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ORIGINAL ARTICLE

Development of Directly Suspended Droplet Micro Extraction Method for Extraction of Organochlorine Pesticides in Water Samples

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KEYWORDS

Organochlorine pesticides
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ABSTRACT: A simple and efficient directly suspended droplet micro extraction in conjunction with gas chromatography-electron capture detector (GC-ECD) has been developed for extraction and determination of organochlorine pesticides (OCPs) from water samples. In this technique a micro drop of 1-dodecanol is delivered to the surface of an aqueous sample while being agitated by a stirring bar in the bulk of solution. Factors relevant to the extraction efficiency were studied and optimized. The optimized extraction conditions were extraction solvent: 1-dodecanol; extraction temperature: 60°C; NaCl concentration: 0.5M; solvent extraction volume: 10 µL; stirring rate: 800rpm and the extraction time: 20 min. The detection limits of the method were in the range of 0.066–1.85 ngL^{-1}, relation standard deviation (n=5) range were 0.102 - 0.964. A good linearity (r^2 ≥0.995) and a relatively broad dynamic linear range (25–2600$ng.L^{-1}$) were obtained and recoveries of method were in the range of 90.729% - 102.343%. Finally, the proposed method was successfully utilized for pre concentration and determination of OCPs in different real samples. We successfully developed a method based on the DSDME technique combined with capillary GC-ECD for the analysis of OCPs in the water samples and compared with the conventional sample preparation method such as LPME.

INTRODUCTION

"Organochlorine pesticides (OCPs) are a broad class of pesticides that were widely used in the 1950s and 1960s, can be divided in to three groups: benzene hexachloride isomers (e.g. lindane), cyclodienes (such as: aldrin, dieldrin, endrin, chlordane, heptachlor, and endosulfan) and DDT or analogues (e.g. methoxy chlor, dicofol, and chlorobenzylate)" [1]. OCPs have been of great concern due to their persistent nature and chronic adverse effect

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on wild life and humans the ban and restriction on the usage of OCPs in developed Countries during the 1970s and 1980s, some developing countries are still using them for agricultural and public purposes because of the low cost and versatility in controlling various insects [2, 3]. Pesticides are mostly moved from agricultural fields to surface water during a surface run-off. The amount lost from fields and transported to surface water depends on several factors, including soil characteristics, topography, weather, agricultural practices, and chemical and environ mental properties of individual pesticides [3,4].

Presence of OCPs in water is strictly regulated by legislation to concentrations ranging between less than 10 and 100ng.L^{-1} [5-7]. One of the primary goals in water analyses for pesticides is to reach determination limits of about 0.1µg.L^{-1} for individual pesticides and 0.5µg.L^{-1} for total concentrations in order to meet the require elements of the European Union (EU) drinking water directives and those of the US National Pesticide Survey [8-10]. Most determinations of OCPs are based on chromatographic methods.

Studies involving the determination of OCPs in environmental matrices often deal with samples with low analyte concentrations containing a high number of interferent Compounds. Simple and highly sensitive analytical techniques are two main factors for detect and quantify pollutants in water at trace levels [11]. To achieve the acceptable level of sensitivity, an enrichment step is needed before the chromatographic analysis. Historically, the initial extraction of OCPs from aqueous samples is performed batch wise (separatory funnel) or continuously using liquid–liquid extraction (LLE) [12]. Large volumes of both aqueous sample (typically, One litter) and high-purity organic extracting solvent (typically dichloromethane) are required, and most of the latter is ultimately discarded as chemically hazardous waste.

Analytical methods that employ smaller volumes of initial sample and/or extracting solvent would be preferable. In recent years, Lu and co-workers [13] developed DSDME as a new sampling method.

In this method, a stirring bar (magnet) is placed at the bottom of a vial containing an aqueous sample and rotated at a speed required to cause a gentle vortex. If a small volume of an immiscible organic solvent is added to the surface of the aqueous solution, the vortex results in the formation of a single droplet at or near the center of rotation. The droplet itself may also rotate on the surface of the aqueous phase, increasing mass transfer. Compared with the other liquid phase microextraction (LPME) techniques based on droplet systems (e.g., single droplet microextraction (SDME)), it provides more flexibility in the choice of the operational parameters, especially the amount of the solvent and the stirring frequency.

