Optimizing of microfunnel-supported liquid-phase microextraction for the extraction of chlorophenoxyacetic acids in seawater samples

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Received: December 2016

Accepted: March 2017

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Abstract

In this study, microfunnel-supported liquid-phase microextraction method (MF-LPME) was optimized and applied for the determination of some chlorophenoxyacetic acids (CPAAs) from natural water samples. The extraction was performed with 540 μL toluene which was retained at the surface of the sample solution using an upside down microfunnel. After the extraction, toluene was moved into the narrow stem of the microfunnel by pushing the device inside the sample. Then, using a microsyringe, it was transferred into micro vial followed by evaporation using a gentle stream of nitrogen. The residual re-dissolved into 50 μL acetonitrile, diluted to 100 μL with deionized water and analyzed applying a high performance liquid chromatography equipped with a UV detector (HPLC-UV). Central composite design (CCD) was used for the optimization of factors influencing the extraction. Under the optimized conditions, the limits of detection were 0.42 and 0.43 ng mL^{-1} for 2,4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), respectively. Preconcentration factors of 66 and 80 were obtained for 2,4-D and MCPA, respectively. The precision of the technique was evaluated in terms of repeatability which was less than 11.1% ($n=5$). The applicability of the proposed method was evaluated by the extraction and determination of CPAAs from some natural water samples.

Keywords: Microfunnel; Liquid-phase microextraction; Chlorophenoxyacetic acids; 2,4-D; MCPA; Seawater

1. Introduction

Because of widespread usage of pesticides, contamination of the environment especially in the

developing countries is steadily growing. Herbicides seem to be one of the most important categories of the available pesticides (Barcelo and Hennion 1997, Leganes and Fernandez-Valiente 1992, Ogg and Young 1991). Chlorophenoxyacetic acids (CPAAs) are an important group of herbicides widespread in

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agriculture, industries, weed-control and forestry (Alan et al. 1991, Ware 2000, Alexander et al. 1985). The CPAAs used in the present study are 2, 4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA), which are being more frequently used in Iran (Yamini and Saleh 2013). Due to their physical and chemical properties and also their water solubility, monitoring of these contaminants in different environmental matrices especially water samples are highly recommended (Grabinska-Sota et al. 2003). Various analytical techniques have been used for determination of CPAAs by solid-phase extraction (SPE), solid phase microextraction (SPME), dispersive liquid-liquid microextraction (DLLME), automated dynamic liquid-liquid-liquid microextraction (DLLLME), dynamic hollow-fiber liquid phase microextraction (DHF-LPME) and ultrasound assisted emulsification microextraction (Yamini and Saleh 2013, Butz et al. 1994, Majzik et al. 2006, Ebrahimi et al. 2016, Wells and Yu 2000, Ding et al. 2000, Rodríguez et al. 2005, Farhadi et al. 2008, Wu et al. 2005, Esrafil et al. 2011, Hassan et al. 2011).

Recently, bell-shaped extraction device assisted liquid-liquid microextraction (BSED-LLME), based on applying a low-density organic solvent was introduced by Čabala and Bursová (2012). In this approach, the extraction of some organic compounds from mineral and drinking water samples was carried out through specially designed bell-shaped extraction device (BSED). The main advantage of this method is simple and rapid manipulation with the extraction solvent and aqueous sample. With approximately the same principle, Saleh et al. introduced a LPME technique based on the use of modified microfunnel device (MF-LPME) for the extraction and determination of antifouling agents in seawater samples (Saleh et al. 2014). The possibility of utilizing large volume of water sample and small volume of extraction solvent resulted in achieving low limits of detection in this method. In MF-LPME,

during the extraction, the wide mouth of the device is tangent to the surface of the sample through edges which leads to a minimum interference in mixing efficiency and maximum possible surface contact between organic solvent droplet and water sample with almost no solvent loss by evaporation. Considering the advantages of this method, in current study, central composite design (CCD) was used to optimize MF-LPME for the preconcentration and determination of MCPA and 2, 4-D in fresh and seawater samples. In this method, the extraction of an aqueous solution with a volume of about 300 mL was carried out with introduction of a rather small volume of solvent less dense than water to the MF device placed on top of the sample surface through the stopper. After the extraction, the organic solvent containing analytes was withdrawn via a syringe and its solvent evaporated by N₂ purging. The residual re-dissolved in acetonitrile, diluted with deionized water and injected into the HPLC for analysis. The influence of different effective variables was investigated and optimized using CCD approach.

