

## Development of Solid Phase Extraction Method for Polycyclic Aromatic Hydrocarbons (PAHs) Determination Using MCM-41-Imi/Br in Water Samples

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**Abstract:** A solid phase extraction (SPE) method for determination of selected polycyclic aromatic hydrocarbons (PAHs) in water samples was developed using MCM-41-Imi/Br. The four model analytes of PAHs were analysed with gas chromatography-mass spectrometry (GC-MS). Effective SPE parameters such as sample loading volume, type of elution and elution solvent volume were studied. The proposed method exhibits good linearity from 10 to 1000  $\mu\text{g mL}^{-1}$ , LODs (0.2-0.4  $\mu\text{g mL}^{-1}$ ) and repeatability (4.4-5.8%). The accuracy of the method as tested in two real water samples at two fortification levels (1 and 50  $\mu\text{g mL}^{-1}$ ) were (85 to 97%) with RSDs (< 6%, n = 3) for all of the selected PAHs in real samples.

**Key words:** Sample preparation • Environmental pollutant • Gas-chromatography-mass spectrometry

### INTRODUCTION

Polyaromatic hydrocarbons (PAHs) are organic compound that mainly consist of two or more fused aromatic rings of carbon and hydrogen atoms. They are generally originated from wide variety of natural and anthropogenic sources [1]. PAHs are formed by pyrolysis of organic matter or incomplete combustion [2]. Due to mutagenic and carcinogenic characteristics of PAHs, they have been listed as priority pollutants by U.S. Environmental Protection Agency (EPA) and the European Union (EU) [3, 4]. PAHs are usually present in environmental water samples at trace levels (nanogram per liter levels and lower), due to their low water solubility and high hydrophobicity [5]. Therefore, monitoring the trace level of PAHs in environmental samples is crucial to ensure that the environment free from PAHs contamination.

Solid phase extraction has been explored extensively for analysis of PAHs in water samples [6-8]. The common SPE sorbent used for the determination of PAHs were octadecylsilane ( $\text{C}_{18}$ ), carbon nanotubes (CNTs) and molecular imprinted polymer (MIPs).

MCM-41 is an example of silica material that has been developed considerable attention as potential sorbent for sample preparation techniques. This is due to their special characteristics such as high specific surface, narrow pore-

size distribution, good thermal stability and moderate hydrophobic character [9]. Despite of the structural advantages of MCM-41, the major drawback as reported in literatures, they are lack of active sites which limits application as adsorbent. Functionalization MCM-41 can overcome this drawback since the amorphous pore walls and rich surface hydroxyl groups of MCM-41 could facilitate the modification of their skeleton and surface [10, 11].

Functionalized MCM-41 was observed to obtain higher stability and better selectivity for the extraction of PAHs in environmental samples [12-14]. Therefore, in this study imidazole (Imi) and 1,2-dibromoethane was immobilized on MCM-41 using a 3-step process to give MCM-41-Imi/Br. MCM-41-Imi/Br was successfully applied as catalyst for the synthesis of styrene carbonate [15]. MCM-41-Imi/Br was further extended its application in this work as sorbent for solid phase extraction for PAHs analysis in water samples. Nevertheless, up to date, no report has been published on the use of MCM-41-Imi/Br as sorbent for the extraction of PAHs in water samples.

### MATERIAL AND METHODS

**Standards and Reagents:** PAHs standards with >99% purity fluorene (FLU), phenanthrene (PHE), fluoranthene (FLA) and pyrene (PYR) were obtained from Sigma

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Aldrich (St. Louis, MO, USA). HPLC grade acetonitrile (MeCN) and methanol (MeOH), dichloromethane, acetone, ethyl acetate, ethanol and hexane were from QReC (99.0%). Stock solutions ( $1000 \mu\text{g mL}^{-1}$ ) and working standard solutions were prepared by diluting the stock solution with methanol (FLU and FLA) and acetonitrile (PHE and PYR), stored at  $4^\circ\text{C}$  when were not in use. Distilled water was distilled using water stills (Favorit W4L, Malaysia).

Water samples (tap water and bottled mineral water) were selected as model real samples. Bottled mineral water was purchased from local market. Tap water was collected from the laboratory. For the recovery analysis, the tap water and bottled mineral water samples were spiked with mixtures of PAH standards at known concentrations and sample pH (pH=7) was adjusted and carefully homogenized. The spiked samples were allowed to stand overnight before the experiment.

