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Doctor Forum

Optimization of Sample Pretreatment for Determination of Polycyclic Aromatic Hydrocarbons in Estuarine Sediments by Gas Chromatography

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Abstract This study examined levels of polycyclic aromatic hydrocarbons (PAHs) in estuarine sediments in Licun (Qingdao, China) by gas chromatography under optimized conditions for sample pretreatment via ultrasonic extraction, column chromatography, and thin layer chromatography. Methanol and dichloromethane (DCM)/methanol (2:1, v/v) were used in ultrasonic extraction, and DCM was used as eluate for column chromatography. The developing system consisted of *n*-hexane and DCM at a ratio of 9:1 (v/v), with DCM as the extraction solvent for PAHs-containing silica gel scraped off the plate. When the spiking level is 100 ng, total recoveries of spiked matrices for four target PAHs (phenanthrene, anthracene, pyrene and chrysene) were 83.7%, 76.4%, 85.8%, and 88.7%, respectively, with relative standard deviation (RSD) between 5.0% and 6.5% (n = 4). When the spiking level is 1000 ng, associated total recoveries were 78.6%, 72.7%, 82.7% and 85.3%, respectively, with RSD between 4.4% and 5.3% (n = 4). The optimized method was advantageous for determination of PAHs in complex matrix due to its effective sample purification.

Key words ultrasonic extraction; thin layer chromatography; estuarine sediment; PAHs

1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in contaminated environments on a global scale. Their mutagenic and carcinogenic characteristics, as well as long residence terms have raised public concern (Jackson *et al*., 1994; Leitea *et al*., 2008). Most PAHs are generated from incomplete combustion of organic materials, automobile exhaust, coal-fired power plants and oil spills. Associated compounds have been detected in various environments such as atmospheric aerosols, waters, sediments, soils and organisms (Dickhut *et al*., 2000; Shi *et al*., 2010; Zhang *et al*., 2010). Among these, sediments are considered as the major reservoir of PAHs. Once discharged into aquatic systems, PAHs preferentially adsorb suspended particulate materials and eventually settle down to sediments. To assess environmental and biological risks of sediment PAHs, it is necessary to establish an effective method convenient for accurate quantification of associated compounds in sedimentary environments, especially those inshore where quantification of PAHs is problematic due to strong land and anthropogenic impacts.

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The PAHs analysis in environmental matrices commonly involves sample pretreatment (extraction, separation and purification) and instrumental measurement. Methods previously used for PAHs extraction include soxhlet extraction, ultrasonic extraction (Li and Li, 1999; Paula *et al*., 2005; Patricia *et al*., 2009), supercritical fluid extraction (Librando *et al*., 2004; Portet *et al*., 2009), accelerated solvent extraction (Berset *et al*., 1999; Li *et al*., 2003), and microwave extraction (Xiong *et al*., 1998). Ultrasonication has been preferentially used due to the high efficiency, low cost and extraction temperature, as well as easy operation (Darryl and Paul, 2005).

Column chromatography using silica or alumina-silica gel as the sorbent is a traditional method for sample purification prior to sediment PAHs analysis. Single column is usually sufficient for purification of samples extracted from deep-sea sediments, but not so for those from inshore sediments. This is because the latter can receive various anthropogenic contaminants combined with a large amount of terrestrial organic complexes. As a consequence, PAHs peaks in the trace of gas chromatography (GC) are commonly overlapped with a 'hump', the so-called unresolved complex mixture (UCM). To avoid UCM interference, column chromatography has been used for further sample purification. In addition, thin layer chromatography (TLC) is a convenient technique commonly used for separation of complex mixtures. For

example, Kim *et al*. (2005) isolated PAHs from environmental samples for compound-specific isotope analysis via column chromatography, high-performance liquid chromatography, and TLC, whereas Liu *et al*. (2005) purified PAHs in atmospheric aerosol samples for compound-specific carbon isotope analysis via alumina-silica gel column and TLC.

