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MEASUREMENT UNCERTAINTY IN THE ANALYSIS OF SELECTED POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL WATER BY MAGNETIC MOLECULARLY IMPRINTED POLYMER-BASED MICRO-SOLID PHASE EXTRACTION- LIQUID CHROMATOGRAPHY

(Ketidakpastian Pengukuran dalam Analisis Hidrokarbon Aromatik Polisiklik Terpilih di dalam Air Persekitaran dengan Pengekstrakan Fasa Pepejal-Mikro berasaskan Polimer Bercetakan Molekul Bermagnet-Kromatografi Cecair)

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Abstract

Sample preparation is important to produce a clean extract to enhance detection sensitivity and minimize instrument maintenance. A micro-solid phase extraction (µ-SPE) technique using magnetic molecularly imprinted polymers (magnetic-MIPs) as the adsorbents combined with micro-high performance liquid chromatography and ultraviolet detection (µ-HPLC-UV) has been developed for the determination of selected polycyclic aromatic hydrocarbons (PAHs), namely benzo[a]pyrene, phenanthrene, fluoranthene and pyrene in environmental water samples. The magnetic-MIPs were characterized using scanning electron microscopy, Brunauer–Emmett–Teller, thermogravimetric analysis and vibrating sample magnetometer to finalize the physical properties of the magnetic-MIPs. The µ-SPE was then optimized using one variable at a time approach to enhance the extraction efficiency. Under the optimal extraction conditions, the feasibility of using magnetic-MIPs as the adsorbents for the extraction of the selected PAHs was proven with a wide linearity range (5-250 µg L^{-1}), good repeatability (relative standard deviation <10%), ultra-trace detection limits (0.01-0.02 µg L^{-1}) and good relative recovery (80.2-119.3%) for the application in the environmental water. The doubt of using the µ-SPE was investigated by calculating the measurement uncertainty. The estimated combined standard uncertainties for the determination of the selected PAHs were in the range of 0.0678-0.0890. It was concluded that the uncertainty of the µ-SPE-µ-HPLC-UV is mainly attributed to its accuracy. Nevertheless, the results showed that the measurement uncertainty of the proposed magnetic-MIPs-based µ-SPE-µ-HPLC-UV was at an acceptable level. The method is beneficial to the routine analysis, especially in providing simple and sensitive determination of the selected PAHs in environmental water. The µ-SPE technique consumes minimal amounts of solvent and traces of adsorbents, which then greatly minimizes the waste and analysis costs in routine analysis.

Keywords: chromatography, measurement uncertainty, molecularly imprinted polymer, PAHs, water

Abstrak

Penyediaan sampel adalah penting untuk menghasilkan ekstrak bersih untuk meningkatkan sensitiviti pengesanan dan meminimumkan penyelenggaraan instrumen. Teknik pengekstrakan fasa pepejal-mikro (μ-SPE) menggunakan polimer bercetakan molekul bermagnet (MIPs bermagnet) sebagai penjerap yang digabungkan dengan kromatografi cecair berprestasi tinggi-mikro dan pengesanan ultraungu (μ-HPLC-UV) telah dibangunkan untuk penentuan hidrokarbon aromatik polisiklik (PAHs) terpilih, iaitu benzo[a]pirena, fenanthrena, fluoranthena dan pirena di dalam sampel air persekitaran. MIPs bermagnet telah dicirikan menggunakan mikroskop elektron pengimbasan, *Brunauer-Emmett-Teller*, analisis termogravimetrik dan magnetometer sampel bergetar untuk memuktamadkan sifat fizikal MIPs bermagnet. μ-SPE kemudiannya dioptimumkan menggunakan pendekatan satu pembolehubah pada satu masa untuk meningkatkan kerberkesanan pengekstrakan. Di bawah keadaan pengekstrakan yang optimum, kebolehlaksanaan menggunakan MIPs bermagnet sebagai penjerap untuk pengekstrakan PAHs terpilih telah dibuktikan dengan julat kelinearan yang luas (5-250 μg L^{-1}), kebolehulangan yang baik (sisihan piawai relatif <10%), had pengesanan ultra-surihan (0.01-0.02 μg L^{-1}) dan pemulihan relatif yang baik (80.2-119.3%), terutamanya untuk aplikasi di dalam air persekitaran. Keraguan menggunakan μ-SPE ini telah disiasat dengan mengira ketidakpastian pengukuran. Anggaran gabungan ketidakpastian piawai bagi penentuan PAHs terpilih adalah dalam julat 0.0678-0.0890. Disimpulkan bahawa ketidakpastian μ-SPE-μ-HPLC-UV disebabkan terutamanya oleh ketepatannya. Namun begitu, keputusan ini menunjukkan bahawa ketidakpastian pengukuran μ-SPE-μ-HPLC-UV berasaskan MIP bermagnet yang dicadangkan berada pada tahap yang boleh diterima. Kaedah ini bermanfaat kepada analisis rutin terutamanya dalam menyediakan penentuan mudah dan sensitif PAHs terpilih di dalam air persekitaran. Teknik μ-SPE hanya menggunakan sedikit pelarut dan penjerap yang dapat meminimumkan sisa dan kos analisis dalam analisis rutin.

