Talanta 98 (2012) 62–68

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of organophosphorus pesticides using dispersive liquid–liquid microextraction combined with reversed electrode polarity stacking mode—micellar electrokinetic chromatography

Phimpha Soisungnoen, Rodjana Burakham, Supalax Srijaranai *

Materials Chemistry Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

ARTICLE INFO

Article history:
Received 30 March 2012
Received in revised form
16 June 2012
Accepted 18 June 2012
Available online 27 June 2012

Keywords:
Organophosphorus pesticide
Micellar electrokinetic chromatography
Reversed electrode polarity stacking mode
Dispersive liquid–liquid microextraction

ABSTRACT

A rapid and sensitive method using two preconcentration techniques, dispersive liquid–liquid microextraction (DLLME) followed by reversed electrode polarity stacking mode (REPSM) was developed for the analysis of five organophosphorus pesticides (OPPs) by micellar electrokinetic chromatography (MEKC). Parameters that affect the efficiency of the extraction in DLLME and preconcentration by REPSM, such as the kind and volume of the extraction and disperser solvents, salt addition, sample matrix and injection time were investigated and optimized. Under the optimum conditions, the enrichment factors were obtained in the range from 477 to 635. The linearity of the method for parathion, azinphos and fenitrithion was in the range of 20–1000 ng mL^{-1}, and for malathion and diazinon in the range of 50–1000 ng mL^{-1}, with correlation coefficients (r^2) ranging from 0.9931 to 0.9992. The limits of detecton (LODs) at a signal-to-noice ratio of 3 ranged from 3 to 15 ng mL^{-1}. The relative recoveries of five OPPs from water samples at spiking levels of 20 and 200 ng mL^{-1} for parathion, azinphos and fenitrithion, and 50 and 500 ng mL^{-1} for malathion and diazinon, were 69.5–103%. The proposed method provided high enrichment factors, good precision and accuracy with a short analysis time.

1. Introduction

The use of pesticides provides benefits for increasing agricultural production. Organophosphorus pesticides (OPPs) are among the most commonly employed pesticides worldwide, because they are less persistent in the environment than other pesticides. However, they can also reach the food chain and may therefore represent a risk to human health. OPPs are very toxic when absorbed by human organisms because of acetylcholinesterase de-activation. In addition, OPPs are known to reduce the activity of neurotransmitters and hence to cause irreversible effects on the nervous system [1,2]. The incorrect uses of OPPs may result in the presence of residues of these compounds in agricultural products such as fruits, fruit juices and vegetables. Moreover, they can also persist in the environment thus contaminating soils as well as surface and ground water [3].

Most analytical methods for OPPs analysis are based on gas chromatography (GC) with flame photometric detector (FPD) [4–7], nitrogen phosphorus detector (NPD) [8,9] or mass spectrometric detector (MS) [10–12] and high performance liquid chromatography (HPLC) with UV detector [13], diode array detector (DAD) [14,15] or MS detector [16,17]. Capillary electrophoresis (CE) combines the advantages of GC resolution and the capability of LC for the separation of compounds. Thus, CE has been accepted as a versatile analytical tool for the determination of a wide variety of pesticides in different types of samples due to its high efficiency, high resolution, short analysis time and low consumption of sample and reagents [18,19]. In principle, the simple mode of CE, capillary zone electrophoresis (CZE), can analyze only ionic or charged analytes, since its separation mechanism is based on the difference in electrophoretic mobilities of analytes. Nowadays, micellar electrokinetic chromatography (MEKC) has been acknowledged as a very powerful separation tool for improving separation efficiency not only neutral analytes but also charged analytes by using a CE instrument without any alteration. In MEKC, an ionic surfactant is used as a psuedostationary phase (PS) that corresponds to the stationary phase in conventional chromatography. The separation mechanism is based on their differential partitioning between aqueous phase and the micelle phase [20,21].

The most widely used detector in CE is the UV photometric detector, since many solutes have UV absorption and the UV

* Corresponding author. Tel.: +66 4320 2222 41x12243; fax: +66 4320 2373.
E-mail address: supalax@kku.ac.th (S. Srijaranai).

0039-9140/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2012.06.043

detector is easily set up and is cost-efficient. The major limitation of UV detection in MEKC is its low sensitivity in term of solute concentration, which is caused by the small sample volume that can be introduced into the capillary and a short optical pathlength equal to the capillary diameter. However, it has already been solved by on-line preconcentration or off-line preconcentration strategies [21].

On-line preconcentration can be performed easily by injection of a large volume of sample solution without any modification of the instrument and the analyte can be focused into the minimum volume inside the capillary. On-line preconcentration strategies have been used in MEKC are sample stacking [18–22], sweeping [23–26] or analyte focusing by micelle collapse (AFMC) [26–28]. In this work, sample stacking namely reversed electrode polarity stacking mode (REPSM) was chosen because it is very simple for the preconcentration of neutral analytes when compared with the other techniques.

Briefly, in REPSM the capillary is conditioned with a micellar background electrolyte and the analytes which are prepared in a low conductivity matrix are then injected as long plugs into the capillary. Then a negative voltage is then placed at the capillary inlet in order to facilitate stacking of the analytes and remove the sample matrix. Once the current reached 97–99% of the predetermined current at this configuration, the polarity is then switched to positive which enable the separation and detection of stacked zones [22].