2. Materials and Methods

2.1. Reagents and chemicals

2, 4-dichlorophenoxyacetic acid (2,4-D) and 4-chloro-2-methylphenoxyacetic acid (MCPA) were purchased from Accu Standards (New Haven, CT, USA). Stock standard solutions of 2,4-D and MCPA were prepared in HPLC-grade acetonitrile purchased from Samchun (Seoul, South Korea). HPLC grade methanol was purchased from Samchun. All standard solutions were stored at 4 °C and protected from light. Toluene, *n*-hexane, *n*-heptane and dichloromethane (DCM) were purchased from Merck (Darmstadt, Germany) and used as extraction solvents. Sodium hydroxide and sodium dihydrogen phosphate were purchased from Merck. Water used in the experiment was purified on a Milli-Q ultra-pure water purification system purchased from

Millipore (Bedford, MA, USA). All the other chemicals used were of reagent grade or of the highest purity available. Plastic and glassware used for the experiments were washed with acetone and rinsed carefully with doubly distilled water.

2.2. MF-LPME procedure

To extract CPAAs using MF-LPME procedure, a volume of 300 mL of an aqueous solution containing the analytes was introduced into a 250 mL Erlenmeyer flask. A small magnetic rod (0.5 cm $\times$ 2.5 cm) was inserted and the flask was closed with a rubber septum cap through which an upside-down glass microfunnel (MF) device passed. The wide mouth of the device was immersed about 1 mm below the surface of aqueous sample and 540 μ L of toluene was added to the surface of sample through the upper narrow stem of the MF device by using a 1 mL syringe (Hamilton, USA). The sample compartment was stirred at 240 rpm for 90 min with an IKA magnetic stirrer (Wilmington, NC, USA). During the extraction process, the organic solvent was trapped in a space between the MF mouth and the surface of the aqueous solution vortex and the analytes were extracted from the aqueous into the organic phase. After the extraction, MF was immersed further into the sample (10 to 15 mm) to push the extraction phase into the narrow stem of the device. The solvent was then transferred into a glass micro vial and evaporated to dryness by a gentle stream of nitrogen gas. The residual re-dissolved into 50 μ L acetonitrile, diluted to 100 μ L with deionized water and introduced into the HPLC for analysis.

2.3. HPLC analysis

Chromatographic analysis was carried out on an Agilent 1100 HPLC system (California, USA). The HPLC system was equipped with a G1379A Micro Vacuum degasser, a G1312A Binary pump, a G1158A six-port two-position injection valve with a

100 μ L sample loop, and an Agilent G1314A variable wavelength detector (VWD). Chromatographic data were recorded and analyzed using ChemStation software (version Rev.A.09.03 [1417]). A C18 column (5 μ m, 4.6 mm $\times$ 250 mm) from Agilent Eclipse plus was applied to separate the analytes under isocratic elution conditions. A Mixture of 25 mmol L⁻¹ phosphate buffer (pH= 3) and acetonitrile (37:63, v/v) with a flow rate of 1.0 mL min⁻¹ was used as the mobile phase. The injection volume was 100 μ L for all standards and samples and detection was performed at wavelength of 240 nm.

