Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction for Determination of Organophosphorus Pesticides in Water Samples by Gas Chromatography-Pulsed Flame Photometric Detection

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A fast and simple method was developed for simultaneous determination of seven organophosphorus pesticides (diazinon, disulfoton, chlorpyrifos, parathion-methyl, fenthion, parathion and quinalphos) with ultrasound-assisted dispersive liquid-liquid microextraction and gas chromatography-flame photometric detection in water samples. Some parameters affecting the extraction efficiency, including the types and volumes of extractant and dispersant, sample pH and ultrasonic time, were systematically evaluated. Under optimum conditions, a mixture of 25 μL tetrachloromethane (extraction solvent) and 0.8 mL acetone (disperser) was rapidly injected into a 5 mL water sample. The mixture was emulsified by ultrasound for 5 min to form a cloudy solution. After centrifuge for 5 min at 3500 rpm, the 1 μL sediment phase was directly injected for GC-PFPD analysis. The method showed good linearity in the range of 0.1-150 μgL^{-1} with a correlation coefficient between 0.9945-0.9995. Enrichment factors varied from 143 to 374 fold. The limits of detection (LOD) (S/N = 3) and limit of quantification (LOQ) (S/N = 10) were 0.01-0.04 and 0.05-0.1 μgL^{-1}, respectively. Recovery studies were carried out at two concentration levels in real samples within the range of 70-110 % and RSD were less than 9.8 % (n = 5).

Keywords: UA-DLLME, Organophosphorus pesticides, GC-PFPD, Water samples.

INTRODUCTION

Organophosphorus pesticides are phosphorus containing organic compounds used as a means of eliminating pests and limiting their adverse effects in agriculture. But the residues of organophosphorus pesticides are toxic substances that contaminate our environment due to their mobile and bioaccumulation abilities[1], with organophosphorus pesticides' derivatives more toxic than their parent forms[2]. Pesticide exposure in the environment and in water is harmful to the health of all organisms. Thus, monitoring pesticide levels, especially in the water, is necessary. Many countries and regions set maximum residue limits (MRLs) for organophosphorus pesticides in drinking water. The European Union (EU) allows a maximum concentration of 0.1 μgL^{-1} of each individual pesticide and 0.5 μgL^{-1} of the sum of pesticides in drinking water[3].

Determination of organophosphorus pesticides in water matrices is usually performed by sample preparation methods such as solid-phase extraction (SPE), single-drop microextraction (SDME) and solid-phase microextraction (SPME) combined with gas chromatography[4-6] or high performance liquid chromatography[7].

Sample preparation is a key factor in the analysis. A novel Sample preparation technique called dispersive liquid-liquid microextraction (DLLME)[8], which is based on a ternary component solvent scheme including extractant, disperser and aqueous sample. Improvements in DLLME were gradually introduced in the years following its introduction, with both the type of extractant and disperser enlarged. Hydrophobic ionic liquids[9] and solvents lighter than water[10] are employed as extractants. Hydrophilic ionic liquids[11] and surfactant[12] (*e.g.*, Triton-100, CTAB *etc.*) are used as dispersers. Dispersive liquid-liquid microextraction was widely adopted due to its simplicity of use, low cost, high enrichment and environmental friendliness. Ultrasound can accelerate the formation of a fine cloudy solution due to the assisting-dissolving and emulsifying effect, which would increase extraction efficiency and reduce equilibrium time. Ultrasound-assisted technique is often used combine with DLLME[13].

The present study develops a reliable sample preparation UA-DLLME coupled with GC-PFPD for determination of trace-level organophosphorus pesticides in water. Various parameters affecting the extraction and enrichment efficiency were evaluated and optimized.

EXPERIMENTAL

Seven standard pesticides were obtained from the Agro-Environmental Protection Institute, Ministry of Agriculture

(China). Each of them was diluted in acetone to prepare a mixed standard solution of 100 mgL^{-1}. A fresh standard solution of organophosphorus pesticides (1 mgL^{-1}) was prepared in acetone and stored at 4 °C.

Chlorobenzene, C$_2$H$_2$Cl$_4$ and CCl$_4$ (analytical grades) were obtained from the Beijing Chemical Factory. Acetonitrile and acetone (analytical grades) were purchased from the Tianjin Chemical Factory. [C$_4$MIM][BF$_4$] (>99 % Purity) and Triton-100 were obtained from the Shanghai Jin-Chun Chemical Co. Ltd.. Doubly-distilled water was filtered with a 0.45 µm filter membrane before use.

