Application of reverse miniaturized dispersive liquid–liquid extraction method for determination of organophosphorus pesticides in water samples by GC/MS

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ABSTRACT

In this work, the application of reverse miniaturized dispersive liquid–liquid extraction techniques for determination of some organophosphorus pesticides (OPPs) in water samples has been evaluated. Reverse miniaturized dispersive liquid–liquid extraction method based on extraction with methanol containing buthyl acetate as a solvent was added sample and after phase separation, buthyl acetate injected to the GC/MS instrument. Gas chromatography/Mass spectrometry (GC/MS) in selected ion storage mode (SIS) was employed for the identification and quantification of diazinon, chlorpyrifos and butachlor. Important parameters affecting both extraction and reverse miniaturized dispersive liquid–liquid extraction procedures were investigated and optimized. Analytical method provides enrichment factors in the range of 11-18 for these pesticides. The calibration plots were linear in the ranges 0.06–15.0 ng mL^{-1}, 0.1-15.0 ng mL^{-1} and 0.4-15.0 ng mL^{-1} and limit of detection was 0.02, 0.03 and 0.14 ng mL^{-1} for diazinon, chlorpyrifos and butachlor, respectively. The developed method was able to detect trace amounts of these pesticides in the water samples.
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KEYWORDS

Organophosphorus pesticides;
Reverse miniaturized dispersive liquid–liquid extraction;
GC/MS;
Preconcentration.

INTRODUCTION

The withdrawn organochlorine pesticides were replaced by organophosphorus pesticides (OPPs) and organonitrogen pesticides (ONPs), which are universally applied because of their low cost, ready availability, wide range of efficacy, ability to combat a large number of pest species, and being less stable in the environment than organochlorine compounds[1]. Thus, in the past few years, organophosphorus compounds have become one of the most widely used classes of pesticides in the world. Organophosphorus pesticides (OPPs) are among the most common pesticides used in industrialized countries. Pesticide monitoring programs and export controls are needed for the protection of consumers and for quality evaluation of commodities. The determination of organophosphorus pesticides (OPPs) in water samples still presents significant prob-

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lems. U.S. environmental protection agency method 525 has a maximum allowable risk level for OPPs in drinking water ranging from 0.001-0.25 mg L^{-1}[2]. To reach the required EU level of 0.1 part-per-billion (ppb) for drinking water and the 1-3 ppb level in surface water, highly sensitive methods are required. Therefore, it is necessary to develop new analytical methods for OPPs determination in a simple and efficient way.

Numerous extraction techniques are available, the usual ones involving liquid-liquid extraction (LLE). LLE relies on the partition of analytes between two immiscible liquids, usually an aqueous solution and an organic solvent. But, even though LLE is relatively simple and inexpensive, it has many drawbacks, among them the need to use large quantities of solvents that are often toxic. In order to eliminate some of the inconveniences restricting the application of LLE, numerous modifications have been developed, mainly miniaturizations of the process in order to reduce the amounts of solvents used[3-5].

A new procedure termed as dispersive liquid–liquid microextraction (DLLME) has received much attention for sample pretreatment[6-8]. The main disadvantage of the DLLME technique is the use of chlorinated solvents as extractant that are heavier than water and more toxic than hydrocarbons. Recently, we reported our experiments for employing low-density extraction solvents in DLLME that we called, miniaturized dispersive liquid–liquid extraction (MDLLE)[9-11]. Traditionally, selective detectors in GC have been used to detect individual classes of GCamenable pesticides, such of organochlorines, organophosphates, and organonitrogens. In recent years, GC/MS has become the primary approach to analyze all classes of GC-amenable pesticides in the same chromatogram. Traditionally, GC/MS was mainly used for confirmation of analytes previously detected by selective detectors, but modern GC/MS instruments are sensitive, easy to use, reliable, and affordable by most laboratories. Another facet in MS analysis involves whether selected ion monitoring (SIM) should be employed to provide lower LOQ and greater selectivity in the analysis of targeted pesticides, or whether full scan MS should be conducted to potentially identify any GC-amenable chemical in the chromatogram. Furthermore, gas chromatography coupled to mass spectrometry (GC/MS) is a powerful tool to separate, identify and quantify OPPs in the different samples.

