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Dispersive liquid-liquid microextraction and liquid chromatographic determination of pentachlorophenol in water

Research Article

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Abstract: A simple and sensitive dispersive liquid-liquid microextraction method for extraction and preconcentration of pentachlorophenol (PCP) in water samples is presented. After adjusting the sample pH to 3, extraction was performed in the presence of 1% W/V sodium chloride by injecting 1 mL acetone as disperser solvent containing 15 μL tetrachloroethylene as extraction solvent. The proposed DLLME method was followed by HPLC-DAD for determination of PCP. It has good linearity (0.994) with wide linear dynamic range (0.1-1000 μg L^{-1}) and low detection limit (0.03 μg L^{-1}), which makes it suitable for determination of PCP in water samples.

Keywords: *Dispersive liquid-liquid microextraction • Herbicide • Pentachlorophenol • Water sample • High performance liquid chromatography*

1. Introduction

Pentachlorophenol (PCP) has been used for over fifty years as a herbicide in agriculture and an effective preservative in wood industry [1]. PCP is classified as the 31st hazardous material in the list of environmental protection agency (EPA) and also it is classified as a highly dangerous material by the world health organization (WHO) [2,3]. PCP is extremely toxic when ingested by humans; the probable oral lethal dose is 50 to 500 mg kg^{-1} for a 70 kg person. Acute inhalation exposure to pentachlorophenol in humans may result in death from effects on the circulatory system and accompanying heart failure. Tests involving acute exposure of animals, such as the LC50 and LD50 tests in rats and mice, have shown pentachlorophenol to have high toxicity from inhalation exposure and extreme toxicity from oral exposure.The maximum contamination level (MCL) for PCP in drinking water is regulated at 1 ppb by EPA and 0.5 ppb by European Union [4] and it has various short and long term harmful effects on human health.

Short term hazards include central nervous system damage while long term hazards include cancer and damage to the liver, kidneys and reproductive system [2]. It can get accumulated in the food chain due to its lipophilicity and contaminates water and soil [5]. So in order to manage environmental pollution and increase the drinking water quality, there is a vital need to develop more sensitive methods for PCP determination. Various analytical methods such as spectrophotometric [6], liquid chromatographic [7-9], gas chromatographic [10] and electrochemical methods [11] have been developed for this purpose.

Despite technological advances in instrumentation of chemical analysis, the resultant sensitivities are

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limited. Sample preparation is an important step in analytical methods and follows two main steps, the first is sample clean-up and the second is preconcentration. So a combination of advanced instruments with novel sample preparation methods has enabled analysis of trace amounts of analytes with higher accuracy. In the last decades design and development of miniaturized alternative methods to the older sample preparation techniques has been one of the most important challenges for analysts. These new methods, which are known as microextraction techniques employ minimum amounts of extraction phases and offer high preconcentration factors [12].

Development of liquid-liquid extraction process started with the liquid-liquid microextraction (LLME) method, presented by Jeannot and Cantwell [13] and single drop microextraction (SDME), developed by He and Lee [14]. They reduced volume of extraction solvent to a single drop. High preconcentrations and minimal exposure to toxic solvents is the main advantage of these methods; but there are some of important disadvantages too. Fast stirring can cause break up in solvent drop, leading to air bubble formation and the extraction process takes a lot of time consuming and in most cases equilibrium can not be achieved in partitioning of the analytes between the samples and extracting phase even after long time. Recently a novel microextraction technique was introduced by Rezaee and coworkers called dispersive liquid-liquid microextraction (DLLME) [15]. DLLME is an extraction technique which employs three phases, disperser solvent, extraction solvent and sample. It is a simple and fast procedure, with low cost and high preconcentration factor and could be used with various instrumental methods such as gas and liquid chromatography and atomic absorption spectrophotometry [16-21] making it superior to the previously reported methods.

In this study, we designed a DLLME method for extraction of PCP from water samples and used it for liquid chromatographic determination in the ppb level.

2. Experimental Procedures

2.1. Chemicals

Pentachlorophenol was purchased from ACROS organics (Geel, Belgium). Glacial acetic acid, sodium chloride, hydrochloric acid, HPLC grade methanol, acetonitrile, acetone and water were all from Merck (Darmstadt, Germany). Tetrachloroethylene and carbon tetrachloride were from AppliChem (Darmstadt, Germany). All chemicals were analytical grade and used without further purification. Standard stock solution of pentachlorophenol (100 mg L^{-1}) was prepared by dissolving appropriate amount of the analyte in methanol. Calibration series were prepared by dilution of stock solution in water.