Kata kunci: kromatografi, ketidakpastian pengukuran, polimer bercetakan molekul, PAHs, air

Introduction

The emergence of increased industrial, rapid urbanization and agricultural development has led to environmental pollution and poor sanitation water [1]. Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that originate from both natural and anthropogenic sources. Dry and wet deposition, road runoff, industrial effluent, leaching from creosote-impregnated wood, petroleum spills and fossil fuel combustion are the main route sources producing PAHs [2]. PAHs are semi-volatile and thus they can travel a long distance in air and water [3]. They are absorbed strongly by waterborne organic and inorganic particles. They may eventually be brought down to the bottom of sediment as a sink in the aquatic system. Even under favorable conditions, the sorbed PAHs will be released into the water as an extended source to threaten the aquatic ecosystem through bioaccumulation in food chains [4]. The ubiquitous presence of PAHs in different environmental compartments greatly opens multiple ways for human exposure to PAHs. The United States Environmental Protection Agency (USEPA) has listed sixteen PAHs as the priority pollutants due to their massive occurrence of toxicological profile and suspected carcinogenicity [5].

To date, liquid-liquid extraction (LLE) and solid phase extraction (SPE) are still the most applied worldwide recognized and accredited techniques for the extraction of environmental pollutants in environmental water. These techniques do not fall in the category of green analysis, and they are tedious and consume large amounts of organic solvents. This indirectly causes environmental pollution, is hazardous to human health and demands extra costs for waste treatment. Therefore, microextraction techniques that are friendlier towards the environment are preferred nowadays, where there is more and more research focusing on greener alternatives.

Magnetic micro-solid phase extraction (μ-SPE) is one of the widely employed microextractions because of its ease of application and low consumption of organic solvents. It is based on the use of magnetic nanoparticles that are composed of a magnetic core, covered with an adsorbent phase to bind analytes of interest. Molecularly imprinted polymers (MIPs) are used as adsorbents due to their specific molecular recognition cavities. MIPs are one of the most promising adsorbents when dealing with complex biological and environmental matrices [6-8]. Integrating both features, namely green μ-SPE and selective MIPs into one technique greatly enhances the extraction performance and simplifies the routine analysis.

Measurement data is produced and used as the basis for product quality determination, environmental pollution and food safety monitoring. All measurements have some degree of uncertainty that may come from all sorts of sources during the determination process. Measurement uncertainty provides an interval within which the true value is likely to fall, thus it is an indication of the reliability of a measurement. Therefore, it is important to report the measurement uncertainty to demonstrate the quality of test results and ensure its validity for any purpose.

In this study, commercial PAHs MIPs were coated on magnetic nanoparticles, particularly iron oxide (Fe_3O_4) to produce magnetic-MIPs. Then, the magnetic-MIPs were characterized physically using a dissecting microscope namely Zeiss Stemi 2000-c Trinocular microscope, scanning electron microscope and Brunauer-Emmett-Teller to analyze the surface morphology and determine the pore volume. The thermal stability of magnetic-MIPs was measured using thermogravimetric analysis and the magnetization properties were analyzed using a vibrating sample magnetometer. The magnetic-MIPs were then employed as the adsorbents in the μ-SPE for the extraction of selected PAHs, namely pyrene, phenanthrene, fluoranthene and benzo[a]pyrene in environmental water. Several extraction parameters such as amounts of magnetic-MIPs, extraction time, types of desorption solvent, volume of desorption solvent and desorption time were thoroughly investigated and optimized to enhance the extraction efficiency of μ-SPE. Next, the μ-SPE technique was validated and applied for the determination of PAHs in environmental water. The sensitivity and selectivity of the recognition site of MIPs were evaluated using different sample matrices. Lastly, the measurement uncertainty of the developed technique was determined to estimate the propagation of errors, mainly from the precision and accuracy involved in the method.

Materials and Methods

Chemical and reagents

The HPLC grade solvents, specifically, acetonitrile (ACN), ethanol (EtOH), methanol (MeOH), isopropanol (IPA) and tetrahydrofuran (THF) were obtained from Merck (Darmstadt, Germany). Iron(III) chloride hexahydrate ($FeCl_3.6H_2O$), iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$), glacial acetic acid and ammonia solution (NH_4OH) were purchased from Sigma-Aldrich (St. Louis, Missouri, United States). The selected polycyclic aromatic hydrocarbons (PAHs) reference standards, namely benzo[a]pyrene (BaP), fluoranthene (FLA), phenanthrene (PHE) and pyrene (PYR) were also supplied by Sigma-Aldrich (St. Louis, Missouri, United States). Commercially available molecularly imprinted polymer (MIP) for PAHs, named RENSA PAH MIP 4003 was obtained from Biotage (Uppsala, Sweden). Lastly, deionized water was obtained from Nano Ultra-pure water system purchased from Thermo Fisher Scientific (New Hampshire, United States).

Preparation of standard and sample solutions

The standard stock solutions (500 mg L^{-1}) of each PAH were prepared by weighing individually 0.005 g of each PAH in a 10 mL volumetric flask and diluting to volume with either ACN (PHE and BaP) or MeOH (FLA and PYR). The mixture working standard solution (50 mg L^{-1}) was prepared weekly by diluting the standard stock solutions with methanol. All standard solutions were stored in darkness at 4 °C when not in use.

Four types of environmental water samples were collected from areas surrounding Universiti Malaysia Terengganu, Kuala Nerus, Terengganu. These included river water, tap water, sea water and rainwater. These samples were collected in glass bottles pre-cleaned with decon and kept in a freezer before analysis. The samples were filtered through Whatman filter paper No. 1 obtained from Sigma-Aldrich (St. Louis, MO, United States) to remove the larger particles before extraction. The environmental water samples were applied in the extraction to imitate real matrices in the relative recovery study.

