

FULL PAPER

Salting out and vortex-assisted dispersive liquid-liquid microextraction based on solidification of floating organic drop microextraction (SO-VADLLME-SFODME) for extraction and determination of polycyclic aromatic hydrocarbons (PAHs) in water and solid samples followed by HPLC

Gholam Basati^a  | Asad Mirzaei^a | Sabah Shiri^{a,b,*}^aDepartment of Clinical Biochemistry, Faculty of Allied Medical Sciences, Ilam University of Medical Sciences, Ilam, Iran^bDepartment of Chemistry, Lorestan University, Khorramabad, Lorestan

In this research the possibility of application of an innovative, simple, economical, selective ability and green (environmentally friendly) method of salting out and Vortex-Assisted dispersive liquid-liquid microextraction based on solidification of floating organic drop by using high performance liquid chromatography with containing UV for pre-concentration, the extraction and determination of polycyclic in water and solid samples followed by HPLC was studied. In this method three integrated and combined approaches have been adopted so as to maximize the advantages of the approach used while minimizing their disadvantages. The effects of experimental variables such as pH, the type and volume of dispersive extracting solvents (μL , pH), the volume of organic drop microextraction (extracting solvent), salt density (concentration), centrifuge condition (time), extraction time and temperature were examined, and the process of optimization was obtained by using a software based on Response Surface Methodology (RSM) and Box-Behnken design [as the experimental model] and Desirability Function (DF). This method has appropriate linear calibration between 0.2 to 800 $\mu\text{g/L}$, and a significant limit of detection coefficient ($r^2 > 0.9991$) and low detection range (between 0.05 to 0.09 $\mu\text{g/L}$). The above method was also applied for the successful determination of polycyclic aromatic hydrocarbons in industrial was water, water and soil samples PAHs) and the percentage of recycling, repeatability and reproducibility, resulting in satisfactory results. Simplicity, being economical, quickness, proper repeatability, less consumption of organic solvent and efficient decomposition are of the principal advantages (merits) of the proposed method for the decomposition and determination of polycyclic aromatic hydrocarbons (PAHs) in water samples.

***Corresponding Author:**

Sabah Shiri

Email: sabahshiri5@yahoo.com

Tel.: +988433343936

KEYWORDS

Liquid-liquid microextraction; polycyclic aromatic hydrocarbons; HPLC.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are fat absorbent organic compositions formed from two or three aromatic cycles, dissipating into the environment through human activities [1-3]. These compounds (solutions) have rather low solvability in water, with a high tendency to absorb particles, dust floating in the street air and colloid [4]. They are decomposed when exposed to ultrasonic light (extraction). Natural disasters such as volcano eruption, forest fire, defective and imperfect combustion of organic materials available in soil and fossil fuels and industrial sources (wastes and residual) such as car industries, power plants, thermal systems consuming oil and coal, cigarette and imperfect combustion of synthetic fuels are among the most important factors creating polycyclic hydrocarbons [5-7].

These toxic compounds transfer into the environment and the atmosphere through the sewage system, causing irreplaceable environmental damages [8].

Humans are exposed to hazard through air breathing as well as consuming agricultural products. The highest density of polycyclic aromatic hydrocarbons is found in the seas; these combinations can be absorbed through eating fish and vegetables and enter the human food cycle, easily [9-11].

These compounds are of primary importance that result in causing cancer and creating high gene-mutation the cancer-creating feature of PAHs is due to bending of these combinations with great a molecule of DNA and RNA. As a result of fat-loving property of PAHs, after entering the body, they accumulate and their harmful and dangerous effects appear after a while [12-15]. In 1970, our environment support agency prepared a list of polycyclic aromatic available in the environment [16], regarded as some of the most toxic polycyclic aromatic hydrocarbons.

Banzo[a]pyrene is more cancerous than other compounds mentioned above [17-18]. This property is so great that its amount in drinking water must not exceed 200 nano/liter.

Due to the breakability property of polycyclic aromatic hydrocarbon compounds, their available amount in the environment is very negligible, and therefore they require a sensitive and a confident method such pre-contrition and extraction of samples before analyzing them via GC-Mass detection [19-21]. The methods of preparation of samples are considered as the first essential phase of chromatography. In the past, sample pre-parathion was usually based on liquid- liquid or solid phase extraction that are time-consuming and need large samples and toxic solvents. Therefore, it is necessary to offer new methods of sample preparation with special merits such as being sensitive, fast, reducing organic solvent volumes, costs, extraction time, being compatible with the environmentally friendly, preventing the sample from was in to during the extraction process, pre-concentration of species before chromatography analysis, improving extraction efficiency process, capability of self-automation and selective ability compared with common old methods [22-24].

Moreover, PAHs determination in the soil needs further process due to low density and complicated infrastures, direct floating drop microextraction and salting out are approaches that have been extensively used in analytical chemistry [25-26].

So far various approaches have been used to determine PAHs, some of which include solid phase microextraction (SAME) and GC-MS, nanocomposite as an absorbent for the dispersive solid-phase extraction, microwave-assisted extraction, ultrasonic extraction, single-drop micro extraction with GC-MS detection, solid-phase micro extraction based on modified single-layer nanocomposite fiber, solid-phase fiber micro extraction, accelerated solvent extraction,

dispersive liquid-liquid micro extraction, solid phase micro extraction by GC-MS, dispersive solid phase micro extraction nano modified iron dust with cholesterol, and nano modified magnetic iron dust with graphene for extraction.

Salting out is regarded as one of the most applied approaches for pre-concentration and extraction of a lot of organic compounds. In this approach organic liquid-based solvents such as EtOH, MeOH, CH₃CN, propanol and *etc.* are used as solvent [25-29].

As water-based solvents and water phase are homogenous, extraction time in this method is highly short because of their extremely high interactive connections between organic and liquid phase. Another advantage of salting out method is its comparability of used solvents with a liquid chromatography device having high efficiency [30].

In this method the solvent is located above and the liquid phases are placed below.

Therefore, collecting low volumes of the water-based solvent is really a difficult task due to its being spread on the sample surface, limiting using this method for large volume samples of the solvent. Collecting solid-phase after the extraction and freezing at ice-bath is used in this method by somehow like hanging solid-phase, which will keep the liquid phase separate without waste, and it is one difficulty associated with solid organic drop microextraction. This is the technique that has been used for the first time [31-33].

The present research is aimed at introducing the solidification off-loading organic drop microextraction by a salting out and Vortex-assisted dispersive liquid-liquid microextraction for extraction of naphthalene, fluorene, phenanthrene, anthracene and pyrene (Figure 1) to remove the obstacles and highlight their advantages [34-35].

