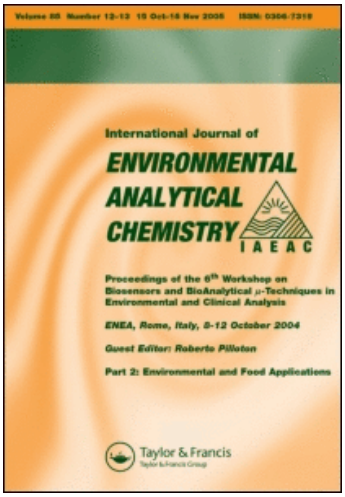

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Application of dispersive liquid-liquid microextraction and reversed phase-high performance liquid chromatography for the determination of two fungicides in environmental water samples

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Online publication date: 11 August 2010

To cite this Article Cheng, Jing , Zhou, Yiwen , Zuo, Mei , Dai, Liping and Guo, Xiaojie(2010) 'Application of dispersive liquid-liquid microextraction and reversed phase-high performance liquid chromatography for the determination of two fungicides in environmental water samples', International Journal of Environmental Analytical Chemistry, 90: 11, 845 — 855

To link to this Article: DOI: 10.1080/03067310903180468

URL: http://dx.doi.org/10.1080/03067310903180468

Intern. J. Environ. Anal. Chem.
Vol. 90, No. 11, 15 September 2010, 845–855

Application of dispersive liquid–liquid microextraction and reversed phase-high performance liquid chromatography for the determination of two fungicides in environmental water samples

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(*Received 8 November 2008; final version received 11 July 2009*)

Dispersive liquid–liquid microextraction (DLLME) has been developed for the extraction and preconcentration of diethofencarb (DF) and pyrimethanil (PM) in environmental water. In the method, a suitable mixture of extraction solvent (50 μL carbon tetrachloride) and dispersive solvent (0.75 mL acetonitrile) are injected into the aqueous samples (5.00 mL) and the cloudy solution is observed. After centrifugation, the enriched analytes in the sediment phase were determined by HPLC-VWD. Different influencing factors, such as the kind and volume of extraction and dispersive solvent, extraction time and salt effect were investigated. Under the optimum conditions, the enrichment factors for DF and PM were both 108 and the limit of detection were 0.021 ng mL^{-1} and 0.015 ng mL^{-1}, respectively. The linear ranges were 0.08–400 ng mL^{-1} for DF and 0.04–200 ng mL^{-1} for PM. The relative standard deviation (RSDs) were both almost at 6.0% ($n = 6$). The relative recoveries from samples of environmental water were from the range of 87.0 to 107.2%. Compared with other methods, DLLME is a very simple, rapid, sensitive (low limit of detection) and economical (only 5 mL volume of sample) method.

Keywords: dispersive liquid–liquid microextraction (DLLME); fungicides; reversed phase-high performance liquid chromatography; environmental water samples

1. Introduction

Pesticides such as fungicides, herbicides and insecticides are widely used for the control of plant diseases and weeds [1]. They impair the terrestrial and aquatic lives owing to their great consumption in agricultural areas. They have entered into the environment by all kinds of routes such as spraying, soil and storage, as well as the discharge of wastewater. Moreover, the application of these compounds and their residues has the possibility of contaminating ground and surface waters, and posing a consequent potential danger to the environment and public health [2]. In order to guarantee public drinking water quality, the European Union Directive on drinking water quality (98/83/EC) established a maximum allowed concentration of 0.1 μg L^{-1} for individual pesticides and of 0.5 μg L^{-1} for total pesticides in drinking water [3]. Therefore, an accurate and efficient monitoring of these pesticides and their residues in environmental water is necessary.

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ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310903180468
http://www.informaworld.com

Diethofencarb [Propan-2-yl N-(3,4-diethoxyphenyl)carbamate (Figure 1a) and Pyrimethanil [4,6-dimethyl-n-phenyl-2-pyrimidinamine (Figure 1b) are widely used fungicides for the control of grape and tomato diseases [4–6]. Discharge of water used to wash grapes and tomatoes often results in contamination of the environmental water. The residue analysis of the environmental water can be challenged because of the trace level of analytes. Therefore, the search for a simple and sensitive method for analysing the two trace fungicides in the environmental water is extremely urgent.