Fabrication of magnetic molecularly imprinted polymers

The fabrication of magnetic-MIPs was adopted from past research with minor modifications on the chemical [9]. $FeCl_3.6H_2O$ (approximately 3.62 g) and $FeSO_4.7H_2O$ (approximately 0.77 g) were dissolved with 50 mL of deionized water in a 250 mL Erlenmeyer flask. The flask was incubated in a beaker containing water at 40 °C and the mixture was vigorously stirred with a hot plate and magnetic stirrer (Corning, United States) for 30 min. Next, the MIPs (0.35 g) were added to 100 mL of 1% (v/v) acetic acid. The resulting solution was then added into the same Erlenmeyer flask and vigorously stirred for 2 h. Next, the ammonia solution (48 mL) was added dropwise over 2 h into the same flask with vigorous stirring. The temperature was maintained at 40 °C until the end of preparation. After the reaction, the product was filtered and washed with deionized water until the filtrate achieved pH 7. The product was then washed with ethanol 3 times. Lastly, the product was dried in an oven at 60 °C for 24 h. The products obtained were iron oxide (Fe_3O_4)-MIPs or magnetic-MIPs.

Characterization of magnetic-MIPs

Physical characterization was performed to observe the morphology of the MIPs deposited on the Fe_3O_4 that functioned as the adsorbent in this study. The morphology of both MIPs and magnetic-MIPs was observed through a disserting microscope namely Zeiss Stemi 2000-c Trinocular Stereo zoom microscope (Germany). Both MIPs and magnetic-MIPs were also scanned with a focused beam of electrons using a scanning electron microscope (SEM) (JEOL 6360 LA, Japan) to obtain information on their surface morphology. The thermal stability of magnetic-MIPs was also investigated by thermogravimetric analysis (TGA) (Mettler-Toledo GmbH TGA/DSC 1, Switzerland). The pore volume and surface area of the magnetic-MIPs were analyzed using Brunauer-Emmett-Teller (BET) (ASAP 2020 Micromeritics, United States). Magnetization properties of magnetic-MIPs were also recorded using a vibrating sample magnetometer (VSM) (Lakeshore 7404, United States).

Magnetic MIPs-based micro-solid phase extraction procedure

The sample solution (10 mL) was pipetted into a 10 mL glass centrifuge tube and magnetic-MIPs (10 mg) were added to the sample solution. The tube was capped and subjected to vortex at 460 rpm for 10 min using a vortex mixer (DLab Scientific, United States). The magnetic-MIPs were then attracted to an external magnet attached to the outer layer of the tube and the sample solution was discarded. Next, the selected PAHs were desorbed from the magnetic-MIPs by sonicating the magnetic-MIPs in THF (120 µL) for 10 min using an ultrasonic bath (Elmasonic, United States). The extract was then filtered through a 0.2 µm nylon syringe filter before quantification using micro-high performance liquid chromatography-ultraviolet detection (µ-HPLC-UV). Figure 1 shows the schematic of the magnetic-MIPs-based micro-solid phase extraction (magnetic-MIPs-µ-SPE) procedure.

Chromatographic conditions

All analyses were performed using µ-HPLC-UV (Agilent Technologies, Milan, Italy). The chromatographic separation of PAHs was carried out on a ZORBAX Eclipse XDB-C18 column (2.1 × 100 mm, 3.5 µm) purchased from Agilent (Italy). The separation was performed using isocratic mobile phase ACN-water (70:30) (v/v) with the column temperature maintained at 30 °C. The flow rate, injection volume and detection wavelength were fixed at 0.2 mL min^{-1}, 2 µL and 254 nm, respectively. Chromatographic data were processed using Agilent ChemStation software.

Optimization of magnetic-MIPs-µ-SPE-µ-HPLC-UV

In this study, several extraction parameters were thoroughly investigated to enhance the analytes' enrichment. The amounts of magnetic-MIPs (5-20 mg), extraction time (1-12 min), types of desorption solvents (MeOH, EtOH, ACN, THF), volume of desorption solvent (50-150 µL of THF) and desorption time (4-12 min) were optimized based on one variable at a time (OVAT) approach and triplicate extraction was performed to finalize the consistency of the results. The OVAT approach involves experiments holding all variables constant and varying only one variable to observe the changes in extraction efficiency.

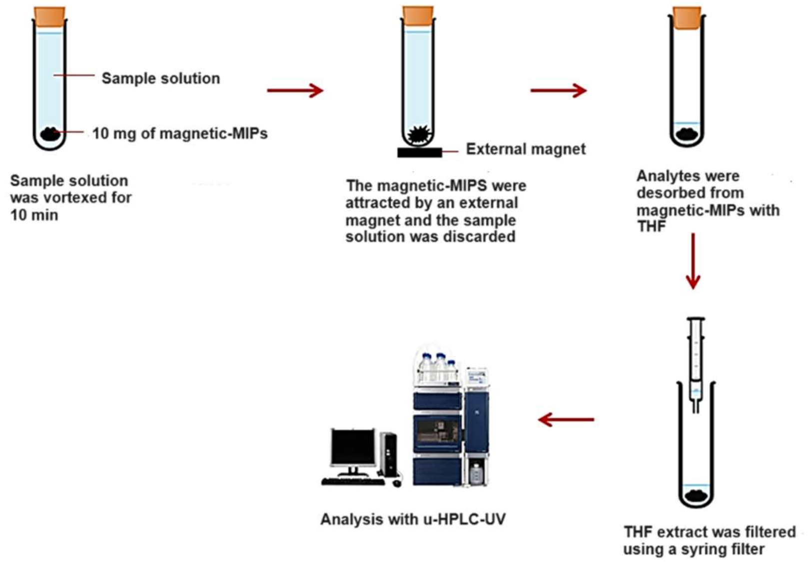

Figure 1. Schematic of magnetic-MIPs-μ-SPE procedure.