For off-line preconcentration methods, several sample preparation methods have been developed for the determination of OPPs, including liquid–liquid extraction (LLE) [29], solid phase extraction (SPE) [19,29,30], solid-phase microextraction (SPME) [3,12,13] and liquid-phase microextraction (LPME) [7,31], Since conventional extraction techniques, such as LLE and SPE, are laborious and time-consuming and need large volumes of samples and toxic organic solvents, much attention is being paid to the development of more efficient environment-friendly extraction techniques, such as SPME and LPME [32]. However SPME is also expensive, its fiber is fragile and has limited lifetime and sample carry-over can be a problem [33]. Recently, LPME has emerged as an attractive alternative for sample preparation because of its simplicity, low cost, and small volume of organic solvents consumed. LPME is based on the miniaturization of the traditional LLE method by greatly reducing the use of organic solvent. Single-drop microextraction (SDME) belonging to a kind of LPME, is a solvent-minimized sample pretreatment procedure and also has been used to analyze OPPs in water samples [31]. However, the disadvantages of SDME are as follows: fast stirring often breaks up the organic solvent drop, air bubbles are easily formed, the extraction procedure is time-consuming and in most cases equilibrium is not easily attained even after a long time [34]. Efforts to overcome these limitations led to the development of dispersive liquid–liquid microextraction (DLLME) with the advantage of short extraction time, simplicity of operation and small amount of solvents used [33].

DLLME is based on ternary solvent component system involving an aqueous phase, a non-polar water immiscible solvent (extraction solvent) and a polar water miscible solvent (disperser solvent). In this technique, fine droplets of the extraction solvent are dispersed into the aqueous phase when an appropriate mixture of both solvents is rapidly injected into aqueous samples. The mixture is then gently shaken and a cloudy solution (water/disperser solvent/extraction solvent) is formed in the tube. After centrifugation, the fine particles of extraction solvent containing the target analytes are separated from the aqueous phase and finally determine by various analytical techniques [35,36]. The performance of DLLME has been demonstrated in the determination of OPPs by chromatographic methods [4,7,37]. However, until now there are very few literatures reporting about the applications of the DLLME in combination with CE for the analysis of pesticides in real samples.

In this work, we propose to develop a new method for the determination of five organophosphorus pesticides (parathion, malathion, diazinon, azinphos and fenitrothion) by MEKC-UV using REPSM and DLLME as on-line and off-line preconcentration techniques. The effects of some important experimental parameters that influence the DLLME and REPSM efficiency were studied.

2. Experimental

2.1. Reagents, chemicals and materials

Pesticide standards (Fig. 1) were purchased from Dr.Ehrenstorfer (Germany) including parathion-methyl, malathion, diazinon, azinphos-methyl and fenitrothion, all 98–99% purify. Stock solution of each pesticide at 100 mg L^{-1} were prepared in methanol and stored at 4 °C. Standard working solutions at various concentrations were prepared daily by an appropriate dilution of the stock solutions with deionized water with a resistivity of 18.2 MΩ cm from RiO_S^{TM} Type I Simplicity 185 (Millipore, USA).

All chemicals were of analytical reagent grade. Sodium dodecyl sulfate (SDS) was purchased from BDH (England). Sodium tetraborate decahydrate (borax) was purchased from Fluka (Germany). Dichloromethane (CH_2Cl_2), acetone, sodium hydroxide, sodium chloride, sodium sulfate, potassium chloride, potassium iodide and boric acid were purchased from Carlo Erba (Italy). Methanol (HPLC grade), acetonitrile (HPLC grade) and hydrochloric acid were purchased from Lab-scan Asia (Thailand). All the solvents were filtered through 0.45 μm filter purchased from Whatman International (Germany) prior to use.

2.2. Dispersive liquid–liquid microextraction procedure

For the DLLME, a 10.00 mL of water sample was placed in a 15 mL screw-cap centrifuge tube. Two milliliter of acetonitrile (as disperser solvent) containing 300 μL of CH_2Cl_2 (as extraction solvent) was rapidly injected into the sample solution. Then, the mixture was shaken by hand for 1 min. A cloudy solution that

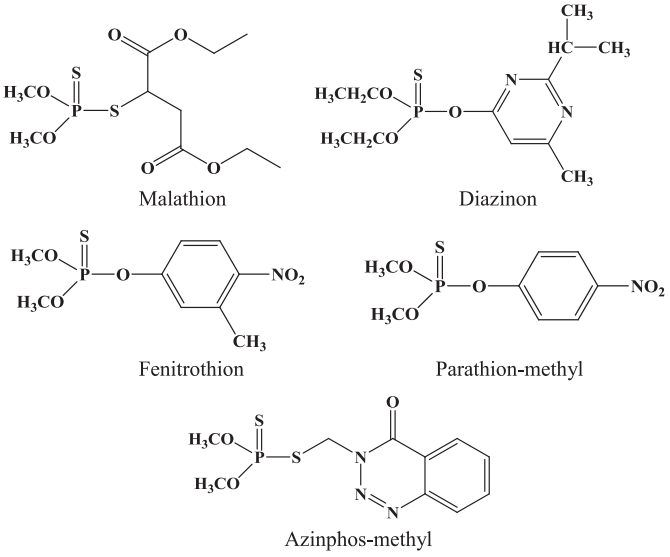

Fig. 1. Structures of the studied pesticides.

consisted of very fine droplets of CH_2Cl_2 dispersed into aqueous sample was formed, and the analytes were extracted into the fine droplets. After centrifugation at 3500 rpm for 5 min, the CH_2Cl_2 phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was transferred into another tube and then evaporated to dryness with nitrogen stream. The residue was dissolved with 250 μL of 15 mM borate buffer and finally analysed by the REPSM–MEKC as described in Section 2.3.

