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Simultaneous Determination of Omethoate and Dichlorvos by Capillary Electrophoresis

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Abstract A capillary electrophoresis method has been developed for the analysis of omethoate and dichlorvos. Response surface methodology (RSM) was used to optimize the separate conditions for simultaneous detection of omethoate and dichlorvos. The results indicated that the optimal conditions for omethoate and dichlorvos determination were; pH 7.64, SDS concentration 67.5 mM, separation voltage 19.0 KV. Quantitative linear ranges were 1.0–300 μg/mL for omethoate and dichlorvos with correlation of 0.9967 and 0.9965, respectively. The limits of detection were 0.046 μg/mL and 0.031 μg/mL for omethoate and dichlorvos, with relative standard deviations (R.S.D.) <3.6%. The value of recoveries for omethoate and dichlorvos were arranged from 94.1% to 106.0%.

Keywords Capillaryelectrophoresis (CE) · Omethoate · Dichlorvos · Response surface methodology (RSM) · Determination

Organophosphorus pesticides (OPs) are essential in modern agriculture, and are widely used to control pests and to increase harvest productivity. However, the extensive use of pesticides has badly impacted on the environment and human health. High-level exposure to these neurotoxins results in acetylcholine accumulation, which interferes with muscular responses, leading to the possibility of death (Ang et al. 2005). The poisoning caused by OPs accounts for 73% of total pesticides poisonings in China, while omethoate and dichlorvos occupied the majority of them (Huang 2001). For this reason, the level of these compounds needs to be monitored priority so that appropriate measures can be taken. Many methods have been developed in the last few years for the determination of OPs, such as gas chromatography (GC) (Xiao et al. 2006), high-performance liquid chromatography (HPLC) (Tomás et al. 2005), ion mobility spectrometry (Mohammad 2006), spectrophotometric (Mathewa et al. 2007) and biosensor (Lin et al. 2006). Among them, GC and HPLC have become the classical methods that were widely used. GC has the advantage of high sensitivity and selectivity for pesticides analysis, has high resolving power and ability to resolve individual analytes. Likewise, many HPLC analysis methods for OPs determination have been reported in the literature over the past 20 years or more (Rial-Otero et al. 2007). However, GC is inadequate to analyze for polar, thermo-labile and low-volatility compounds. For example, dichlorvos cannot be analyzed without a preliminary derivatization step (Shigeaki et al. 2007). Meanwhile, most

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of HPLC require multi-step sample preparation and complicated procedures. The high price of detector along with their expensive maintenance considerably boots the expansion of the usage of HPLC. For such cases CE became an alternative for OPs determination.

CE yields high-speed, high-resolution, low consumption of sample and reagents, and has the capable of automation and miniaturization. CE has been applied to a number of biological separation problems, but only a few articles report applications to pesticides or environmental samples (Chang and Liao 2002; Ana et al. 2005; Cheng et al. 2007; Javier et al. 2005; Carlos et al. 2006; Pérez-Ruiz 2005). To our knowledge, there were few reports for simultaneous determination of omethoate and dichlorvos by CE. The aim of this study was to develop a rapid, sensitive and quantitative assay for the simultaneous determination of omethoate and dichlorvos by CE. Optimization of parameters by RSM and validation of this method were investigated, and the application of analyzing these two OPs in soil was demonstrated.

Materials and Methods

All experiments were performed using a Beckman P/ACE MDQ capillary electrophoresis system equipped with a diode array detector. Separation was carried out on a 42.8 cm long fused-silica capillary (36 cm effective length to the detector), 75 μm internal diameter and 375 μm of external diameter. To optimize the separation and to obtain a more stable baseline, ca. 1 mm of the polyimide coating was removed by burning both ends of the capillary and then polishing them.

All reagents were of analytical reagent grade and ultrapure water was used during all the process. Pesticide standards dichlorvos (National Standard Number of China: GSB G23023-92) and omethoate (National Standard Number of China: GSB G23013-92), were obtained from Agro-Environmental Protection Institute of Ministry of Agriculture (Tianjin, China).

Stock standard solutions (100 μL/mL) of omethoate and dichlorvos were prepared by dissolving in acetone. These solutions were stored in the dark at 4°C. Working solutions of lower concentration were freshly obtained by dilution with acetone. Borate, phosphate, citrate, acetate and barbitone buffers were prepared by dissolving these reagents in ultrapure water and adjusting to the desired pH with sodium hydroxide or hydrochloric acid solutions. All solutions were filtered through a 0.45 μm membrane filter, and then degassed by sonication and evacuation.