3. Results and Discussion

3.1. Type of extraction solvent

The type of extraction solvent is a crucial parameter affecting the performance of the LPME method. Many of the solvents suitable for LPME have densities lower than water (Saleh et al. 2009) and their extraction efficiency could be evaluated in the MF-LPME method. In this study a number of different organic solvents less dense than water including: toluene, *n*-hexane, *n*-heptane and the mixture of DCM and *n*-hexane (3:7 v/v) were selected and examined for MF-LPME of target analytes. A series of spiked sample solutions were extracted using 400 μ L of each extraction solvent. The extraction was performed according to the procedure mentioned in 2.2. The highest peak areas were obtained by toluene and as a result toluene was selected for subsequent experiments.

3.2. Experimental design

Central composite design (CCD) has been widely used for the optimization of different microextraction methods. In this work, CCD was used (the Design-Expert 7.0.0, DOE software, Stat-Ease, Inc., Minneapolis, MN, USA) to optimize four effective

MF-LPME parameters including the volume of extraction solvent, sample pH, extraction time and salt concentration. It comprises a factorial design (2^f) augmented with ($2f$) star point, where f is the number of factors to be optimized, and with (n) central points. The star points are situated at $+\alpha$ and $-\alpha$ from the center of the experimental domain. In order to establish the rotatability of the experimental design, α was set at $\alpha = \sqrt[4]{2f} = \pm 2$ (Montgomery 2001, Johansson et al. 2009, Sarabia and Ortiz 2009). The total number of design point needed (N) is determined by the following equation:

$$N = 2^f + 2f + n \quad (1)$$

In this study, in total, 30 experiments in 3 blocks were performed for four factors ($n=6$). According to the design, each of the four factors (A, B, C and D) was studied at five levels which were shown in Table 1. A quadratic regression model, on the basis

of a multiple linear regression (MLR), should be applied to relate the responses and the variables. The second order polynomial with the most reasonable statistics, that is higher F and R values and low standard error were considered as the satisfactory response surface model to fit the experimental data. This model is represented in Eq. (2); include four main effects, one factor interaction effect and one curvature effect.

$$\log_{10}(R) = -0.33 - 0.47A + 0.060B + 0.13C - 7.811E-3D - 0.073AD - 0.098A^2 \quad (2)$$

Where R is the sum of relative peak areas for the analytes as a function of A (sample pH), B (volume of extraction solvent), C (salt concentration (w/v)) and D (extraction time). The analysis of variance (ANOVA) table was used to evaluate the model and the significance of the effects (Table 2).

Table 1: Factors, their symbols and levels for central composite design

Factor	Symbol	Level				
		$-\alpha$	-1	0	+1	$+\alpha$
Sample pH	A	1	2	3	4	5
Volume of extraction solvent (μL)	B	200	300	400	500	600
Salt Concentration (w/v)	C	0	5	10	15	20
Extraction time (min)	D	30	50	70	90	110

Table 2: Analysis of variance (ANOVA) for central composite design

Source	Sum of squares	d.f. ^a	Mean Square	F-Value	p-value	
Prob > F						
Block	0.037	2	0.018			
Model	6.18	6	1.03	105.87	< 0.0001	significant
A	5.30	1	5.30	544.07	< 0.0001	
B	0.087	1	0.087	8.96	0.0069	
C	0.44	1	0.44	44.77	< 0.0001	
D	1.464E-003	1	1.464E-003	0.15	0.7020	
AD	0.084	1	0.084	8.67	0.0078	
A ²	0.28	1	0.28	28.60	< 0.0001	

Residual	0.20	21	9.734E-003			
Lack of Fit	0.19	18	0.011	2.08	0.3009	not significant
Pure Error	0.015	3	5.063E-003			
Total	6.42	29				