Validation of magnetic-MIPs-μ-SPE-μ-HPLC-UV

Validation was carried out to assess the viability of the method, which included linearity, relative recovery, limit of detection (LOD) and limit of quantification (LOQ). The LOD and LOQ were calculated based on signal-to-noise 3:1 and 10:1, respectively.

Measurement uncertainty

Measurement uncertainty (MU) is a measure of the quality and confidence of results for decision-making. MU is important to identify any errors that occur during the experiments and thus the estimation of MU in a method can reduce the errors that exist in data for future analysis. The equation of calculation of the analyte concentration is shown in Equation (1).

The path in calculating the analyte concentration and the error sources were identified and summarized in Figure 2, a fishbone diagram. Several parameters affecting the analyte concentration originated from the preparation of the standard and sample solutions, calibration of glassware and weighing balance, precision and accuracy.

The estimation of the individual uncertainty component was impracticable and tedious. Therefore, all sources were reconciled into bias (systematic errors) and precision (random errors) [10]. The combined standard uncertainty is therefore based on Equation (2).

$$[\mathrm{PAH}] = \frac{(\text{Peak area} \quad)}{\mathrm{m}} \tag{1}$$

where, [PAH] = concentration of the selected PAH, c = y-intercept of linear regression, m = slope of linear regression.

$$\mu^2(\mathrm{y}) = \mathrm{s}^2(\text{precision}) + \mu^2(\text{bias}) \tag{2}$$

where, μ^2 = combined standard uncertainty; s = precision uncertainty; μ = bias uncertainty.

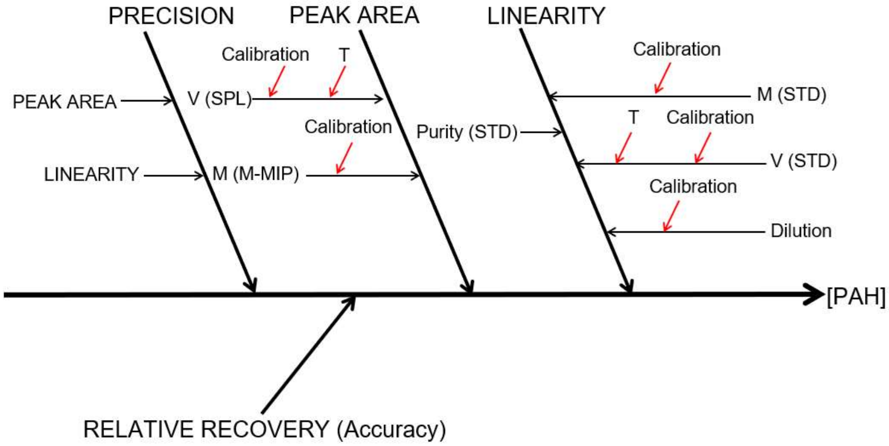

Figure 2. The fishbone diagram indicates error sources in magnetic-MIPs-µ-SPE-µ-HPLC-UV. Abbreviation: standard (STD); sample (SPL); temperature (T); magnetic-MIP (M-MIP); mass (M); volume (V)

The precision uncertainty was obtained from the pooled relative standard deviation (RSD) calculated from the repeatability of the test results (replicate number, n=3) using different analytes spiked at different concentrations using different sample matrices. The pooled RSD equation is shown in Equation (3).

The bias uncertainty was obtained from the relative recovery results, which was also the accuracy study. The uncertainty of bias was calculated using Equation (4) and Equation (5).

$$\text{Pooled RSD}^2 = \sqrt{\frac{(n_1-1)\ \text{x RSD}_1{}^2+(n_2-1)\ \text{x RSD}_2{}^2+\cdots}{(n_1-1)+(n_2-1)+\cdots}} \tag{3}$$

where, pooled RSD^2 = pooled relative standard deviation and n = the number of data.

$$\mu(\text{bias}) = \sqrt{\text{RMSbias}^2 + \text{RMS}\mu\_\text{ref}^2} \tag{4}$$

$$\text{RMSbias}^2 = \sqrt{(x_1-1)^2 + (x_2-1)^2 + \cdots} \tag{5}$$

where, µ(bias) = bias uncertainty, RMSbias^2 = bias in reference standard, RMSµ_ref^2 = reference material uncertainty, RMSbias^2 = bias in reference standard and x = the mean of relative recovery results.

Results and Discussion

Fabrication and characterization of magnetic-MIPs

Magnetic-MIPs were fabricated and submitted for morphological characterization using a dissecting microscope namely Zeiss Stemi 2000-c Trinocular Stereo zoom microscope and SEM. The magnetic-MIPs were placed on a petri dish and viewed under a fluorescence microscope at x100 magnification. The dissecting microscope image (Figure 3a) shows the non-homogeneous particle size distribution of MIPs. Nevertheless, it tallied with the manufacturer's analysis, as the particle sizes of MIPs were in the range of 32 to 100 µm.