Presently, there is a lack of research regarding sample purification for inshore sediment PAHs analysis. This study developed a simple and effective purification procedure to determine PAHs levels in highly-contaminated inshore sediments. The associated experimental conditions were optimized and a combined method of column chromatography and TLC was proposed. This method was further tested by GC determination of sediment PAHs in the Licun estuary, Qingdao, China.

2 Materials and Methods

2.1 Study Site and Sample Collection

Sediment samples were collected from the intertidal zone of the Licun estuary in Qingdao (N 36.16º, E 120.37º) in May 2010 (Fig.1). Top 2 cm sediments were taken at low tide, immediately transported to field laboratory and kept frozen prior to further processing.

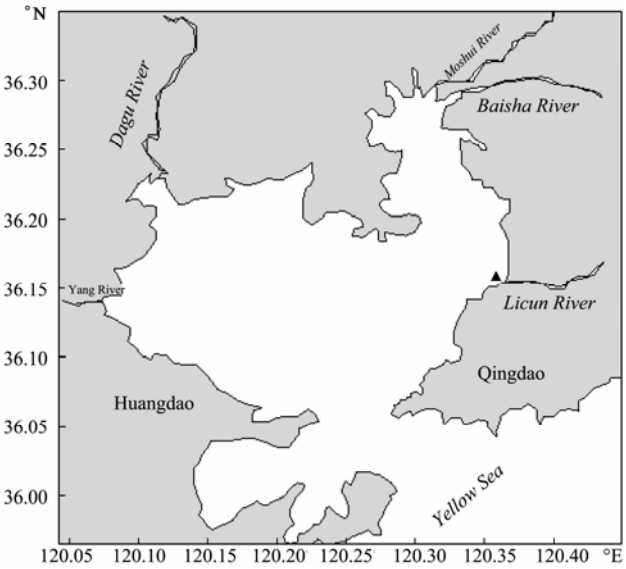

Fig.1 Location of the study area and sampling site.

2.2 Chemicals and Materials

Dichloromethane (DCM) and *n*-hexane (HPLC grade) were purchased from Dikma Technologies Inc., China; methanol, toluene, chloroform, and tetrachloromethane (HPLC grade) from Tianjin Shield Chemicals Co. China; acetone (AR, redistilled before use) from Yantai Sanhe Chemicals Co. China; and silica TLC plates (G type，200×100×1 mm) from Anhui Liangchen Silicon Source Material Co. China. Silica gel (100-200 mesh, Qingdao Oceanic Chemical Factory, China) used for column chromatography was activated by heating at 180℃ for 12 h. Anhydrous sodium sulfate (Na_2SO_4, Sinopharm Chemical Reagent Co. Ltd) was heated at 450℃ for 4 h and stored in a tightly-capped glass bottle. Copper powders (Fisher Scientific) were activated in 7 $molL^{-1}$ HCl and successively rinsed with distilled water, acetone and *n*-hexane. Standards were prepared using phenanthrene, anthracene, pyrene, chrysene and hexamethylbenzene (Aldrich Chem Co., purity >98%). Associated stock solutions were prepared in DCM (500μg mL^{-1}) and kept in dark at 4℃.

Glasswares were washed with detergent, rinsed with distilled water and heated at 450℃ for >4 h before use.

2.3 Sample Extraction and Purification

Sediment samples were freeze-dried at –40℃, ground and sieved to remove large particles and debris. Sulfur present in samples was removed by adding 1 g activated copper powder to 4 g sediment. Ultrasonic extraction of the mixture was performed with solvent for several times. Sediment extracts were repetitively dried via rotary evaporation and re-dissolved in 1 mL *n*-hexane for three times prior to column chromatography.

The extract was dehydrated using a 7 g silica-gel- column with 2 g anhydrous Na_2SO_4 on the top. Aliphatic hydrocarbons were eluted from the column using 20 mL *n*-hexane, followed by elution of aromatic hydrocarbons using 20 mL solvent. The aliphatic fraction was discarded, whereas aromatic fraction was concentrated for further purification via TLC.

