A green dispersive liquid phase microextraction technique based on the solidification of switchable hydrophilic fatty acid for determination of polynuclear aromatic hydrocarbons in aqueous samples

Mahsa Sadat Hosseininejad, Hakim Faraji and Ahmad Jamshidi

ABSTRACT

A green dispersive liquid phase microextraction approach based on the solidification of switchable hydrophilic fatty acid as an extraction phase has been developed for the determination of 16 priority polynuclear aromatic hydrocarbons (PAHs) in aqueous samples. In this study, the centrifugation step was omitted by the applying salting-out phenomenon. The influence of main variables on the efficiency of the procedure was studied by chemometric methods. Under optimal conditions, the completion time for extraction was less than 1 min, and the detector response was linear in the range of 0.1–250 μg L^{-1}. Limit of detection and limit of quantitation were estimated as the concentration range of 0.01–0.14 μg L^{-1} and 0.03–0.47 μg L^{-1}, respectively. The precision consists of repeatability and reproducibility, which were determined by calculating the relative standard deviation percent; their values were less than 7.2% and 10.5%, respectively. Applicability of the developed procedure was successfully evaluated for the analysis of PAHs in different water samples.

Key words | aqueous samples, centrifugeless, green analytical chemistry, green dispersive liquid phase microextraction, polynuclear aromatic hydrocarbons

Mahsa Sadat Hosseininejad
Hakim Faraji (corresponding author)
Department of Chemistry, Varamin-Pishva Branch,
Islamic Azad University,
Varamin 338177489,
Iran
E-mail: *hakimfaraji@yahoo.com*

Ahmad Jamshidi
Department of Novel Drug Delivery Systems,
Iran Polymer and Petrochemical Institute,
P. O. Box 14185/458, Tehran,
Iran

HIGHLIGHTS

- A green microextraction approach to analyze polynuclear aromatic hydrocarbons in water samples.
- Overcoming the challenges of conventional dispersive liquid–liquid microextraction and its various species.
- Reducing the organic waste, diminishing energy consumption and enhancing the operator safety.
- Replacing toxic reagents with safer ones, and making it easy to automate the process.
- One step closer to green/sustainable analytical chemistry by using chemometrics.

INTRODUCTION

The green analytical chemistry (GAC) framework, a branch of green chemistry, was initially defined by L. H. Lawrence as 'the use of analytical chemistry techniques and methodologies that reduce or eliminate solvents, reagents, preservatives and other chemicals that are hazardous to human health or the environment and that may also enable faster and more energy efficient analysis without compromising performance criteria' (Faure *et al.* 2014). Sample pretreatment is an essential step prior to instrumental determination for trace or ultra-trace concentration of pollutants in complex matrices. This step, including sample collection and storage, separation, extraction, isolation and pre-concentration, takes up to 80% of the analysis time for a significant number of analytical methods, and also is the biggest consumer of solvents and other chemicals (Kaljurand & Koel 2011; Koel 2016).

Hence, pioneering efforts have also being made to overcome or, at least, improve the GAC challenges. These endeavors have focused on the use of chemometrics and statistics methods for the decrease of the number of experiments and samples, the use of less toxic solvents, preferably natural reagents, the reduction of chemical

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consumption, application of integrated analytical systems for enhancement of analytical efficiency, and the miniaturization of formal sample preparation methods to meet the GAC requirements (Tobiszewski *et al.* 2012). In this way, microextraction techniques were introduced as miniaturization versions of conventional solid phase extraction (SPE) and liquid–liquid extraction (LLE) for greening sample preparation. Solid phase microextraction (SPME), liquid phase microextraction, and their modifications were developed to improve the significant drawbacks of traditional extraction techniques (Kokosa 2015).

In 2006, Assadi and colleagues introduced dispersive liquid–liquid microextraction (DLLME), which displayed many benefits, such as speed, simplicity, relative cheapness, low solvent consumption, and high efficiency (Rezaee *et al.* 2006). DLLME was extensively used for sample preparation of organic and inorganic analytes in different matrices (Campillo *et al.* 2017). However, there are some drawbacks to using this method, e.g., limitations of using chlorinated extracting solvents, requiring a second solvent for the dispersion, the difficulty in collecting the organic solvent after phase separation, and the centrifugation step, which are not in agreement with the new trends of GAC. These shortcomings have led to an attempt to develop the classical procedure modalities (Gilberto-Primel *et al.* 2017).