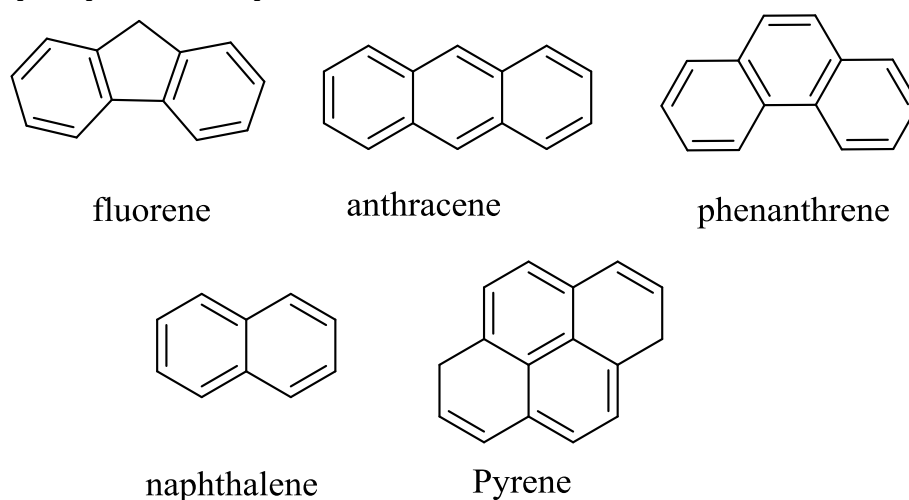


FIGURE 1 Polycyclic aromatic hydrocarbon (PAHs) chemical structure

PBD, BBD and ANOVA were utilized to evaluate the effects of independent variables to optimize variables affecting. Response surface methodology, as a further approach, helping to optimize variables and to show the effects of factors on the experimental results, has also been used. Besides, it depicts the interaction between variables. In designing

experiments through response surface methodology, we can obtain the most information by the fewest number of experiments [36-38].

Designing experiments to determine objectives and variables affecting them is conducted and then goals and objectives are expressed as a mathematical equation.

The accurate number of optimized variables experimented with mathematical equations.

To achieve the goals and variables, selecting experiments were carried out at first, and then optimization variables were chosen by using a response surface approach [39-43].

Experimental

Chemicals and reagents

Naphthalene, fluorene, phenanthrene, anthracene, pyrene, acetone, methanol, ethanol, acetonitrile, ammonium sulfate, sodium chloride, magnesium sulfate, sodium sulfate, potassium dihydrogen phosphate, 1-undecanol, 1-dodecanol and 1-decanol were purchased from Merck (Darmstadt, Germany). A stock standard solution of phenol, naphthalene, fluorene, phenanthrene, anthracene and pyrene, were obtained by dissolving PAHs reagent in the water. Working standard solutions were prepared daily by diluting the stock standard solution with double distilled water (DDW) to the required concentrations.

Instrumentation

Analysis of naphthalene, fluorene, phenanthrene, anthracene and pyrene was done using the HPLC system (model platin blue, Knauer, Germany) equipped with a UV-detector (Well chrome, K-2600; Knauer), and a reverse-phase C₁₈ column (length ID 100 × 3 mm, particle size, 3 μm, packing material Eurospher (II) 100-3 C₁₈, vortex plus column, KNAUER) operating at a wavelength of 283 nm, dual solvent pump (model LC-10Avp) and aerodyne model platin blue injector with 1 μL was used for separation. The mobile phase was made up of methanol and phosphate buffer (35:65, v/v) adjusted to pH 5.9. The flow rate was set at 0.9 mL min⁻¹. The pH measurement was done with a 780 pH meter (Metrohm, Switzerland) equipped

with a combine Ag/AgCl glass electrode. A Vortex (Biosan model V-1 PLUS, Republic of Latvia) was used in the extraction procedure.

SO-VADLLME-SFODME procedure

Salting out and vortex-assisted dispersive liquid-liquid micro extraction based on solidification of floating organic drop micro extraction method includes 4 steps (Figure 2). Initially 10 ml of liquid solution containing PAHs (naphthalene, phenanthrene, anthracene and pyrene, and fluorene) was prepared and then 400 μL of ethanol (distributing phase) including 60 μL of 1-dodecanol (extracting solvent) with pressure was injected very quickly into the solution from the top by an injector and it was vortexed for 6 minutes. 1.4 ml of salt solution of sodium sulfate 10% (w/v) was added to the solution very fast, and vortexed for 5 minutes at a speed of 3000 rpm, and 1-dodecanol at a high level of aqueous sample was observed due to low density. At the next step, designed lace hook made of stainless steel was put in the experiment tube to transfer into ice bath. After 6 minutes, organic solvent 1-dodecanol became solid as the result of low melting temperature (24 °C) and following taking out the rich, solid, and metal net from polycyclic aromatic hydrocarbon samples were put into a container with 1.5 ml capacity and to 10 μL ethanol diluted, and then nearly 1 μL was injected to HPLC by Hamilton syringe and with a high efficiency (Figure 2).

We used drinking (mineral) water bottles without doing any preparation work on it, but sewerage water after passing from filtering paper with being distilled twice in a proportion of 1:1 was diluted in the Ilam petrochemical company and near the petrochemical company, respectively. Soil samples were initially prepared by ethanol solvent, for 5 minutes and was used in section (*SO-VADLLME-SFODME procedure*), after being softened and diluted in water.

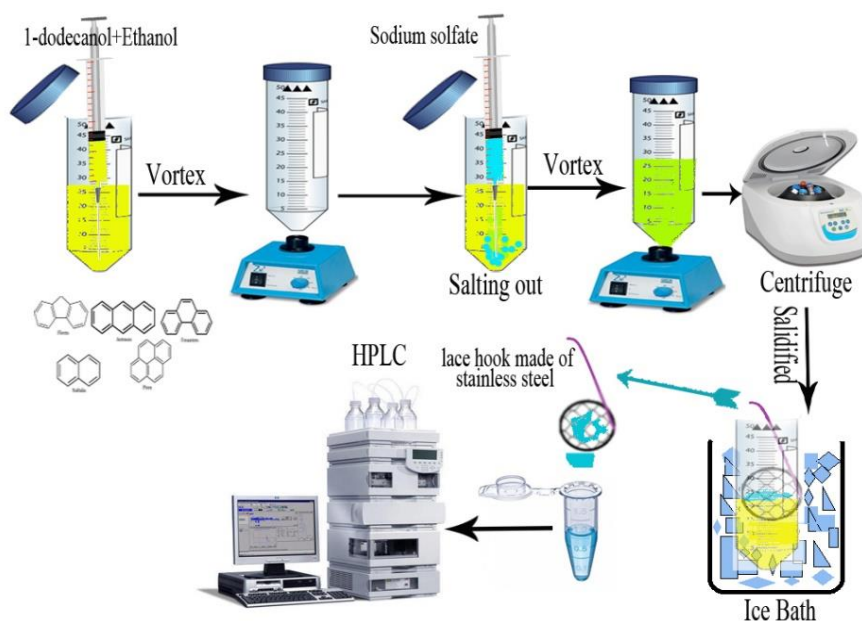


FIGURE 2 salting out and vortex-assisted dispersive liquid-liquid micro extraction based on solidification of floating organic drop

Results and discussion

Optimization of the SO-VADLLME-SFODME

In order to enhance extraction efficiency, the effects of various factors, especially the type and volume of organic extractor solvent, the volume of organic dispersive solvent and salt, type and its density, the speed of riling the sample and centrifuge time were studied separately.

Selecting the type of organic extracting solvents

To optimize the type of extracting solvent in the method, selecting an appropriate extracting solvent from 1-dodecanol, 1-decanol and 1-undecanol is an essential step. Extracting an appropriate solvent in this method has such characteristics as follows: Immiscibility with water, being less solvent dielectric constant, being economical and available, being stable with few fluctuations and changes, high extracting efficiency, having a lower density than that of water, and a melting point at a range between 10-30 °C (somewhat near our room temperature), and

being toxic at a very low level. Considering these characteristics, we selected 1-dodecanol with a density of 83% gr/ml and a melting point ranging between 10-24 °C as the extracting solvent in identical conditions.

