Arabian Journal of Chemistry (2016) 9, 38–42

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Miniaturized counter current liquid–liquid extraction for organophosphorus pesticides determination

Jalal Hassan [a,*], **Masoumeh Sarkouhi** [b]

[a] *Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*
[b] *Department of Chemistry, Faculty of Science, Tarbiat Modares University, Tehran, Iran*

Received 10 October 2012; accepted 5 February 2013
Available online 16 February 2013

KEYWORDS

GC/FID;
MCCLLE;
Organophosphorus pesticide;
Preconcentration

Abstract In the present work, a simple, rapid, sensitive and economical sample pre-treatment technique; miniaturized counter current liquid–liquid extraction was developed for the determination of organophosphorus pesticide (OPP) residue in water samples and compared with conventional dispersive liquid–liquid microextraction. Gas chromatography–flame ionization detector (GC/FID) was used for OPP quantification. Two OPPs (diazinon and malathion) were selected as model compounds and the proposed methods were carried out for their preconcentration from water samples. The presented method was based on dispersive liquid–liquid extraction with methanol containing butyl acetate as a solvent with density lower than water. After phase separation, butyl acetate was injected into the GC/FID instrument. The linearity was obtained in the concentration range of 0.4–1000.0 μg L^{-1} and correlation coefficients were in the range of 0.999–0.997 for diazinon and malathion, respectively. The limits of detection (LODs), was based on signal-to-noise ratio (S/N) of 0.1 μg L^{-1} for two pesticides.

Introduction

Water contamination due to the wide variety of pesticides used in agriculture is a global environmental pollution problem. Organophosphorus pesticides (OPPs) are widely found in water resources. They are released into the environment from manufacturing, transportation, and agricultural applications (Farajzadeh et al., 2009). In environmental samples the analyte is often present in only trace concentrations and the matrix is complicated. In order to reach at sub-μg L^{-1} levels of detection, an efficient extraction and preconcentration technique is required (Zhao et al., 2007). Traditional solvent extraction has been used for many years as the basic, powerful method of concentration. However, it requires large amounts of organic solvents and nowadays attention is focused on techniques that are environmentally friendly and reduce the cost of analysis by reduction or complete elimination of organic solvents (Ojeda and Rojas, 2009; Sarafraz-Yazdi and Amiri, 2010). In

---

* Corresponding author. Tel.: +98 21 61117027; fax: +98 21 66438324.
E-mail addresses: jalalhassan@ut.ac.ir, jalalhassan1355@yahoo.com (J. Hassan).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2013.02.003

recent years, the development of fast, precise, accurate and sensitive methodologies has become an important issue. Liquid phase microextraction (LPME) has been developed as a minimized-solvent-based pretreatment method. (Pusvaskiene et al., 2009; Moinfar and Milani Hosseini, 2009). Homogeneous liquid–liquid extraction (HLLE) and dispersive liquid–liquid microextraction (DLLME) are novel environmentally benign sample-preparation techniques, possessing obvious advantages of simple operation with a high enrichment factor, low cost, and low consumption of organic solvent (Kocurova et al., 2012). In DLLME, the appropriate mixture of extraction and disperser solvents is rapidly injected by a syringe into an aqueous sample containing the analytes of interest. DLLME employs a mixture of a high-density solvent (extractant) and a water miscible, polar solvent (disperser). Acetone, methanol and acetonitrile can be used as dispersers, whereas chlorinated solvents such as chlorobenzene, carbon tetrachloride and tetrachloroethylene are useful as extractants. Other non chlorinated solvents such as undecanol, 1-dodecanol, 2-dodecanol and n-hexadecane can also be used. In practice, a cloudy solution forms, resulting from the formation of fine droplets of the extraction solvent, which disperse in the sample solution and after centrifuging, the fine droplets settle at the bottom of the conical test tube. Sample preparation plays an important role in the field of pesticide residue analysis. In all HLLE and DLLME methods, analyte was extracted from the aqueous phase, but our team introduced a novel miniaturized HLLE for extraction of polycyclic aromatic hydrocarbons and pesticides from the methanolic phase by *n*-hexane as a solvent of lower density than water (Hassan et al., 2010; Shamsipur and Hassan, 2010) called low density miniaturized homogenous liquid–liquid extraction (LDMHLLE). The development of miniaturized methodologies that combines high throughput analysis, low cost, and environmental sustainability, is of great current concern. The aim of this study was to develop a new version of our previous method that we can call it as miniaturized counter current liquid–liquid extraction (MCCLLE) (in this method sample phase and extracting solvent move against each other) for determination of OPPs by GC/FID.

