

Determination of Chlorophenols in Marine Sediments Using Reverse Phase Dispersive Liquid-Liquid Microextraction Method Coupled with High Performance Liquid Chromatography

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Abstract:

In this study, Reverse phase dispersive liquid-liquid micro extraction (RP-DLLME) method coupled with high performance liquid chromatography (HPLC) have been successfully developed for pre-concentrate of ultra-trace amounts of 2-chlorophenol and 2,4-dichlorophenol from marine sediments after primary ultrasonic extraction. In this method, alkaline water was chosen as extraction solvent instead of organic solvent and other factors including pH, extraction time, type and volume of disperser solvents were optimized. Under optimal conditions, linear ranges for 2-chlorophenol and 2,4-dichlorophenol were between 0.001-2 mg.Kg⁻¹ and 0.2-2 mg.Kg⁻¹, respectively. The preconcentration factor of 101, 102 and the relative standard deviation (n=5) 5.9, 3.3 as well as limit of detection 0.01, 0.006 µg.kg⁻¹ were obtained for 2-chlorophenol and 2,4-dichlorophenol, respectively. Finally, using the high sensitivity, low organic solvent consumption and waste generation procedure, 2-chlorophenol and 2,4-dichlorophenol contents in marine sediments from several locations in Chabahar Bay (southeast Iran) were estimated at 0.21-2.18 mg.Kg⁻¹ and 0.68-2.55 mg.Kg⁻¹, respectively.

Keywords: Chlorophenols, marine sediments, Reverse phase dispersive liquid-liquid microextraction, HPLC.

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1. Introduction

Phenols, and particularly chlorophenols (CPs), belong to the class of compounds especially harmful to the environment. They are widely distributed in the environment due to their industrial use as intermediates in the production of chlorinated pesticides, fungicides, wood preservatives and application in the preparation of adhesives (Afghan, 1989). CPs; and their presence in the natural environment is of particular interest. CPs have been included in both the U.S. Environmental Protection Agency (EPA) and the European Community (EC) priority pollutants lists due to their toxicity, persistence and health effects (EPA, 1984). Sediments or suspended solids are good adsorbents of CPs due to their high surface area and surface activities. Accumulation of CPs to relatively high concentrations can subsequently negatively affect aquatic life (Tolgyessy, 2009). Therefore, utilization of suitable analytical methods for determining these pollutants in solid matrices is necessary. In general the extraction of phenolic compounds, e.g., CPs, from solid matrices is not straightforward due to the strong binding of them with soil organic matters (Alonso, 1998). Due to the importance of monitoring their levels in the environment, the analysis of phenolic compounds in soil and sediment samples has been extensively studied. A great variety of sample pre-treatment techniques such as Soxhlet extraction (Alonso, 1998; Paasivirta, 1990), ultrasonic solvent extraction (Czaplicka, 2001), accelerated solvent extraction (Wennrich, 2000), microwave assisted extraction (wennrich, 2000; Morales, 2005) and supercritical fluid extraction (Naeeni, 2012; Liu, 1993) as well as pressurized liquid extraction (Richter, 1996) have been applied in the analysis of CPs in solid matrices. Since, the majority of these methods require large volumes of poisonous organic solvents, large amounts of samples, and a long extraction time and manipulation, much consideration in sample preparation has been centralized on miniaturization of the liquid–liquid extraction techniques to provide considerable reduction of extraction time as well as organic solvent consumption. Nowadays, a number of alternative separation methods, such as cloud-point extraction (Santana, 2004), single-drop micro extraction (Saraji, 2005), homogeneous liquid–liquid microextraction (Cabuk, 2014) and dispersive liquid–liquid micro extraction (DLLME) (López-Darias, 2010; Nassiri, 2014; Salgueiro-González, 2012) which can be applied in the case of liquid samples have been developed.

Recently, we have successfully developed a new generation of dispersive liquid–liquid micro extraction (DLLME), termed as reversed-phase (RP) DLLME for the preconcentration of trace amounts of total phenol from sediment samples as a prior step to its enhanced determination by UV–Vis spectrophotometry (Ziyaadini, 2016). In the proposed RP-DLLME technique, the solvent polarity reverses in the ordinary DLLME and the toxic solvents replaces with water. In this procedure, a small volume of water is dispersed in organic solvent with the aim of a moderately polar solvent as the disperser. Therefore, the sedimented phase will be an aqueous micro-drop that can be directly injected into analytical instruments.

