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Determination of polycyclic aromatic hydrocarbons in waters by ultrasound-assisted emulsification-microextraction and gas chromatography–mass spectrometry

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ABSTRACT

An ultrasound-assisted emulsification-microextraction (USAEME) procedure was developed for the extraction of US EPA 16 polycyclic aromatic hydrocarbons (PAHs) in 10 mL of water samples, with subsequent determination by gas chromatography–mass spectrometry (GC–MS). After determination of the most suitable solvent and solvent volume, several other parameters (i.e., extraction time, centrifugation time and ionic strength of the sample) were optimized using a 2^3 factorial experimental design. Limits of detection ranged from 0.001 to 0.036 $\mu g\,L^{-1}$. The developed procedure was applied to fortified distilled water with different fortification levels (0.5, 2 and 5 $\mu g\,L^{-1}$). Recoveries were over 92% and relative standard deviations of the recoveries were below 8%. The efficiency of the USAEME was compared with traditional liquid–liquid extraction (LLE) and solid-phase extraction on real water samples (i.e., tap water, well water and surface (lake) water as well as domestic and industrial wastewaters). The USAEME showed comparable efficiencies especially with LLE. The developed USAEME was demonstrated to be robust, viable, simple, rapid and easy to use for the determination of PAHs in water samples by GC–MS.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that originate from different emission sources, mainly associated with human activities, such as the incomplete combustion of fossil fuels, industrial processes or the use of motor vehicles. The determination of PAHs in environmental samples is an important topic because of their toxicity to humans and deteriorative effects on soil organisms and plants [1].

In order to determine these pollutants by a suitable chromatographic technique, an extraction or a pre-concentration step is necessary. Liquid–liquid extraction (LLE) is probably the most widely used method for the extraction of PAHs from aqueous samples [2,3]. However, LLE needs relatively large volumes of organic solvents and is a time-consuming as well as a labor-intensive method. Solid-phase extraction (SPE) has been used as an alternative method to LLE for the extraction of PAHs from water samples because it uses less solvent and is less time-consuming than LLE. However, SPE is a relatively expensive method [3].

Therefore, in recent years, solid-phase microextraction (SPME) [4] and different modes of liquid–liquid microextraction, such as single drop microextraction (SDME) [5,6], hollow fiber liquid phase microextraction (LPME) [7], dispersive liquid–liquid microextraction (DLLME) [8,9] and ultrasound-assisted emulsification-microextraction (USAEME) [10–15], have been developed in order to establish an efficient and economical sample preparation method.

SPME is based on the partitioning of analytes between sample matrixes and the polymer-coated fibre. The main drawbacks of the SPME method are that SPME fibres are rather expensive and have a limited lifetime. In addition, sample carry-over has been frequently reported for SPME method [16]. Compared to the SPME, SDME has many advantages including no sample carry-over, wide selection of available solvents, low cost, simplicity and ease of use, minimal solvent use, short pre-concentration time, requiring no conditioning (as is the case with the fibre in the SPME), no need for instrument modification, etc. Nevertheless, some drawbacks, such as instability of droplet and relative low precision were reported for SDME method [17]. The advantages of the DLLME method are rapidity, low cost and high enrichment factors. However, general drawbacks of this method are difficulty to automate and requirement of using a disperser solvent, which usually decreases the partition coefficient of analytes into an extraction solvent [8,18].

In order to eliminate the disadvantages of these methods, a new extraction technique termed as ultrasound-assisted emulsification-microextraction (USAEME) was developed by

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Regueiro et al. [10]. This method combines the microextraction system and ultrasonic radiation in one step. Ultrasonic radiation is a powerful tool for acceleration of various steps in the analytical process for solid and liquid samples [19–22]. Especially, this type of energy has great help in the extraction of organic and inorganic compounds from the environmental samples [23]. Additionally, ultrasonication offers several advantages that make it an ideal method for pre-treating a large number of samples. These advantages include high extraction efficiency, lower equipment cost, ease of operation and lower extraction temperature, etc. Therefore, ultrasound assistance is being used more and more in analytical chemistry, enabling different steps in the analytical process, particularly in sample preparation [24]. In USAEME, the application of ultrasonic radiation facilitates the emulsification phenomenon and accelerates the mass-transfer process between two immiscible phases. This improves the extraction efficiency in a minimum time [24,25]. But, the main drawback of this method is difficulty to automate.

According to the literature, USAEME has been applied to determine the polybrominated diphenyl ethers [limits of detection (LOD): 1–2 ng L^{-1}] [11], polychlorinated biphenyls (LOD: 14–30 ng L^{-1}) [12], organochlorine pesticides (LOD: 0.002–0.016 μg L^{-1}) [13] and phenolic preservatives (LOD: 3.90–27.5 ng L^{-1}) [15] in water samples. Saleh et al. [14] also used this method for the extraction of selected 10 PAHs from water samples, with subsequent determination by gas chromatography with flame ionization detection (GC-FID). They reported that the recoveries of their method for studied PAHs were in the range of 59.2–90.5% and LOD were between 0.02 and 0.05 μg L^{-1}. However, using special-made centrifuge tubes was necessary in their method because the low density extraction solvents (i.e., toluene, 1-octanol, 1-undecanol, 1-dodecane and 1-dodecanol) were used.

In the present study, a new USAEME procedure was developed by using classical centrifuge tubes for the extraction of EPA 16 PAHs in water samples, followed by GC–MS determination. The efficiency of the developed method was also compared with LLE and SPE methods on the real water samples.