^a Degree of freedom

Values of “Prob > F ” less than 0.10 indicate model terms are significant. The quality of model fitting was expressed by the coefficient of determination, R^2 , and adjusted R^2 values, which were 0.9680 and 0.9589, respectively. From the ANOVA summary, the model was found to be significant, with a p -value less than 0.0001. The lack-of-fit (LOF) p -value of 0.3009 indicates that the LOF was not significant relative to the pure error. According to the Eq. (2), the factors affect the response in the following order: pH of sample solution > amount of salt in aqueous solution > volume of extraction solvent. Volume of extraction solvent and concentration of salt in water sample have positive effect on the response, but

sample pH has negative effect. It means that, increasing in concentration of NaCl (salting-out effect) and extraction solvent volume lead to enhancement of the extraction efficiency. Fig. 1 demonstrates three-dimensional plots (3D) of the response surface model. As shown in Figure 1, concurrently decreasing of sample pH and increasing of extraction time within the investigated ranges enhances the performance of the microextraction process. Since the neutral and more hydrophobic form of CPAAs (pKa values of 2.73 and 3.07 for 2, 4-D and MCPA, respectively) are dominant in acidic pH, higher extraction efficiencies are expected at lower pH values (Figure 1).

Fig. 1: Demonstration of three-dimensional (3D) plot of the response surface model

According to the results from the optimization study, the optimal conditions were obtained as follows: sample pH, 1.5; volume of extraction solvent, 540 μL ; salt (NaCl), 15% (w/v) and extraction time, 90 min. The results show that a good agreement exists between the predicted values by the model and the experimental values at the points of interest.

3.4. Method validation

3.4.1. Analytical performance

In order to validate the applicability of the proposed MF-LPME method, the figures of merit including preconcentration factors (PFs), linear ranges (LRs), limit of detections (LODs) and limit of quantifications (LOQs) for the extraction of CPAAs in spiked aqueous sample were investigated under optimum conditions. The results are shown in Table 3.

The linearity was observed over the range of 1.40-1000 ng mL^{-1} for 2, 4-D and 1.43-1000 ng mL^{-1} for MCPA with regression coefficients (R^2) better than 0.997. The LODs, calculated at a signal-to-noise (S/N) ratio of 3, were 0.42 and 0.43 ng mL^{-1} for 2, 4-D and MCPA, respectively. The PF values were 66 and 80 for 2, 4-D and MCPA, respectively.

The precision of the method was evaluated in terms of repeatability which was less than 11.1% ($n = 5$). A comparison of the present method with previously published methods for extraction of chlorophenoxyacetic acid herbicides is summarized in Table 4 (Rodríguez Pereiro et al. 2004, Quintana et al. 2007, Saraji and Farajmand 2008, Tsai and Huang 2009). Figures of merit obtained by MF-LPME are comparable with dispersive liquid-liquid-liquid microextraction coupled with liquid chromatography-UV detection (DLLME-LC-UV) (Tsai and Huang 2009). Although, techniques coupled with gas chromatography-mass spectrometry detection (GC-MS) have very low LODs (in the range of ng L^{-1}) (Quintana et al. 2007, Saraji and Farajmand 2008), determination of most of the herbicides using GC-MS needs a derivatization step which increases the cost and time of analysis. In addition, solid-phase microextraction (SPME) and stir-bar sorptive extraction (SBSE) suffer from possible memory effect problems. Elimination of the instability of extraction solvent drop during the extraction process and capability of analysis of large volumes of samples are significant features of the proposed MF-LPME in contrast to single-drop microextraction (SDME) method.

Table 3: Figures of merit for the MF-LPME of target analytes

Analyte	Linearity		LOD ^a	LOQ ^b	RSD%	PF ^c
	LR (ng mL^{-1})	R^2	(ng mL^{-1})	(ng mL^{-1})	($n=5$)	
2,4-D	1.40-1000	0.997	0.42	1.40	11.1	66
MCPA	1.43-1000	0.996	0.43	1.43	10.4	80

^a Limit of detection ($\text{LOD}=3\times S_b/m$, where S_b is the standard deviation of the blank and m is the slope of the calibration curve).

^b Limit of quantification ($\text{LOQ}=10\times S_b/m$)

^c Preconcentration factor.