The aim of this study was to develop a new version of our previous method that called reverse dispersive liquid–liquid extraction (RDLLE) coupled to GC/MS for OPPs determination in water samples (Because of extraction of OPPs by addition of aqueous phase samples to co-solvent containing extracting solvent, we can call it reverse miniaturized dispersive liquid–liquid extraction (RMDLLE)).

EXPERIMENTAL

Chemicals and reagents

Analytical grade Methanol, n-hexane, cyclohexane, ethyl acetate, butyl acetate, hexyl acetate and NaCl were purchased from Merck (Darmstadt, Germany) and were used without further purification. Deionized water prepared on a Direct-Q 3 UV with a pump system (Millipore, Molshein, France). All OPPs (diazinon, chlorpyrifos and butachlor) prepared from Ehrnestorfer (Augsburg, Germany). Each stock solution was prepared at a concentration of 100 mg L^{-1} in methanol and stored in a refrigerator (4 °C) until use. The working solution was prepared by appropriate dilution of the stock solution with the methanol.

Apparatus

The analysis was performed using a Varian CP-3800 gas chromatograph coupled to ion-trap mass spectrometer (Varian Saturn 2200). Separations were carried out in a HP-5 MS (95% polydimethylsiloxane, 5% polyphenyl) fused-silica capillary column (20 m × 0.32 mm i.d. and 1.2 μm film thickness). The injector temperature was 250 °C and 2.0 μL of the sample was injected manually in the splitless mode. Helium (99.999% purity) was obtained from Roham Gas Company (Tehran, Iran) and was used as carrier gas at constant flow of 1.0 mL min^{-1}. The temperature program used for the chromatographic separation was as follows: 50 °C for 2 min, temperature increase at 25 °C min^{-1} to 100 °C and hold for 2 min, and then temperature increase at 5 °C min^{-1} to 280 °C where it was finally held for 2 min. Ion source was at ionization mode with electron impact at 70 eV and the analysis was done in the SIS (selected ion storsge) mode. The ion source,
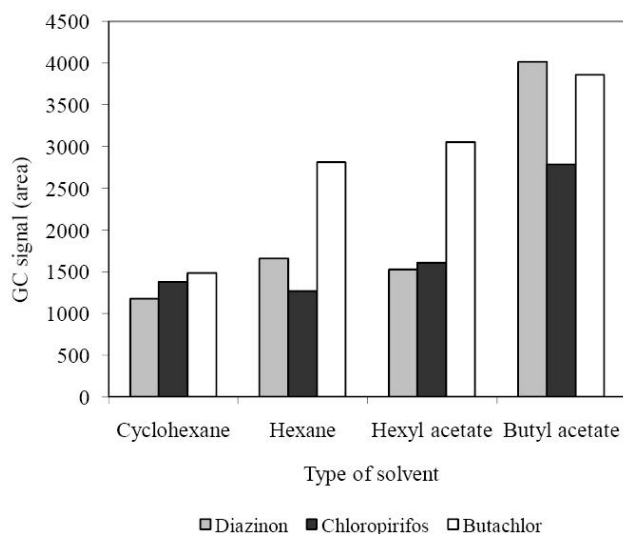
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trap, manifold and transfer line temperatures were maintained at 150, 150, 40 and 300 °C, respectively. Specific conditions for each analyte are listed in TABLE 1. A parent ion was chosen for each compound by taking the m/z and relative abundance of parent ions as high as possible in order to increase sensitivity. The GC/MS based on the SIS mode was used for quantifications. A Varian Workstation 5 software was used for data collection and processing.

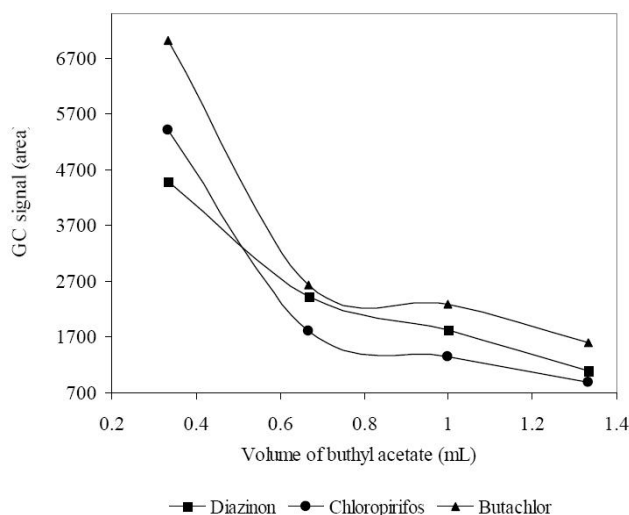## Low density miniaturized reverse dispersive liquid–liquid extraction procedure

