

Salting out and vortex-assisted dispersive liquid–liquid microextraction based on solidification of floating organic drop microextraction as a new approach for simultaneous determination of phenol and chlorophenols in water samples

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Abstract

In the present research, salting out and vortex assisted dispersive liquid–liquid microextraction based on solidification of floating organic drop microextraction (SO-VADLLME-SFODME) for the extraction and determination of phenol and chlorophenols in water samples followed by HPLC have been studied through a novel, simple, low-cost, selectable and environment friendly (so called green) method. Three approaches have been combined in a way that their advantages have been used while reducing their deficiencies. The effects of various empirical parameters such as pH, type and volume of dispersive solvent, the volume of extracted organic solvent, salt concentration, the condition of centrifuge, and mixing time were studied and optimal conditions were achieved by using Mini-Tab software and Respond Surface Methodology (RSM) based upon Box-Behnken Design (BBD) and Desirability Function (DF). Under the optimum conditions, calibration curves were linear in the range of 0.1 to 1000 μgL^{-1} , limit of detections (LODs) were in the range of 0.06 to 0.34 μgL^{-1} and $r^2 > 0.9916$, respectively. The method was also utilized to measure phenol and chlorophenols in various water samples successfully resulted in extraction recoveries ranged from 92.8 to 103.9%.

Keywords: Chlorophenols; microextraction; salting out; solidification.

Introduction

Nowadays, polluting water resources with phenol and phenol compounds is one of the most serious environmental challenges, causing a serious danger and threat to human health due to its highly poisonous ingredients [1,2]. Phenol and chlorophenols are among aromatic compounds as well as one of

benzene derivatives available in the nature while being extremely poisonous, as well. Phenol is a colorless material, absorbing moisture with a crystal order that changes into pink through oxidization when exposed to the air. Having been dissolved in water, it gives water a very little acid property. As a result of their wide use in such

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industrial processes as production of plastic, paint, pharmacological, pesticide, herbicides, fungicides painting chlorine and etc. are widely distributed in natural water resources [3-5]. Chlorophenols can be found in water, especially in drinking water; chlorophenols and some other phenolic compounds, considered as the most poisonous and cancerous factor by Environmental Protection Agency (EPA), are listed and issued among strong pollutants materials [6,7]. The presence of phenol and its derivatives in water and sewage system which create a threat to human and animal lives and the environment, due to its poisonous nature, has raised a critical concern [8,9]. Phenol compounds while presenting chlorine in water create complexes with an unpleasant flavor and fumes. This substitution with chlorine not only increases flavor and fume but creates toxic effects as well [10,11]. Chlorophenols are very durable and long lasting compounds which could be found in wastes and slops of industries in great quantities. Chlorophenols are among organic compounds formed by using one or more chlorine atoms on a phenol ring. As a result, there are 19 different types of chlorophenols [12,13]. The solubility of chlorophenols in water is low, but meanwhile this solvability decreases by adding the number of chlorine in their molecular structure. Moreover, these atoms decrease the process of biological dissolubility of chlorophenol compounds [14]. Most chlorophenols are poisonous and cancerous; their dissolubility is too difficult with high durability in water. Burning, emancipation in the air, desorption, wet oxidization, electro chemical oxidization, biological and advanced chemical oxidization are the most important ways of purification and

elimination of phenol compounds [15,16]. Considering the fact that dissolubility of phenol compounds notably chlorophenols in environmental samples are very low, a sure and sensitive approach, simple preconcentration process and extracting samples before analysis by using such approaches as chromatography is essential. To measure phenol and chlorophenol compounds, various analytical methods have been utilized as follows: gas chromatography–mass spectrophotometry [9,17], spectrophotometry [18], electrochemical sensor for 4-chlorophenol determination based on molecularly imprinted polymer [19], application of on-line solid-phase extraction with molecularly imprinted polymer [20], chromatography [21], electrochemical [22], salting out assisted–liquid extraction with dispersive liquid–liquid microextraction [23], sample preparation approach of primary samples which constitutes the essential phase in chromatography analysis over the past years, preparation of the sample commonly based upon liquid–liquid extraction or extractions of solid-phase, which were time-consuming and needed a sample with a large volume and poisonous solvents. Therefore, it is necessary to offer new approaches to prepare a sample with special advantages including fastness, sensitiveness, decreasing the volume of organic solvent, its low-cost, reducing extraction time, and being environmentally friendly (with sound environmental policies) [24-29]. Salting out is among the most practical and applied ways of preconcentration and the extraction of many organic compounds. In this approach, organic liquid friendly solvents such as ethanol, acetonitrile, aston, methanol, propanol and etc. are used as extraction solvent.

Organic solvents can be extracted from liquid phase by adding mineral and organic salt. As organic liquid solvent mixture and aqueous phase are homogenous, extraction time in this approach is very short because of extremely high contact level between organic and liquid phases. Another advantage of salting out extraction is the adaptability of used organic solvents to liquid chromatography with high efficiency [30-31].

In this approach, liquid solvent is located on top and liquid phases on the bottom of extraction instrument. Collecting volumes of less liquid solvent is, therefore, difficult due to its emancipation at sample level, which, in turn, results in utilizing high volumes of liquid solvent. Collecting solid phase after extraction and freezing at ice bath is one of the problems of solid organic micro extraction in which solid phase hook is separated without any waste and with liquid phase. This technique has been used for the first time.

The present research aims at introducing microextraction of floating organic solid drop using dispersive liquid–liquid microextraction assisted by salting out and vortex to extract phenol and chlorophenols (2-chlorophenols (2-CP), 4-chlorophenols (4-CP), 2, 4 and 6-trichlorophenol (2, 4, 6-TCP)), meanwhile our purpose is eliminating the limitation of the three mentioned approaches and marking as well as enhancing their advantages.

