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Contributed Paper

Determination of Phenanthrene and Fluorene by MCM-41-Dispersive Micro-solid Phase Extraction Combined with Gas Chromatography-Mass Spectrometry

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ABSTRACT

A simple and rapid technique termed MCM-41-dispersive micro-solid phase extraction (MCM-41-D-μ-SPE) combined with gas chromatography-mass spectrometry (GC-MS) was utilized for the analysis of polycyclic aromatic hydrocarbons (PAHs) in water samples. Fluorene and phenanthrene were selected as model compounds. Three parameters, namely type of desorption solvent, extraction time and volume of desorption solvent were investigated. In the optimized procedure, MCM-41 (5 mg) was dispersed in water sample (10 mL) in a centrifuge tube and the mixture was agitated vigorously for 2 min followed by centrifugation (5 min) at 3800 rpm. The sedimented adsorbent was dried under nitrogen gas flow and treated with 60 μL of acetone as desorption solvent. 1 μL of final solution was injected into the GC-MS for analysis. This method showed good linearity in the range of 0.3-200.0 μg/L with coefficient of determination (r^2) between 0.9973-0.9980. The limits of detections (LODs) were in the range of 0.07-0.16 μg/L. The proposed extraction method provided acceptable recoveries (79.5-107.6 %) with relative standard deviations (RSDs) in the range of 1.7-9.4%. The method was successfully applied to the determination of fluorine and phenanthrene in tap water and lake water samples.

Keywords: dispersive micro-solid phase extraction, MCM-41, gas chromatography-mass spectrometry, polycyclic aromatic hydrocarbons

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic organic pollutants made up of at least two fused benzene rings. PAHs (fluorene, fluoranthene, phenanthrene, and pyrene) are widely found in water resources. Since 1976, they have been known as mutagens or carcinogens towards human health when they are entering via the contamination of drinking water and vegetables [1]. PAHs compounds are ubiquitous in the environment and can be formed naturally, but they are also derived from anthropogenic activities such as incomplete combustion, pyrolysis of organic matters and vehicular emmisions [2-3]. Fluorene and phenanthrene are among compounds classified as "priority pollutants" by the US EPA and the action and risk levels of fluorine and phenanthrene are described in the Dutch Government Quality Standards for the assessment soild and water contaminations [4]. The determination of PAHs in environment matrices is complicated due to their trace level presence and losses incurred during sample handling, extraction, etc. [5]. Thus, the development of appropriate sample preparation is crucial to remove major interferences and to concentrate the target analytes prior to instrumental analysis.

Conventional extraction methods that have been developed for the analysis of PAHs include solid phase extraction (SPE) [6], liquid-liquid extraction (LLE), liquid-phase microextraction (LPME) [1] and solid phase microextraction (SPME) [7]. However, large quantities of organic solvents are required in LLE and environmentally hazardous waste produced which has contradicted the green chemistry concept [8]. Minimum amount of organic solvent used in SPE compared with LLE, however, SPE requires pretreatment and further necessary toxic organic solvent for the sample elution step [1]. The latter two methods require are complicated procedures and high cost. To overcome the shortcomings, new sample preparation method termed dispersive micro-solid phase extraction (D-μ-SPE) has been introduced. It has received considerable attention since it is rapid and effective, requires minute amounts of chemical solvent, little waste effluent produced, and hence support the concept of green chemistry [9].

In this study, MCM-41-D-μ-SPE was carried out for the extraction of selected PAHs, namely fluorene and phenanthrene in real water samples. Hexagonally ordered mesoporous silica, MCM-41 is considered to be promising adsorbent owing to its characteristics of large surface area, uniform pore structure and huge pore volume with unique mechanical, chemical and thermal stability [10]. MCM-41 has been successfully applied as adsorbent for the extraction of pharmaceutical compounds from biological matrices [11-12]. The proposed MCM-41-D-μ-SPE method was optimized for PAHs analysis in terms of selection of desorption solvent, extraction time and volume of desorption solvent. The optimized method offers rapid analysis with simple extraction steps and it was applied to the analysis of PAHs in lake and tap water samples.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The selected PAHs used in this study, namely fluorene and phenanthrene (Figure 1) were of analytical grade purchased from Sigma-Aldrich (USA). Acetone as desorption solvent was purchased from Merck, Germany. Double-distilled water used for preparation of aqueous solution was at least 18 MΩ purified by Nano ultra-pure water system (Barnstead, USA). The standard stock

solutions of 200 mg/L of the analytes were prepared by dissolving in acetonitrile (phenanthrene) and methanol (fluorene), respectively and were stored in refrigerator prior to use. The working standard solutions of lower concentrations were prepared weekly by diluting standard stock solution with methanol.

