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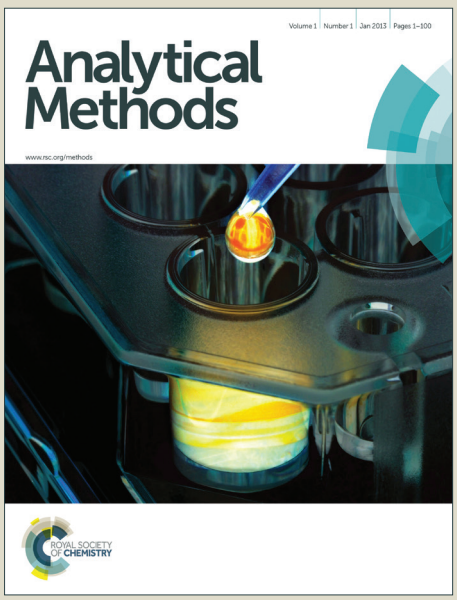

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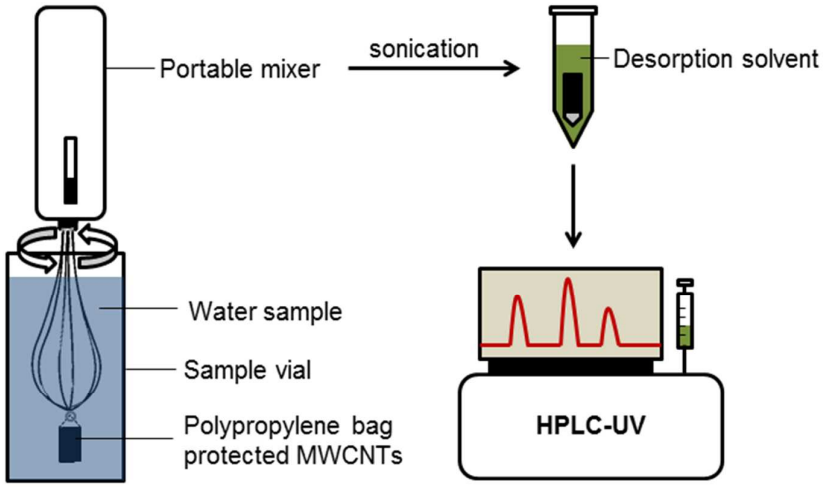

PORTABLE MICRO-SOLID PHASE EXTRACTION FOR THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN WATER SAMPLES

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Abstract

This work describes for the first time the application of portable micro-solid phase extraction (μ-SPE) for the determination of polycyclic aromatic hydrocarbons (PAHs). In this technique, a battery-operated electric whisk stirrer combined with μ-SPE device was employed to provide agitation of the sample solution and facilitate the pre-concentration of the target analytes. The μ-SPE device consisted of multi-walled carbon nanotubes (MWCNTs) packed in polypropylene (PP) membrane. The performance of the μ-SPE sampling coupled with high performance liquid chromatography-ultraviolet detection (HPLC-UV) was evaluated for the analysis of five target PAHs (fluorene, anthracene, fluoranthene, pyrene, benzo[*a*]pyrene) in water. Important μ-SPE parameters were studied and the optimal extraction conditions were 30 min of extraction time, 10 min of desorption

time, isopropanol as the conditioning and desorption solvent and no addition of salt. The developed portable μ-SPE method provided good linearity in the concentration range of 0.1 - 100 μg/L for fluorene, anthracene, fluoranthene, pyrene and 1 - 100 μg/L for benzo[*a*]pyrene with good coefficients of determination (r^2) (0.9975 - 0.9989), low limits of detection (0.01 - 0.59 μg/L), acceptable intra-day precisions (3.5 - 6.2% for on-site analysis) and acceptable relative recoveries (77.3 - 107.2%). The portable-μ-SPE combined with HPLC-UV was successfully applied to the determination of targeted PAHs in selected water samples. The proposed sample preparation technique proved to be simple, cost effective, easy-to-operate and feasible for both off-site and on-site analyses.

Keywords: Portable micro-solid phase extraction; On-site analysis; Polycyclic aromatic hydrocarbon; Water samples

1 Introduction

For years, analysis of chemical contaminations in the environment has been a great concern. The overall analytical scheme is crucial as some of the pollutants are very toxic for living organisms even in very low doses [1]. However, two-thirds of the total analysis time largely involve sampling and sample pretreatment processes and this has prompted scientists to develop methods that can facilitate the whole analytical procedures [2, 3]. On-site sample preparation has been a key factor in the pursuit of this objective. It may reduce errors, and eliminates the probability of sample change and time delays associated with both sample storage and transport, resulting in more accurate and more precise analytical data.