GC conditions: A gas chromatograph (Varian, CP-3800) equipped with a split/splitless injector system and a pulsed flame photometric detector was used for analysis. Pesticide separation was conducted with a Rtx-1701 capillary column (30 m × 0.25 mm i.d., 0.25 m film thickness, Restek. U.S). The temperature program commenced at 120 °C, then raised by 30 °C min^{-1} to 180 °C and was held for 0 min, after which it was raised by 10 °C min^{-1} to 220 °C and held for 2 min. Finally, the temperature was increased by 10 °C min^{-1} to 270 °C and kept at this temperature for 2 min. The total time required for one GC run was 15 min.

Other operating conditions were as follows: Injection port temperature was 250 °C and 1 µL of the sample was injected in a splitless mode. The detector temperature was held at 300 °C. High-purity nitrogen (99.999 %) was used as carrier gas, with Hydrogen and zero air (99.999 %) as an oxidant for PFPD (Kunming Messer Gas Products Co.LTD, Kunming, China) with a flow rate of 120 mL min^{-1}.

Sample preparation: Four water samples were collected from Panlong river and Dianchi lake (Kunming, China). All samples were filtered with a 0.45 µm filter membrane and analyzed within 24 h.

Optimized UA-DLLME procedure: A 5 mL aliquot of the water sample (blank or spiked) was transferred in 10 mL screw cap glass tubes with a conic bottom. A mixture of 25 µL CCl$_4$ and 0.8 mL acetone was rapidly injected into the sample to form the ternary solvent system. The mixture was vortexed for 1 min and then further emulsified by ultrasound for 5 min to produce a fine, cloudy solution. Finally, the cloudy solution was centrifuged for 5 min at 3500 rpm and a droplet sediment formed at the bottom of the centrifuge tube. 1 µL sediment was directly injected for GC-PFPD analysis.

RESULTS AND DISCUSSION

Selection of extraction solvent: The type of extraction solvent used significantly affects the extraction efficiency. In this study, three kinds of solvent-chlorobenzene (C$_6$H$_5$Cl), tetrachloroethane (C$_2$H$_2$Cl$_4$) and tetrachloromethane (CCl$_4$) were evaluated. 30 µL of each extractant along with 1 mL of acetone were added to 5 mL of sample solution. The extraction efficiency was based on the average peak area counts of each analyte for 3 replicates. As indicated in Fig.1, CCl$_4$ has the highest extraction efficiency for most of the organophosphorus pesticides. Therefore, CCl$_4$ was selected as the extractant for this work.

Selection of disperser solvent: Miscibility in extraction solvent and aqueous phase is key for the selection of the dispersive solvent. Acetone, acetonitrile, hydrophobic ionic [C$_4$MIM][BF$_4$] and surfactant Triton-100 were used for this
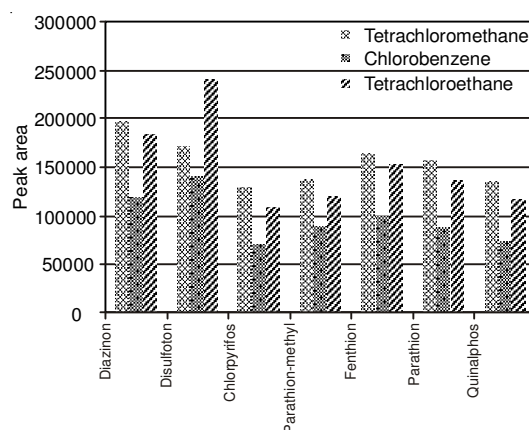

Fig. 1. Comparison of average peak area response using different extractive solvents (n = 3)

purpose in the present experiment. The results (Fig. 2) show that acetone produces the highest extraction efficiency for all pesticides, which may be due to its higher dispersing capability for the extractant and relatively lower loss of the analytes. Additionally, it is less toxic and comparatively cheaper. It was chosen as the optimum dispersive solvent.
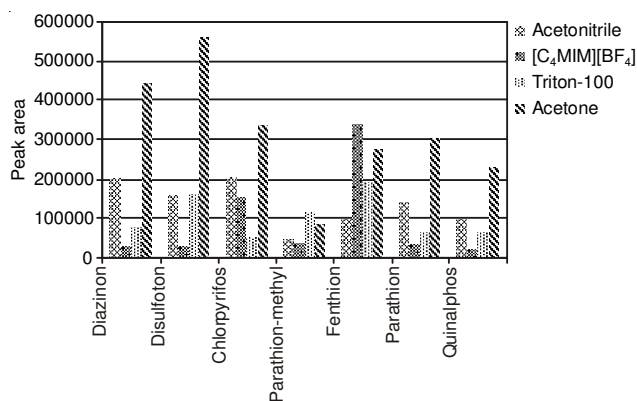