Experimental

Reagents and materials

All chemicals were of reagent grade and were used without further purification. Analytical grade methanol, chloroform, butyl acetate, toluene, benzene, diethyl ether and methyl-t-butyl ether and sodium chloride were purchased from Merck (Darmstadt, Germany). Diazinon, malathion and chlorpyrifos were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solution of pesticides was prepared at a concentration of 1000.0 mg L^{-1} in methanol. The working solutions were prepared at an appropriate concentration from stock solutions and stored at −20 °C. Intermediate stock solution of pesticide was prepared at a concentration of 100.0 mg L^{-1} in methanol and stored in a refrigerator (4 °C) until use. The working solution was prepared by an appropriate dilution of the stock solution with the methanol. Chlorpyrifos was prepared at 50.0 mg L^{-1} in extraction solvent and used as internal standard. Purred water was prepared on a Direct-Q 3 UV with a pump system (Millipore, Molshein, France).

Apparatus

The extracted compounds were analyzed on a 7890 A agilent gas chromatograph coupled to flame ionization detector (Agilent Technologies, Avondale, PA, USA) and 2 μL of the sample was injected into the splitless mode at 250 °C into a 30 m × 0.25 mm × 0.25 μm DB-5 MS capillary column and operated by Chemstation Software (Agilent Technologies). The temperature program used for the chromatographic separation is as follows: 50 °C for 2 min, temperature increase at 25 °C min^{-1} to 100 °C and held for 2 min, and then temperature increase at 20 °C min^{-1} to 270 °C where it was finally held for 4 min. The carrier gas was helium (99.999%) and was kept at a constant flux of 1.0 mL min^{-1}. The FID temperature was maintained at 290 °C and hydrogen gas was generated by a hydrogen generator (Dominick Hunter, United Kingdom) for FID at a flow rate of 30 mL min^{-1}. The flow rate of zero air (99.999%, Air Products, UK) for FID was 400 mL min^{-1}.

Counter current liquid–liquid extraction procedure

500.0 μL of methanol containing 100.0 μL of butyl acetate was placed in a 10 mL dry narrow neck volumetric flask. By adding 10.0 mL of aqueous sample into volumetric flask, butyl acetate was separated at the top of volumetric flask and was drawn out by a Hamilton syringe and transferred to a conical vial and 2.0 μL of butyl acetate was injected into GC/FID for quantitative analysis.

Dispersive liquid–liquid microextraction procedure

For the DLLME, a 10.00-mL aliquot of aqueous sample was placed in a 10-mL screw-cap glass tube with conical bottom. 500.0 μL of methanol containing 100.0 μL $CHCl_3$ was injected rapidly into the sample solution by 1.00-mL syringe and then the solution is vortexed for 5 s. A cloudy solution which consisted of very fine droplets of $CHCl_3$ that dispersed into aqueous sample was formed, and the analytes were extracted into the fine droplets. After centrifugation at 3500 rpm for 5 min, the $CHCl_3$ phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was completely transferred to another test tube with conical bottom using 100 μL syringe and 2.0 μL of the $CHCl_3$ phase injected into the GC system for analysis.

Results and discussion

The theory of DLLME and MCCLLE is similar to that of LLE. Thus, the equations that describe the effects of several parameters on the efficiency of the proposed method are similar to those of LLE. The partition coefficient (K) is defined as the ratio of the analyte concentration in the extracting phase ($C_{\mathrm{eq}}^{\mathrm{ex}}$) and the final concentration of the analyte in the source sample solution ($C_{\mathrm{eq}}^{\mathrm{sam}}$):

$$K = \frac{C_{\mathrm{eq}}^{\mathrm{ex}}}{C_{\mathrm{eq}}^{\mathrm{sam}}} \tag{1}$$

$$E_r = \frac{n_{\mathrm{ex}}}{n_{\mathrm{ini}}} = \frac{C_{\mathrm{eq}}^{\mathrm{ex}} V_{\mathrm{ex}}}{C_{\mathrm{ini}}^{\mathrm{sam}} V_{\mathrm{sam}}} \tag{2}$$