To optimize variables affecting the efficiency of chlorophenols extraction, we used Mini-Tab software and the way of surface respond based on Plackett–Burman designs (PBD) and Box–Behnken design (BBD) as well as variance analysis (ANOVA) to evaluate the effects of independent variables. Response Surface Methodology (RSM) further helps to find optimal state of

factors while showing how factors can affect the results of experiments (the scope of our study). Besides, we have considered the mutual effects of variables. In designing experiments of RSM, we can obtain a large amount of information with the fewest number of experiments [32-34]. To achieve this goal, we used sifting experiments at first, and then determined the effects of various parameters on extraction efficiency in different conditions (circumstances), utilizing surface response survey and optimizing variables.

Experimental

Chemicals and reagents

Phenol, 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 2,4,6-trichlorophenol (2,4,6-TCP), acetone, methanol, ethanol, acetonitrile (ACN), ammonium sulfate, sodium chloride, sodium sulfate, magnesium sulfate, potassium dihydrogen phosphate, 1-undecanol, 1-decanol and 1-dodecanol were purchased from Merck (Darmstadt, Germany). A stock standard solution of phenol, 2-CP, 4-CP, 2, 4, 6-TCP was obtained by dissolving phenols reagent in 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 phenol, 2-CP, 4-CP, and 2,4,6-TCP 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 275 nm, dual solvent pump (model LC-10Avp)

and aerodyne model platin blue injector with 1 μ L was used for separation. The mobile phase was made up of methanol and phosphate buffer (35:65, v/v) adjusted to pH 4.5. 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-1PLUS, Republic of Latvia) was used in the extraction procedure.

SO-VADLLME-SFODME procedure

Initially 10 mL of solution (including phenol, 2-CP, 4-CP, 2, 4, 6-TCP 100 μ g/L) was prepared and the pH=4.5 is adjusted to the desired value with the aid of the appropriate buffer solution. then, 380 μ L of methanol (distributing phase) including 40 μ L 1- undecanol (extraction solvent) with pressure were injected into the solution very fast from top and it was vortexes for 2 minutes. At the next step, 1300 μ L from salt solution of ammonium sulfate 10% (w/v) was quickly added to solution (a), it was vortexes for 2 minutes and then centrifuged for 4 min at a speed of 3000 rpm, and 1-undecanol at high level of aqueous sample was observed due to

low density; in the next stage, firstly designed lace hook made of stainless steel was put in experiment tope like a hook and then the experiment tube was put to ice bath. After 6 minutes, organic solvent 1-undecanol became solid due to low melting temperature (24 °C) and following taking out the rich, solid, and metal net from phenol and chlorophenols, it was transferred into container with 1/5 mL capacity and to 10 μ L ethanol diluted. Finally, nearly 10 μ L injected to HPLC (Figure 1).

Samples Preparation

We collected samples of drinking water and industrial sewerage respectively from laboratories, Ilam petrochemical company and river water near the petrochemical company, and we bought mineral water bottles. Moreover, drinking water without preparation was used. Sewerage water after passing from filtering paper with being distilled twice in a proportion of 1:1 was diluted. Prepared samples were preserved in a refrigerator before being used. Finally 10 mL from each sample was studied under the mentioned extraction method.

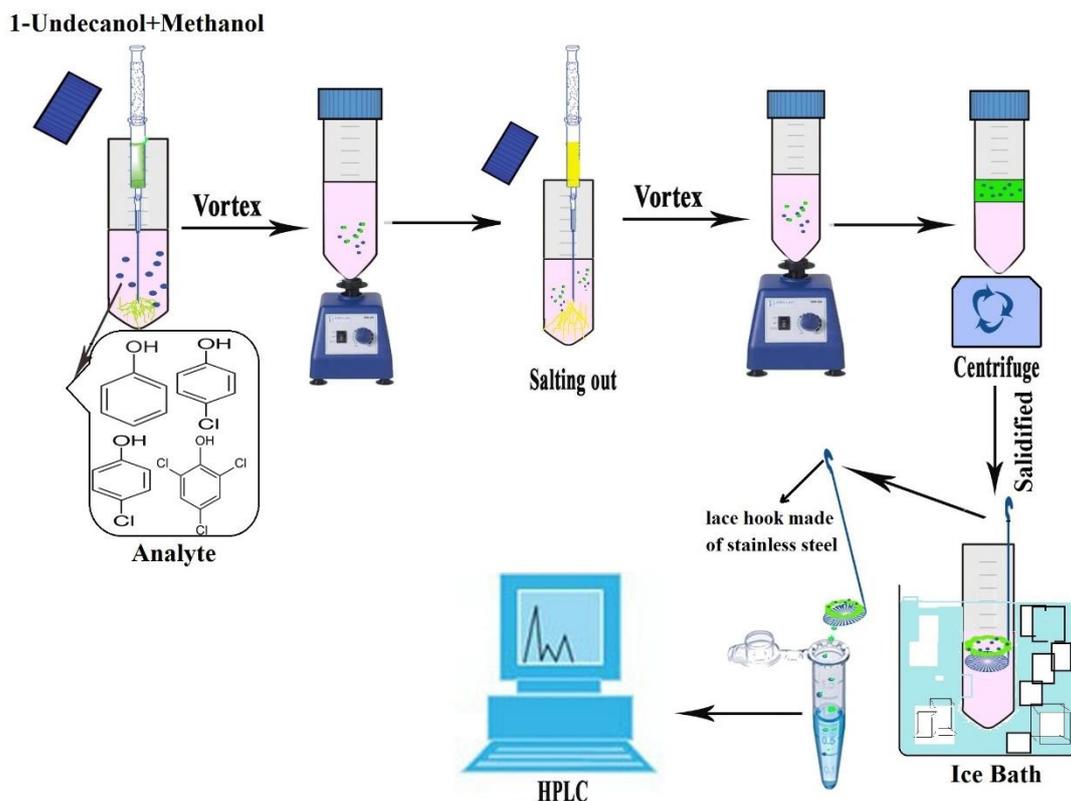


Figure 1. Schematic salting out and vortex- assisted dispersive liquid–liquid microextraction based on solidification of floating organic drop microextraction method

Results and discussion

Optimization of the SO-VADLLME-SFODME

To enhance extraction efficiency, the effects of various especial kinds and volumes of organic solvent extractor, the type and volume of organic dispersive, solvent salt type and its density, the speed of sample riling and centrifuge time separately were studied.