2. Experimental

2.1. Reagents and solvents

All chemicals used were of analytical grade. The EPA 16 PAHs mixed standard, including naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLO), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), benzo[a]anthracene (BaA), chrysene (CHR), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (IcdP), dibenzo[a,h]anthracene (DahA), benzo[g,h,i]perylene (BghiP), was obtained from Accustandard Co. (New Haven, CT, USA). Internal standard (1,2,3,4-tetrachloronaphthalene, (1,2,3,4-TCNAP)) and four surrogate standards (ACE-d_{10}, PHE-d_{10}, CHR-d_{12}, and perylene-d_{12}) were also obtained from Accustandard Co. Solvents of residue grade purity including dichloromethane, chloroform, bromoform, 1,2-dichlorobenzene, n-hexane, methanol and ethyl acetate were obtained from Merck Co. (Darmstadt, Germany). Sodium chloride and sodium sulfate were also obtained from Merck Co. Octadecyl (C_{18}) SPE cartridges were obtained from J&T Baker (Deventer, Holland).

Standard stock solution of 16 PAHs, internal standard and surrogates were prepared at 1 μg mL^{-1} level in methanol for each compound. All solutions were stored in the dark at 4 °C. Working solutions were prepared by dilution of standard stock solution with distilled water. In order to avoid any further evolution, the working solutions were prepared again every 4 h.

2.2. The optimized ultrasound-assisted emulsification-microextraction (USAEME) procedure

A 10 mL water sample was placed in a 10 mL glass-centrifuge tube. As an extraction solvent, chloroform (100 μL) was added into the water sample and mixed. The resulting mixture was immersed into an ultrasonic bath (frequency 35 kHz, 320W, Super RK 510, Sonorex, Bandelin, Germany) for 15 min at 25 °C. During the sonication, the solution became turbid due to the dispersion of fine chloroform droplets into the aqueous bulk. The emulsification phenomenon favoured the mass-transfer process of PAHs from the aqueous bulk to the organic phase. The emulsion was centrifuged at 1344 × *g* for 5 min to achieve the phase separation. After centrifugation, chloroform (65 μL) was removed from bottom of the tube by using a 100 μL Hamilton syringe (Hamilton Bonaduz AG, Bonaduz, Switzerland) and transferred into a micro vial. After 10 ng of internal standard (1,2,3,4-TCNAP, 10 μL of 1 ng μL^{-1} solution) was added into the micro vial, GC–MS analysis was performed as described in Section 2.5.

2.3. LLE and SPE

LLE procedure was adopted from US EPA Method 3510C [26]. Water sample (200 mL) was placed in a 250 mL separatory funnel. The extraction was carried out three times with 20 mL of dichloromethane. The extracts were combined and dried with anhydrous sodium sulfate. The resulting extract was concentrated to <1 mL using rotary evaporator (Buchi B-160 Vocabox, Flawil 1, Switzerland) and gentle nitrogen stream. After 100 ng of internal standard (1,2,3,4-TCNAP, 100 μL of 1 ng μL^{-1}) was added into the extract, its volume was completed to 1 mL and transferred into the vial. Then, GC–MS analysis was performed as described in Section 2.5.

SPE procedure was carried out as described by Aydin et al. [3]. Octadecyl (C_{18}) SPE cartridge was used for the extraction of PAHs from water sample. The cartridge was consecutively washed with 10 mL of methanol and 8 mL of n-hexane/ethyl acetate (5:3, v/v). Then, it was conditioned with 10 mL of methanol and 2× 5 mL of distilled water. Water sample (200 mL) was passed through the cartridge under vacuum. After the cartridge was dried for 10 min by maintaining vacuum, elution of PAHs from the cartridge was carried out with 10 mL of n-hexane/ethyl acetate (7:3, v/v). The rest of the procedure including concentration and addition of internal standard into the extract and its transfer into the vial was the same as in LLE. Then, GC–MS analysis was performed as described in Section 2.5.

2.4. Real water samples

The optimized USAEME was examined on the real water samples, including tap water, well water, surface (lake) water, domestic and industrial wastewater samples. Tap water was taken from the laboratory and well water came from deep-ground water in Konya (Turkey). Surface (lake) water was taken from Cavuscugol in Ilgın (Konya, Turkey). Domestic and industrial wastewater samples were taken from the sewage system in residential area and industrial zone in Konya (Turkey), respectively. The sampling of the real samples was performed according to standard methods [27]. All samples were collected free of air bubbles in glass containers and they were stored in the dark at 4 °C until analysis. The analyses of the samples were carried out within 4 h after sampling. Tap water and well water samples were analysed without previous treatment or filtration. The surface (lake) water, domestic and industrial wastewater samples were filtered through 0.45 μm pore size membrane filters before the extraction procedures.

Table 1
GC–MS analysis of PAHs: retention times of studied compounds, ions monitored and method linearity ranges.