2.3. Electrophoresis procedure

All CE experiments were performed on a Beckman P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Singapore), equipped with a diode array detector (DAD). Uncoated fused-silica capillaries with 75 μm i.d. were purchased from Beckman Coulter (Singapore). The effective length was 30 cm and the total length was 40 cm.

Before first use, fused-silica capillary was washed (20 psi) for 5 min with 0.1 M HCl, 3 min with water, 5 min with 0.1 M NaOH, 3 min with water and 5 min with running buffer (separation electrolyte). The capillary conditioning was done every time prior to use with 0.1 M NaOH for 5 min at 20 psi, followed with water for 5 min, and finally with running buffer for 5 min. To achieve a good reproducibility, the capillary was flushed between runs with 0.1 M NaOH for 5 min at 20 psi, then with water for 3 min, and finally with the running buffer for 5 min.

For REPSM–MEKC procedure, the capillary was first filled with the separation electrolyte. Then the large plug of sample was hydrodynamically injected for 90 s at 0.5 psi. A high voltage (−17 kV) was then applied and the electrical current was monitored to control the removal sample matrix from the capillary. When the current became 97–99% of the value obtained with running buffer, the voltage was turned off and the polarity was reversed to run the separation (+17 kV). The separation was carried out at 25 °C and at +17 kV with diode array dection at 200 nm, using 15 mM borate buffer at pH 9.5 containing 40 mM SDS and 10% methanol as running buffer.

3. Results and discussion

3.1. Optimization of DLLME procedure

In order to obtain the optimum extraction conditions, enrichment factor (EF) and extraction recovery (ER) were used to evaluate the extraction efficiency under different experimental parameters. The enrichment factor and the extraction recovery were deduced according to Eqs. (1) and (2) as follows [33]:

$$EF = \frac{C_{sed}}{C_0} \quad (1)$$

where EF, C_{sed} and C_0 are the enrichment factor, the analyte concentration in the sediment, and the initial analyte concentration in the sample, respectively.

$$EF = \frac{C_{sed}V_{sed}}{C_0V_{aq}} \times 100 \quad (2)$$

where ER (%), V_{sed} and V_{aq} are the extraction recovery, the volume of the sediment phase, and the volume of the aqueous phase, respectively.

There are different factors that affect the extraction process including type of extraction and disperser solvents, volume of extraction and disperser solvents, and extraction time. In DLLME, extraction time has little effect on the extraction efficiency. The reason for this is that the extraction solvent can be dispersed after the formation of the cloudy solution, the transfer migration of the analytes from aqueous phase to extraction phase can be very fast, and equilibrium state can be subsequently achieved very quickly [35]. Therefore, in this study, the extraction time was kept constant at 5 min. In this experiment, 10.0 mL of water spiked with 20 ng mL^{-1} each of OPPs was used to study the extraction performance of DLLME under different experimental conditions.

3.1.1. Selection of extraction and disperser solvents

The selection of an appropriate extraction solvent is a major parameter for DLLME process. The extraction solvent should meet the following requirement: it should have a higher density than water, a low solubility in water, high extraction capacity for the target analytes and form a stable two-phase system in the presence of a disperser solvent when injected to an aqueous solution. Generally, the solvents with density higher than water are mainly halogenated hydrocarbons [35]. In this study, dichloromethane (CH_2Cl_2) was selected as the extraction solvent because it was commonly used in conventional LLE technique. In another study [38] showed that carbon tetrachloride (CCl_4) or chloroform ($CHCl_3$) had more extraction efficiency than CH_2Cl_2. However, carbon tetrachloride and chloroform were not investigated in our study because only dichloromethane is allowed to be used in our country.

On the other hand, the selection of a disperser solvent is limited to solvents such as acetone, methanol and acetonitrile, which are miscible with both water and the extraction solvents and could form a cloudy state when injected with the organic extractant into water [35]. With CH_2Cl_2 as the extraction solvent, the use of acetone or acetonitrile as disperser solvent could produce a two-phase system. The effect of these solvents on extraction recovery is given in Fig. 2. As a result, acetonitrile gave the best extraction recovery. Consequently, acetonitrile was selected for subsequent studies.