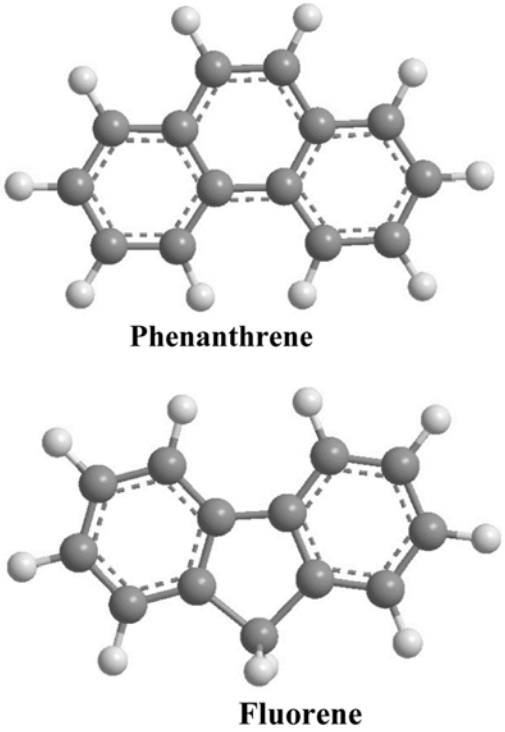

Figure 1. 3D-structures of selected polycyclic aromatic hydrocarbons.

2.2 GC-MS Operating Conditions

An Agilent Technology 6890N gas chromatography (GC) equipped with a mass spectrometer (MS) (Milan, Italy) was employed for the analysis of PAHs. High-purity helium gas was used as carrier gas with a flow rate of 1.0 mL/min and nitrogen was used as the make-up gas at constant velocity of 52.7 mL/min. The injection port was held at 250°C and used in the splitless mode. Separation was conducted on a HP5-MS capillary column, 25 m × 0.25 mm I.D. with 0.25 µm film thickness which also known as Ultra 2. The oven temperature programmed was as follows: initial 170°C (held 1 min) and then ramped at 20°C/min to 250°C, held for 0 min and finally ramped at 35°C/min^{-1} to 280°C, and held for 1 min. The MS temperature was maintained at 280°C. Chromatographic data was interpreted using Agilent Chemstation Software.

2.3 MCM-41-Dispersive Micro-Solid Phase Extraction

The MCM-41-D-µ-SPE procedure was shown in Figure 2. MCM-41 powder (5 mg) was dispersed into double-distilled water sample (10 mL) in a 15 mL centrifuge tube and the mixture was spiked with selected PAHs. The mixture was agitated vigorously for 2 min using vortex mixer. Subsequently, the adsorbent was isolated from the solution by centrifugation (3800 r/min, 5 min). After centrifugation, the mixture was separated into two phases; liquid phase (upper) and solid phase (bottom). The supernatant (liquid phase) was discarded and the adsorbent (solid phase) was dried under nitrogen (N_2) gas flow to remove water. Acetone (60 µL) was added to desorb the analytes from the adsorbent. After 5 min of incubation, the adsorbent was filtered by a Millipore Millex-GN Nylon 0.45 µm filter device (Agilent, Santa Clara, USA) from the desorption solvent into 1.5 mL vial. A portion (1.0 µL) of the solution was injected into GC-MS using a GC microsyringe for analysis.

