

ORIGINAL ARTICLE

Homogeneous Liquid-Liquid Microextraction via Flotation Assistance Method as a Sensitive and Efficient Sample Preparation Method for Determination of Diazinon in Water Samples

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KEYWORDS

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ABSTRACT: In the present study a sensitive method for extraction of diazinon in water samples was developed using homogeneous liquid-liquid microextraction via flotation assistance (HLLME-FA). Toluene at microliter volume level was used as extraction solvent. The main advantages of method are high enrichment factor, high recovery, good repeatability and extraction solvent volume at μL level. The influence of several variables on the extraction efficiency was investigated. In the proposed method, the calibration graph was linear in the range of 0.5 - 100 $\mu\text{g L}^{-1}$ with a detection limit of 0.2 $\mu\text{g L}^{-1}$. The relative standard deviation (RSD) for four replicate measurements of diazinon was 6.5%. The proposed method is fast, simple, and sensitive without any need for stirring and centrifugation and applied to real water samples, successfully.

INTRODUCTION

Organophosphorus pesticides (OPPs) are a class of insecticides, several of which are highly toxic. Until the 21st century, they were among the most widely used insecticides available. Thirty-six of them are presently registered for use in the United States, and all can potentially cause acute and subacute toxicity. OPPs are used in agriculture, homes, gardens and veterinary practices [1]. Many OPPS are potent nerve agents, functioning by

inhibiting the action of acetylcholinesterase (AChE) in nerve cells. They are one of the most common causes of poisoning worldwide, and are frequently intentionally used in suicides in agricultural areas. OPPs can be absorbed by all routes, including inhalation, ingestion, and dermal absorption. Even at relatively low levels, organophosphates may be hazardous to human health [2, 3].

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Therefore, determination and monitoring of OPPs in water samples is important.

Current methods have applied various extraction and clean-up techniques for OPPs in samples, including liquid-liquid extraction (LLE) [4] solid-phase extraction (SPE) [5] and matrix solid-phase dispersion (MSPD) [6]. However, in LLE multiplication of extraction steps to obtain optimum output is necessary and use of large volumes of organic solvents, which is cost and toxic. Moreover, Emulsion's difficulties, hinders the full recovery of the extract. In the past decade, solid-phase microextraction (SPME) and liquid-phase microextraction (LPME) techniques were developed and applied for extraction of OPPs [7-9].

Dispersive liquid-liquid microextraction (DLLME) is a recent microextraction technique first developed by Rezaee et al. [10]. It allows the simultaneous extraction and preconcentration of analytes into a micro-volume of extracting solvent based on a ternary solvent system involving an aqueous phase, a nonpolar water immiscible high-density solvent that acts as extraction phase, and a disperser solvent, which is often polar and water miscible [10-15].

Assadi et al. [16] applied DLLME for extraction OPPs from water samples. However, the extraction solvent is limited in the solvents, which have higher density than water and all of them are toxic and environment - unfriendly. Recently, homogeneous liquid-liquid microextraction via flotation assistance (HLLME-FA) method reported for the determination of polycyclic aromatic hydrocarbons (PAHs) in soil and water samples [17, 18].

The present investigation has centered on the development of a reliable method for determination of diazinon in water samples. We for the first time used the new preconcentration method of HLLME-FA, combined with GC for the determination of diazinon in water samples. Influence of extractant and homogeneous solvents,

as well as ionic strength, on the performance of the method is discussed in detail.

MATERIALS AND METHODS

Chemicals and reagents

Diazinon and sodium chloride of the highest purity available from Merck (Darmstadt, Germany) were used. N-hexane, n-heptane, toluene, 1-octanol as extraction solvents and methanol, acetone and acetonitrile as homogeneous solvents were obtained from Merck. A stock standard solution of diazinon (1000 mg L^{-1}) was prepared in methanol. Working standard solutions were prepared in doubly distilled water. Youngling ultra pure water purification system (Aqua MaxTM-ultra, Korea) was used for purification of water.

Instrumentation

Separation, identification and quantification were carried out on an Agilent GC-7890 system equipped with flame ionization detector. Helium (with 99.999% purity) was used as carrier (flow rate= 2.0 mL min^{-1}). The inlet was operated in the split less mode with a split ratio of 1:5. Separation of pesticide was carried out using DB5, $25\text{m} \times 0.32\text{mm i.d.}$ and $0.25 \text{ }\mu\text{m}$ film thicknesses from SGE (Victoria, Australia) capillary column. The injector and detector temperatures were set at $250 \text{ }^\circ\text{C}$ and $270 \text{ }^\circ\text{C}$, respectively. The GC oven was kept at $150 \text{ }^\circ\text{C}$ for 2 min then raised to $200 \text{ }^\circ\text{C}$ at $5 \text{ }^\circ\text{C min}^{-1}$ and held for 5 min.

