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ORIGINAL ARTICLE

Gas chromatograph–mass spectrometry determination of carcinogenic naphthalene, anthracene, phenanthrene and fluorene in the Bangsai river water of Bangladesh

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Abstract A sensitive and fast method is described that solid phase extraction (SPE) using dichloromethane, followed by gas chromatograph-mass spectrometry for the determination of environmentally carcinogenic naphthalene, anthracene, phenanthrene and fluorene (NAPF) from the Bangsai river water near the Dhaka mega city of Bangladesh. The method was applied to identify and quantify the carcinogenic NAPF in water samples collected from surface and 30 cm depth of water. Methanol (50 ml) pretreated and filtered water samples were applied directly to a C_{18} SPE column. The carcinogenic NAPF were extracted with dichloromethane and the NAPF concentration was obtained to be 0.39 to 54.98 ppm. The factors influencing SPE *e.g.*, absorbent types, sample load volume, eluting solvent and temperature, were investigated. A cartridge containing a C_{18} absorbent and using solvent gave a better performance for the extraction of NAPF from the Bangsai river water samples. Average recoveries exceeding 75% could be achieved for toluene at 25 °C with a 2.6% RSD.

1. Introduction

Carcinogenic organic compounds, *e.g.*, naphthalene, anthracene, phenanthrene, chrysene, pyrene, fluorene (PAHs) and its derivatives, are important environmental contaminants because of their high toxicity and widespread occurrence. They are present in aviation fuel (gasoline) and are widely used as industrial solvents and raw materials for the production of different commodities (Klist, 1993). Naphthalene, anthracene, phenanthrene, chrysene, pyrene, fluorene (PAHs) are among compounds designated as "priority pollutants" by

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the US EPA and the action and risk levels of naphthalene, anthracene, phenanthrene, chrysene, pyrene, fluorene (PAHs) are described in the Dutch Government Quality Standards for the assessment soil and water contaminations (EQSS, 1991). The determination of PAHs in environment matrices is difficult because of their trace level presence and losses incurred during sample handling, extraction etc. Presently there has been considerable interest in the development of SPE column for the clean-up all the suspended materials, extraction and preconcentration of liquid samples (Berrueta, et al. 1995). The applications of SPE are found in different areas, such as soils and sediments (Loconto, 1991; Zhang and Pawliszyn 1995), industrial effluents (Deans, et al. 1993) and water samples (Saner, et al. 2007; Auther, et al. 1992). This metod has been used for the extraction of PAHs (Mency, et al. 1998; Redondo, et al. 1993) and pesticides (Murry, 2001) from soils and sediments. A few applications of SPE have also appeared for organic components in the PAHs analysis of water samples; a cartridge containing C_{18} absorbent was used to extract PAHs from industrial effluents (Deans, et al. 1993) and benzene and toluene from seawater (Saner, et al. 2007). On the other hand solid phase micro extraction (SPME) was also applied to isolate BTEX from water samples either by direct absorption from the liquid (Auther, et al. 1992) or *via* headspace sampling (Mency, et al. 1998), the limitation of SPME method for the determination of sulfur based volatile organic compounds (VOCs) has been reported (Tan et al. 2000). The VOCs analysis from air and water samples by open-tubular, wall coated columns (Stone and Taylor, 2000), solvent trapping (Charles et al, 2001) and semi-VOC from air by atmospheric pressure chemical ionization mass spectrometry (Hossain, et al. 1999; Motaleb, 1999) were also described.

The Bangsai river is very importance because of this presence in the Dhaka mega city, the capital of Bangladesh, and have an enormous impact on socio-economic activities of the country. House-hold, workshop and road washing as well as oil spillage are common features on the river at different places. As a result, the water of this river is being polluted awasomely by various organic compounds, especially polycyclic aromatic hydrocarbons. Now a day, different kinds of aromatic solvents are increasingly used in industry (Klist, 1993) and the wastes are sometimes disposed of in the aquatic environment, which becomes increasingly contaminated. Continuous monitor the contaminants, we determined the concentrations of various normal and polycyclic aromatic hydrocarbons in Buriganga river water (Motaleb and Abedin, 1999) and pesticides in soils (Saner, et al. 2007) by using GC with liquid - liquid extraction and SPE methods, respectively. Motaleb et al. 1999 had also reported on the development of a GC-MS chromatography method for the identification, determination and characterization of anionic detergents in various rivers water.

The present paper describes the concentration levels of naphthalene, anthracene, phenanthrene and fluorene (NAPF) in the Bangsai river water samples collected at two depths from the three different sampling spots near the Dhaka mega city. And the sampling spots are: Savar, Dhaka Export Processing Zone (DEPZ) and Bank Colony. Also discussed are the recoveries of PAHs using different absorbents for SPE columns and factors influencing SPE absorption, such as the sample load volume, eluting solvent and temperature.

