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Determination of phenolic compounds in industrial wastewaters by gas chromatography after extraction and preconcentration by microextraction procedure

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Abstract

This paper presents an efficient, simple, and fast method for the derivatization, extraction, and preconcentration of several phenolic compounds (phenol, *o*-, *m*- and *p*-cresol, 4-chlorophenol, and 2-nitrophenol) from wastewater samples and analysis of those samples by gas chromatography-flame ionization detection. In this method, initially the phenolic compounds are derivatized with acetic anhydride in an alkaline pH. In the following, the derivatized analytes are extracted into mL–volume of acetonitrile during homogeneous liquid–liquid extraction and further enrichment of the analytes are accomplished by their extraction into µL–volume of 1,1,2-trichloroethane through dispersive liquid–liquid microextraction step. Effective parameters controlling the performance of the proposed method such as type and volume of derivatization agent and catalyst, type and volume of extraction/disperser solvent in homogeneous liquid–liquid extraction, and type and volume of extraction solvent and salt addition in dispersive liquid–liquid microextraction are optimized. Under optimum conditions linear range of the proposed method was obtained 0.7–4000 µg L^{-1}. Limits of detection and quantification were in the ranges of 0.07–0.20 and 0.23–0.70 µg L^{-1}, respectively. Enrichment factors and extraction recoveries were ranged from 220 to 440 and 44 to 88%, respectively.

Keywords: Dispersive liquid–liquid microextraction, homogenous liquid–liquid extraction, gas chromatography, derivatization, phenolic compounds, wastewater samples.

1. Introduction

Phenols are aromatic components which contain one or more hydroxyl groups that are attached to an aromatic ring. The chemical properties of phenols are unique and are used widely in industry as precursors and components of numerous chemicals in the production of plastics, dyes, drugs, pesticides, antioxidants, paper, and petrochemical products (Nielson *et al.*, 1991). Owing to the increasing production and application of these compounds, they are found in ground waters, rivers, and drinking waters (Visscher *et al.*, 1996). Due to their toxicity, carcinogenicity and persistence, some of them have been included in the lists of priority pollutants of several countries and are required to be determined (Puig and Barcelo, 1996; Commission of the European Communities, 1990). High-performance liquid chromatography (Ou *et al.*, 2006), electrochemical techniques (Gan *et al.*, 2016; Gan *et al.*, 2019; Gan *et al.*, 2017; Kim *et al.*, 2015), capillary electrophoresis (Fu *et al.*, 2002), and gas chromatography (GC) (Zhou *et al.*, 2005) have been commonly used among other analytical approaches for the trace-level analysis of phenols. However GC with flame ionization detector (FID) (Ghorbanpour *et al.*, 2014; Sarafraz *et al.*, 2012; Farajzadeh *et al.*, 2014), electron capture detector (Bagheri and Saraji, 2001) or mass spectrometry (MS) (Faraji *et al.*, 2009) is preferred to the rest, because of its benefits such as high sensitivity and resolution, fast separation, and low cost (2001; Rodriguez *et al.*, 1997). Extraction of phenolic compounds from an aqueous solution into an organic phase is difficult due to polar nature of them (Pierce, 1968; Halket and Zaikin, 2004). Also, these compounds due to formation of hydrogen bond with the stationary phase of GC column have broad peaks. To resolve the problems of phenolic compounds analysis by GC and to enhance their extractability from an aqueous solution, a derivatization step prior to GC analysis is essential (Ballesteros *et al.*, 1990). For this purpose acetylation in an alkaline aqueous solution by means of acetic anhydride is a simple, cheap and efficient procedure (Rodriguez *et al.*, 1996; Llompart *et al.*, 2002; Sojo and Djauhari, 1999; Turnes *et al.*, 1996). Because of low concentrations of phenolic compounds in the aqueous solutions, sample pretreatment as well as preconcentration of the analytes are crucial steps. Aqueous samples containing phenols were prepared, to separation and preconcentration of the analytes before chromatographic analysis. For this purpose, various methods have been proposed. Liquid–liquid extraction (LLE) (Faraji *et al.*, 2009) and solid phase extraction (SPE) (Zhao *et al.*, 2009) are commonly used as sample

preparation methods before analysis of phenolic compounds in aqueous samples. These methods are basic sample preparation techniques for a diverse range of samples, but LLE is time-consuming, expensive and hazardous to health due to the high volume of potentially toxic solvents used. Additionally SPE cartridges need pretreatment and still require organic solvents for washing and elution steps. Another extraction method is homogeneous liquid–liquid extraction (HLLE) that extracts the desired solutes existing in a homogeneous aqueous solution into a water-immiscible solvent formed by each kind of phase separation phenomenon (Kujawski *et al.*, 2014). To overcome the limitations of SPE technique, solid phase microextraction (SPME) (2005; Shang *et al.*, 2014), headspace solid-phase microextraction (Bagheri *et al.*, 2008), and stir bar sorptive extraction (Hu *et al.*, 2013) methods were presented as miniaturized SPE techniques. Generally, they are expensive and their fibers (in SPME) are fragile, furthermore, they have a limited lifetime and also the possibility to create sample carryover. To overcome the problems of SPME, liquid phase microextraction (LPME) methods were introduced. Two types of LPME are single-drop LPME (Saraji and Bakhshi, 2005), and hollow fiber LPME (Villar *et al.*, 2012). In 2006, Assadi and coworkers developed a novel LPME technique termed dispersive liquid–liquid microextraction (DLLME) (Rezaee *et al.*, 2006), which consists of a ternary component solvent system. DLLME is a simple and rapid technique with great advantages of low sample volume, low cost, and relatively high enrichment factors.