Most of the disadvantages mentioned above were improved by developing modifications (Hashemi *et al.* 2017). Currently, ionic liquids (Asensio-Ramos *et al.* 2011), supramolecular solvents (Ebrahimpour *et al.* 2014), surfactants (Moradi *et al.* 2011), non-halogenated solvents with a density lower than that of water (Faraji & Mohammad-Ali 2012), or switchable solvents with some of the significant advantages over the chlorinated organic solvents are considered as alternatives to conventional organic solvents (Naeemullah *et al.* 2016). Some techniques, including magnetic stirring (Ranjbari & Hadjmohammadi 2015), ultrasound irradiation (Ahmadvand *et al.* 2015), vortex agitation (Jouyban *et al.* 2016), and air pumping have been introduced into DLLME to replace the common disperser solvents (Faraji *et al.* 2013). In order to simplify collecting the extracting solvent after phase separation, solidification of the floating organic drop (Ahmadi-Jouibari *et al.* 2014), and some modified vessels were used (Leong *et al.* 2014). Generally, several approaches, including solvent de-emulsification (Liang *et al.* 2013), gas flotation (Molaei *et al.* 2015), salt induction (Mirparizi *et al.* 2017), and magnetic retrieval have been already used to eliminate the centrifugation step (Wu *et al.* 2016). To the best of the authors' knowledge, there are no previous reports to overcome or to improve all the shortcomings of the formal DLLME method. Hence, the aim of this study is to develop a green microextraction procedure, according to GAC principles, for simultaneously improving the challenges of the DLLME.

In this survey, some strategies were adopted for making the conventional DLLME method greener. In order to overcome the limitations of using the routine solvents, a medium-chain saturated fatty acid named switchable hydrophilic solvent was employed. This solvent has a freezing point near the ambient temperature, so can rapidly solidify at low temperatures as an extraction phase. The dispersion procedure was also performed by varying the pH of solution. In this manner, the hydrophobic fatty acid was ionized to a hydrophilic form with surfactant properties. Therefore, the extraction phase was dispersed into the sample solution in the absence of dispersive solvent. To omit the centrifugation step, micro-emulsions of the fatty acid were then separated from aqueous phase by using the salting-out effect. Eventually, with the purpose of facilitating the extracting phase collection after the phase separation, the floating extraction solvent was solidified and a bell-shaped collection device (BSCD) was used (Čabala & Bursová 2012). Chemometrics and multivariate analysis have also been used for development of GAC.

The new method is named green dispersive liquid phase microextraction (G-DLPME) based on the solidification of switchable solvent and salting-out effect. The effects of various experimental variables on extraction efficiency were evaluated by focusing on the chemometrics. The proposed method coupled with high performance liquid chromatography–fluorescence detection (HPLC-FLD) was developed and validated for determination of 16 priority polynuclear aromatic hydrocarbons (PAHs), designated by the US Environmental Protection Agency (EPA), in aqueous samples (EPA 1999).

EXPERIMENTAL

Materials and reagents

An EPA standard mixture of 16 priority PAHs, which contains 2,000 mg L^{-1} of acenaphthene (Ace), acenaphthylene (Acy), anthracene (Ant), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[ghi]perylene (BgP), benzo[a]pyrene (BaP), chrysene (Chr), dibenz[a,h]anthracene (DBA), fluoranthene (Flt), fluorene (Flu), indeno[1,2,3-cd]pyrene (Ind), naphthalene (Nap), phenanthrene (Phe), and pyrene (Pyr), was supplied

by Sigma-Aldrich Co. LLC (Bellefonte, PA, USA). All chemicals with the purity higher than 99.0% were obtained from Merck Chemicals (Darmstadt, Germany).

A standard stock solution, 20.0 μg mL^{-1}, in acetonitrile was prepared. Working solutions were prepared daily by diluting standard stock solution with ultrapure water, produced on a Milli-Q water purification system (Millipore, Bedford, MA, USA), to attain the needed PAHs concentration and were stored at 4°C.

The tap water sample was obtained from the laboratory of Islamic Azad University, Varamin branch. The river water sample was taken from Jajrood River. The wastewater sample was collected from an industrial zone near Varamin. The well water sample was obtained from a farm in Varamin. The samples were filtered through 0.45-μm membrane filters to remove suspended particulate matter and stored at 4°C before performing the extraction procedures.

Instruments and software

The analysis was performed using an Agilent 1200 LC system equipped with a fluorescence detector, binary pump, and a 20 μL injection loop manual injector (see supplementary material Table S1, available with the online version of this paper). A homemade BSED was created based on the reported work (Čabala & Bursová 2012).

All data analysis, modeling processing and design of experiments were performed and evaluated using Minitab, version 17.