3.1.2. Effect of extraction solvent volume

In order to study the effect of the extraction solvent volume on the performance of the presented DLLME procedure, the volume of CH_2Cl_2 was varied in the range 200–400 μL with a constant volume of acetonitrile 1.5 mL (disperser solvent). With less than 200 μL of CH_2Cl_2, no two-phase system was observed. Fig. 3 illustrates the effect of volume of the extraction solvent (CH_2Cl_2) on the extraction recovery. It was observed that the extraction

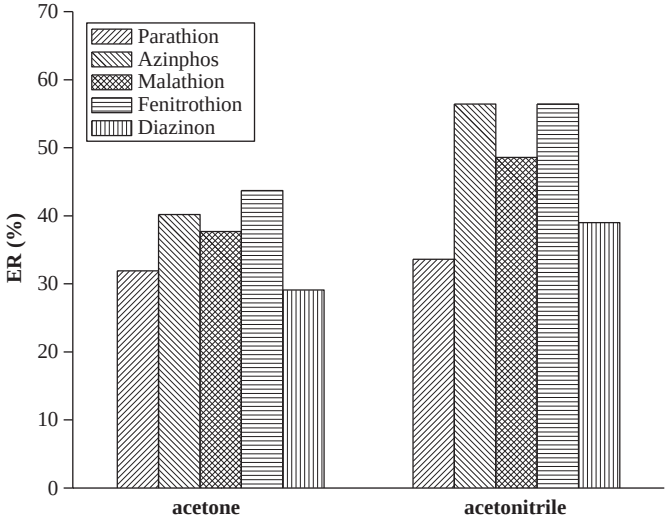

Fig. 2. Effect of different disperser solvents on the extraction recovery of the organophosphorus pesticides. Extraction conditions: sample volume, 10.0 mL; extraction solvent, 200 μL CH_2Cl_2; disperser solvent volume, 1.5 mL.

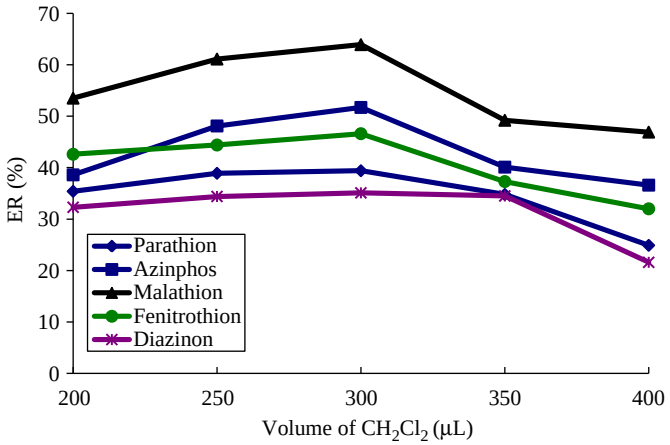

Fig. 3. Effect of the volume of the extraction solvent (CH_2Cl_2) on the extraction recovery of the organophosphorus pesticides. Extraction conditions: sample volume, 10.0 mL; disperser solvent, 1.5 mL acetonitrile.

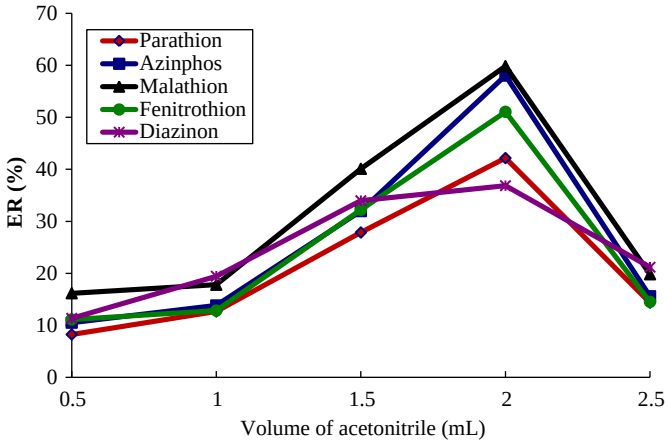

Fig. 4. Effect of the volume of the disperser solvent (acetonitrile) on the extraction recovery of the organophosphorus pesticides. Extraction conditions: sample volume, 10.0 mL; extraction solvent, 300 μL CH_2Cl_2.

recovery was increased with increased volume of CH_2Cl_2 from 200 to 300 μL; after that it decreased by increasing the volume of CH_2Cl_2. Thereby, 300 μL of CH_2Cl_2 was chosen as the optimal volume for extraction solvent.

3.1.3. *Effect of disperser solvent volume*

The influence of the volume of the disperser solvent (acetonitrile) was investigated by changing its volume to 0.5, 1.0, 1.5, 2.0 and 2.5 mL, respectively. The results (Fig. 4) indicated that the extraction efficiency increased first and then decreased by increasing the volume of acetonitrile for all OPPs. The reason could be that at a low volume of acetonitrile, a cloudy state could not be well formed, thus giving a low recovery. Whereas, at a larger volume of acetonitrile, the solubility of the pesticides in water was increased, leading to a decreased extraction efficiency because of a decrease in distribution coefficient [32,38]. Based on the experimental results in Fig. 5, 2.0 mL of acetonitrile was chosen.

