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Chemometric optimization of dispersive suspended microextraction followed by gas chromatography–mass spectrometry for the determination of polycyclic aromatic hydrocarbons in natural waters

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ABSTRACT

A dispersive suspended microextraction (DSME) method coupled with gas chromatography–mass spectrometry (GC–MS) was developed and validated for the simultaneous determination of ten polycyclic aromatic hydrocarbons in real water samples. The optimization of the method was achieved with a 2^{7-4} Plackett–Burman design, while the significant factors were optimized using a central composite design (CCD). The parameters that were studied included the sample volume, organic solvent volume, extraction time, restoration time and organic solvent. The optimum experimental conditions for the proposed method comprised 4.3 mL of the water sample, 93 μL of toluene as the extraction solvent, a 104-s extraction time and a 10-min restoration time. The recoveries varied from 70 to 111%. Chrysene was the least recovered compound, while anthracene displayed the highest extraction efficiency. The analytical method (DSME) was shown to be linear ($R^2 > 0.993$) over the studied range of concentrations, exhibiting satisfactory precision (RSD% < 10.6%) and reaching limits of detection between 8 and 46 ng L^{-1}.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that belong to a huge family of molecules containing two or more aromatic rings [1]. PAH residues can be found in natural water samples [2,3], in the atmosphere (outdoor [4] and indoor [5]), in soils [6,7] and in many other matrices because of their wide distribution throughout the environment. Additionally, PAHs can be found in living organisms, as well as sediments [8], which work as natural filters in the sea. PAHs are of special concern because they present toxic, carcinogenic and mutagenic properties [1].

The most important anthropogenic sources of PAHs are emissions from vehicle exhaust, asphalt pavement and heating appliances. However, various natural sources also contribute to PAH levels, such as incomplete combustion processes at high temperatures and pyrolytic processes involving fossil fuels such as peat, coal and petroleum [9]. Forest fires have also drawn attention because they are a major natural source of polycyclic aromatic hydrocarbons (PAHs) through incomplete combustion [10]. Finally volcanic activity and biosynthesis by bacteria and plants are other natural sources of PAHs but contrary to previous sources, they contribute small amounts to the environment [11].

Once emitted, PAHs can undergo long-range transport and significantly enhance the near ground PAH concentrations in surrounding countries under certain meteorological conditions [12].

The contamination of surface and ground waters with PAHs concerns many researchers around the world. Numerous analytical methods based on extraction techniques have been developed in recent years. Methods based on liquid–liquid extraction (LLE) were the first to allow determination of PAH residues in water samples [13]. The most common method for the extraction of PAHs from water samples is solid phase extraction (SPE) [2,9,14-18]. The most recent traditional method for the extraction of PAHs is solid phase microextraction (SPME) [19–22].

In recent years, new microextraction methods have been developed to make analysis faster and cheaper by using smaller volumes of solvents. These techniques include single-drop microextraction (SDME) [23,24], ultrasound-assisted emulsification-microextraction (USAEME) [25,26], dispersive liquid–liquid microextraction (DLLME) [27–31], vortex-assisted liquid–liquid microextraction (VALLME) [32,33], and directly suspended droplet microextraction (DSDME) [34–37].

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In 2011, Yang et al. [38] developed a new extraction method called dispersive suspended microextraction (DSME). The method is based on high speed agitation of a small amount of organic solvent with an aqueous sample. In this method, two critical steps are involved: the extraction step and the restoration step. During the extraction step, target analytes are extracted into the extraction solvent, while during the restoration step, the organic and aqueous phases are separated. After the separation of the two phases, the target analytes are injected into GC–MS for further analysis.

Because of the high speed of the magnetic stirring bar in the extraction step, the extraction solvent forms fine droplets. This can significantly increase the contact surface between the two phases and minimize the extraction time. The organic phase forms in the top-center position of the vortex and it can be collected because it has a lower density than the aqueous phase. Dispersive suspended microextraction has overcome several of the disadvantages of other liquid-phase microextraction methods while retaining many of their advantages [38].

Most of the studies dealing with analytical methods make use of the traditional one-factor-at-a-time (OFAT) approach by examining the effect of each parameter individually while holding all others constant. However, the results of this univariate analysis show inadequate optimization toward response(s). Moreover, OFAT approach is costly in the sense of time and reagents, and not that efficient. There is now increasing recognition that hereditary malpractice ought to be replaced by sound chemometric methods such as response surface methodology (RSM) based on statistical design of experiments (DOE). Such statistical analyses are more efficient, since they account for interaction effects between the studied variables and determine more accurately the combination of levels that produces the optimum of the analytical method.

With the above in mind, the aim of this study is two-fold. The first is to exploit the analytical utility of DSME for the determination of PAHs in natural water samples, according to our knowledge for the first time, followed by gas chromatography–mass spectrometry (GC/MS). The second is to evaluate and optimize the method with the aid of response surface methodology and experimental design. For this reason, a Plackett–Burman factorial design as well as a central composite design (CCD) were employed for the determination of the optimized experimental conditions for the DSME extraction of analytes from natural water samples.