Microextraction procedure

The experiments were performed using 5.0 mL of sample solution spiked with the analytes at concentration of 10.0 μg L^{-1} PAHs. The pH value of the sample was adjusted to 2.0 by using sulfuric acid (77 μL). An aliquot of 149 μL of sodium decanoate as extraction phase was rapidly injected into the sample for the formation of a homogeneous phase of micellar solution. The cloudy mixture was transferred into an SPE column containing 3 g of NaCl, and after 30 s, it was gently passed at a flow rate of 2.5 mL min^{-1} through the salt layer. Due to the critical increase of ionic strength in the contact surface area of salt with sample solution, the salt-induced phase separation phenomenon leads the movement of the tiny droplets of the extracting solvent up through the sample solution, and thereby the organic phase is compiled and floated as a separate layer on top of the homogeneous solution. Then the widened BSED end was submerged into the sample solution, and the organic layer containing the analytes was automatically pressed into the BSCD. Then the floated organic phase was solidified by transferring the column with the BSCD into an ice water bath for 3 minutes. Afterwards, the frozen decanoic acid, which was cautiously transferred to a conic vial, immediately melted at ambient temperature. Finally, analysis was performed by injecting 20.0 μL of the latter solution into the HPLC system.

RESULTS AND DISCUSSION

Optimization of the procedure

In this study, seven independent variables – the type of fatty acid, the hydrogen donor volume, extraction phase volume, extraction temperature, ionic strength, pH value, and flow rate of the sample solution through the SPE column containing 3.0 g NaCl – were investigated. Firstly, a univariate data analysis approach was used for choosing the optimal type of fatty acid and hydrogen donor. Then a two-steps multivariate strategy containing Plackett–Burman design (PBD) for screening and central composition design (CCD) for optimization were employed to select the other main optimal variables.

Selection of the type of fatty acid

The main objective of this study is to eliminate the use of conventional halogenated and hazardous solvents, and to facilitate the collection of the solvent after phase separation in order to achieve the maximum extraction efficiency. Thus it is of utmost importance to select a proper fatty acid as an extracting solvent. To achieve this purpose, chosen solvents must meet some requirements, such as high affinity for target species, switchable behavior, low solubility in water, compatibility with the LC-FLD technique, not interfering with chromatographic peaks of target analytes, and having a melting point near the room temperature (Faraji & Mohammad-Ali 2012). In this research three different fatty acids, dodecanoic acid, decanoic acid and undecanoic acid, were evaluated and optimized using the one-variable-at-a-time approach (see supplementary material Figure S1, available with the online version of this paper). A one-way analysis of variance (ANOVA) was conducted to compare the effect of extracting phase type on the extraction efficiency. The *p*-value for the extraction phase recovery ANOVA was less than 0.05. This result demonstrated that the mean

differences between the extraction efficiency of the fatty acids were statistically significant (see supplementary material Table S2, available online). The graph of the Tukey simultaneous confidence intervals displayed that the confidence interval for the difference between the means of undecanoic acid and decanoic acid was −9.09 to −0.91 (see supplementary material Figure S2, available online). This range does not include zero, which indicates that the difference between these means is significant. The confidence intervals for the remaining pairs of means all include zero, which indicates that the differences are not significant. Thus, decanoic acid was chosen because it has lower melting point and higher extraction efficiency than dodecanoic acid.

Screening design

In the next step, the PBD was applied as a screening method to evaluate the significant variables (i.e. volume of extraction phase, extraction temperature, volume of sulfuric acid (hydrogen donor), ionic strength, and flow rate of the sample solution), and to estimate the main effects of those variables on method performance through a multivariable approach. Subsequently, a total number of 24 experiments (containing four replicates at central points for assessing the experimental error) were randomly carried out. Details of the experimental design, including the code used, and the low and high levels of each factor are given in the supplementary material (Table S3, available online). ANOVA was conducted on the design to measure the significance of the model. Analysis of ANOVA results demonstrated that the model terms are significant (see supplementary material Table S4, available online). The results showed that extraction phase volume, extraction temperature, ionic strength, and acid volume have significant effects on PAHs relative recovery ($p < 0.05$), while the other variable does not significantly affect extraction efficiency (Figure 1(a)). The normal probability plot displayed the negative significant effect of ionic strength (Figure 1(b)). This can be explained by the fact that by increasing the ionic strength from 0% to 10%, the solution viscosity would increase, which makes mass transfer and interaction between analytes and the solvent difficult (this happens due to the increase of the solution viscosity by increasing ionic strength from 0% to 10%, which makes it difficult for mass to transfer and for analytes and solvent to interact together). Eventually extraction efficiency would be decreased. In this case, the subsequent experiments were carried out at a sample flow rate 2.5 mL min^{-1}, and without salt addition.

