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Off-line Preconcentration Coupled With On-line Stacking Methods in Enhancing Detection of Selected Pesticides Using Capillary Electrophoresis

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Abstract: - In this study, off-line preconcentration which involves the usage of solid phase disc extraction (SPDE) was used as an enrichment step in enhancing the detection of selected pesticides with capillary electrophoresis (CE). Micellar electrokinetic chromatography (MEKC) with stacking with reverse migrating micelles (SRMM) is the mode used in improving the limit of detection (LOD) of the analytes. The selected pesticides chosen are organophosphorous pesticides (OPPs) which include dicrotophos, monocrotophos and phosphamidon. This study was carried out by spiking 1 µg and 0.5 µg of each pesticide into 250 mL of pond water before undergoing sample enrichment with SPDE thus achieving a concentration of 4 ppb and 2 ppb respectively. Recovery and repeatability studies of the SPDE method were also carried out as a means of method validation. From the results, the recovery for 1 µg of spiked pesticide was in the range of 96.2-132% while at 0.5 µg it was at 74-117%. The repeatability of the extraction was higher for lower concentrations in the range of 10-12%RSD as compared to higher concentration within 0.81-5.36%RSD. The LOD for the three analytes has been reduced by 250 times with dicrotophos (0.6 ppb), monocrotophos (1.56 ppb) and phosphamidon (0.88 ppb) when SPDE was coupled with on-line preconcentration technique that is SRMM.

KeyWords*:* MEKC, organophosphorous pesticides, solid phase extraction and stacking

Introduction

Organophosphorous (OPPs) pesticides are generally esters, amides or thiol derivatives of phosphoric, phosphonic, phosphorothioic or phosphonothioic acids [1]. These class of OPPs were chosen as they are not easily detectable in biological samples and aqueous conditions due to the alkylating activity of organophosphate esters as they are very reactive. OPPs work by inhibiting the acetychlolinesterase enzyme (AChE) in the nervous system and subsequent accumulation of toxic levels of endogenous acetylcholine (ACh) in nervous tissue and effector organs in both insects and mammals (2). In modern agricultural practices, excessive application of pesticides has caused serious environmental problems. The leaching of pesticides into surface and groundwater is through bypass flow either in solution or suspended in colloidal matter. This can cause poor pest control, crop injury and increased loss of pesticides or accumulation of pesticides in the soil. Thus greater accuracy and sensitivity in detecting these pesticides in environmental samples is required.

These led to the development of many analytical methods for monitoring these compounds. In the case of determination of pesticides in water, a sample pretreatment step includes the analyte enrichment as well as the removal of matrix components. This is commonly carried out using solid-phase extraction (SPE) in the form of cartridges and disc devices. SPE is commonly used in extraction of pesticides [3-5]. SPE benefits from low intrinsic costs, shorter processing times, low solvent consumption, simpler processing procedures and easier automation [6]. SPE has been commonly used in pesticide multiresidue analysis such as in fruits and vegetables [7, 8], eggs [9] and wine [10] with gas chromatography detection methods.

There have also been coupling of SPE with on-line stacking methods in capillary electrophoresis in order to lower the detection sensitivity especially in environmental analysis of pesticides using SPE catridges [11-15]. Süsse *et. al.* [16] employed a two-step enrichment process (SPE combined with REPSM stacking) which an enrichment factor of 5000-10000. Detection limits were reduced greatly to 0.04 ppb in the analysis of various carbamates and organophosphate pesticides. Automated SPE with MEKC-UV detection was used in the determination of organophosphorous pesticides in water, vegeatables and grain achieving a enrichment factor of 250 [17]. In this study, solid phase extraction in the form of discs were used in these two step enrichment process of three hydrophilic organophosphorous pesticides which are dicrotophos, monocrotophos and phosphamidon

with the use of online stacking which is stacking with reversed migrating micelles (SRMM). Stacking with reverse migrating micelles (SRMM) is performed by preparing the sample in a low conductivity matrix and introduced hydrodynamically at the cathodic end and then separation voltage is applied at negative polarity at the injection end [18-20]. SRMM was applied to the trace analysis of multi-residue pesticides in water and vegetables with off-line solid-phase extraction achieving and LOD of 0.1 µg/L [21]. SRMM has been able to reduce the LOD of melatonin to 30ng/mL and was applied to the analysis of this hormone in human serum [22]. Thus due to its good performance, SPE-SRMM analysis of water sample containing a certain amount of phosphamidon, dicrotophos and monocrotophos was evaluated based on reproducibility and limit of detection (LOD) values.