2. Experimental

2.1. Chemicals and reagents

Polycyclic aromatic hydrocarbons, namely, naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benz[*a*]anthracene (B[*a*]A), and chrysene (Chry), were purchased from Supelco (Bellefonte, Pennsylvania, USA). Toluene, hexane, isooctane, *p*-xylene, methanol and dichloromethane were supplied by Labscan (Dublin, Ireland), and benzene was supplied by Merck (Darmstadt, Germany). All solvents and reagents were analytical grade. An individual stock solution of each compound was prepared (1000 mg L^{-1}) in methanol:dichloromethane (1:1), and a standard mixture solution of all target analytes was prepared in the same solvent at a concentration of 1000 μg L^{-1}. The water sample used for optimization was collected from the Louros River springs (Epirus, NW Greece) and did not contain PAH residues above the limit of detection. Moreover, six environmental water samples, including river water, and lagoon water samples, were selected (Epirus, NW Greece) to examine the applicability of the proposed method.

2.2. Apparatus

Analyses were performed using a Trace GC Ultra instrument (Thermo Scientific, Austin, Texas, USA) coupled to an ISQ mass spectrometer controlled by a computer running XCalibur software. The separation was performed using a TR-5 column with a film thickness of 0.25 μm (30 m × 0.25 mm i.d., Thermo Fisher Scientific, Austin, Texas, USA). Helium (purity > 99.999 vol%, Air Liquide, Greece) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The GC oven temperature program was as follows: initial temperature of 60 °C for 1 min, 6 °C min^{-1} to 175 °C (held for 4 min), 3 °C min^{-1} to 235 °C, and finally 8 °C min^{-1} to 300 °C (held for 7 min). The injector was set at 220 °C in the splitless mode. The temperatures of the ion source and the interface were set at 220 °C and 250 °C, respectively. The mass spectrometer was operated in the electron ionization mode at an ionization energy of 70 eV. In the selected-ion monitoring (SIM) acquisition mode, the target ions were monitored at different time windows defined by the corresponding retention times. Three ions of each analyte were chosen according to the characteristic features of the mass spectra obtained in the full-scan mode and through comparison with the NIST library. The quality criteria adopted for the retention times of the analytes as well as the relative intensities of the selected ions were within the tolerances established by the 2002/657/EC directive concerning the performance of analytical methods and the interpretation of results [39]. The retention times as well as the identification and quantification ions selected for the target compounds are shown in Table 1. Fig. 1 depicts a typical DSME/GC–MS chromatogram obtained from a spiked (10 μg L^{-1}) natural water sample.

2.3. Dispersive suspended microextraction (DSME) procedure

For the DSME procedure (Fig. 2), 4.3 mL of aqueous sample was placed in a 10-mL crimp-top glass vial sealed with an open centered aluminum cap and with a PTFE-gray butyl septum. When needed, the sample was fortified with a solution of target compounds to obtain the desired final concentration (10 μg L^{-1}). A 93-μL aliquot of toluene, taken with a 100 μL single channel pipette (Gilson, Middleton, Wisconsin, USA, accuracy ≤ 0.8 μL), was added as the extraction solvent to the surface of the aqueous sample. A magnetic micro stirring bar (10 mm × 3 mm o.d., VWR, Arlington Heights, Illinois, USA) was placed at the bottom of the vial. The sample vial was placed on a magnetic stirrer to stir the sample.

The DSME procedure first involved an extraction step, followed by a restoration step. In particular, the stirring speed was set at 1500 rpm (extraction speed) for 104 s (extraction time) and then was reduced to 800 rpm (restoration speed). In the extraction step, a cloudy solution was formed, and the analytes in the water sample were extracted into fine toluene droplets. When the stirring speed was adjusted to 800 rpm, the restoration step began. At this stirring speed, a gentle vortex was formed and the toluene droplets began to coalesce. After 10 min (restoration time), the toluene had separated from the aqueous phase, and an organic droplet (85–87 μL) was formed in the bottom center of the vortex. Finally, after the restoration step, 1.5 μL of the organic phase was collected with a 10 μL micro syringe (Hamilton, Reno, Nevada, USA) with a small hub removable needle (26s gauge, 2 in, point style 2) and injected into the GC–MS for further analysis.

3. Results and discussion

3.1. Preliminary experiments

First, an appropriate extraction solvent for the DSME technique was selected to recover the studied compounds. It was important

Table 1
Retention times, molecular weights and target ions for the GC–MS analysis of the target compounds.

Compound	Cas number	Time window (min)	Retention time (min)	Molecular weight ($g\,mol^{-1}$)	Quantification and identification ions (m/z)
Naphthalene	91-20-3	5-14.5	11.32	128.18	**128**, 127, 51
Acenaphthylene	208-96-8	14.5-20.5	17.35	152.19	**152**, 76, 151
Acenaphthene	83-32-9		18.04	154.21	**154**, 152, 76
Fluorene	86-73-7		20.13	166.22	**166**, 165, 82
Phenanthrene	85-01-8	20.5-37	25.49	178.22	**178**, 152, 89
Anthracene	120-12-7		25.86	178.22	**178**, 152, 89
Fluoranthrene	206-44-0		34.37	202.26	**202**, 200, 101
Pyrene	120-00-0	37-57.3	36.05	202.26	**202**, 200, 101
Benz[a]anthracene	56-55-3		45.52	228.29	**228**, 226, 114
Chrysene	218-01-9		45.74	228.29	**228**, 226, 114

for the extraction solvent to fulfill the following criteria: (i) to be insoluble in water to form two different phases in solution and facilitate phase separation, (ii) to exhibit a lower density than water to rise above the aqueous phase, (iii) to have a high extraction capability toward the target analytes and (iv) to display good gas chromatographic behavior. The solvents that were studied and met these requirements were toluene, hexane, isooctane, *p*-xylene and benzene. The results revealed that toluene and benzene had the highest extraction efficiency (expressed as recoveries) compared to the other tested solvents (Fig. S1, Supplementary information); these were therefore selected for further examination in the experimental design.