The possibility of applying larger volumes of organic solvents in this method also makes it a useful technique to match with HPLC in addition to GC. In 2008, Sarafraz-Yazdi et al. developed the method and combined DSDME with HPLC for the determination of diclofenac. The same authors also used DSDME with GC with flame ionization detection (GC-FID) for the determination BTEX compounds [13] and of two tricycle antidepressant (TCA) drugs, amitriptyline and nortriptyline [14].

DSDME is very simple and free from cross contamination, the equilibration time is reached quickly, and supporting materials are not required. The main disadvantage of the method is the difficulty of taking out the small amount of suspended droplet from the solution (<5 µL). Using a micro syringe, exact collection of the micro drop is impossible and some water may be transferred into the syringe, and that can create problems for some instruments (e.g., GC-ECD). To overcome this problem, Yemeni et al. [15], introduced a new sampling

method based on solidifying the floating organic droplet. In this method, we address the challenges associated with DSDME, while maintaining the associated benefits, by utilizing a rotating vial instead of a stir bar for mixing samples. Using organic solvent, and organo chlorine pesticides a water pollutant, as a model organic compound the operation characteristics and analysis capability of this novel micro extraction method were investigated.

In this study using a simple and efficient directly suspended droplet micro extraction in conjunction with gas chromatography-electron capture detector (GC-ECD) extraction of OCPs from water polluted samples.

MATERIALS AND METHODS

Reagent

All OCPs (heptachlor, lindane, dieldrin, p,p′-DDT, p,p′-DDE, methoxychlor) were purchased from Fuluka, 1-undecanol, 2-dodecanol, 1-dodecanol, n-hexadecane, Toluene, n- hexane and cyclo hexane reagent sodium chloride, HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). All aqueous solution prepared with deionized water. Proper amount of each OCP was dissolved in 20 ml methanol to obtain a stock standard solution with a concentration of 200 $mg.L^{-1}$. Fresh six sample standard solution, which any containing one OCP with concentration of 2 $mg.L^{-1}$ was prepared in acetonitrile and stored at $4^{\circ}C$.

Instrumentation

Separation, identification and quantification were carried out on a Chrompack CP-9001 (Chrompack, Middleburg, Nether- lands) gas chromatograph system equipped with an electron capture detector. Helium and nitrogen (with 99.999% purity) were used as carrier gas (flow rate=1mL.min $^{-1}$) and make-up gas (flow rate=30mL.min $^{-1}$), respectively. The inlet was operated in the split mode with a split ratio of 1/15. Separation of OCPs was carried out using a CPSil8 CB fused silica capillary column (Chrompack, Middleburg, Netherlands) specialized for pesticides separation (50 m×0.25 mm I.D., 0.25 µm film thicknesses). The injector and detector temperature were set at 280 and 300 $^{\circ}C$, respectively. The GC oven was kept at 50 $^{\circ}C$ for 5min then raised to 200 $^{\circ}C$ at 10 $^{\circ}C$ min $^{-1}$ and held for 5min, finally raised to 250 $^{\circ}C$ at 5 $^{\circ}C$ min $^{-1}$ and held for 10 min. The total run time was 40 min. All chromatograms were recorded and processed by the Maestro software, version 2.4. Stirring the solution was carried out with a magnetic heater-stirrer (Heidolph MR 3001K). A simple water bath placed on the heater stirrer was used for controlling the temperature of the sample solutions. All injections were carried out using a 10 µL model 701N micro syringe (Reno, NV, USA).

Analytical procedure

Twenty ml of aqueous solution of OCPs ($2\mu g.L^{-1}$) were transferred in to a 21 mL vial. Ten µl of 1-dodecanol were delivered to the surface of solution using the micro syringe, the vial was sealed and then the magnetic stirrer was turned on. In this method stirring bar (3 mm) was used in this extraction procedure, 10 μl flat – cut syringe (Hamilton, England). Stirrer bar (magnet) was placed within the sample the magnetic stirrer was turned on and set to 800 rpm for stirring the extraction mixture. The stirrer bar was kept rotating smoothly in order to form a steady vortex. Then, the screw cap was kept closed during the extraction process. After 20 min, the screw cap was removed and a portion of the organic droplet was drawn out by micro syringe and then injected into the GC –ECD system for analysis (Figure 1).