The TLC was performed on a 200×100 mm glass plate coated with 1.0 mm-thick silica gel. Concentrated aromatic fraction was transferred to the TLC plate as a thin band. The plate was developed with solvent, with PAHs band identified by comparison with standard samples under short UV radiation (254 nm). The PAHs-containing silica gel was scraped off the plate and extracted with solvent via ultrasonication. The extract was dried via rotary evaporation, re-dissolved in *n*-hexane. The solution was gently dried under N_2, with 50μL n-hexane and 50 μL hexamethylbenzene added as internal standards for quantitative PAHs analysis.

2.4 GC Conditions

Quantitative PAHs analysis was performed on a Shimadzu 2010 plus GC equipped with a FID detector and a HP-5 capillary column (30 m×0.32 mm i.d., 0.25μm film thicknesses). The oven temperature first remained at 60℃ for 2 min, then was increased to 290℃ following a three-ramping mode: first increased at 10℃ min^{-1} to 160℃ and held for 1 min; then increased at 2℃min^{-1} to 240℃ and held for 1 min; finally increased at 20℃ min^{-1} to 290 ℃ and held for 5min. The flow rate of carrier gas (high-purity N_2, >99.995%) was 1.5 $mLmin^{-1}$.

3 Results and Discussion

3.1 Optimization of Ultrasonic Extraction

Dried sediments were washed twice with acetone and

once with methanol, then heated at 450℃ for 4 h to remove organic matter. Samples were supplemented with PAHs standard solutions to obtain 250 ng g^{-1} phenanthrene, anthracene, pyrene or chrysene, then extracted ultrasonically following procedures listed in Table 1. Mean recoveries of four target PAHs with different solvent mixtures were compared for procedures A–D. Procedures D and E were performed to investigate effects of extraction duration and cycles on PAHs recoveries.

Mean PAHs recoveries and associated relative standard deviations (RSD) following different extraction procedures (A–E) are summarized in Table 1. Comparison of extraction efficiencies of A–B and C–D indicated that PAHs recoveries increased with methanol content in the ultrasonication solvent. The recoveries further increased when the number of extraction cycles increased from two to three (D and E, Table 1). The optimized ultrasonic procedure involved 10 min extraction in 20 mL methanol and 5 min extraction in 10 mL methanol-DCM mixture (1:2, v/v) for three times.

3.2 Optimization of Elution Solvent for Column Chromatography

Table 2 shows mean PAHs recoveries and associated RSD using different elution solvents for aromatic fraction from column chromatography. Apparently, mean recoveries of four target PAHs all increased with increasing polarity of associated solvent. Despite the highest mean PAHs recoveries, elution with DCM-acetone mixture (4:1, v/v) resulted in an eluate containing substantial impurities, thus was not suitable for further TLC purification. In this study, we selected DCM as the elution solvent for aromatic fraction from column chromatography.

Table 1 Mean PAHs recoveries following different extraction procedures

Procedure	Solvent (s) (50 mL)	Volume (mL) × time (min) × number of cycles	Mean recoveries and RSD (%, n = 4)			
			Phe	Anth	Pyr	Chry
A	Methanol-DCM (1:4, v/v)	25×15×2	65.6 (6.7)	72.9 (6.4)	70.8 (5.3)	73.1 (4.8)
B	Methanol-DCM (1:2, v/v)	25×15×2	73.5 (5.2)	82.4 (6.8)	75.7 (4.9)	74.5 (4.2)
C	Methanol Methanol-DCM (1:2, v/v)	10×10×1 20×10×2	88.6 (5.2)	85.3 (5.6)	89.2 (5.4)	90.3 (5.2)
D	Methanol Methanol-DCM (1:2, v/v)	20×10×1 15×10×2	92.4 (4.2)	89.6 (5.8)	93.8 (4.9)	93.6 (4.2)
E	Methanol Methanol-DCM (1:2, v/v)	20×10×1 10×5×3	92.6 (4.9)	90.7 (4.9)	94.2 (4.0)	95.8 (3.3)