Analytical techniques used in determination of pesticides are mainly high-performance liquid chromatography (HPLC) or gas chromatography (GC) coupled to selective detection systems, such as electron-capture detection (ECD), nitrogen-phosphorous detection (NPD) or mass spectrometric detection (MS) [7–12]. However, the residues of the title fungicides cannot be analysed in water matrices without some preliminary sample preparation which is one of the most important steps in the analytical process. Traditionally, liquid–liquid extraction (LLE) has been employed. However, LLE is time-consuming and involves large volumes of toxic organic solvents. In order to overcome these problems, various extraction techniques have been developed. Among these techniques, solid-phase extraction (SPE) and solid-phase microextraction (SPME) are often used. However, SPME is expensive, its fibre is fragile and has limited lifetime, and sample carry-over could be a problem [13].

Recent research has been oriented towards the development of efficient, economical and miniaturised sample preparation methods. Consequently, dispersive liquid-liquid microextraction (DLLME) has been developed by Assadi and his co-workers. The advantages of the DLLME method are simplicity of operation, rapidity, low cost, high recovery and enrichment factors. In this method, the appropriate mixture of extraction solvent and dispersive solvent is injected into aqueous sample rapidly by syringe and a cloudy solution is formed. The analytes in the sample are extracted into the fine droplets of extraction solvent. After extraction, phase separation is performed by centrifugation and

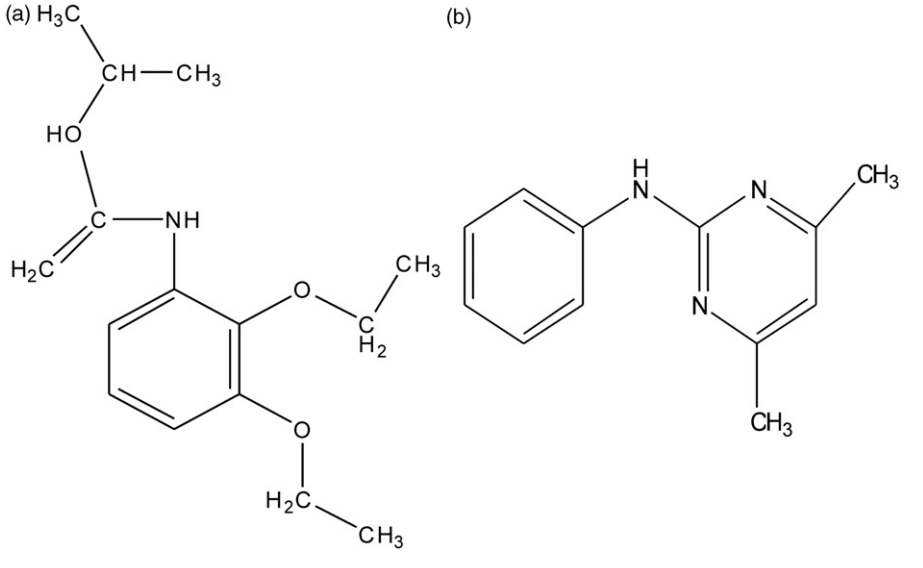

Figure 1. (a) diethofencarb [Propan-2-yl N-(3,4-diethoxyphenyl) carbamate]. (b) pyrimethanil [4,6-dimethyl-n-phenyl-2-pyrimidinamine].

the enriched analytes in the sediment phase are determined by chromatographic or spectrometric methods. This method has been applied for the determination of trace organic pollutants and metal ions in the environmental samples [14–20]. The method is also used for the determination of antioxidants, polycyclic aromatic hydrocarbons (PAHs), chlorophenols (CPs), organophosphorus pesticides (OPPs), chlorobenzenes (CBs), trihalomethanes (THMs), phthalate esters (PEs), polybrominated diphenyl ethers (PBDEs) in water samples [21–28].

The purpose of this work was to employ DLLME as an extraction and enrichment technique coupled to HPLC-VWD to determine the two representative fungicides in environmental water samples. The effects of different experimental conditions on extraction of the two fungicides were investigated. The results show that DLLME is a simple, rapid and sensitive method that can be used in monitoring residues of fungicides in environmental water samples.

2. Experimental

2.1 *Reagents and standards*

Diethofencarb (98.0%) and pyrimethanil (98.0%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All of the reagents used as extraction solvent in this experiment (dichloroethane, dichloromethane, carbon tetrachloride and chlorobenzene) were of analytical grade and were redistilled at least three times. Acetonitrile, methanol, acetone and ethanol as dispersive solvents (HPLC/SPECTRO) were obtained from Tedia (Fair Lawn, New Jersey, USA). Deionised water was purified with a Millipore Mill-Q plus System (Bedford, MA, USA). Natural water from East Lake (Wuhan, PR China) and South Lake (Wuhan, PR China) and tap water from our laboratory were collected for the work. All the solvents and water samples were filtered through a 0.45 μm membrane to eliminate particulate matter before analysis. Stock solutions were prepared by dissolving 0.1 g regents in methanol, and diluting to 100 mL, the standard working solutions were daily prepared by dilution of stock standard solution with distilling water to the required concentrations.