Materials and Methods

Envi-Disk^{TM} octadecyl (C_{18}) disks (47 mm i.d., 0.5 mm thick) were supplied by Supelco (Bellefonte, PA, USA). Samples were extracted with a Sigma Aldrich vacuum manifold system hooked up to the Aspirator A-3S supplied by Eyena, Tokyo Rikakikai Co. Ltd. ACS grade dichloromethane was obtained from J. T. Baker Inc. (New Jersey, USA) while AR Grade anhydrous sodium sulphate was obtained from Fisher Chemicals (UK). Sodium dodecyl sulphate (SDS) was from Fischer Scientific (Loughborough, UK) while di-sodiumhydrogen phosphate was from Riedel-de Haen (Seelze, Germany). Sulphuric acid (1.0 M) was obtained from J. T. Baker (New Jersey, USA) while sodium hydroxide pellets were from Riedel-de-Haen, Seelze, Germany. HPLC Grade methanol were obtained from J.T. Baker, California, USA . While for the organophosphorous pesticides (OPPs), three types of hydrophilic pesticides were chosen which were phosphamidon, dicrotophos and monocrotophos, analytical grade (AR) grade obtained from Dr. Ehrenstorfers GmbH laboratory (Augsburg, Germany). Stock solutions of these pesticides were prepared in methanol at 1000 ppm. Phosphate buffer was adjusted to a pH 2.3 with 0.1 M sulphuric acid. Deionised water was obtained from Milipore UltraPure Water System purified up to 18 MΩ.

All experiments were conducted on a capillary electrophoresis system (220V Agilent Capillary Electrophoresis System, Hanover, Germany) equipped with a diode array detector with the optimum wavelength set at 225 nm. Data acquisition and system control was carried out by the 3D-CE ChemStation Software by Agilent Technologies. Standard bare fused silica capillaries (Polymicro Technologies, Phoenix, Arizona, USA) with 48.5 cm total length, 40 cm effective length and 50 µm i.d. were utilized to perform the separation. Injection offset was set at 4 mm. Hydrodynamic injection at 50 mbar for 1s was used. Polypropylene vials (Agilent Technologies, Hanover, Germany) of 1 mL were used to place samples, buffers and other solutions in the electrophoretic systems. All samples and buffer solutions were degassed before use and filtered through a 0.45 µm Nylon filter disc (Whatman).

All samples and buffer solutions were filtered through a 0.45 µm Nylon filter disc (Whatman). The final buffering solution pH was measured by *Cyberscan 500* pH meter from Eutech Instruments Pte. Ltd. (Singapore). Samples were introduced hydrodynamically at constant pressure 50 mbar, 50 s. Separation voltage remain constant at 25 kV at negative polarity as acidic buffer is being used.

MEKC Separation Conditions and Conditioning of the Capillary

All experiments were conducted at a constant temperature of 25 ºC ± 1ºC at constant wavelength of 225 nm. At the beginning of each day, the capillary was flushed for 10 min with 0.1 M NaOH to activate the silanol groups of the capillary followed by 10 min of deionized water followed by 10 min of running buffer. Before each sample injection, the capillary was rinsed for 5 min with 0.1M NaOH, followed by 5 min with deionised water before flushing with 5 min of running buffer. With separation in acidic buffers, continuous flushing with deionised water for approximately 15 minutes in between runs was necessary to clean the capillary properly with the usual EOF characteristics. At the end of the day, the capillary was flushed with 20 min of water followed by 20 min of air. Both ends of the capillary were dipped in deionized water before shutting down for the day.

Stacking With Reverse Migrating Micelles in the Analysis of OPPs

Standards were prepared in water with concentrations 10, 20 and 30 ppm and injected at 50 mbar for 50 s at the cathodic end. Then separation voltage is applied at -25kV at negative polarity.

Sample Preparation

Before extraction, 250 mL pond water was spiked with 1µg and 0.5 µg of a mixture of pesticides both in separate 250 mL volumetric flasks in order to investigate the recovery of the method. Procedure blanks were also prepared with pond water in 250 mL volumetric flasks.