Table 2 Mean PAHs recoveries using different elution solvent for aromatic fraction from column chromatography

Elution solvent	Recoveries and RSD (%, n = 4)							
	Phe		Anth		Pyr		Chry	
n-hexane-DCM (3:2, v/v)	68.8	4.27	78.4	4.18	76.4	3.34	78.4	3.98
n-hexane-DCM (1:1, v/v)	83.2	4.18	80.7	4.02	85.8	3.22	83.9	3.83
DCM	92.2	4.03	88.4	3.93	96.0	3.09	94.9	3.74
DCM-acetone (4:1, v/v)	101.4	4.97	99.6	4.76	100.6	3.92	95.0	4.57

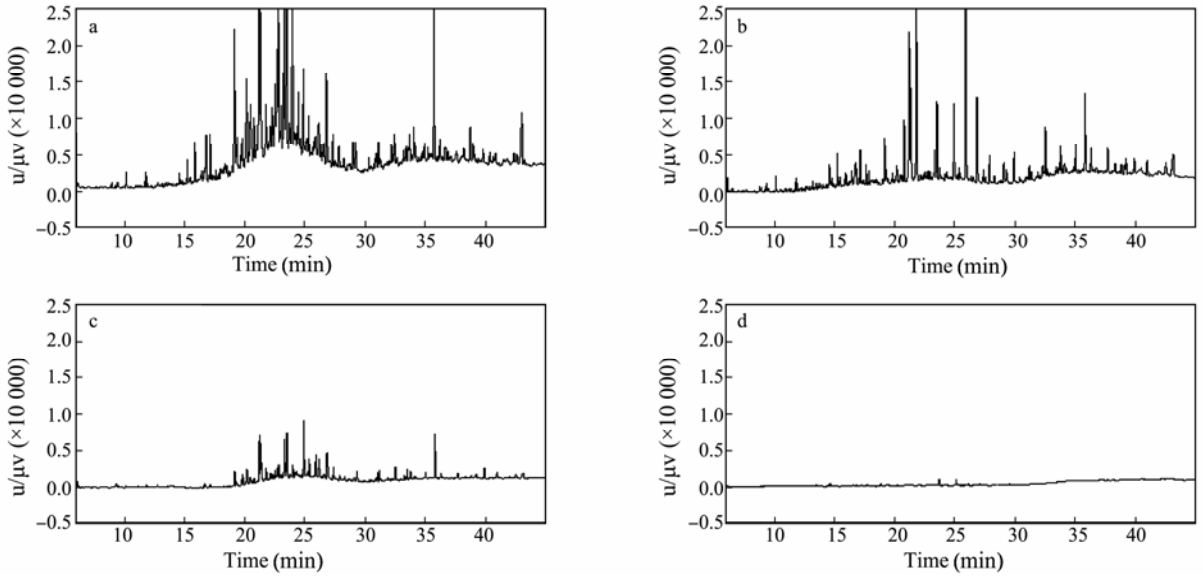

Fig.2 Chromatograms of the extracts from non-washed TLC plate (a), and those from TLC plates washed with ethyl acetate for one time (b), DCM-methanol mixture (1:1, v/v) for one time (c) and DCM-methanol mixture (1:1, v/v) for one time and methanol for another time (d).

3.3 Thin Layer Chromatography

3.3.1 Purification of TLC plates

Due to the presence of various impurities (Fig.2a), TLC plates were prewashed before use. The capability of various solvents and associated mixtures to remove impurities from TLC plates is shown in Fig.2b–c. A large amount of impurities remained in the plate when prewashed once with ethyl acetate (Fig.2b); less impurities persisted in the plate when prewashed once with DCM-methanol mixture (1:1, v/v) (Fig.2c); no impurity peaks were detectable in the plate when prewashed twice with DCM-methanol mixture (1:1, v/v) and methanol, respectively (Fig.2d).