The aim of this work was to introduce a simple, fast and efficient analytical method for the derivatization, extraction and determination of some phenolic compounds in aqueous samples. In this method, initially the phenolic compounds are derivatized and then extracted by an HLLE method. In the HLLE step, acetonitrile (ACN) is used as an extraction solvent. Organic phase was separated by addition of a salt. The separated layer is used as a dispersant solvent in the following DLLME step and more enrichment is achieved. Effective parameters such as type and volume of derivatization agent and catalyst, reaction time of derivatization, type and volume of extraction/disperser solvent in HLLE, type and volume of extraction solvent in DLLME step, etc will be optimized.

2. Experimental

2.1. Apparatus and chromatographic conditions

Chromatographic analysis was carried out using a Shimadzu GC-2014 gas chromatograph (Kyoto, Japan) comprising an FID and a splitless/split injector. Separation of the analytes was performed on an HP-5 MS (5% polydiphenyl, 95% polydimethyl siloxane) capillary column (30 m × 0.25 mm i.d., with a 0.25 µm film thickness) (Agilent Technologies, CA, USA) Helium (99.999%, Gulf Cryo, United Arab Emirates) was employed as the carrier gas at a constant linear velocity of 30 cm s^{-1} and make up gas at a flow rate of 30 mL min^{-1}. The injector temperature was constant at 220 °C. Injections (1 µL) were done in a splitless/split mode (sampling time of 1 min and split ratio of 1:10). The oven temperature was regulated as follows: initial temperature 40 °C (held for 2 min), elevated to 190 °C at a rate of 10 °C min^{-1} and then to more clean up the column enhanced to 220 °C and held for 5 min. The FID temperature was fixed at 220 °C. A hydrogen generator (OPGU-1500S, Shimadzu, Japan) at a flow rate of 40 mL $min^{-1}$was used to generate hydrogen gas for FID. The flow rate of air for FID was 300 mL min^{-1}. A Hettich centrifuge model D-7200 (Germany) was used for accelerating phase separation. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out on an Agilent 6890N gas chromatograph equipped with a 5973 mass-selective detector (Agilent Technologies, CA, USA). The separation was carried out on an HP-5 MS capillary column (30 m × 0.25 mm i.d., and film thickness of 0.25 mm) (Hewlett–Packard, Santa Clara, USA). Helium was used as carrier gas at a flow rate of 1.0 mL min^{-1}. Temperatures of injector and detector and as well as column temperature regulating were the same as used in GC-FID analysis.

2.2. Reagents and solutions

All studied analytes (phenol, *o*-cresol, *m*-cresol, *p*-cresol, 4-chlorophenol, and 2-nitrophenol) with a purity of >98% were purchased from Merck (Darmstadt, Germany). Deionized water was from Ghazi Company (Tabriz, Iran). Acetic anhydride was obtained from Merck as a derivatization agent. The tested compounds as the catalyst in derivatization reaction (pyridine, picoline, pipyridine, and cyclohexyl amine) were purchased from Merck. Chloroform, 1,2-dibromoethane (1,2-DBE), carbon tetrachloride, and 1,1,2-trichloroethane (1,1,2-TCE) tested as the extraction solvent in DLLME step were from Merck. ACN as the extraction/dispersant solvent was from Merck. Appropriate amounts of aforementioned phenols were dissolved in ACN in order to preparation of a mixture standard solution of the phenolic compounds at a concentration of 1000 mg L^{-1} (each analyte). Diluted solutions were prepared daily from the standard solution by adding deionized water. A mixture standard solution of the derivatized analytes was prepared by adding 20 µL picoline and 100 µL acetic anhydride into 1 mL 1,1,2-TCE containing 1000 mg L^{-1} of each phenolic compound. This solution was injected into the separation system each day (three times) for quality control, and the obtained peak areas were used in the calculation of enrichment factors (EFs) and extraction recoveries (ERs).

2.3. Samples

In order to assess the ability of the proposed method in analysis of the compounds of interest in aqueous samples, the method was applied for determination of the selected phenolic compounds in some wastewater samples. For this purpose, wastewater samples were collected from treatment plants of petrochemical and refinery units. Input and final output of petrochemical unit were obtained from Tabriz Petrochemical Company (Tabriz, Iran). Output of desalination unit and final output of refinery were collected from Tabriz Refinery. The input of the petrochemical wastewater was prepared at a dilution ratio of 1:1 from

deionized water before applying the proposed method. Other samples were used without any dilution.