$$P_f = \frac{C_{\mathrm{eq}}^{\mathrm{ex}}}{C_{\mathrm{ini}}^{\mathrm{sam}}} \tag{3}$$

where, C_{eq}^{ex} is the final concentration of analyte in the extracting phase, C_{eq}^{sam} and C_{ini}^{sam} are the final and initial analyte concentrations in the source phase, respectively. n_{ex} is the moles of the analyte extracted into the extracting phase and n_{ini} is the total moles of the analyte, V_{ex} and V_{sam} are the final (separated) volume of the extracting phase and initial volume of sample, respectively, E_r is the extraction recovery and P_f is the preconcentration factor.

Type of co-solvent extraction solvent

For the MCCLLE method, the co-solvent should be miscible with the extracting phase as well as sample. The following parameters will influence the efficiency of the MCCLLE: kind of extraction and co-solvent, volume of extraction solvent and co-solvent and salt effect. It is important to note that after addition of water to acetonitrile, methanol, and acetone as co-solvent, butyl acetate cannot form a distinct water immiscible phase with acetonitrile or acetone and only methanol can form a distinct water immiscible phase with butyl acetate. Thus, methanol was selected as the co-solvent.

The criteria of solvent selection in this technique include lower density than water, high enrichment factor, low water solubility and environmentally friendly. For these investigations, butyl acetate, toluene, benzene, diethyl ether and methyl-t-butyl ether were examined as extraction solvents. The experimental results revealed that, among the solvents tested, only butyl acetate can be quickly and completely separated, while other solvents tested cannot be separated from methanol after water addition. Thus, butyl acetate was selected as the extraction solvent.

Effect of volume of extracting solvent

Some primary experiments showed that the best phase separation can be achieved at methanol:water volume ratio of 6:4 and for methanol:water volume ratio of greater than 6:4, butyl acetate cannot be separated, while methanol:water volume ratios in the range of 1:20–6:4 have no effect on volume of separated butyl acetate; however, the highest sensitivity of the method was achieved at a methanol:water volume ratio of 1:20. (It is important to note that methanol contains 100 μL of butyl acetate in all experiments). The peak area (concentration) of the OPPs decreased with increasing volume of extracting solvent and fraction of total analyte transferred to the organic phase (extraction efficiency) increased with increasing volume of extracting solvent.

Salt effect

The effect of salt addition on extraction efficiency was examined by adding a different amount of NaCl (0–10%, w/v) under other constant experimental conditions. By increasing the ionic strength from 0 to 10%, the volume of the separated phase increased due to the decrease in aqueous solubility of the extraction solvent in the presence of salt, which consequently resulted in the decrease of concentration of OPPs. Therefore, all the extraction experiments were carried out without addition of salt.

Time of extraction

In this research, a series of extraction times ranging from 0 to 10 min were studied with other experimental conditions remaining constant. The results showed that the variations of peak area versus extraction time were not remarkable. This is the remarkable advantage of this technique over other extraction techniques, such as single-drop microextraction solid phase microextraction, stirbar adsorption extraction.

Analytical performance

In order to validate the developed methods, linearity, correlation coefficient, detection limits, were tested using spiked

Table 1 Limit of detections, regression equations, correlation coefficients and dynamic linear ranges, for DLLME and MCCLLE.

Method	Pesticide	P_f	R^2	DLR (μg L^{-1})	LOD (μg L^{-1})
DLLME	Diazinon	202	0.9999	0.5–1000.0	0.20
	Malathion	204	0.9965	0.5–1000.0	0.15
MCCLLE	Diazinon	213	0.9992	0.40–1000.0	0.13
	Malathion	221	0.9973	0.40–1000.0	0.12

P_f: Preconcentration factor; R^2: Correlation coefficient; DLR: Dynamic linear range; LOD: Limit of detection; DLLME: Dispersive liquid–liquid microextraction; MCCLLE: Miniaturized counter current liquid–liquid extraction.

Table 2 Recovery for OPPs from water samples at different concentration levels.