**Instrumentation:** A Elite-5 MS fused capillary GC column ( $30 \text{ m} \times 0.32 \text{ mm (i.d.)} \times 0.25 \mu\text{m}$  film thickness) was used with an Clarus 600 GC system which comprised of an Clarus 600 MS were used for separation and quantification of PAHs. Helium (99.9%) was used as carrier gas at a flow rate of  $1.0 \text{ mL min}^{-1}$ . The injection port temperature was set at  $250^\circ\text{C}$  while the detector temperature was set at  $300^\circ\text{C}$ . The GC temperature profile was initially started at  $80^\circ\text{C}$  (held 1 min), ramped at  $15^\circ\text{C min}^{-1}$  and finally increased up to  $280^\circ\text{C}$  (held 5 min). Manual injection ( $1 \mu\text{L}$  sample) was carried out under splitless mode with a solvent delay of 3.00 min. For the MS conditions, SCAN mode was used with source temperature of  $230^\circ\text{C}$ , quad temperature of  $150^\circ\text{C}$ , transfer line temperature at  $280^\circ\text{C}$  and multiplier voltage auto tune voltage at 17 kV. Quantitative calculations of PAHs were made based on peak area of PAHs obtained from GC-MS.

**Preparation of MCM-41-Imi/Br:** MCM-41-Imi/Br was prepared as per reported in the publication [15].

**SPE Procedure:** SPE manifold (Supelco, Bellefonte, PA, USA) was used to carry out the extractions. 100 mg of MCM-Imi/Br was manually packed into an empty 3-mL polypropylene SPE tube (Supelco, Shanghai, China) with PTFE frits (Supelco, Shanghai, China) placed above and below the sorbent. The sorbent was preconditioned with  $1 \times 5 \text{ mL}$  of MeOH followed by  $1 \times 10 \text{ mL}$  distilled water. Then,  $15 \text{ mL}$  of spiked water sample with mixture of PAHs spiked at desired concentration was passed through. Retained analytes in the cartridge were eluted with  $5 \text{ mL}$

acetonitrile. Eluted fraction was dried under a stream of gentle nitrogen gas till complete dryness before it was reconstituted in  $100 \mu\text{L}$  acetonitrile.  $1 \mu\text{L}$  sample was subjected into GC-MS analysis.

## RESULTS AND DISCUSSIONS

**SPE Optimization:** Three effective SPE parameter namely sample loading volume, eluting solvent type and eluting solvent volume were studied in order to obtain the optimum SPE design. The sorbent (MCM-41-Imi/Br) was kept constant at 100 mg for entire of the study. Sample loading volume is important in determining the loading capacity of SPE sorbent and to determine the overall time required by the sorbent to reach equilibrium with analyte. Sample volumes (5-20 mL) were examined. Figure 1 shows that increasing sample volume increases the peak area of PAHs extracted. However, beyond 15 mL the peak area of PAHs extracted started to decrease significantly. This is probably due to the breakthrough of sorbent being exceeded and the active sites in MCM-Imi/Br were occupied by PAHs and decrease the adsorption of PAHs onto their surface [16]. Therefore, 15 mL was selected as the optimum sample volume.

In SPE, eluting solvent should be strong enough to elute all of the selected analyte from the sorbent to obtain optimum enrichment. Seven eluting solvents of different polarities namely acetonitrile, ethyl acetate, methanol, ethanol, acetone, hexane and dichloromethane were investigated to determine the best elution solvent. Figure 2 indicated that acetonitrile (MeCN) being the most polar solvent was the most effective eluting solvent as it gave the highest peak for all the selected PAHs.

Eluting solvent volumes from 1 to 10 mL MeCN were studied to obtain the minimum but sufficient volume needed to elute the retained PAHs in sorbent. Figure 3 shows that, on increasing solvent volume from 1 mL to 5 mL, the peak area of PAHs increased. The highest peak areas for the PAHs were obtained at 5 mL of MeCN. Therefore, 5 mL MeCN was selected as the optimum eluting solvent volume. Therefore, the optimized parameters obtained for the extraction of PAHs using MCM/Imi-Br sorbent were 15 mL sample loading volume and 5 mL MeCN as the eluting solvent and were used for entire of this study.

**Method Validation:** To validate the applicability of the proposed SPE method using the MCM-41-Imi/Br sorbent, linearity, limit of detections (LODs) at a signal to noise ratio of 3 ( $\text{S/N}=3$ ) and limit of quantifications (LOQs) at a

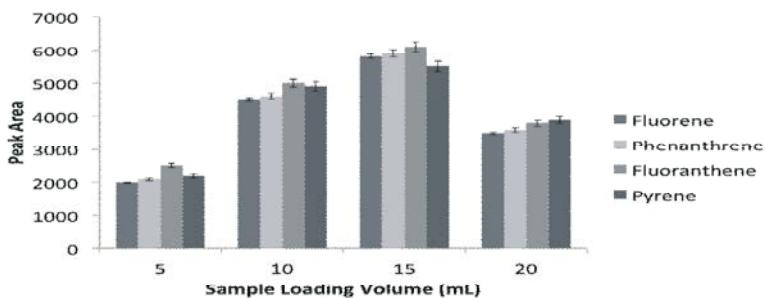


Fig. 1: Effect of sample loading volume on the extraction efficiency of PAHs using SPE- MCM-41-Imi/Br