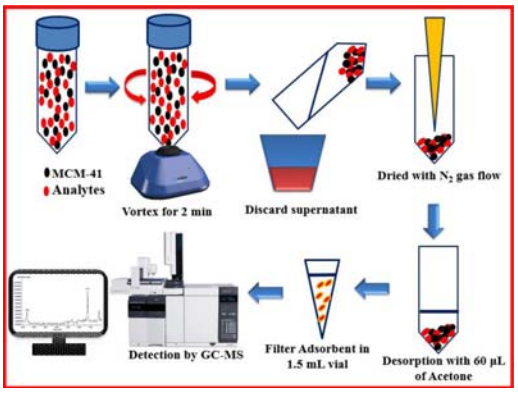

Figure 2. Schematic diagram of sample pre-concentration procedure.

2.4 Sample Preparation

The optimized MCM-41-D-µ-SPE method was applied to the analysis of water samples, namely tap water and lake water samples obtained from the Universiti Teknologi Malaysia, Johor Bahru campus. The water samples were filtered through 0.45 µm Whatman nylon filter membrane to remove any particles and the samples were stored in refrigerator (4°C) prior to use.

3. RESULTS AND DISCUSSION

3.1 Optimization of Extraction Conditions

In order to optimize the extraction conditions of the D-µ-SPE of PAHs from water samples, several important analytical factors that can potentially affect the extraction efficiency were investigated. The parameters involved were desorption solvent, extraction time and volume of desorption solvent.

3.2 Selection of Desorption Solvent

In this study, three different desorption solvents were evaluated i.e. methanol, acetonitrile and acetone. Desorption solvent was used to desorb the analyte from the adsorbent. The result shows that acetone exhibited the highest desorption efficiency compared to methanol and acetonitrile due to highest peak area obtained (Figure 3). This observation explained the porous material used, MCM-41 is reversed phase sorbent and more readily to retain non-polar PAHs [9]. Since acetone has a less polar characteristic than methanol and acetonitrile, it is useful for desorption of relatively non-polar analytes such as fluorine and phenanthrene [13]. Besides, being a highly polar solvent, methanol showed less desorption efficiency of analytes. Therefore, acetone was selected as the desorption solvent in subsequent experiments.

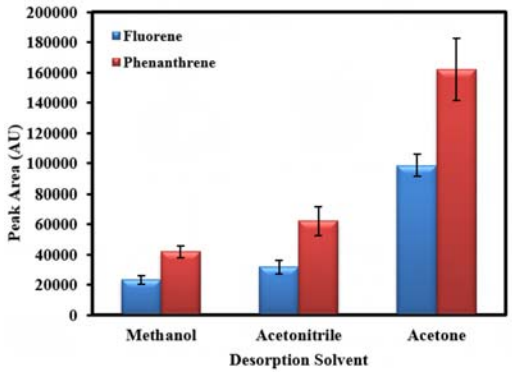

Figure 3. Effect of desorption solvent on MCM-41-D-µ-SPE. Conditions: concentration of PAHs: 0.20 mg/L, sample volume: 10 mL, stirring rate: 3800 rpm, extraction time: 30 s, volume of desorption solvent: 100 µL.

3.3 Effect of Extraction Time

In order to determine the effect of the extraction time on the extraction efficiency, MCM-41-D-µ-SPE procedures were carried out by varying the vortex time from 1 to 4 min. The result shows that the peak area decreased with increasing extraction time (Figure 4). Extraction time of 1 min was not chosen as its efficiency of extraction was not as high as in 2 min due to insufficient extraction time for selected PAHs to be adsorbed on adsorbent.

Extraction time of 2 min gave the highest peak area and provided highest sensitivity. Thus, extraction time of 2 min was chosen as optimum condition and employed in all subsequent experiments.

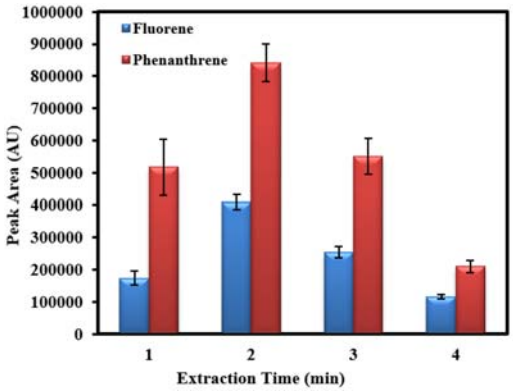

Figure 4. Effect of extraction time on MCM-41-D-μ-SPE. Conditions: concentration of PAHs: 0.20 mg/L, sample volume: 10 mL, stirring rate: 3800 rpm, volume of desorption solvent: 100 μL.