PAHs	Retention times (min)[a]	Ions (*m*/*z*) monitored for quantitation	Ions (*m*/*z*) monitored for confirmation	Concentration ranges (ng μL^{-1})	R^2
NAP	8.43	128	129, 127	0.01–10	1.000
ACY	11.14	152	153, 151	0.01–10	1.000
ACE-d_{10}	11.42	164	162, 160	0.01–10	1.000
ACE	11.48	153	154, 152	0.01–10	0.999
FLO	12.65	166	165, 167	0.01–10	1.000
PHE-d_{10}	15.53	188	189, 180	0.01–10	0.999
PHE	15.62	178	176, 179	0.01–10	1.000
ANT	15.84	178	179, 176	0.01–10	1.000
1,2,3,4-TCNAP	19.08	266	264, 268	–	–
FLA	21.43	202	203, 200	0.01–10	1.000
PYR	22.61	202	203, 200	0.01–10	1.000
BaA	30.85	228	226, 229	0.01–10	1.000
CHR-d_{12}	30.91	240	236, 241	0.01–10	0.999
CHR	31.06	228	226, 229	0.01–10	1.000
BbF	38.30	252	253, 250	0.01–10	0.999
BkF	38.49	252	253, 250	0.01–10	0.999
BaP	40.35	252	253, 250	0.01–10	0.999
Perylene-d_{12}	40.68	264	260, 265	0.01–10	0.999
IcdP	47.24	276	277, 274	0.01–10	0.998
DahA	47.54	278	279, 276	0.01–10	0.998
BghiP	48.43	276	277, 138	0.01–10	0.999

[a] Obtained under described temperature program.

2.5. GC–MS conditions

The determination of PAHs was carried out using a gas chromatograph (GC, Agilent 6890N, Agilent Technologies, Palo Alto, CA, USA) equipped with MS (Agilent 5973, Agilent Technologies, Foster City, CA, USA). The features and operating conditions of GC–MS system were as follows: GC, equipped with programmed temperature vaporizing (PTV) injector, DB-5 MS 5% phenylmethyl siloxane fused silica capillary column (30 m length, 0.25 mm i.d. and 0.25 μm film thickness) and helium (purity 99.999%) as carrier gas at constant flow-rate of 1.9 mL min^{-1}. PTV program was as follows: 80 °C, 12 °C s^{-1} to 350 °C and hold at 350 °C for 2 min. Injections were performed by an Agilent 7683 B Series automatic injector (Agilent Technologies, Palo Alto, CA, USA). The temperature of the ion source and MS transfer line were adjusted at 170 and 280 °C, respectively. The GC oven temperature was as follows: initial temperature 60 °C for 4 min, 15 °C min^{-1} to 160 °C, 3 °C min^{-1} to 300 °C, hold at 300 °C for 10 min (run time: 67.33 min). MS detector was operated in selected ion monitoring (SIM) mode. Ions monitored for the quantification and confirmation of PAHs and corresponding retention times are given in Table 1. The quantitation of 16 PAHs and surrogates were performed using the internal standard method. Analytical curves were drawn using eight pure PAH standards in methanol within the concentration range between 0.01 and 10 ng μL^{-1}. Each calibration standard contained the internal standard (1,2,3,4-TCNAP) at level of 0.1 ng μL^{-1}. The coefficients of determination (R^2) for all studied PAHs were between 0.998 and 1.000 (see Table 1).

3. Results and discussion

3.1. Selection of organic solvent

For selection of a suitable extraction solvent to be used in the USAEME method, some factors should be considered. First, it is convenient that the extraction solvent remain at the bottom of the centrifuge tube after phase separation. Hence, the extraction solvent should be denser than water and water immisible [11]. Second, the chosen organic solvent must have good affinity for target compounds. Finally, it should have excellent gas chromatographic behavior [28].

Considering these factors, dichloromethane, 1,2-dichlorobenzene, chloroform and bromoform were examined in the preliminary experiments. Ten millilitre aliquots of distilled water fortified with 2 μg L^{-1} of each PAH were extracted by using 100 μL of each solvent in ultrasonic bath for 5 min. Emulsification was observed in all cases with the exception of dichloromethane. Dichloromethane was completely dissolved in the aqueous solution (solubility in water: 13 mg mL^{-1}) [29]. Similar observation was reported for the USAEME of synthetic musk fragrances, phthalate esters and lindane [10], polybrominated diphenyl ethers [11], polychlorinated biphenyls [12] and organochlorine pesticides [13] from an aqueous solution. The extraction efficiencies of PAHs from water with 1,2-dichlorobenzene, chloroform and bromoform are presented in Fig. 1. The highest recoveries of PAHs were obtained with chloroform. Refs. [10–13] reported a detailed explanation for such results by considering the dipole moment of the extraction solvents. The solvent hydrophobicity expressed as octanol/water partition coefficient (log Kow) decreases in the order 1,2-dichlorobenzene (log Kow = 3.38) > bromoform (log Kow = 2.38) > chloroform (log Kow = 1.97) [29]. Therefore, it was expected that 1,2-dichlorobenzene and bromoform had higher affinity towards PAHs than chloroform. However, contrary result was obtained. A possible explanation for this result might be related to surface tension of the examined solvents (at 20 °C, bromoform: 41.5 mN m^{-1}, 1,2-dichlorobenzene: 37 mN m^{-1}, chloroform: 27.5 mN m^{-1}) [29]. A lower surface tension of chloroform would enable a higher cavitation under ultrasound irridation and hence, a higher efficiency in emulsion formation [10]. Therefore, chloroform was selected as extraction solvent for further experiments.