1.0 mL of butyl acetate added to 2.0 mL methanol (1:2) and used as the extraction solvent. In a typical extraction experiment, 1.0 mL of butyl acetate solution was placed in a dried 10 mL volumetric flask and then, 8.0 mL of water sample was transferred to volumetric flask rapidly. The mixture was gently shaken. After this process, butyl acetate (extraction solvent) was separated at the top of volumetric flask. The butyl acetate was drawn out by a Hamilton syringe (0.30 mL) and transferred to a conical vial and 2.0 μL was injected to GC/MS.
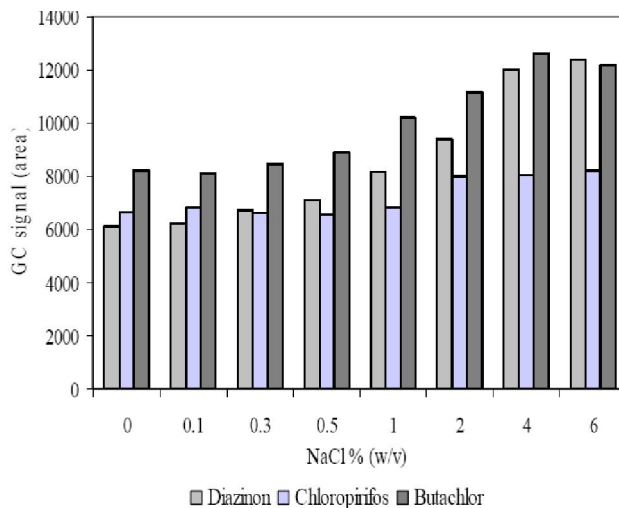
RESULTS AND DISCUSSION

In this study, reverse miniaturized dispersive liquid–liquid extraction (RMDLLE) technique combined with GC/MS was developed for the determination of some
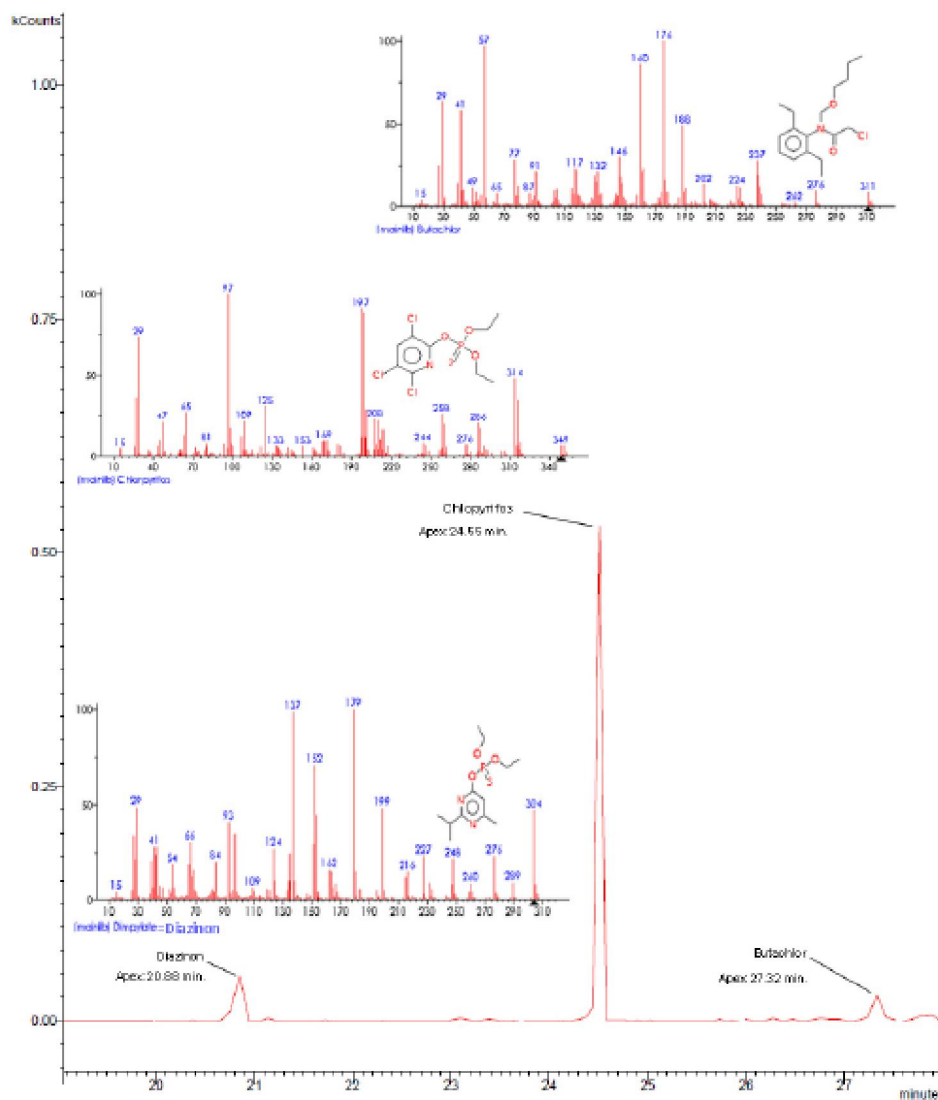