Selecting the type of organic extracting solvents

One of the most important steps to optimize the type of extracting solvent in the method was mentioned as an appropriate extracting solvent from 1-undecanol, 1-decanol and 1-dodecanol. To choose appropriately, the extracting solvent should meet such characteristics as follows: being immiscible with water, possessing less solvent dielectric constant, being economic and available,

being stable and having fewer changes and fluctuations, high extracting efficiency, having lower density than water density, with a melting point at a range from 10 to 30 °C (somewhat near our room temperature), and low-level of toxic. Considering these characteristics, we selected 1-undecanol, with a density of 0.83 gr/mL and a melting point between 22-24 °C as the extracting solvent in similar conditions. The results are shown in Figure 2 indicating that 1-undecanol offers higher extraction efficiency compared to the other solvents. Figure 2 shows the effects of type of organic extracting solvent with a melting temperature at a degree near room temperature on the efficiency of phenol extraction 2- CP, 4- CP, 2, 4, 6-TCP under the same condition.

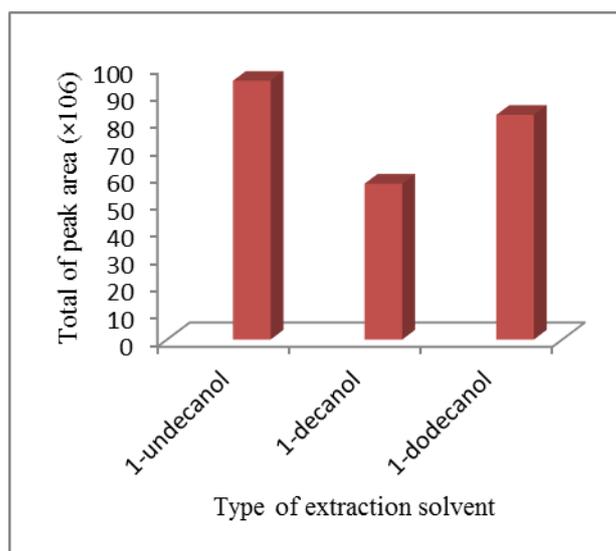


Figure 2. The type of organic extracting solvents effect on the extraction of phenol, 2- CP, 4- CP, 2, 4, 6-TCP under identical conditions

The effects of dispersive solvent

Before choosing the type of dispersive solvent, some measures such as being non-toxic breakability in water phase and economies of scale, based on solvents like acetonitrile, acetone, methanol and ethanol were taken and studied. To achieve this aim, a certain volume of liquid solution containing

samples were injected into liquid in an identical condition by Hamilton syringe and with pressure (0.5 mL of each solvent including a certain volume of 1-undecanol at first). Then, considering the results, we were led to select methanol with the highest productivity and efficiency as the optional dispersive solvent (Figure 3).

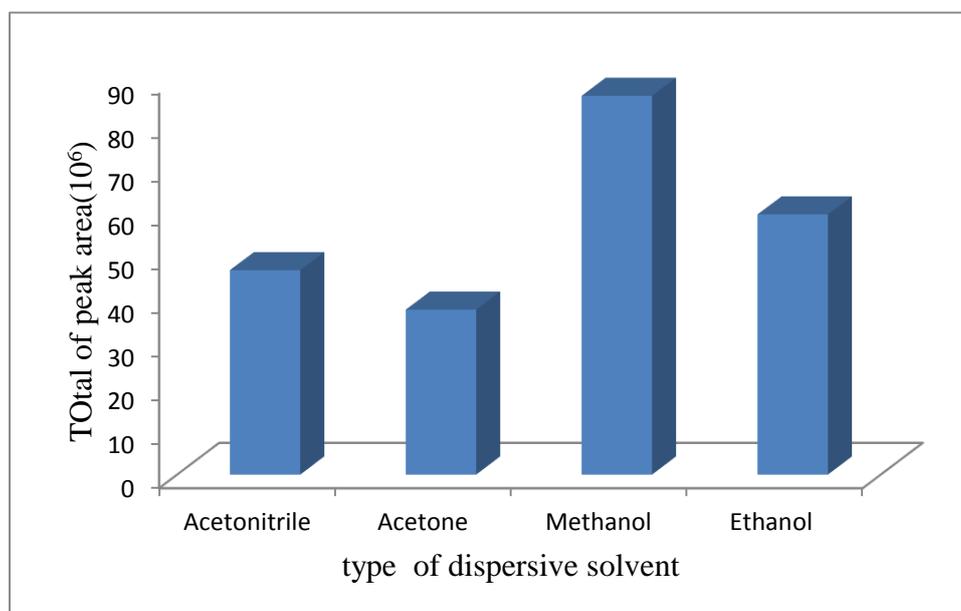


Figure 3. The type of dispersive organic solvents effect on the extraction of phenol, 2- CP, 4- CP, 2, 4, 6-TCP under identical conditions

The effect of salt type

Ions of salt, being surround by water molecules, reduces breakability degrees of different types in water phase, this, in return, causes distribution of types to increase in organic phase. To select salt, the following elements must be taken into consideration [39]. 1-Salt solubility in an organic solvent must be very negligible. 2-Salt solubility must be extremely high in aqueous phase. To select appropriate salt, the extraction efficiency of ammonium sulfate, sodium chloride, sodium sulfate, magnesium sulfate and potassium

dihydrogen phosphate were investigated. As the solubility of the mentioned types of salt in water are different from each other, to achieve the highest extraction efficiency, the same volumes of a solvent with 1 mL in (10 % w/v) were added in similar conditions and based upon the above mentioned method. Figure 4 indicates that compared to the other types of salt ammonium, sulfate has a greater effect on extraction efficiency of phenol and chlorophenols which might be related to more solubility of ammonium sulfate in water and its better salting out.