Performing on-site sample preparation is a good practice since it has been demonstrated that analytes are more stable in the extraction phase compared to the natural matrix [3]. Solid phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) are the most commonly used sample preparation methods based on solid phase extraction. However, there are some limitations associated with both methods. Firstly, the use of large size magnetic stirrer that requires alternating current power supply in conventional laboratory process is inconvenient to carry for on-site purposes. Secondly, commercially available SBSE are incompatible with sampler jaws (such as electric drill jaws) [4, 5]. These two limitations can largely be resolved by extending the applications to the on-site analysis. Sigma-Aldrich Supelco has launched a commercial SPME portable field sampler (Supelco TM) with 100 μm polydimethyl-siloxane (PDMS) fiber. In order to be more cost-effective, different approaches using home-made SPME and SBSE field samplers have been developed [4, 6, 7]. Both methods employed similar extraction device in which the solid phase was usually held by the drill bit of the electric drill. However, these methods apparently are unable to remove matrix interferences and provide sample pre-concentration simultaneously. As the matrix refers to all components in the sample apart from the analyte(s) of interest, the matrix can have a

consequential effect on the quality of the results obtained [8, 9]. In order to address the issue, membrane protected sorbent techniques have been introduced. Both micro-solid phase extraction (μ-SPE) and solid phase membrane tip extraction (SPMTE) utilize sorbents held in polypropylene (PP) membrane envelope as the extraction medium. The membrane works effectively for filtration of the matrix components due to the small pore size of the membrane that act as extraction barrier between adsorbent and sample matrices [10]. Compared to conventional SPE, these approaches require much less organic solvent and smaller amounts of sorbent such as MWCNTs [11, 12].

Carbon nanotubes have tremendous applications in various fields of chemical analysis. The increasing number of works involving MWCNTs is due to its extraordinary large surface area which also makes them potentially useful as sorbent materials [13, 14]. Current literature review clearly shows that MWCNTs present an adequate sorption capacity for the extraction of various organic compounds (pesticides, drugs, phthalate esters, aromatic compounds etc.) [15-18]. However, to the best of our knowledge, there has been no report on on-site application of μ-SPE.

In this work, a hand-held battery-operated portable stirrer was developed for the extension of a μ-SPE technique in field sampling. The device comprised a miniature PP bag containing MWCNTs and the bag was attached to the bottom of the stirrer using a fine stainless steel wire. The μ-SPE technique eliminates the need of sample filtration and pertinent sorbents can be utilized according to the target analytes while agitation of sample solutions was provided by the whisk mixer itself. The feasibility of the designed portable μ-SPE sampling approach combined with HPLC-UV was evaluated for the determination of five PAHs as model compounds.

2 Experimental

2.1 Reagents and materials

The PAH standards, fluorene (FLU), anthracene (ANT), fluoranthene (FLT), pyrene (PYR) and benzo[*a*]pyrene (BaP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions (1000 mg/L of each analytes) were prepared separately by dissolving the compounds in acetonitrile (ANT, PYR and BaP) and methanol (FLU and FLT) and stored at 4°C in the dark. Working solutions of standard mixture were prepared by dilution in methanol. Analytical grade sodium chloride was purchased from Bendosen (Selangor, Malaysia). HPLC-grade tetrahydrofuran (THF), isopropyl alcohol (IPA), methanol (MeOH), and acetonitrile (ACN) were purchased from Merck (Darmstadt, Germany). Milli-Q water (resistance ≥ 18.2 MΩ) was produced by Milli-Q system (Millipore, Bedford, MA, USA). Q3/2 Accurel 2E HF (R/P) PP backbone PTFE sheet (157 μm thickness, 0.2 μm pore size) was purchased from Membrana (Wuppertal, Germany). MWCNTs (purity > 95%, outside diameter 8-15 nm, average length 50 μm) were obtained from Sun Nanotech (Jiangxi, China). A Daiso battery-operated electric mixer (Johor, Malaysia) with a replaceable rotating whisk was purchased from a local store and used as a portable stirrer.