The results, shown in Figure 3, indicate that 1-dodecanol offer higher efficiency compared to the other solvents. Using various organic solvents to obtain drops with the same volume and higher extraction efficiency of undecanol may be due to the following reasons:

1) Aromatic hydrocarbons are polycyclic and among solvents mentioned as 1-dodecanol has lower polarity.

The lower the dielectric constant of a solvent is, the less interaction with water, it has, and therefore it would be easier to separate from the aqueous phase.

The effects of salt type being surrounded by molecules of water, ions of salt, reduce breakability degrees of different types in the water phase. To select salt, the following elements must be taken into consideration, (i) salt solubility in an organic solvent must be very negligible. (ii) Salt solubility must be extremely high in aqueous phase.

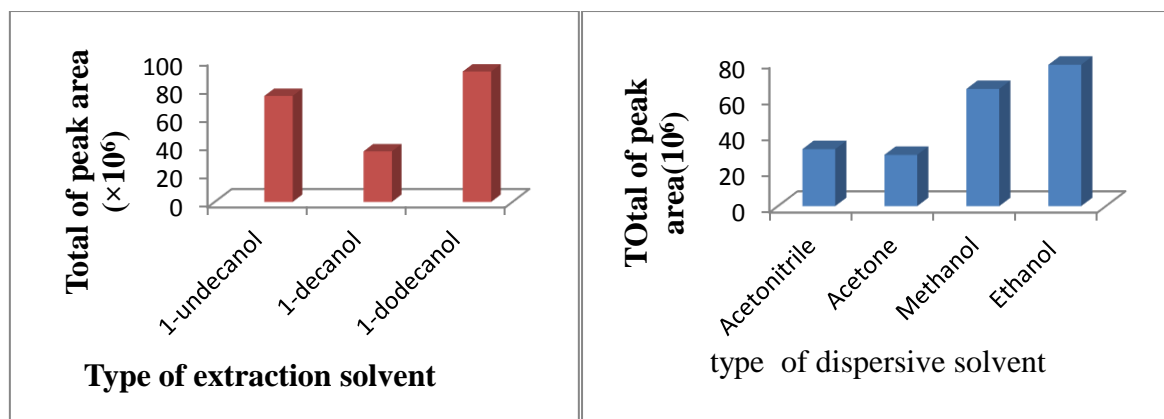


FIGURE 3 the effects of organic extracting solvent with frustration temperature point at a room limits on the extraction efficiency of naphthalene, phenanthrene, anthracene and pyrene and FT under the identical condition

To pick out appropriate salt, the extraction efficiency of ammonium sulfate (solubility in water 44% gram in 100 ml, and the temperature of 20 °C), sodium chloride, (solubility in water 39% gr in 100 ml) and magnesium sulfate (solubility in water 35.1 ml, at the temperature of 20 °C) were studied. As the solubility of the above-mentioned salts in the water is different to obtain the highest extraction productivity, the same volumes of 5% (w/v) in an identical condition were

added based on the method expressed in section.

Figure 4 shows that compared with the other two types of salt, sodium sulfate has high efficiency in the polycyclic aromatic extraction. This can be due to the more solubility of ammonium sulfate in water and its better salting out effects. Therefore, sodium sulfate was selected for further/the next experiments.

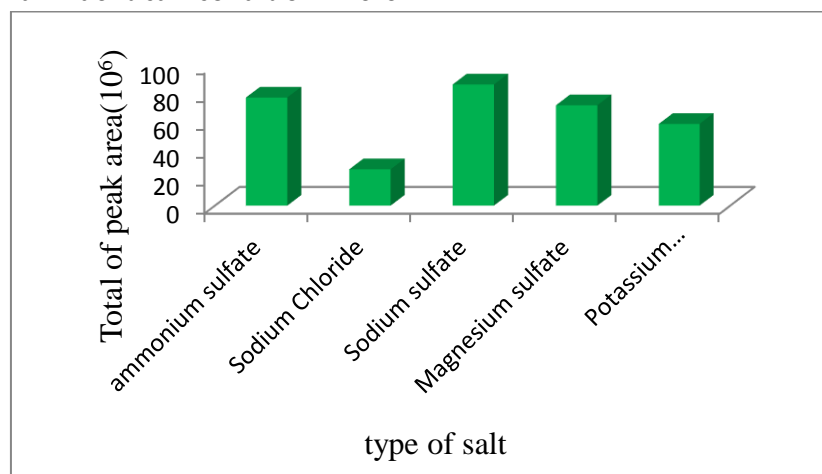


FIGURE 4 The effects of salt type on the extraction efficiency of naphthalene, phenanthrene, anthracene and pyrene and fluorene under identical conditions

Before picking out the effects of the type of dispersive solvent two measures, namely being nontoxic breakability in the water phase and economic benefits, based on using solvents like acetonitrile, acetone, methanol and ethanol should be taken. To achieve this

goal, a certain amount of liquid solution solvents containing 60 μ L of each dispersive solvent at certain volumes of 1-undecanol at first was injected by Hamilton syringe with pressure.

As a result of the experiment, ethanol was selected as the optimal dispersive solvent with the highest productivity and efficiency (Figure 5).

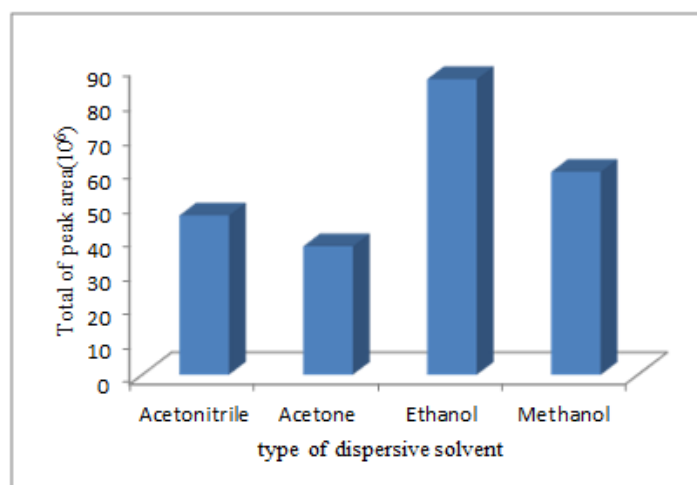


Figure 5 demonstrates the effects of various dispersive solvent on the efficiency of the polycyclic aromatic hydrocarbon extraction.

FIGURE 5 The effects of dispersive solvent type on the efficiency of naphthalene, phenanthrene, anthracene and pyrene and fluorene on extraction efficiency under the identical condition

Plackett-Burman Design (PBD)

Plackett-Burman Design is a powerful instrument with fast searching for main and key variables in multi variable systems and due to its simplicity, it could also be one of highly useful approaches in the initial process of optimization.

Using symbols of +1 (or +), -1 (or -) for upper and lower variables respectively is the most common approach to show the levels of variability. The symbol of zero represents central point's variables. Among 7 variables for solvent extracting volume of salt (μL), the volume of salt with 10% (w/v), the volume of dispersive solvent (μL), vortex extraction time (the first phase) and in the second phase vortex extraction and centrifuge time, variable affecting extraction efficiency were riddled by using PBD and optimized through Response Surface Methodology LRM and Box-Bohnken Design (BBD). In Table 1, levels of variables their relevant code, the number of required experiments and the results obtained for each experiment are reported. Selecting these variables is based on SO-VADLLME-SFODME method and their

relevant levels have also been in accordance with initial experiments. To reduce the effects of uncontrollable variables, experiments were carried at random.