Figure 3b and Figure 3c show the SEM images of both MIPs and magnetic-MIPs at x1000 magnification, respectively. The results indicated that well-shaped particles with an estimated diameter distribution from 10 µm to 100 µm were obtained for magnetic-MIPs. The MIPs had spherical shapes and smooth surfaces, and the magnetic-MIPs also possessed similar spherical shapes even after being deposited on the magnetite. MIPs consist of a large surface area leading to more effective imprinting sites [11]. The SEM images of both materials revealed that the particle size distribution was maintained even after deposition of MIPs on the magnetite. This proved that magnetic-MIPs were successfully prepared for the extraction of selected PAHs in this study.

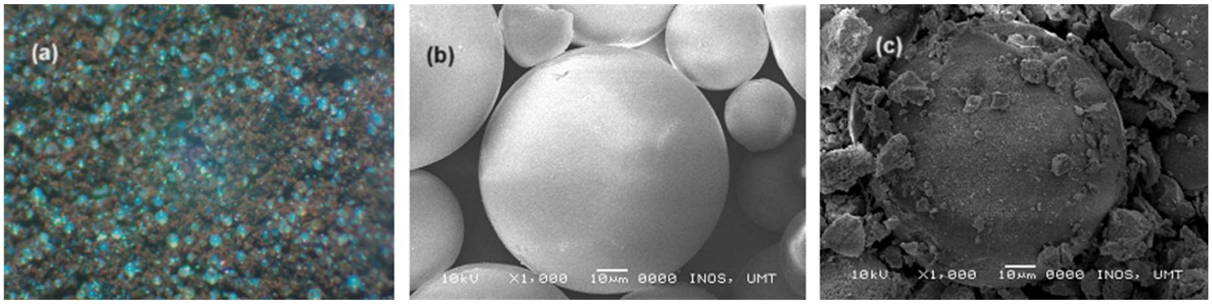

Figure 3. (a) Microscope image of magnetic-MIPs at x100 magnification, (b) SEM images of MIPs and (c) magnetic-MIPs at x1000 magnification

TGA was used to analyze the thermal stability of magnetic-MIPs in the temperature range of 30 °C to 800 °C. There were two stages of degradation behaviour observed on magnetic-MIPs (Figure 4a). The weight loss in the first stage was described as the loss of absorbed and bound trace water between 30 °C and 70 °C. Another weight loss occurred between 330 °C and 400 °C, which was due to the decomposition of organic parts on magnetic-MIPs [12]. The extraction using magnetic-MIPs as the adsorbents in this study was performed at room temperature and thus the magnetic-MIPs were stable throughout the analysis.

The information on pore volume and surface area of adsorbents is important to ensure repeatable and effective adsorption of analyte in trace analysis. The pore volume and surface were determined using BET technique. The pore volumes and surface areas for MIPs and magnetic-MIPs were determined as 1.37 $cm^3 g^{-1}$ and 710 $m^2 g^{-1}$, and 0.38 $cm^3 g^{-1}$ and 224 $m^2 g^{-1}$, respectively. The decline in both pore volume and surface area of magnetic-MIPs indicated that magnetite had occupied parts of the MIPs due to the deposition of magnetite on MIPs. The reduction in surface area for active sites and cavities in MIP might reduce the analytes' adsorption and similar results were also reported in previous studies [13,14]. However, the magnetic-MIPs had greatly simplified the collection of MIPs after the extraction process, and the magnetic-MIPs were still capable of detecting ultra-trace target analytes after a thorough optimization was performed.

VSM analysis showed that the magnetic-MIPs possessed supermagnetism properties. This proved that magnetic-MIPs could be attracted and completely actuated to the external magnet after extraction. Magnetic-MIPs reached a saturation magnetization value at 9.1025 emu g^{-1} with a coercivity of 62.194 G (Figure 4b). The saturation of magnetization value observed for magnetic-MIPs might be less than the pure Fe_3O_4 nanoparticles reported [15]. This could be due to the limited particle surface impact. The magnetic saturation value decreased because of the coating effect into the layer and some of the MIPs might be trapped in the Fe_3O_4 contributing to the degradation of the magnetism moment [16]. This result showed that magnetic-MIPs were successfully prepared and had good magnetism properties that could be applied in μ-SPE for rapid removal of target compounds from sample solution.

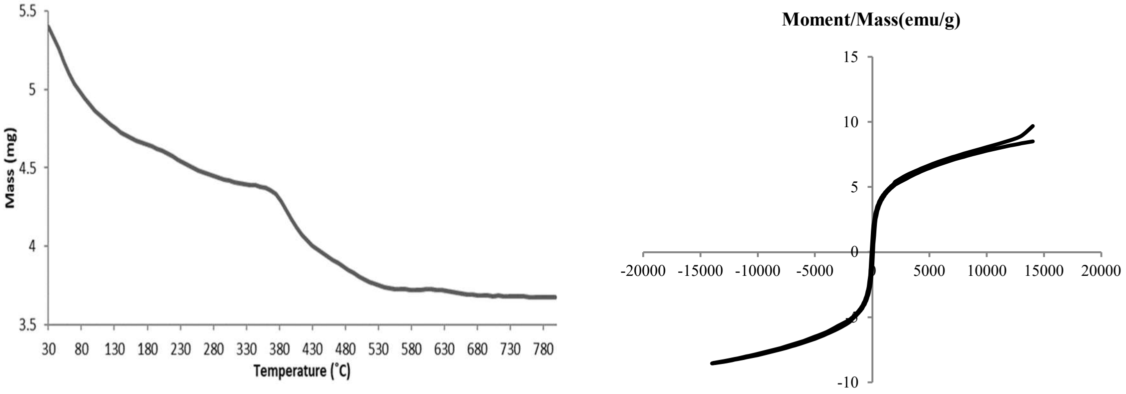

Figure 4. (a) TGA thermogram of magnetic-MIPs, (b) magnetization curves of magnetic-MIPs at room temperature