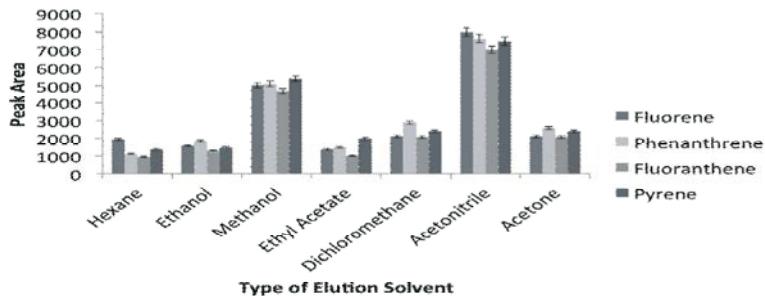


Fig. 2: Effect type of eluting solvent on the extraction efficiency of PAHs using SPE- MCM-41-Imi/Br

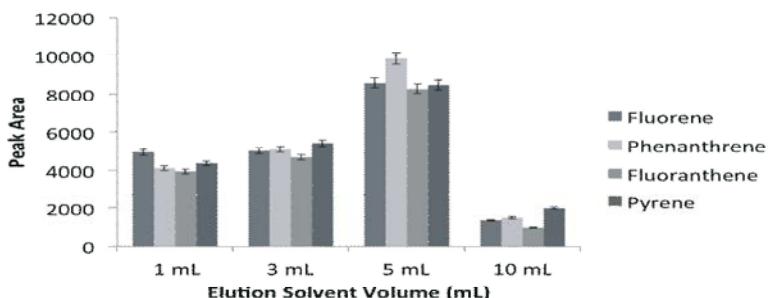


Fig. 3: Effect of eluting solvent volume on the extraction efficiency of PAHs using SPE-MCM-41-Imi/Br

signal to noise ratio of 10 ( $S/N = 10$ ) were assessed using the optimum SPE conditions (100 mg MCM-41-Imi/Br as SPE clean up sorbent, 15 mL sample loading volume and 5 mL acetonitrile eluting solvent). Blank sample analysis was also performed for comparison purposes. The validation of the SPE method was carried out by spiking the four selected PAHs in water sample at five different concentrations from 10 to 1000  $\mu\text{g L}^{-1}$  to produce a five point calibration. Three replicates were made for each of the concentration to determine the relative standard deviations (RSDs). The linearity was determined by plotting a calibration graph of analyte concentration versus peak area.

The SPE method was found to be linear over the range of 10 to 1000  $\mu\text{g L}^{-1}$  with coefficient of determination,  $r^2 > 0.9986$ . The LODs obtained were 0.4, 0.3, 0.2, 0.4  $\mu\text{g L}^{-1}$ , respectively for fluorene (FLU), phenanthrene (PHE), fluoranthene (FLA) and pyrene

(PYR) while the limit of quantifications (LOQ) obtained was in range of 0.9 to 1.8  $\mu\text{g L}^{-1}$ . LODs achieved in the present work were slightly lower compared to the current maximum residue levels (MRL) as established by World Health Organization (WHO) International Standards for drinking water which was imposed to be lesser than 0.0002  $\text{mg L}^{-1}$  [4].

Repeatability of the proposed SPE-MCM-Imi/Br method was evaluated by performing three repeated extractions in the same day while the reproducibility of the method was tested by repeating three extractions per day for five consecutive days (making a total of 15 extractions). Both inter- and intra-day extractions were carried out with mixture of PAHs each spiked at 50  $\mu\text{g L}^{-1}$ . The RSDs for intra-day ( $n = 3$ ) and inter-day ( $n = 15$ ) extraction were 4.4-4.9% and 6.5-7.7%, respectively. Table 1 summarises the analytical performance of SPE-MCM-Imi/Br in determination of PAHs.