A 10 g portion of the soil sample, 5 g of sodium chloride and 50 mL of acetonitrile were homogenized together at 12,000 rpm for 1.0 min in a homogenizer. Then 10 g of anhydrous sodium sulfate was added and the mixture was shaken for ~60 min. A 10 mL sample of the extraction solution was transferred into a Xask and concentrated to near-dryness in a rotary-evaporator at 35°C, then dissolved in 5 mL of acetone (Song et al. 2007).

In the present study, we used Box-Behnken design (Box et al. 1978; Souza et al. 2005) to estimate three most important factors for omethoate and dichlorvos separation. The design was carried out in a randomized sequence, and the migration times and peak widths were measured. Resolution (Rs) was used to assess the optimization of the experimental result.

Results and Discussion

Five different buffers were tested: borate, phosphate, citrate, acetate and barbitone, all adjusted to the pH range 6.0–8.5. Since phosphate buffer provided the best results as regards peak symmetry and differences in the migration times, this buffer was selected for further studies.

The migration times for the two OPs obtained using a running electrolyte containing 20 mM phosphate buffer and 60 mM SDS decreased with increasing pH. Meanwhile, it was noted that the migration order for the two pesticides remained unchanged throughout the range of pH values examined. The best results in terms of separation were achieved at pH 7.6 when resolution of the critical pair was the greatest.

Surfactants SDS have proved to be the best surfactant for OPs separation (Pérez-Ruiz 2005). To determine SDS concentration on OPs separation, SDS concentration range 10–80 mM was added to the phosphate buffer.

The migration time of each pesticide increased with increasing SDS concentration because of stronger interaction with micelles. Meanwhile, the resolution of the two pesticides increased with the increment of the SDS concentration, shown in Fig. 1. The resolution reached the

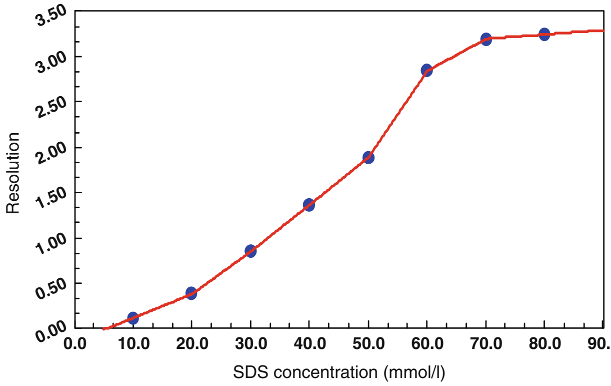

Fig. 1 The relationship between SDS concentration and the resolution

greatest when the SDS concentration was 80 mM. The SDS concentration selected in the background electrolyte was 60 mM, which gave the best base line separation for the two OPs.

The effect of applied voltage on the determination of the two OPs was studied at a constant temperature of 25°C and using an electrophoretic solution containing 60 mM SDS and 20 mM phosphate buffer at pH 7.6. In these experiments, we evaluated the effects of voltage from 5 to 25 kV.

An increase in applied voltage led to shorter migration times, sharper peaks and higher efficiencies. However, high voltage led to the short resolution for omethoate and dechlorvos separation, shown in Fig. 2. The best separation was achieved with a voltage of 20 kV (current, 94.5 µA).

Based on above experiments, buffer pH, SDS concentration and separation voltage were delineated as crucial variables with significant effects on the two OPs separation. RSM was used to establish the optimum conditions for OPs separation. The experimental design and statistical analysis were performed by using SAS software 8.2 (SAS Institute, Cary, NC, USA). Experiments were based on Box-Benhnken design with quadratic model employed to study the combined effect of three independent variables, i.e. pH, SDS concentration and voltage. The three variables pH (X_1), SDS concentration (X_2) and voltage (X_3) studied at low, middle and high levels, were designated as, −1, 0 and +1, respectively. A description of these three variables is given in Table 1. The relation between the coded values and actual values were: $X_1 = (A-7.6)/0.4$, $X_2 = (B-60)/20$, $X_3 = (C-20)/5$.

For the three-level three-factorial Box-Behnken experimental design, a total of 15 experimental runs, shown in Table 2 are needed. Data from Box-Benhnken design were analyzed by using SAS System to yield analysis of variance (ANOVA). Regression analysis were conducted for fitting the model and to examine the statistical significance of the model terms. The results of the second order response surface model fitting in the form of ANOVA were given in Table 3.