The influence of the ionic strength on the efficiency of the DSME was evaluated with the addition of NaCl with concentration in the range of 0–3.5% (w/v). In all cases enrichments factors were slightly decreased with increasing ionic strength. It is supposed that this observation could be attributed to the formation of a physical barrier across the aqueous and organic phase interface, which prevents the mass transfer of analytes into the extraction solvent [38].

3.2. Experimental design

To evaluate the main factors affecting the efficiency of the DSME method, i.e., the sample volume, extraction solvent volume, extraction time, restoration time and extraction solvent, a design with two steps (screening and optimization) was used to screen the optimal experimental conditions. For this purpose, the STATISTICA 7.0 (StatSoft Inc., Tulsa, USA) statistical package was used to generate the experimental matrix and to evaluate the results.

3.2.1. Plackett–Burman design

An experimental Plackett–Burman design was created to determine the main factors affecting the extraction efficiency (expressed as recoveries). This design was applied to evaluate the main effects of the following five real factors plus two dummy variables: the sample volume (V_s), extraction solvent volume (V_d), extraction time (T_{ext}), restoration time (T_{res}) and extraction solvent (Solv). Overall, the experimental design included eight experiments with one replication for each. In total, 16 experiments were carried out (Table S1, Supplementary information).

Analysis of variance (ANOVA) was performed to examine whether the studied experimental factors were significant in the performance of the proposed method. An effect was considered significant when it was above the standard error at the 95% confidence level ($p < 0.05$). According to the Pareto chart and analysis of variance, three factors were significant and were evaluated in the central composite design for further analysis (Fig. S2, Supplementary information). Specifically, the extraction solvent volume ($p = 0.041$) and extraction time ($p = 0.004$) had a positive effect, while the sample volume ($p = 0.011$) had a negative effect. According to the obtained results in the investigated experimental domain (Table S1, Supplementary information), the restoration

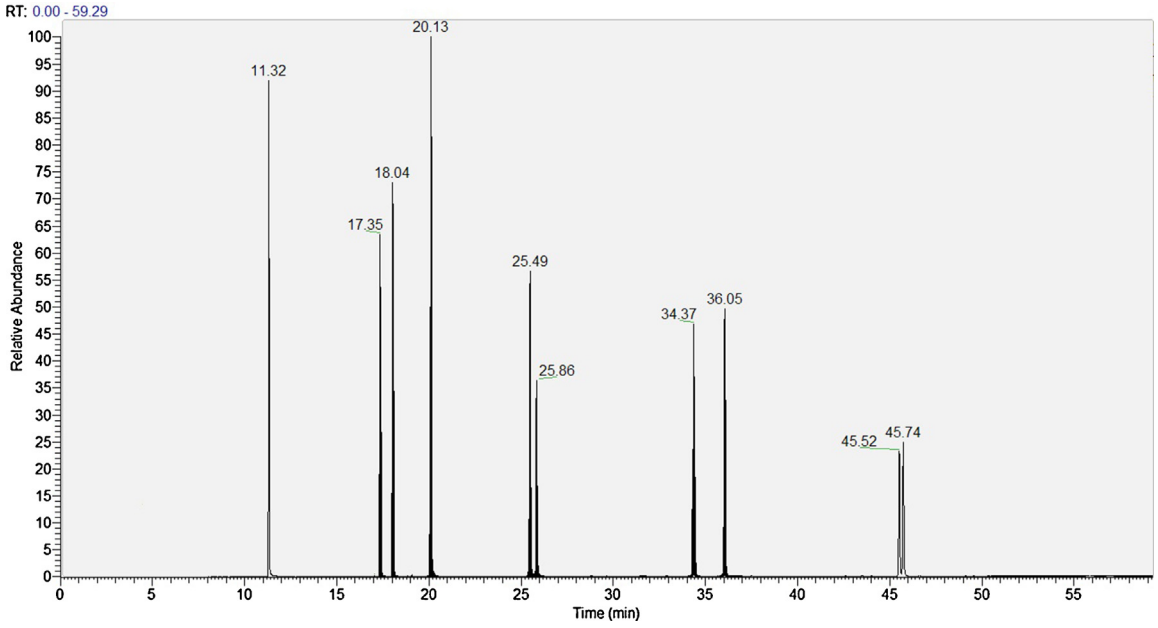

Fig. 1. Typical DSME/GC–MS chromatogram obtained from a spiked ($10\,\mu g\,L^{-1}$) natural water sample.