2.2 HPLC-UV analysis

The PAHs were identified and quantified using a Agilent Technologies G4288C HPLC system with ultraviolet detection (Agilent Technologies, Santa Clara, California, USA). Chromatographic separations of PAHs were carried out on a Zorbax SB-C18 column (2.1 × 100 mm, 3.5 μm) (Agilent, USA). The separations were performed using isocratic mobile phase acetonitrile-water (80:20) (v/v) at a flow rate of 0.2 mL/min. Detection wavelength and injection volume was fixed at 254 nm and 2 μL, respectively. Chromatographic data were processed using Agilent Chemstation software.

2.3 *Preparation of portable μ-SPE device*

The portable μ-SPE sampling equipment consisted of two main parts, namely a battery operated electromotor mixer and a μ-SPE device containing MWCNTs enclosed in a miniature home-made PP membrane bag. A stainless steel whisk (10 cm × 2 cm) was fixed onto the hand-carry electric stirrer. Maximum stirring speed of up to 1080 rpm was achieved using this mixer. The speed was kept constant throughout the run and was determined by a portable Omnitech DT2234C^{+} intelligent digital photo tachometer (Kuching, Malaysia). The mini-electromotor was powered by two AA NiMH 1.25 V rechargeable batteries (Energizer Recharge Universal).

The PP membrane miniature bag was prepared according to Basheer *et al*. [2] with slight modification. Briefly, a piece of PP membrane was cut into a shape of a home-base (2.0 cm × 0.7 cm for the bottom square with a 0.5 cm high triangle on top). The flat edge of the membrane was equally folded to form a smaller house-shaped membrane bag. Portable impulse heat sealer was used to heat-seal the edge of the longest flap. MWCNTs (4 mg) were introduced into the house-shaped membrane bag through the remaining open end, using a micropipette tip and a glass pestle tip, which was then heat-sealed to secure the sorbent.

Prior to use, each μ-SPE device was cleaned by ultrasonication in acetone for 5 min and stored in the selected conditioning solvent until use. For extraction, the prepared house-shaped bag containing sorbent was hung at the bottom of the stirrer using stainless steel wire. The portable μ-SPE device was exposed to the samples solution during the extraction (Fig. 1) and it was operated by the electromotor instead of using the conventional magnetic stirrer to accomplish the agitation of the solution. The device was used for both laboratory off-site μ-SPE analysis as well as on-site field sampling.

2.4 *Environmental water samples*

On-site sample preparation of environmental water samples source were carried out at different locations in the Universiti Teknologi Malaysia Johor Bahru campus involving three different types of water samples, namely lake water, river water and farm water at the UTM Educational and Research Agriculture farm. The in-laboratory extractions were carried out on the above-mentioned water samples. All samples were used without any pre-treatment or filtration.

2.5 *Micro-Solid Phase Extraction*

2.5.1 *Off-site sampling/ Laboratory sampling*

Off-site water samples were collected in Teflon bottles pre-cleaned with acetone and covered with aluminium foil. The samples were stored in the dark at 4°C for 3 days until analysis. Spiked ultrapure water samples were prepared in the laboratory. MWCNTs (4 mg) were packed in the house-shaped membrane. The miniature PP bag was conditioned with isopropanol prior to the extraction. During extraction of the targeted PAHs, the hand-held stirrer was clamped to a retort stand with the stirrer blades and the μ-SPE device completely immersed in the sample solution (30 mL). The solution was continuously agitated at 1080 rpm for 30 min. After extraction, the PP bag was removed, rinsed in high purity water and dabbed dry with lint-free tissue. The bag was then placed inside a 500 μL safe-lock tube. Isopropanol (IPA) (200 μL) was added to the tube and desorption of analytes was via conventional ultrasonication using a Bransonic 3510E-DTH ultrasonic cleaner (Branson Ultrasonics, Danbury, USA) for 10 min. After desorption, 2 μL of the extract was injected into HPLC-UV for analysis.

2.5.2 *On-site sampling*

For on-site extraction, 30 mL of lake water was processed as described in off-site sampling (pH and salinity of sample solution were not adjusted). After extraction, the PP bag was placed inside a safe-lock tube and then placed in an ice-chilled box before transported to the laboratory for desorption process and HPLC-UV determination. The procedure was carried out in triplicates. At the end of each extraction, the used whisk was washed with acetone to avoid contamination.