3.4 Effect of Desorption Solvent Volume

In order to evaluate the effect of the desorption solvent on enrichment factor, the volume of acetone was varied in the range of 40 to 100 μL. The result shows an increasing trend in the peak area with decreasing volume of acetone, from 100 to 40 μL (Figure 5). In a preliminary experiment, 40 μL of acetone was used to desorb the analyte but the result was inconsistent as most of the acetone readily evaporated. The maximum enrichment was obtained using 60 mL of acetone that is when "the desorption solvent"-to-"adsorbent amount" ratio was 12:1 (volume/amount) [9]. Minute amount of organic solvent required has supported the concept of green chemistry. Thus, the desorption volume of 60 μL was used and employed in all subsequent experiments, as it is the lowest volume used to fully submerge the MCM-41 adsorbent during desorption [14].

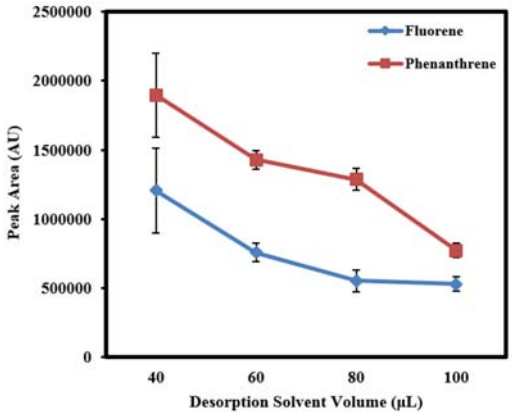

Figure 5. Effect of volume of desorption solvent on MCM-41-D-μ-SPE. Conditions: concentration of PAHs: 0.20 mg/L, sample volume: 10 mL, stirring rate: 3800 rpm, extraction time: 2 min.

3.5 Method Validation

The proposed MCM-41-D-μ-SPE method was validated by carrying out the extraction of selected PAHs under optimized conditions: 60 μL of acetone as desorption solvent as well as 2 min duration of extraction time. The method shows excellent linearity with coefficients of determination (r^2) of 0.9980 and 0.9973, and good limits of detection (LODs) of 0.16 μg/L (fluorene) and 0.07 μg/L (phenanthrene) as well as good limits of quantification (LOQs) of 0.24 μg/L (fluorene) and 0.52 μg/L (phenanthrene) (Table 1).

Table 1. Correlation coefficients, LOD and LOQ of selected PAHs using MCM-41-D-μ-SPE coupled with GC-MS.

PHA	Coefficient of determination, r^2	LOD (μg/L)	LOQ (μg/L)
Fluorene	0.9980	0.16	0.52
Phenanthrene	0.9973	0.07	0.24

The analytical characteristics of the proposed MCM-41-D-μ-SPE method were compared with other reported methods (Table 2). The Soxhlet extraction, SBSE and LPME methods resulted in excellent sensitivity for PAHs, however, it took a relatively long extraction time for each analysis (>25 min). The LOD and recovery of MCM-41 was comparable to those other reported methods. The use of 5 mg of the adsorbent and the organic solvent (60 μL) have added incentives in MCM-41-D-μ-SPE as an alternative microextraction technique for PAHs.

Table 2. Comparison of the MCM-41-D-μ-SPE with published methods for extraction of PAHs in water samples.

Analysis methods	Dynamic linear range, μg/L	LOD, μg/L	Enrichment factor	References
Soxhlet extraction-GC-MS	0-500	Nanogram per liter level	-	[15]
SBSE-HPLC-FD	0.002-0.05	0.0002-0.0015	-	[16]
Ultrasound assisted emulsification microextraction-GC-MS	-	0.001-0.036	-	[17]
AF-LPME-GC-MS	0.1-200	0.01-0.04	57-106	[1]
MCM-41-D-μ-SPE	0.3-200	0.07-0.16	7.5-29	This work

3.6 Real Sample Analysis

In order to investigate the applicability of the proposed microextraction method, MCM-41-D-μ-SPE was applied to the determination of selected PAHs in selected water samples, tap and lake water. The non-spiked water samples were analyzed in triplicate and the representative chromatograms are shown in Figure 6 and Figure 7. In initial experiments, neither fluorene nor phenanthrene was detected in the real water samples.