Optimization of magnetic-MIPs-μ-SPE

Several extraction variables were thoroughly investigated to optimize the magnetic-MIPs-μ-SPE of selected PAHs, namely PHE, FLA, PYR and BaP from water samples. RENSA PAHs MIPs were chosen as the adsorbents in this study due to their selective molecular recognition abilities, which can eliminate the clean-up procedure treated for complicated sample matrices [17]. In this study, the MIPs were deposited on Fe_3O_4 to simplify the collection of MIPs after the extraction. This could eliminate the filtration steps, as well as preventing the loss of microscopic MIPs. The optimization study was performed using deionized water samples that were spiked with each selected PAH at 100 μg L^{-1}. Triplicate extractions were carried out for each variable to ensure results consistency and repeatability.

Amounts of magnetic-MIPs and extraction time

The amounts of magnetic-MIPs were examined to investigate the capacity of magnetic-MIPs in extracting selected PAHs from 10 mL of water sample. In this study, the amounts of magnetic-MIPs in the range of 5 to 20 mg were tested. Based on Figure 5a, the peak areas for all PAHs increased dramatically when magnetic-MIPs were added from 5 to 10 mg in the 10 mL of spiked water sample. However, the decline in peak areas was recorded beyond 10 mg. This meant that the amounts of adsorbents applied achieved the equilibrium mass transfer to lead to the most favorable enrichment [18]. The drop could be due to the agglomeration of magnetic-MIPs occurring in the confined volume, where the relative strength of the van der Waals forces bound the adsorbent particles together [19]. This phenomenon resulted in extraction inefficiency because of the lack of binding sites available for the adsorption. Therefore, 10 mg of magnetic-MIPs were applied in the subsequent optimization experiments.

The effect of extraction time was evaluated by monitoring the peak areas of target analytes from 1 to 12 min of extraction time. The extraction was performed by vortexing the sample solution. The extraction time determines the extraction performance that is dependent on the adsorption equilibrium of the analytes between the sample solution and the adsorbent [20]. The peak areas showed steady increases from 1 to 10 min for all analytes, except for BaP (Figure 5b). There was no significant difference ($p>0.05$) in the peak areas of BaP extracted for 8 min and 10 min. The system achieved complete equilibrium at 10 min. Thereafter, a steep decline in peak areas was observed because of the back-diffusion of analytes from the adsorbent [18]. For this reason, 10 min was chosen as the time to achieve the complete extraction.

Type of desorption solvent, volume of desorption solvent and desorption time

The binding sites in MIPs are mainly dependent on the size, structure and functional groups of the template molecules that have the most similarity with target analytes [21]. This allows MIPs to possess selective recognition of target analytes and hence the polarity of desorption solvents is important to offer a good polarity match to the target analytes for an efficient desorption [22]. A solvent that can dissolve the target analyte and has a strong affinity to the target analytes is therefore influential. Four polar organic solvents that varied in polarity index (P'), namely ACN (P'=5.8), EtOH (P'=5.2), MeOH (P'=5.1) and THF (P'=4) were investigated in this study. The most effective desorption (Figure 5c) was recorded by the THF. THF possessed the lowest P' and this sustained the release of the hydrophobic PAHs from the imprinted cavities. Therefore, the THF was chosen as the desorption solvent to proceed with the following optimization experiments.

The effect of desorption volume in the range of 50 to 150 µL was investigated. The peak areas increased gradually (Figure 5d) when the selected PAHs were desorbed with 50 to 120 µL of THF, except for BaP, which showed no significant difference ($p>0.05$) in the peak areas extracted using 100 µL and 120 µL of THF. However, when 150 µL of THF was used, a decline in peak areas was observed. This could be due to the dilution effect [23]. Since the 120 µL was sufficient to submerge the magnetic-MIPs and offered better extraction efficiency for selected PAHs, the best-configured volume was therefore chosen to be 120 µL.

Desorption process is an equilibrium-dependent process, thus sufficient time is allocated to the desorption process to optimize the release of analytes from the adsorbents [24]. In this study, desorption time from 4 to 12 min was explored and assisted by ultra-sonication. An enhancement in peak areas of selected PAHs (Figure 5e) was observed when the selected PAHs were desorbed from magnetic-MIPs with THF from 4 to 10 min. When the desorption was performed for longer than 10 min, the peak areas of the selected PAHs diminished. This could be due to the re-adsorption of the analytes on the adsorbents [25]. The 10 min desorption time was therefore chosen for the magnetic-MIPs-µ-SPE.