3.1.4. *Effect of salt addition*

The salting-out effect is an important parameter in microextraction such as SPME and LPME. Generally, the addition of salt

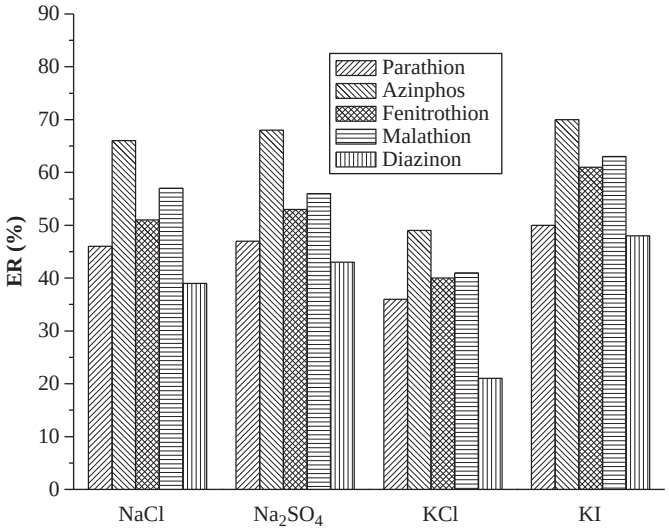

Fig. 5. Effect of different salt addition on the extraction recovery of the organophosphorus pesticides. Extraction conditions: sample volume, 10.0 mL; extraction solvent, 300 μL CH_2Cl_2; disperser solvent, 2.0 mL acetonitrile.

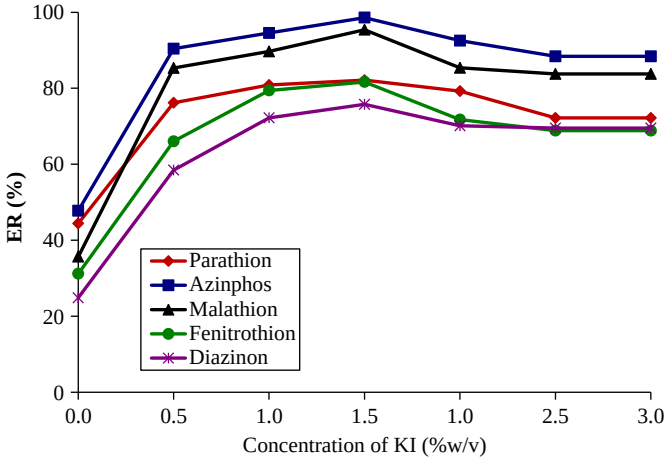

Fig. 6. Effect of salt addition (KI) on the extraction recovery of the organophosphorus pesticides. Extraction conditions: sample volume, 10.0 mL; extraction solvent, 300 μL CH_2Cl_2; disperser solvent, 2.0 mL acetonitrile.

results in increasing ionic strength, decreases the solubility of analytes in the aqueous solution and enhances their partitioning into the organic phase, which is favorable for reaching high recovery [34–35]. In order to examine salt influence of DLLME of the OPPs, the extraction was performed in the presence of different salts. Sodium chloride, commonly salt used to study the effect of ionic strength, sodium sulfate, potassium chloride and potassium iodide were studied. In Fig. 5, the results showed that potassium iodide was the suitable salt because it gave highest extraction efficiency and it showed the best baseline when injected into REPSM–MEKC system (data not shown). Potassium iodide was used in further experiments.

The amount of salt is also a major parameter affecting ionic strength. To evaluate the effect of the quantity of salt, the extraction efficiency was studied with the different concentrations (0–3%, w/v) of potassium iodide. Fig. 6 depicts the dependence of extraction recovery upon the content of KI, respectively. It can be seen that the extraction efficiency increased with the increasing of KI concentration up to 1.5% and remained almost constant at higher concentration. It could be explained that

extraction efficiency increased due to salting out, whereby water molecules form hydration spheres around the ionic salt molecules that reduce the concentration of water available to dissolve the analyte molecules, thereby driving the additional analytes into the organic droplets [39]. Based on this result, 1.5% of potassium iodide was added to the aqueous samples for further studies.

3.2. Optimization of MEKC separation

In order to separate five pesticides (parathion-methyl, malathion, diazinon, azinphos-methyl and fenitrothion), analytical parameters including buffer concentration, SDS concentration, pH of buffer and methanol content were optimized by direct hydrodynamic injection (5 s at 0.5 psi) of a mixture of the five pesticides dissolved in water (10 mg L^{-1} each).

3.2.1. Effect of the buffer and SDS concentration

Buffer concentration has significant effect on the separation performance because it can influence the Joule heating, the electroosmotic flow (EOF) and the current produced in the capillary. Borate buffer, the most commonly used buffer system in CE at high pH, was chosen as the running buffer in this work. In order to obtain the best separation of five OPPs, the influence of borate concentration on the separation was tested by changing the concentrations from 5 to 20 mM. The results demonstrated that with increased concentration of borate, the migration time was increased, and the complete separation was obtained at concentration higher than 10 mM. On the other hand, high borate concentration resulted to increase Joule heating which could cause an increased baseline noise [38]. As a result, 15 mM borate was selected for subsequent investigations.

The effect of SDS concentration was studied by varying from 30 to 60 mM. The result indicated that migration time increased with increased SDS concentration because of the probability of partitioning into the micelle increased at higher SDS concentration [20]. Giving as overall consideration of both resolution and analysis time, 40 mM SDS was selected for further studies.

3.2.2. Effect of the buffer pH

The acidity of the running buffer affects the EOF in untreated fused silica capillary, and therefore, will influence the migration time and separation efficiency of analytes [38]. In this study, the running buffers of 15 mM borate containing 40 mM SDS and 10% methanol at different pH values (8.5–10) were examined. It was found that the migration time of analytes was increased when pH was increased, and the complete separation could be achieved at pH higher than 9.5. Therefore, pH 9.5 was chosen for further studies.