Fig. 2. Comparison of average peak area response using different dispersive solvents (n = 3)

Optimization of extraction and disperser solvent volume: Various volumes of CCl$_4$ were added to 1 mL acetone. The results (Fig. 3) show that the peak area decreased with increasing volume of CCl$_4$ from 20 to 35 µL. This is expected due to the dilution effect of the analytes at a higher volume of extraction solvent. Although less extractant volume (12 µL) was reported in organophosphorus pesticides' analysis in water[14], it was difficult to collect the sediment when the initial volume of extraction solvent was less than 25 µL in our experimental conditions. Relative standard deviation (RSD) was decreased when extraction solvent volume was increasing. To maintain a high enrichment factor and ensure enough sediment phases for further chromatographic analysis, 25 µL was selected as the best volume of extraction solvent to be used in further experimentation.

In order to study the effect of volume on extraction performance, different volumes of acetone in the range of 0.5-2.5 mL were investigated. A decrease in extraction efficiency was observed at higher disperser volumes, likely due to enhanced solubility of the analytes in the aqueous/acetone phase (Fig. 4). A volume of 0.8 µL was found to be optimum.
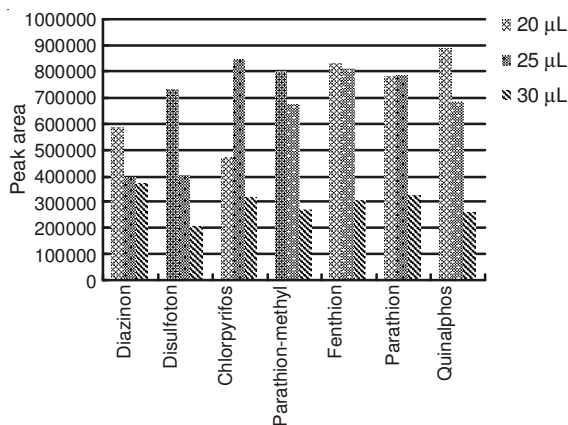
Fig. 3   Comparison of average peak area response using different volumes of extractive solvents (n = 3)
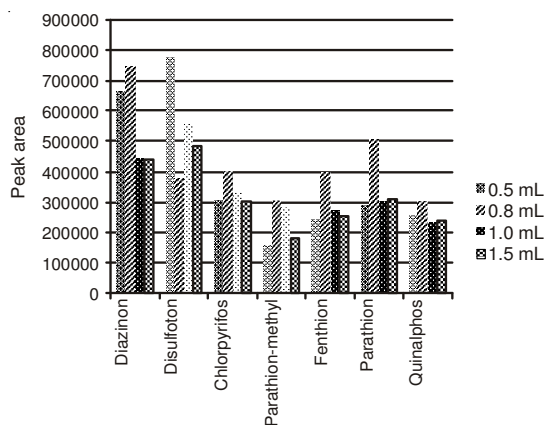

Fig. 4.   Comparison of average peak area response using different volumes of dispersive solvents (n = 3)

Fig. 5. Effect of pH value on extraction (n = 3)
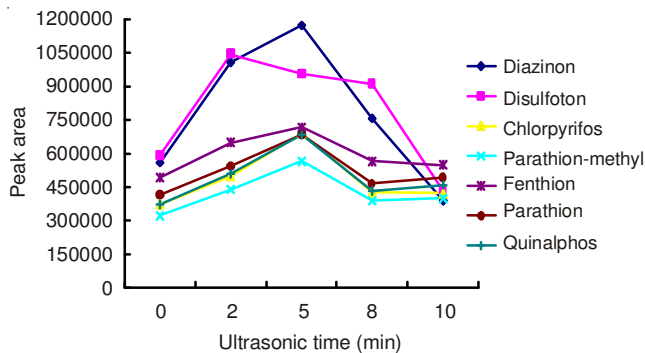
Fig. 6. Effect of ultrasonic time on extraction (n = 3)

Effect of pH: Most of the organophosphorus pesticides are less stable in alkaline solutions than in acid ones. Thus, in the present study, the pH was adjusted in a range of 2-6 using acetic acid (1 mol L^{-1}). Fig. 5 demonstrates that pH value has no significant effect on extraction performance for most of the analytes. Therefore, keeping the initial pH level was suitable for the UA-DLLME procedure.