Validation of magnetic-MIPs-µ-SPE-µ-HPLC-UV

Several parameters were performed for validation, which included establishing linearity range, LOD, LOQ and relative recovery to ensure the viability of the magnetic-MIPs-µ-SPE-µ-HPLC-UV technique for the analysis of selected PAHs in environmental water. Linearity in the range of 5 to 250 µg L^{-1} with correlation coefficients, $r \geq 0.9951$ was established experimentally for the quantification of the selected PAHs. The LODs (0.01-0.02 µg L^{-1}) and LOQs (0.04-0.06 µg L^{-1}) revealed that the µ-SPE-µ-HPLC-UV using the magnetic-MIPs as the adsorbents was capable to offer ultra-trace detection of selected PAHs in water samples.

Relative recovery was performed rather than absolute recovery because the µ-SPE technique is a non-exhaustive extraction technique that depends on the equilibrium time. Environmental samples namely river water, tap water, seawater, and rainwater were chosen to investigate the feasibility of the magnetic-MIPs-µ-SPE for the extraction of selected PAHs in these sample matrices. Each sample was spiked with selected PAH at concentrations of 5, 25 and 100 µg L^{-1}, respectively. Blank samples were analyzed to ensure the blank concentration was deducted from the spiked samples. However, the blank samples chosen were all free from the selected PAHs. The blank magnetite was also used to extract the spiked deionized samples randomly, where the peak areas obtained were negligible. The proposed technique could recover the ultra-trace levels of the selected PAHs spiked in the environmental samples with an acceptable average of relative recoveries in the range of 80.2-119.3% (Table 1) with repeatability <10%. This revealed that magnetic-MIPs with reduced surface area compared to core MIPs were still capable of offering good accuracy in determining the PAH residues in environmental water samples. The matrix effects demonstrated by these environmental water samples were insignificant when the proposed magnetic-MIPs-µ-SPE was applied for the extraction of the selected PAHs in the samples. The technique offers a simple and fast procedure and therefore it can be applied as a greener alternative technique for the analysis of PAHs in environmental water samples.