In this context, the aim of this study was to optimization of the RP-DLLME Method Coupled with High Performance Liquid Chromatography (HPLC) for determination of 2-chlorophenol and 2,4-dichlorophenol in marine sediments of Chabahar Bay.

2. Experimental Section

2.1. Chemical and Reagents

A 1000 mg/L standard solution of 2-chlorophenol and 2,4-dichlorophenol (Merck, Germany) was prepared by dissolving in n-hexane-dichloromethane (50:50, v/v). Working solutions were prepared daily by dilution of this stock solution. Dichloromethane, n-hexane were purchased from Fluka (Buchs, Switzerland) and were used after cleaning up with ultrapure water. Analytical grade ethanol, acetone and acetonitrile (Merck) were used as received.

2.2. Instruments and Conditions

The used HPLC system was from Knauer (Berlin-Zehlendorf, Germany) and consisted of a EA4300F smartline pump, fitted with a smartline autosampler 3950, a UV detector 2500 (at 254 nm) and a 250 × 4.6mm Eurospher 100-5 C18 with precolumn. Chromatographic data handling was performed using ChromGate V3.1.7 software. The separations and determinations of CPs were carried out with a mobile phase of (63:37) acetonitrile:water solution at a flow rate of 0.5 mL min⁻¹ isocratically. A centrifuge Centurion Scientific k3 series model k241R was used to accelerate the sedimentation of the rich phase in RP-DLME procedure. A 100 µL Hamilton syringe (Hamilton Company, Nevada) was used for phase separation of the collected sediments. A TPS pH-meter model wp-80 (Brisbane, Australia) equipped with a glass combination electrode was used for pH measurements.

2.3. Sediments Collection

Marine sediments were collected from different parts of the Chabahar bay coastline (Fig. 1) at winter 2017. All samples were transferred in labeled bags and placed in a cooler packed with ice during transportation to the laboratory. They were freeze-dried, passed through a 0.2 mm- sieve, and carefully preserved in glass bottle with Al-foil cover at 4 °C until laboratory analysis.



Fig. 1. Locations of coastal Chabahar Bay sampling sites (1: 25°0.355048 N, E 60°0.602569, 2: 25°0.3094833 N, E 60°0.624362, 3: 25°0.313012 N, E 60°0.616975, 4: 25°0.292438 N, E 60°0.601179, 5: 25°0.35961 N, E 60°0.311075.)

2.4. Ultrasonic Extraction

The primary extraction of analyte from the sediments was carried out exactly the same as our previous work (Ziyaadini, 2016) by the standard of EPA method 3550C, so that 1 g of the dried sediment samples was added to 5.0 mL of n-hexane- dichloromethane (50:50, v/v) and the samples were then sonicated for a further 15 min by an ultrasonic extractor. After centrifugation at 4000 rpm for 10 min, the supernatant was decanted in to 15 mL conical glass sample tube and protected for the next step.

2.5. RP-DLLME Procedure

An amount of 5.0 mL of standard solution (containing 1mg/L of 2-CP and 2,4-DCP in n-hexane-dichloromethane (50:50, v/v)) or real sample (5.0 mL of the solution, which have been prepared in the previous step) was placed in the glass test tube with a lengthened conical bottom. In a microvial, 100 μ L 0.7 M NaOH solution (as extraction solvent) and 300 μ L Acetone (as disperser) were mixed, and the mixture was rapidly injected into the diluted sample by a 2-mL polyethylene syringe. So, the extraction solvent (water) was dispersed into the sample as very fine droplets to form cloudy solution. The solution centrifuged for 5 min at 4000 rpm. The sedimented phase was completely transferred to a micro vial using a 100- μ L micro syringe and 20 μ L of the alkaline aqueous phase was injected to HPLC after pH adjustment by addition of hydrochloric acid solution.