In order to assess the effectiveness of the extraction method, the recoveries of the PAHs in the real samples were studied by spiking 100 μg/L of each PAHs in water samples. The relative recoveries were calculated as the percentage of mean of target analytes found after extraction (derived from the plotted standard addition calibration curve) against the concentration spiked in the sample (n = 3). Table 3 displays the relative recoveries of the spiked samples individually. In general, the relative recoveries were in the range of 79.5-107.6% with RSDs of <10%. In spiked technique, high recoveries in analysis of samples usually correspond to high accuracy.

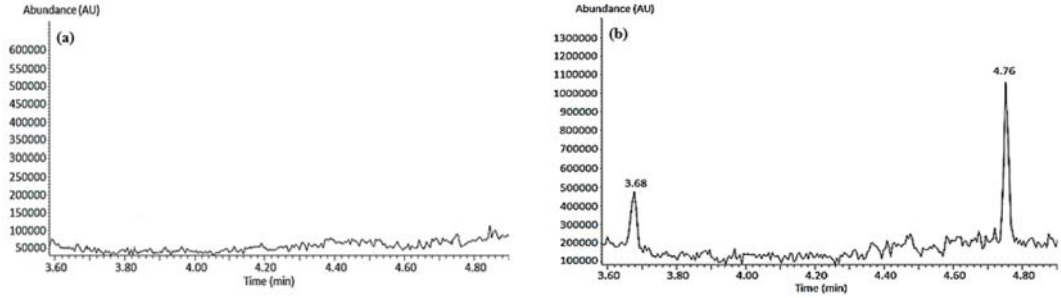

Figure 6. GC-MS chromatogram of PAHs obtained by MCM-41-D-μ-SPE under optimized conditions (a) non-spiked lake water (b) spiked lake water at 100 μg/L.

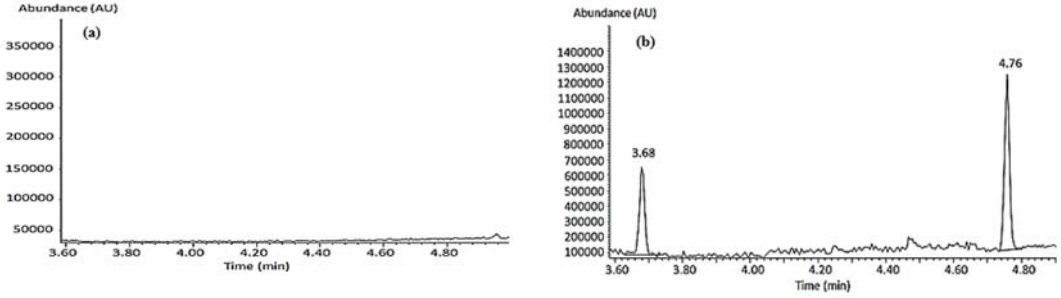

Figure 7. GC-MS chromatogram of PAHs obtained by MCM-41-D-μ-SPE under optimized conditions (a) non-spiked tap water (b) spiked tap water at 100 μg/L.

Table 3. Percent recovery and percent relative standard deviation of the samples by MCM-41-D-μ-SPE.

Water	PAHs	% Recovery	% RSD (n=3)
Tap water	Fluorene	90.4	4.0
	Phenanthrene	107.6	1.7
Lake water	Fluorene	79.5	9.4
	Phenanthrene	88.0	7.5

4. CONCLUSIONS

A simple and rapid method based on MCM-41-D-μ-SPE coupled with GC-MS was developed for the determination of PAHs in real water samples. Parameters included selection of desorption solvent, extraction time and desorption solvent volume were examined throughout this study. The MCM-41-D-μ-SPE method shows acceptable sensitivity with satisfactory recoveries of PAHs in real water samples. The LODs were 0.07 and 0.16 μg/L while the LOQs were 0.24 to 0.52 μg/L for fluorene and phenanthrene, respectively. The recovery was in the range 79.5-107.6%. The relative standard deviations were in the range 1.7-9.4%. This method has the merits of minute amounts of organic solvent requirement and thus supports the green chemistry concept.

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