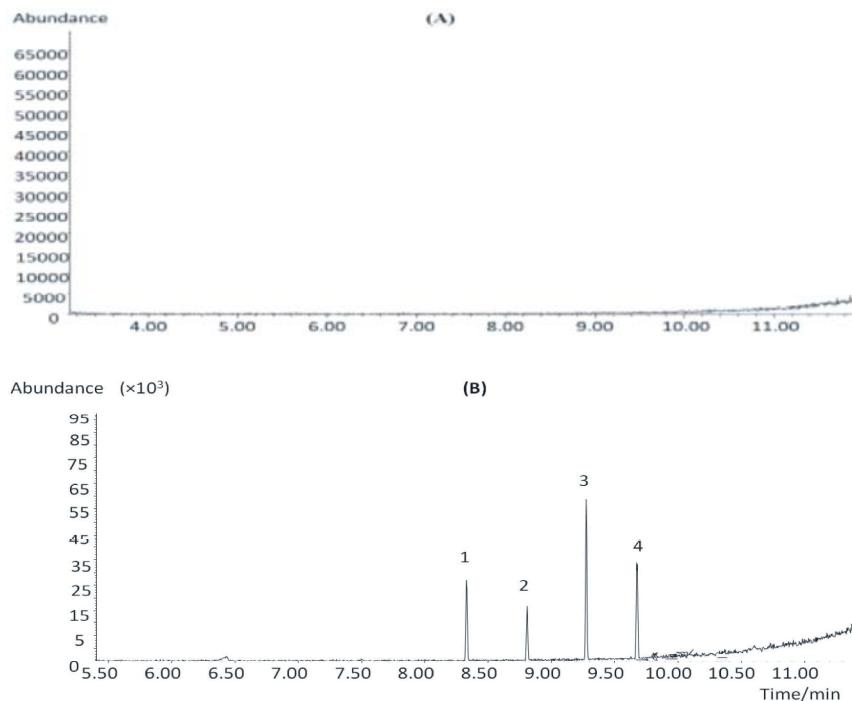


Fig. 4: Chromatogram obtained by SPE-MCM-Imi/Br with GC-MS analysis of PAHs from (a) unspiked and (b) spiked ( $50 \mu\text{g L}^{-1}$ each PAHs) tap water samples in scan mode with solvent delay of 3 min GC-MS. Peaks: (1) Fluorene, (2) Phenanthrene and (3) Fluoranthene, (4) Pyrene

Table 1: Analytical performances of the SPE-MCM-41-Imi/Br method for the determination of PAHs

SPE-MCM-41-Imi/Br					
PAHs	Correlation of determination ( $r^2$ )	*RSD (n=3) (%)	*RSD (n=15) (%)	*LOD ( $\mu\text{g L}^{-1}$ )	<sup>b</sup> LOQ ( $\mu\text{g L}^{-1}$ )
FLU	0.9987	4.4	6.5	0.4	1.8
PHE	0.9988	5.1	7.2	0.3	1.2
FLA	0.9985	5.5	7.7	0.2	0.9
PYR	0.9982	4.9	6.8	0.4	1.8

Linearity range: 10-1000  $\mu\text{g L}^{-1}$

<sup>a</sup>LOD: S/N=3

<sup>b</sup>LOQ: S/N=10

\*Spiked Level- 50  $\mu\text{g L}^{-1}$  (individual PAHs)

Table 2: Analysis of spiked PAHs in tap water and bottled mineral sample using SPE- MCM-Imi/Br

Analyte	Recovery ( $\pm$ RSD%, n=3)			
	Tap Water		Bottled Mineral Water	
	1 $\mu\text{g L}^{-1}$	50 $\mu\text{g L}^{-1}$	1 $\mu\text{g L}^{-1}$	50 $\mu\text{g L}^{-1}$
FLU	89 (3.2)	95 (4.8)	85 (3.8)	97 (4.2)
PHE	88 (3.9)	97 (4.9)	88 (3.7)	98 (4.9)
FLA	87 (3.6)	97 (5.3)	89 (3.9)	95 (5.3)
PYR	85 (3.1)	93 (5.6)	90 (3.9)	97 (5.6)

**Real Sample Analysis:** The reliability of the proposed SPE-MCM-Imi/Br-GC-MS method in the determination of the selected PAHs was evaluated by employing the method to two real water samples namely tap water and bottled mineral water. Blank extractions of water

samples were carried out for comparison purpose. Pre-analysis on the real samples showed no detection of the PAHs selected. Recovery analysis was carried out by spiking the selected PAHs at two fortification levels ( $1 \mu\text{g L}^{-1}$  and  $50 \mu\text{g L}^{-1}$ ) to assess matrix effect using the developed SPE-MCM-Imi/Br method at different concentrations. The recovery was evaluated by relating the recovered amount of PAHs versus the added amount in the spiked samples. The recovery obtained at spiking levels of  $1 \mu\text{g L}^{-1}$  and  $50 \mu\text{g L}^{-1}$  ranged from 85 to 97% with RSDs <6% (Table 2). Figure 4 shows the typical chromatograms of extracted PAHs from (A) unspiked and (B) spiked ( $50 \mu\text{g L}^{-1}$ ) tap water sample using the developed SPE-MCM-Imi/Br method prior to GC-MS analysis.

## CONCLUSION

MCM-Imi/Br has been successfully used as sorbent for SPE. The LODs achieved ( $0.2\text{--}0.4 \mu\text{g L}^{-1}$ ) were slightly lower compared to the current MRLs established by World Health Organization (WHO) International Standards for water samples making the developed method applicable for determination of PAHs in real water samples: even at low ppb levels.

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