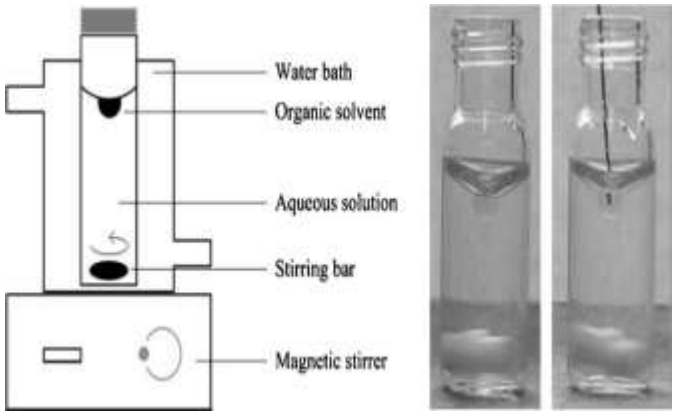

Figure 1.Schematic DSDME method

RESULTS AND DISCUSSION

Selection of organic solvent

To achieve acceptable selectivity and extraction efficiency, it is necessary to choose a suitable organic solvent. For this purpose the organic solvent should be a very low solubility in water to avoid dissolution in the aqueous sample and also was low vapor pressure to prevent loss during extraction, Based on this consideration, six solvent: 1-undecanol, 2-dodecanol, 1-dodecanol, n-hexadecane, Toluene, n- hexane and cyclohexane were tested under extraction condition: solvent drop volume: 10 µL; stirring rate:720rpm; extraction time: 20 min; NaCl concentration: 0.5 M; temperature extraction: 25 °C .The results represented at Figure 2. It can be seen that, 1-dodecanol show that the best efficiency for the extraction solvent in this investigation.

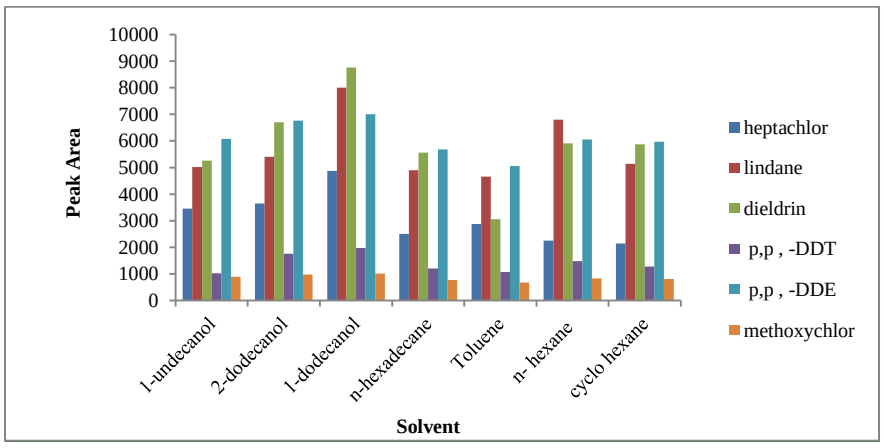

Figure 2. Extraction efficiency of different organic solvent for OCPs Extraction

Sample solution temperature

Temperature is a main parameter affecting extraction efficiency. In most of LPME works, a temperature raise has led to higher enrichment factors [16]. This process facilitates mass transfer of the analyte from sample to the organic solvent and increases the efficiency of the extraction. The effect of sample solution temperature on the extraction efficiency was studied in the range of 25–80 ˚C by floating a 1-dodecanol micro drop for 20 min on the surface of the water samples. Experimental results are shown in Figure 3. Clearly, by increasing the temperature, the extraction efficiency increased for all of the analytes. However, high temperatures (>60 ˚C) can lose the drop size dramatically and create over-pressurization in the sample vial which makes the extraction system unstable. Therefore, in further experiments the sample vial temperature was held at 60 ˚C.