2.4. Derivatization and extraction procedure

Five mL aqueous sample or deionized water spiked with 1 mg L^{-1} of each analyte or sample solution was placed in a 12-mL glass test tube. Forty µL picoline was added as a catalyst for derivatization reaction. For derivatizing of the selected phenolic compounds, 50 µL acetic anhydride was added and the resulting mixture was agitated with hand for 30 s. In the following, 2 mL ACN containing 25 µL 1,1,2-TCE was added and a uniform solution resulted. Then to initiate a two-phase separation, 1.5 g NaCl was dissolved into it and centrifuged for 1 min at 7000 rpm. In this step 1.0 ± 0.05 mL organic phase was collected at top of the tube. In the DLLME step to increase analytes enrichment, the collected organic phase in the first step was removed by a 2-mL glass syringe and rapidly injected into 5 mL deionized water containing 0.4 g NaCl placed into a 10–mL test tube with conical bottom. A cloudy solution was formed, that resulted from dispersion of the tiny droplets of 1,1,2-TCE into the aqueous solution due to dissolving ACN in water and the derivatized analytes were extracted and concentrated into 1,1,2-TCE. Then, the resultant solution was centrifuged for 5 min at 7000 rpm, which led to sedimentation of the dispersed droplets of the extractant at the bottom of the tube. In the centrifugation step 10 ± 0.5 µL of the organic phase was sedimented. 1 µL of the organic phase was withdrawn and injected into the separation system for analysis.

2.5. Calculation of EFs and ERs

Two main parameters, namely EF and ER, have been employed for evaluating the proposed method. The EF is defined as the ratio of the analyte concentration in the sedimented phase (C_{sed}) to the initial concentration of the analyte (C_0) in the sample:

$$\mathrm{EF} = C_{\mathrm{sed}} / C_0 \qquad (1)$$

C_{sed} is calculated by comparison of the peak areas obtained by direct injection of the standard solution of the derivatized analytes with those obtained by injection of the extractant after performing the proposed method. The ER is defined as the percentage of the total analyte amount (n_0) which is extracted into the sedimented phase (n_{sed}):

$$\mathrm{ER} = n_{\mathrm{sed}} / n_0 \times 100 = (C_{\mathrm{sed}} \times V_{\mathrm{sed}}) / (C_0 \times V_{\mathrm{aq}}) \times 100 = \mathrm{EF} \times V_{\mathrm{sed}} / V_{\mathrm{aq}} \times 100 \qquad (2)$$

Where V_{sed} and V_{aq} are volumes of the sedimented phase in DLLME step and aqueous solution, respectively.

3. Results and discussion

In this method, initially the selected phenolic compounds are derivatized by acetic anhydride. In the following, the derivatized analytes are extracted and preconcentrated by coupling HLLE and DLLME procedures. Indeed, the HLLE method provides extraction of the analytes from the aqueous sample and the DLLME stage results in enrichment of the analytes by transferring them into µL–volume of an extraction solvent. To obtain optimum conditions, more effective parameters on the derivatization and extraction efficiencies are investigated.

3.1. Optimization of derivatization step

3.1.1. Type and volume of catalyst

Derivatization of phenolic compounds by acetylation is usually carried out in an alkaline medium. In this procedure, a basic agent acts as a catalyst. For this purpose, four basic agents including picoline, pyridine, pipyridine, and cyclohexyl amine were tested. The obtained results showed that the selected analytes were not derivatized in the presence of pipyridine. As it can be seen from Figure 1, picoline gives the highest efficiency among the other basic catalyst used. Therefore picoline was selected as the catalyst for the further experiments. In the following, to achieve the optimized volume of picoline, varied volumes of it within the range of 6–60 µL were tested. Considering the obtained results, analytical signals were higher in the case of 40 µL picoline compared to other volumes. Therefore 40 µL was selected as the optimum volume of picoline in the subsequent stages of the optimization process. It seems that volumes less than 40 µL were not enough and in the cases of volumes higher than 40 µL, the sedimented phase volume increased which led to reduced analytical signals due to diluting effect.

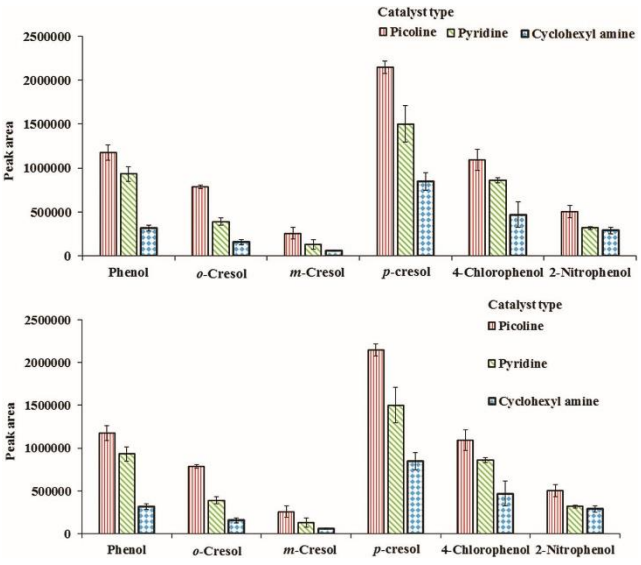

Figure 1. Study of catalyst type on the derivatization efficiency. Extraction conditions: aqueous phase, 5 mL deionized water spiked with 1 mg L^{-1} of each phenolic compounds; catalyst volume, 20 µL; acetic anhydride volume, 100 µL; acetonitrile volume, 2 mL; extraction solvent, chloroform (70 µL); concentration of NaCl, 30%, *w/v*; centrifuging rate, 7000 rpm; centrifuging time, 1 min; volume of the collected phase used in DLLME step, 1.0 mL; aqueous phase in DLLME step, 5.0 mL deionized water; centrifuging rate in DLLME step, 7000 rpm; and centrifuging time in DLLME step, 5 min. The error bars indicate the maximum and minimum of three repeated determinations

3.1.2. Derivatization reaction time

To select the optimal derivatization reaction time, different times (0, 0.5, 1.0, 2.0, 4.0, 5.0, 7.0 and 10 min) were tested. In this study, the reaction time is defined as the interval spent after mixing the aqueous solution containing the selected analytes with the derivatization reagent (acetic anhydride) and just before adding of the extraction/disperser solvent (ACN). The obtained results in Figure 2 show that the reaction time has no significant effect on the analytical signals. Indeed derivatization of the analytes is very fast. Therefore, the subsequent experiments were carried out without applying excess time for derivatization step.