2.6 *Validation of method*

For validation of the portable μ-SPE method, calibration curves were constructed for the off-site analysis of sample solution containing a mixture of five PAHs at concentrations of 0.1–100 μg/L (FLU, ANT, FLT and PYR) and 1 - 100 μg/L (BaP). The quantifications were carried out based on the peak area of each PAH. The method was evaluated for its linearity, limit of detection (LOD) and limit of quantification (LOQ), extraction recovery, and precision. The analytical results for on-site extraction and off-site extraction were compared to validate the feasibility of the on-site sampling.

3 Results and discussion

3.1 *Optimization of portable μ-SPE*

In order to evaluate the performance of the portable μ-SPE device, optimization procedures were performed on spiked water sample at concentrations of 10 μg/L (FLU, ANT,

FLT and PYR) and 50 µg/L (BaP). All the parameters for optimization were studied in the laboratory (off-site analysis).

3.1.1 Optimization of sample volume and organic solvent conditioning

Sample volume is important in determining the loading capacity of MWCNTs. The effect of sample volumes in the range of 10 – 35 mL was investigated. It was found that lower sample volumes gave poorer extraction efficiencies (Fig. 2(A)). It was noted that solutions of < 20 mL experienced reduced surface contact between analytes and adsorbent due to vortex flow created by the whisk rotation, thus decreasing the adsorption of analytes. The results also indicated that sample volume of 30 mL gave the highest extraction efficiency for most of the PAHs studied. This volume is adequate to allow the adsorbent to extract the analytes effectively. No significant increase was observed with further increase in sample volume which suggested that the adsorption sites of the adsorbent have reached saturation. Sample volume of 30 mL was therefore chosen as the optimum sample volume and applied in the subsequent experiments.

As PP membrane and MWCNTs are hydrophobic in nature, different types of organic solvents were evaluated for conditioning and enhancing the wettability of the µ-SPE device. Organic solvents with various polarities and water-miscible properties (methanol, acetonitrile, tetrahydrofuran and isopropanol) were used. The µ-SPE device was immersed in corresponding solvents for 2 min and then rinsed with high-purity water. The results (Fig. 2B) showed that isopropanol gave highest extraction efficiencies for three of the analytes (FLT, PYR and BaP) compared to the other conditioning solvents investigated. This result suggested that wetting of the PP bag with slightly non-polar organic solvent improved the interactions between the hydrophobic membrane and MWCNTs with samples solution. The impact of the membrane material as a sorbent itself was not investigated since it has been

reported previously that PP membrane does not have significant influence on analytes adsorption [12].

3.1.2 Optimization of desorption solvents and desorption time

Four different organic solvents namely methanol, acetonitrile, tetrahydrofuran and isopropanol were investigated for use in the desorption of PAHs from the MWCNTs. The best desorption efficiencies were obtained when isopropanol and tetrahydrofuran were used as desorption solvent in comparison with other solvents (Fig. 2(C)). Due to the strong adsorption of hydrophobic PAHs on MWCNTs, only non-polar solvent can disrupt the interactions and desorb the PAHs from the MWCNTs [19]. Tetrahydrofuran and isopropanol are more hydrophobic compared to methanol and acetonitrile as they have lower polarity index and dielectric constant. This goes according to "like dissolves like" aphorism, which stated that substances will dissolve best in solvent with similar chemical characteristics [20].Isopropanol was chosen as the desorption solvent since it displayed a greater capacity to enhance BaP (which is listed as priority pollutant of unsubstituted PAHs according to the US-EPA) solubility compared to tetrahydrofuran [21].

Analytes were desorbed from MWCNTs using ultrasonication of the extraction device in organic solvent as sonication can break the interactions between PAHs and MWCNTs. Desorption times in the range of 5 to 20 min were studied. It was observed that 10 min of sonication was required for the analytes to be completely desorbed from the MWCNTs except for BaP (data not shown). This might be due to the higher affinity of BaP towards MWCNTs compared to the other analytes. No significant increase in peak area of PAHs was observed when desorption times of > 10 min were used. Therefore, 10 min of desorption time with ultrasonication was adopted as the optimum desorption conditions.