2.2 *Instrumentation*

An Agilent 1100 HPLC equipped with a manual injection and variable wavelength detector (VWD) were used to determine the amount of the residue of the two fungicides in water samples. The analytes were separated on a Zorbax Eclipse XDB-C_8 column (150 mm × 4.6 mm, 5 μm particle size). All injections were performed manually with 20.0 μL sample loop. The mobile phase was a methanol/water (75 : 25, v/v) and the flow rate was 0.6 mL min^{-1}. The detection wavelength was 254 nm and the column temperature was 40°C. An 80-2 centrifuge (Changzhou Guohua Electric Appliance Co.Ltd, PR China) was used for centrifuging. Screw-cap glass test tubes with conical bottoms (used as extraction vessels) were heated at 500°C in a furnace (carbolite, UK; model CWF 1200) to remove organic compounds.

2.3 *Dispersive liquid-liquid microextraction procedure*

DLLME was performed as follows: 5.00 mL of aqueous sample containing diethofencarb and pyrimethanil were placed into a 10 mL screw-cap glass test tube with conical bottom.

A mixture of acetonitrile (0.75 mL, as dispersive solvent) and carbon tetrachloride (50.0 μL, as extraction solvent) was rapidly injected into the above mentioned aqueous sample by a 1.00 mL syringe. After gentle shaking, a milky cloudy solution (water/acetonitrile/carbon tetrachloride) was formed in the test tube (the cloudy state was stable at least for 1 hr). In this step, the analytes in the aqueous sample were extracted into the fine droplets of carbon tetrachloride. The mixture was centrifuged for 5.0 min at 3000 rpm. The dispersed fine particles of extraction phase were sedimented in the bottom of conical test tube. The sediment phase (20.0 μL) was removed using a 25 μL microsyringe and injected into HPLC for analysis.

3. Results and discussion

3.1 *Optimisation of DLLME*

In DLLME, enrichment factor (EF) and extraction recovery (ER) were used to evaluate the extraction efficiency in order to obtain the optimised extraction conditions. The enrichment factor was defined as the ratio between the analytes' concentration in the sediment phase (C_{sed}) and the initial concentration of analytes (C_0) within the sample:

$$EF = \frac{C_{sed}}{C_0} \tag{1}$$

The C_{sed} was acquired from the calibration graph of direct injection of diethofencarb and pyrimethanil standard solution in the extraction solvent.

The extraction recovery was defined as the percentage of the total analytes' amount (n_0) which was extracted to the sediment phase (n_{sed}).

$$ER = \frac{n_{sed}}{n_0} \times 100 = \frac{C_{sed} \times V_{sed}}{C_0 \times V_{aq}} \times 100 \tag{2}$$

where V_{sed} and V_{aq} are the volumes of sediment phase and sample solution, respectively.

3.1.1 *Selection of extraction solvent*

The selection of an appropriate extraction solvent is very important for the DLLME process. The selection of extraction solvent must obey the following principles: it has higher density than water; good chromatographic behaviour; extraction capability of interested compounds; low solubility in water; and can form a two phase system (cloudy solution with very fine droplets in the presence of a dispersive solvents with very fine droplets) when injected to an aqueous solution. Among the solvents with density higher than water (mainly chlorinated solvents), CH_2Cl_2 (1.32 g mL^{-1}), CH_3CHCl_2 (1.25 g mL^{-1}), CCl_4 (1.59 g mL^{-1}) and C_6H_5Cl (1.10 g mL^{-1}) were studied. On the other hand, the selection of a dispersive solvent is limited to solvents dissolved in both water and extraction solvents, such as methanol, acetonitrile, ethanol and acetone. In this study, CH_2Cl_2, CH_3CHCl_2, CCl_4 and C_6H_5Cl (50 μL) as extraction solvents and methanol, acetonitrile, ethanol and acetone (1.0 mL) as dispersive solvents were taken into consideration. For CH_2Cl_2 and C_6H_5Cl, their chromatographic peak cannot be separated from the analytes' peaks. So it is not necessary to investigate them as extraction solvents for the four dispersive solvents. While with CH_3CHCl_2 as an extraction solvent, the sedimented phase removed to the HPLC has much more consumption volume, and CCl_4

was found to give the better extraction efficiency for both of the analytes studied. Hence CCl_4 was selected as the extraction solvent for subsequent experiments.