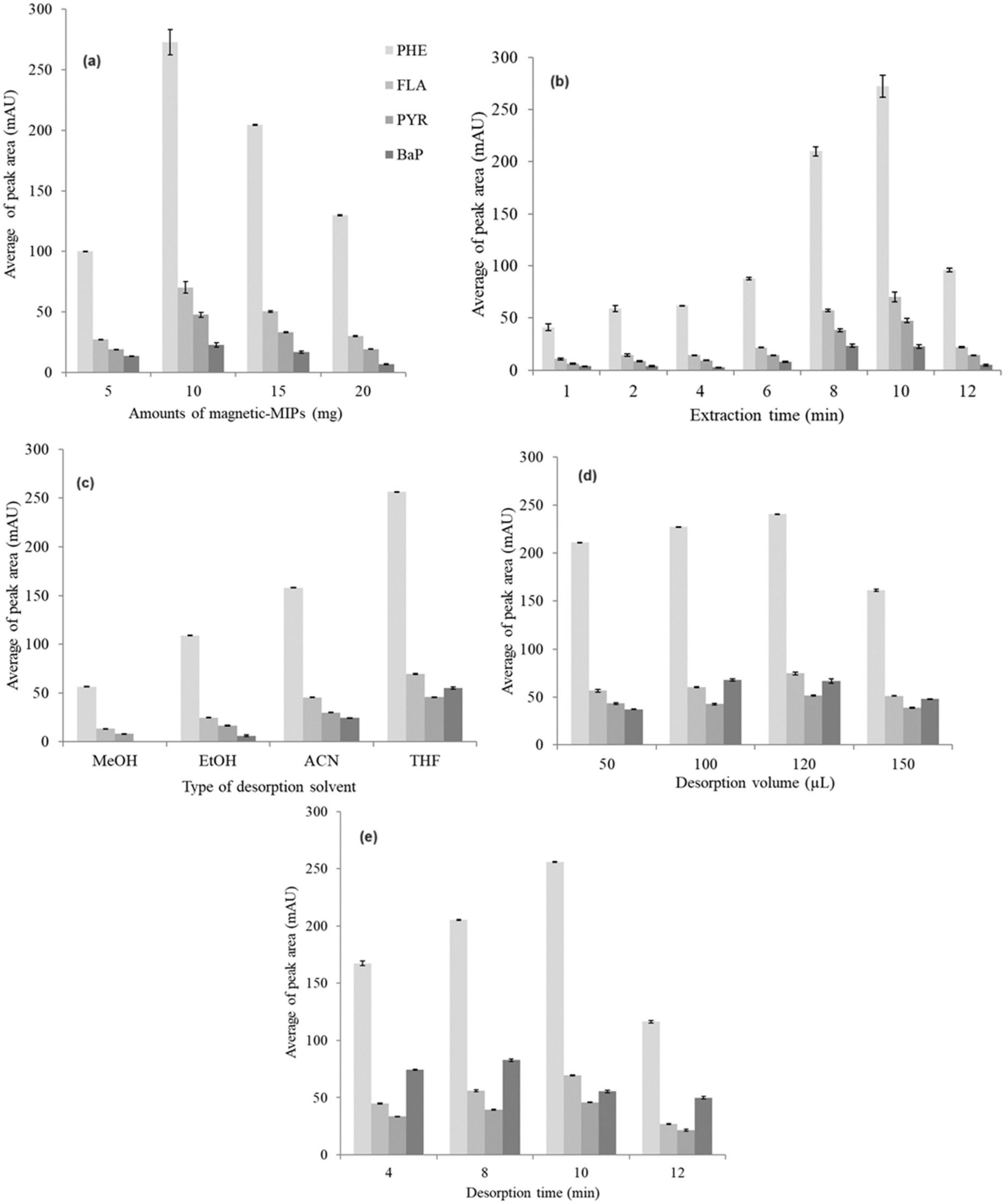

Figure 5. Effect of (a) amounts of magnetic-MIPs, (b) extraction time, (c) type of desorption solvent, (d) desorption volume and (e) desorption time on the extraction efficiency of μ-SPE for the extraction of selected PAHs in spiked deionized water (n = 3 in each case). Initial extraction conditions applied before the optimization were 10 min of extraction time and desorption by 100 μL of acetonitrile for 10 min until an optimal condition was adopted from the experiments. Error bars represent the standard deviations

Table 1. Relative recovery study using magnetic-MIPs as the adsorbent in µ-SPE technique for the extraction of selected PAHs in environmental water samples

Samples	PAH	Average of Relative Recovery, % (RSD%)		
		5.0 µg L^{-1}	25.0 µg L^{-1}	100 µg L^{-1}
River water	PHE	109.6 (0.5)	83.8 (0.5)	106.0 (2.3)
	FLA	113.5 (0.3)	83.1 (0.6)	107.6 (2.1)
	PYR	95.4 (6.7)	81.6 (1.4)	104.6 (0.6)
	BaP	98.4 (0.3)	93.8 (1.5)	108.1 (2.7)
Tap water	PHE	98.0 (1.4)	88.8 (2.2)	99.8 (2.9)
	FLA	92.2 (0.6)	87.4 (2.6)	95.7 (2.7)
	PYR	93.3 (3.4)	98.1 (1.1)	113.9 (2.9)
	BaP	80.2 (4.5)	92.5 (2.9)	96.3 (2.5)
Seawater	PHE	85.5 (10.3)	105.7 (2.3)	92.8 (3.4)
	FLA	84.7 (3.0)	98.3 (4.5)	90.4 (6.5)
	PYR	84.1 (8.0)	94.0 (2.0)	90.8 (3.8)
	BaP	92.0 (5.7)	110.4 (2.8)	109.3 (3.1)
Rainwater	PHE	119.3 (1.7)	108.0 (2.1)	106.9 (1.5)
	FLA	93.3 (2.3)	102.0 (0.9)	105.4 (1.4)
	PYR	116.2 (4.7)	109.7 (3.2)	107.5 (1.7)
	BaP	110.6 (2.3)	104.1 (1.2)	113.0 (1.3)

Measurement uncertainty of magnetic-MIP-µ-SPE-µ-HPLC-UV for the determination of selected PAHs in environmental water

In this study, the combined MU was estimated according to the top-down approach that was best for testing laboratories. The actual dispersion of test results from the validation experiments was observed and included the precision or repeatability, bias uncertainty from reference standard materials, and accuracy or relative recovery results that contributed as the error sources to the uncertainties associated with the outcome. The implementation of the suggested approach was performed using the validation data obtained from the relative recovery study using four types of environmental water samples. In general, the MU in the determination of the concentration of the selected PAHs was associated with systematic and random errors in the method. The combined standard uncertainties for the determination of the PHE, FLA, PYR and BaP were 0.0812, 0.0678, 0.0890, and 0.0778, respectively.

The results showed that the main source contributing to the combined standard uncertainties was the accuracy test performed during method validation, which was termed relative recovery study. The relative recovery study was performed using four different environmental water samples with each spiked at 5, 25 and 100 µg L^{-1}. The relative recovery was calculated relative to the spiked deionized water. This could be the main factor causing the moderate relative standard uncertainty in accuracy because the deionized water was cleaner as compared to other environmental water samples. However, the relative recovery results were still accepted as proposed in the Association of Official Agricultural Chemists validation guidelines, where the recovery studied in micrograms per liter (µg L^{-1}) levels shall be in the range of 80 to 110% and 60 to 115% for 100 µg L^{-1} and 10 µg L^{-1} spiking level, respectively [26] to demonstrate negligible matrix effects. Therefore, the relative recovery average in the range of 80.2-119.3% achieved in this study was satisfactory. In conclusion, the MU of the proposed magnetic-MIP-µ-SPE-µ-HPLC-UV for the determination of selected PAHs in environmental water samples was acceptable.

Conclusion

The integration of magnetism and selective recognition of PAHs into magnetic-MIPs corresponds to a quick and effective way to isolate the selected PAHs from the sample solution by applying an external magnetic field.

The proposed µ-SPE procedure is simple, sensitive and fulfils the green analysis concept in minimizing the use of organic solvents and reducing waste disposal costs. In addition, this technique applies minimal extraction solvent, which is beneficial in the enrichment of analytes. In fact, it is economical and eco-friendly. In conclusion, the combined standard uncertainty for the determination of the selected PAHs is satisfactory and proves that the magnetic-MIP-µ-SPE-µ-HPLC-UV is feasible to be applied as a routine testing procedure for the analysis of selected PAHs in environmental water. The estimation of measurement uncertainty in the analysis revealed that the main source of uncertainty was the accuracy test performed during method validation. By identifying sources of uncertainty, researchers can take steps to reduce uncertainty and improve the accuracy and reliability of the measurements over time.

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