2.6. HPLC Analysis

Separations were carried out at room temperature. Different mobile phases were used to optimize the efficiency of separation of CPs. When the mobile phase was 100% methanol, the overlap phenomenon occurred between the two CPs and the solvent signal. In order to improve the separation efficiency, an appropriate ratio was used to increase the polarization of the mobile phase. The best separation was done in the mixture of 63/37 ratio (acetonitrile/water). Therefore, this ratio was chosen as the optimal mobile phase. The mobile phase was constantly degassed using helium sparging and used at a flow-rate of 0.5 mL/min. The detections were performed at 295 nm.

3. RESULTS AND DISCUSSION

3.1. Optimization of RP-DLLME parameters

3.1.1. Selection of Extraction Solvent

Phenolic compounds are present as hydrophilic salt forms in alkaline condition (Liu, 2013), thus they can be extracted to the alkaline aqueous phase from organic phase. Utilization of alkaline water as extraction solvent is preferable compared to extraction with organic solvents; at a first regard, it can be considered as a clean-up step after Ultrasound-assisted extraction (UAE). After UAE, analytes can be extracted and preconcentrated by RP-DLLME to microliter amount of alkaline water. In order to evaluate the effect of extraction solvent volume and concentration on the extraction efficiency of CPs, 50, 100, 125 and 250 μL of aqueous NaOH Solutions with different concentrations (0.1–1.2 M) were used to extract the spiked blank sample, and the corresponding results are shown in Fig. 2. A significant improvement in the Extraction Recovery was found as the concentration of NaOH was increased up to 0.7 M and then the Extraction Recovery did not increase with a further increase in the concentration of NaOH. It was also observed that the extraction recovery has been increased with increasing solvent volume up to 100 μL . Extraction recovery has been decrease above 100 μL . Based on these observations, a volume of 100 μL was used for further experiments.

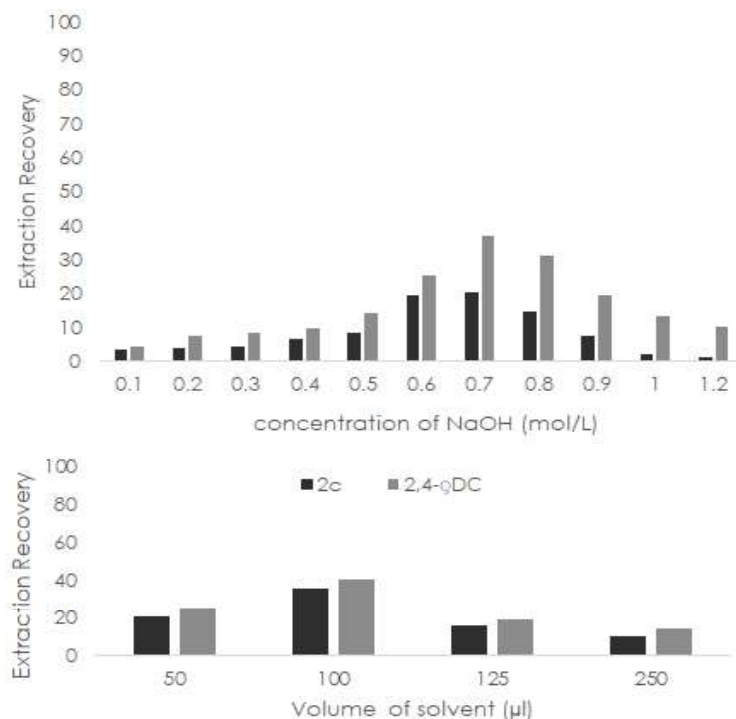


Fig. 2. Effect of extraction Solvent (Concentration & Volume)

3.1.2. Selection of Disperser Solvent

In RP-DLLME, water is dispersed as very fine droplets in the organic phase with the purpose of a moderately polar disperser. The miscibility of the disperser solvent with the extraction solvent and the organic phase is the main necessity. Thus, as seen in Fig. 3 three organic solvents such as ethanol, acetonitrile and acetone were investigated as dispersers. A comparison between extraction recoveries showed that the best extraction recovery was yielded when acetone was used as disperser solvent. Hence, further experiments were performed using acetone as a disperser solvent.