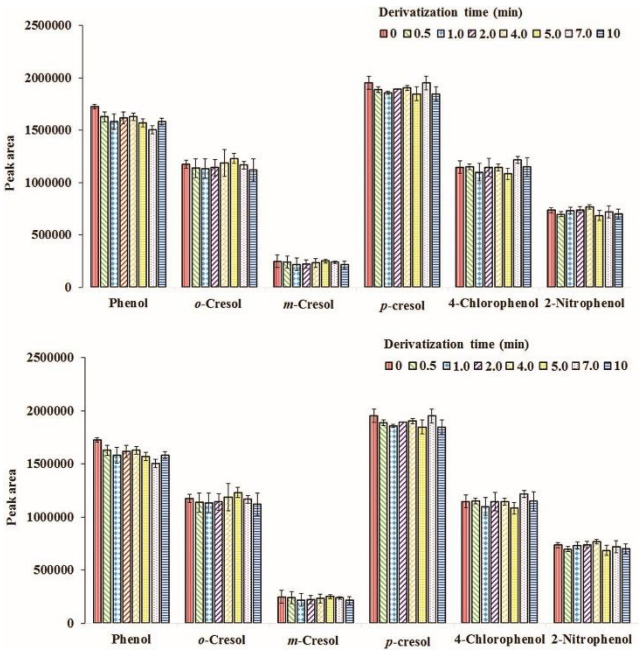

Figure 2. Study of derivatization reaction time. Extraction conditions: catalyst, picoline (40 µL); other conditions are the same as used in Figure 1. The error bars indicate the maximum and minimum of three repeated determinations

3.1.3. Derivatization reagent volume

To evaluate the effect of acetic anhydride volume on the derivatization efficiency, different volumes of the reagent (0, 5, 10, 20, 50, 70, 80, 100, and 120 µL) were tested. The obtained results (Figure 3) show that the peak areas increase up to 50 µL, and then remain constant till 80 µL and partially decrease at high volumes. It can be concluded that an inadequate derivatization of the analytes is obtained at low volumes (<50 µL) of acetic anhydride. On the other hand, at high volumes (>80 µL) of the derivatization agent, the volume of the sedimented phase increased which led to dilution of the analytes. It seems that in this case a portion of acetic anhydride is dissolved in ACN in HLLE step and transferred to DLLME procedure. Therefore 50 µL was selected as the optimum volume of acetic anhydride in the subsequent stages of the optimization process.

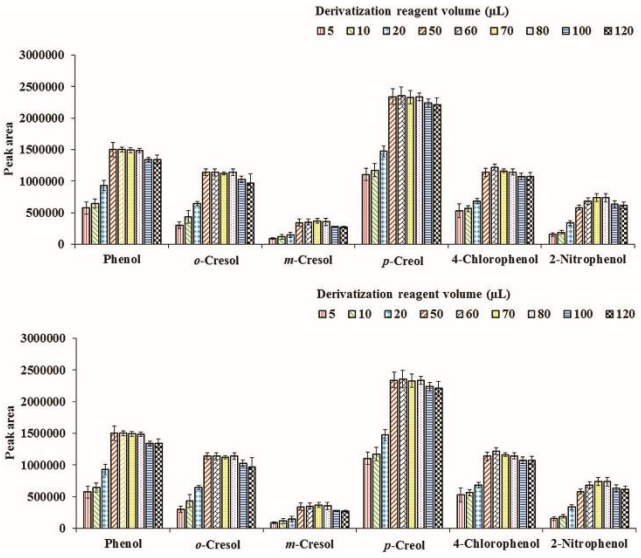

Figure 3. Influence of derivatization reagent volume. Extraction conditions: the same as used in Figure 2 without applying extraction time for derivatization step. The error bars indicate the maximum and minimum of three repeated determinations

3.2. Optimization of extraction procedures

3.2.1. Type and volume of disperser/extraction solvent in the HLLE stage

Selection of a suitable extraction solvent for the extraction of the derivatized phenolic compounds from the aqueous solution is an important parameter in this method. In this work, the extraction solvent used in HLLE step acts as a disperser solvent in the next DLLME step. This solvent is selected on the basis of its miscibility with the organic phase (extraction solvent of DLLME) and aqueous phase (to form a homogenous solution), its ability to produce a tow-phase system upon adding a salt, and its high extraction efficiency for the compounds of interest from the aqueous solution. Among the tested solvents (methanol, acetone, ACN, and THF), ACN was selected by providing the above-mentioned factors.