Extraction

The reservoir and SPE disc were prerinsed with 20 mL dichloromethane (DCM) to remove residues of environmental matrices and impurities from the manufacturing processes and then placed under vacuum for 5 min. To the disc, 25 mL methanol was added which was left in contact with the disc for 3 min to condition its hydrophobic surface. The vacuum was applied and the methanol drawn through. Before the disc went dry, 50 mL of deionized water was added to the reservoir. This critical step is to ensure that the surface of the disc did not go dry. Before all the water

was drawn through the disc, the sample (250 mL) was added. After the entire sample had been drawn through, the disc was air-dried for 5 min. A test tube was placed in the filter flask for sample extract collection. The elution solvent used was 20 mL of methanol. Sodium sulphate is then used to dry the extract from any moisture before the extract was transferred to a round bottom flask and the solvent evaporated to 1 mL with a rotary evaporator before being concentrated under nitrogen stream to dryness. The concentrated extract is then reconstituted to a final volume of 1 mL with deionised water.

Validation of the Method

The method using Envi-Disk^{TM} octadecyl (C_{18}) disks in the extraction step was validated with 250 mL pond water spiked with 1 µg of a mixture containing dicrotophos, monocrotophos and phosphamidon while another batch contained 0.5 µg of the same compounds as in the previous mixture. Thus, after dilution in 250 mL of water would give a 250 dilution factor giving 4 ppb and 2 ppb respectively. Accuracy expressed as recovery (in %), precision (reproducibility, % RSD), obtained from three repetitions of the analysis in different series of the samples.

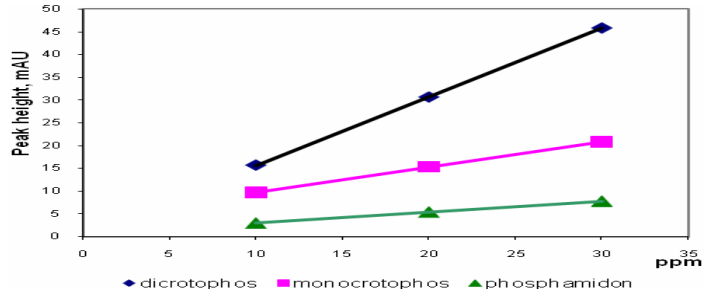
Results and Discussion

Calibration Lines, r^2 and LODs of Stacking with Reverse Migrating Micelles

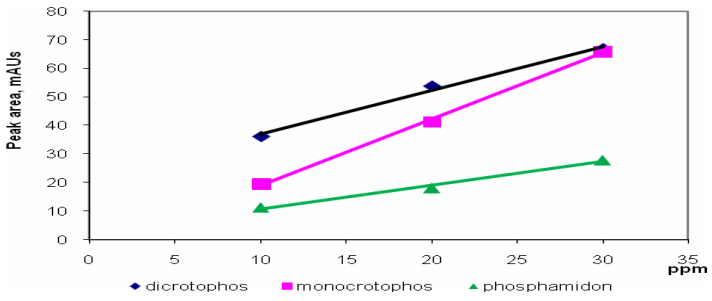
LODs were calculated based on peak area and peak height using calibration curves obtained from three standard mixtures (10, 20 and 30 ppm). The calibration curves based on peak areas and peak heights are shown in Figure 1A and Figure 1B and their respective linear equations, r^2 and LODs are shown in Table 1. Three electropherograms from each mixture of OPPs are shown in Figure 2.

Table 1: Equation of calibration curves, r^2, LODs (for S/N = 3) on the basis of calibration curves in Figure 1.

		Dicrotophos	Monocrotophos	Phosphamidon
Peak Area	Equation	y = 1.5331x + 21.69	y = 2.3252x - 4.3489	y = 0.8289x + 2.534
	r^2	0.9926	0.9987	0.9896
	LOD, ppm	3.66	1.50	4.35
Peak Height	Equation	y = 1.5036x + 0.6266	y = 0.5592x + 4.1214	y = 0.2402x + 0.5414
	r^2	1	= 0.9999	1
	LOD, ppm	0.15	0.39	0.22



(A)



(B)

Figure 1: Calibration curves based on (A) peak height and (B) peak areas for the separation of hydrophilic OPPs in SRMM-MEKC. Separation buffer contained 20 mM phosphate (pH 2.3), 10 mM SDS and 10% v/v methanol; applied potential -25kV; hydrodynamic sample injection for 50 s at 50 mbar.