3.1.2 *Selection of dispersive solvent*

For the DLLME method, the selection of dispersive solvents should obey the following principles: they must be miscible with both water and the extraction solvents and have good chromatography behaviour; the analytes must be dissolved in the dispersive solvents. Therefore, methanol, acetonitrile, ethanol and acetone were tested as dispersive solvents and the effect of these solvents on the performance of DLLME was investigated. The chromatographic peak of acetone cannot be separated from the peak of the analytes completely, so acetone was not chosen as a dispersive solvent. In order to maintain the identical volume of sendimented phase removed to HPLC for analysis, the kind of dispersive solvent and the volume of CCl_4 must be changed simultaneously. The experiments were performed using 1.0 mL of ethanol, methanol and acetonitrile containing 36.0, 42.0, 42.0 and 50.0 μL of CCl_4, respectively. Under these conditions, the volume of the sedimented phase was identical (25 ± 1 μL). The effect of different dispersive solvents on the EF of diethofencarb and pyrimethanil are shown in Figure 2. It was shown that EF for all analytes are the highest using acetonitrile as the dispersive solvent, so acetonitrile was selected as a dispersive solvent in this work.

3.1.3 *Effect of extraction solvent volume*

In order to study the effect of extraction solvent volume on the extraction efficiency, different volumes of CCl_4 (40.0–90.0 μL at 10 μL interval) and a constant volume of

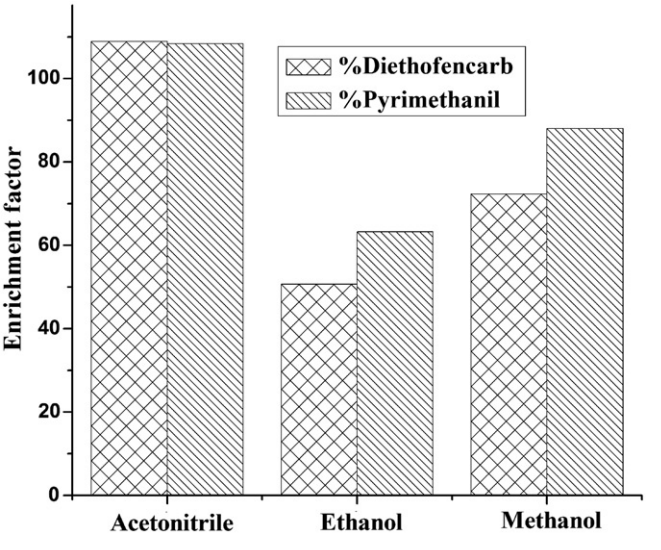

Figure 2. Effect of different dispersive solvent on the enrichment factor of diethofencarb and pyrimethanil. Extraction conditions: sample volume, 5.0 mL; dispersive solvent volume, 1.0 mL; extraction solvent, CCl_4; concentration of diethofencarb and pyrimethanil: 20 ng mL^{-1}, 10 ng mL^{-1}; room temperature.

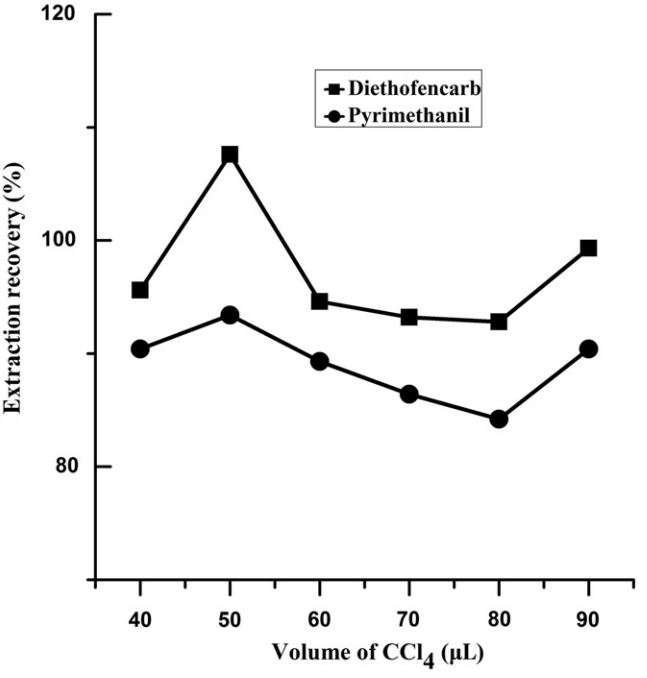

Figure 3. Effect of the volume of extraction solvent (CCl_4) on the enrichment factor of diethofencarb and pyrimethanil. Extraction conditions: as in Figure 2; dispersive solvent (acetonitrile) volume: 1.0 mL.