Optimization design

Two-level designs only support linear models of responses and are unable to supply information about optimum level or any non-linear relationships. Thus a design with more levels was necessary. The variables with significant effect established by PBD results were further studied. A response surface method design based on CCD was employed for optimization of the extraction phase volume (A), extraction temperature (B), and sulfuric acid volume (C). The factors, their levels, symbols and design matrix for CCD are illustrated in the supplementary material (Table S5, available online). In this study, overall, the matrix of CCD included 20 experiments (eight cube points, six center points in cube and six axial points), which were performed in random order. Analysis of variance was used to evaluate the statistical significance of the model. The results showed that the regression of the model was significant ($p < 0.05$), while the lack of fit was insignificant, $p > 0.05$ (see supplementary material, Table S6, available online). Moreover, a full quadratic model is the response regression model, which could be formulated by Equation (1):

$$Y = -557.0 + 3.4778A + 18.247B + 1.349C - 0.0136A^2 - 0.2274B^2 - 0.0071C^2 + 0.0072AB + 0.0037AC - 0.0204BC \quad (1)$$

The coefficient of determination (R^2), as a measure of the variation around the mean, was 99.89%, showing a good correlation between the experimental data and the fitted model. According to Equation (1), three-dimensional two-factor response surface plots were developed for providing graphical representation of the interactions, and for determination of optimal conditions of each variable for the maximum relative recovery of the total PAHs (Figure 2). A glance at the graphs reveals that the best results were obtained in about 149 μL of the extraction phase, sulfuric acid volume of 77 μL and an extraction temperature of 39 °C. As is presented in the surface plot, the extraction efficiency has been gradually increased to about 96% while the extraction phase volume was increased from 57 to 149 μL, and then it has been gradually decreased (Figure 2(a) and 2(b)). This could be assigned to the fact that, according to the principle of LLE, the analyte migration rate into the organic phase micro droplets is directly related to the surface area between the two liquid phases, and is conversely related to the organic phase volume. Although an increase in the extraction phase volume causes a rise in the