To study effect of ACN volume on the extraction efficiency, different volumes (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL) were tested. The volumes of the separated phase in the cases of 1.0, 1.5, 2.0, 2.5, and 3.0 mL were 0.2, 0.6, 1.0, 1.6, and 2.0 mL, respectively. Also no separated phase was obtained when 0.5 mL ACN was used. It is noted that in the 0.2 and 0.6 mL, the collected phase volume was reached to 1 mL with pure ACN and then applied in DLLME procedure. Also, in the cases of 1.6 and 2.0 mL collected phase, only 1 mL of them was utilized for the following DLLME step. Based on the achieved results (Figure 4), ERs increase till 2 mL, and then decrease to corresponding amounts at higher volumes of ACN. Therefore 2 mL was selected as the optimum volume of ACN.

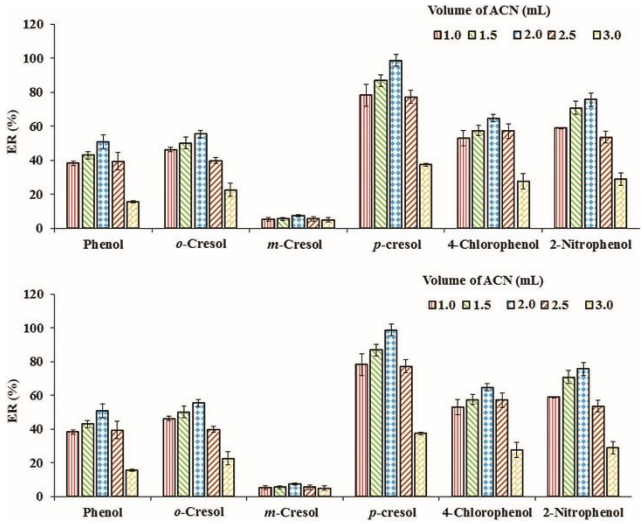

Figure 4. Study of ACN volume. Extraction conditions: the same as used in Figure 3, except 50 µL acetic anhydride was used as derivatization agent. The error bars indicate the maximum and minimum of three repeated determinations

3.2.2. Selection of extraction solvent kind and volume in DLLME stage

One of the important and effective parameters on the extraction efficiency of the proposed method is the extraction solvent kind in DLLME. The extraction solvent should have the following features: immiscible in water, high ability to extract the analytes, good chromatographic behavior, and preferably higher density than water. For this purpose four organic solvents including chloroform, 1,2-DBE, 1,1.2-TCE, and carbon tetrachloride were tested. To obtain a same sedimented phase volume (50 ± 2 µL), 100 µL chloroform, 82 µL 1,2-DBE, 93 µL carbon tetrachloride, and 70 µL 1,1.2-TCE were used. As it is shown in Figure 5, 1,1,2-TCE is a proper extraction solvent for this stage since it provides high analytical response among the other tested solvents. Therefore, it was selected as the extraction solvent.

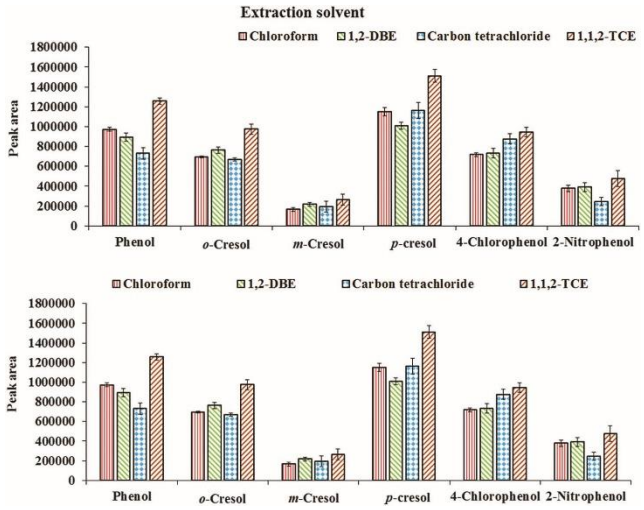

Figure 5. Selection of extraction solvent in DLLME step. Extraction conditions: the same as used in Figure 4, except 2 mL ACN was used in HLLE step. The error bars indicate the maximum and minimum of three repeated determinations

Volume of the extraction solvent can affect repeatability of the results and EF by changing volume of the sedimented phase. To study the effect of this parameter \different volumes of 1,1,2-TCE (30, 40, 50, 60, 70, and 80 µL) were examined. The obtained results showed that by increasing volume of the extraction solvent, the analytical signals decreased. It is noted that by increasing the volume of 1,1,2-TCE from 30 to 80 µL, the volume of the sedimented phase increased from 10 to 60 µL. Therefore, 30 µL was selected as the suitable volume of the extraction solvent in order to obtain high EFs.

3.2.3. Investigation of ionic strength effect in DLLME

Generally, salt addition can have multiple effects on the extraction efficiency which have been addressed as follows: (1) Solubility of the analytes in aqueous phase decreased and their extraction into organic phase enhanced which improves extraction efficiency, (2) solubility of the extraction solvent in aqueous phase was decreased which leads to increase in volume of the sedimented organic phase, and (3) viscosity of the aqueous phase was increased which leads to decrease in diffusion coefficients of the analytes and low ERs are obtained. To investigate the effect of salt addition, varied values of sodium chloride within the range of 0, 4, 8, and 12%, *w/v* were investigated. To access a same volume of the precipitated phase (10 ± 0.5 µL), 30, 27, 25, and 20 µL of the extraction solvent (1,1,2-TCE) were used for 0, 4, 8, and 12%, *w/v,* of salt, respectively. The results (Figure 6) indicate that the extraction efficiency increases up to 8%, *w/v*, and then decreases at high concentrations of the salt. Therefore 8%, *w/v,* NaCl was selected for the further studies.