3.2. Effect of solvent volume

To increase the sensitivity of the USAEME method, different volumes of chloroform in the range of 50–300 μL were examined. A chloroform volume of 50 μL was completely dissolved in the aqueous bulk. As shown in Fig. 2 increasing the chloroform volume from 100 to 300 μL resulted in a decrease of detector response for all PAHs. This observation might be attributed to dilution effect. Similar results for the effect of solvent volume in different liquid–liquid microextraction systems were reported by Zhao and Lee [30], Tor and Aydin [6], Rezaei et al. [31], Regueiro et al. [10] and Fontana

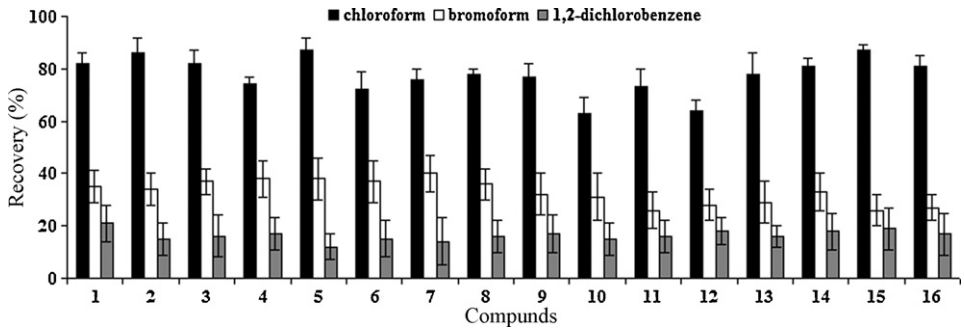

Fig. 1. Recoveries of PAHs extracted from fortified distilled water samples with different organic solvents ($n = 8$). Extraction conditions: sample volume, 10 mL; fortification level, 2 μg L^{-1}; extraction solvent volume, 100 μL; extraction time, 5 min; centrifugation time, 5 min (1344 × *g*); without addition of NaCl; temperature, 25 °C. (1, NAP; 2, ACY; 3, ACE; 4, FLO; 5, PHE; 6, ANT; 7, FLA; 8, PYR; 9, BaA; 10, CHR; 11, BbF; 12, BbF; 13, BaP; 14, IcdP; 15, DahA and 16, BghiP).

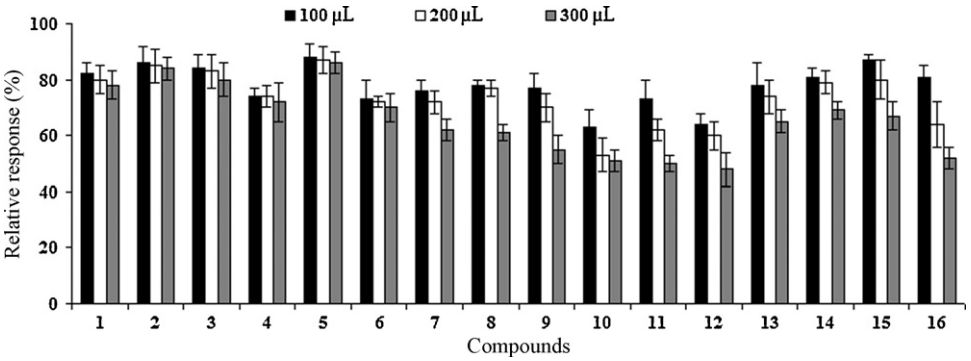

Fig. 2. Relative detector responses for PAHs extracted from fortified distilled water samples with different volumes of chloroform ($n = 8$). Extraction conditions: extraction solvent, chloroform; sample volume, 10 mL; fortification level, 2 μg L^{-1}; extraction time, 5 min; centrifugation time, 5 min (1344 × *g*); without addition of NaCl; temperature, 25 °C. (1, NAP; 2, ACY; 3, ACE; 4, FLO; 5, PHE; 6, ANT; 7, FLA; 8, PYR; 9, BaA; 10, CHR; 11, BbF; 12, BbF; 13, BaP; 14, IcdP; 15, DahA and 16, BghiP).

et al. [11]. Therefore, in the present study, 100 μL of chloroform was selected for further optimization experiments. After extraction with 100 μL of chloroform, recovered volume of chloroform was (65 ± 3 μL). This result also corroborated that collection of chloroform was reproducible.

3.3. Factorial design

The factorial experimental design reduces the time needed for the optimization of an investigated procedure and overall costs [12]. Therefore, after selection of the most suitable extraction solvent (chloroform) and its volume (100 μL), the other factors affecting the efficiency of the USAEME, such as extraction time (denoted as X_1), centrifugation time (denoted as X_2) and ionic strength of the sample (denoted as X_3), were optimized by a factorial experimental design at two levels (2^3). The lower and higher level for each factor was signed as "−" and "+", respectively. The corresponding levels (low and high level) for factors X_1–X_3 were 5 and 15 min, 5 and 10 min and 0 and 0.1 g mL^{-1}, respectively. The experimental design matrix is constituted as shown in Table 2. A full 2^3 design would have required eight experiments, which were duplicated in order to calculate the residual error. The experiments were performed in a randomized order to avoid any systematic error.

After processing the data by analysis of variance (ANOVA) using Tool Pak in Microsoft Excel, the ANOVA tables were constructed to test the significance of the effect of each factor on the extraction efficiency. At significance level of 5%, the factor with *F*-value over critical *F*-value (5.318) has a significant effect on the extraction efficiency.

The extraction time (X_1) was a significant factor with positive effect on the extraction of all PAHs. In other words, the USAEME for 15 min gave better result than 5 min. For the present study, the extraction time interval was defined as the time elapsed between addition of chloroform and the end of the sonication stage. In this study, an increase in the extraction time from 5 to 15 min improved the recoveries of all PAHs. Therefore, 15 min was chosen as extraction time for further studies.