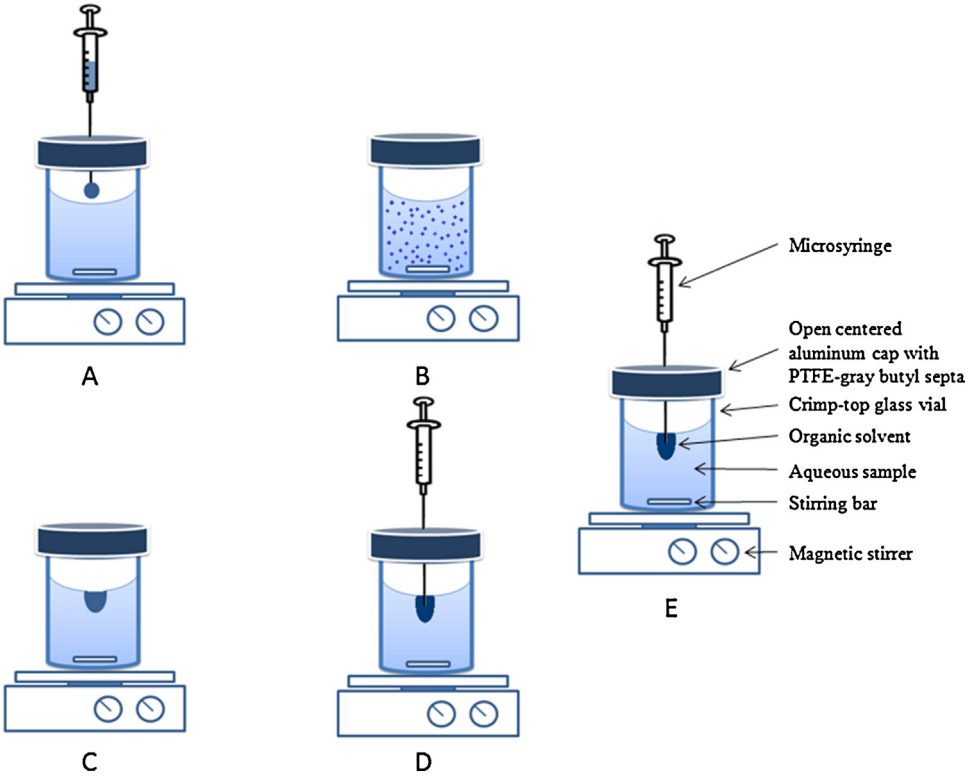

Fig. 2. Schematics procedure of DSME: (A) set up; (B) extraction step; (C) restoration step, (D) sampling, (E) parts of device.

time ($p = 0.215$) and solvent ($p = 0.301$) had no significant impact on the extraction yield and thus were kept fixed for further analysis. In particular, toluene was chosen as the extraction solvent because it separates rapidly from water, and it is more quickly recovered compared to benzene. In addition, the restoration time was selected to be 10 min because by this time the organic phase was separated from the aqueous phase. Phase separation of toluene could be achieved at lower restoration time, however, the recovered phase volume displayed a higher variability.

3.2.2. Central composite design

Following the results of the previous design, the next step was to optimize the analytical method for the remaining three factors by employing a central composite design (CCD). Seventeen experiments were required in this design with three central points. The conditions set in each experiment are listed in Table 2.

The main effects, interaction effects, and quadratic effects were evaluated through analysis of variance (ANOVA) at a spiked concentration of 0.01 mg L^{-1}. The lack of fit, which measures the failure of the model to represent the data in the experimental domain at points that are not included in the regression [40], was also evaluated and shown to be not significant (p value = 0.057), indicating the good response of the model. A summary of the ANOVA is included in Table 3.

From the pareto chart (Fig. S3, Supplementary information), it can be observed that all linear, interaction and quadratic effects were significant. Data analysis at the 95% confidence level permitted an expression (Eq. (1)) to be defined in terms of the significant coded factors:

$$Y(R\%) = 86.69 - 7.91_{V_s} - 6.09^2_{V_s} + 3.16_{V_d} - 0.39^2_{V_d} - 1.38_{T_{ext}} - 1.93^2_{T_{ext}} - 4.66_{V_s \times V_d} - 1.96_{V_s \times T_{ext}} + 2.25_{V_d \times T_{ext}} \quad (1)$$

where V_s is the sample volume, V_d is the extraction solvent volume and T_{ext} is the extraction time.

The reflected interaction between the sample volume and the extraction solvent (toluene) volume (Fig. S4, Supplementary information) showed that the highest extraction efficiency (recoveries) was obtained at a sample volume of 4.1–4.4 mL and at the highest levels of toluene (V_d). Under these conditions, the extraction solvent (toluene) was more efficiently dispersed in the aqueous solution (resulting in the highest extraction yield of the target analytes) and then restored to form the organic droplet. This observation was also confirmed by the fact that the same amount of toluene (85–87 μL) was recovered at the end of the phase separation process, irrespective of the sample volume. Considering the interaction between the toluene volume (V_d) and the extraction time (Fig. S4, Supplementary information), when a large extraction volume was used, a longer extraction time was required. However, for a fixed V_d, a sample volume close to 4.3 mL and an extraction time above 100 s produced the highest extraction yields (Fig. S4, Supplementary information). It should be noted that extraction times longer than 110 s were insufficient due to the inability of toluene to be restored to a single droplet after the extraction.

The overall optimal extraction conditions were calculated by the software (99.9% average recovery of the target analytes) to comprise a 4.3 mL sample volume, a 93 μL organic solvent volume, a 104 s extraction time, toluene as the organic solvent and a 10-min restoration time.