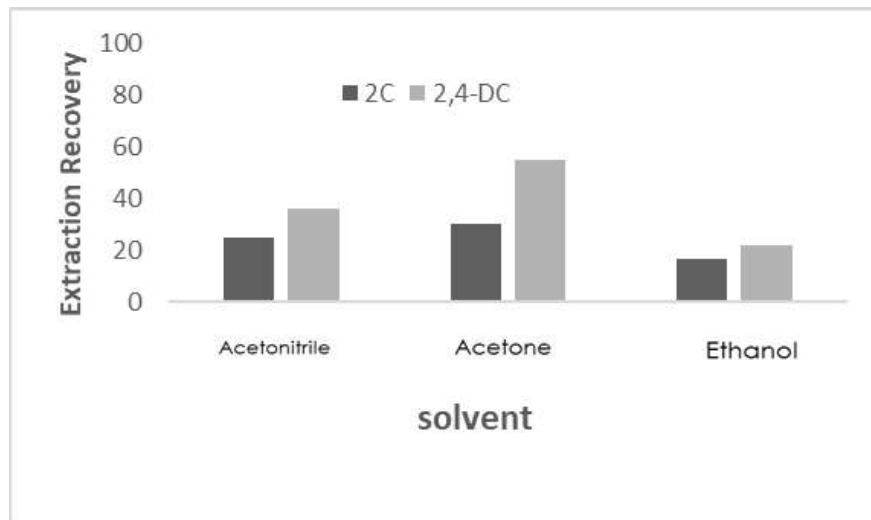


Fig. 3. Selection of Disperser Solvent

3.1.3. Effect of Disperser Solvent Volume

The volume of disperser solvent have an effect on the amount of solvent dispersion in the aqueous phase as well as the extraction efficiency and enrichment factor. To specify the optimal volume of acetone, experiments were performed with different volumes (100–800 μL). As stated by the results illustrated in the below figure, 300 μL was selected as the optimal volume of the disperser solvent to get maximal extraction efficiency.

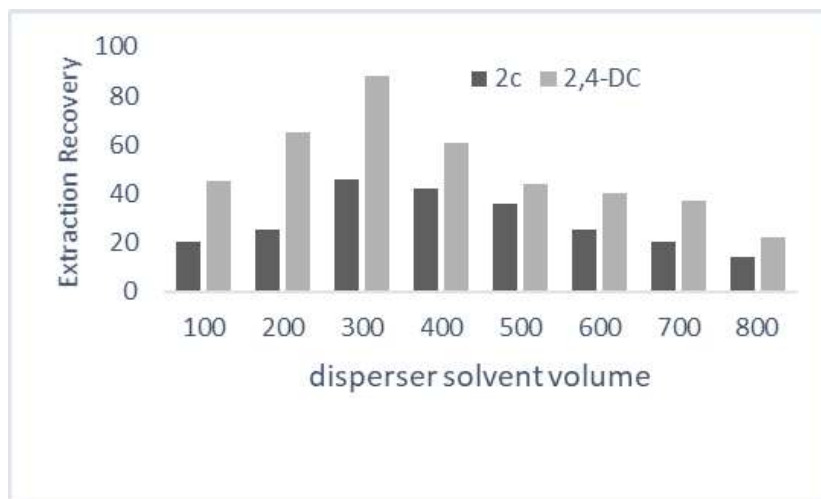


Fig. 4. Effect of Disperser Solvent Volume

3.1.4. Effect of Extraction Time

The effect of extraction time was tested in the range of 1–10 min with fixed experimental conditions. The obtained results (Fig. 5) shows that the best extraction recovery occurred at 5 min. Ultimately 5 min was applied to all experiments.