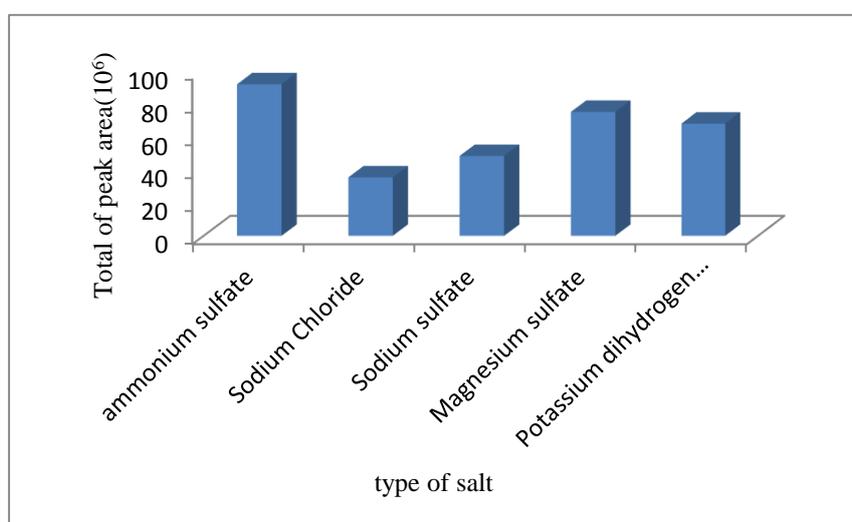


Figure 4. The type of salt effect on the extraction of phenol, 2- CP, 4- CP, 2, 4, 6-TCP under identical conditions

Plackett–Burman designs (PBD)

Plackett–Burman design (PBD) is a powerful instrument with a fast searching method for investigating important and key variables in multi variables systems; due to its simplicity, it can also be one of the really useful approaches in the initial process of optimization [35]. Among 7 variables of solvent extracting volume (μL) including pH, the volume of salt with 10%w/v, the volume of dispersive solvent (μL), Vortex extraction time (first phase) and the second phase of vortex extraction time and centrifuge time, effective variables on extraction

efficiency were riddled by using PBD and then optimized through Response Surface Methodology (RSM) and BBD. Variables' levels, their relevant codes, the number of required experiments and the observed response for each experiment are reported in Table 1. Selecting these variables based on SO-VADLLME-SFODME method and their relevant levels have also been conducted on initial experiments. In order to minimize the effect of non-controlled variables, to estimate errors and repeatability, two central points were used. The obtained results from Table 1 were analyzed using Pareto

chart. Pareto chart indicates the fact that variables affecting the results of the experiment are as follows: the volume of dispersive extracting solvents (μL), pH, the volume of salt solution with 10% w/v concentration, the volume of dispersive (μL), which passes from broken lines showing a 95% confidence level; the three remaining variables

(first phase of vortex extraction time and vortex time of extraction, second phase, and centrifuge time) don't have any significant relation with the result of the experiment. As a result, these variables were kept at low levels of 2, 2 and 4 min to conduct the next experiments.

Table 1. Levels of variables their relevant codes, the number of required experiments and results obtained for each experiment in plaket – Barman design so as to extract phenol, 2-CP, 4-CP and 2, 4, 6 –TCP

Factors	Levels		
	Low (-1)	Central (0)	High (+1)
(X ₁) solvent extracting volume (μL)	20	60	100
(X ₂)pH	2	5	8
(X ₃) volume of dispersive solvent (μL)	200	350	500
(X ₄) volume of salt (ammonium Sulfate) with 10% w/v	0.5	1	1.5
(X ₅) Vortex extraction time (first phase)(min)	2	6	10
(X ₆) Vortex extraction time (second phase)(min)	2	6	10
(X ₇) Centrifuge time (min)	4	7	10

Run	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	Total peak Area($\times 10^6$)
1	100	2	500	1.5	2	10	4	50.4
2	100	8	500	0.5	10	10	4	39.6
3	20	8	200	0.5	2	10	10	32.6
4	100	2	200	0.5	10	10	10	31.3
5	20	2	200	1.5	10	10	4	65.3
6	20	2	500	1.5	10	2	10	85.6
7	100	2	500	0.5	2	2	10	46.1
8	20	2	200	0.5	2	2	4	64.3
9- Cp	60	5	350	1.0	6	6	7	72.6
10	20	8	500	0.5	10	2	4	54.5
11	100	8	200	1.5	10	2	10	28.1
12	20	8	500	1.5	2	10	10	59.1
13- Cp	60	5	350	1.0	6	6	7	69.2
14	100	8	200	1.5	2	2	4	50.4

^{CP} Centre point

Box-Behnken design (BBD)

Box-Behnken design as an experimental model is expressed in Equation 1. This is a multi-variable with second order equation based on incomplete factorial design with vast applications in determining optimal conditions of the experiment. Each independent variable is defined at three levels according to 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 shows decomposable response (total peak area), B_0 is model constant, B_i , B_{ii} and B_{ij} / linear coefficients, second order and interaction between variables, respectively. Dependent values X_1 , X_2 , X_3 and X_4 represent coded factors of independent variables. In fact, this process of optimization consists of 3 steps: 1- Conducting designed

experiments. 2- Estimating coefficients within a mathematical model. 3- Predicting results and evaluating the quality of established model.

We used 3 central points in this scheme, based on the number of variables and central points in accordance with the Equation 2, and 27 experiments were considered [36].

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

where in Equation 2, N is the number of experiments, K is the number of variables and C_P shows the number of central point, then, assessing the properness of the empirical model as well as drawing optimization figures and diagrams were made using an appropriate mathematical model, statistical analysis of data while utilizing Mini-Tab software. After determining significant variables in PBD optimal conditions, we used the factors to obtain BBD with 4 variables including the volume of organic extracting solvent (μL) (X_1), and pH (X_2), the volume of dispersive solvent (X_3) and salt concentration of ammonium sulfate (10%w/v) (X_4). The experiments related to BBD were made randomly so that we could prevent uncontrollable errors. Obtained responses based on designing experiments using Pareto diagram were reviewed and analyzed (Figure 5). As it is observed, the linear effects of passing line through vertical dotted area, showing 95% confidence interval calculated from the experiment results, was significant such as four discussed variables. The reported results in the Pareto chart of PBD, consistent with Pareto of BBD, linear effect (L) and

second order (Q) of every variable except for the volume of dispersive solvent, are significant as well.