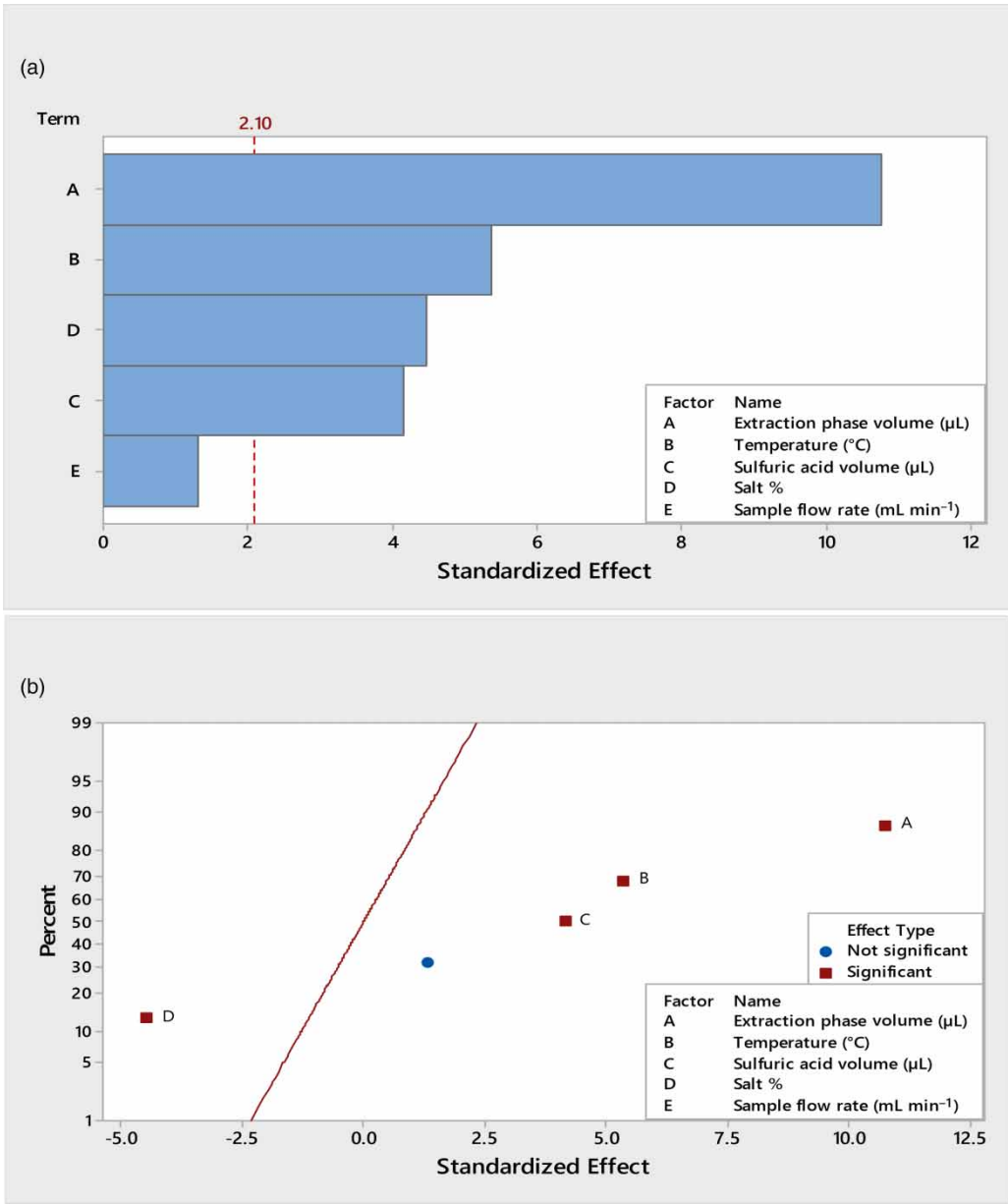

Figure 1 | (a) Pareto charts of the main effects obtained from the Plackett–Burman design. (b) Normal probability plot of standardized effects at $p = 0.05$.

interfacial area following the extraction efficiency, further increase in the solvent volume leads to decrease in the extraction efficiency.

On the other hand, Figure 2(a) and 2(c) suggest that the relative recovery was slightly increased by raising the temperature up to 39 °C, but further increase in the temperature attenuated the extraction efficiency. This could be expected, because the Henry's constants and diffusion coefficients are improved by increasing the temperature, so mass transfer is also enhanced and equilibrium time would be decreased. On the other hand, increase in the temperature of solution leads to reduced gas solubility and growth of gas bubble formation, which can cause quantification problem and lack of repeatability (Hou & Lee 2002; Shariati-Feizabadi *et al.* 2003). The experimental results in Figure 2(b) and 2(c) display that pH alteration has significant effect on the extraction efficiency. The practical strategy in this study is based on the fast and instantaneous conversion of hydrophilic sodium decanoate into hydrophobic form. Hence, to maximize solvent accessible surface area without the use of dispersive solvent, and to facilitate the extraction phase separation, the pH of solution should be less than the pK_a