2.2. Instrumentation

An Agilent 1100 series high performance liquid chromatography apparatus (Agilent Technologies, USA), equipped with a quaternary pump, degasser, column thermostat and diode array detector was used. Separation was carried out on a ZORBAX Eclipse XDB-C18 column (150 mm × 4.6 mm I.D., 5 µm particle size). The analyte was eluted with a mixture of 20% acetate buffer (pH = 3.8) and 80% acetonitrile at a flow rate of 1 mL min^{-1}. The diode array detector and column thermostat were set at 303 nm and 25ºC, respectively. ChemStation software was used for data acquisition and processing.

A Hettich centrifuge model MIKRO 22R (Hettich, Germany) was used for separation of the dispersed phase. A Metrohm 744 pHmeter (Metrohm, Switzerland) equipped with a glass electrode was used for pH adjustments.

2.3. Dispersive liquid-liquid microextraction procedure

A 5.00 mL water sample (pH adjusted in 3) including 1% W/V sodium chloride was placed in a 10 mL glass tube with conical bottom. Acetone (1 mL) as disperser solvent, containing 15 µL tetrachloroethylene as extraction solvent was injected rapidly into the sample solution and the resultant cloudy solution containing fine droplets of extraction solvent was then centrifuged for 5 min at 5000 rpm (rotor radius, 6 cm). The sedimented phase was separated by a Hamilton syringe and its volume adjusted at 100 µL with extraction solvent in another tube and 20 µL of this solution was injected to the HPLC system. It must be noted that by optimizing the microextraction process, especially after salt addition the volume of sedimented phase increased to 21 µL. So, in the final protocol of microextraction there is no need for diluting the sedimented phase.

2.4. Preconcentration factor and extraction recovery in DLLME

The preconcentration factor and extraction recovery in DLLME have been defined previously by Rezaee *et al.* [15]. Preconcentration factor (or enrichment factor) is the ratio of the analyte concentration in the sedimented phase (C_{sed}) and the initial concentration of analyte within the sample (C_0).

$$PF \text{ or } EF = \frac{C_{sed}}{C_0} \quad (1)$$

The extraction recovery (ER) is defined as the percentage of the total analyte amount (n_0) which was extracted to the sedimented phase (n_{sed}).

$$ER = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed} \times V_{sed}}{C_0 \times V_{aq}} \times 100 \quad (2)$$

where V_{sed} and V_{aq} are the volumes of sedimented phase and sample solution, respectively.

3. Results and Discussion

3.1. Selection of extraction and disperser solvents

As was described before, DLLME is a three phase system and the nature of disperser and extraction solvents play major role in microextraction efficiency. A main point in disperser solvent selection is its miscibility in both the organic phase (extraction solvent) and aqueous phase (sample solution).

In the case of extraction solvent, it must be denser than water, have ability to extract analytes and have good chromatographic behavior. In this case, ability of methanol, acetonitrile and acetone (1 mL) as disperser solvents were studied in presence of tetrachloroethylene and carbon tetrachloride (50 µL) as extraction solvents. Results shown in Fig. 1, indicating acetone as disperser solvent and tetrachloroethylene as extraction solvent provided maximum recovery of 60.1% with preconcentration factor of 63.

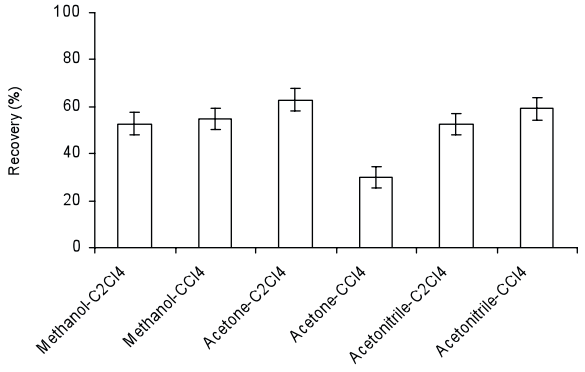

Figure 1. Effect of disperser solvent and extraction solvent nature on extraction recovery (analyte concentration 1 mg L^{-1}, volume of disperser solvents and extraction solvents were 1 mL and 50 µL, respectively, pH = 3, n = 3).