HLLME-FA Procedure

Acetone (0.5 mL), as homogeneous solvent, contains $50.0 \text{ }\mu\text{L}$ toluene, as extraction solvent was injected into the home-designed extraction cell (Figure 1). A 22.0 mL of saline water sample was added into the cell. Using air flotation, the organic solvent was collected on the top of the solution. After separation of the two phases, doubly distilled water were added into the glass tube fixed on

the side of the vial. 2.0 μL of the collected organic solvent was injected into the GC-FID instrument.



Figure 1. Structure of home-designed extraction cell

RESULTS AND DISCUSSION

HLLME-FA combined with GC-FID was developed for determination of diazinon in water samples. The effects of different parameters were performed using one variable at a time method.

Selection of extraction solvent

Selection of the optimum extraction solvent is one of the most important tasks in method development in HLLME-FA. Solvent properties both required and de-

sired include 1- high extraction efficiency of the analyte 2- immiscibility with water 3- lower density than water. 1-octanol, n-hexane, n-heptane and toluene were considered. The results (Figure 2) revealed that toluene has the higher extraction efficiency in comparison with the other tested solvents. It is probably, because of higher solubility of the analyte in toluene. Therefore, toluene was selected as the extraction solvent.

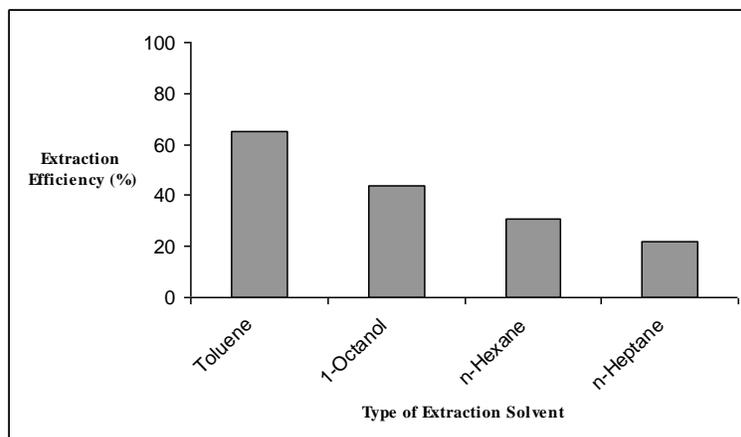


Figure 2. Effect of type of extraction solvent on the extraction efficiency

Selection of homogeneous solvent

The miscibility of homogeneous solvent in aqueous and organic phase (extraction solvent) is an essential point for its selection. Acetone, acetonitrile and methanol have been successfully used for this purpose. Variations of extraction recoveries by using different homogeneous solvents are not remarkable, thus, acetone is selected because of low toxicity and low cost.

Selection of extraction and homogeneous solvent volumes

In this extraction method, the preconcentration factor is strongly dependent on the volume of the collected toluene phase. The relationship among the volume of added toluene and the preconcentration factor is shown in Figure 3. As can be seen, the extraction of diazinon is quantitative at 50.0 μL of toluene. Hence, In order to achieve maximum preconcentration factor, 50.0 μL of added toluene was selected.

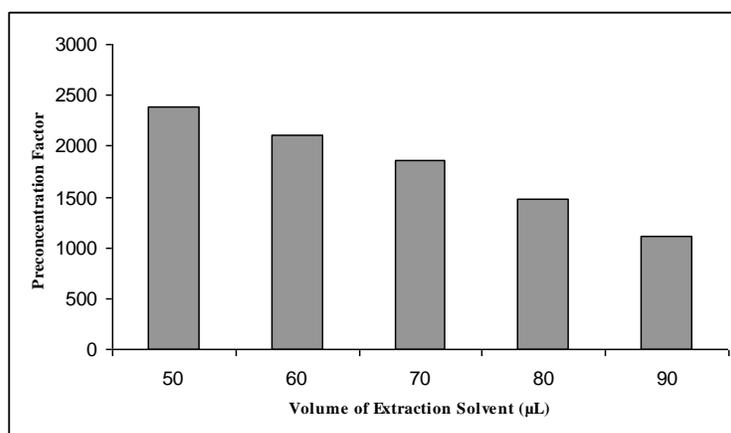


Figure 3. Effect of volume of extraction solvent on the preconcentration factor

In order to investigate the effect of homogeneous solvent volume on the extraction efficiency, various volumes of acetone (0.5, 1.0, 1.5 and 2.0 mL) containing 50 μL of toluene were tested (Figure 4). Increasing the volume of acetone resulted in decreased extraction efficien-

cy. This was probably due to the increased solubility of the diazinon in water as the volume of acetone was increased. Thus, 0.5 mL of acetone was chosen as the optimum volume of the homogeneous solvent.