We also used variance analysis to evaluate the obtained results. The results from variance analysis (ANOVA) of BBD have been reported in Table 2. The p-values in Table 2 reveal that, with 95% confidence level, certain terms in the model are significant ($p\text{-value} < 0.05$), while some others are not ($p\text{-value} > 0.05$). When the $P\text{-value} > 0.05$, it means lack of fit (LOF), which is equal to 0.2035 here, implying the fact that the model designed to process experimental data has been appropriate. In addition, the quality of the fit of the polynomial model equation was explained by the coefficient of determination ($R\text{-squared} = 0.9845$ and $R\text{-adjusted} = 0.9665$). Also the linear relationship between the predicted values by the model and the experimental values is observable (Figure 6). As it has been reported in Table 2, determinant coefficient of the model is $r^2 = 0.9845$, showing great ability of the model to process the empirical data.

Moreover, the quality of the fit of the polynomial model equation was explained by the coefficient of determination ($R\text{-squared} = 0.9845$ and $R\text{-adjusted} = 0.9665$). The following compound statement equation illustrates the relationship between dissoluble answer of total peak area (Y) and significant variables. The interaction between variables has been depicted in three dimensions (Figure 7).

Table 2. ANOVA results obtained by Box-behnken design

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	5.303E+15	14	3.788E+14	54.55	< 0.0001	significant
(X ₁) -Volume of extracting solvent(μL)	1.472E+15	1	1.472E+15	211.97	< 0.0001	
(X ₂) -pH	2.350E+14	1	2.350E+14	33.84	< 0.0001	
(X ₃) -Volume of dispersive solvent (μL)	3.010E+14	1	3.010E+14	43.35	< 0.0001	
(X ₄) -volume of salt (ammonium Sulfate) with 10% w/v	4.750E+14	1	4.750E+14	68.41	< 0.0001	
X ₁ X ₂	1.588E+14	1	1.588E+14	22.86	0.0004	
X ₁ X ₃	8.100E+11	1	8.100E+11	0.1167	0.7386	
X ₁ X ₄	1.243E+14	1	1.243E+14	17.90	0.0012	
X ₂ X ₃	1.296E+13	1	1.296E+13	1.87	0.1969	
X ₂ X ₄	1.703E+14	1	1.703E+14	24.53	0.0003	
X ₃ X ₄	3.906E+13	1	3.906E+13	5.63	0.0353	
X ₁ ²	8.546E+14	1	8.546E+14	123.07	< 0.0001	
X ₂ ²	1.489E+15	1	1.489E+15	214.42	< 0.0001	
X ₃ ²	8.681E+14	1	8.681E+14	125.02	< 0.0001	
X ₄ ²	9.481E+12	1	9.481E+12	1.37	0.2653	
Residual	8.333E+13	12	6.944E+12			
Lack of Fit	7.962E+13	10	7.962E+12	4.30	0.2035	not significant
Pure Error	3.707E+12	2	1.853E+12			
Total SS	5.386E+15	26				
R ²	0.9845					
Adjusted R ²	0.9665					
Predicted R ²	0.9133					

The quadratic model to predict the extraction efficiency in terms of actual factors is as follows:

$$Y = +7.387 \times 10^7 - 1.107 \times 10^7 X_1 - 4.425 \times 10^6 X_2 + 5.008 \times 10^6 X_3 + 6.292 \times 10^6 X_4 - 6.300 \times 10^6 X_1 X_2 - 4.500 \times 10^5 X_1 X_3 - 5.575 \times 10^4 X_1 X_4 - 1.800 \times 10^6 X_2 X_3 - 6.525 \times 10^6 X_2 X_4 + 3.125 \times 10^6 X_3 X_4 - 1.266 \times 10^7 X_1^2 - 1.671 \times 10^7 X_2^2 - 1.276 \times 10^7 X_3^2 - 1.333 \times 10^6 X_4^2$$

In the next step, to study and review normal values of the reminder of decomposition, the obtained empirical results were analyzed. The results showed that the errors had been distributed according to normal distribution function, and moreover, no specific pattern was observed in the diagram. This fact showed the lack of fit (LOF) which allows a specific systematic error in the used model. In addition, the linear relation between values of total areas of couriers was

measured, and the used empirical model showed that benefits of linear regression R² is equal to 98.45 indicating that more than 98 percent extraction efficiency are related to dependent variables and only less than 2% of these changes cannot be explained by the predicted model. Therefore, high value of R² is another sign of properness of the suggested model, used in the present study. To pick out the optimal value of each variable, we used desirability function (DF). Based on the results reported, the highest value of total areas of peaks and the lowest value of it are respectively as follows: 8.33×10^7 and 2.6×10^7 in this context desirability 1 for the highest value of total peak areas, zero for lowest value and 0.5 for average result ($5.445 \cdot 10^7$) are regarded.