In order to estimate errors and repeatability two central points were used. The results obtained from Table 1 were analyzed by using a Pareto chart (Pareto optimization).

Figure 6, Pareto chart, implies the fact that variables affecting the result of the experiment include:

The volume of dispersive organic extracting solvents (μL), pH, the volume of salt solution with 10% w/v concentration, which passes through the broken lines showing a 95% confidence level. The three remaining variables, i.e. vortex extraction time, had no significant correlation with effect on the results of the experiment. It is probable that each variable has a positive or negative effect on decomposable response. Positive effect means decomposable response increases with a change in (an increase) the volume of the given variable and vice versa, and negative effect represents a decrease in decomposable response with changing the

volume of variable. The vortex extraction time (the first phase) and the second phase of vortex extraction time as well as centrifuge time has a negligible positive effect on extraction efficiency. As a result, these variables were kept at low level of 2 minutes to conduct the next experiments, but the first phase of vortex extraction time and the

second phase of it centrifuge time were held at a mid-level of 6 minutes. Increasing salt volume caused an improvement in extraction time through salting out effect, though it did not have a significant effect. Adding salt to solution sample led to an increase in viscosity of aqueous phase while caused a decrease in transferring mass of species.

TABLE 1 levels of variables, their relevant codes, the number of required experiments and results obtained for each experiment in Plaket-Barman design to extract naphthalene, phenanthrene, anthracene and pyrene and fluorene

Factors	Levels		
	Low (-1)	Central (0)	High (+1)
(X ₁) Solvent extracting volume (μL)	10	50	100
(X ₂) pH	2	5	10
(X ₃) Volume of dispersive solvent (μL)	150	375	600
(X ₄) Volume of salt (sodium Sulfate) with 10%w/v	0.5	1.25	2
(X ₅) Vortex extraction time (first phase)(min)	2	5	8
(X ₆) Vortex extraction time (second phase)(min)	2	5	8
Centrifuge time (min))X ₇ (2	6	10

Run	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	Total peak Area(×10 ⁶)
1	100	2	600	2.00	2	8	2	57.6
2	100	10	600	0.50	8	8	2	37.9
3	10	10	150	0.50	2	8	10	49.6
4	100	2	150	0.50	8	8	10	41.3
5	10	2	150	2.00	8	8	2	76.3
6	10	2	600	2.00	8	2	10	79.1
7	100	2	600	0.50	2	2	10	47.1
8	10	2	150	0.50	2	2	2	74.3
9- Cp	55	6	375	1.25	5	5	6	82.4
10	10	10	600	0.50	8	2	2	64.1
11	100	10	150	2.00	8	2	10	27.6
12	10	10	600	2.00	2	8	10	61.1
13- Cp	55	6	375	1.25	5	5	6	78.2
14	100	10	150	2.00	2	2	2	42.1

Figure 6 Pareto chart was obtained from Box-Behnken for the extraction of naphthalene, phenanthrene, anthracene and pyrene and fluorene: (positive effect) and (negative effect).

(x₁) stands for solvent extracting volume (μL), pH (x₂) represents the volume of dispersive solvent (μL), (x₃) shows the volume/concentration of salt, sodium sulfate (w/v), (x₄) indicates vortex extraction time (the first phase), and (min) x₅ shows vortex

extraction time (the second phase) (min) (x₆) and demonstrate centrifuge time (min) (x₇)

Box-Behnken Designs (BBD)

Box-Behnken Designs experimental model is usually expressed as an Equation 1. This is in from of a multi variable second order equation based on an incomplete factorial design with vast applications to determine optimal conditions of the experiment, each in accordance with the cited values of codes.

$$Y = \beta_0 + \sum \beta_i \cdot X_i + \sum \beta_{ii} \cdot X_i^2 + \sum \beta_{ij} \cdot X_i X_j + \varepsilon \quad (1)$$

In this equation y (dependent variable) indicates decomposable response (total peak area), B is model constant, β_i , β_{ii} and β_{ij} show linear, second order and interaction between variables, respectively. Independent variables x_1 , x_2 , x_3 and x_4 represent coded factors of independent variables. As a matter of fact, these processes of optimization contain 3 steps:

- 1-Conducting designed experiments,
- 2-estimating coefficients in a mathematical model, and
- 3-Predicting results and evaluating the quality of established model. Here in this experiment (Scheme) we used 3 central points based on the number of variables and central points in accordance with the Equation 2.

$$N = 2K(K-1) + C_p \quad (2)$$

Where N represents the number of experiments, K is the number of variables and C_p stands for central points.

In Table 2 the results of experiments carried out by Box-Behnken design, including variable level, their relevant codes, the number of required experiments and the obtained rescue for each experiment are reported. To reduce the effects of uncontrollable variables, experiments were conducted randomly. After selecting an appropriate mathematical model, and statistical analysis of data, we studied the properness of the empirical model as well as drawing optimization and diagrams by using Mini-Tab and Design Expert software's. After identifying and determining significant variables in placket-Burman design optimal conditions, we used a four-variable PBD, including the volume of organic extracting solvent (μL) (x_1), pH (x_2), the volume of dispersive solvent (x_2), and salt concentration of Sodium sulfate (10% w/v), respectively, (x_4), and coded factors of independent

variables. In order to prevent uncountable errors, the experiments related to BBD were carried out randomly. In Table 2, the name of variables, their relevant codes and levels have been offered. In the last column of the Table 2, experiment results in terms of total peak areas of naphthalene, phenanthrene, anthracene and pyrene and fluorene as well as predicted peak areas are reported.

Obtained results from Table 2 were reviewed and analyzed by using a Pareto diagram.

As it is observed, the linear effect of the line crossing through the vertical dotted area, showing the confidence interval of 95%, on experiment results such as four discussed variables, was significant (meaningful). The four variables included:

- Solving extracting volume (μL) (x_1)
- pH (x_2)
- volume of dispersive solvent (μL) (x_3)
- volume/ concentration of (Sodium sulfate) with 10% w/v (μL) (x_4)

The reported results in the Pareto chart of PBD were consistent with those of Pareto of BPD (Figure 6). Linear effect (L) and second order (Q) of each variable except for the volume of dispersive solvent were significant as well.

We used variance analysis to evaluate the results obtained from schedule (Table 4). The results from analysis (ANOVA) of BBD is reported in Table 3. The P values in Table 2 reveals that, with confidence level certain terms in the model are significant (P values <0.05), though some others are not (P values >0.05). When the P value is greater than 0.05, it means lack of fitness (LOF), which is equal to 0.2035 here, showing that the model designed to process experimental data has been appropriate. As reported in Table 2, the coefficient of determination (r^2) is 0.9845, indicating ability of the model to process the empirical (data strong and significant correlation between variables).