3.1.3 Optimization of salt addition

Salting-out effect is often associated with the ionic strength of the aqueous phase. Increasing ionic strength may increase the transfer of analytes from sample solution to the adsorbent phase since the purpose of salt addition is to reduce the solubility of analytes in the aqueous sample. The salting-out effect on extraction efficiency was determined by the addition of sodium chloride in the range of 0 to 15% (w/v) to the sample solution. It was found that there was a negative effect on the peak areas of analytes with the addition of sodium chloride (data not shown). The addition of NaCl did not contribute in extraction of PAHs from aqueous solution since PAHs are non-polar and have low solubility in water. In a similar previous work, Hou and Lee reported that no change in extraction efficiency was observed when NaCl was added to the sample solution [22]. High salt concentration also increased the viscosity of sample solution which decreased the diffusion rate of analytes [23]. Therefore, no addition of salt was adopted in this study.

3.1.4 Optimization of extraction time

The effect of extraction time was studied since mass transfer is a time-dependent process. A period of time is needed for the system to achieve equilibrium. Extraction time profiles in the range of 10 to 50 min were examined to determine the equilibrium time required for PAHs adsorption from the sample solution to the MWCNTs. It was found that the extraction efficiencies of PAHs reached a plateau at 30 min and no significant increase in the peak areas was observed when the extraction time exceed 30 min (Fig. 2D). Instead, a slight decrease was observed for some analytes beyond 30 min. Hence, 30 min was selected as the optimum extraction time

3.2 Method validation of the proposed method

In order to validate the analytical performance of the portable μ-SPE, analyses were carried out to obtain the calibration curves and determine the limits of detection (LOD) and limits of quantification (LOQ) for the analytes using spiked deionized water as shown in Table 1. Calibration curves were constructed by plotting peak area versus concentration based on six concentration levels in the range of 0.1 - 100 μg/L for FLU, ANT, FLT and PYR and 1 - 100 μg/L for BaP. Triplicate analyses were performed for each concentration. Good linearities were obtained with coefficients of determination (r^2) in the range of 0.9975 to 0.9989 and relative standard deviations (% RSD) of 4.8% to 7.1%. The LODs and LOQs of the method were calculated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. The LODs obtained for the analytes were in the range of 0.01 to 0.59 μg/L while the LOQs were in the range of 0.05 – 1.96 μg/L.

3.3 Real water samples analysis

In order to assess the feasibility of the portable μ-SPE approach, three environmental water samples were extracted on-site using extraction conditions identical to the off-site analysis. The concentration of targeted PAHs detected in the water samples were summarized as in Table 2. The river water samples in with no PAHs detected were spiked with the target analytes to give final concentrations of 10 μg/L (FLU, ANT, FLT and PYR) and 50 μg/L (BaP). The average relative recoveries (ratios of peak areas of analytes from spiked river water and spiked ultrapure water extraction, for off-site and on-site analysis) were in the range of 77.3 to 104.1 % with RSDs of < 10.0% (intra-day, $n = 3$). This clearly indicates that the results are reproducible and the matrix effects were reduced as this method provides a membrane works effectively for pre-filtration of the matrix components along with pre-concentration simultaneously. A statistical *t*-test was also performed to verify the equivalence of the two sampling modes as described in Table 3. The *p*-values in independent *t*-test for

comparison of on-site and off-site μ-SPE were more than 0.05 for most cases (FLU, FLT, PYR and BaP) confirmed that these two modes of extraction were comparable. Fig. 3 shows chromatograms of extracted spiked and non-spiked river water sample under identical extraction conditions.

3.4 Comparison with other published method

A comparison between the portable μ-SPE method and other reported methods based on sample pre-treatment techniques of PAHs in environmental water samples are given in Table 4. In general, comparable recoveries and LODs were demonstrated by the portable μ-SPE and previously published methods [24-27]. Although the agarose film liquid phase microextraction (AF-LPME) and SPE showed lower LODs, these methods required longer extraction times [25] and involved tedious multi-step analysis), respectively [26]. As for headspace solvent drop microextraction-HPLC- fluorescence detection (HS-SDME-HPLC-FLD), its main disadvantage is the instability of the droplet which may result in dissolution and of the drop [27]. Thus, the proposed portable μ-SPE method implies a great advantage over other sample pre-treatment approaches, which could be employed for on-site sampling.