Samples	Recovery (±RSD)			
	DLLME		MCCLLE	
Tap water	Diazinon	Malathion	Diazinon	Malathion
	<LOD	<LOD	<LOD	<LOD
Spiked tap water at 1.0 μg L^{-1}	110 (±10)	110 (±8)	105 (±9)	110 (±15)
Spiked tap water at 5.0 μg L^{-1}	96 (±11)	98 (±7)	98 (±8)	96 (±9)
Spiked tap water at 10.0 μg L^{-1}	102 (±11)	99 (±13)	98 (±5)	101 (±6)
Spiked tap water at 20.0 μg L^{-1}	106 (±8)	98 (±7)	99 (±8)	106 (±11)
Karoon river	<LOD	<LOD	<LOD	<LOD
Spiked Karoon river at 5.0 μg L^{-1}	96 (±8)	110 (±3)	105 (±4)	110 (±7)

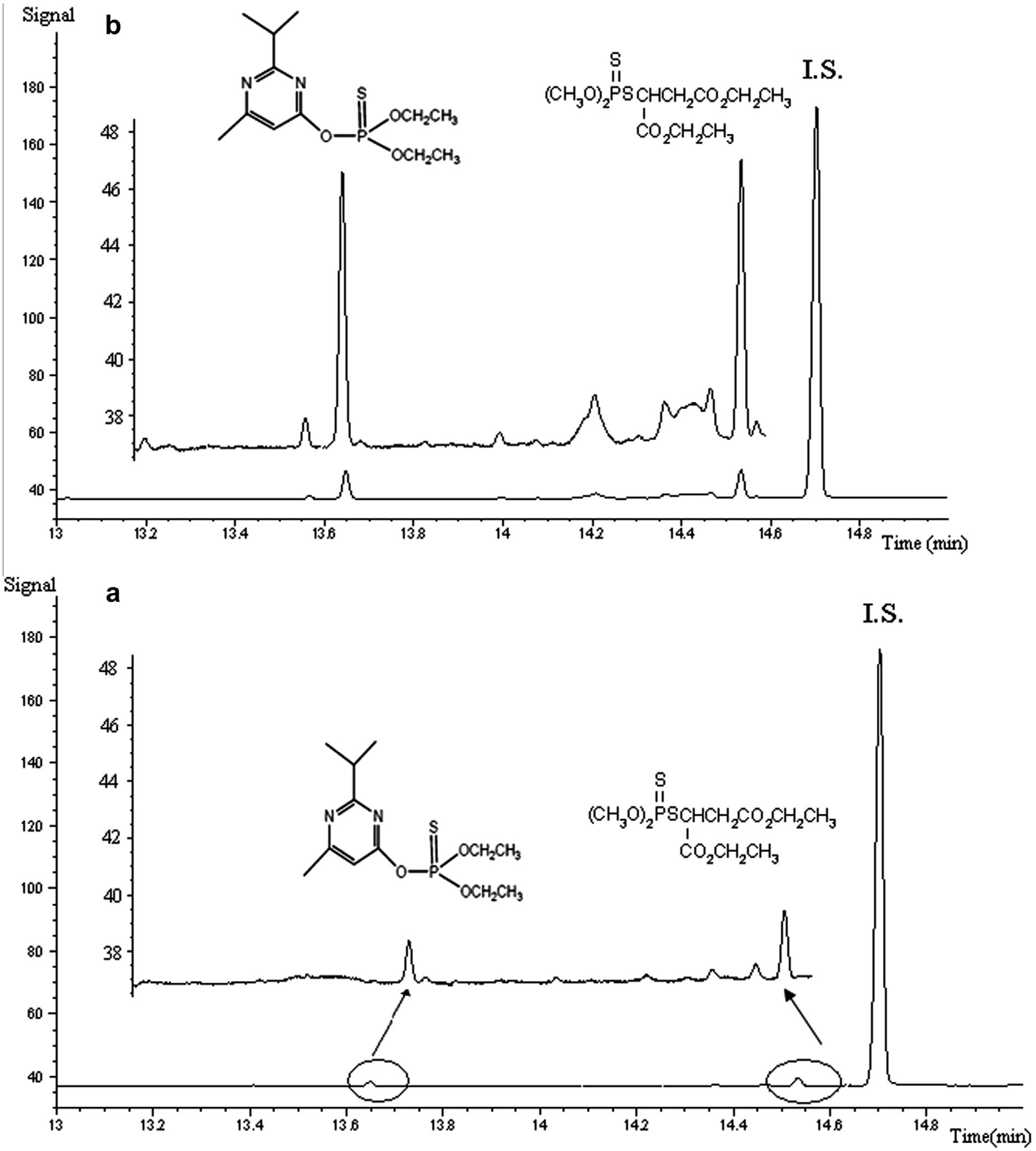

Figure 1 Chromatogram of of OPPs for a tap water spiked at 1.0 μg L^{-1} (a) and 10.0 μg L^{-1} (b) by MCCLLE (I.S. = internal standard). Conditions: sample volume 10.0 mL, 500 μL of methanol containing 100 μL butyl acetate.

samples. Good linear relationships were obtained over the concentration with the correlation coefficient (R^2) > 0.99 for two methods (Table 1). As can be seen, according to obtained results there is no significant difference between MCCLLE and DLLME. The proposed MCCLLE and DLLME were applied to the determination of trace amounts of OPPs in water samples, using the internal calibration method. To evaluate the applicability and accuracy of the proposed method in real samples, two kinds of water samples Karoon River (Ahvaz), and tap water (Tehran) collected and were analyzed using the proposed methods. Tap water was spiked with 1.0, 5.0, 10.0 and 20 μg L^{-1} and river water spiked at 5.0 of diazinon and malathion, respectively in order to assess possible matrix effects for MCCLLE. The relative recoveries of diazinon and malathion from tap water were in the range of 96–110, and from river water were in the range of 105–110, respectively. Each treatment was in triplicate, and the results are shown in Table 2. Fig. 1 shows the chromatogram obtained by MCCLLE-GC/FID for a water sample when (b) spiked with diazinon and malathion at 1.0 μg L^{-1} (a) and 10 μg L^{-1} (b) level.

Conclusions