The enrichment factor (EFs), which was defined as the ratio of the analyte concentration in the extraction solvent after the extraction process and the concentration of analyte in water before the extraction process, was used to evaluate the extraction yield under different experimental conditions. Enrichment factors (EFs) were in the range of 35–55.

3.3. Analytical performance of DSME

The figures of merit of the proposed method were evaluated with the determination of the limits of detection (LODs) and

Table 2
Central composite design showing factors, codes and levels.

Factors	Levels			Star point ($\alpha = 1.682$)	
	Low (−1)	Central (0)	High (+1)	$-\alpha$	$+\alpha$
(V_s) sample volume (mL)	4.25	4.5	4.75	4.08	4.92
(V_d) extraction solvent volume (μL)	72	80	88	66.5	93.5
(T_{ext}) extraction time (s)	85	100	115	75	125

Runs	V_s	V_d	T_{ext}	Average recovery (R%)
1	−1	−1	−1	56.9
2	−1	−1	+1	86.4
3	−1	+1	−−1	77.3
4	−1	+1	+1	86.9
5	+1	−1	−1	80.6
6	+1	−1	+1	91.8
7	+1	+1	−1	96.1
8	+1	+1	+1	78.2
9	$-\alpha$	0	0	79.9
10	$+\alpha$	0	0	86.7
11	0	$-\alpha$	0	69.3
12	0	$+\alpha$	0	67.2
13	0	0	$-\alpha$	82.2
14	0	0	$+\alpha$	91.8
15	0	0	0	65.6
16	0	0	0	82.9
17	0	0	0	79.5

quantification (LOQs), the intra-day and inter-day precision, the trueness expressed as R% and the linear range; they were calculated to evaluate the analytical performance of the method under optimum conditions.

3.3.1. Limit of detection

The limits of detection (LODs) and limits of quantification (LOQs) of the proposed method were based on a signal-to-noise ratio of 3 ($S/N \geq 3$) and 10 ($S/N \geq 10$), respectively. The detection limit of the method ranged from 8 to 46 ng L^{-1}, while the quantification limits ranged between 24 and 138 ng L^{-1}.

3.3.2. Precision

The intra-day repeatability (RSD_r%, $n = 5$) and inter-day reproducibility (RSD_R%, $n = 5 \times 3$ days) were expressed by calculating the relative standard deviation (RSD%). Experiments were carried out by extracting a water sample spiked at 10 μg L^{-1}. The intra-day repeatability ranged between 3.7 and 10.6%, while the inter-day reproducibility ranged between 3.3 and 10.9% (Table 4).

3.3.3. Linear range

To investigate the linearity of the method, extractions of seven spiked water samples were used. Each concentration was injected in triplicate. The linear determination coefficients ranged between 0.993 and 0.999 (Table 4). The linear range for each compound is listed in Table 4.

3.3.4. Method trueness

The trueness of the method was evaluated by recovery studies. The following equation was used to calculate the recovery:

$$\text{Analytical recovery } \% = \frac{c \text{ analyte found}}{c \text{ analyte added}} \times 100\%$$

where c analyte found is the analyte concentration found in the spiked water sample, and c analyte added is the theoretical spiked analyte concentration.

The water sample used for the recovery studies did not contain PAH residues above the limit of detection. The recoveries were calculated at three different concentrations. Water samples were spiked at concentrations of LOQ, 5 × LOQ and 10 × LOQ for each compound. Table 4 shows the results from these concentration levels.

3.4. The influence of the addition of humic acid on the DSME extraction efficiency

Humic matter can reduce the amount of extractable organic compounds and/or interfere in their analysis. The influence of humic acid on the extraction efficiency (expressed as recoveries)

Table 3
ANOVA results obtained by central composite design.

	Sum of Squares (SS)	Degrees of freedom	Mean Squares (MS)	F-value	p-Value
V_s(L)	854.510	1	854.5096	9876	0.0001
V_s(Q)	416.762	1	416.7619	4817	0.0002
V_d(L)	136.616	1	136.6164	1579	0.0006
V_d(Q)	1.700	1	1.7004	20	0.0473
T_{ext}(L)	25.846	1	25.8456	299	0.0033
T_{ext}(Q)	40.637	1	40.6372	470	0.0021
V_s(L) by V_d(L)	173.358	1	173.3583	2004	0.0005
V_s(L)by T_{ext}(L)	30.641	1	30.6414	354	0.0028
V_d(L)by T_{ext}(L)	40.542	1	40.5420	469	0.0021
Lack of fit	7.295	5	1.4589	17	0.0569
Pure error	0.173	2	0.0865		
Total SS	1712.590	16			

(V_s) = sample volume, (V_d) = extraction solvent volume and (T_{ext}) = extraction time.

Table 4
Quality factors for the analytical performance of DSME.