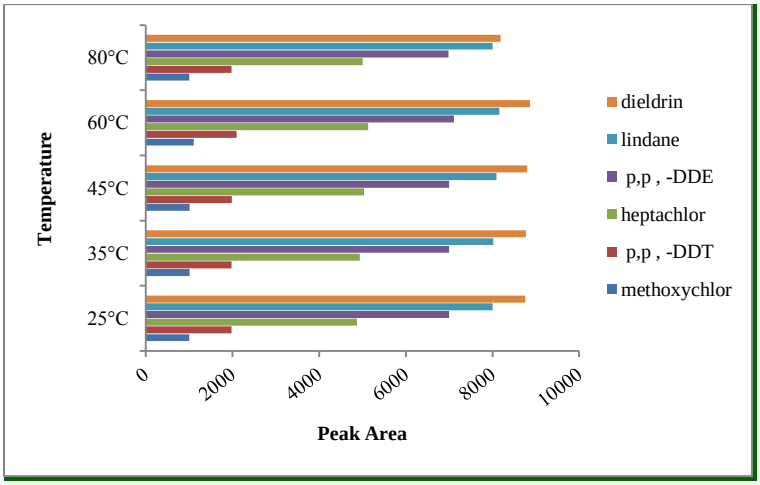

Figure 3. Extraction efficiency at different temperature for OCPs Extraction

Effect of ionic strength

Addition of salt to the sample may have several effects on the extraction efficiency for polar compounds. Extraction is usually enhanced by increasing salt concentration (salting out effect). It was assumed that apart from the salting-out effect, the presence of salt causes a second effect and changes the physical properties of the Nernst diffusion film and thus reducing the rate of diffusion of the target analytes in to the micro drop (salting-in effect) [17,18]. The effect of NaCl concentration (ranging from 0 to 2M) under Extraction condition: type solvent: 1-dodecanol; extraction time: 20 min; stirring rate: 720 rpm and temperature extraction: 60°c, was investigated and the extraction efficiency was monitored. Results obtain represented in Figure 4.

According to the bar, the addition of ionic strength increases the transport of the analytes to the extracting microdrop up to 0.5 M, but above 0.5 M, the salting-in effect was observed and the extraction efficiency for all further analytes decreased. Therefore, the NaCl concentration was adjusted at 0.5 M in all further experiments.

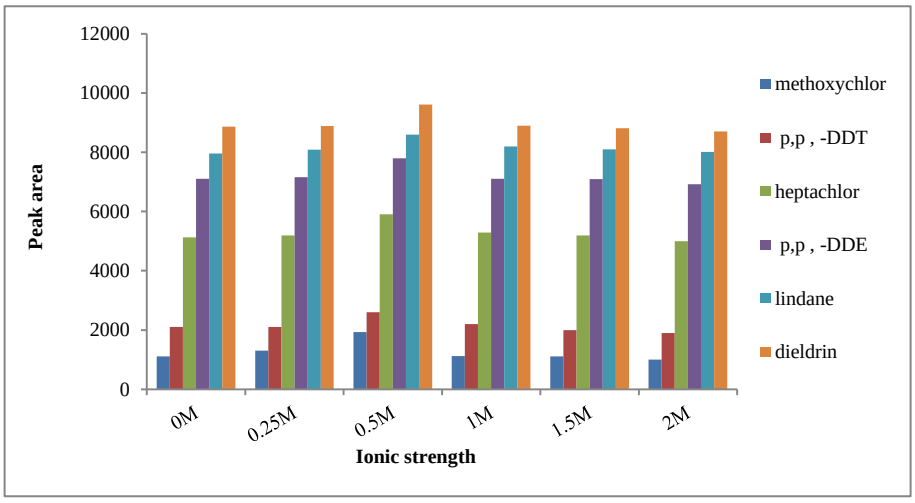

Figure 4. Effect of ionic strength on the OCPs Extraction

Organic solvent volume

For investigation the effect of organic solvent volume on the analytical signal, some experiments were performed by increasing the micro drop volume from 6 to 20 µL. The results are shown in Figure 5. As it was expected, increases in the volume of the micro drop (up to 10µL) resulted in negligible increases in the extraction efficiency. However, at larger volumes, extraction efficiency decreased. By increasing the organic solvent volume, both surface area and volume of organic drop were increased. The influence of organic solvent volume, therefore, originates from the integrated influence of two factors, justifying why the GC response enhances with increasing organic solvent volume up to 10µL and decreases afterward [19].