3.2.3. Effect of organic modifier

The addition of organic solvents, such as methanol, to the running buffer could improve resolution because they could cause a difference in affinity between micelles and analytes which decreasing of the aqueous phase polarity. This fact facilitated the dissolution of neutral compounds in aqueous phase and their separation could be increased [40]. In this work, the effect of methanol content was investigated by changing from 0 to 20% (v/v). When the methanol content was increased, the resolutions between the analytes were improved but with increased migration time. Consequently, 10% of methanol was selected for the experiment due to the best result of both resolution and analysis time.

Based on the above optimizations, the optimum separation electrolyte was the mixture of 15 mM borate buffer at pH 9.5 containing 40 mM SDS and 10% methanol.

3.3. Optimization of REPSM procedure

The poor sensitivity normally obtained by CE-UV can be improved by on-line preconcentration strategies called stacking techniques. The use of these techniques allows the introduction of larger sample volumes into the capillary. The criterion of these techniques is the sample should be dissolved in an appropriate matrix (normally with lower conductivity than that of the separation electrolyte). With the purpose of increasing the sensitivity of the determination of these pesticides, the REPSM technique was used in this study. In this case, both the injection time and the sample matrix were optimized. In order to obtain a sample matrix with low conductivity and to provide a sensitivity increase as large as possible, different solvents i.e., water and various concentrations of borate buffer (separation buffer without SDS and methanol) were investigated. The highest sensitivity was achieved by dissolving the analytes in 15 mM borate solution at pH 9.5. By using this solution, the sample could be injected in the capillary up to 90 s at 0.5 psi. In this case, the reversal time (the time need to eliminate the matrix of the sample by applying voltage at negative polarity) was 1.0 min. The larger injection times resulted in peak distortion and overlap and also need larger reversal times. Fig. 7A and B show the electropherograms of the separation of five pesticides under the optimum separation (MEKC) and REPSM–MEKC conditions, respectively. It can be seen that the complete separation of five OPPs was achieved within 14 min with good resolutions and providing an approximately 20-fold preconcentration after using REPSM.

3.4. Analytical characteristics and method validations

The analytical performance features and the validation for the proposed method including linearity, limits of detection (LODs, $S/N=3$), repeatability (intra-day precision), and reproducibility (inter-day precision) were determined. The results are summarized in Table 1. The linearity was observed in the range 20–1500 ng mL^{-1} with the correlation coefficient (r^2) ranging from 0.9931 to 0.9992. The LODs ranged between 3 and 15 ng mL^{-1}. Precisions of the proposed DLLME–REPSM–MEKC method was evaluated in terms of intra-day and inter-day, by extracting the

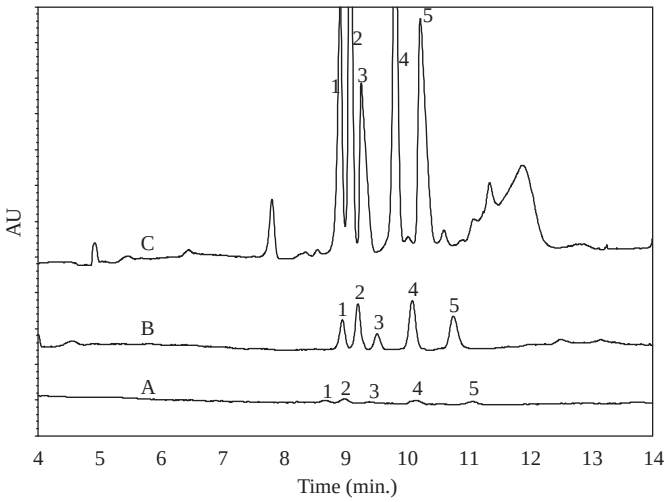

Fig. 7. Electropherograms of the studied organophosphorus pesticides obtained from without preconcentration (MEKC) (A) and with on-line preconcentration (REPSM–MEKC) (B) and with off-line and on-line preconcentration (DLLME–REPSM–MEKC) (C). Separation electrolyte: 15 mM borate buffer at pH 9.5 containing 40 mM SDS and 10% methanol. Total length of the capillary 40 cm (30 cm effective length); voltage, +17 kV; UV detection at 200 nm. Peak assignment: 1 parathion, 2 azinphos, 3 malathion, 4 fenitrothion, 5 diazinon.

Table 1
Analytical performance for OPPs obtained from DLLME–REPSM–MEKC.

Pesticide	Linearity (ng mL^{-1})	r^2	LOD (ng mL^{-1})	Intra-day (n=5), %RSD		Inter-day (n=5 × 3 day), %RSD	
				Time	Peak area	Time	Peak area
Parathion	20–1000	0.9931	5	0.8	3.6	1.3	6.9
Azinphos	20–1000	0.9942	5	0.8	2.8	1.3	3.4
Malathion	50–1000	0.9977	15	0.7	2.3	1.1	5.1
Fenitrothion	20–1000	0.9992	3	0.9	2.1	1.4	4.1
Diazinon	50–1000	0.9982	10	0.8	2.9	1.4	6.2

Table 2
Enrichment factors obtained from off-line and on-line preconcentration method.