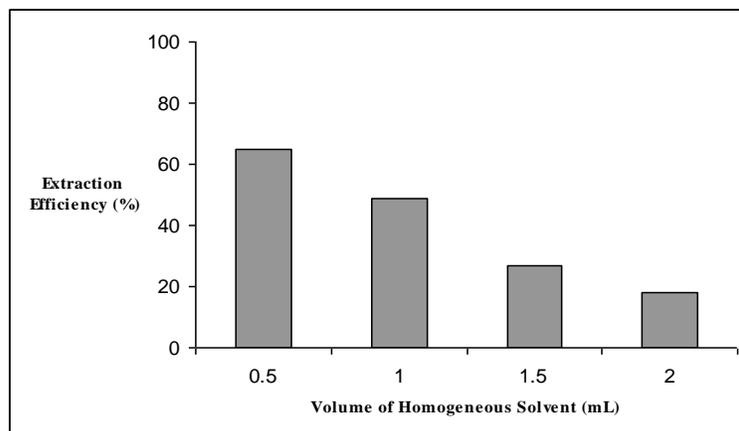


Figure 4. Effect of volume of homogeneous solvent on the extraction efficiency

Effect of salt addition

The effect of the addition of salt on the extraction efficiency was studied by adding NaCl (0.5–3 M) into the aqueous solution. The results obtained (Figure 5) showed that increasing the amount of salt up to 1.5 M, resulted in enhanced the extraction efficiency. However,

further addition of salt decreased the extraction efficiency. Therefore, the salting out effect increases the extraction efficiency up to 1.5 M of NaCl. Consequently, 1.5 M of NaCl was used in subsequent experiments.

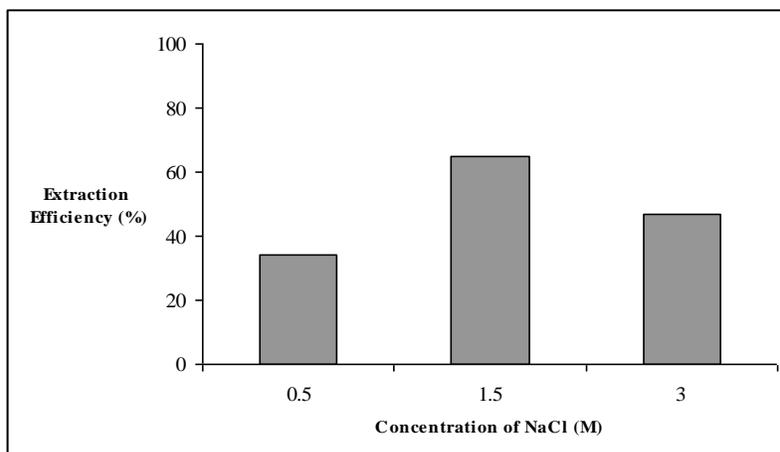


Figure 5. Effect of NaCl concentration on the extraction efficiency

Quantitative analysis

The characteristics of calibration curve were obtained under optimized conditions. Linearity was in the range of 0.5 to 100 $\mu\text{g L}^{-1}$ for diazinon with coefficients of determination (r^2) of 0.9992. Calibration curve was plotted between peak area and concentrations of diazinon. The relative standard deviations (RSD%, $n=4$) of diazinon was 6.5%. The limit of detection (LOD), ($S/N=3$) was 0.2 $\mu\text{g L}^{-1}$.

Comparison of the proposed method with solid-phase microextraction (SPME) [19] and single-drop microextraction (SDME) [20] for extraction and determination of diazinon indicates that this novel method has a short extraction time for determination of diazinon. Quantitative results of proposed method such as detection limit and linear range are comparable with SPME and SDME methods without using sensitive detector such as FPD. Moreover, SPME is expensive, its fiber is fragile and has limited life-time and sample carry-over can be a problem and disadvantages of the SDME method are as

follows: fast stirring would tend to break up the organic drop, air bubble formation, extraction is time-consuming. Quantitative results of proposed method such as detection limit and linear range are comparable with DLLME method [16] without using sensitive detector such as FPD. In addition, the main advantages of the proposed method are this novel method does not need centrifugation to separate the organic phase and it is possible to the usage of low-density extraction solvents. The proposed method used organic solvent with less toxicity. Finally, it can be concluded that the proposed method is an efficient, rapid, simple and cheap microextraction method that can be a complement technique for DLLME method that have been used with organic solvents more dense than water for determination of diazinon in water samples.