Table 4: Comparison of analytical performance data of proposed method with other methods applied for the analysis of chlorophenoxyacetic acid herbicides

Method	Herbicides	LR (ng mL ⁻¹)	LOD (ng mL ⁻¹)	RSD% (n=5)	PF	Ref.
SPME/ GC-MS ^a	2,4-D, MCPA	30-1000	1-2	2.9-7	-	Rodríguez Pereiro et al. 2004
SBSE ^b / GC-MS	2,4-D	0.16-1.66	0.066	12-13	18-36	Quintana et al. 2007
SDME ^c / GC-MS	2,4-D, MCPA	0.01-0.24	0.007-0.024	3.7-7.7	77-84	Saraji and Farajmand 2008
DLLLME/ LC-UV ^d	2,4-D, MCPA	0.16-1000	0.13-1.31	4.2-12.2	-	Tsai and Huang 2009
MF-LPME/ LC-VWD	2,4-D, MCPA	1.40-1000	0.42	10.4-11.1	66-80	This work

^a Solid-phase microextraction/gas chromatography-mass spectrometry.^b Stir-bar sorptive extraction.^c Single-drop microextraction.^d Dispersive liquid-liquid-liquid microextraction/liquid chromatography-ultraviolet detection.

3.4.2. Real sample analysis

MF-LMPE was applied for determining of target compounds in different environmental samples including river water (Rasht, Iran), tap water (Tehran, Iran) and seawater (Caspian Sea) samples. All samples were collected in amber glass container and maintained in dark at 4 °C until analysis. To evaluate the accuracy and matrix effect of the present method, the relative recoveries of the CPAAAs were

investigated in spiked real samples at analyte concentration of 50 ng mL⁻¹ and the results were depicted in Table 5. The results showed that the relative recoveries were in the ranges of 89% to 110%, indicated that the matrices of natural water samples had no significant effect on the extraction efficiency of this method.

Figure 2 shows the HPLC-UV chromatograms of sea water samples before (a) and after (b) spiking at the concentration of 50 ng mL⁻¹ of each CPAA.

Table 5: Results obtained from analysis of some natural water samples.

Real samples	Analyte	Initial concentration (ng mL ⁻¹)	Added (ng mL ⁻¹)	Found (ng mL ⁻¹)	RR% ^a	RSD%
Tap water	2,4-D	nd ^b	50	51.8	103.0	7.4
	MCPA	nd	50	52.0	104.0	24.8
River water	2,4-D	nd	50	46.6	93.4	17.7
	MCPA	nd	50	44.6	89.0	17.7
Sea water 1	2,4-D	nd	50	46.6	93.2	11.7
	MCPA	nd	50	45.5	90.9	5.7
Sea water 2	2,4-D	nd	50	54.9	110.0	18.2
	MCPA	nd	50	55.0	110.0	13.1

^a Relative recovery (RR%=(C_{found}-C_{initial}/C_{add})×100).^b Not detected.

Fig 2: Typical HPLC-UV chromatograms of seawater before (a) and after (b) spiking at the concentration of $50 \mu\text{g L}^{-1}$ of each CPAA

4. Conclusion

In this study, microfunnel-supported liquid-phase microextraction method was optimized for the determination of some chlorophenoxyacetic acids from natural water samples. The possibility of utilizing large volume of water sample and small volume of extraction solvent resulted in achieving low limits of detection in this method. In MF-LPME, during the extraction, the wide mouth of the device is tangent to the surface of the sample through edges which leads to a minimum interference in mixing

efficiency and maximum possible surface contact between organic solvent droplet and water sample with almost no solvent loss by evaporation. The study and the optimization of the experimental variables were performed using a central composite design and response surface methodology. Finally, good LODs and linearity and acceptable repeatability offered by MF-LMPE method make it as a feasible alternative to the standard techniques, such as SPE, SPME and other kind of LPME for the extraction of CPAAs residues in natural water samples.

Acknowledgements

We thank Iranian National Institute for Oceanography and Atmospheric Science (INIOAS) for providing lab space and analytical instruments for performing this research.

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