Pesticide	Enrichment factors		
	DLLME	REPSM–MEKC	DLLME–REPSM–MEKC
Parathion	33.1	14.4	477
Azinphos	31.0	15.7	488
Malathion	34.7	18.3	635
Fenitrothion	26.0	18.7	485
Diazinon	29.8	19.3	576

Table 3
Recoveries obtained from the determination of OPPs in spiked water samples.

Pesticide	Spiked (μg mL^{-1})	Tap water		Surface water	
		Found (μg mL^{-1})	Relative recovery (%)	Found (μg mL^{-1})	Relative recovery (%)
Parathion	0	ND	–	ND	–
	0.02	0.017	86.7	0.017	82.8
	0.20	0.192	96.1	0.151	75.7
Azinphos	0	ND	–	ND	–
	0.02	0.016	77.8	0.017	84.8
	0.20	0.180	90.2	0.174	87.1
Malathion	0	ND	–	ND	–
	0.05	0.048	96.4	0.051	103
	0.50	0.434	86.7	0.486	97.3
Fenitrothion	0	ND	–	ND	–
	0.02	0.015	74.8	0.014	69.5
	0.20	0.186	92.8	0.177	88.5
Diazinon	0	ND	–	ND	–
	0.05	0.042	83.0	0.037	74.6
	0.50	0.426	85.2	0.419	83.8

ND: not detected.

OPPs standard at the concentration of each pesticide at 50 ng mL^{-1} in the same day and on the three consecutive days. The results (Table 1) show an acceptable precision in all cases with intra-day RSD values below 3.6% and inter-day values within 6.9%.

Moreover, the comparison of the enrichment factors for DLLME, REPSM–MEKC and the combination technique of DLLME with REPSM–MEKC were investigated. As a result, in Table 2 and Fig. 7C, compared with the sensitivity obtained from MEKC (Fig. 7A), the DLLME–REPSM–MEKC method provided more than 477-fold sensitivity enhancement with acceptable resolution and good reproducibility. The above results demonstrated that the proposed DLLME–REPSM–MEKC method markedly improved the detection sensitivity compared with conventional MEKC and also REPSM–MEKC.

3.5. Evaluation of method performance

To evaluate the accuracy and applibility of the proposed method, the extraction and determination of the five OPPs in water samples were performed. To check the interferences due to the matrix, these water samples were spiked with the standard solution of target analytes at various concentrations with three replicate experiments. The results are given in Table 3. It was found that the relative recoveries for the OPPs in water samples were in the range 69.5–103%. Fig. 8 shows the electropherograms of the extract OPPs from tap water sample before and after spiking with five OPPs standard. It could be seen that no any matrices interfered the separation.

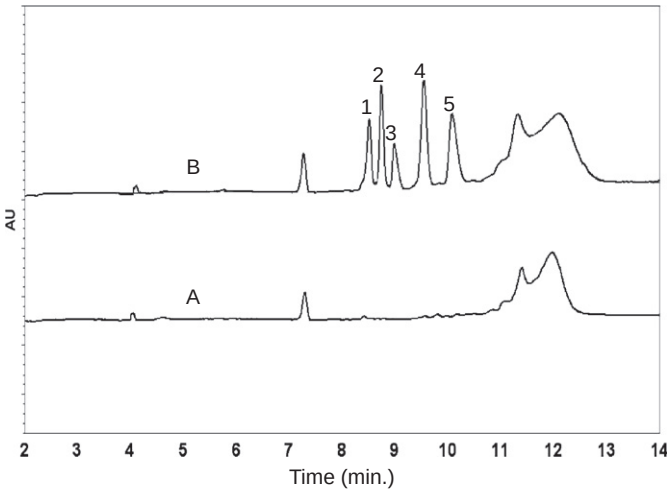

Fig. 8. Electropherograms for unspiked (A) and spiked (B) water samples by DLLME–REPSM–MEKC. Separation conditions and peak identifications are the same as in Fig. 7. Extraction conditions: sample volume, 10.0 mL; extraction solvent, 300 μL CH_2Cl_2; disperser solvent, 2.0 mL acetonitrile; salt addition, 1.5% KI.

4. Conclusion

In this work, a new method has been developed for the analysis of five organophosphorus pesticides by combining DLLME, an off-line prconcentration with an on-line preconcentration procedure of REPSM–MEKC method. The results demonstrated that the proposed method has high enrichment factors, good precision and accuracy with a short analysis time. Moreover, DLLME method can offer advantages of speed, simplicity and low consumption of organic solvent when compared with the other extraction methods.

Acknowledgments

This research was supported by a grant under the program Strategic Scholarships for Frontier Research Network for the Join Ph.D. Program Thai Doctoral Degree from the Office of the Higher

Education Commission, Thailand. We also thank the Rajamungala University of Technology Isan (RMUTI) for partial support.