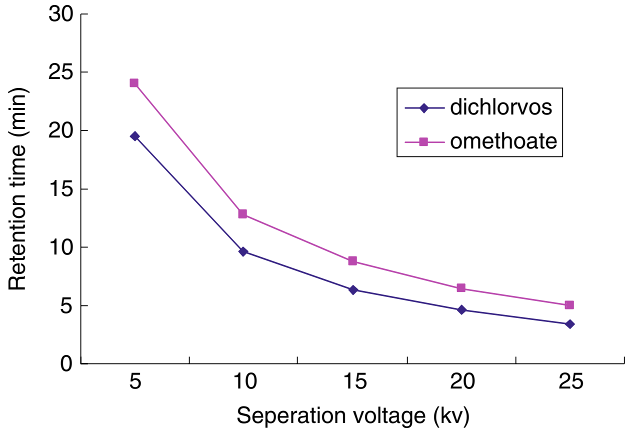

Fig. 2 The relationship between separation voltage and retention time

Table 1 Levels and codes of Box-Behnken experimental design

Factor	Code		Code level		
	Code	None code	−1	0	1
Buffer pH	X_1	A	7.2	7.6	8.0
SDS concentration (mmol/L)	X_2	B	40	60	80
Separation voltage	X_3	C	15	20	25

Table 2 Experimental values of Box-Behnken design

Runs	Factors			Rs
	A	B	C	
1	−1	−1	0	1.254
2	−1	1	0	2.251
3	1	−1	0	1.299
4	1	1	0	2.364
5	0	−1	−1	1.368
6	0	−1	1	1.356
7	0	1	−1	2.812
8	0	1	1	2.675
9	−1	0	−1	2.132
10	1	0	−1	2.754
11	−1	0	1	1.927
12	1	0	1	2.013
13	0	0	0	3.262
14	0	0	0	3.263
15	0	0	0	3.263

Table 3 displayed the retrogressive mean square analysis. The probability was 0.00154 ($p < 0.01$), which indicated that the prominence and reliability of retrogressive equation were significant. High value of R^2 (0.9761) indicated a high dependence and correlation between the observed and the predicted values of response.

The surface and contour plots of resolution versus the interactive effect of pH, SDS concentration and voltage were presented in Fig. 3. The shapes of the response surfaces and contour plots indicate the nature and the extent of the interaction between different factors. The more nearly

Table 3 Analysis of variance for the regressive model

Source	Degree freedom	Sum of square	Mean square	*F*-value	Probability >F	R^2 value
Model	9	7.3054	0.8117	22.7192	0.00154	0.9761
Error	5	0.1786	0.0357			
Total	14	7.4840				