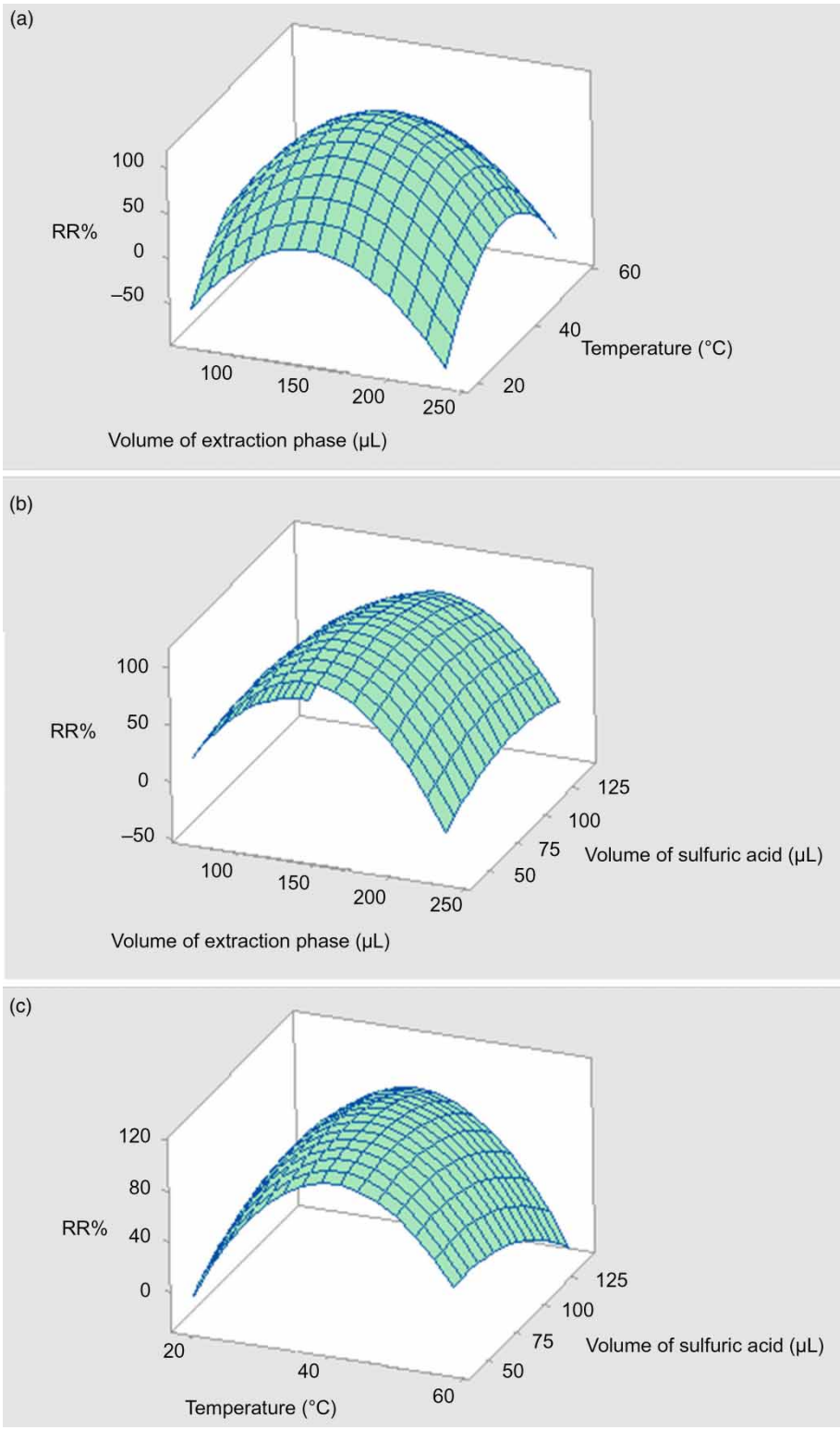

Figure 2 | Response surface plots on the sum of the peak area of PAHs as affected by the volume of extraction phase, volume of sulfuric acid, and extraction temperature: (a) surface plot of percentage relative recovery (RR%) vs extraction phase volume (µL) and temperature (°C) at a constant sulfuric acid volume (µL), (b) surface plot of RR% vs extraction phase volume (µL) and sulfuric acid volume (µL) at a constant temperature (°C), (c) surface plot of RR% vs temperature (°C) and sulfuric acid volume (µL) at a constant extraction phase volume (µL).

of decanoic acid. According to the results, the optimum amount of the sulfuric acid to reach this purpose was 77 μL. In accordance with the overall optimization study results, 149 μL sodium decanoate, 77 μL sulfuric acid, extraction temperature 39 °C, without salt addition and 2.5 mL min^{-1} sample flow rate were finally selected as the optimum conditions for extraction of the analytes in the next experiments.

Method validation study

Validation of the procedure was statistically studied pursuant to the International Conference on Harmonization (ICH) guidance document (ICH 2005), and based on validation procedure described in the previous study (Faraji & Helalizadeh 2017). Calibration model, linearity range, detection and quantitation limit, accuracy, precision (repeatability and reproducibility) were studied as the analytical characteristics.

Calibration functions were performed through the standard calibration curves supplied with 15 different concentrations of the analytes schemed as relative response vs concentration, giving the function model and coefficient of determination for each calibration curve (Table 1). Each calibration level was made in triplicate. Linearity was evaluated checking the suitability of function model by calculation of Fisher variance (*F*-test), where the function model is appropriate to demonstrate the considered data due to some significant lack of fit, if the ratio $F_{critical}/F_{experimental}$ value is higher than 1.0. The coefficients of determination above 0.9918 for all PAHs as well as the results of *F*-test confirmed that linear function models were significant (Table 1).

The analytical sensitivity was evaluated using the estimation of limit of detection (LOD) and limit of quantitation (LOQ) (Faraji & Helalizadeh 2017). As illustrated by the results presented in Table 2, LODs and LOQs for the PAHs were in the range 0.01–0.14 and 0.03–0.47 μg L^{-1}, respectively.

The accuracy was evaluated by comparing the percentage relative recovery (RR%) of the analytes in a sample spiked at low, medium and high concentrations levels. The results were statistically determined by comparative analysis (*t*-test), at 95% level of confidence, organizing the null hypothesis as H_0: $\bar{R}_{exp} = 100\%$ and the alternative hypothesis as H_1: $\bar{R}_{exp} \neq 100\%$. $\bar{R}_{exp}$ is the average experimental recovery. The null hypothesis (H_0) is accepted at significance level α if t_{exp} does not exceed the critical value at the level α (Faraji & Helalizadeh 2017). The results showed no statistical difference ($p > 0.05$), hence $t_{exp} < t_{(0.025,\ N-1)}$