4 Conclusions

In the present study, a new portable battery-operated electric whisk stirrer combined with μ-SPE approach for both off-site and on-site analysis has been developed. For the first time, μ-SPE was successfully applied for on-site environmental water sample preparation. The on-site technique showed good linearity, high relative recoveries, acceptable relative standard deviation and comparable limits of detection with other reported methods. Home-made μ-SPE device demonstrated to be simple, cost-effective, and easy-to-operate. The

whisk on the stirrer provided sample agitation during extraction while the PP membrane and the protected MWCNTs acted as sample pre-filtration and pre-concentration device simultaneously, which together created a unique merit for field analysis. In addition, the approach only required a small amount of sorbents and low consumption of organic solvents. After extraction, only the membrane bag was brought to the laboratory for further analysis, thus, eliminating the need of large volumes water samples for laboratory analysis and discard the usage of AC power supply during extraction. Being an alternative technique for conventional laboratory solid phase extraction, the portable μ-SPE proved to be feasible for both off site and on-site analysis.

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Tables

Table 1: Quantitative data of portable μ-SPE for the determination of five selected PAHs

	Linearity (μg/L)	Coefficient of Determination (r^2)	% RSD (*n* = *3*)	LOD (μg/L)	LOQ (μg/L)
Fluorene	0.1 - 100	0.9975	7.1	0.06	0.20
Anthracene	0.1 - 100	0.9988	4.8	0.01	0.05
Fluoranthene	0.1 - 100	0.9982	5.6	0.05	0.18
Pyrene	0.1 - 100	0.9989	5.4	0.05	0.16
Benzo[*a*]pyrene	1.0 - 100	0.9988	6.3	0.59	1.96

Table 2: Determination of PAHs in environmental water samples by on-site μ-SPE

	Residue level (μg/L) (% RSD, *n* = *3*)		
	Farm water	River water	Lake water
Fluorene	Detected[a]	nd[b]	nd
Anthracene	0.11 (7.3)	nd	0.19 (6.9)
Fluoranthene	Detected	nd	Detected
Pyrene	0.16 (5.5)	nd	nd
Benzo[*a*]pyrene	nd	nd	nd

[a] Detected = LOD ≤ result < LOQ.

[b] nd, non-detected or less than detection limit.

Table 3: Relative recovery (%) and method precisions (RSD %, n = 3) of off-site and on-site μ-SPE (n = 3)

	Average relative recovery (%)[a], (% RSD, n = 3)		t-test
	Off-site analysis	On-site analysis	(p-value)
Fluorene	98.6 (6.1)	99.7 (4.3)	0.50
Anthracene	99.7 (2.7)	107.2 (3.5)	0.03
Fluoranthene	77.3 (5.6)	80.9 (6.2)	0.15
Pyrene	98.3 (6.4)	89.2 (4.2)	0.06
Benzo[a]pyrene	90.1 (7.2)	88.4 (4.5)	0.46

[a] Percentage of peak area of analyte from extracts of spiked river water samples versus spiked ultrapure water, with both samples spiked at 10 μg/L for fluorene, anthracene, fluoranthene and pyrene and 50 μg/L for benzo[a]pyrene.

Table 4: Comparison of LODs obtained by different reported analytical approaches in the determination of PAHs in water samples

Analysis method*	Dynamic linear range (µg/L)	LOD (µg/L)	References
TiO_2-µ-SPE-HPLC-UV	0.2 – 100 and 1.0 – 100	0.026 – 0.82	[24]
AF-LPME–GC–MS	0.1 – 200	0.01 – 0.04	[25]
SPE-HPLC-UV	0.04 – 100	0.005 – 0.058	[26]
HS–SDME–HPLC–FD	0.01 – 50	0.004 – 0.247	[27]
Off/On-site µ-SPE-HPLC-UV	0.1 – 100 and 1 – 100	0.014 – 0.588	This work

* Abbreviations = TiO_2-µ-SPE: titanate-micro solid phase extraction; AF-LPME: agarose film-liquid phase microextraction; HS: headspace; SDME: solvent drop microextraction; HPLC: high performance liquid chromatography; UV: ultraviolet; FD: fluorescence detection; GC: gas chromatography; MS: mass spectrometry.

Figure captions

Fig. 1. Schematic of portable μ-SPE system

Fig. 2. Optimization of off-site μ-SPE; (A) effect of sample volume, (B) effect of μ-SPE bag conditioning solvent, (C) effect of desorption solvent type, (D) effect of extraction time. Extraction conditions are as described in the text. Error bars represent standard deviation, $n = 3$.