Compound	R^2[a]	RSD_r[b] (%)	RSD_R[c] (%)	LOD (ng L^{-1})	LOQ (ng L^{-1})	Linear range (ng L^{-1})	Enrichment factors	Recovery[d] (%)	Recovery[e] (%)	Recovery[f] (%)
Naphthalene	0.993	7.3	6.7	8	24	25–500	52	106	108	107
Acenaphthylene	0.998	5.0	4.6	13	39	50–750	51	104	105	104
Acenaphthene	0.999	3.7	3.3	12	36	50–750	44	89	97	98
Fluorene	0.998	4.3	5	13	39	50–750	43	86	93	94
Phenanthrene	0.996	5.1	4.7	18	54	75–1000	45	92	97	96
Anthracene	0.998	10.6	10.9	24	72	75–1000	55	111	113	114
Fluoranthrene	0.995	6.5	6.7	16	48	50–750	50	101	103	105
Pyrene	0.996	4.5	4.7	14	42	50–750	47	96	102	93
Benz[*a*]anthracene	0.998	6.5	8.2	46	138	150–1500	38	77	92	90
Chrysene	0.999	5.2	3.8	41	123	150–1500	35	70	89	91

[a] Linear coefficient of determination.
[b] Intra-day precision, $n = 5$.
[c] Inter-day precision, $n = 5$.
[d] Concentration level = LOQ for each compound.
[e] Concentration level = 5 × LOQ for each compound.
[f] Concentration level = 10 × LOQ for each compound.

Table 5
Target compounds found in real water samples.

Compound	SS1 River	SS2 River	SS3 River	SS4 River	SS5 Lagoon	SS6 Lagoon
Naphthalene	nd	nd	nd	nd	nd	nd
Acenaphthylene	nd	nd	nd	nd	0.357	nd
Acenaphthene	nd	nd	nd	nd	nd	nd
Fluorene	nd	nd	0.443	0.449	nd	nd
Phenanthrene	nd	nd	nd	0.393	nd	nd
Anthracene	nd	nd	nd	0.385	nd	nd
Fluoranthrene	nd	nd	0.359	0.358	nd	nd
Pyrene	nd	nd	nd	0.263	nd	nd
Benz[*a*]anthracene	nd	nd	nd	nd	nd	nd
Chrysene	nd	nd	nd	0.256	nd	nd

SS: sample station; nd: not detected.

of the target analytes in the DSME protocol was therefore investigated. The recoveries of the analytes (LOQ concentration for each analyte) spiked at different concentrations of humic acid (12.5, 25 and 50 mg L^{-1}) were determined with the DSME/GC–MS procedure. Table S2 (Supplementary information) shows the recoveries obtained at different concentrations of humic acid for the analytes studied. For the majority of the analytes, similar recoveries were obtained in the presence of low and high humic acid concentrations. This suggests that the presence of organic matter has little influence on the detection of the analytes.

3.5. Comparison with other methods

A statistical comparison of the developed DSME method with DLLME [41] was performed to verify the agreement of the two results at 95% confidence level ($n = 5$). For simplicity, the variance was considered to be constant among the various samples. The samples were fortified at 250 ng L^{-1} to ensure the robustness of the measurements. Neither the individual *F*-test nor the *T*-test revealed that statistically significant differences were not found.

The proposed method was also compared to other microextraction methods with regard to PAHs determination in aqueous samples (Table S4, Supplementary information). Compared to SPME [20] and HSPME [42] extraction methods, the proposed DSME method is attained faster with an extraction/restoration process no longer than 12 min. Although DSME was equally compared to the sensitivity and LODs of DLLME [41], which displayed an extraction process of few seconds, the limitation of both methods was the use of toxic extraction solvents. Recently, Tseng et al., [43], showed that UDSA-DLLME and WLSEME are viable alternative techniques for sample pre-concentration in terms of performance and speed with minor limitations to sensitivity.

3.6. Real samples analysis

The proposed method was applied to real water sample analysis. Different types of water samples obtained from six sample stations were used, including river and lagoon sources. Among the target analytes, seven PAHs were detected in three of a total of six sample stations. Specifically, six PAHs were detected in one sample station, while three sample stations were totally free from PAH residues. Table 5 shows the results collected from the real water analysis.

4. Conclusions

In the present study, for the first time, directly suspended microextraction (DSME) coupled with GC–MS was evaluated for the simultaneous determination of PAH residues in real water samples. This simple, rapid and inexpensive extraction method fulfilled analytical validation criteria. The sensitivity and linear dynamic range were relevant to environmental water analysis, and accurate measurement of the compounds of interest in surface waters was achieved.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.06.019.