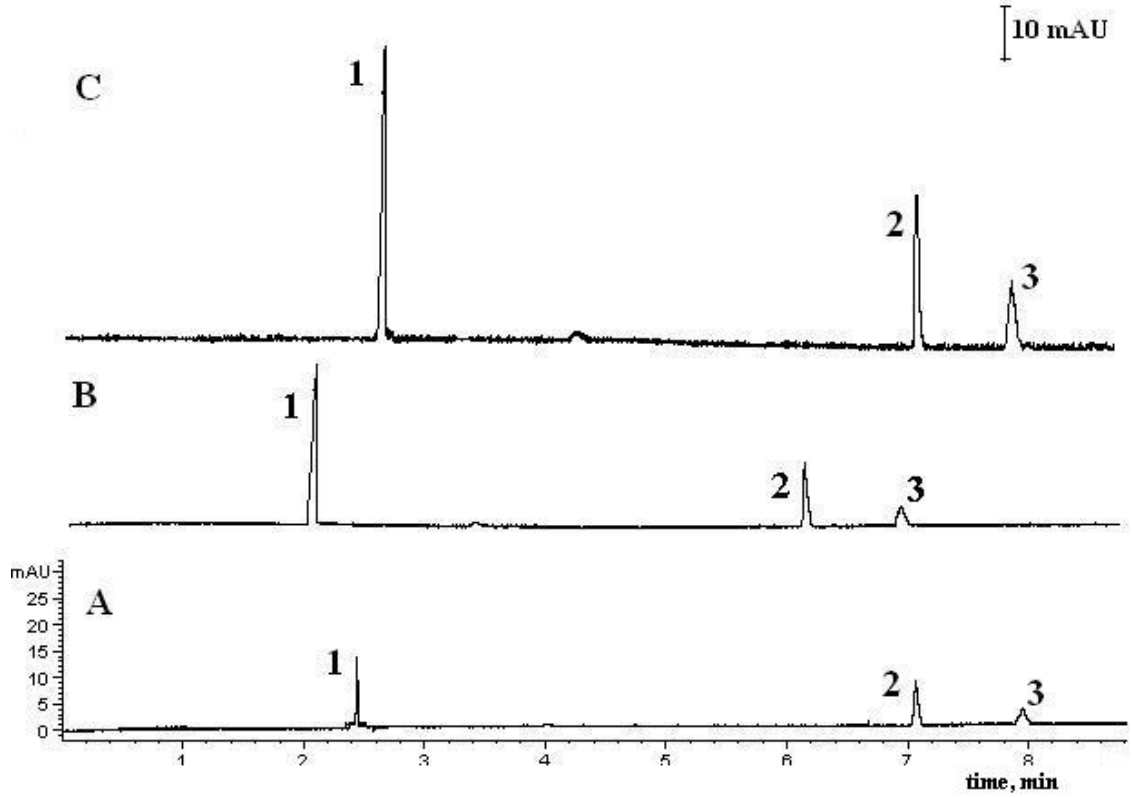


Figure 2: Separation of hydrophilic OPPs at mixture (A) 10 ppm, (B) 20 ppm and (C) 30 ppm for (1)dicrotophos, (2) monocrotophos and (3) phosphamidon. Separation conditions remain the same as in Figure 1.

Based on Table 1, this shows that the linearity and LOD for all analytes based on peak areas were much poorer as compared to peak height. High LODs may be attributed to lower regression values as compared to those obtained using peak heights which maybe due to the analytes being prone to peak broadening. Therefore in further studies, calibration curves based on peak heights would be used. Dicrotophos gave the lowest LOD (0.15 ppm) while monocrotophos gave the highest LOD (0.39 ppm). The LODs remained in the sub-ppm region thus off-line preconcentration is needed in order to improve on sensitivity.

Reproducibility, N

RSDs of migration time, peak height and peak areas are given in Table 2. The RSDs for all analytes for migration time, peak height and peak area all fall within the reasonable range below 5%. This shows that SRMM under acidic conditions is stable and gives good reproducibility. A study conducted by Quirino *et. al.* [9] showed SRMM giving poor reproducibility in the range of 5-7% for migration time which was due to difference in local electroosmotic flow between the sample zone and background solution (BGS) zone. In this study, this issue did not cause a major problem in the migration time reproducibility.

Table 2: Intraday RSD of migration time (min), peak area (mAUs) and peak height (mAU) in the separation of dicrotophos, monocrotophos and phosphamidon at three replicates each at 10 ppm.

ANALYTES	RSD, n=3		
	Migration time	Peak area	Peak Height
Dicrotophos	1.10	4.36	2.14
Monocrotophos	1.40	4.79	4.68
Phosphamidon	1.71	3.72	1.42

The maximum concentration of individual pesticides in drinking water allowed by the European Community legislation is 0.1 µg/L [17]. Therefore, analyte preconcentration prior to MEKC determination is required in order to reach sensitivity below the legal limits. To provide a reference value, blank pond water was passed through the SPE discs too and no peak signals were detected. Figure 3 is the electropherogram of blank runs and spiked pond water (1 µg and 0.5 µg).