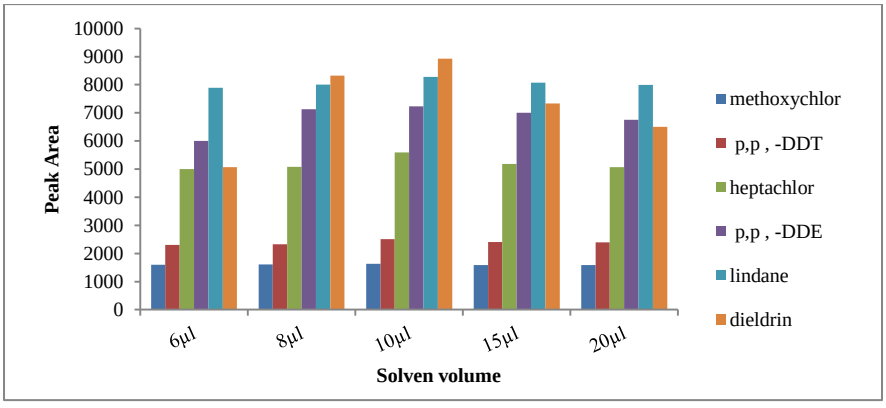

Figure 5. Effect of different solvent volume on the OCPs Extraction

Stirring rate

A high stirring rate reduces the necessary time to reach thermodynamic equilibrium and thus increases extraction efficiencies significantly. Based on the film theory of convective diffusion mass transfer for LPME system, at steady state, the aqueous phase mass-transfer coefficient increased with increases in the stirring speed (rpm) because faster stirring speed can be decreased the thickness of the diffusion film in the aqueous phase. Therefore, agitation produces an enhancement in extraction efficiency [20- 22]. The obtained results support this explanation. In this study, the samples with volumes of 20 mL were agitated at different stirring rates between ranges of 650-1000 rpm with a 14 mm×0.4 mm stirring bar on a stirrer plate as shown in Figure 6. The relative peak area of all analytes increases by increasing in the stirring rate up to 800 rpm. Higher stirring rates (>800) were not used because of spattering damaged the micro drop. Hence, for further studies, a stirring rate of 800 rpm was chosen.

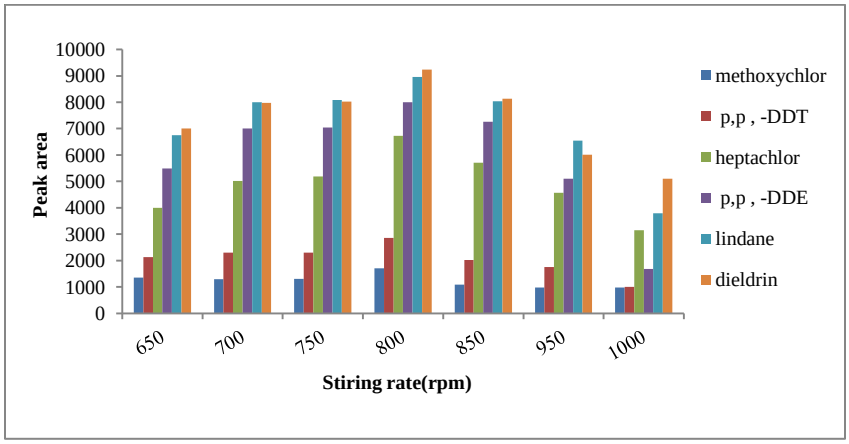

Figure 6. Effect of different stirring rate on the OCPs Extraction

Extraction time

Like DSDME, LPME is not an exhaustive extraction method under the real conditions and for optimum repeatability of the extraction, it is necessary to choose an extraction time during which equilibrium between aqueous and organic phase is reached. The amount of analyte extracted at a given time depends upon the mass transfer of analyte from the aqueous phase to the organic phase. This procedure requires a period for equilibrium to be established. Normally the time for establishing equilibrium is selected as the extraction time [23]. The effect of time was examined in the range of 5–30 min at the optimized experimental conditions. The peak areas increased with extraction time up to 20 min (Figure 7). After 20min, the extraction system reached a steady state and no dramatic increase in peak areas was observed with additional extraction time. Therefore, extraction period of 20 min was chosen to obtain a reasonable sensitivity. Although the extraction time was relatively long, the sheer smallness of the developed LPME system (DSDME) makes parallel extraction a most attractive.