2. Materials and methods

2.1. Chemicals

Naphthalene, anthracene, phenanthrene, and fluorene (NAPF) standards were purchased from Sigma–Aldrich Company with purity 99.9%. Dichloromethane (DCM) (BDH, UK), methanol and water (Merck, Germany) were of HPLC grade. Anhydrous sodium sulfate (Merck, Germany) was cleaned by heating at 200 °C before use. Silica gel (60–120 mesh, Loba, India) was activated at 400 °C for 12 h. prior to use. The C_{18}, C_8 and PH SPE cartridge were obtained from Supelco Ltd.

2.2. Instrument and operating conditions

GC–MS was carried out using total ion monitoring mode on a Varian 3800 gas chromatograph interfaced to a Varian Saturn ion trap 2200 GC–MS. The temperatures of transfer line and ion source were 280 and 275 °C, respectively. Ions were obtained by electron ionization mode. The VF-5 capillary column (30 m length, 0.25 mm I.D., 0.25 μm film thickness) was used. A 20% split injection mode was selected with a solvent delay time of 3 min. with injection volume 1 μl. The initial column temperature was started at 50 °C for 1 min, programmed at 8 $°C\ min^{-1}$ to 200 °C and heated until 300 °C at 10 $°C\ min^{-1}$. Injection port was set at 250 °C. Helium was used as carrier gas with a flow-rate of 1.0 $ml\ min^{-1}$. Molecular ions were monitored for identification. Mass range: 40–500 *m*/*z*.

2.3. Preparation of standard

Calibration curves for the samples, treated according to the described analytical procedure, were made using the SIM mode. The reference compounds, naphthalene, anthracene, phenanthrene and fluorene (NAPF) were used as markers. The markers were accurately weighed and dissolved in dichloromethane to produce a series of concentrations. Standard calibration curves were established by plotting the peak areas against different concentrations of the reference compound (varying from 1.25 to 10 μg/ml). The external standard method was used for quantification of the markers in the Bangsai river water extract.

2.4. Bangsai river water samples

Contaminated river water samples were collected in 1-1 dark amber-color glass bottles on August 7, 2009 from the three different sampling spots, the names of which are mentioned as above, of near the Dhaka mega city. Cleaned bottles were rinsed with simple water prior to sample collection. The locations of the sampling points of the Bangsai river are shown in Fig. 1. Ten samples at the surface and 10 at a depth of 30 cm were collected. The distance of the sample collection point from the river bank was about 2 m. At first, the bottle was lowered slowly into the water and its cork was opened by hand, marked accordingly in centimeter at the desired depth. When the bottle was filled with water, it was closed and drawn up carefully. Then 100 ml of water was discharged from the glass bottle. At the same time 10% $CuSO_4$ was added as a stabilizing

Figure 1 Map of Bangsai river showing the locations of the sampling stations and collection points of water samples. Sampling collection points 30 cm depth.

agent (Hossain et al., 1999) into the water samples, closed by the cork and shaken vigorously by the hand.

2.5. Extraction of markers (NAPF) from synthetic water and lake water samples

A 10 ml volume of a synthetic sample was extracted with 10 ml of dichloromethane in a 30 ml vial; the layers were allowed to separate. Prior to SPE work, 2–4 ml of the organic layer was removed and stored in a sealed glass vial at 4 °C. The extraction of a river water sample was carried out essentially according to a reported method (Hossain et al., 1999). Each 10 ml water sample was shaken vigorously for 30 min with 50 ml of dichloromethane at 4 °C. The aqueous was separated and extracted again with 25 ml of dichloromethane. The combined extracts were then stored at 4 °C for SPE. Prior to direct use of the water samples in SPE, the sample (200 ml) was filtered by a 0.45 µm nylon filter paper.

2.6. Solid-phase extraction

The column was activated with 3 ml of methanol and pre-equilibrated with 3 ml of demonized water. The river water sample (10 ml) was loaded on the column at 3 ml/min. Elution was carried out with two portions of 2 ml aqueous 1% methanol. Finally, solutes were eluted with two aliquots of 2 ml of dichloromethane. Similar elution profiles were obtained for recovery experiments. Dichloromethane (5%) was added as an internal standard prior to a GC–MS analysis. Passing samples through a dryer containing sodium sulfate only eliminates trace amount of water.