References

[1] S. Orecchio, V. Papuzza, Levels, fingerprint and daily intake of polycyclic aromatic hydrocarbons (PAHs) in bread baked using wood as fuel, J. Hazard. Mater. 164 (2009) 876–883.
[2] J.N. Brown, B.M. Peake, Determination of colloidally-associated polycyclic aromatic hydrocarbons (PAHs) in fresh water using C18 solid phase extraction disks, Anal. Chim. Acta 486 (2003) 159–169.
[3] M.J. Gómez, S. Herrera, D. Solé, E. García-Calvo, A.R. Fernández-Alba, Spatio-temporal evaluation of organic contaminants and their transformation products along a river basin affected by urban, agricultural and industrial pollution, Sci. Total Environ. 420 (2012) 134–145.
[4] A.M. Mastral, M.S. Callén, J.M. López, R. Murillo, T. Garcı´a, M.V. Navarro, Critical review on atmospheric PAH. Assessment of reported data in the Mediterranean basin, Fuel Process. Technol. 80 (2012) 183–193.
[5] R. Barro, J. Regueiro, M. Llompart, C. Garcia-Jares, Analysis of industrial contaminants in indoor air: Part 1. Volatile organic compounds, carbonyl compounds, polycyclic aromatic hydrocarbons and polychlorinated biphenyls, J. Chromatogr. A 1216 (2009) 540–566.
[6] M. Llompart, K. Li, M. Fingas, Headspace solid phase microextraction (HSSPME) for the determination of volatile and semivolatile pollutants in soils, Talanta 48 (1999) 451–459.
[7] K. Li, M. Mk Landriault, M. Fingas, Llompart, Accelerated solvent extraction (ASE) of environmental organic compounds in soils using a modified supercritical fluid extractor, J. Hazard. Mater. 102 (2003) 93–104.
[8] A.J. King, J.W. Readman, J.L. Zhou, Dynamic behaviour of polycyclic aromatic hydrocarbons in Brighton marina, UK, Mar. Pollut. Bull. 48 (2004) 229–239.
[9] E. Martinez, M. Gros, S. Lacorte, D. Barceló, Simplified procedures for the analysis of polycyclic aromatic hydrocarbons in water, sediments and mussels, J. Chromatogr. A 1047 (2004) 181–188.
[10] S.D. Choi, Time trends in the levels and patterns of polycyclic aromatic hydrocarbons (PAHs) in pine bark, litter, and soil after a forest fire, Sci. Total Environ. 470–471 (2014) 1441–1449.
[11] P. Babula, O. Vodicka, V. Adam, M. Kummerova, L. Havel, J. Hosek, I. Provaznik, H. Skutkova, M. Beklova, R. Kizek, Effect of fluoranthene on plant cell model: Tobacco BY-2 suspension culture, Environ. Exp. Bot. 78 (2012) 117–126.
[12] Y. Zhang, H. Shen, S. Tao, J. Ma, Modeling the atmospheric transport and outflow of polycyclic aromatic hydrocarbons emitted from China, Atmos. Environ. 45 (2011) 2820–2827.
[13] D.M. Brum, R.J. Cassella, A.D. Pereira-Netto, Multivariate optimization of a liquid–liquid extraction of the EPA-PAHs from natural contaminated waters prior to determination by liquid chromatography with fluorescence detection, Talanta 74 (2008) 1392–1399.
[14] S. Zhang, Q. Zhang, S. Darisaw, O. Ehie, G. Wang, Simultaneous quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and pharmaceuticals and personal care products (PPCPs) in Mississippi river water, in New Orleans, Louisiana, USA, Chemosphere 66 (2007) 1057–1069.
[15] J. Ma, R. Xiao, J. Li, J. Yu, Y. Zhang, L. Chen, Determination of 16 polycyclic aromatic hydrocarbons in environmental water samples by solid-phase extraction using multi-walled carbon nanotubes as adsorbent coupled with gas chromatography–mass spectrometry, J. Chromatogr. A 1217 (2010) 5462–5469.
[16] L. Oliferova, M. Statkus, G. Tsysin, O. Shpigun, Y. Zolotov, On-line solid-phase extraction and HPLC determination of polycyclic aromatic hydrocarbons in water using fluorocarbon polymer sorbents, Anal. Chim. Acta 538 (2005) 35–40.
[17] J. Jin, Z. Zhang, Y. Li, P. Qi, X. Lu, J. Wang, J. Chen, F. Su, Enrichment of polycyclic aromatic hydrocarbons in seawater with magnesium oxide microspheres as a solid-phase extraction sorbent, Anal. Chim. Acta 678 (2010) 183–188.
[18] V. Khalili-Fard, K. Ghanemi, Y. Nikpour, M. Fallah-Mehrjardi, Application of sulfur microparticles for solid-phase extraction of polycyclic aromatic hydrocarbons from sea water and wastewater samples, Anal. Chim. Acta 714 (2012) 89–97.
[19] R.A. Doong, S.M. Chang, Y.C. Sun, Solid-phase microextraction for determining the distribution of sixteen US Environmental Protection Agency polycyclic aromatic hydrocarbons in water samples, J. Chromatogr. A 879 (2000) 177–188.
[20] A.J. King, J.W. Readman, J.L. Zhou, Determination of polycyclic aromatic hydrocarbons in water by solid-phase microextraction–gas chromatography–mass spectrometry, Anal. Chim. Acta 523 (2004) 259–267.
[21] H. Bagheri, E. Babanezhad, A. Es'Haghi, An aniline-based fiber coating for solid phase microextraction of polycyclic aromatic hydrocarbons from water followed by gas chromatography-mass spectrometry, J. Chromatogr. A 1152 (2007) 168–174.
[22] E. Rianawati, R. Balasubramanian, Optimization and validation of solid phase micro-extraction (SPME) method for analysis of polycyclic aromatic hydrocarbons in rainwater and stormwater, Phys. Chem. Earth 34 (2009) 857–865.