Fig. 3. HPLC chromatograms for (a) on-site μ-SPE extract of unspiked water sample, and (b) μ-SPE extract of spiked water sample at 10 μg/L except for BaP (50 μg/L). μ-SPE conditions: 4 mg of MWCNTs; conditioning solvent: isopropanol; sample volume: 30 mL; extraction time: 30 min; desorption solvent: 200 μL of isopropanol; desorption time: 10 min; no salt addition. Peak identifications: (1) fluorene, (2) anthracene, (3) fluoranthene, (4) pyrene and (5) benzo[*a*]pyrene.

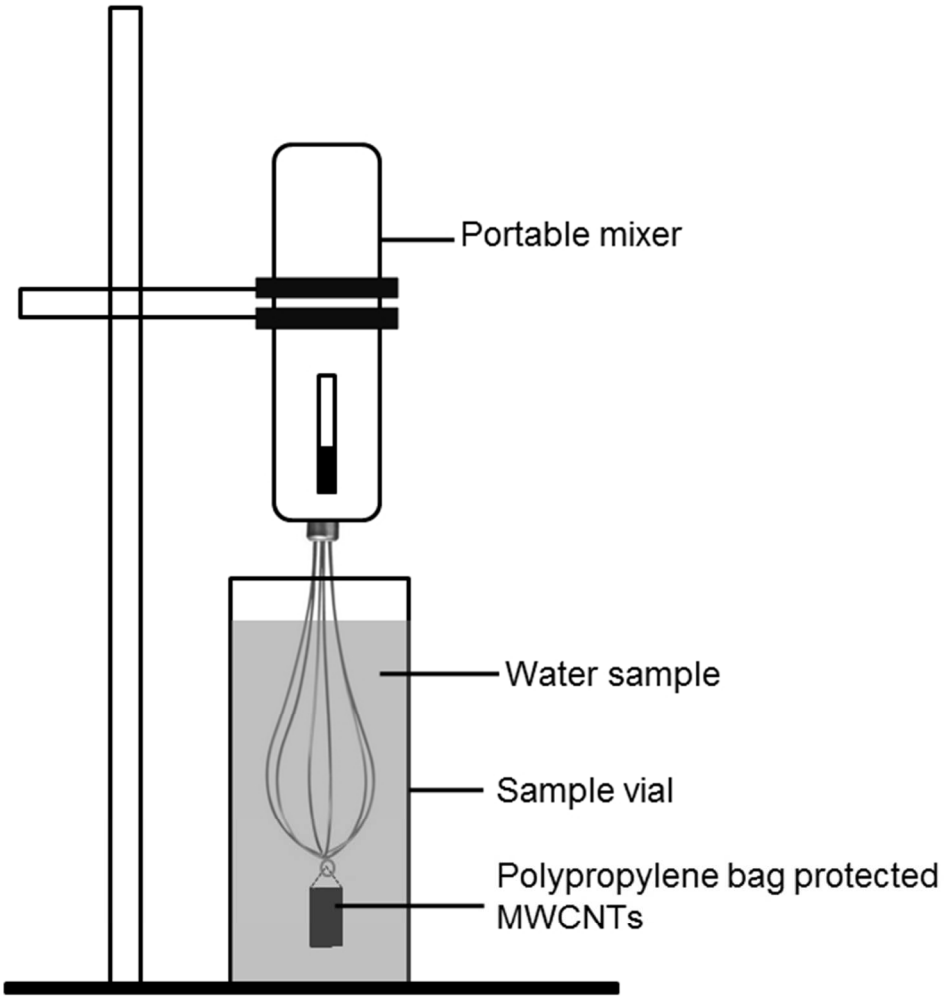

Fig. 1. Schematic of portable μ-SPE system
182x190mm (96 x 96 DPI)