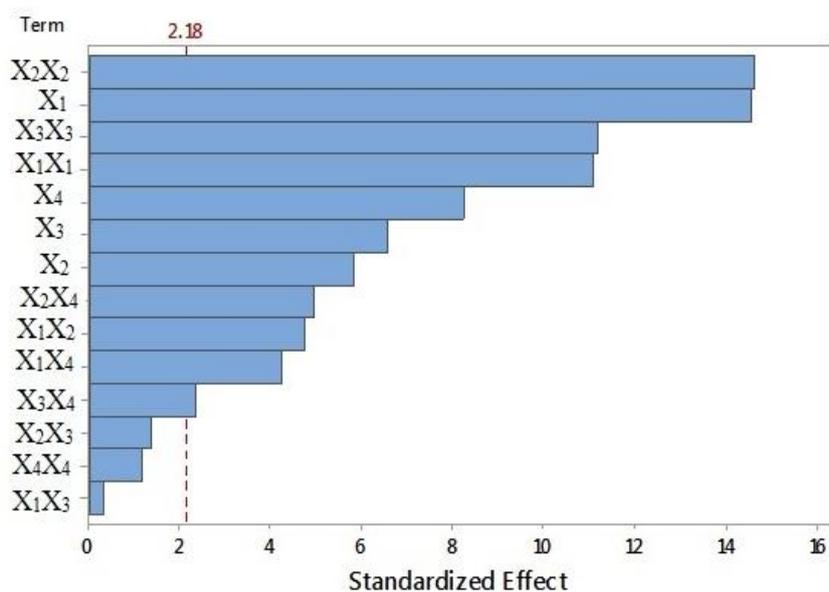


Figure 5. Pareto chart obtained from Box-Behnken design for the extraction phenol, 2-CP, 4-CP and 2, 4, 6-TCP, (X₁) the volume of organic extracting solvent, (μL), (X₂) pH, (X₃) the volume of dispersive extracting solvent(μL), (X₄) volume of salt (ammonium sulfate) with 10% w/

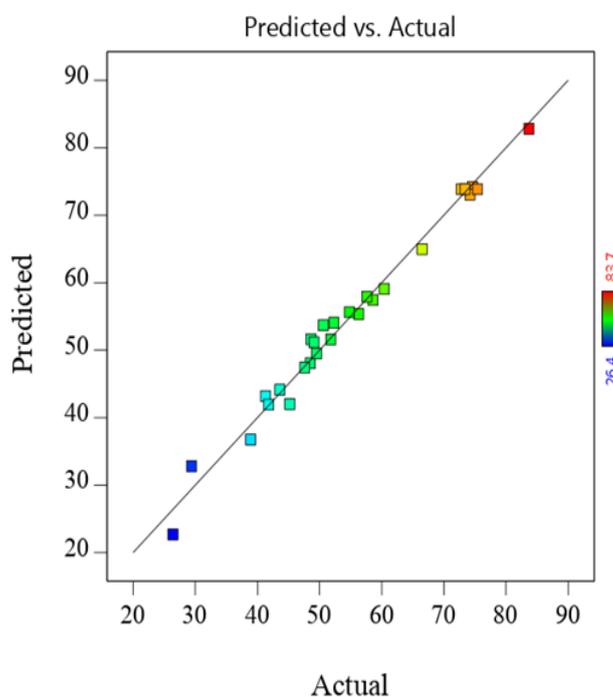


Figure 6. Shows the linear relationship to between of the predicted values by the model and the experimental values

The results obtained from desirability function (Figure 8) indicates that by increasing the volume of organic extracting solvent (μL) from 20 to 100 μL, the extraction efficiency decreases. In volumes less than 40 μL,

extraction efficiency is low due to insufficiency of solvent volumes for types. In volumes more than 40 μL, extraction efficiency is also decreasing because of an increase in volume, and thereby diluting analytes concentration.

The effects of volume of dispersive solvent on extraction efficiency were studied at a range from 200 to 500 μL . The results of Figure 8 indicate an increase in dissoluble up to 380 μL of dispersive solvent. The reasons for the reduction in extraction efficiency when using fewer volumes less than 380 μL is that the solution still has capacity and maintainability, as well as eliminating more analytes. As a result, by increasing dispersive solvent, more analytes transfer to extraction phase and therefore the volume of 380 μL was chosen as the optimal volume in Figure 8; changes in dissoluble response in line with the change in salt concentration have also been shown. As we can see, along with increasing salt concentration, the effect of salting out

the analytes will also rises. For this reason, with increasing salt concentration, salt interaction with water becomes more and thereby more analytes are transferred to the extraction phase. Considering the diagram to 1.3 mL volume, ammonium sulfate of 10% w/v, increases and from 1.3 mL volume onward, extraction efficiency with an increase in constant salt concentration has changed, in this way, we can select optimal level at 1.3 mL. Figure 8 shows changes in pH from 2 to 8 as pH increase up to 4, extraction efficiency increases too and, from pH =5 onward, we observe response reduction and the most response decomposition response is achieved at pH = 4.5.

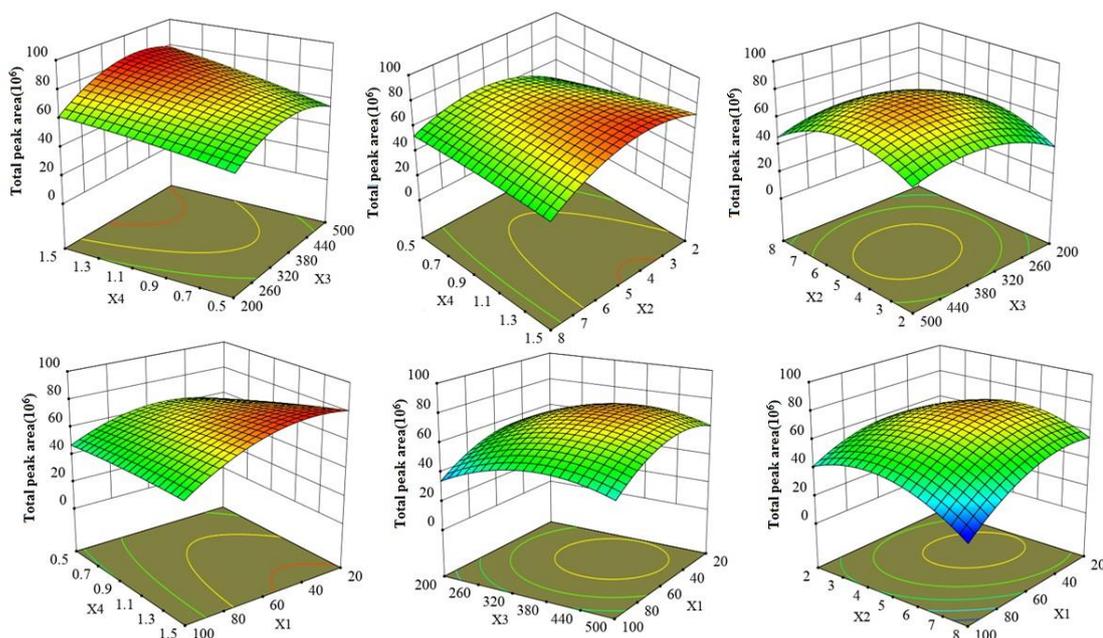


Figure 7. Three dimensional diagrams in BBD to extract phenol, 2-CP, 4-CP and 2, 4, 6-TCP, (X_1) the volume of organic extracting solvent, (μL), (X_2) pH, (X_3) the volume of dispersive extracting solvent (μL), (X_4) volume of salt (ammonium sulfate) with 10% w