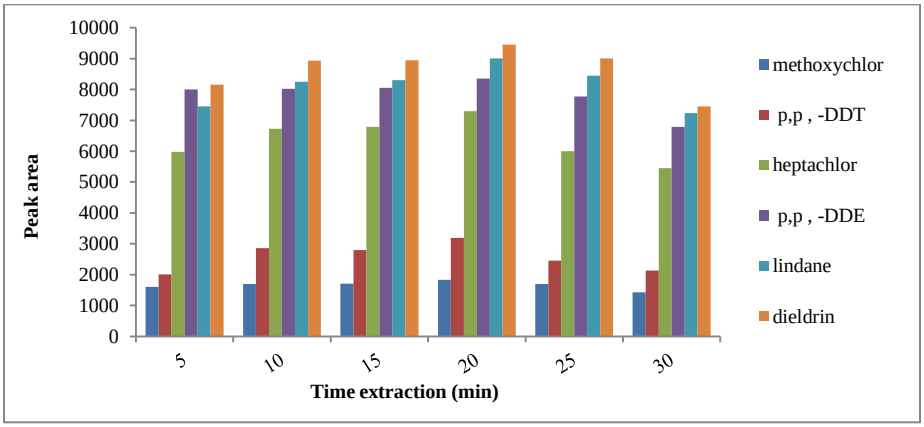

Figure 7. Effect of different time extraction on the OCPs Extraction

Evaluation of the method performance

A chromatogram of the standard solution of analytes at 2μgL^{-1} concentrations after utilizing the proposed method with a 10μL micro drop of 1-dodecanol was shown in Figure 8a. Calibration curves were drawn using 6 spiking levels of OCPs in the concentration range of 25–2600 ng L^{-1}. For each level three replicate extractions were performed at optimal conditions (extraction time: 30 min, drop volume: 8 μL, stirring rate: 800 rpm, sample temperature: 60 ◦C, sample volume: 20M and ionic strength: 0.5 M of NaCl). The corresponding regression equations, correlation coefficients(r^2), dynamic linear ranges (DLRs) and the limit of detections (LODs), relation standard deviation$(RSD)_{n=5}$ based on, three times of signal to noise ratio, were calculated and summarized in Table 1, a series of standard solutions 25-2600ngL^{-1} in 1-dodecanol were prepared and 2 μL of them were injected into GC.

Table1. Figures of merit of the proposed DSDME-GC-ECD method for extraction and determination of OCPs

Analyte	LOD[a] (ng.L^{-1})	LOQ[b](ng.L^{-1})	r^2	Regression equation	DLR(ng.L^{-1})	RSD%	Recovery%
p,p' -DDT	0.379	1.266	0.996	A= 1.6132C_1 - 41.298	50-2600	0.203	101.1276
heptachlor	1.857	6.192	0.996	A= 1.1165C_2 + 174.58	50-850	0.363	90.7290
p,p' -DDE	0.188	0.627	0.995	A= 3.7953C_3 + 125.72	50-2600	0.102	95.921
lindane	0.066	0.222	0.996	A= 0.9468C_4 - 0.5393	25-2600	0.142	100.754
dieldrin	0.259	0.863	0.995	A = 4.5787C_5+ 154.98	75-2600	0.121	102.343
methoxychlor	0.893	2.798	0.995	A = 1.0486C_6 - 34.087	50-850	0.964	100.987

a :limit of detection b: limit of quantitation

Real water analysis

To assess the applicability of the method to real samples, the proposed method was applied to the extraction and determination of OCPs from real water samples. River and agricultural water samples were collected in glass bottles. Tap water sample was collected after allowing the water to flow for 10–20 min. All of water samples were stored in a fridge at 4 ˚C till analysis time. Water samples were filtered before analysis, using 0.45 µm cellulose acetate membrane filters to eliminate particulates. The chromatogram DSDME–GC-ECD of three water samples represented in Figure 8 (b, c and d). The results for tap and river water showed that they were free of OCP contamination. In the agricultural water sample, DDT, heptachlor, lindane and methoxychlor were detected (Table 2). A comparison between the proposed DSDME technique and literature values for SPME [26], LPME-SFO [25], DLLME-SFO [27], USAEME [24] and SDME [28] methods for the extraction of OCPs are presented in Table 3. LOD, LOQ and RSD for the proposed method are better than other methods. Precision of the proposed method is better and is comparable with other methods.