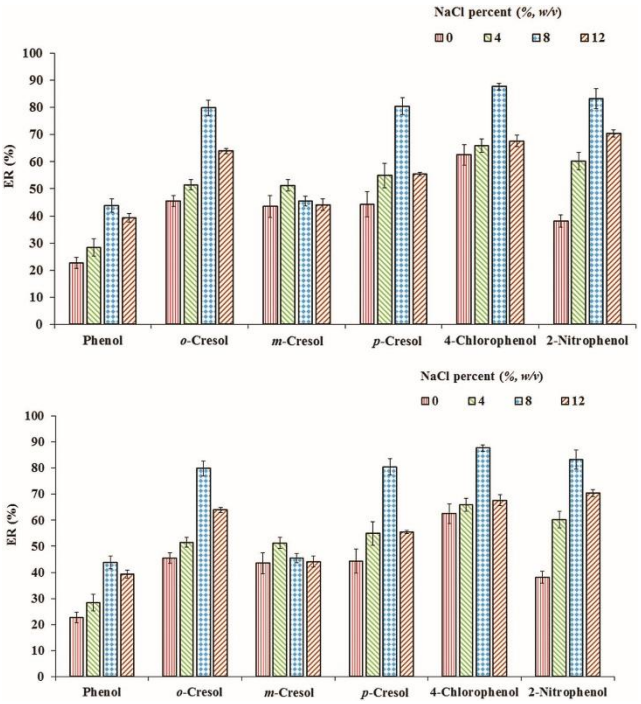

Figure 6. Study of ionic strength in DLLME. Extraction conditions: the same as used in Figure 5, except 30 µL 1,1,2-TCE was used. The error bars indicate the maximum and minimum of three repeated determinations

3.2.4. Evaluation of analytical performance of the method

The performance of the proposed method in analysis of the selected phenolic compounds was assayed under the obtained optimum conditions by calculation of linear range (LR), coefficient of determination, limit of detection (LOD), limit of quantification (LOQ), precision expressed as relative standard deviation (RSD%), EF, and ER. These results are summarized in Table 1. According to the results, the RSD values are equal or less than 8% for intra- and inter-day precisions which indicate that an acceptable repeatability for the developed technique is achievable. The calibration graph is linear in the broad concentration ranges for all selected analytes with coefficients of determination higher than 0.996. The LODs and LOQs calculated on the basis of signal to noise ratio (S/N) of 3 and 10, respectively, ranged from 0.07–0.20 and 0.23–0.70 μg L^{-1}, respectively. The EFs and ERs are between 220 and 440 and 44 and 88%, respectively. Good repeatability, high EFs and ERs, and low LODs and LOQs are main advantages of the proposed method.

Table 1. Quantitative features of the proposed method for the selected phenolic compounds

Analyte	LR[a]	R^{2}[b]	LOD[c]	LOQ[d]	EF ± SD[e]	ER ± SD[f]	RSD (%)[g]	
							Intra-day	Inter-day
Phenol	0.33–4000	0.998	0.10	0.33	220 ± 20	44 ± 4	5	6
o-Cresol	0.26–4000	0.996	0.08	0.26	400 ± 15	80 ± 3	4	5
m-Cresol	0.70–4000	0.998	0.20	0.70	225 ± 7	45 ± 3	6	7
p-Cresol	0.33–4000	0.998	0.10	0.33	400 ± 20	80 ± 4	5	7
4-Chlorophenol	0.23–4000	0.998	0.07	0.23	440 ± 15	88 ± 3	4	5
2-Nitrophenol	0.26–4000	0.998	0.08	0.26	415 ± 30	83 ± 6	8	8

[a]*Linear range (μg L^{-1})*

[b]*Coefficient of determination*

[c]*Limit of detection, S/N = 3 (μg L^{-1})*

[d]*Limit of quantification, S/N = 10 (μg L^{-1})*

[e]*Mean enrichment factor ± standard deviation, (n = 3)*

[f]*Mean extraction recovery ± standard deviation, (n = 3)*

[g]*Relative standard deviation (n = 6, C = 50 μg L^{-1}) for intra-day and (n = 4, C = 50 μg L^{-1}) for inter-day precisions*

3.2.5. Real sample analysis

To demonstrate the performance of the proposed method, it was applied to the determination of the target analytes in four wastewater samples including input and output of treatment plant of Tabriz Petrochemical Company, output of the desalination unit and final output of refinery (both from Tabriz Refinery). After extracting the analytes with the proposed method and their determination by GC-FID, the analytes concentrations were calculated by standard addition method and shown in Table 2. The typical GC-FID chromatograms of blank, standard solution (200 mg L^{-1} of each derivatized analyte), output of the desalination unit refinery wastewater, input of the petrochemical wastewater, final output of refinery wastewater, and final output of petrochemical wastewater are shown in Figure 7. According the obtained results, none of the analytes were detected in final output of petrochemical wastewater and final output of refinery wastewater. While in the output of the desalination unit, some peaks are observed at retention times of the analytes that they can be related to phenol, *o*-cresol, *m*-cresol-, *p*-cresol, and 4-chlorophenol. Also, in the input of petrochemical wastewater sample two peaks are observed at retention times of phenol and *m*-cresol.