3.3.2 Optimization of TLC developing solvent

The R_f value of associated compound is critical for selection of TLC developing solvent (R_f = b/a, b is the distance between the origin center and spot center; a is the distance between the origin center and solvent front). For better developing results, R_f values ranging from 0.3 to 0.7 are preferentially used. The R_f values of four target PAHs in different developing systems were determined by applying mixed PAHs standard solution onto the TLC plate. Results showed that the R_f values of phenanthrene, anthracene and pyrene were at similar levels, resulting in a single wide band upon development. By comparison, the R_f value of chrysene was substantially smaller. These indicated that all developing systems tested were suitable for TLC separation of PAHs, except for the mixture of *n*-hexane-toluene (3:2, v/v). To minimize the toxicity to human and the environment, we selected the mixture of *n*-hexane-DCM (9:1, v/v) as the developing system (Table 3).

Table 3 R_f values of PAHs in different developing solvents

Developing solvent (v/v)	R_f value	
	Phe, Anth and Pyr	Chry
n-hexane: toluene (3:2)	0.73-0.76	0.65
n-hexane: DCM (7:3)	0.66-0.69	0.56
n-hexane: chloroform (9:1)	0.55-0.58	0.45
n-hexane: DCM (9:1)	0.49-0.52	0.40
n-hexane: CCl_4 (10:1)	0.34-0.37	0.26

3.3.3 Optimization of extraction conditions for TLC silica gel

The eluted PAHs fraction from column chromatography was concentrated and applied onto the prewashed TLC plate, then developed with a mixture of *n*-hexane-DCM (9:1, v/v). Two PAHs bands were scraped off the TLC plate and extracted with solvent via ultrasonication.

To achieve higher recoveries, extraction conditions with different solvents were optimized. Results showed that DCM extraction yielded highest PAHs recoveries (Table 4). Increases in solvent volume slightly increased or did not significantly impact associated PAHs recovery. Increased solvent volume also prolonged concentration duration. Finally, we selected 20 mL DCM as the optimal conditions for solvent and volume.

3.4 Comparison of Purification Effects

GC traces of samples purified by column chromatography and column chromatography-TLC are presented in Fig.3. Results showed that the latter method decreased unwanted peaks and avoided co-elution of PAHs.

Table 4 PAHs recoveries (%) under different extraction conditions

PAHs	*n*-hexane: DCM (2/1, v/v)			*n*-hexane: DCM (1/1, v/v)			DCM		
	15 mL	20 mL	25 mL	15 mL	20 mL	25 mL	15 mL	20 mL	25 mL
Phe	76.8	80.1	84.3	82.7	87.5	90.5	90.3	93.3	93.4
Anth	70.2	74.1	77.6	75.9	79.4	83.2	88.1	90.8	90.3
Pyr	82.7	87.3	89.0	88.6	91.2	93.7	93.4	95.8	95.4
Chry	80.6	88.5	91.1	90.8	93.3	93.9	93.7	95.6	95.6