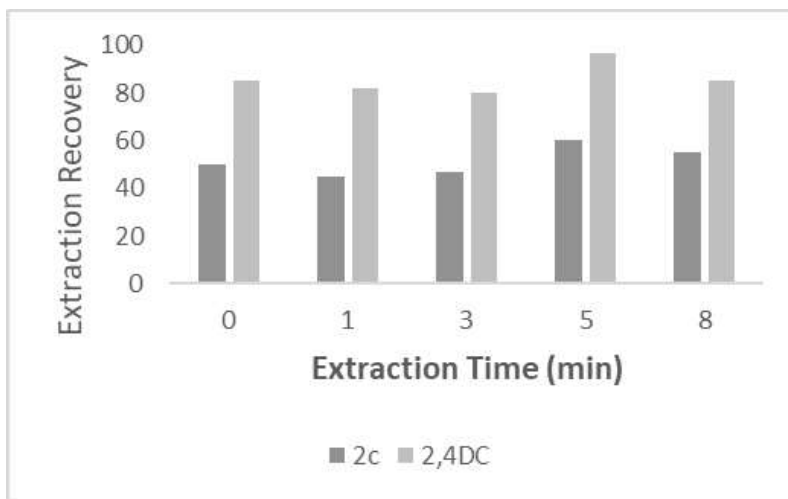


Fig. 5. Effect of Extraction Time

3.1.5. Effect of Centrifugation

Like in some extraction techniques, centrifuge time and speed are other parameters whose influences need to be taken into account. Here, centrifugation speed from 1000-5000 rpm in different time (3-10 min) was studied. According to the results (Fig. 6), the extraction recovery was improved from 1000 to 4000 rpm, in 5 minutes while further increasing exhibited no obvious effect on the extraction recovery. Therefore, the speed of 4000 rpm in 5 min was selected as the optimum speed and time of centrifugation.

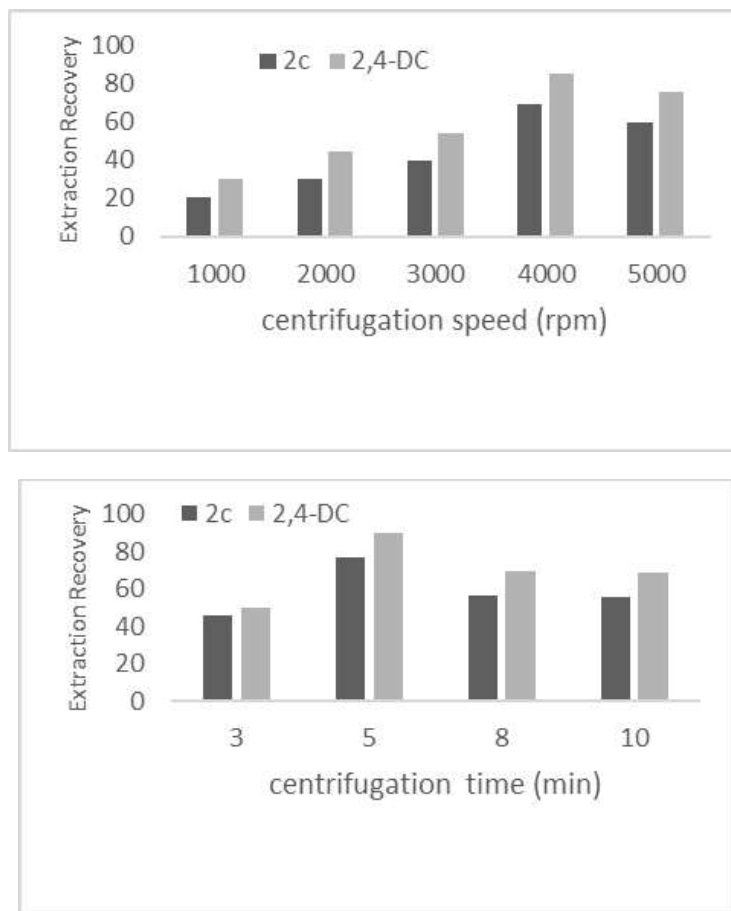


Fig. 6. Effect of Centrifugation (Speed and Time)

3.1.6. Evaluation of Method Performance

To evaluate the efficiency of RP-DLLME coupled with HPLC, all conditions were optimized for reaching high extraction recoveries as well as preconcentration factors (PFs). The PF value was calculated according to the following equation:

$$PF = C1/Ci$$

Where C1 is the concentration of analytes (mg/kg) in the sedimented aqueous phase after extraction with UAE-RP-DLLME method and Ci stands for the initial concentration of spiked analytes (mg/kg) into 1.0 g of marine sediment samples.