To confirm the obtained results, all samples were analyzed by GC-MS after performing the proposed method on the mentioned samples. The obtained typical total ions current (TIC) chromatogram for output of desalination unit of refinery along with the mass data are shown in Figure 8. The mass data confirmed the presence of the mentioned analytes in the samples. Matrix effect was studied through "added-found" method. For this purpose the samples were spiked at three different concentrations (50, 100, and 500 μg L^{-1} of each analyte) and analyzed by the proposed method. The obtained peak areas were compared with the corresponding peak areas in the chromatogram of deionized water added the same concentrations. The results of this comparison as relative recoveries are summarized in Table 3. As a result, matrix effect was only observed in input of petrochemical wastewater. To solve this problem, after testing different dilution ratios, input of the petrochemical wastewater was diluted at a ratio of 1:1 with deionized water to reduce its matrix effect.

Table 2. Analytes' contents of the samples determined by the proposed HLLE-DLLME-GC-FID method

Analyte	Mean concentration of the analyte (μg L^{-1}) ± standard deviation (n = 3)			
	Input of petrochemical wastewater	Final output of petrochemical Wastewater	Output of desalination unit of refinery wastewater	Final output of refinery wastewater
Phenol	2049 ± 113	ND[a]	788 ± 43	ND
o-Cresol	ND	ND	243 ± 11	ND
m-Cresol	31 ± 2	ND	109 ± 6	ND
p-Cresol	ND	ND	91 ± 5	ND
4-Chlorophenol	ND	ND	74 ± 3	ND
2-Nitrophenol	ND	ND	ND	ND

[a]*Not detected*

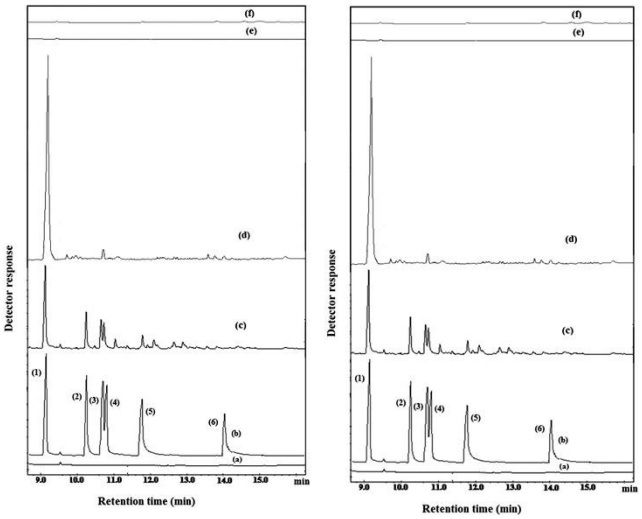

Figure 7. GC-FID chromatograms of: (a) blank, (b) standard solution of the derivatized phenolic compounds in 1,1,2-TCE (200 mg L^{-1}, each phenolic compound), (c) output of desalination unit of refinery, (d) input of the petrochemical wastewater, (e) final output of refinery wastewater, and (f) final output of petrochemical wastewater. All chromatograms, except (b) were obtained by applying the extraction method and injection 1 µL of the sedimented organic phase into GC-FID. In chromatogram (b) direct injection (1 µL) was used. Peaks identification: (1) phenol, (2) *o*-cresol, (3) *m*-cresol, (4) *p*-cresol, (5) 4-chlorophenol, and (6) 2-nitrophenol

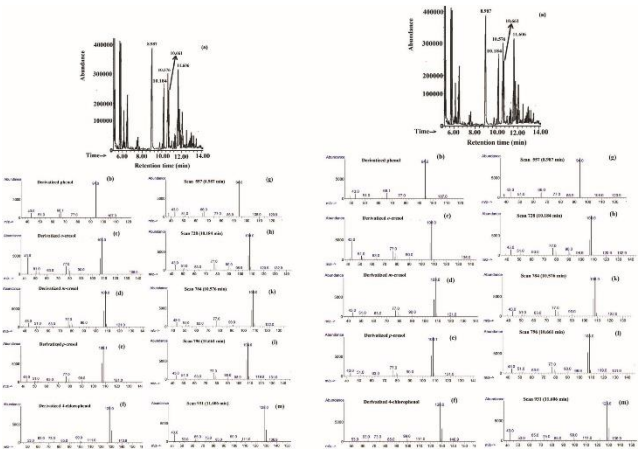

Figure 8. (a) GC-TIC-MS of output of desalination unit of refinery after performing the proposed method and mass spectra of derivatized (b) phenol, (c) *o*-cresol, (d) *m*-cresol, (e) *p*-cresol, and (f) 4-chlorophenol, and scans (g) 557 (retention time 8.987 min), (h) 728 (retention time 10.184 min), (k) 784 (retention time 10.576 min), (l) 796 (retention time 10.661 min), and (m) 931 (retention time 11.606 min)

Table 3. Study of matrix effect in the studied samples. Analytes' contents of the samples were subtracted. All samples were used without dilution, except input of petrochemical wastewater which was diluted 1:1 with deionized water