The centrifugation time (X_2) was not a significant factor for all PAHs. Namely, 5 min of centrifugation was adequate to break down the emulsion, hence the phase separation was achived. Therefore, 5 min was selected as centrifugation time.

The effect of the ionic strength on the extraction efficiency was evaluated by increasing NaCl concentration of sample from 0 to 0.1 g mL^{-1}. The ionic strength of the sample (X_3) was a significant factor with negative effect on the extraction of all PAHs. In this study, increasing NaCl concentration in the water sample from 0 to 0.1 g mL^{-1} decreased the extraction recovery of all

Table 2
Design matrix for factorial design and average recoveries of PAHs for the effect of parameters on the USAEME method.

Experiment no	Codified parameters			Average recovery (%)
	X_1 (min)	X_2 (min)	X_3 (g mL^{-1})	
#1–9	–	–	–	78
#2–10	+	–	–	99
#3–11	–	+	–	84
#4–12	+	+	–	97
#5–13	–	–	+	40
#6–14	+	–	+	47
#7–15	–	+	+	43
#8–16	+	+	+	51

X_1: extraction time, X_2: centrifugation time, X_3: ionic strength of the sample (addition of salt, NaCl, into the sample).

Table 3
Comparison of limits of detection (LOD) of 16 PAHs in water samples analysed by optimized USAEME-GC–MS method (extracting solvent chloroform; solvent volume: 100 μL; extraction time: 15 min at 25 °C, centrifugation time: 5 min) and by methods reported in the literature: SPME-GC–MS [34], Dynamic LPME-HPLC-UV [35], floating drop LPME-GC-FID [36], DLLME-GC-FID [8] and USAEME-GC-FID [14].

PAHs	LOD ($\mu g\,L^{-1}$) (present study)	LOD (SPME-GC–MS) ($\mu g\,L^{-1}$)[a] [34]	LOD (dynamic LPME-HPLC-UV) ($\mu g\,L^{-1}$)[b] [35]	LOD (floating drop LPME-GC-FID) ($\mu g\,L^{-1}$)[c] [36]	LOD (DLLME-GC-FID) ($\mu g\,L^{-1}$)[d] [8]	LOD (USAEME-GC-FID)[e] [14]
NAP	0.001	0.003	–[f]	–	0.010	0.020
ACY	0.023	0.003	–	–	0.010	0.020
ACE	0.021	0.006	–	–	0.007	0.050
FLO	0.022	0.002	–	–	0.008	0.020
PHE	0.030	0.017	–	0.070	0.009	0.050
ANT	0.031	0.020	–	0.100	0.009	0.050
FLA	0.028	0.001	0.450	0.370	0.010	0.050
PYR	0.015	0.001	0.600	0.190	0.010	0.050
BaA	0.029	0.029	–	0.240	0.010	–
CHR	0.013	0.005	0.400	0.430	0.010	0.050
BbF	0.028	0.027	0.350	0.120	–	–
BkF	0.024	0.013	0.450	0.350	–	–
BaP	0.029	0.018	0.500	0.450	0.020	–
IcdP	0.036	0.021	–	1.670	–	–
DahA	0.032	0.014	–	0.840	–	–
BghiP	0.029	0.012	–	1.490	0.030	–

[a] SPME-GC–MS: solid-phase microextraction-GC–MS.
[b] Dynamic LPME-HPLC-UV: dynamic liquid phase microextraction-HPLC-UV.
[c] Floating drop LPME-GC-FID: floating drop liquid phase microextraction-GC-FID.
[d] DLLME-GC-FID:dispersive liquid-liquid microextraction-GC-FID.
[e] USAEME-GC-FID: ultrasound-assisted emulsification-microextraction-GC-FID (method used extraction solvents which had lower density than the water).
[f] Not available.

PAHs. The obtained result was in agreement with relevant references [10–13,32], which explained the effect of ionic strength of an aqueous sample on the emulsification phenomenon. Therefore, further experiments were performed without addition of NaCl into the samples.

Additionally, interaction between the extraction and centrifugation times ($X_1 \cdot X_2$) was significant with positive effect on the efficiency of USAEME. However, interactions between the extraction time and ionic strength of the sample ($X_1 \cdot X_3$) and centrifugation time and ionic strength of the sample ($X_2 \cdot X_3$) were significant with negative effect. As a result, the optimum conditions for USAEME of PAHs from water were as follows: chloroform as an extraction solvent, solvent volume: 100 μL; extraction time: 15 min at 25 °C with no addition of NaCl and centrifugation time: 5 min.

Finally, effect of repetition of developed USAEME on the recoveries of all PAHs should be examined. Therefore, developed USAEME was carried out three times by adding 100 μL of fresh chloroform into the same fortified samples, and recoveries were calculated. From the first USAEME, the recoveries of PAHs were above 94% with relative standard deviations (R.S.D.) below 8% ($n = 6$). However, in the extract from second and third repetition, negligible recoveries (≤2% with R.S.D. ≤ 3%, $n = 6$) were obtained. Hence, in order to obtain satisfactory recoveries of PAHs, USAEME should be performed once.

3.4. Evaluation of the performance of the developed procedure

The LOD and limits of quantification (LOQ) for 16 PAHs determined according to published guidelines at a signal-to-noise ratio (S/N) of 3 and 10, respectively [33], were given in Table 3.