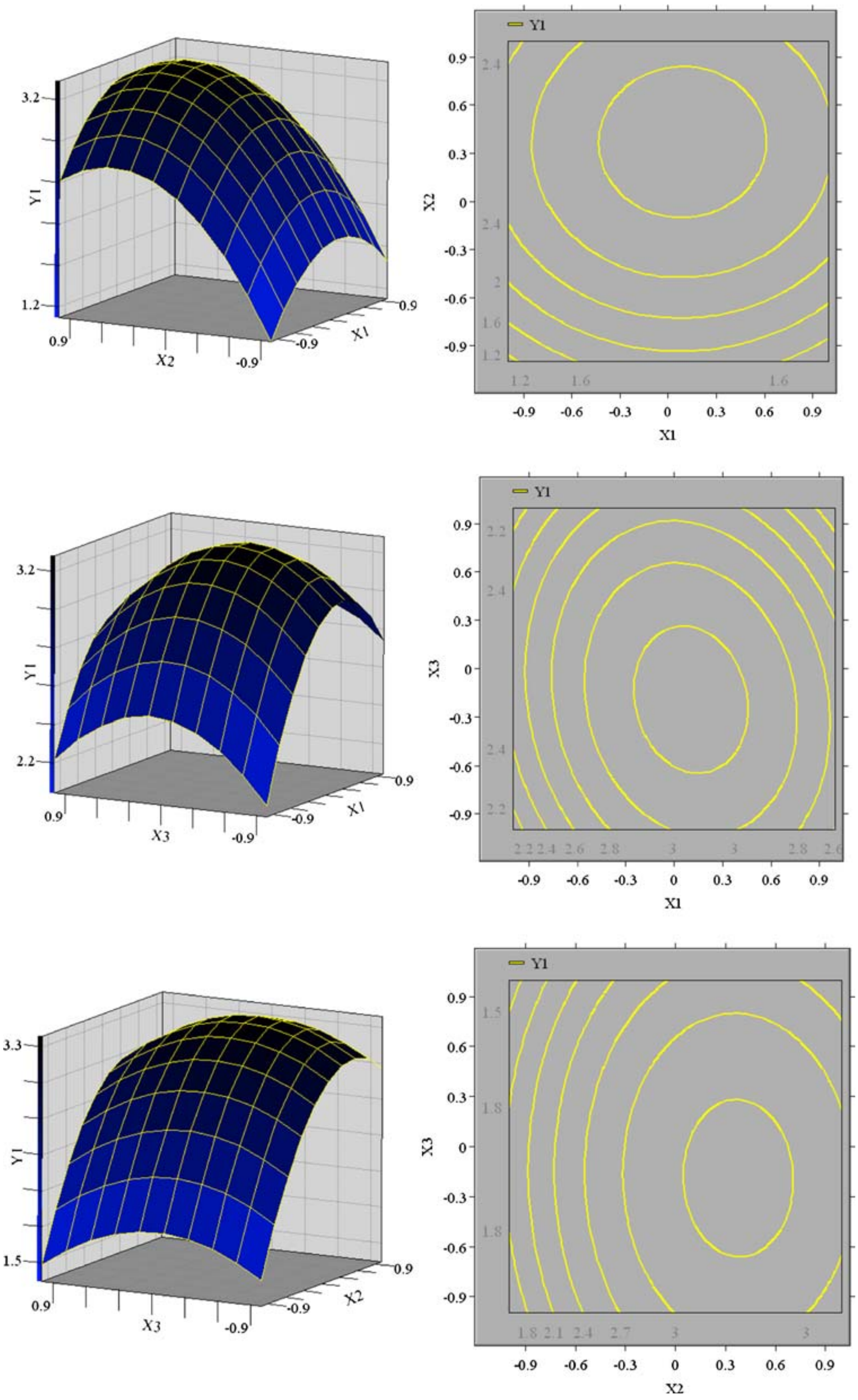

Fig. 3 The interaction between buffer pH, voltage and SDS concentration

linear the response, the smaller the magnitude of interactions and vice versa. Less prominent or negligible interactions are shown by the circular nature of the contour plots, while prominent interactions are shown by the elliptical nature of the contour plots. Therefore, the non-elliptical nature of these contour plots showed that there was no interaction between buffer pH and SDS concentration. Meanwhile, the interaction effects of pH and voltage, SDS concentration and voltage were significant.

By applying multiple regression analysis on the experimental data, the experimental results were fitted with a second-order polynomial equation. Thus, a mathematical regression model for resolution fitted in the coded factors was given as following:

$$\begin{aligned} Y = {} & 3.262667 + 0.10825X_1 + 0.603125X_2 - 0.136875X_3 \\ & - 0.658458X_1X_1 + 0.017X_1X_2 - 0.134X_1X_3 \\ & - 0.812208X_2X_2 - 0.03125X_2X_3 - 0.397708X_3X_3 \end{aligned}$$

Then explain the partial differential coefficient to X_1, X_2, X_3, respectively, three equations were gained as below.

$$0.10825 - 1.316916X_1 + 0.017X_2 - 0.134X_3 = 0$$
$$0.603125 + 0.017X_1 - 1.624416X_2 - 0.03125X_3 = 0$$
$$-0.136875 - 0.134X_1 - 0.03125X_2 - 0.795416X_3 = 0$$

From the above three equations we known the extreme point: pH was 7.64 ($x_1 = 0.10792$), SDS concentration was 67.5 mM ($x_2 = 0.37636$), separation voltage was 19.0 KV ($x_3 = -0.20505$). Under this condition the theoretical resolution value was 3.379. Then three demonstrative experiments were completed and the value of resolution was 3.384, 3.383 and 3.384, respectively (shown in Fig. 4). It illuminated that this model was of reliability.

The assay was calibrated in the range of 1.0–300 µg/mL both for omethoate and dichlorvos (at least 10 samples covering the whole range of concentrations were used). External calibration was used to obtain the linear regression equations and coefficients of correlation (presented in Table 4). The linearity curves were defined by the following equations: $y = 13415.2x + 153$, $R^2 = 0.9967$ for omethoate and $y = 6752.4x - 528.7$, $R^2 = 0.9965$ for dichlorvos, where y was the peak area of the analyte and x the pesticide concentration expressed in µg/mL. The satisfactory coefficients of correlation confirmed that the omethoate and dichlorvos responses were linear over the concentration range studied.