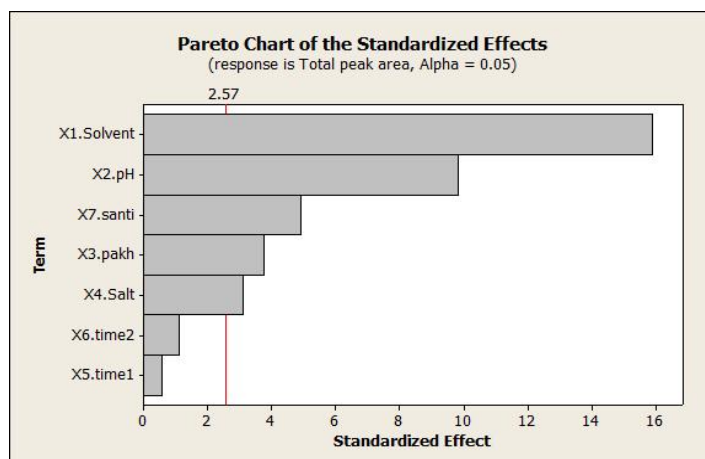


FIGURE 6 Obtained Pareto diagram in BBD to extract naphthalene, phenanthrene, anthracene and pyrene and fluorene

TABLE 2 ANOVA results obtained by Box-Behnken design for extraction of naphthalene, phenanthrene, anthracene and pyrene and fluorene

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7.627E+15	20	3.814E+14	1088.42	< 0.0001	significant
A- Volume of extracting solvent(μL) - X_1	1.298E+15	1	1.298E+15	3704.03	< 0.0001	
B- pH -(X_2)	9.168E+13	1	9.168E+13	261.66	< 0.0001	
C- Centrifuge time (min)) X_3	5.382E+14	1	5.382E+14	1536.18	< 0.0001	
D- Volume of dispersive solvent (μL) -(X_4)	7.331E+14	1	7.331E+14	2092.20	< 0.0001	
E- X_5 -Salt	1.398E+14	1	1.398E+14	399.09	< 0.0001	
X_1X_2	3.306E+13	1	3.306E+13	94.36	< 0.0001	
X_1X_3	1.225E+11	1	1.225E+11	0.3496	0.5609	
X_1X_4	4.900E+11	1	4.900E+11	1.40	0.2508	
X_1X_5	4.692E+13	1	4.692E+13	133.92	< 0.0001	
X_2X_3	4.000E+10	1	4.000E+10	0.1142	0.7390	
X_2X_4	5.550E+13	1	5.550E+13	158.41	< 0.0001	
X_2X_5	1.332E+13	1	1.332E+13	38.02	< 0.0001	
X_3X_4	6.864E+14	1	6.864E+14	1959.16	< 0.0001	
X_3X_5	9.923E+12	1	9.923E+12	28.32	< 0.0001	
X_4X_5	1.166E+14	1	1.166E+14	332.90	< 0.0001	
X_1^2	1.774E+15	1	1.774E+15	5062.71	< 0.0001	
X_2^2	2.053E+15	1	2.053E+15	5860.45	< 0.0001	
X_3^2	3.102E+14	1	3.102E+14	885.32	< 0.0001	
X_4^2	4.916E+14	1	4.916E+14	1403.19	< 0.0001	
X_5^2	3.501E+14	1	3.501E+14	999.34	< 0.0001	
Residual	7.007E+12	20	3.504E+11			
Cor Total	7.634E+15	40				
Lack of Fit	8.82000E+12	4	8.82000E+12	0.81	0.670	not significant
R²	0.9991					
Adjusted R ²	0.9982					
Predicted R ²	0.9247					
Pure Error	8.82000E+12	1	8.82000E+12			

In addition, the quality of the fit of polynomial model equation shows the interaction between decomposable responses of total peak area (Y) and significant variables.

$$Y = +7.860 \times 10^7 + 9.006 \times 10^6 X_1 - 2.494 \times 10^6 X_2 + 5.800 \times 10^6 X_3 - 6.769 \times 10^6 X_4 + 2.956 \times 10^6 X_5 - 2.875 \times 10^6 X_1 X_2 - 1.750 \times 10^5 X_1 X_3 - 3.500 \times 10^5 X_1 X_4 - 3.425 \times 10^6 X_1 X_5 + 1.000 \times 10^7 X_2 X_3 + 3.725 \times 10^6 X_2 X_4 - 1.825 \times 10^6 X_2 X_5 - 1.310 \times 10^7 X_3 X_4 + 1.575 \times 10^6 X_3 X_5 - 5.400 \times 10^6 X_4 X_5 - 2.393 \times 10^7 X_1^2 - 2.575 \times 10^7 X_2^2 - 1.001 \times 10^7 X_3^2 - 1.260 \times 10^7 X_4^2 - 1.063 \times 10^7 X_5^2$$

The interaction among naphthalene, phenanthrene, anthracene, pyrene and fluorene has been demonstrated in three dimensional diagrams (Figure 7).

Considering the value of P-value in the analysis table and three imensional diagrams, we have demonstrated significant interaction between variables

L_2 linear and Q_2 : second order derivatives volume of organic extracting solvent (μL) (x_1), pH (x_2), volume of dispersive solvent (μL) (x_3), = volume of salt concentration (Sodium sulfate) with 5% w/v (μL) (x_4), demonstrate centrifuge time (min) (x_7).

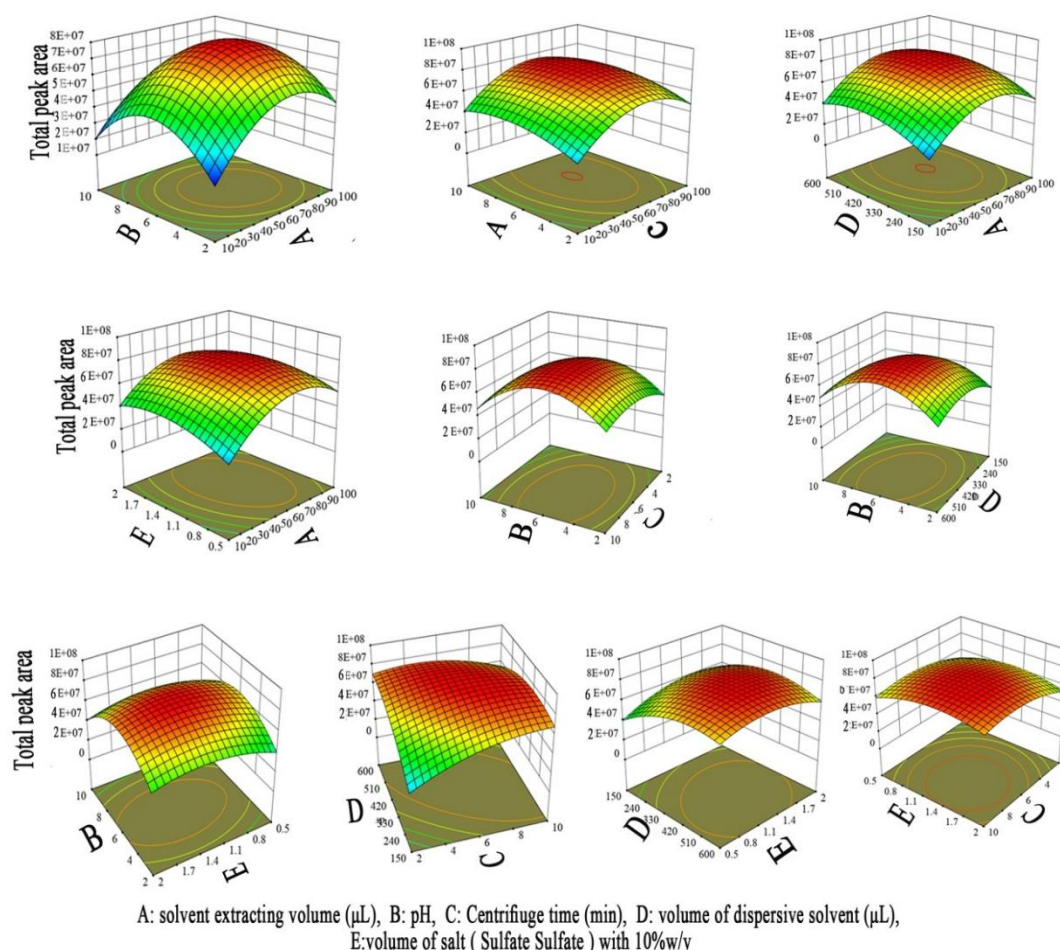


FIGURE 7 three dimensional diagrams in BBD to extract naphthalene, phenanthrene, anthracene and pyrene and fluorene. (x_1) Volume of extracting solvent (μL), x_3 volume of dispersive solvent (μL), (x_4) salt concentration (Sodium sulfate) with 5% w/v (μL)

In the next step, empirical data were obtained to evaluate normal distribution the reminder decompositions.

