak area (y) against the concentration (x) of each PAH was applied. The equations, correlation coefficients (R^2) and detection limits (MDL) are listed in Table 4. Note that MDLs are expressed by 3 times of signal to noise ratio (n=7) in blank, which is usually adopted as an IUPAC criterion. It is obvious that the concentrations of each PAH were in a very good linear relationship with its peak areas in a certain concentration range, with MDLs between 0.75ng·L^{-1} and 10.99 ng·L^{-1}. Based on quintic measurements of a standard mixture, it was also shown that RSDs for retention time and peak area were 0.048% to 0.140% and 1.32% to 4.38%, respectively (detailed data not shown), which is precise enough for HPLC determination of trace organic compounds.

To further ascertain the precision and accuracy of the method, the recoveries for a spiked blank and a spiked matrix (sediment sample) at a level of 10 ng·mL^{-1} were determined (Table 5). Spiking recoveries in the range of 54.82% to 94.70% with RSDs of 3.02% to 23.22% for a spiked blank, and 61.20% to 127.08% with RSDs of 7.61% to 26.93% for a spiked matrix basically satisfy the US EPA criterion for analysis of trace components, except for naphthalene, acenaphthene and fluorene which are highly volatile.

It is well known that the spiking recoveries may not be representative for a natural sample (Miège *et al*., 1998). Therefore, a NIST standard reference material (SRM 1941b, Organics in Marine Sediment) was tested to further evaluate the precision and accuracy of the methods. The recoveries and RSDs for the 15 PAHs were in the range of 50.79% to 83.78% and 5.24% to 21.38% (n=4), respectively (Table 6). Low recoveries for SRM 1941b might indicate that a portion of PAHs were not extractable simply by organic solvents from the sediment matrix. We also noticed that the recovery of naphthalene for spiked samples (Table5) and standard reference sediment (Table 6) was significantly higher than that for the clean-up procedure (Table 3), obviously because of high loss of volatile naphthalene in the step of clean-up.

Table 4 Retention times, regression equations, correlation coefficients and method detection limits

PAHs	Retention time min	Regression equation* (n=7)	Concentration range ng mL^{-1}	R^{2}*	MDL* (ng L^{-1}, n=7)
Naph	10.37±0.01	y = 11.248x + 355.77	20-5000	0.9994	8.28
Ace+Flu	17.01±0.01	y = 10.944x + 20.853	4-1000	0.9997	3.11
Phe	18.05±0.02	y = 2.9609x + 1.8659	2-500	0.9999	10.52
Anth	19.32±0.02	y = 38.117x + 136.42	2-500	0.9995	0.75
Fla	22.70±0.02	y = 2.9634x + 20.075	4-1000	0.9997	10.99
Pyr	24.14±0.02	y = 8.4718x - 2.5819	2-500	0.9997	3.83
Chry	29.89±0.03	y = 5.7029x + 6.6495	2-500	0.9999	5.51
BaA	30.48±0.03	y = 6.4747x + 19.732	2-500	0.9997	4.16
BbF	37.31±0.03	y = 4.0746x + 4.3498	4-1000	0.9999	9.40
BkF	38.14±0.03	y = 18.583x + 10.694	2-500	0.9999	1.88
BaP	39.31±0.02	y = 22.385x + 50.115	2-500	0.9998	1.12
DahA	43.64±0.02	y = 5.9794x + 3.7151	4-1000	0.9999	5.42
BghiP+InP	45.18±0.02	y = 10.218x - 24.464	2-500	0.9999	2.99

Note: * y: peak area; x: concentration; R^2: linear correlation coefficient; MDL: method detection limit.