Figure 2 : Extraction efficiency of various butyl acetate volumes for extraction of the OPPs by RMDLLE (Conditions: sample volume 8.0 mL; OPPs concentration: 10.0 ng mL^{-1})

Figure 3 : Extraction efficiency of various butyl acetate for extraction of the OPPs in the presence of different concentration of NaCl by RMDLLE (Conditions: sample volume 8.0 mL; OPPs concentration: 10.0 ng mL^{-1}; volume of butylacetate solution: 1.0 mL)

OPPs in water samples. Operating in Selected Ion Storage (SIS) modes can increase the sensitivity and selectivity of this instrument. Before applying the proposed RMDLLE to the extraction and determination of OPPs in water samples, several experiments with spiked samples of the OPPs were carried out in order to select the optimum conditions including type and volume of extracting solvent, thermodynamic behavior, extraction time and salt addition for the extraction process. In the experiment, 8.0 mL of deionized water spiked with 10.0 ng mL^{-1} each of the OPPs was used to study

Figure 1 : Extraction efficiency of different extraction solvents evaluated for extraction of the OPPs by RMDLLE (Conditions: sample volume 8.0 mL; OPPs concentration: 10.0 ng mL^{-1}; volume of butylacetate solution: 1.0 mL)

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Figure 4 : Chromatogram obtained by RMDLLE/GC–MS analysis of an rganophosphorus-spiked water sample in SIS mode. Extraction conditions: 10.0 mL of water sample containing 5.0 ng mL^{-1} OPPs extracted to 0.33 mL butyl acetate

TABLE 1 : Selected ions and time windows for GC/MS analysis of the selected pesticides

Window	Acquisition time (min)	Organophosphorus pesticide	m/z range	Parents ion (m/z)	Most abundant ion	Quantification ions (m/z)
1	20.0-23.70	Diazinon	92-307	304	179	304,199,179,152,137,93
2	23.70-26.0	Chloropyrifos	96-315	314	97	314,197,125,97
3	26-27.6	Butachlor	56-277	276	176	276,237,188,176,160,57

the extraction performance under different experimental conditions. The theory of RMDLLE is similar to that of LLE and can find elsewhere[12].

Selection of the extraction solvent

For these investigations, four low-density solvents (n-hexane, cyclohexane, butyl acetate and hexyl acetate) differing in polarity and water solubility were tested. Experiments in triplicate were performed using a deionized water sample spiked with 10.0 ng mL^{-1} concentrations of OPPs. Butyl acetate, followed by hexyl acetate, n-hexane and cyclohexane, has the highest extraction efficiency as shown in Figure 1 It seems the acetic esters group of organic solvents benefits the extraction of the selected pesticides. The reason for this could be that butyl acetate has a more similar po-