The results in Figure 8 show that the errors were distributed according to normal distribution Function, while no specific

pattern is observed in the diagram. This fact shows lack of fit (LOF), a specific systematic error in the used model. The linear relation between values of total peak area was measured and the used empirical model showed that the linear regression coefficient r^2 (Determination regression coefficient r^2) is equal to 99.91, implying that 99 percent of

extraction efficiency values is related to [in] dependent variables and only 2% these changes cannot be explained by the prediction model. High values of r^2 is therefore another confirming sign of properness of suggested (empirical) model, used in the present study.

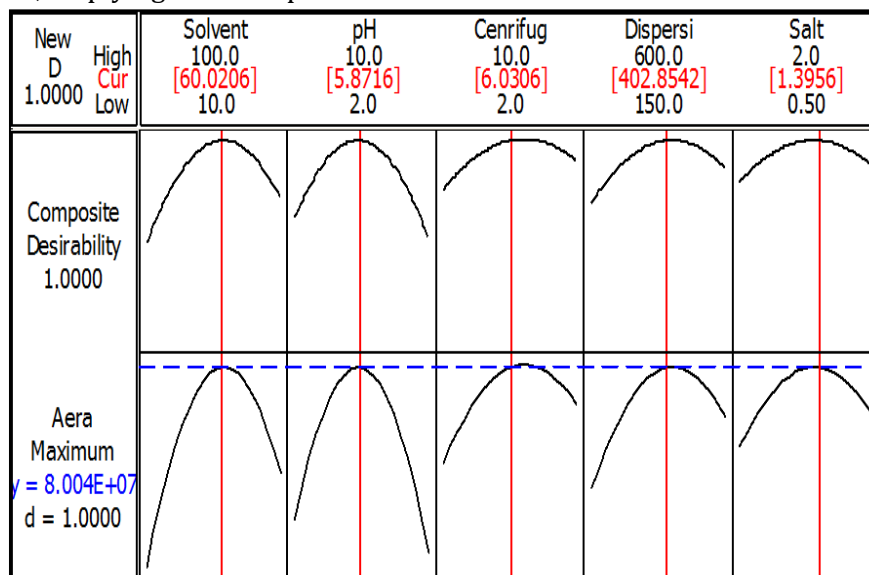


FIGURE 8 Normal distribution diagram for the reminder (decompositions), to extract naphthalene, phenanthrene, anthracene and pyrene and fluorene

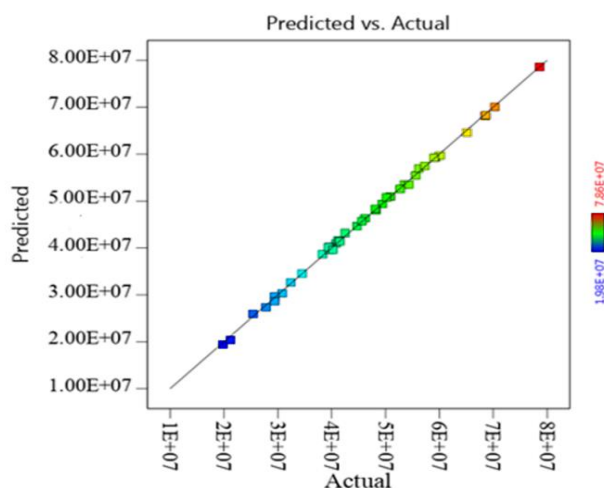


FIGURE 9 The linear relationship between the predicted values measured by the empirical suggested model

To obtain the optimal value of each variable we used the desirability function. According to the results reported in Table 2, the highest and the lowest values of total peak areas are 2.7×10^6 and 82.4×10^6 ,

respectively. And thereby, desirability¹ is set to the highest value total peak area, zero for the lowest and 0.5 for an average result (58.48×10^6).

The results obtained from desirability function in Figure 2 show that by increasing the volume of organic extracting solvent (μL) from 10 to 100 μL the extraction efficacy decreases and in volumes less 60 (μL) less than 10 μL , extraction efficacy is also low, due to insufficiency of solvent volumes for (various) types; in volumes more than 10 μL this variable is decreasing as well, because of an increase in volume and there by diluting analytes concentration.

The effects of the volume of dispersive solvent on extraction efficiency were examined in a range from 150 to 600 μL . The results in Figure 2 illustrate an increase in (dissoluble) results up to 403 μL of dispersive solvent. The reason for this reduction in extraction efficiency while using volumes less than 403 μL is that the solution still has the capacity and maintainability as well as eliminating more analysts. Desirability function diagrams are proper and the most total peak areas that are obtained using solvent extracted (1-undecanol, 60 μL), dispersive solvent (403 μL), salt volume (1.4 ml), sodium sulfate (5% w/v) and pH = 5.9.

To check whether there is consistency between optimal results with obtained from desirability function and empirical or not, repeated experiments were also carried out. Obtained retrieval values demonstrate consistency and coincidence between theory and empirical experiments, with the error level less than 4.6%.

To evaluate the method used in the present research, we calculated the figures of merit such as linear ranges (LRs), limits of detection (LODs), limits of quantification (LOQs), enrichment factors (EFs) and r^2 . The results achieved, are reported in Tables 3 and 4.

Calibration curves for naphthalene, phenanthrene, anthracene (at a concentration level of 10) and for pyrene and fluorene, at concentration levels of arc were depicted.

To calculate the enrichment factor and limit of detection, equations of (1) and (2) were used respectively.

Moreover, to calculate the limits of the quantifications, equation (1) has been used.

In above elongation m shows the slope of the calibration curve and S_b stands for the standard deviation.

TABLE 3 Analytical figures of merit, proposed So-VAPLLME method

Analyte	LRs ($\mu\text{g L}^{-1}$) ^a	LOD ^a ($\mu\text{g L}^{-1}$)	LOQ ^b ($\mu\text{g L}^{-1}$)	r^2	EF	RSD% (within day, n=5)	RSD% (between day, n=5)
Fluorene	0.5-800	0.09	0.20	0.9961	186	4.1	4.7
Anthracene	0.5-800	0.07	0.23	0.9969	162	3.8	4.9
Fenantern	0.2-600	0.05	0.25	0.9934	139	3.9	5.2
Pyrene	0.2-600	0.06	1.09	0.9947	147	2.8	4.3

^aDetection limits are calculated as signal to noise ratio of three times. (LOD = $3S_b/m$)

^blimits of quantification (LOQ = $10S_b/m$) ('s' and m are the standard deviation and the slope of each calibration curve, respectively).

Analysis water, vegetables and fruits samples

The volume of recycling of naphthalene, phenanthrene, anthracene, pyrene and fluorine were analyzed by So-VAPLLME-SFO DME method and by adding various amounts of standard solution of procyclic aromatic organic hydrocarbons. As for the sample from tap water, mineral water industrial sewerage

water, river and irrigation water for vegetables and fruits were evaluated. To calculate the recycling percentage, we used the formula 1-3, the recycling percentage of procyclic aromatic hydrocarbons.