Table 3 also presents the reported LOD found in the literature for the determination of PAHs in water samples when using SPME-GC–MS [34], dynamic liquid phase microextraction (dynamic LPME) coupled with high performance liquid chromatography-ultraviolet detection (HPLC-UV) [35], floating drop LPME-GC-FID [36], DLLME-GC-FID [8] and USAEME-GC-FID [14]. Table 3 corroborated that the LOD obtained with USAEME in presented study is generally better than those obtained with dynamic LPME-HPLC-UV, floating drop LPME-GC-FID and USAEME-GC-FID. For some PAHs, i.e., PYR, CHR, BghiP, etc., the optimized procedure shows comparable LOD with DLLME-GC-FID. However, LOD of SPME-GC–MS are better than those reported in this study.

The recoveries of PAHs from distilled water fortified with 0.5 $\mu g\,L^{-1}$ of each compound ranged from 92 ± 7 to 98 ± 6% ($n = 8$). Comparable results were obtained at fortification levels of 2 $\mu g\,L^{-1}$ (recoveries between 94 ± 5% and 102 ± 8%, $n = 8$), and of 5 $\mu g\,L^{-1}$ (recoveries between 94 ± 8% and 105 ± 6%, $n = 8$). The recoveries at three different fortification levels were not significantly different ($p > 0.05$) indicating the high efficiency of developed USAEME method for extraction of PAHs from water.

3.5. Real water analysis

Real water samples are expected to represent very complex matrices. Therefore, in order to study possible matrix effects, developed USAEME method was applied to both unfortified and fortified (2 $\mu g\,L^{-1}$ of each PAH and surrogates including ACE-d_{10}, PHE-d_{10}, CHR-d_{12}, and perylene-d_{12}) real water samples, including tap

Table 4
Comparison of PAH mass concentrations (mean value and standard deviation) determined in real surface and waste water samples by optimized USAEME procedure and LLE and SPE procedures.

	Concentration ($\mu g\,L^{-1}$) ($n = 4$)		
	Optimized USAEME method	LLE method	SPE method
Surface (lake) water			
NAP	0.14 ± 0.02	0.12 ± 0.04	0.08 ± 0.04
CHR	0.35 ± 0.08	0.31 ± 0.06	0.21 ± 0.05
IcdP	0.22 ± 0.06	0.23 ± 0.07	0.12 ± 0.04
Domestic wastewater			
NAP	0.10 ± 0.06	0.10 ± 0.05	0.05 ± 0.04
ACY	0.12 ± 0.05	0.13 ± 0.05	0.07 ± 0.05
BghiP	0.45 ± 0.06	0.43 ± 0.07	0.28 ± 0.06
Industrial wastewater			
NAP	0.35 ± 0.06	0.30 ± 0.05	0.23 ± 0.07
PHE	0.45 ± 0.05	0.43 ± 0.04	0.25 ± 0.08
BghiP	0.25 ± 0.06	0.20 ± 0.04	0.15 ± 0.08

Table 5
Comparison of extraction efficiency (mean value and standard deviation) of 16 PAHs and PAH surrogates from fortified water samples (fortification concentration: 2 μg L^{-1}, $n = 4$) by optimized USAEME procedure and LLE and SPE procedures.

	Recovery (%)								
	Tap water			Well water			Surface (lake) water		
	Optimized USAEME method	LLE method	SPE method	Optimized USAEME method	LLE method	SPE method	Optimized USAEME method	LLE method	SPE method
NAP	95 ± 5	81 ± 4	68 ± 7	94 ± 5	90 ± 4	70 ± 6	NC[a]	NC	NC
ACY	99 ± 5	86 ± 3	78 ± 8	100 ± 5	87 ± 3	79 ± 7	99 ± 7	88 ± 3	80 ± 8
ACE	96 ± 6	85 ± 2	76 ± 9	93 ± 4	87 ± 5	75 ± 7	98 ± 8	87 ± 5	74 ± 6
FLO	94 ± 4	90 ± 4	71 ± 8	92 ± 4	93 ± 4	74 ± 8	92 ± 9	82 ± 3	76 ± 6
PHE	98 ± 6	98 ± 5	61 ± 7	95 ± 5	96 ± 4	64 ± 7	96 ± 7	86 ±	68 ± 5
ANT	95 ± 7	96 ± 3	74 ± 9	94 ± 6	90 ± 3	75 ± 6	93 ± 9	83 ± 4	76 ± 8
FLA	100 ± 7	101 ± 4	75 ± 9	95 ± 3	98 ± 5	78 ± 8	98 ± 8	87 ± 3	80 ± 9
PYR	98 ± 5	101 ± 5	72 ± 8	94 ± 6	98 ± 3	73 ± 9	98 ± 7	86 ± 5	78 ± 8
BaA	102 ± 4	105 ± 6	78 ± 7	94 ± 7	108 ± 6	80 ± 8	103 ± 6	93 ± 3	82 ± 8
CHR	96 ± 5	102 ± 7	75 ± 6	92 ± 5	107 ± 5	78 ± 6	NC	NC	NC
BbF	97 ± 3	103 ± 5	76 ± 8	94 ± 6	105 ± 4	81 ± 5	100 ± 7	102 ± 4	83 ± 6
BkF	99 ± 6	106 ± 5	74 ± 6	95 ± 8	102 ± 2	78 ± 6	101 ± 9	101 ± 5	80 ± 8
BaP	100 ± 5	102 ± 4	80 ± 6	96 ± 6	104 ± 4	82 ± 7	102 ± 7	92 ± 3	85 ± 7
IcdP	103 ± 3	108 ± 3	82 ± 8	98 ± 4	108 ± 5	81 ± 6	NC	NC	NC
DahA	96 ± 4	107 ± 5	81 ± 9	95 ± 5	104 ± 3	84 ± 8	100 ± 6	104 ± 4	82 ± 8
BghiP	100 ± 5	101 ± 4	83 ± 7	99 ± 4	104 ± 2	80 ± 8	102 ± 5	99 ± 3	84 ± 9
ACE-d_{10}	100 ± 5	88 ± 3	78 ± 4	102 ± 5	90 ± 6	90 ± 6	100 ± 4	89 ± 5	73 ± 4
PHE-d_{10}	100 ± 4	98 ± 4	70 ± 5	101 ± 4	98 ± 4	98 ± 5	100 ± 3	88 ± 3	74 ± 5
CHR-d_{12}	98 ± 4	103 ± 4	80 ± 5	100 ± 6	99 ± 5	99 ± 4	101 ± 4	100 ± 3	82 ± 6
Perylene-d_{12}	101 ± 5	104 ± 3	78 ± 6	102 ± 3	101 ± 3	101 ± 4	103 ± 6	103 ± 5	80 ± 6