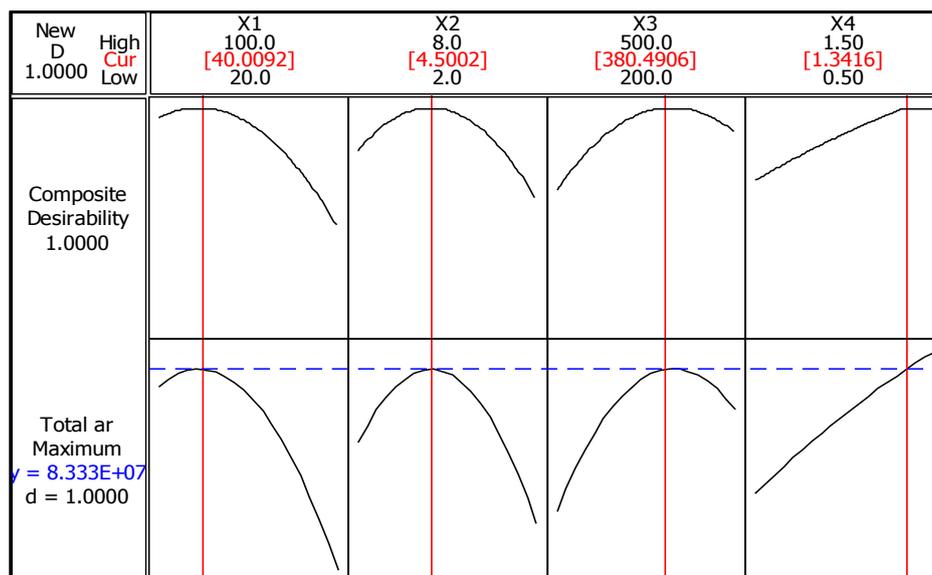


Figure 8. Desirability function to extract phenol, 2-CP, 4-CP and 2, 4, 6-TCP, (X_1) the volume of organic extracting solvent, (μL), (X_2) pH, (X_3) the volume of dispersive extracting solvent (μL), (X_4) volume of salt (ammonium sulfate) with 10% w

Desirability function diagrams show that achieving desirability 1 and the most total area at peaks is the time when using 40 μL of solvent extracted (1-undecanol), 380 μL from dispersive solvent, pH 4.5 and salt volume of 1.3 mL salt, ammonium sulfate (10% w/v) is achieved. In order to check whether there is consistency between optimal results obtained from desirability function and empirical experiments or not, 5 repeated experiments were carried out. The obtained retrieval values show consistency and coincidence between theory and empirical experiments, with the error less than 4.6 %.

Evaluation of figures of merit value method

To evaluate the method used in this research, we calculated the figures of

merit such as linear ranges (LRs), limits of detection (LODs), limits of quantification (LOQs), coefficients of determination (r^2), reproducibility (intraday precision), repeatability (intraday precision), extraction recoveries (ER %), enrichment factors (EFs) and precision employed in validating SO-VADLLME-SFODME method. EFs were calculated based on the following Equation:

$$EF = \frac{C_f}{C_i}$$

where C_f , 1-undecanol and C_i , aq are the final concentration of analytes in 1-undecanol rich phase and initial concentration of them in aqueous solution, respectively. The results are summarized in Tables 3 and 4.

Table 3. Analytical figures of merit of the proposed SO-VADLLME-SFODME method for determination and extraction phenol, 2-CP, 4-CP and 2, 4, 6-TCP

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)
phenol	0.1-1000	0.06	0.20	0.9933	165	3.7	4.2
2-CP	0.1-1000	0.07	0.23	0.9970	157	3.5	5.1
4-CP	1-1000	0.75	0.25	0.9918	148	4.6	4.9
2, 4, 6-TCP	1-1000	0.34	1.09	0.9916	136	3.9	4.1

^aDetection limits are calculated as signal to noise ratio of three times. ($\text{LOD} = \frac{3s_b}{m}$).

^bLimits of quantification ($\text{LOQ} = \frac{10s_b}{m}$) ('s' and m are the standard deviation and the slope of each calibration curve, respectively).

HPLC-UV chromatograms of the water, wastewater and standard solution are shown in Figure 9 after applying the purposed method. As can be seen, the peaks of phenol, 2-CP, 4-CP and 2, 4, 6-TCP respectively have appeared at retention times 2.2, 5.9, 8.6 and 11.1

min with acceptable disconnection and without any interference. In the river water (Ilam river) and wastewater sample phenol, 2-CP and 4-Cp were recognized, respectively. In Tap water (Ilam, Iran) and natural mineral water samples, none of species was observed.