References

[1] H. Tsoukali, G. Theodoridis, N. Raikos, I. Grigoratou, J. Chromatogr. B 822 (2005) 194–200.
[2] G.F. Jia, C.G. Lv, W.T. Zhu, J. Qiu, X.Q. Wang, Z.Q. Zhou, J. Hazard. Mater. 159 (2008) 300–305.
[3] C.G. Zambonin, M. Quinto, N. De Vietro, F. Palmisano, Food Chem. 86 (2004) 269–274.
[4] S. Berijani, Y. Assadi, M. Anbia, M.M. Hosseini, E. Aghaee, J. Chromatogr. A 1123 (2006) 1–9.
[5] E. Fuentes, M.E. Báez, R. Labra, J. Chromatogr. A 1169 (2007) 40–46.
[6] M.R. Khalili-Zanjani, Y. Yamini, N. Yazdanfar, S. Shariati, Anal. Chim. Acta 606 (2008) 202–208.
[7] S. Moinfar, M.M. Hosseini, J. Hazard. Mater. 169 (2009) 907–911.
[8] E. Ballesteros, M.J. Parrado, J. Chromatogr. A 1029 (2004) 267–273.
[9] X. Zhu, J. Yang, Q. Su, J. Cai, Y. Gao, J. Chromatogr. A 1092 (2005) 161–169.
[10] Y.R. Tahboub, M.F. Zaater, Z.A. Al-Talla, J. Chromatogr. A 1098 (2005) 150–155.
[11] L. Amendola, F. Botrè, A.S. Carollo, D. Longo, L. Zoccolillo, Anal. Chim. Acta 461 (2002) 97–108.
[12] C. Gonçalves, M.F. Alpendurada, J. Chromatogr. A 1026 (2004) 239–250.
[13] C. Cháfer-Pericás, R. Herráez-Hernández, P. Campíns-Falcó, J. Chromatogr. A 1141 (2007) 10–21.
[14] C.P. Sanz, R. Halko, Z.S. Ferrera, J.S. Rodríguez, Anal. Chim. Acta 524 (2004) 265–270.
[15] M.P. García de Llasera, M.L. Reyes-Reyes, Food Chem. 114 (2009) 1510–1516.
[16] H.G.J. Mol, R.C.J. van Dam, O.M. Steijger, J. Chromatogr. A 1015 (2003) 119–127.
[17] P. Salm, P.J. Taylor, D. Roberts, J. de Silva, J. Chromatogr. B 877 (2009) 568–574.
[18] C. Malina-Mayo, J. Hernández-Borges, T.M. Borges-Miquel, M.Á. Rodríguez-Delgado, J. Chromatogr. A 1150 (2007) 348–355.
[19] A. Juan-García, Y. Picó, G. Font, J. Chromatogr. A 1073 (2005) 229–236.
[20] J.P. Quirino, S. Teraba, J. Chromatogr. A 791 (1997) 255–267.
[21] J.B. Kim, S. Terabe, J. Pharm. Biomed. Anal. 30 (2003) 1625–1643.
[22] Z. Liu, P. Sam, S.R. Sirimanne, P.C. McClure, J. Grainger, D.G. Patterson Jr., J. Chromatogr. A 673 (1994) 125–132.
[23] J.P. Quirino, S. Teraba, Science 282 (1998) 465–468.
[24] J.P. Quirino, J.B. Kim, S. Teraba, J. Chromatogr. A 965 (2002) 357–373.
[25] A.T. Aranas, A.M. Guidote Jr., P.R. Haddad, J.P. Quirino, Talanta 85 (2011) 86–90.
[26] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, J. Chromatogr. A 1217 (2010) 386–393.
[27] J.P. Quirino, P.R. Haddad, Anal. Chem. 80 (2008) 6824–6829.
[28] J.P. Quirino, J. Chromatogr. A 1214 (2008) 171–177.
[29] J.J. Jiménez, J.L. Bernal, M.J. del Nozal, C. Alonso, J. Chromatogr. A 1048 (2004) 89–97.
[30] M. Mezcua, A. Agüera, J.L. Lliberia, M.A. Cortés, B. Bagó, A.R. Fernández-Alba, J. Chromatogr. A 1109 (2006) 222–227.
[31] F. Ahmadi, Y. Assadi, S.M.R. Milani-Hosseini, M. Rezaee, J. Chromatogr. A 1101 (2006) 307–312.
[32] Q. Wu, X. Zhou, Y. Li, X. Zang, C. Wang, Z. Wang, Anal. Bioanal. Chem. 393 (2009) 1755–1761.
[33] M. Rezaee, Y. Assadi, M.R.R. Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1–9.
[34] L. Fu, X. Lui, J. Hu, X. Zhao, H. Wang, X. Wang, Anal. Chim. Acta 632 (2009) 289–295.
[35] X.H. Zang, Q.H. Wu, M.Y. Zhang, G.H. Xi, Z. Wang, J. Chin., Anal, Chem 37 (2) (2009) 161–168.
[36] M.I. Pinto, G. Sontag, R.J. Bernardino, J.P. Noronha, Microchem. J. 96 (2010) 225–237.
[37] E. Zhao, W. Zhao, L. Han, S. Jiang, Z. Zhou, J. Chromatogr. A 1175 (2007) 137–140.
[38] S. Zhang, C. Li, S. Song, T. Feng, C. Wang, Z. Wang, Anal. Methods 2 (2010) 54–62.
[39] M.B. Melwanki, W.S. Chen, H.Y. Bai, T.Y. Lin, M.R. Fuh, Talanta 78 (2009) 618–622.
[40] A. Farran, S. Ruiz, C. Serra, M. Aguilar, J. Chromatogr. A 737 (1996) 109–116.