Real water analysis

In order to evaluate the application of the proposed method, the diazinon content was determined in the three types of water samples. To assess matrix effects, each sample was spiked with diazinon standard, then preconcentrated, and determined by the proposed procedure and each determination was repeated for three times. The recoveries from these samples at the spiking

level of $5.0 \mu\text{g L}^{-1}$ showed that the matrices of the analyzed water samples had little effect on the HLLME-FA procedure for determination of diazinon. The results obtained for determination of diazinon in the three types of water samples are given in Table 1. Figure 6 show the chromatograms obtained for the well water samples before and after spiking of diazinon.

Table 1. Determination of diazinon in the three different water samples.

Sample	Concentration of diazinon ($\mu\text{g L}^{-1}$)	Added diazinon ($\mu\text{g L}^{-1}$)	Found diazinon ($\mu\text{g L}^{-1} \pm \text{RSD}$, n=3)	Relative recovery (%)
Tap water ^a	n.d. ^d	5.0	4.6 ± 5.7	92
Well water ^b	n.d.	5.0	4.5 ± 7.3	90
River water ^c	n.d.	5.0	4.4 ± 9.7	88

^aThe water was taken from Tonekabon Branch, Islamic Azad University (Tonekabon, Iran).

^bThe water was collected from well in Tonekabon (Tonekabon, Iran).

^cThe sample was collected from Langrud River (Gilan, Iran).

^dNot detected.

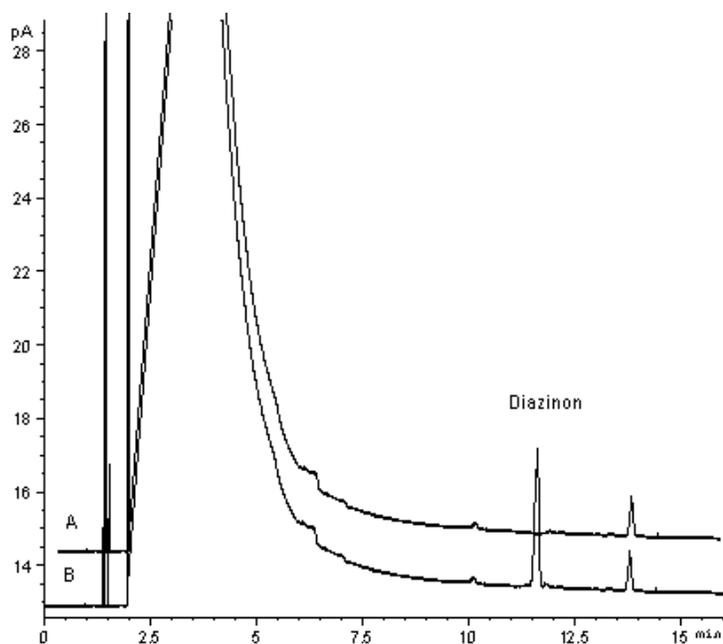


Figure 6. GC-FID chromatograms of diazinon in river water, before spiking (A) and after spiking with $5.0 \mu\text{g L}^{-1}$ of diazinon (B) using proposed method combined with GC-FID under optimum conditions.

CONCLUSIONS

A homogeneous liquid-liquid microextraction via flotation assistance procedure combined with GC-FID detection was presented for concentration of diazinon from water samples. The method is simple, rapid and inexpensive. In this method sample preparation time as well as consumption of toxic organic solvents was minimized without affecting the sensitivity of the method. No matrix effect was observed when the proposed HLLME-FA technique was applied to water samples spiked with the analyte. Finally, HLLME-FA provides high preconcentration factor and low LOD within very short time for the analyte in water sample.