3.2. Effect of extraction solvent volume

Volume of extraction solvent is another important parameter for obtaining higher preconcentration factor and better extraction efficiency. So effect of this parameter was studied using solutions containing different volumes of tetrachloroethylene (10, 15, 20, 30, 50, 70 and 100 µL) in acetone. Fig. 2 shows that increase in extraction solvent volume causes an increase in the analyte recovery which extends from 40% (tetrachloroethylene volume, 10 µL) to 60% (tetrachloroethylene volume ≥ 15 µL).

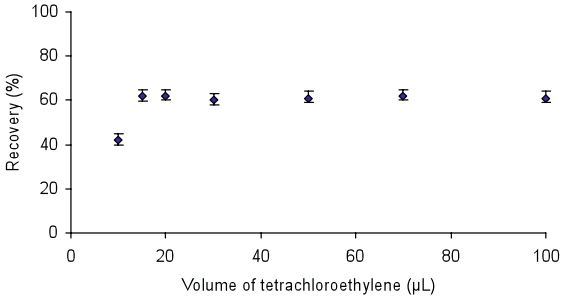

Figure 2. Effect of extraction solvent volume on extraction recovery (analyte concentration 1 mg L^{-1}, volume of disperser solvent was 1 mL, pH = 3, n = 3).

It is obvious that the volume of sedimented phase (measured by Hamilton syringe at 25 ± 1ºC) increases with increase in extraction solvent volume, while the preconcentration factor of the analyte decreases. As shown in Fig. 3 by increasing the volume of tetrachloroethylene from 10 to 100 µL, the volume of sedimented phase increases from 3 to 93 µL and preconcentration factor decreases to 22 (Fig. 4). Thus 15 µL was selected as the best extraction solvent volume.

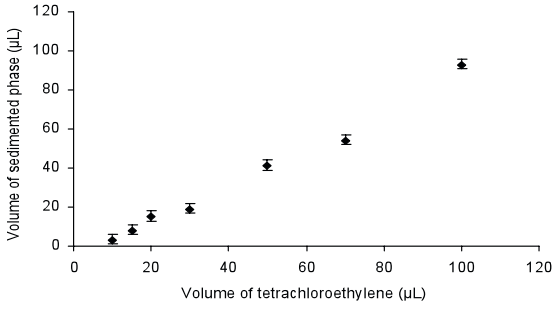

Figure 3. Effect of extraction solvent volume on volume of sedimented phase (analyte concentration 1 mg L^{-1}, volume of disperser solvent was 1 mL, pH = 3, n = 3).

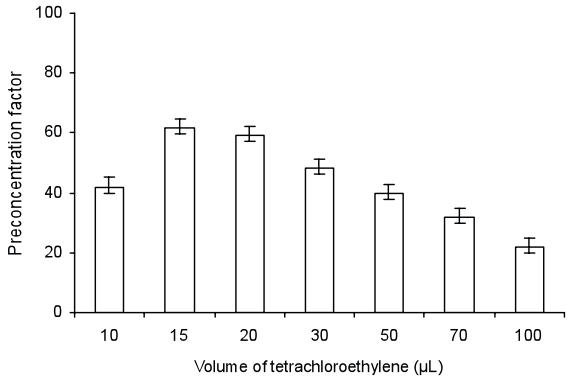

Figure 4. Effect of extraction solvent volume on preconcentration factor (analyte concentration 1 mg L^{-1}, volume of disperser solvent was 1 mL, pH = 3, n = 3).

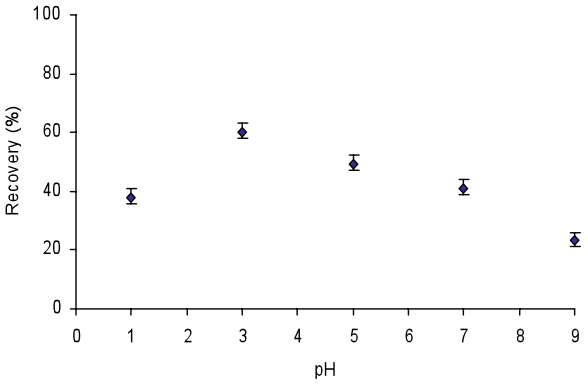

Figure 6. Effect of solution pH on extraction recovery (analyte concentration 1 mg L^{-1}, volume of disperser solvent and extraction solvent were 1 mL and 15 µL, respectively, n = 3).