	Recovery (%)					
	Domestic wastewater			Industrial wastewater		
	Optimized USAEME method	LLE method	SPE method	Optimized USAEME method	LLE method	SPE method
NAP	NC[a]	NC	NC	NC	NC	NC
ACY	NC	NC	NC	96 ± 6	82 ± 5	76 ± 8
ACE	93 ± 5	94 ± 3	75 ± 8	94 ± 8	81 ± 3	73 ± 8
FLO	91 ± 4	99 ± 5	77 ± 7	92 ± 4	88 ± 5	72 ± 8
PHE	90 ± 6	105 ± 6	69 ± 8	NC	NC	NC
ANT	92 ± 7	101 ± 4	75 ± 7	92 ± 7	89 ± 4	71 ± 7
FLA	93 ± 7	106 ± 3	82 ± 7	90 ± 5	94 ± 3	75 ± 8
PYR	90 ± 5	104 ± 4	79 ± 6	90 ± 6	95 ± 4	73 ± 7
BaA	92 ± 5	107 ± 3	84 ± 5	93 ± 6	106 ± 3	79 ± 8
CHR	89 ± 6	103 ± 2	80 ± 8	91 ± 7	100 ± 5	73 ±5
BbF	90 ± 7	102 ± 2	85 ± 6	92 ± 5	101 ± 4	78 ± 5
BkF	93 ± 8	100 ± 4	83 ± 8	94 ± 4	99 ± 3	79 ± 6
BaP	91 ± 7	101 ± 4	87 ± 7	90 ± 6	98 ± 4	80 ± 7
IcdP	90 ± 5	100 ± 3	85 ± 8	90 ± 7	100 ± 3	78 ± 8
DahA	88 ± 7	103 ± 4	84 ± 6	90 ± 5	100 ± 5	76 ± 7
BghiP	NC	NC	NC	NC	NC	NC
ACE-d_{10}	98 ± 4	90 ± 4	80 ± 5	100 ± 6	88 ± 5	82 ± 6
PHE-d_{10}	97 ± 5	103 ± 4	82 ± 4	101 ± 4	90 ± 4	79 ± 6
CHR-d_{12}	96 ± 6	105 ± 3	86 ± 6	98 ± 5	101 ± 3	80 ± 5
Perylene-d_{12}	98 ± 7	101 ± 5	85 ± 6	97 ± 5	99 ± 4	86 ± 6

[a] NC: not considered because this compound was detected in real surface (lake) water samples.

water, well water and surface (lake) water as well as domestic and industrial wastewater samples. Because surface water, domestic and industrial wastewater samples were filtered through the membrane filter before extraction, these samples did not affect the volume of chloroform recovered after extraction. The analyses of the unfortified tap water and well water samples showed that they were free of PAHs contamination. However, "NAP, CHR and IcdP", "NAP, ACY and BghiP" and "NAP, PHE and BghiP" were determined in the surface water, domestic and industrial wastewater samples, respectively.

The efficiency of USAEME was also compared with those involving LLE and SPE on the unfortified and fortified real water samples. The USAEME procedure showed comparable results especially with LLE for unfortified real water samples (Table 4). After all real water samples were fortified with 2 μg L^{-1} of each PAH and surrogates, they were analysed by using developed method. As seen in Table 5, the recoveries of 16 PAHs were in the range of 88–101% with R.S.D. below 9% ($n = 4$). LLE showed comparable recoveries (81–108% with R.S.D. below 7%, $n = 4$). However, the recoveries obtained from SPE were in the range of 61–87% with R.S.D. below 9% ($n = 4$).

The recoveries of surrogates ranged from 96(±6) to 103(±6)% ($n = 4$) (Table 5). When all recoveries of 16 PAHs and surrogates from the fortified real water samples were gauged against absolute limits of 70% and 130% [37], it was seen that developed USAEME method gave satisfactory results. The results also showed that efficiency of the developed method was higher than that of SPE method. Moreover, the developed method showed comparable efficiency with LLE (Table 5). It should also be emphasized that optimized USAEME is not a time-consuming procedure. Furthermore, it needs much lower volumes of extraction solvent than LLE and SPE and it is not necessary to concentrate the sample for GC analysis.