Under optimal conditions linear ranges, limit of detections (LOD) and PFs were investigated. As shown in Table 1, the linear ranges for 2-CP and 2,4-DCP were between 0.001-2 mg.Kg-1, and 0.2-2 mg.Kg-1, respectively. The preconcentration factor of 101, 102

and the relative standard deviation (n=5) 5.9, 3.3 were obtained for 2-CP and 2,4-DCP, respectively. These results confirmed the potential of the procedure to quantify trace amounts of CPs in marine sediments with adequate repeatability and sensitivity.

Table 1. Analytical Figures of Merit

Analyts	RSD	PF	LOD ($\mu\text{g}\cdot\text{kg}^{-1}$)	Calibration Curve	R ²	linear ranges ($\text{mg}\cdot\text{kg}^{-1}$)
2-CP	5.9	101	0.01	$y= 29.935x + 3.2523$	0.9946	0.001-2
2,4-DCP	3.3	102	0.006	$y= 26.614x + 6.8354$	0.9942	0.2-2

A comparison between the proposed method and other results reported in the literature for extraction and determination of phenol and its derivatizations in solid matrices was made, whose values are listed in Table 2. Compared to the other extraction methods presented in this table, UAE–RP-DLLME has provided lower LODs at short extraction times. As it can be seen from the results, the applicability of the proposed method provides a viable alternative to other extraction techniques.

Table 2. Comparison of the Proposed Method with Other Methods for Extraction and

Determination method	%RSD	Extraction recovery (%ER)	EF	LOD ($\mu\text{g}\cdot\text{kg}^{-1}$)	Reference
LC-DID-CPE	10	100	-	1.2-12.7	(Santana, 2004)
MAME-HPLC	<10	100	-	2000-20000	(Santana, 2005)
HPLC-DLLME	<7.6	68-82	25-30	0.5-2	(Naeeni, 2012)
GC-MS-DLLME	<6.7	84-88	-	10	(Daneshvand, 2015)
UV-Vis-RP-DLLME	4.8	105	33	15	(Ziyaadini, 2016)
HPLC-RP-DLLME	<5.9	77-90	101-102	0.006-0.01	This work

Determination of Phenol in Sediment Samples

3.1.7. Analysis of Real Samples

In order to validate the newly developed procedure to real samples, CPs content in marine sediments obtained from several locations in the Chabahar Bay (southeast Iran) was determined. In all stations, the calibration curve method was applied for determining the 2-CP and 2,4-DCP in sediment samples which are mentioned in Table 3. The results showed that the contents of CPs in the five stations were 0.21-2.18 mg.Kg⁻¹ and 0.68-2.55 mg.Kg⁻¹ for 2-CP and 2,4-DCP respectively. There are several sources of chlorophenol pollution in the Chabahar Bay, including the industrial and city wastes, effluents from marine transportation, and maintenance of boats and ships in coastal areas.

Table 3. Determination of 2-CP and 2,4-DCP in Marine Sediments of Chabahar Bay by RP-DLLME Procedure

Stations	Concentration (mg.Kg ⁻¹)	
	2-CP	2,4-DCP
S1	1.06	2.30
S2	< LOQ	0.95
S3	2.18	2.55
S4	< LOQ	< LOQ
S5	0.21	0.68

4. Conclusions

The present study indicates the suitability of RP-DLLME coupled with HPLC for extraction of ultra-trace amounts of 2-CP and 2,4-DCP from sediment samples. One of the most significant advantages of this method is using an alkaline water instead of organic solvent; and so it can be regarded as an environmental friendly procedure. This technique was optimized and then under optimum conditions, the limit of detection, the linearity range, relative standard deviation and enrichment factor of method were obtained. Finally, using this method, CPs content in marine sediments from several locations in the Chabahar Bay (southeast Iran) was determined. This new procedure gives simple and rapid preconcentration of CPs from marine sediments within high preconcentration factors and recovery, a lower limit of detection compared to other techniques. Furthermore, it will be enforceable in a variety of solid samples such as biological samples for instance tissues of animals and plants in further studies in this area.

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