In Table 4, recovery percentage (%) of polycyclic aromatic hydrocarbons at 3 concentration level 10, 200 and 400 $\mu\text{g/L}$ for

naphthalene, phenanthrene, anthracene and pyrene and fluorene as well concentration levels of 10,200 and 400 $\mu\text{g/L}$ for phenanthrene and anthracene are reported.

To obtain the accurate level of proposed method, formula 4 was used. The results indicate more than 88.2% of mentioned compositions (solutions) is recovery by the method mentioned, with on error of less than 2.9%.

Recovery of polycyclic aromatic hydrocarbons from aqueous (water), vegetables and fruits samples are listed in Table 4.

Chromatograms of water, vegetables and fruits samples, after applying the method proposed are displayed in Figure 10. According to obtained chromatograms and values reported in Table 4, some of solutions, studied in this research, in waste water and river water were detected.

The results showed that among vegetables and fruits, cabbage and cauliflower were polluted with a number of polycyclic aromatic hydrocarbons.

The volume found in cabbage and cauliflowers were less than limits of qualification (LOQ) so-VADLLME-SFODE method.

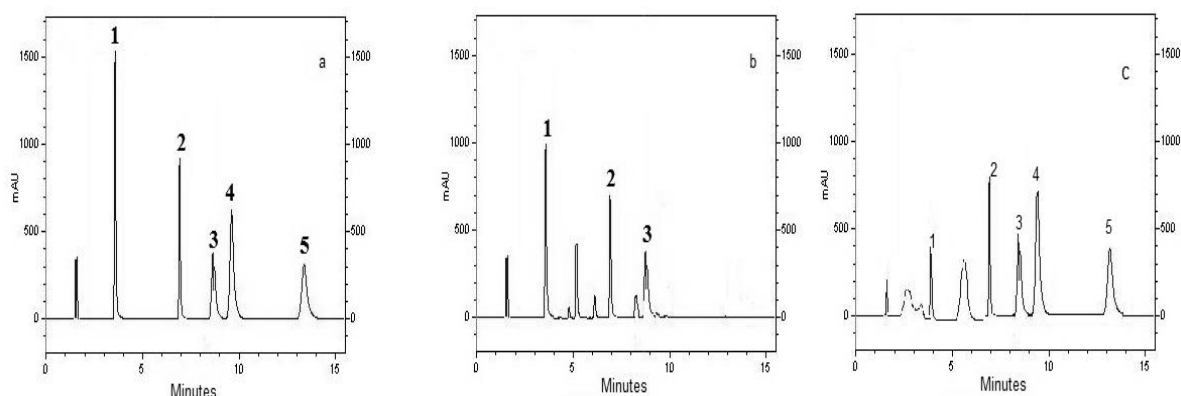


FIGURE 10 The chromatogram of (a) standard of analytes (1; naphthalene), (2; pyrene), (3; phenanthrene), (4; fluorene), (5; anthracene) (b) Tap water (Ilam river); and (c) Wastewater, spiked at $200 \mu\text{g L}^{-1}$ of each, after SO-VADLLME-SFODME method under optimal conditions naphthalene, naphthalene, anthracene, pyrene and fluorine

Extraction conditions: Using $60 \mu\text{L}$ from solvent, pH 5.9 and salt volume of 1.4 mL salt, (1-undecanol), $403 \mu\text{L}$ from dispersive sodium sulfate (5% w/v)

TABLE 4 Recoveries and RSD (%) ($n = 3$) obtained for different water and wastewater samples. Spiked of Naphthalene, naphthalene, anthracene, pyrene and fluorine

sample	Compound	Amount found ($\mu\text{g L}^{-1} \pm \text{SD}^a$)	Recoveries (RSD%) Amount add ($\mu\text{g L}^{-1}$)		
			10	200	400
Tap water (Ilam, Iran)	Naphthalene	6.8	96.7(3.1)	98.6(4.2)	99.3(3.5)
	Fluorene	7.7	95.5(4.3)	97.1(3.1)	96.4(3.2)
	phenanthrene	9.8	96.3(3.1)	99.5(2.9)	94.7(5.2)
	Anthracene	ND	88.2(3.8)	96.6(4.2)	96.8(4.7)
	Pyrene	ND			
Wastewater Collected from a municipal wastewater treatment plant	Naphthalene	6.4	95.3(4.8)	99.1(4.2)	98.1(3.8)
	Fluorene	9.7	97.8(5.0)	97.2(4.1)	97.5(3.1)
	phenanthrene	12.8	98.6(3.2)	97.6(4.3)	98.2(4.1)
	Anthracene	3.8	98.8(4.8)	95.8(3.8)	93.7(4.7)
	Pyrene	6.5	97.7(3.8)	93.8(4.3)	93.5(4.7)

Determination of low concentrations of PAHs in water samples

Conclusion

Upper space-solid-phase micro extraction, soxcole, solid phase micro extraction and gel-agarose- liquid phase micro extraction for polycyclic aromatic hydrocarbons micro extraction have been reported. The efficiency of salting out liquid-liquid extraction (SALLE) is observed.

Combined with Vortex-assisted dispersive liquid-liquid micro extraction based on solidification of floating organic drop method has been applied (used) as a simple, fast and accurate method for determination of polycyclic aromatic hydrocarbons. The advantages of this method compared with solidification of floating organic drop micro extraction and salting out approach are as follows:

1. Using a solvent compatible with the environment (an environment friendly method) and liquid chromatography with high and effective performance.
2. Being faster in micro extraction and reducing extraction time of salting out and Vortex-assisted dispersive liquid-liquid micro extraction based on solidification of floating organic drop micro extraction
3. Collecting the organic extracting solvent easily, by using an innovative and simple net as well as preventing wasting of solvent and integrating liquid;
4. further enhancing extraction efficiency compared with other methods;
5. having the merit of salting out and dispersive micro extraction method;
6. not using surfactants; and
7. integrating three extracting methods together to increase extraction efficiency.

Acknowledgements

The authors would like to thank Medical University of Ilam, Iran, for financial support to accomplish this project.

Orcid:

Gholam Basati: <https://orcid.org/0000-0002-4739-4567>

References

- [1] M. Hoseini, M. Yunesian, R. Nabizadeh, K. Yaghmaeian, R. Ahmadkhaniha, N. Rastkari, S. Parmy, S. Faridi, A. Rafiee, K. Naddafi, *Environ. Sci. Pollut. Res.*, **2016**, *23*, 1820-1832. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [2] X. Wang, J. Feng, Y. Tian, C. Luo, M. Sun, *J. Chromatogr. A*, **2018**, *1550*, 1-7. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [3] I. Tolosa, S.J. de Mora, S.W. Fowler, J. Villeneuve, J. Bartocci, C. Cattini, *Mar. Pollut. Bull.*, **2005**, *50*, 1619-1633. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [4] M. Qiao, W. Qi, H. Liu, J. Qu, *Sci. Total Environ.*, **2014**, *481*, 178-185. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [5] R. Kobayashi, R. Okamoto, R. Maddalena, N. Kado, *Environ. Res.*, **2008**, *107*, 145-151. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [6] L. Fereya, N. Delaunay, D.N. Rutledge, A. Huertag, Y. Raould, P. Gareil, J. Vial, *J. Chromatogr. A*, **2013**, *1302*, 181-190. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [7] V.G. Zuin, L. Montero, C. Bauer, P. Popp, *J. Chromatogr. A*, **2005**, *1091*, 2-10. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [8] A. Morzik, Z. Piotrowska-Seget, S. Labuzek, *Pol. J. Environ. Stud.*, **2003**, *12*, 15-25. [[Google Scholar](#)], [[Publisher](#)]
- [9] N. Kazerouni, R. Sinha, C.H. Hsu, A. Greenberg, N. Rothman, *Food Chem. Tox.*, **2001**, *39*, 423-436. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [10] M.S. Hassanvand, K. Naddafi, S. Faridi, R. Nabizadeh, M.H. Sowlat, F. Momeniha A. Gholampour, M. Arhami, H. Kashani, A. Zare, S. Niazi, N. Rastkari, S. Nazmara, M. Ghani, M. Yunesian, *Sci. Total Environ.*, **2015**, *527*, 100-110. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [11] H.R. Pohl, N. Roney, S. Wilbur, H. Hansen, C.T. De Rosa, *Chemosphere*, **2003**, *53*,

- 183-197. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [12] W.C. Tsai, S.D. Huang, *J. Chromatogr. A*, **2009**, *1216*, 7846-7850. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [13] S. Casal, E. Mendes, J. Fernandes, M. Oliveira, M. Ferreira, *J. Chromatogr. A*, **2004**, *1040*, 105-114. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [14] S.K. Sagiv, M.M. Gaudet, S.M. Eng, P.E. Abrahamson, S. Shantakumar, S.L. Teitelbaum, P. Bell, J.A. Thomas, A.I. Neugut, R.M. Santella, M.D. Gammon, *Environ. Res.*, **2009**, *109*, 287-291. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [15] National Toxicology Program, NTP 11th Report on Carcinogens, Rep Carcinog, **2004**, *11*, 1-A32. PMID: 19826456. [[Publisher](#)]
- [16] W.B. Wilson, A.A. Costa, H. Wang, A.D. Campiglia, J.A. Dias, S.C.L. Dias, *Microchem. J.*, **2013**, *110*, 246-255. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [17] R. Kurihara, F. Shiraishi, N. Tanaka, S. Hashimoto, *Environ. Toxicol. Chem.*, **2005**, *24*, 1984-1993. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [18] H.F. Nassar, N. Tang, A. Toriba, F.K. Abdel-Gawad, K. Hayakawa, *Int. J. Sci. Eng. Res.*, **2015**, *8*, 1983-2006. [[Pdf](#)], [[Google Scholar](#)], [[Publisher](#)]
- [19] S. Yang, X. Fang, L. Duan, S. Yang, Z. Lei, X. Wen, *Spectrochim. Acta A*, **2015**, *148*, 72-77. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [20] C.H. Lee, Y. Shin, M.W. Nam, K.M. Jeong, J. Lee, *Talanta*, **2014**, *129*, 552-559. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [21] A. Frankenberger, R.R. Brooks, M. Hoashi, *Anal. Chim. Acta*, **1991**, *246*, 359-363. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [22] X. Zhu, Z. Zhu, S. Wu, *Microchim. Acta*, **2008**, *161*, 143-148. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [23] M. Guñez, C. Bazan, L.D. Martinez, S. Cerutti, *Microchem. J.* **2018**, *139*, 164-173. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [24] M.H. Hosseini, M. Rezaee, H.A. Mashayekhi, S. Akbarian, F. Mizani, M.R. Pourjavid, *J. Chromatogr. A*, **2012**, *1265*, 52-56. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [25] L. Yangcheng, L. Quan, L. Guangsheng, D. Youyuan, *Anal. Chimica Acta*, **2006**, *566*, 259-264. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [26] D. Mekiki, N. Kalogerakis, E. Psillakis, *Chromatographia*, **2006**, *63*, 85-89. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [27] C.H. Hung, H.P. Ho, M.T. Lin, C.Y. Chen, Y.Y. Shu, M.R. Lee. *J. Chromatogr. A*, **2012**, *1265*, 1-6. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [28] X. Ma, P. Huang, X. Dang, Y. Ai, D. Zheng, H. Chen, *Microchem. J.* **2019**, *146*, 1026-1032. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [29] L.O. Santos, J.P. Dos Anjos, S.L.C. Ferreira, J.B. de Andrade, *Microchem. J.*, **2017**, *133*, 431-440. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [30] L. Guo, H.K. Lee, *J. Chromatogr. A*, **2013**, *1286*, 9-15. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [31] M. Tutino, A. Di Gilio, A. Laricchiuta, G. Assennato, G. de Gennaro, *Chemosphere*, **2016**, *161*, 463-469. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [32] W.Y. Zhang, Y. Sun, C.M. Wang, C.Y. Wu, *Anal. Bioanal. Chem.*, **2011**, *401*, 1685-1693. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [33] X. Liu, Y. Ji, Y. Zhang, H. Zhang, M. Liu, *J. Chromatogr. A*, **2007**, *1165*, 10-17. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [34] A. Albinet, E. Leoz-Garziandia, H. Budzinski, E. Villenave, J.L. Jaffrezo, *Atmos. Environ.*, **2008**, *42*, 43-54. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [35] L. Kocúrová, I.S. Balogh, J. Šandrejová, V. Andruch, *Microchem. J.*, **2012**, *102*, 11-17. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [36] M. Rezaee, Y. Yamini, M. Faraji, *J. Chromatograph. A*, **2010**, *1217*, 2342-2357. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]
- [37] Z. Yan, J. Yuan, G. Zhu, Y. Zou, C. Chen, S. Yang, S. Yao, *Analytica. Chimica. Acta.*, **2013**, *780*, 28-35. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[38] Y. Jia, Y. Zhao, M. Zhao, Z. Wang, X. Chen, M. Wang, *J. Chromatogr. A*, **2018**, *1551*, 21-28.

[[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[39] W. Wang, R. Ma, Q. Wu, C. Wang, Z. Wang, *J. Chromatogr. A*, **2013**, *1293*, 20-27.

[[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[40] M. Gupta, A.K.K.V. Pillai, A. Singh, A. Jain, K.K. Verma, *Food Chem.*, **2011**, *124*, 1741-1746. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[41] Y. Cai, Y.E. Cai, Y. Shi, J. Liu, S. Mou, Y. Lu, *Microchim Acta*, **2007**, *157*, 73-79.

[[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[42] S.L.C. Ferreira, R. Bruns, H. Ferreira, G. Matos, J. David, G. Brandao, E.G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N.L. Dos Santos, *Anal. Chim. Acta*, **2007**, *597*, 179-186. [[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

[43] M.J. Wang, Y.L. Xie, Z.J. Chen, S.J. Yao, *J. Appl. Polym. Sci.*, **2010**, *117*, 3001-3012.

[[crossref](#)], [[Google Scholar](#)], [[Publisher](#)]

How to cite this article: Gholam Basati, Asad Mirzaei, Sabah Shiri*. Salting out and Vortex-Assisted Dispersive Liquid-Liquid Microextraction Based on Solidification of Floating Organic Drop Microextraction (SO-VADLLME-SFODME) for Extraction and Determination of Polycyclic Aromatic Hydrocarbons (PAHs) in Water and Solid Samples Followed by HPLC. *Eurasian Chemical Communications*, 2021, 3(10), 726-742.

Link:

http://www.echemcom.com/article_136886.html