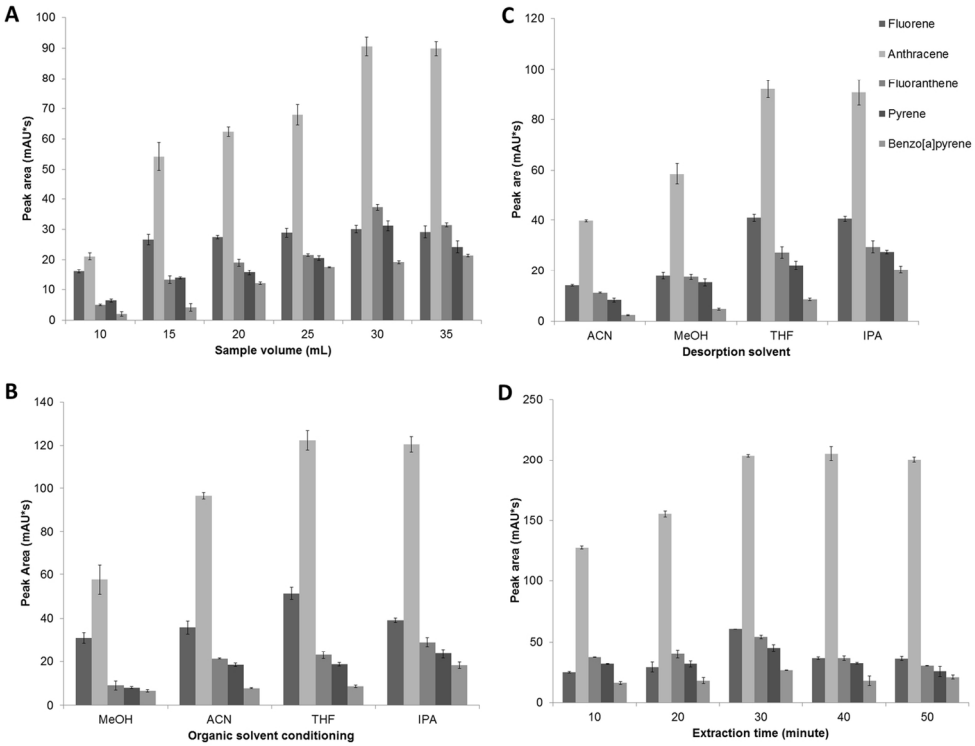

Fig. 2. Optimization of off-site μ-SPE; (A) effect of sample volume, (B) effect of μ-SPE bag conditioning solvent, (C) effect of desorption solvent type, (D) effect of extraction time. Extraction conditions are as described in the text. Error bars represent standard deviation, n = 3.
138x105mm (300 x 300 DPI)

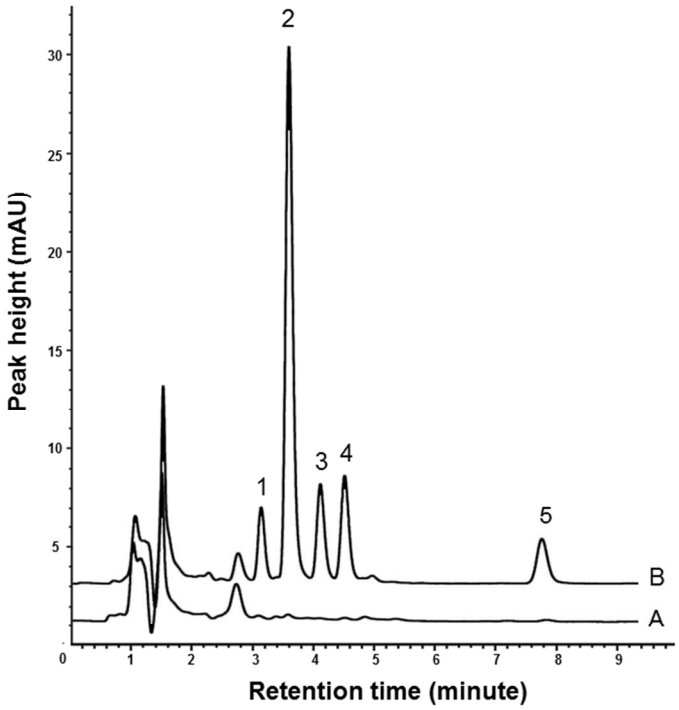

Fig. 3. HPLC chromatograms for (a) on-site μ-SPE extract of unspiked water sample, and (b) μ-SPE extract of spiked water sample at 10 μg/L except for BaP (50 μg/L). μ-SPE conditions: 4 mg of MWCNTs; conditioning solvent: isopropanol; sample volume: 30 mL; extraction time: 30 min; desorption solvent: 200 μL of isopropanol; desorption time: 10 min; no salt addition. Peak identifications: (1) fluorene, (2) anthracene, (3) fluoranthene, (4) pyrene and (5) benzo[a]pyrene.
254x190mm (96 x 96 DPI)