Effect of ultrasonic time: Ultrasonic time in a range of 0-10 min was adopted to evaluate the effect on the extraction ratio. The results show that average peak area increased gradually in the first 5 min, but decreased and then smoothed in later time periods. This may be explained as the volatilization loss of the analytes and extractant increase with the extension of ultrasonic time. Thus, in the experiment, 5 min is the ideal time (Fig. 6) for the ultrasound assistance process.

Methods performance: Under the optimum condition, validation parameters of the methods, including the linearity, precision, repeatability, enrichment factors and limits of detection, were investigated using spiked doubly-distilled water samples. The regression equations and linearity range for each pesticide is shown in Table-1. Coefficient of determination (r) ranged from 0.9940 to 0.9990. The extraction recoveries and enrichment factors were 71-93 % and 143-374, respectively. The LOD (S/N = 3) obtained were in the range of 0.01-0.04 µgL^{-1} and the LOQ (S/N = 10) were from 0.05 to 0.1 µgL^{-1}.

Real water analysis: The river and lake water samples (each 2 portions) were extracted and analyzed using the developed method. The results (Table-2) revealed that they were free of organophosphorus pesticides contamination. To study the effect of sample matrix and the accuracy of the method, two different concentrations of pesticide standards were spiked into the sample and the recovery was calculated. Recovery rates were in the range of 70-110 %, RSD = 9.8. Fig. 7 shows the chromatograph which demonstrates that the real river and lake water matrices have minor effects on the developed method. The method was thereby proven reliable and can be used for the trace analysis of organophosphorus pesticides residues in water samples.

| TABLE-1 |||||||
| PARAMETERS OF THE UA-DLLME-GC METHOD |||||||
Organophosphorus pesticides	Linear equation	R	LR (µg L^{-1})	EF	ER (%)	LOD (µg L^{-1})	LOQ (µg L^{-1})
Diazinon	Y = 1.743267e + 007x − 2.322002e + 003	0.9986	0.1-80	289	83	0.02	0.06
Disulfoton	Y = 2.867219e + 007x − 5.717741e + 004	0.9945	0.1-80	374	71	0.02	0.07
Chlorpyrifos	Y = 5.705419e + 006x + 2.644155e + 003	0.9954	0.1-150	276	72	0.03	0.09
Parathion-methyl	Y = 6.677403e + 006x − 4.077595e + 003	0.9990	0.1-150	175	81	0.02	0.07
Fenthion	Y = 8.456542e + 006x − 1.238325e + 004	0.9962	0.1-80	196	89	0.04	0.10
Parathion	Y = 7.190335e + 006x − 2.414060e + 003	0.9995	0.1-100	207	92	0.01	0.05
Quinalphos	Y = 6.633667e + 006x − 4.006799e + 003	0.9992	0.1-100	143	93	0.02	0.07
r: Correlation coefficient; LR: Linear range; EF: Enrichment factors; ER: Extraction recovery; LOD: Limit of detection; LOQ: Limit of quantification						

TABLE-2
AVERAGE RECOVERY (n = 5), RSD (n = 5) OF ORGANOPHOSPHORUS PESTICIDES SPIKED RIVER AND LAKE WATER

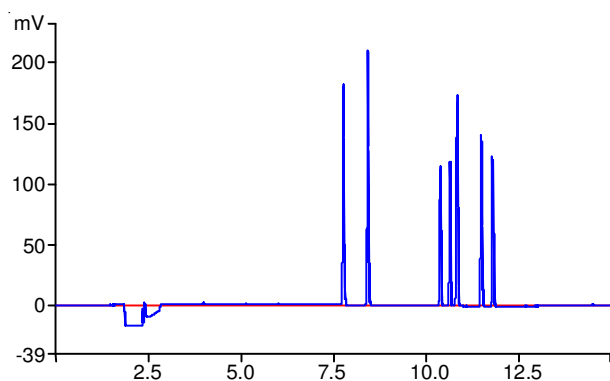| Organophosphorus pesticides	Spiked (µg L⁻¹)	River water			Lake water		
		Found (µg L⁻¹)	Recovery (%)	RSD (%)	Found (µg L⁻¹)	Recovery (%)	RSD (%)
Diazinon	0	ND			ND		
	0.5	0.54	108.0	6.3	0.53	106.0	7.2
	5.0	4.6	92.0	5.6	5.1	102.0	8.0
Disulfoton	0	ND			ND		
	0.5	0.35	70.0	7.5	0.37	74.0	8.9
	5.0	3.6	72.0	6.8	3.7	74.0	7.3
Chlorpyrifos	0	ND			ND		
	0.5	0.46	92.0	5.4	0.48	96.0	6.8
	5.0	4.7	94.0	4.6	4.5	90.0	7.1
Parathion-methyl	0	ND			ND		
	0.5	0.41	82.0	4.2	0.43	86.0	6.3
	5.0	4.2	84.0	3.9	4.1	82.0	7.5
Fenthion	0	ND			ND		
	0.5	0.42	84.0	6.3	0.45	90.0	6.4
	5.0	4.3	86.0	5.1	4.4	88.0	7.6
Parathion	0	ND			ND		
	0.5	0.51	102.0	9.8	0.55	110.0	9.1
	5.0	5.2	104.0	7.9	5.3	106.0	8.6
Quinalphos	0	ND			ND		
	0.5	0.48	96.0	6.9	0.48	96.0	7.3
	5.0	4.7	94.0	7.2	5.1	102.0	8.4