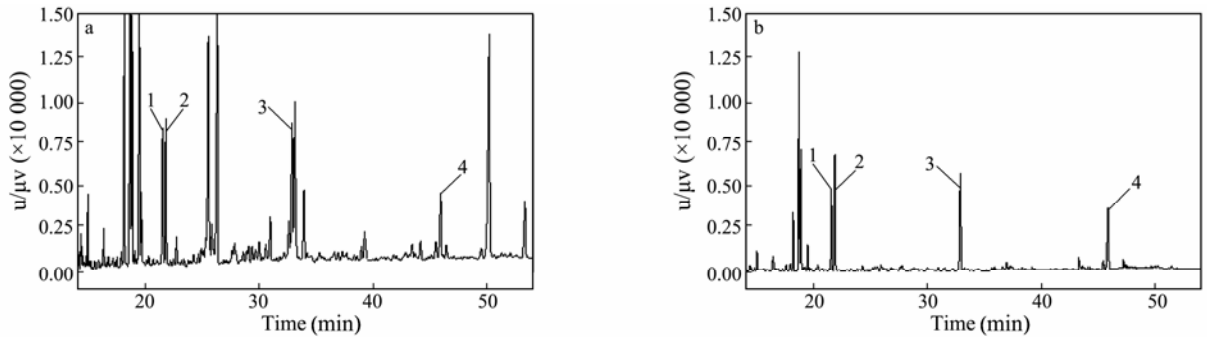

Fig.3 GC traces of sample purified by column chromatography (a) and column chromatography-TLC (b). (1- phe; 2-anth; 3-pyr; 4-chry).

3.5 Method Blank, Standard Equations and Total Matrix-Spiking Recovery

Under optimized conditions, a blank test demonstrated that the procedure and reagents used in this study did not impose strong interference on PAHs analysis.

Mixed standard solutions were prepared for quantification of PAHs in sediment samples. As shown in Table 5,

the GC area ratio varied linearly with mass concentration between 5 and 80μg mL^{-1} (R^2 >0.999).

For determination of matrix-spiking recoveries, 1.0 mL target contaminant (1 and 0.1 $\mu g \cdot mL^{-1}$) was added to 4 g freeze-dried sediment. Spiked samples were analyzed using our proposed method. The overall PAHs recoveries ranged from 72.7 to 88.7%, with RSDs between 4.4 and 6.5% (Table 6).

To evaluate the proposed method, we determined levels of phenanthrene, anthracene, pyrene and chrysene in sediments from the Licun estuary in Qingdao, China. Results were 569, 72, 408 and 230 ng g^{-1}, respectively (on dry-weight basis).

Liu *et al* (2005) developed an extraction method suitable for purification of aerosol sample. However, most inshore sediments suffer from substantial interferences such as organic matters. Kim *et al*. (2005) used gel permeation chromatography without column chromatography and TLC, but the associated cost was high. By comparison, the optimized method proposed in this study is advantageous due to the low cost and simple operation. Results indicated that our method is accurate and reliable, thus can be used for determination of PAHs in complex matrix.

Table 5 Standard regression equations and correlation coefficients for the four target PAHs

Target PAHs	Regression equation	R^2
Phe	$y=0.05x-0.0026$	0.9993
Anth	$y=0.342x+0.0124$	0.9995
Pyr	$y=0.0373x-0.0027$	0.9993
Chry	$y=0.0534x-0.019$	0.9993

Note: y is the area ratio of A_i/A_s, where A_i is the peak area of a PAH and A_s the peak area of internal standard. x is the mass concentration (μg mL^{-1}) of the PAH.

Table 6 Recoveries and RSDs of target contaminants for spiked matrix ($n=4$)

PAH	PAHs in matrix (ng)	Spiking amount (ng)	Recovery (%)	RSD (%)
Phe	2276	1000.0	78.6	5.3
		100.0	83.7	6.1
Anth	288	1000.0	72.7	5.1
		100.0	76.4	6.5
Pyr	1632	1000.0	82.7	4.8
		100.0	85.8	5.0
Chry	920	1000.0	85.3	4.4
		100.0	88.7	5.6

4 Conclusions

This work proposed a purification protocol for determination of PAHs in estuarine sediments by gas chromatography. Conditions were optimized for crucial steps of the combined method. Methanol and DCM/methanol (2/1, v/v) were used in ultrasonic extraction, DCM was used as eluate for column chromatography, n-hexane/DCM (9/1, v/v) was selected as the developing system, and 20 mL of DCM was selected as extraction solvent after the silica gel containing PAHs was scraped off the plate. After these pretreatments the UCM of the PAH fractions was wiped off. Test with the Licun estuarine sediments in Qingdao showed recoveries of spiked matrices for four target contaminants between 72.7% and 88.7%. Results in the present workindicate that the proposed method is suitable for determination of PAHs in sedimentary environments receiving organic complexes.

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