dispersive solvent (acetonitrile, 1.0 mL) were tested. Figures 3 and 4 show the EF and ER curves of volume of CCl_4. Thereby, 50.0 μL CCl_4 was used as the extraction solvent in subsequent experiments.

3.1.4 *Effect of dispersive solvent volume*

The effect of dispersive solvent volume on the extraction efficiency was studied by adding different volumes of acetonitrile into the sample solution. Figure 5 shows the results that the EF increased with the increase of the volume of acetonitrile when it is less than 0.75 mL and reduction was observed after the volume exceeds 0.75 mL. The reason for this is that acetonitrile cannot disperse CCl_4 effectively at low volume, therefore the cloudy solution is not completely formed. With the increase in the volume of acetonitrile, the amount of CCl_4 disolved in the high volume of acetonitrile increases the optimal volume of acetonitrile in this experiment. So the optimal volume of acetonitrile was chosen as 0.75 mL.

3.1.5 *Effect of extraction time and centrifuging time*

In DLLME, extraction time is defined as interval time between injecting the mixture of dispersive solvent (acetonitrile) and extraction solvent (CCl_4) in aqueous samples, and before centrifuging. To investigate the effect of extraction time, the extraction time of 0, 5, 10, 15 and 25 min were studied. Figure 6 shows that the features of the variations of EF versus extraction time are not remarkable. It reveals that the DLLME method is time-independent. It is the reason that the transition of analytes from aqueous phase to

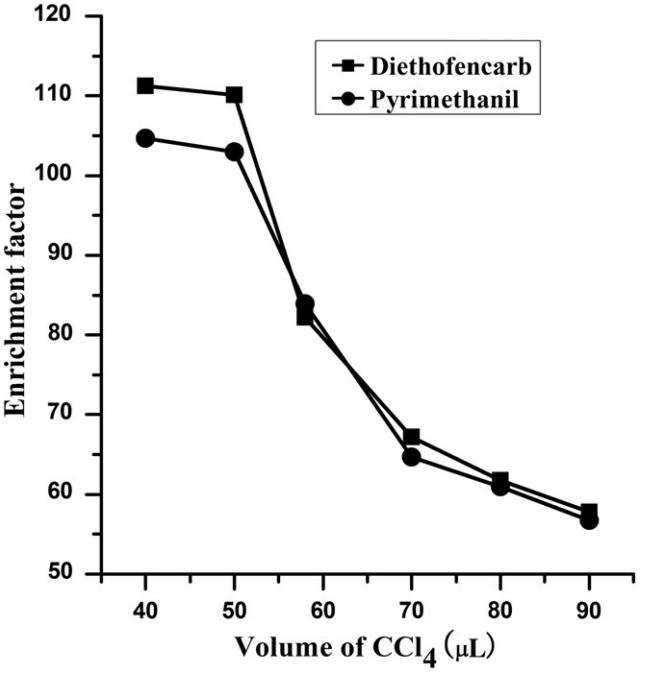

Figure 4. Effect of the volume of extraction solvent (CCl_4) on the extraction recovery of diethofencarb and pyrimethanil. Extraction conditions: as in Figure 3.