Table 1 | Analytical calibration data and linearity

Compound	[a]t_r (min)	[b]LR (μg L^{-1})	Slope	Intercept	[c]R^2	[d]F_{exp}	[e]EF
Nap	3.11	0.150–200	0.1827	0.3083	0.9991	4.21	238
Acy	4.21	0.15–200	0.2944	0.1185	0.9928	2.83	214
Ace	4.76	0.15–200	0.1471	0.2304	0.9981	1.97	205
Flu	4.98	0.10–150	0.2634	0.2171	0.9952	3.05	371
Phe	5.49	0.150–200	0.3182	0.0711	0.9943	2.11	169
Ant	6.18	0.20–200	0.5462	−0.0835	0.9918	4.02	110
Flt	6.81	0.50–250	0.1607	0.0091	0.9991	2.61	268
Pyr	7.43	0.15–200	0.0831	0.1426	0.9985	2.08	137
BaA	7.96	0.10–150	0.1295	−0.0127	0.9976	1.86	114
Chr	9.35	0.150–200	0.1938	−0.0318	0.9953	2.47	186
BbF	9.82	0.10–150	0.2967	0.0629	0.9994	3.15	162
BkF	11.24	0.10–150	0.1356	0.0094	0.9927	2.26	171
BaP	11.81	0.10–150	0.1182	0.0341	0.9980	2.02	155
DBA	12.57	0.10–150	0.0774	−0.1033	0.9995	1.88	94
BgP	13.74	0.10–150	0.1121	0.0614	0.9991	2.43	192
Ind	14.48	0.10–150	0.1507	0.0826	0.9959	3.50	128

[a]t_r is retention time. [b]LR is linear range. [c]R^2 is the determination coefficient. [d]F_{exp} is the ratio of residual variance to squared pure error, critical $F_{(0.05,\ 42,\ 30)} = 1.79$ is the critical value of *F* with (I − 3) = 42 and (I − L) = 30 degrees of freedom at 95% confidence level, where I is the number of calibration samples (45) and L the number of concentration levels (15). [e]EF is enrichment factor.

Table 2 | Sensitivity (LOD and LOQ), accuracy, and precision results

	Sensitivity		Accuracy			Precision	
Compound	**LOD (μg L^{-1})**	**LOQ (μg L^{-1})**	**Spiked (μg L^{-1})**	**RR% (±SD) ($n = 3$)**	**t_{exp}**	**Repeatability (RSD%, $n = 10$)**	**Reproducibility (RSD%, $n = 3$)**
Nap	0.04	0.13	0.50	96.31 (0.37)	2.11	5.5	6.9
			1.00	95.98 (0.42)		2.8	5.8
			2.00	98.15 (0.53)		6.1	7.1
Acy	0.03	0.10	0.50	97.14 (0.91)	1.47	4.4	8.0
			1.00	100.08 (1.12)		5.7	7.4
			2.00	99.24 (0.64)		4.9	6.3
Ace	0.03	0.12	0.50	98.82 (0.93)	1.52	6.1	5.8
			1.00	96.53 (0.68)		7.2	6.5
			2.00	95.61 (1.09)		5.3	7.6
Flu	0.01	0.03	0.10	97.91 (0.94)	1.75	6.0	7.3
			0.5	98.51 (0.88)		3.6	6.4
			1.0	99.02 (1.19)		5.7	8.1
Phe	0.04	0.13	0.50	101.11 (0.52)	1.94	3.4	7.9
			1.00	104.01 (1.13)		6.7	6.2
			2.00	98.17 (0.61)		2.9	9.5
Ant	0.06	0.20	0.50	98.14 (0.83)	1.26	4.1	7.0
			1.00	99.37 (1.04)		5.0	6.3
			2.00	98.05 (0.95)		3.4	7.7
Flt	0.14	0.47	1.0	99.21 (0.46)	0.89	2.9	8.1
			2.0	99.53 (0.86)		3.7	7.5
			4.0	98.28(1.20)		5.3	5.6
Pyr	0.02	0.08	0.50	97.80 (0.94)	1.12	6.5	8.0
			1.00	96.12 (0.88)		4.8	7.9
			2.00	100.93 (1.19)		5.6	6.6
BaA	0.01	0.03	0.10	95.88 (1.52)	1.09	3.6	8.6
			0.5	98.14 (1.06)		4.1	6.4
			1.0	98.17 (0.75)		6.2	7.6
Chr	0.04	0.14	0.50	104.04 (0.91)	1.61	5.8	5.6
			1.00	97.43 (0.81)		4.6	7.3
			2.00	98.91 (0.59)		6.0	6.9
BbF	0.01	0.03	0.10	102.73 (0.63)	1.99	7.0	8.8
			0.5	101.31 (0.86)		5.3	7.4
			1.0	97.61(1.32)		6.4	8.5
BkF	0.01	0.03	0.10	99.07 (0.76)	2.20	4.8	5.4
			0.5	96.16 (1.52)		3.5	7.2
			1.0	101.94 (1.29)		5.7	6.9
BaP	0.01	0.03	0.10	99.13 (1.45)	1.28	1.9	7.5
			0.5	96.38 (066)		3.7	6.4
			1.0	98.71 (0.71)		4.9	5.9
DBA	0.01	0.03	0.10	101.14 (0.83)	1.41	7.1	10.5
			0.5	99.43 (1.04)		2.8	4.9
			1.0	101.05 (0.95)		6.2	6.8
BgP	0.01	0.03	0.10	97.82 (0.46)	1.53	5.4	5.6
			0.5	99.53 (0.86)		4.9	9.8
			1.0	103.61(1.20)		2.9	7.1
Ind	0.02	0.07	0.50	97.80 (0.94)	1.17	3.5	8.6
			1.00	98.16 (0.88)		1.7	6.2
			2.00	101.39 (1.19)		4.7	7.9