Table 5 Spiking recoveries and RSDs of PAHs (n=4) and concentrations of PAHs in a marine sediment sample

PAHs	Spiking amount ng	Spiked blank		Spiked matrix		Content in sediment sample (ng g^{-1})
		Average recovery %	RSD %	Average recovery %	RSD %	
Naph	50	54.82	23.22	62.54	26.93	5.58
Ace+Flu	10	65.57	18.27	61.20	17.36	3.67
Phe	5	72.59	10.44	81.37	11.88	27.75
Anth	5	74.13	9.56	113.41	21.53	8.95
Fla	10	77.92	6.73	125.39	13.80	67.26
Pyr	5	73.50	5.66	120.47	9.79	100.88
Chry	5	84.05	10.79	107.59	11.85	39.63
BaA	5	71.18	5.01	127.08	7.61	33.21
BbF	10	81.73	8.57	104.81	10.41	72.39
BkF	5	94.70	3.02	95.47	8.77	16.28
BaP	5	83.12	5.74	108.73	12.10	18.85
DahA	10	91.19	6.21	93.31	14.60	18.49
BghiP +InP	5	87.94	7.12	96.56	9.20	23.69

Table 6 Recoveries and RSDs of PAHs for SRM 1941b (n=4)

PAHs	Nominated content ng g^{-1}	Content detected by using this method ng g^{-1}				Average recovery %	RSD %
Naph	848±95	450.14	296.12	494.55	482.01	50.79	21.28
Ace+Flu	85±15	46.41	36.54	48.28	51.09	53.63	13.88
Phe	406±44	243.02	245.20	278.03	282.64	64.59	8.02
Anth	184±18	124.84	116.08	138.75	135.75	70.03	8.08
Fla	651±50	460.22	422.82	468.08	477.46	70.22	5.24
Pyr	581±39	393.46	359.51	418.06	486.24	71.31	12.94
Chry	291±31	226.81	210.45	241.19	240.96	78.99	6.34
BaA	335±25	258.27	228.28	255.47	266.56	75.27	6.58
BbF	453±21	330.94	284.24	324.47	336.37	70.42	7.42
BkF	225±18	176.94	159.09	190.49	198.66	80.57	9.54
BaP	358±17	275.12	251.66	298.33	317.22	79.77	9.95
DahA	53±10	40.57	33.90	39.35	39.21	72.18	7.75
BghiP+InP	307±45	241.19	233.21	271.73	282.63	83.78	9.23

In summary, the method established is suitable for HPLC determination of PAHs in marine sediments with adequate sensitivity, precision and accuracy.

3.5 Determination of PAHs in Marine Sediments

The methods developed in this study were applied to determine the contents of the 15 PAHs in a surface sediment sample from Hongshiya in the Jiaozhou Bay area of Qingdao, China. Data are listed in the last column of Table 5. Among the 15 PAHs, the contents of fluoranthene, pyrene, chrysene, benzo[a] anthracene and benzo[b] fluoranthene were higher than 30 ng g^{-1}. In contrast, those of naphthalene, acenaphthene+fluorene and anthracene were less than 10ng g^{-1}. Acenaphthylene was not reported because it cannot be detected with fluorescence detection.

4 Conclusions

This work presented a method for determination of PAHs in marine sediments by HPLC with fluorescence detection, emphasizing the optimization of the extraction solvents and the clean-up procedures. For a wet sediment sample, the extraction efficiency of MeOH-DCM mixture was higher than that of DCM. In the followed chromatographic column clean-up procedure, elution directly with DCM-hexane (2:3, v/v) mixture is preferable in view of both eliminated interference and simplified experimental procedures. The recoveries of purification were in the range of 30.68% to 63.74% (2 or 3-rings PAHs) and 82.59% to 93.72% (high-molecular-weight PAHs), respectively.

The major advantages of this method are the low consumption of hazardous and toxic organic solvents and the elimination of additional preprocessing steps. The precision and accuracy were assessed with spiked samples and a NIST standard reference material. The results showed that the method is accurate and reliable with high sensitivity and can be used for determination of PAHs in marine sediment samples. Based on the optimized conditions, the level of PAHs in the sediment samples collected from Hongshiya in the Jiaozhou Bay area of Qingdao was found to be between 10.0 and 30.0 $ng\,g^{-1}$.

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