4. Conclusion

The results from the presented study indicated that the developed USAEME method could be efficiently used as a sample preparation technique for the determination of EPA 16 PAHs in water samples by GC–MS. The developed method is precise, reproducible, rapid and easy for the analyses of water samples. It also requires only small volumes of extraction solvent and sample materials.

References

[1] ATSDR (Agency for Toxic Substances and Disease Registary, Division of Toxicology/Toxicology Information Branch), Toxicological Profile for Polycyclic Aromatic Hydrocarbons (PAHs), ATSDR, Atlanta, USA, 1995, pp. 11–111.
[2] A. Tor, Y. Cengeloglu, M.E. Aydin, M. Ersoz, H. Wichmann, M. Bahadir, Fresen. Environ. Bull. 12 (2003) 732–735.
[3] M.E. Aydin, H. Wichmann, M. Bahadir, Fresen. Environ. Bull. 13 (2004) 118–123.
[4] D.A. Lambropoulou, I.K. Konstantinou, T.A. Albanis, J. Chromatogr. A 1124 (2006) 97–105.
[5] A. Tor, J. Chromatogr. A 1125 (2006) 129–132.
[6] A. Tor, M.E. Aydin, Anal. Chim. Acta 575 (2006) 138–143.
[7] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 999 (2003) 145–153.
[8] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1–9.
[9] S. Berijani, Y. Assadi, M. Anbia, M.R. Milani Hosseini, E. Aghaee, J. Chromatogr. A 1123 (2006) 1–9.
[10] J. Regueiro, M. Llompart, C. Garcia-Jares, J.C. Garcia-Monteagudo, R. Cela, J. Chromatogr. A 1190 (2008) 27–38.
[11] A.R. Fontana, R.G. Wuilloud, L.D. Martínez, J.C. Altamirano, J. Chromatogr. A 1216 (2009) 147–153.
[12] S. Ozcan, A. Tor, M.E. Aydin, Anal. Chim. Acta 647 (2009) 182–188.
[13] S. Ozcan, A. Tor, M.E. Aydin, Water Res. 43 (2009) 4269–4277.
[14] A. Saleh, Y. Yamini, M. Faraji, M. Rezaee, M. Ghambarian, J. Chromatogr. A 1216 (2009) 6673–6679.
[15] J. Regueiro, M. Llompart, E. Psillakis, J.C. Garcia-Monteagudo, C. Garcia-Jares, Talanta 79 (2009) 1387–1397.
[16] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 22 (2003) 56–574.
[17] L. Xu, C. Basheer, H.K. Lee, J. Chromatogr. A 1152 (2007) 184–192.
[18] F. Pena-Pereira, I. Lavilla, C. Bendicho, Spectrochim. Acta B 64 (2009) 1–15.
[19] E. Priego-López, M.D. Luque de Castro, Chromatographia 57 (2003) 513–518.
[20] M.E. Aydin, A. Tor, S. Ozcan, Anal. Chim. Acta 577 (2006) 232–237.
[21] A. Tor, M.E. Aydin, S. Ozcan, Anal. Chim. Acta 559 (2006) 173–180.
[22] S. Ozcan, A. Tor, M.E. Aydin, Anal. Chim. Acta 640 (2009) 52–57.
[23] J. Mierzwa, Y.C. Sun, M.H. Yang, Anal. Chim. Acta 355 (1997) 277–282.
[24] M.D. Luque de Castro, F. Priego-Capote, Analytical Applications of Ultrasound, Elsevier, Amsterdam, 2006.
[25] M.D. Luque de Castro, F. Priego-Capote, Talanta 72 (2007) 321–334.
[26] US EPA Method 3510C, Separatory Funnel Liquid–Liquid Extraction (Revision 3-December, 1996), SW846 CH 4.2.1.
[27] APHA/AWWA/WEF, in: L.S. Clesceri, A.E. Greenberg, A.D. Eaton (Eds.), Standard Methods for the Examination of Water and Wastewater, 21st ed., American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC, 2005.
[28] A. Tankeviciute, R. Kazlauskas, V. Vickackaite, Analyst 126 (2001) 1674–1677.
[29] HSDB (Hazardous Substances Data Bank), National Library of Medicine, National Toxicology Information Program, HSDB, USA, 2006.
[30] L. Zhao, H.K. Lee, J. Chromatogr. A 919 (2001) 381–388.
[31] F. Rezaei, A. Bidari, A.P. Birjandi, M.R.M. Hosseini, Y. Assadi, J. Hazard. Mater. 158 (2008) 621–627.
[32] T.J. Mason, J.P. Lorimer, Applied Sonochemistry: Uses of Power Ultrasound in Chemistry and Processing, Wiley VCH Verlag GmbH, Weinheim, 2002.
[33] L.H. Keith, W. Crummett, J. Deegan, R.A. Libby, J.K. Taylor, G. Wentler, Anal. Chem. 55 (1983) 2210.
[34] A.J. King, J.W. Readman, J.L. Zhou, Anal. Chim. Acta 523 (2004) 259–267.
[35] L. Hou, H.K. Lee, J. Chromatogr. A 976 (2002) 377–385.
[36] M.R. Khalili Zanjani, Y. Yamini, S. Shariati, J.A. Jönsson, Anal. Chim. Acta 585 (2007) 286–293.
[37] S Environmental Protection Agency (EPA), QA/QC Guidance for Sampling and Analysis of Sediments, Water and Tissues for Dredged Material Analysis, EPA, 1995.