Table 4. Recoveries and RSD (%) (n = 3) obtained for different water and wastewater samples. Spiked of phenol, 2-CP, 4-CP and 2, 4, 6-TCP

Sample	Compound	Amount found ($\mu\text{g L}^{-1} \pm \text{SD}^a$)	Recoveries (RSD%) Amount add ($\mu\text{g L}^{-1}$)		
			10	200	500
Tap water (Ilam, Iran)	phenol	ND	97.7(4.1)	98.6(4.3)	97.5(3.8)
	2-CP	ND	95.5(4.5)	97.1(3.1)	96.4(3.2)
	4-CP	ND	96.3(3.1)	99.5(2.8)	95.7(4.2)
	2, 4, 6-TCP	ND	92.8(3.8)	95.6(4.8)	94.8(3.7)
Natural mineral water	phenol	ND	94.3(4.7)	98.0(3.2)	97.1(2.8)
	2-CP	ND	96.8(4.0)	96.3(3.1)	98.5(5.1)
	4-CP	ND	100.6(3.2)	97.6(4.3)	95.2(4.3)
	2, 4, 6-TCP	ND	96.7(3.8)	93.8(3.3)	94.7(3.7)
River water (Ilam river)	phenol	20.4	99.3(4.7)	101.3(3.9)	99.4(3.5)
	2-CP	12.6	103.9(5.3)	98.6(3.3)	98.9(4.7)
	4-CP	ND	96.3(2.3)	99.9(3.2)	96.2(3.6)
	2, 4, 6-TCP	ND	97.4(2.9)	96.6(4.7)	95.6(4.6)
Wastewater Collected from a municipal wastewater treatment plant	phenol	32.1	102.5(3.6)	97.4(4.3)	102.6(2.9)
	2-CP	18.9	98.3(4.2)	101.5(5.2)	100.3(4.3)
	4-CP	6.8	98.7(3.4)	99.4(4.1)	98.6(2.9)
	2, 4, 6-TCP	ND	95.1(3.4)	96.4(3.7)	96.8(2.8)

^aStandard deviation

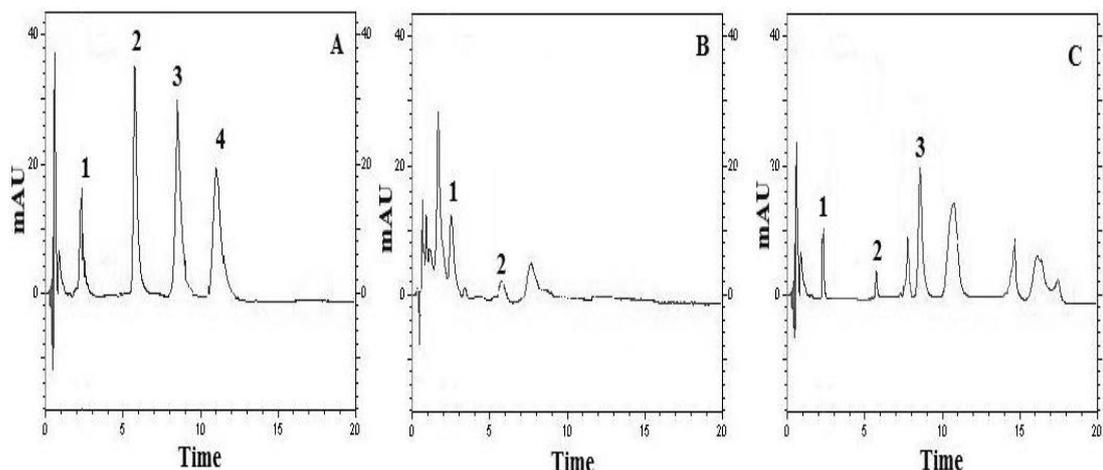


Figure 9. The chromatogram of (A) standard of analytes (1; phenol), (2; 2-CP), (3; 4-CP), (4; 2,4,6-TCP); (B) River water (Ilam river); and (C) Wastewater, spiked at $200\mu\text{g L}^{-1}$ of each, after SO-VADLLME-SFODME method under optimal conditions

Comparison of V-D- μ -SPE with other reported methods

In Table 5, linear dynamic ranges (LDRs), limits of detection (LODs), limits of quantification (LOQs), correlation of determination (r^2), the relative standard deviation (RSD%) and extraction recoveries (ER %) were compared with molecularly imprinted polymer (MIP) and PDDA-functionalized graphen- based sensors [19], salting out liquid-liquid extraction

(SALLE) combined with dispersive liquid-liquid microextraction (DLLME) [23], solid-phase microextraction method with micellar desorption HPLC [36], ionic liquid based in situ solvent formation microextraction coupled to thermal desorption-GC/MS [38], to extraction phenol, 2-CP, 4-CP and 2, 4, 6-TCP were reported. As can be seen, the figures of merit of SO-VADLLME-SFODME method are the same or even better than other methods.

Table 5. Comparison of SO-VADLLME-SFODME method with reported methods for the determination of phenol, 2-CP, 4-CP and 2, 4, 6-TCP

Method	LDR	LOD	R^2	R (%)	RSD (%)	Ref
MIP and NIP-based sensors	$0.8\text{-}100\mu\text{molL}^{-1}$	$0.3\mu\text{molL}^{-1}$	0.999	>97.7	>1.1	[19]
SALLE- DLLME	$1\text{-}300\mu\text{L}^{-1}$	$0.15\text{-}0.22\mu\text{gL}^{-1}$	0.993	>94.8	>4.8	[23]
SPME-MD-HPLC	$1\text{-}200\mu\text{gL}^{-1}$	$1.1\text{-}5.9\mu\text{gL}^{-1}$	0.993	>88.8	<15	[37]
Ionic liquid-TD-GC/MS	$0.25\text{-}50\mu\text{gL}^{-1}$	$0.06\text{-}0.44\mu\text{gL}^{-1}$	>0.990	>72.0	>4.5	[39]
SO-VADLLME-SFODME	$0.1\text{-}1000\mu\text{gL}^{-1}$	$0.06\text{-}0.75\mu\text{gL}^{-1}$	>0.9916	>92.8	<5.3	Proposal

Conclusion

In this study, the method of salting out and vortex-assisted dispersive liquid-liquid microextraction based on solidification of floating organic drop method has been used as an easy, simple, fast and accurate approach to measure phenol and chlorophenols. The advantages of this approach compared

with salting out and solidification of floating droplets microextraction are using an environment friendly solvent (compatible) with the environment use of high-performance liquid chromatography as well as being faster as a method of micro extraction and reducing extraction time, easy collecting of organic extracting solvent

for solidification of floating organic drop micro extraction by using an innovative and simple net, preventing from wasting of solvent and integrating liquid phase, increasing extraction efficiency, more than other approaches, benefiting from salting out method and also dispersive micro extraction, not using surfactants and finally integrating three extraction approaches into an approach to enhance extraction efficiency.

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