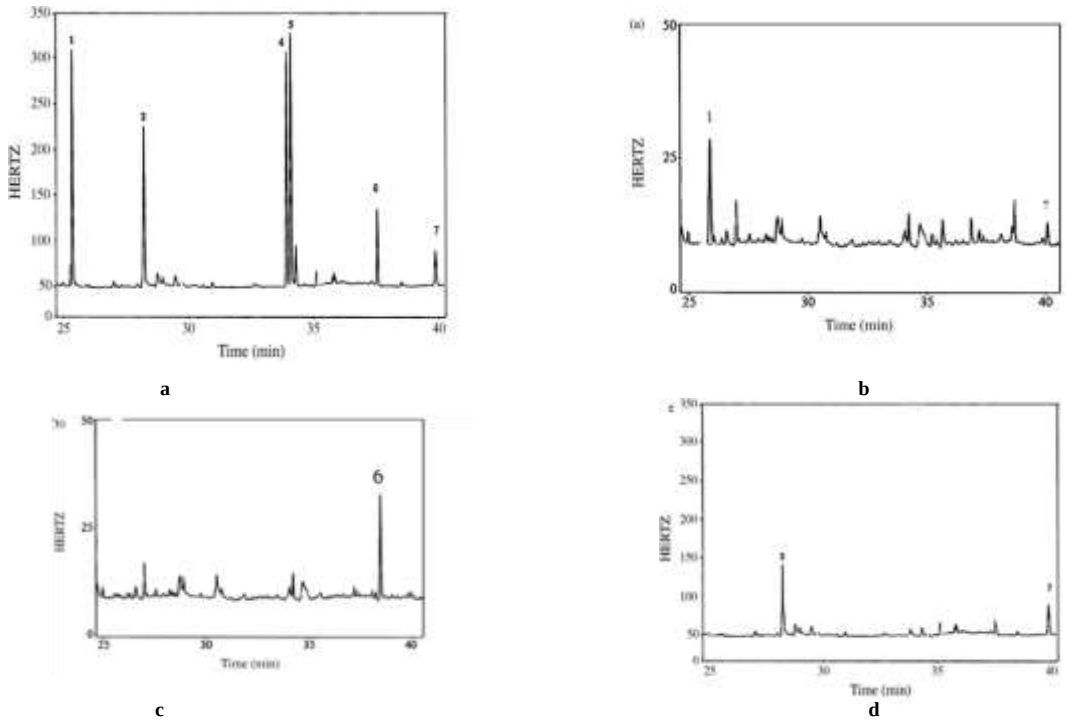

Figure 8. Chromatograms of (a) standard solution of OCPs (b), agricultural water (Dehloran, Iran), (c) agricultural water obtained (Gol- Gol, ilam, Iran) and (d) water rive (Semarea, ilam, Iran) by using proposed DSDME method combined with GC-ECD. (1) Lindan; (6) p, p´ -DDT; (7) methoxychlor.

Table 2. The results obtained from analysis of real samples

Samples	Methoxychlo	p,p' -DDT	heptachlor	p,p' -DDE	Lindane	dieldrin
River water (GolGol, ilam, Iran): concentration of OCPs(ng.L^{-1})	N,D [a]	N.D	N.D	N.D	N.D	N.D
Tap water (Ilam, Iran) concentration of OCPs(ng.L^{-1})	0.034	N.D	N.D	N.D	0.056	N.D
Agricultural water (Dehloran city, Ilam, Iran): concentration of OCPs(ng.L^{-1})	N.D	0.0123	N.D	N.D	N.D	N.D
water river(Semarea)(ng.L^{-1})	0.016	N.D	0.019	N.D	N.D	N.D

a : not detection

Table 3. Comparison of different micro extraction methods for investigation OCPs in water samples

Methods	Detection system	LOD(ng.L^{-1})	LOQ(ng.L^{-1})	RSD (%)	Recovery(%) in waters	Ref
USAEME	MS	0.8-10	-	2.70-12.40	-	**24**
SPME	MS	0.04-0.41	-	3.2-11.3	70.0-112.6	**26**
LPME-SFO	ECD	8-19	0.9931-0.9992	4.90-7.20	-	**25**
DLLME-SFO	ECD	11 -110	-	5.8-8.88	82.9–102.5	**27**
SDME	MS	22-101	-	5.9-9.9	-	**28**
Proposed method	ECD	0.379- 1.857	0.222-2.798	0.10-0.96	90.73-102.34	-

CONCLUSION

This paper has outlined the successful development and application of a method based on the DSDME technique combined with capillary GC-ECD for the analysis of OCPs in the water samples and compared with the conventional sample preparation method such as LPME. The current method has numerous advantages such as simplicity, low cost, ease of operation, no possibility of sample carry-over and short analysis time. Good linearity (25–2600ngL^{-1}), high sensitivity and repeatability of results were obtained. In addition, the method requires only small volume of organic extract ants; therefore, it is an environment friendly approach to the LPME. The performances of this procedure for the extraction of OCPs from different water with various matrices were excellent.

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