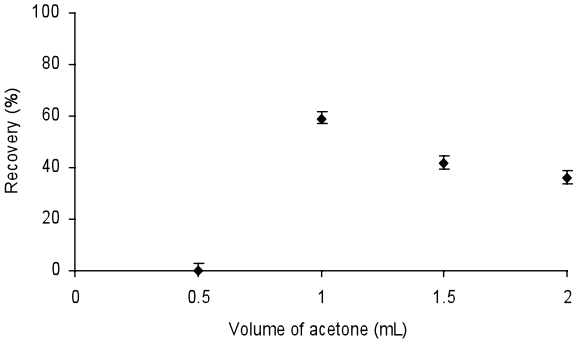

Figure 5. Effect of disperser solvent volume on extraction recovery (analyte concentration 1 mg L^{-1}, volume of extraction solvent was 15 µL, pH = 3, n = 3).

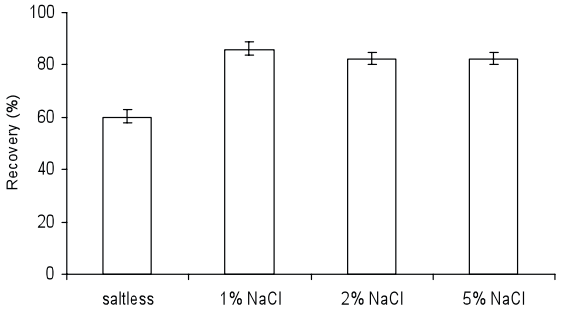

Figure 7. Effect of salt addition on extraction recovery (analyte concentration 0.1 mg L^{-1}, volume of disperser solvent and extraction solvent were 1 mL and 15 µL, respectively, pH = 3, n = 3).

3.3 Effect of disperser solvent volume

Variation in the volume of disperser solvent causes changes in volume of the sedimented phase and can affect recovery and preconcentration factor. So selection of the optimum volume for the disperser solvent is necessary. For this purpose, various volumes of acetone containing 15 µL tetrachloroethylene were used. Fig. 5 shows variation of the analyte recovery by variation in disperser solvent volume. In the case of 0.5 mL, no sedimented phase was formed and by using 1 mL acetone the maximum recovery of 60% was obtained.

3.4. Effect of extraction time

In all of the extraction methods extraction time is an important factor in achieving equilibrium between two phases. Extraction yield can be increased with longer extraction times, which provides longer contact time between the extracting phase and sample. In DLLME, extraction time is defined as interval time between injection of solvent (mixture of disperser and extraction solvent) and beginning of sample centrifuge. In this study, extraction times ranging from 0 to 60 min were examined. Results show that time has no effect on extraction efficiency. It was concluded that fine dispersed drops of extraction solvent provide large contact surface with the sample which is enough for extraction of the PCP with acceptable yield.

3.5. Effect of solution pH

PCP is a weak acid with pKa value of 4.74 and it can be found in aqueous solutions in molecular and dissociated forms according to the solution pH. In this step effect of solution pH on the amount of extracted PCP was investigated in the range of 1-9. As shown in Fig. 6, extraction recovery is maximum at pH = 3.

3.6. Effect of salt addition

In extraction methods salt addition was used in order to improve the extraction efficiency. In DLLME salt addition has dual effect. Increase in salt concentration causes an increase in volume of the sedimented phase and on the other hand increases the amount of extracted analytes

Table 1. Quantitative characteristics of the proposed method.

Compound	Calibration curve equation[a]	r^2 [b]	LOD [c] ($\mu g\ L^{-1}$)	LDR [d] ($\mu g\ L^{-1}$)	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	RSD % [e]
PCP	A = 1.1754C - 6.91	0.994	0.03	0.10-1000	5.00	5.10	1.23

[a] *A = Peak area, C = concentration in μg L⁻¹*
[b] *r^2 = Square of correlation coefficient*
[c] *Limit of detection*
[d] *Linear dynamic range*
[e] *Relative standard deviation for three replicate microextraction and determination of PCP (n = 3)*

due to the salting out effect. In this study DLLME was performed for extraction of spiked PCP (100 μg L^{-1}) from aqueous solutions containing 0, 1, 2 and 5% W/V sodium chloride and results are shown in Fig. 7.