[23] S. Shariati-Feizabadi, Y. Yamini, N. Bahramifar, Headspace solvent microextraction and gas chromatographic determination of some polycyclic aromatic hydrocarbons in water samples, Anal. Chim. Acta 489 (2003) 21–31.
[24] Y. Wu, L. Xia, R. Chen, B. Hu, Headspace single drop microextraction combined with HPLC for the determination of trace polycyclic aromatic hydrocarbons in environmental samples, Talanta 74 (2008) 470–477.
[25] S. Ozcan, A. Tor, M.E. Aydin, Determination of polycyclic aromatic hydrocarbons in waters by ultrasound-assisted emulsification-microextraction and gas chromatography–mass spectrometry, Anal. Chim. Acta 665 (2010) 193–199.
[26] A. Saleh, Y. Yamini, M. Faraji, M. Rezaee, M. Ghambarian, Ultrasound-assisted emulsification microextraction method based on applying low density organic solvents followed by gas chromatography analysis for the determination of polycyclic aromatic hydrocarbons in water samples, J. Chromatogr. A 1216 (2009) 6673–6679.
[27] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, Development of an ionic liquid based dispersive liquid–liquid microextraction method for the analysis of polycyclic aromatic hydrocarbons in water samples, J. Chromatogr. A 1216 (2009) 6356–6364.
[28] H. Xu, Z. Ding, L. Lv, D. Song, Y.Q. Feng, A novel dispersive liquid–liquid microextraction based on solidification of floating organic droplet method for determination of polycyclic aromatic hydrocarbons in aqueous samples, Anal. Chim. Acta 636 (2009) 28–33.
[29] M.I. Leong, C.C. Chang, M.R. Fuh, S.D. Huang, Low toxic dispersive liquid–liquid microextraction using halosolvents for extraction of polycyclic aromatic hydrocarbons in water samples, J. Chromatogr. A 1217 (2010) 5455–5461.
[30] L. Guo, H.K. Lee, Low-density solvent-based solvent demulsification dispersive liquid–liquid microextraction for the fast determination of trace levels of sixteen priority polycyclic aromatic hydrocarbons in environmental water samples, J. Chromatogr. A 1218 (2011) 5040–5046.
[31] J. Casado, R. Nescatelli, I. Rodríguez, M. Ramil, F. Marini, R. Cela, Determination of benzotriazoles in water samples by concurrent derivatization–dispersive liquid–liquid microextraction followed by gas chromatography–mass spectrometry, J. Chromatogr. A 1336 (2014) 1–9.
[32] I.P. Román, A. Mastromichali, K. Tyrovola, A. Canals, E. Psillakis, Rapid determination of octanol–water partition coefficient using vortex-assisted liquid–liquid microextraction, J. Chromatogr. A 1330 (2014) 1–5.
[33] A. Papadopoulou, I.P. Román, A. Canals, K. Tyrovola, E. Psillakis, Fast screening of perfluorooctane sulfonate in water using vortex-assisted liquid–liquid microextraction coupled to liquid chromatography–mass spectrometry, Anal. Chim. Acta 691 (2011) 56–61.
[34] F. Pena-Pereira, I. Costas-Mora, I. Lavilla, C. Bendicho, Rapid screening of polycyclic aromatic hydrocarbons (PAHs) in waters by directly suspended droplet microextraction-microvolume fluorospectrometry, Talanta 89 (2012) 217–222.
[35] A. Sarafraz-Yazdi, S. Raouf-Yazdinejad, Z. Es'haghi, Directly suspended droplet microextraction and analysis of amitriptyline and nortriptyline by GC, Chromatographia 66 (2007) 613–617.
[36] A. Sarafraz-Yazdi, F. Mofazzeli, Z. Es'haghi, Directly suspended droplet three liquid phase microextraction of diclofenac prior to LC, Chromatographia 67 (2008) 49–53.
[37] A. Sarafraz-Yazdi, A.H. Amiri, Z. Es'haghi, Separation and determination of benzene, toluene, ethylbenzene and o-xylene compounds in water using directly suspended droplet microextraction coupled with gas chromatography-flame ionization detector, Talanta 78 (2009) 936–941.
[38] Z.H. Yang, Y. Liu, Y.L. Lu, T. Wu, Z.Q. Zhou, D.H. Liu, Dispersive suspended microextraction, Anal. Chim. Acta 706 (2011) 268–274.
[39] Commission Decision 2002/657/EC of 12 August 2002, Off. J. Eur. Commun. L221 (2002) 8–36.
[40] G.N. Rallis, V.A. Sakkas, V.A. Boumba, T. Vougiouklakis, T.A. Albanis, Determination of organochlorine pesticides and polychlorinated biphenyls in post-mortem human lung by matrix solid-phase dispersion with the aid of response surface methodology and desirability function, J. Chromatogr. A 1227 (2012) 1–9.
[41] M. Rezaee, Y. Assadi, M-R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, Determination of organic compounds in water using dispersive liquid–liquid microextraction, J. Chromatogr. A 1116 (2006) 1–9.
[42] D. Djozan, Y. Assadi, Monitoring of Polycyclic Aromatic Hydrocarbons in Water Using Headspace Solid-Phase Microextraction and Capillary Gas Chromatography, Microchem. J. 63 (1999) 276–284.
[43] W.-C. Tseng, P.-S. Chen, S.-D. Huang, Optimization of two different dispersive liquid–liquid microextraction methods followed by gas chromatography–mass spectrometry determination for polycyclic aromatic hydrocarbons (PAHs) analysis in water, Talanta 120 (2014) 425–432.