$t_{(0.025,\ 8)} = 2.30$.

(N is the number of test samples), and the accuracy of the method was confirmed (Table 2).

The precision was estimated by repeatability and reproducibility, and was expressed in relative standard deviation percent (RSD%). The repeatability was computed by assaying 10 replicates on the same day, and reproducibility by assaying three replicates each day for 3 days (Table 2). The precision was acceptable; all attained values were below the maximum defined limits. The results presented in Table 2 confirm the acceptability of the precision, where repeatability values ranged between 1.7 and 7.2% and the reproducibility values ranged from 4.9 to 10.5% (Faraji & Helalizadeh 2017). ANOVA was also applied to the obtained data over 3 days to evaluate the reproducibility. The results demonstrate that *p*-value was smaller than 0.05 at the 95% confidence level.

Analysis of real water samples

Applicability of the developed procedure was surveyed for the analysis of PAHs in tap water, river water, wastewater and well water samples under the optimal extraction procedure. The concentrations of the analytes determined in different real samples are summarized in the supplementary material (Table S7, available online). Although the PAHs were not detected in tap and river water samples, their existence was confirmed in the well water and especially wastewater sample. Naphthalene, fluorene, phenanthrene, and pyrene were determined in both samples of well water and wastewater. The results showed that acenaphthylene concentration has the highest amount (84.60 $\mu g\ L^{-1}$) and fluoranthene concentration has the lowest amount (1.91 $\mu g\ L^{-1}$) among wastewater sample analytes. Relative recovery values were statistically assessed by comparative analysis (*t*-test), at 95% level of confidence for evaluating the systematic error of the proposed method. The lack of systematic error and bias was confirmed by the results displayed in the supplementary material (Table S7) ($p > 0.05$, $t_{exp} < 2.30$).

Comparative study

The analytical characteristics of the present technique of PAHs analysis were compared with other microextraction techniques, especially different modalities of DLLME method, that were previously published. The results presented in the supplementary material (Table S8, available online) illustrate that the G-DLPME approach provides LODs and enrichment factors (EFs) superior or comparable to other procedures. The proposed approach is a greener, safer, more cost-effective, and more environmentally friendly approach compared to the other methods presented in the supplementary material (Table S8).

CONCLUSION

A green microextraction approach as a sustainable alternative to classical DLLME has been developed. The proposed technique was employed to overcome the challenges of conventional DLLME. The procedure was abbreviated G-DLPME. The pre-concentration/microextraction was performed based on the solidification of switchable hydrophilic fatty acid as an extraction phase. The dispersion procedure was also carried out by varying the pH of solution. The centrifugation step was omitted by applying the salting-out phenomenon. The extraction phase separated from aqueous sample was simply collected by employing a homemade device. The chemometric methods in three steps were used for optimization of the independent variables. The sample preparation in this method is unique, and more user-friendly compared to previous methods published in the literature because of eliminating disperser solvent and the centrifugation step. Moreover, other shortcomings, such as limitations of using toxic extracting solvents and the difficulty in collecting the organic solvent after phase separation as common problems of the classic DLLME method, have been masterly overcome. The LOD of the method is 0.01–0.14 $\mu g\ L^{-1}$ at a signal-to-noise ratio of 3. The sensitivity, precision, and accuracy of the method are statistically reliable. The modified DLLME technique provides a good performance and a wide dynamic range for quantification of the analytes. Due to the reasons mentioned above, this method will be qualified for routine analysis of trace levels of 16 PAHs. The developed method also makes it much easier to completely automate the conventional DLLME technique. Consequently, G-DLPME procedure complies at least with six principles of the green or sustainable analytical chemistry, comprising decreasing the sample size, easy automation of the process, reducing the organic waste, diminishing energy consumption, replacing toxic reagents with safer ones, and enhancing the operator safety, which were recommended by Galuska (Gałuszka *et al.* 2013). In this study, chemometrics have also been applied to support the development of green analytical microextraction.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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