As can be seen, extraction recovery reaches 86% in the presence of 1% W/V sodium chloride (volume of sedimented phase was 21 μL in comparison with 8 μL for saltless solution). Thus, for further studies microextraction in the presence of 1% W/V salt solution is recommended.

3.7. Effect of temperature

Temperature is one of the parameters which may affect extraction efficiency. Effect of temperature on extraction of the PCP from aqueous solutions was investigated by adjusting the solution temperatures to 25, 40, 50, 60 and 70ºC.

Results show that increase in solution temperature decreases the amount of analytes extracted. It may be due to several reasons; the first is probability of decrease in distribution coefficient of the PCP by increasing temperature [16], the second reason is evaporation of extraction and disperser solvents and decrease in volume of sedimented phase and the third reason may be increasing the miscibility of the solvents with increasing temperature. Thus, 25ºC was selected as optimum temperature.

Table 2. Comparison of proposed method with other methods reported in literature.

Method	LOD ($\mu g\ L^{-1}$)	LDR ($\mu g\ L^{-1}$)	Reference
Spectrophotometry	0.50 - 1.16	1 - 10	[6]
MIP-SPE-HPLC	0.006	0.05 - 500	[7]
Electroanalytical method	5.50	16.5 - 16500	[11]
Proposed method	0.03	0.1 - 1000	

Table 3. Results of real sample analysis.

Sample	Added ($\mu g\ L^{-1}$)	Found ($\mu g\ L^{-1}$)	Recovery(%) ± SD[b]
Tap water	-	ND[a]	-
Tap water	2.0	1.88	94.37 ± 4.26
Well water	-	ND	-
Well water	2.0	1.80	90.0 ± 3.93

[a] *ND = Not detected*
[b] *Average ± standard deviation (n = 4)*

3.8. Reproducibility of the proposed method

In order to evaluate reproducibility of the method three replicate determinations were carried out and the relative standard deviation (RSD %) was calculated. The relative standard deviation of PCP determined by DLLME-HPLC-DAD method is 1.23% (Table 1), which indicates that the proposed method is reproducible.

3.9. Quantitative characteristics of the proposed method

After optimization of all parameters, quantitative characteristics of the proposed method were studied. These included calibration curve equation, correlation coefficient, limit of detection (LOD, defined as 3×S/N) and linear dynamic range (LDR).

The results are summarized in Table 1. The high correlation coefficient (0.994), low detection and quantification limits (0.03 and 0.10 μg L^{-1}, respectively) and wide linear dynamic range (0.1 - 1000 μg L^{-1}) makes the proposed method suitable for quantification of PCP. Some characteristics of previously reported methods are also summarized in Table 2 for comparison.

3.10. Analysis of real samples

In order to evaluate efficiency of the proposed method in monitoring of low levels of PCP, its levels in tap and well water were investigated. Results are summarized in Table 3. Typical chromatograms of real sample and PCP standard solution are presented in Fig. 8.

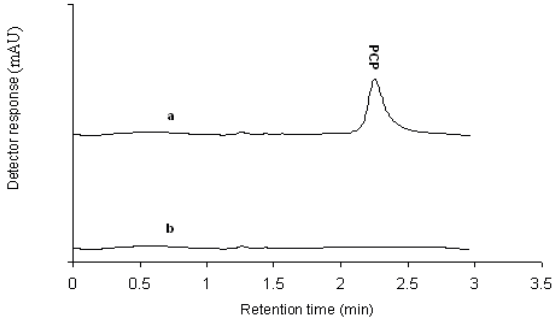

Figure 8. Chromatograms of extracted PCP by proposed DLLME method, (a) standard solution of PCP, 5 μg L^{-1}; (b) tap water sample.

4. Conclusions

In this study a dispersive liquid-liquid microextraction method for extraction of trace amounts of PCP from water samples before its determination by HPLC is presented. The proposed method is simple, cheap, fast and sensitive. Minimum exposure to the toxic organic solvents makes it very safe for analysts.

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