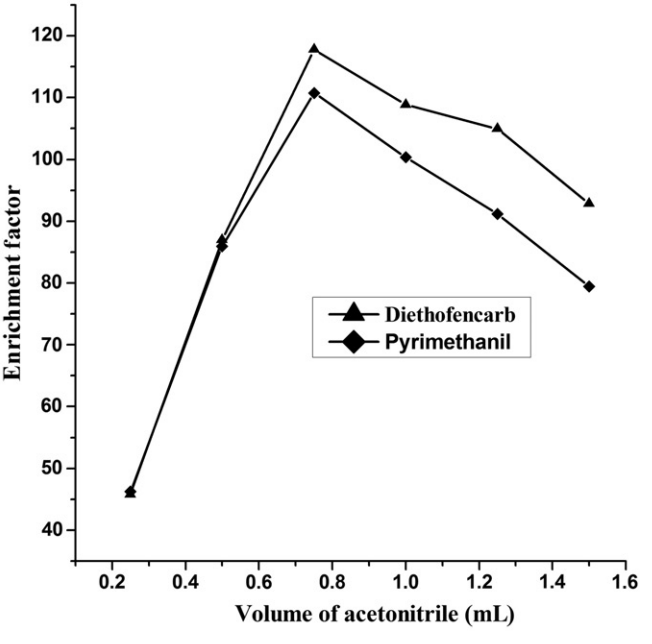

Figure 5. Effect of the volume of dispersive solvent (acetonitrile) on the enrichment factor of diethofencarb and pyrimethanil. Extraction conditions: as in Figure 4; extraction solvent (CCl_4) volume: 50.0 μL.

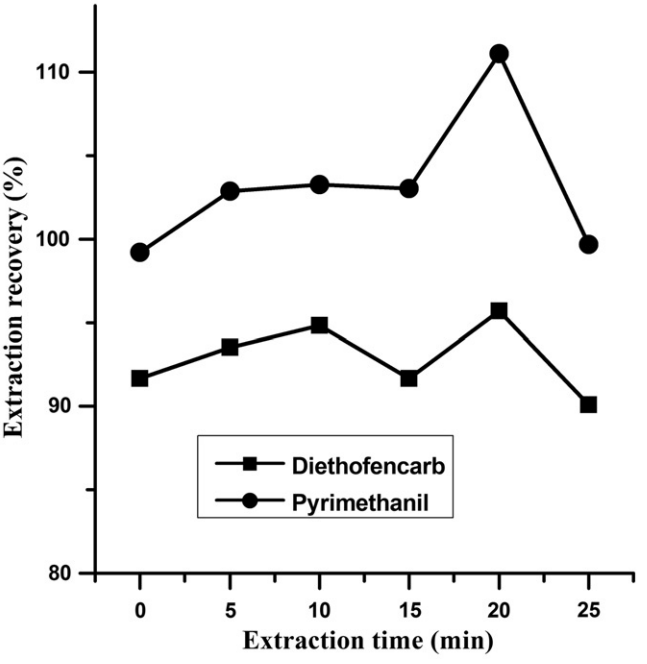

Figure 6. Effect of extraction time on the enrichment factor of diethofencarb and pyrimethanil. Extraction conditions: as in Figure 5; dispersive solvent (acetonitrile) volume: 0.75 mL.

extraction solvents is very fast due to the large contact surface area between the extraction solvent and the aqueous phase during the formation of cloudy solution. Therefore, the equilibrium state is achieved quickly. This is the most important advantage of the DLLME technique.

The centrifuging time was also studied from 1 min to 30 min with the rotation speed of 3000 rpm. Figure 7 shows that the EF for analytes reached the maximum at 5 min and remained unchanged with a further increase of the centrifuging time to 30 min. So the extraction time and centrifuging time for subsequent analysis were fixed at 0 min and 5 min, respectively.

3.1.6 *Effect of ionic strength*

Various experiments were carried out by adding different amounts of NaCl (0–5% (w/v)) to investigate the effect of ionic strength on the extraction efficiency of DLLME. It was found that the features of the variations of EF versus the amount of NaCl from 0 to 5%(w/v) are not remarkable. The ER decreases by increasing the amount of NaCl from 0 to 5% because of decreasing the solubility of extraction solvent in the aqueous phase. As a result, all the extraction experiments were carried out without the addition of salt.

3.2 *Evaluation of the DLLME method*

Chromatograms obtained for a real sample (lake water) before and after DLLME extraction under the optimal conditions are shown in Figure 8.