Based on Table 3, the spiking at higher concentration (1.0 µg) gave better recovery for the three analytes in the range of 96.2-132%. While at 0.5 µg the recovery was lower in the range of 74-117%. Based on the results, this proves that SPDE is

sufficient for quantitative analysis at low concentrations as after being diluted in 250 mL of water, the concentration of the spiked analytes were in the ppb range 2-4 ppb. The repeatability of the extraction was higher for lower concentrations in the range of 10-12%RSD as compared to higher concentration within 0.81-5.36%RSD. The preconcentration factor calculated from spiking into 250 mL of pond water to reconstituting to 1 mL is 250. Thus the LOD for the three analytes has been reduced by 250 times with dicrotophos (0.60 ppb), monocrotophos (1.56 ppb) and phosphamidon (0.88 ppb). The limit attained with off-line SPDE in combination with SRMM-MEKC is still not sufficient for OPPs analysis for water samples set by European Commision (0.1 µg/L). however, this is the first study on hydrophilic OPPs using SRMM-MEKC and further studies in reducing the LOD needs to be investigated but beyond the scope of the current work.

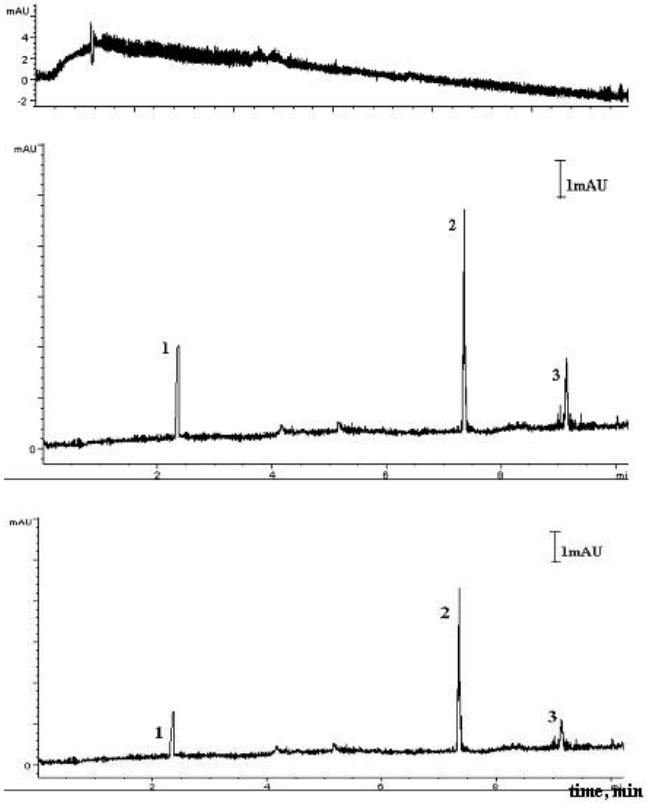


Figure 3: Electropherogram of extracted blank pond water (A) and of dicrotophos (1), monocrotophos (2) and phosphamidon (3) at (B) 0.5 ppm and (C) 1 ppm which is the concentration after reconstituting to a final volume of 1 mL. Separation buffer contained 20 mM phosphate (pH 2.3), 10 mM SDS and 10% v/v methanol; applied potential -25kV; hydrodynamic sample injection for 50 s at 50 mbar.

Table 3: Recovery and repeatability of extraction

Pesticide	Spike µg	Mean µg	Recovery (%)	Repeatability (%RSD)
Dicrotophos	0.50	0.37	74.0	10.0
	1.0	0.96	96.2	4.7
Monocrotophos	0.5	0.56	112.0	12.0
	1.0	1.04	104.4	0.81
Phosphamidon	0.5	0.59	117.0	10.9
	1.0	1.32	132.0	5.36

Conclusion

SPE discs have been successful in the quantitative extraction of three hydrophilic OPPs from water samples with pond water as the sample matrix for method validation purposes with a total extraction time of 35 minutes with recoveries in the range of 74-117% at the lowest spiking concentration. The combining of SPE disc extraction with SRMM-MEKC has successfully detected both analytes at very low concentrations by lowering their detection limits by 250 times. Furthermore, the usage of SPDE is sufficient for quantitative analysis at low concentrations whereby the concentration of the spiked analytes were in the range of 2-4 ppb.

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