2.7. Concentration of response factor and concentration of component

The relative response factor of a component (R_F) to the internal standard of DCM is given by

$$R_F = C_{DCM}/A_{DCM} \times A_C/C_C$$

where C_{DCM} and C_C represent the concentrations of DCM and the component analyte, respectively, in terms of µg/ml. The term A_{DCM} and A_C indicate the peak area for DCM and the component analyte, respectively. The response factors for all components were calculated as mentioned above, and the concentration of each component (C_C) was calculated as follows:

$$C_C = C_{DCM}/A_{DCM} \times A_C/R_F$$

3. Results and discussion

3.1. GC–MS optimization

The GC–MS system used was optimized before the organic compounds measurement. Separations were achieved with different temperature programs. A good separation of NAPF constituents, including dichloromethane (DMC), was obtained under the following conditions: Injector temperature, 250 °C; Column oven temperature, 50 °C for 1 min, programmed at 8 °C min^{-1} to 200 °C and heated until 300 °C at 10 °C min^{-1}. Helium was used as carrier gas with a flow-rate of 1.0 ml min^{-1}. Fig. 2 is GC–MS chromatograms of the standard solution and water samples from the different sampling spots, showing that components and DCM were well resolved.

The linearity of the GC–MS response was also demonstrated by injecting the working standard solutions into the GC–MS instrument. Table 1 depicts the calibration graph of the peak area versus the concentration. The GC–MS gave the good linearity of the response for the detection of NAPF constituents. Hence, it was decided that the above conditions could be used for the determination of toluene from the river water samples.

3.2. Presence of PAHs in the Bangsai river water samples

Table 2 summarizes the concentration of naphthalene, fluorene, phenanthrene and anthracene, predominantly present in the river water samples, analyzed by SPE–GC–MS. The presence of PAHs was not detected using the experimental conditions described previously, although trace levels of naphthalene were found. To detect the other constituents of the PAHs family, an increased volume of 10 ml of water samples

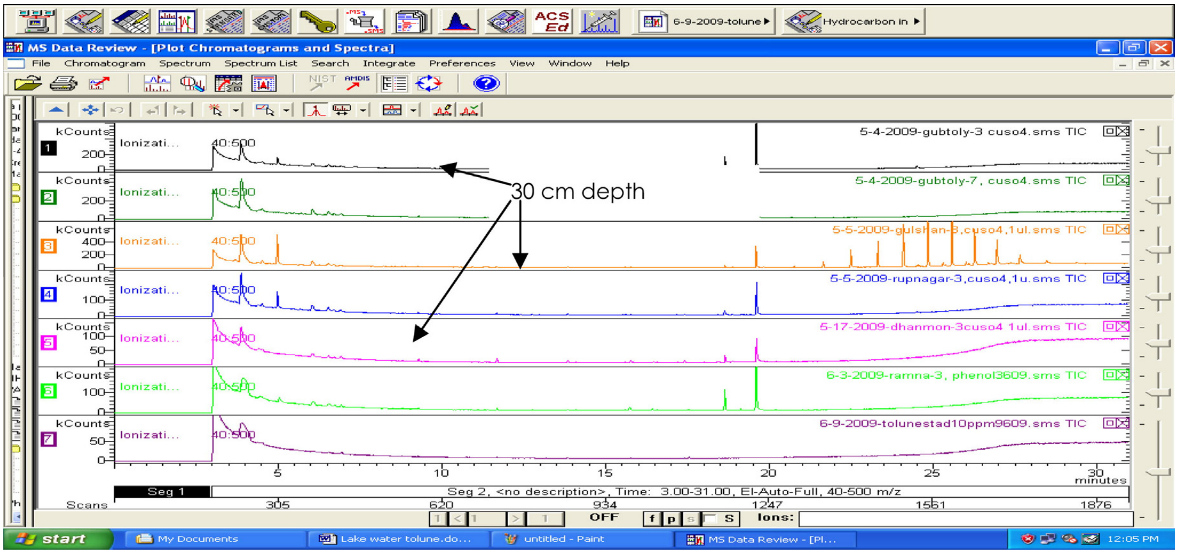

Figure 2 A typical GC–MS chromatograms for standard solution and water samples from different sampling spots. Column: VF-5 (l. 30 m, I.D. 0.25, film thickness 0.25 μm); delay: 3 min; temperature program: 50 °C(1) → 200 °C (8 °C) → 300 °C (10 °C); injector temperature: 250 °C; split: 20%; carrier gas: He; flow rate: 1 ml min^{-1}.

Table 1 Analytical characteristics of calibration curves for the standard markers.

Constituent	Ret. time (min)	Range (μg/ml)	Regression equation	Detection limit (μg/ml)	R^2	Precision (%) (SD[a]) (n = 5, 10 ng/μl)	
						Inter-day	Intra-day
Naphthalene	7.981	2–1000	$0.1291x - 0.3250$	2.3	0.9999	0.65	0.30
Fluorene	18.341	2.5–1000	$0.0698x - 0.0243$	2.0	0.9988	0.70	0.41
Phenanthrene	19.78	2–1000	$0.1950x + 0.3032$	2.5	0.999	1.12	0.98
Anthracene	19.86	2.1–1000	$0.1863x + 0.8219$	2.1	0.9978	0.65	0.81

[a] SD = standard deviation.