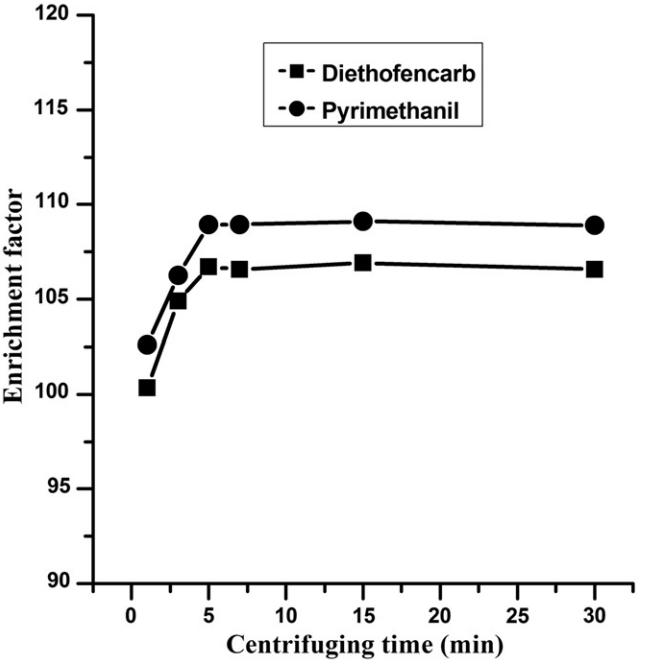

Figure 7. Effect of centrifuging time on the enrichment factor of diethofencarb and pyrimethanil. Extraction conditions: as in Figure 6; extraction time: 0 min.

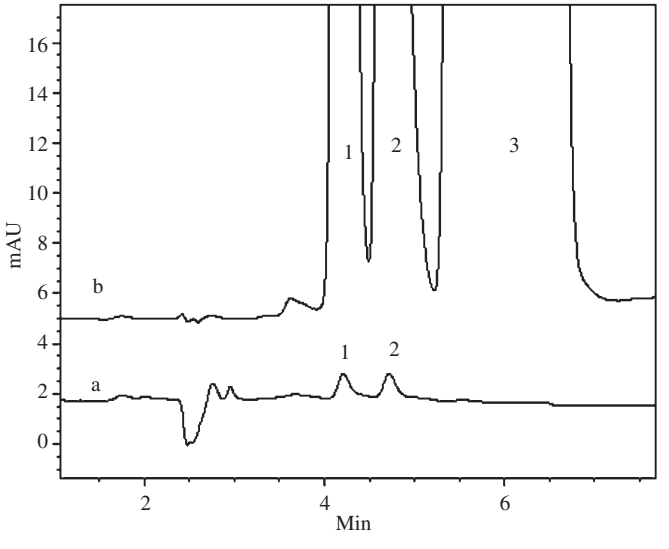

Figure 8. Chromatograms obtained for the spiked lake water sample before (a) and after (b) DLLME extraction under the optimal conditions. Peak: 1 – Diethofencarb, 2 – Pyrimethanil, 3 – Extraction solvent. Extraction conditions: as in Figure 7; centrifuging time: 5 min.

Table 1. Features of the DLLME method.

Compound	Linear range ($ng\,mL^{-1}$)	R	LOD $ng\,mL^{-1}$	R.S.D. (%)	EF
Diethofencarb	0.08–400	1	0.021	5.2	108.8
Pyrimethanil	0.04–200	0.9993	0.015	6.4	108.4

Table 2. Analytical results in real water samples.

Sample	Compound	Found ($ng\,mL^{-1}$)	Add ($ng\,mL^{-1}$)	Recovery (%)
Tap water	*Diethofencarb*	nd	8	107.2%
	Pyrimethanil	nd	4	93.1%
East lake water	*Diethofencarb*	1.7	8	87.0%
	Pyrimethanil	nd	4	86.8%
South lake water	*Diethofencarb*	8.5	8	90.1%
	Pyrimethanil	10.7	4	99.7%

nd: not detected.

3.2.1 *Feature of the method*

Table 1 shows linear range, precision (RSD), enrichment factor, and limits of detector (LODs) of the DLLME method for all target compounds. As can be seen, good linearities were observed for the target analytes, with the correlation coefficient r ranging from 0.9993 to 1.0000. LODs are obtained at the lowest detectable concentration causing the signal-to-noise ratio (S/N) of 3. The precisions of this method, determined by consecutively extracting aqueous samples six times (spiked at $C_{DF}:C_{PM} = 4\,ng\,mL^{-1}$: $2\,ng\,mL^{-1}$), varied from 5.2 to 6.4%.

3.2.2 *Real water samples analysis*

East Lake water (Wuhan, China), South Lake water (Wuhan, China) and tap water from our laboratory were analysed using the proposed method. Recovery testing was carried out with $8\,ng\,mL^{-1}$ and $4\,ng\,mL^{-1}$analytes, respectively, spiked in the water samples. The results are given in Table 2. As can be seen, recoveries were from 86.8% to 107.2%. It indicates the feasibility of the DLLME method for determining the two fungicides simultaneously in water samples.