ND: not detected

Fig. 7. Chromatogram of free pesticide river water spiked at a concentration level of 0.05 µg mL⁻¹ of each organophosphorus pesticides. The order is as follows: diazinon (t_R = 7.76), disulfoton (t_R = 8.40), chlorpyrifos (t_R = 10.37), parathion-methyl (t_R = 10.63), fenthion (t_R = 10.84), parathion (t_R = 11.49) and quinalphos (t_R = 11.79)

Compared with the methods previously reported (Table-3), the DLLME process requires less extraction time and the LOD is lower. Consuming less solvent and high enrichyment factors are two significant advantages of DLLME in analyzing trace components.

TABLE-3
COMPARISON OF PROPOSED UA-DLLME-GC METHOD WITH OTHER METHODS OF DETERMINATION OF ORGANOPHOSPHORUS PESTICIDES IN WATER

Methods	Linearity (µg L⁻¹)	Extraction time (min)	LOD (µg L⁻¹)	RSD (%)	Ref.
LPME-GC-FPD	0.10-100	20	0.01-0.04	3.5-8.9	15
SPME-GC-FPD	0.50-100	30	0.05-0.3	< 8	6
SDME-GC-FPD	0.50-50	20	0.21-0.56	1.7-10	5
DLLME-GC-PFPD	0.10-100	1	0.01-0.04	< 9.8	This work

Conclusion

In this work, a simple, rapid and sensitive sample preparation, the UA-DLLME procedures, followed by a GC-PFPD was proposed and evaluated for the analysis of seven organophosphorus pesticides in water samples. The DLLME process makes it possible to selectively determine trace analyses in samples due to good repeatability, recovery and high enrichment. An ultrasound-assisted process was applied to accelerate the formation of a fine cloudy solution and markedly increased extraction efficiency.

REFERENCES

1. A. Beyer and M. Biziuk, *Ecol. Chem. Eng.*, **14**, 35 (2007).
2. M. Jokanovic, *Toxicol. Lett.*, **190**, 107 (2009).
3. The European Drinking Water Directive, 98/83/EEC, European Union, Brussels (1998).
4. E. Ballesteros and M.J. Parrado, *J. Chromatogr. A*, **1029**, 267 (2004).
5. Q. Xiao, B. Hu, C. Yu, L. Xia and Z. Jiang, *Talanta*, **69**, 848 (2006).
6. Z. Yao, G. Jiang, J. Liu and W. Cheng, *Talanta*, **55**, 807 (2001).
7. P. Liang, L. Guo, Y. Liu, S. Liu and T. Zhang, *Microchem. J.*, **80**, 19 (2005).
8. M. Rezaee, Y. Assadi, M.-R. Milani Hosseini, E. Aghaee, F. Ahmadi and S. Berijani, *J. Chromatogr. A*, **1116**, 1 (2006).
9. L. He, X. Luo, H. Xie, C. Wang, X. Jiang and K. Lu, *Anal. Chim. Acta*, **655**, 52 (2009).
10. V. Vickackaite and E. Pusvaskiene, *J. Sep. Sci.*, **32**, 3512 (2009).
11. T. Mao, B. Hao, J. He, W. Li, S. Li and Z. Yu, *J. Sep. Sci.*, **32**, 3029 (2009).
12. M. Moradi, Y. Yamini, A. Esrafili and S. Seidi, *Talanta*, **82**, 1864 (2010).
13. L. Guo and H.K. Lee, *J. Chromatogr. A*, **1235**, 1 (2012).
14. S. Berijani, Y. Assadi, M. Anbia, M.-R. Milani Hosseini and E. Aghaee, *J. Chromatogr. A*, **1123**, 1 (2006).
15. M.R. Khalili-Zanjani, Y. Yamini, N. Yazdanfar and S. Shariati, *Anal. Chim. Acta*, **606**, 202 (2008).