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REFERENCES

1. Worek F., Aurbek N., Wetherell J., Pearce P., Mann T., Thiermann H., 2008. Inhibition, reactivation and aging kinetics of highly toxic organophosphorus compounds: pig versus minipig acetylcholinesterase. *Toxicology*. 244, 35-41.
2. Vidair C.A., 2004. Age dependence of organophosphate and carbamate neurotoxicity in the postnatal rat: extrapolation to the human. *Toxicol Appl Pharmacol*. 196, 287-302.
3. Barata C., Solayam A., Porte C., 2004. Role of B-esterases in assessing toxicity of organophosphorus (chlorpyrifos, malathion) and carbamate (carbofuran) pesticides to *Daphnia magna*. *Aquat Toxicol*. 66, 125-139.
4. Buonasera K., Dorazio G., Fanali S., Dugo P., Mondello L., 2009. Separation of organophosphorus pesticides by using nano-liquid chromatography. *J Chromatogr A*. 1216, 3970-3976.
5. Ballesteros E., Parrado M.J., 2004. Continuous solid-phase extraction and gas chromatographic determination of organophosphorus pesticides in natural and drinking waters. *J Chromatogr A*. 1029, 267-273.
6. Albero B., Sanchez-Brunete C., Tadeo J.L., 2003. Determination of organophosphorus pesticides in fruit juices by matrix solid-phase dispersion and gas chromatography. *J Agric Food Chem*. 51, 6915-6921.
7. De Pasquale C., Jones A., Charlton A., Alonzo G., 2005. Use of SPME extraction to determine organophosphorus pesticides adsorption phenomena in water and soil matrices. *Int J Environ Anal Chem*. 85, 1101-1115.
8. Berhanu T., Megersa N., Solomon T., Jonsson J.A., 2008. A novel equilibrium extraction technique employing hollow fiber liquid phase microextraction for trace enrichment of freely dissolved organophosphorus pesticides in environmental waters. *Int J Environ Anal Chem*. 88, 933-945.
9. Zhao E.C., Han L.J., Jiang S.R., Wang Q.X., Zhou Z.Q., 2006. Application of a single-drop microextraction for the analysis of organophosphorus pesticides in juice. *J Chromatogr A*. 1114, 269.
10. Rezaee M., Assadi Y., Milani Hosseini M.R., Aghaee E., Ahmadi F., Berijani S., 2006. Determination of organic compounds in water using dispersive liquid-liquid microextraction. *J Chromatogr A*. 1116, 1-9.
11. Rezaee M., Yamini Y., Faraji M., 2010. Evolution of dispersive liquid-liquid microextraction. *J. Chromatogr. A* 1217, 2342-2357.
12. Rezaee M., Yamini Y., Shariati S., Esrafil A., Shamsipur M., 2009. Dispersive liquid-liquid microextraction combined with high-performance liquid chromatography-UV detection as a very simple, rapid

and sensitive method for the determination of bisphenol A in water samples. *J Chromatogr A*. 1216, 1511-1514.

13. Mashayekhi H.A., Abroomand-Azar P., Saber-Tehrani M., Waqif S.H., 2010. Rapid determination of carbamazepine in human urine, plasma samples and water using DLLME followed by RP-LC. *Chromatographia* 71, 517-521.

14. Mashayekhi H.A., Abroomand-Azar P., Saber-Tehrani M., Waqif S.H., 2011. Rapid and sensitive determination of biphenyl and biphenyl oxide in water samples using dispersive liquid-liquid microextraction followed by gas chromatography. *Int J Environ Anal Chem*. 91, 516-524.

15. Mashayekhi H.A., Rezaee M., Sadeghi Garmaroudi S., Montazeri N., Ahmadi S.J., 2011. Rapid and sensitive determination of benzaldehyde arising from benzyl alcohol used as preservative in injectable formulations solution using dispersive liquid-liquid microextraction followed by gas chromatography. *Anal Sci*. 27, 865-868.

16. Berijani S., Assadi Y., Anbia M., Milani Hosseini M.R., Aghaee E., 2006. Dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric detection: very simple, rapid and sensitive method for the determination of

organophosphorus pesticides in water. *J Chromatogr A*. 1123, 1-9.

17. Haji Hosseini M., Rezaee M., Mashayekhi H.A., Akbarian S., Mizani F., Pourjavid M.R., 2012. Determination of polycyclic aromatic hydrocarbons in soil samples using flotation assisted homogeneous liquid-liquid microextraction. *J Chromatogr A*. 1265, 52-56.

18. Haji Hosseini M., Rezaee M., Akbarian S., Mizani F., Pourjavid M.R., Arabieh M., 2013. Homogeneous liquid-liquid microextraction via flotation assistance for rapid and efficient determination of polycyclic aromatic hydrocarbons in water samples. *Anal Chim Acta*. 762, 54-60.

19. Su P.G., Huang S.D., 1999. Determination of organophosphorus pesticides in water by solid-phase microextraction. *Talanta* 49, 393-402.

20. Ahmadi F., Assadi Y., Milani Hosseini M.R., Rezaee M., 2006. Determination of organophosphorus pesticides in water samples by single drop microextraction and gas chromatography-flame photometric detector. *J Chromatogr A*. 1101, 307-312.