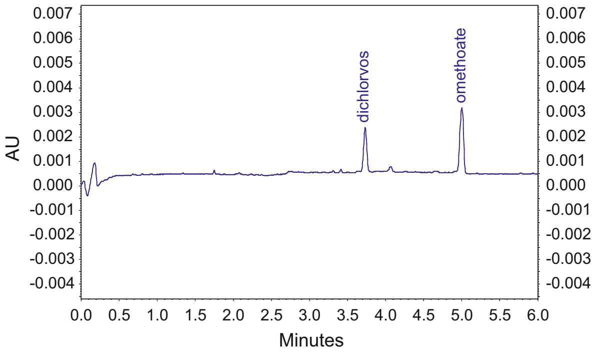

Fig. 4 Capillary electrophoresis of the omethoate and dichlorvos standards (1.0 µg/mL) under the optimum conditions (pH 7.64, SDS concentration 67.5 mM, separation voltage 19.0 KV)

The precision of the proposed method is expressed in terms of relative standard deviation (R.S.D.). The intra-day precision was tested with six repeated injections of two sample solutions containing the analytes at two concentration levels. The R.S.Ds. for the peak area were 2.87% and 3.08% at the 2.0 µg/mL level and 1.96% and 1.86% at the 4.0 µg/mL level for omethoate and dichlorvos, respectively. The inter-day precision of the method was studied by analyzing the identical samples at the 10.0 µg/mL level, injected three times every day, on 5 consecutive days. The R.S.Ds. for the peak area were 3.54% for omethoate and 2.52% for dichlorvos, which demonstrated that this method was of good repeatability.

The LOD was evaluated from five independent samples, which were spiked to produce a peak height for each of these compounds close to three times the base line noise (Baker 1995). The LODs for omethoate and dichlorvos, on the basis of a signal-to-noise ratio of 6 (S/N = 6), were 0.046 µg/mL for omethoate and 0.031 µg/mL for dichlorvos (shown in Table 4).

Limit of detection was estimated according to six times of signal-to- noise.

In order to test the accuracy of the proposed method, omethoate and dichlorvos standard solutions were added into the soil. These samples were analyzed using the sample preparation and electrophoretic procedures described above. As observed in Table 5, satisfying results were achieved. Recoveries were calculated against the external standards at the lower and upper concentrations injected in a sequence for each sample.

Soil was sprayed with omethoate and dichlorvos, and then detected according to the preparation procedure 2 h later. Typical electropherogram of the sample was shown in Fig. 5. The contents of omethoate and dichlorvos were determined by external standard method and were calculated as 1.782 and 1.171 µg/mL, respectively.

This paper described the simultaneous determination of omethoate and dichlorvos by CE. The best approach

Table 4 The regression equations and the detection limits

Analytes	Regression equation	Correlation coefficient	Linearity	Limit of detection
Omethoate	$Y = 153 + 13415.2x$	0.9967	1.0–300 µg/mL	0.046 µg/mL
Dichlorvos	$Y = -528.7 + 6752.4x$	0.9965	1.0–300 µg/mL	0.031 µg/mL

Table 5 Recoveries of omethoate and dichlorvos at different levels

Analytes	Added amount (μg/mL)	Found amount (μg/mL)	Recovery (%)	R.S.D. (%) (n = 5)
Omethoate	2.0	2.12	106.0	2.87
	4.0	3.87	96.7	1.96
	10.0	9.41	94.1	3.54
Dichlorvos	2.0	1.95	97.5	3.08
	4.0	4.21	105.2	1.86
	10.0	9.58	95.8	2.52

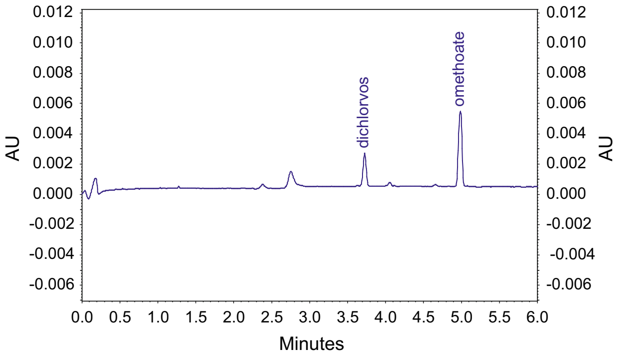

Fig. 5 Capillary electrophoresis of omethoate and dichlorvos (soil sample)

involved with 20 mM phosphate buffer (pH 7.6) and 67.5 mM SDS as electrophoretic electrolyte. Under the optimum conditions, omethoate and dichlorvos were separated completely and good linearity, reproducibility and recovery results were obtained. Their LODs were 0.046 μg/mL and 0.031 μg/mL, respectively. The efficiency of the method was demonstrated by the satisfactory results obtained in the determination of these pesticides in soil.

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