Compared to the existing extraction methods, the proposed MCCLLE method possesses several advantages with respect to DLLME including: simplicity, rapidity, and no need to centrifuging for phase separation, its requirement of only small volumes, use of solvent with density lower than water and ability for coupling with chromatographic methods. Moreover, since the addition of NaCl was found to have no measurable effect on the extraction efficiency, the method can be safely applied to complicated matrices.

References

Farajzadeh, M.A., Seyedi, S.E., Shalamzari, M.S., 2009. Dispersive liquid–liquid microextraction using extraction solvent lighter than water. J. Sep. Sci. 32, 3191–3200.

Hassan, J., Farahani, A., Shamsipur, M., Damerchili, F., 2010. Rapid and simple low density miniaturized homogeneous liquid–liquid extraction and gas chromatography/mass spectrometric determination of pesticide residues in sediment. J. Hazard. Mater. 184, 869–871.

Kocurova, L., Balogh, I.S., Sandrejova, J., Andruch, V., 2012. Recent advances in dispersive liquid–liquid microextraction using organic solvents lighter than water: a review. Microchem. J. 102, 11–17.

Moinfar, S., Milani Hosseini, M.R., 2009. Development of dispersive liquid–liquid microextraction method for the analysis of organophosphorus pesticides in tea. J. Hazard. Mater. 169, 907–911.

Ojeda, C.B., Rojas, F.S., 2009. Separation and preconcentration by dispersive liquid–liquid microextraction procedure: a review. Chromatographia 69, 1–11.

Pusvaskiene, E., Januskevic, B., Prichodko, A., Vickackaite, V., 2009. Simultaneous derivatization and dispersive liquid–liquid microextraction for fatty acid GC determination in water. Chromatographia 69, 271–276.

Sarafraz-Yazdi, A., Amiri, A., 2010. Liquid-phase microextraction. Trends Anal. Chem. 29, 1–14.

Shamsipur, M., Hassan, J., 2010. A novel miniaturized homogenous liquid–liquid solvent extraction-high performance liquid chromatographic-fluorescence method for determination of ultra traces of polycyclic aromatic hydrocarbons in sediment samples. J. Chromatogr. A 1217, 4877–4882.

Zhao, E., Zhao, W., Han, L., Jiang, J., Zhou, Z., 2007. Application of dispersive liquid–liquid microextraction for the analysis of organophosphorus pesticides in watermelon and cucumber. J. Chromatogr. A 1175, 137–140.