4. Conclusion

The application of the DLLME-HPLC-VWD method for the simultaneous determination of the two fungicides has been evaluated in this study. The optimal conditions of extraction techniques have been obtained. The established method can be applied to determine the concentration of the two fungicides in real water samples. The recoveries of the two fungicides studied in water are from 86.8% to 107.2%. Adequate repeatability, good linearity and the low detection limits demonstrated that the method is sensitive and accurate for quantitative analysis of the two fungicides in real water samples and can be used in conventional analysis.

Acknowledgements

The authors want to thank the Wuhan's Science and Technology programme (No 200760423155) for providing financial support to this study.

References

[1] O. Lode, O.M. Eklo, B. Holen, A. Svensen, and Å.M. Johnsen, Sci. Total Environ. **160/161**, 421 (1995).
[2] E. Ballesteros and M.J. Parrado, J. Chromatogr. A **1029**, 267 (2004).
[3] EU Council Directive on the Quality of Water Intended for Human Consumption, 98/83/CE, 1998.
[4] L.M. Ravelo-Pérez and J. Hernández-Borges, J. Chromatogr. A **1185**, 151 (2008).
[5] D.T. Likas, N.G. Tsiropaulos, and G.E. Miliadis, J. Chromatogr. A **115**, 208 (2007).
[6] S. de Melo Abreu, P. Caboni, P. Cabras, A. Alves, and V.L. Garau, J. Chromatogr. A **1103**, 362 (2006).
[7] C. Coscollà, V. Yusà, P. Martí, and A. Pastor, J. Chromatogr. A. **1200**, 100 (2008).
[8] Q.X. Zhou, J.P. Xiao, and Y.J. Ding, Anal. Chim. Acta **602**, 223 (2007).
[9] S. Polati, M. Bottaro, P. Frascarolo, F. Gosetti, V. Gianotti, and M.C. Gennaro, Anal. Chim. Acta **579**, 146 (2006).
[10] D. Liu, P. Wang, W. Zhou, X. Gu, and Z. Chen, Anal. Chim. Acta **555**, 210 (2006).
[11] S. Agarwal, S.G. Aggarual, and P. Singh, Talanta **65**, 104 (2005).
[12] H.-J. Pan and W.-H. Ho, Anal. Chim. Acta **527**, 61 (2004).
[13] A. Tor and M.E. Aydin, Anal. Chim. Acta **575**, 138 (2006).
[14] F. Ahmadi, Y. Assadi, S.M.R. Milani Hosseini, and M. Rezee, J. Chromatogr. A. **1101**, 307 (2006).
[15] R.R. Kozani, Y. Assadi, F. Shemirani, M.R.M. Hosseini, and M.R. Jamali, Talanta. **72**, 387 (2007).
[16] M. Miró and E.H. Hansen, Anal. Chim. Acta **591**, 46 (2007).
[17] L. Farina, E. Boido, F. Carrau, and E. Dellacassa, J. Chromatogr. A **1157**, 46 (2007).
[18] D. Nagaraju and S.D. Huang, J. Chromatogr. A. **1161**, 89 (2007).
[19] E.Z. Jahromi, A. Bidari, Y. Assadi, M.R.M. Hosseini, and M.R. Jamali, Anal. Chim. Acta **585**, 305 (2007).
[20] N. Shokoufi, F. Shemirani, and Y. Assadi, Anal. Chim. Acta **597**, 349 (2007).
[21] M.A. Farajzadeh, M. Bahram, and J.A. Jönsson, Anal. Chim. Acta **591**, 69 (2007).
[22] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, and S. Berijani, J. Chromatogr. A **1116**, 1 (2006).
[23] N. Fattahi, Y. Assadi, M.R. Milani Hosseini, and E.Z. Jahromi, J. Chromatogr. A **1157**, 23 (2007).
[24] S. Berijani, Y. Assadi, M. Anbia, M.R. Milani Hosseini, and E. Aghaee, J. Chromatogr. A **1123**, 1 (2006).
[25] R.R. Kozani, Y. Assadi, F. Shemirani, M.R.M. Hosseini, and M.R. Jamali, Talanta **72**, 387 (2007).
[26] R.R. Kozani, Y. Assadi, F. Shemirani, M.R. Milani Hosseini, and M.R. Jamali, Chromatographia **66**, 81 (2007).
[27] H. Farahani, P. Norouzi, R. Dinarvand, and M.R. Ganjali, J. Chromatogr. A **1172**, 105 (2007).
[28] Y. Li, G. Wei, J. Hu, X. Liu, X. Zhao, and X. Wang, Anal. Chim. Acta **615**, 96 (2008).