Table 2 SPE of Bangsai river water samples at 4 °C (C_{18} column, sample volume 10 ml).

Name of sampling spot	Concentration of components in Bangsai river water							
	Surface water				30 cm Depth			
	Conc. of naphthalene (μg/ml)	Conc. of fluorene (μg/ml)	Conc. of phenanthrene (μg/ml)	Conc. of anthracene (μg/ml)	Conc. of naphthalene (μg/ml)	Conc. of fluorene (μg/ml)	Conc. of phenanthrene (μg/ml)	Conc. of anthracene (μg/ml)
Savar	1.78	0.39	ND*	0.142	1.21	0.22	ND	0.099
DEPZ	2.10	0.55	ND	0.109	1.88	0.32	ND	0.078
Bank Colony	3.09	0.23	ND	0.09	1.78	0.21	ND	0.034

* ND = not detectable

was directly applied to the SPE at 4 °C. An appreciable amount of naphthalene was obtained in the river water samples, while the other constituents such as fluorene, phenanthrene and anthracene were present in a tiny amount. Fig. 2 shows a representative GC–MS chromatogram of the standard solution and lake water samples. The chromatograms in Fig. 2 also correspond to the surface and 30 cm depth of water, collected from the Bangsai river near the Dhaka mega city of Bangladesh. These were obtained when 2 μl SPE eluted samples were injected into the GC–MS and showed a similar chromatographic elution pattern with different magnitudes of the naphthalene compound peak. Blank experiments were performed prior to sample injection.

3.3. Selection of adsorbents and eluting solvent

To select the suitability of adsorbents and eluting solvents, the percentage of recovery of PAHs constituents was investigated using C_{18}, C_{18} and pH cartridges with dichloromethane and chloroform solvents. The recoveries were obtained when 2 ml portions of standard toluene solution were passed through different SPE columns and eluted with two portions of 2 ml of dichloromethane or chloroform at 4 °C. The recovery results are presented in Table 3. The extractions were performed simultaneously for each solvent.

Regardless of the solvents used, higher recoveries were obtained for fluorene, phenanthrene and anthracene. This

Table 3 Recovery of naphthalene from different SPE columns eluted with dichloromethane and chloroform at a temperature of 4 °C.

Recoveries (%) ± standard deviation (SD)[a]		
Solvent	SPE column	Naphthalene
Dichloromethane	C_{18}	75.5 ± 2.5
	C_8	68.3 ± 2.1
	pH	65.8 ± 3.1
Chloroform	C_{18}	71.2 ± 2.9
	C_8	62.9 ± 2.8
	pH	63.7 ± 3.6

[a] SD = standard deviation.

may have been due to a less evaporative loss of the two components because of their higher boiling point. Moreover, in comparison between dichloromethane and chloroform solvents, it was observed that slightly better recoveries were obtained when dichloromethane was used as the eluting solvent (Table 3). This is probably due to a more non-polar interaction between a bonded phase and the dichloromethane system.

3.4. Effect of the sample load volume

The effect of the sample volume on the SPE recovery is one of the most important factors, because the SPE performance is affected by the amount of sample loaded on a particular column. The break through volume of a C_{18} column (500 mg, 3 ml capacity) was determined by passing a number of PAHs standard solutions to a volume of up to 10 ml. Known masses of the analytes were introduced. There were no appreciable changes in the recovery rates up to a sample volume of 8 ml. The percentage of recovery of naphthalene and fluorene decreases more rapidly than other PAHs. This observation confirms the fact that other PAHs possess higher breakthrough volumes than naphthalene and anthracene. Similar break through volumes were obtained for C_8 and phenyl substituted SPE columns. These are not shown.

3.5. Factors affecting the SPE performance investigations

3.5.1. Temperature

To investigate the effect of the temperature on SPE performance, a cartridge containing C_{18} material was employed with dichloromethane solvent at a temperature of 20 and 4 °C. An effect of the temperature on the SPE recovery of PAHs constituents was found to occur Table 3. It has been observed that both liquid-liquid and solid-phase extractions provided slightly recoveries when experiments were carried out at a temperature of 4 °C. The relative standard deviations (RSD) were calculated for each of the PAHs constituents and temperature. At 20 °C, the RSD of recoveries were between 3.3% and 4.0%, however, at 4 °C improved recoveries of the constituents were achieved with RSD values of 2.7–3.7%.

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