Analyte	Mean relative recovery (%) ± standard deviation (n = 3)			
	Input of petrochemical wastewater	Output of desalination unit of refinery wastewater	Final output of refinery wastewater	Final output of petrochemical wastewater
All samples were spiked with each analyte at a concentration of 50 µg L^{-1}				
Phenol	75 ± 4	89 ± 5	99 ± 5	88 ± 5
o-Cresol	94 ± 4	71 ± 3	95 ± 4	94 ± 4
m-Cresol	73 ± 4	90 ± 6	100 ± 6	89 ± 6
p-Cresol	80 ± 4	83 ± 4	88 ± 4	93 ± 5
4-Chlorophenol	88 ± 4	89 ± 4	100 ± 4	72 ± 3
2-Nitrophenol	81 ± 6	99 ± 8	92 ± 7	95 ± 8
All samples were spiked with each analyte at a concentration of 100 µg L^{-1}.				
Phenol	81 ± 4	97 ± 5	95 ±5	90 ± 5
o-Cresol	98 ± 4	78 ± 3	95 ± 4	92 ± 4
m-Cresol	97 ± 6	87 ± 6	96 ± 6	96 ± 6
p-Cresol	95 ± 5	97 ± 5	91 ± 4	93 ± 5
4-Chlorophenol	97 ± 4	97 ± 4	90 ± 4	92 ± 4
2-Nitrophenol	89 ± 7	92 ± 7	98 ± 8	86 ± 7
All samples were spiked with each analyte at a concentration of 500 µg L^{-1}.				
Phenol	100 ± 5	81 ± 4	96 ± 5	97 ± 5
o-Cresol	95 ± 4	87 ± 4	86 ± 4	100 ± 4
m-Cresol	99 ± 6	98 ± 6	92 ± 6	99 ± 6
p-Cresol	97 ± 5	98 ± 5	97 ± 5	99 ± 5
4-Chlorophenol	80 ± 4	90 ± 4	95 ± 4	92 ± 4
2-Nitrophenol	70 ± 7	88 ± 7	96 ± 7	94 ± 8

3.2.6. Comparison of the proposed method with other approaches

For this purpose analytical characteristics of the proposed method including LOD, LR, RSD, and EF were compared with those of other relevant methods for determination of the phenolic compounds in aqueous samples. These results are summarized in Table 4. The current method exhibits low or comparable RSDs with others. The LODs of the proposed method are lower than those of other methods. In addition wide linear range was observed for calibration curve of all analytes. High EF is another advantage of the method compared to other approaches.

Table 4. Comparison of the presented method with other methods used in determination of different phenolic compounds

Analyte	Sample	LR[a] (μg L^{-1})	LOD[b] (μg L^{-1})	EF[c]	RSD (%)[d]	Method	Ref.
2-Nitrophenol	Water samples	50–300	10	336	1.48	Hollow fiber-based three-phase LPME-CE[e]	(Sanaji *et al.*, 2010)
Phenol	Wastewater	5–10000	1.38	–	1.65	LPME-GC-FID[f]	(Zhang and Marzban, 2010)
o-Cresol		5–10000	1.97	–	3.58		
m-Cresol		5–10000	1.34	–	0.96		
Phenol	Wastewater	5–200	1.3	30	14.8	DLLME-HPLC-DAD[g]	(Saraji *et al.*, 2010)
2-Nitrophenol		0.5–500	0.4	97	16.6		
4-Chlorophenol	Water samples	4–400	2	383	4.7	DLLME-derivatization-GC-ECD[h]	(Fattahi *et al.*, 2007)
Phenol	Aqueous samples	0.33–4000	0.10	220	5	Derivatization-HLLE-DLLME-GC-FID[i]	This work
o-Cresol		0.26–4000	0.08	400	4		
m-Cresol		0.70–4000	0.20	225	6		
p-Cresol		0.33–4000	0.10	400	5		
4-Chlorophenol		0.23–4000	0.07	440	4		
2-Nitrophenol		0.26–4000	0.08	415	8		

[a]*Linear range*
[b]*Limit of detection*
[c]*Enrichment factor*
[d]*Relative standard deviation*
[e]*Hollow fiber-based three phase liquid-phase microextraction-capillary electrophoresis*
[f]*Liquid-phase microextraction-gas chromatography- flame ionization detection*
[g]*Dispersive liquid–liquid microextraction-high performance liquid chromatography–diode array detector*
[h]*Dispersive liquid–liquid microextraction-derivatization-gas chromatography-electron capture detector*
[i]*Derivatization-homogeneous liquid–liquid extraction-dispersive liquid–liquid microextraction-gas chromatography-flame ionization detector*

4. Conclusions

In this study, initially the studied phenolic compounds in aqueous samples were derivatized with acetic anhydride and then extracted and preconcentrated by coupling HLLE and DLLME methods. The derivatization process used in this study have some advantageous such as effective derivatization of the phenolic compounds and saving time. This method benefits the advantages of both HLLE and DLLME methods. Evaluation of the proposed method by its applying on real samples demonstrated that this method is a powerful analytical technique which provides high extraction efficiency, short extraction time, simplicity of operation, low cost, and low consumption of organic solvents. Accordingly, this method is appropriate for precise and accurate determination of the studied phenolic compounds in aqueous samples.

Abbreviations: DLLME, Dispersive liquid–liquid microextraction; EF, Enrichment factor; ER, Extraction recovery; FID, Flame ionization detector; GC, Gas chromatography; HLLE, Homogeneous liquid–liquid extraction; LOD, Limit of detection; LOQ, Limit of quantification; LPME, Liquid phase microextraction; MS, Mass spectrometry; RSD, Relative standard deviation.

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