TABLE 2 : Analytical parametres of the RMDLLE-GC/MS method

Pesticide	Retention time (min)	Regression equation	R2	MRL (ng mL$^{?1}$)	LOD (ng mL$^{?1}$)	Mean extraction recovery ± SD (n = 3)
Diazinon	20.88	Area = 318C + 150	0.994	0.06-15.0	0.02	87 ± 6
Chlorpyrifos	24.555	Area = 198C + 9.95	0.997	0.1-15.0	0.03	54 ±3
Butachlor	27.354	Area = 220.9C - 305	0.999	0.4-15.0	0.14	70 ± 5

Maximum regression level

TABLE 3 : Recovery of the OPPs by RMDLLE-GC/MS method

Pesticide	Spiked level (ng mL^{-1})	Tap water		River water	
		Found (ng mL^{-1})	Recovery% (±RSD)	Found (ng mL^{-1})	Recovery% (±RSD)
Diazinon	-	<LOD	-	<LOD	-
	2.0	1.95	97.5 (± 4)	2.06	103.0 (± 2)
	5.0	5.06	101(± 3)	5.1	102.0 (± 5)
Chlorpyrifos	-	<LOD	-	<LOD	-
	2.0	1.86	93.0 (± 5)	1.91	95.5 (± 4)
	5.0	4.91	98.2 (± 4)	4.95	99.0 (± 5)
Butachlor	-	<LOD	-	<LOD	-
	2.0	1.83	91.5 (± 6)	1.94	97.0 (± 2)
	5.0	4.89	97.8 (± 2)	5.07	101.4 (± 3)

larity with the OPPs. Thus, the best extraction solvent appeared to be the butyl acetate.

Volume of extracting solvent

To increase the sensitivity of the RMDLLE procedure, butylacetate solution volume was studied in the range of 1.0-3.0 mL (containing 0.33-1.33 mL butylacetate). The results show the peak area (concentration) of the analytes decreased with increasing volume of buthyl acetate in the studied range due to increase in volume of buthyl acetate (OPPs were diluted). Figure 2 shows, higher extraction efficiency was obtained using 1.0 mL extracting solvent. Thus, 1.0 mL of butylacetate solution (0.67 mL methanol + 0.33 mL buthyl acetate) was chosen in this work.

Selection of the co-solvent

The miscibility of co-solvent in both organic (extraction solvent) and water sample solution is the main point of selection for a co-solvent. Therefore, acetone, acetonitrile and methanol were introduced for this purpose. A series of spiked sample solutions (10.0 ng mL$^{"1}$) was extracted by using 2.00 mL of each co-solvent containing 1.0 mL butylacetate as an extraction solvent. The results indicate that acetonitrile give a broad peak

that interfered in OPPs analysis and thus can not use as a co-solvent. Also, the results showed methanol and acetone the same percent recoveries of the analytes obtained. Methanol was selected as the co-solvent in the subsequent extractions because of its availability and low cost.

Salt addition

The effects of NaCl addition (0–6% w/v) on extraction efficiency were assessed with RMDLLE method. The results show peak areas of OPPs were increased with increasing the NaCl concentration up to 4% and then were approximately level off and volume of but the collected phase does not increase (Figure 3.). And because of decreasing the solubility of OPPs in aqueous phase in the presence of salt, extraction efficiency was increased (salting out effect).

Analytical performance

The calibration curves, were obtained by injecting extracted solvent from 8.0 mL of water samples that were fortified with variable concentrations of the diazinon, chlorpyrifos (5.0–100.0 ng mL^{-1}), and butachlor (50.0–2000.0 ng mL^{-1}) by RMDLLE method. All calibration curves were linear with correla-

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tion coefficients better than 0.998. The calculated limits of detection of RMDLLE at a signal-to-noise ratio of 3, were 0.02, 0.03 and 0.14 ng mL^{-1} for diazinon, chlorpyrifos, and butachlor, respectively.

The method was evaluated using tap water samples obtained from Tehran and river water from north of Iran. These samples analyzed by using the procedure described previously. The results are shown in TABLE 2. The results showed that concentration of the studied pesticides in tap water is lower than LODs of the method.

CONCLUSIONS

Low density miniaturized homogenous liquid-liquid solvent extraction is a simple, rapid, precise, reproducible, inexpensive, green method for extraction of pesticides from aqueous samples. The combination of RMDLLE with GC/MS can be achieved very low limits of detection for determination of pesticides in aqueous samples. The main advantage of the described methods compared with a traditional method is the significant reduction of the required volume of organic solvent.

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