JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2004,** *52,* 5791−5795 **5791**

Application of Micellar Electrokinetic Capillary Chromatography to the Analysis of Uncharged Pesticides of Environmental Impact

ANTONIO SEGURA CARRETERO,* CARMEN CRUCES-BLANCO, SONIA CORTACERO RAMÍREZ, ALEGRÍA CARRASCO PANCORBO, AND ALBERTO FERNÁNDEZ GUTIÉRREZ

Department of Analytical Chemistry, Faculty of Science, University of Granada, Granada, Spain

A test mixture of five pesticides and metabolites (naphthalene acetamide, carbaryl, 1-naphthol, thiabendazole, and carbendazime) has been investigated by capillary electrophoresis with an ultraviolet diode array detector. These compounds were separated in <10 min by micellar electrokinetic capillary chromatography (MEKC). MEKC was performed in 30 mM ammonium chloride/ammonia buffer (pH 9.0) containing 15 mM sodium dodecyl sulfate. The lowest detection limit was obtained for the insecticide carbaryl (0.22 μg mL^{-1}) and the highest for its metabolite 1-naphthol (1.13 μg mL^{-1}). This method was applied to the analysis of the pesticides in cultivated vegetables such as cucumbers, which were extracted with a liquid−liquid extraction procedure, obtaining recovery percentages ranging from 90.1 to 110.2%.

KEYWORDS: Micellar electrokinetic capillary chromatography; pesticides; cultivated vegetables

INTRODUCTION

In recent years, capillary electrophoresis (CE) has become a versatile and powerful tool for numerous types of analysis (*1*−*4*), especially for substances that are unstable at higher temperatures. CE is a powerful microanalytical technique based on electrophoretic separation in narrow capillaries. The high speed of analysis, high resolution, and sensitivity make CE an attractive technique to separate a wide range of compounds. Initially, only charged analytes were studied by the capillary zone electrophoresis methodology (CZE) (*5*), but the high separation efficiency of CE became accessible also for the separation of neutral compounds with the introduction of another mode of operation called micellar electrokinetic capillary chromatography (MEKC) (*6*). In MEKC, an ionic surfactant is added to the background electrolyte in a concentration higher than the so-called critical micelle concentration (cmc). The most commonly used surfactant, in this mode of operation, is sodium dodecyl sulfate (SDS), which has a fairly low cmc (8 mmol L^{-1} in pure water), at which level the conductivity of the electrolyte can be kept low. Charged micelles are formed in the solution that migrate differently from the aqueous phase and thus as a pseudostationary phase. When an electric field is applied, a separation can be obtained for compounds that distribute differently between the micellar and aqueous phases.

Pesticides are important chemicals for achieving high agricultural yields, but heavy pesticide application has raised concerns about pollution of the Earth's hydrology systems, as well as the agricultural fields and the fruits and vegetables being grown under these conditions, especially in greenhouses. Thus, analytical techniques with a high degree of sensitivity and selectivity are needed to control the quality of the different types of environmental and food substances (*7*).

Gas chromatography (*8*) and high-performance liquid chromatography (*9*) have been established for years as the techniques of choice for the analysis of pesticides. However, these methods are expensive, use high volumes of toxic solvents, have high separation times, and sometimes need the development of an extremely complex gradient for the separation. For this reason the application of CE for the analysis of agrochemicals is gaining in popularity. MEKC methodology has been widely used in the determination of neutral substances such as dichlorophenols (*10*), herbicides (*11*−*14*), and fungicides (*15*).

The aim of this work is to demonstrate the applicability of MEKC to the analysis of a complex mixture of pesticides of uncharged nature using UV diode array detection. This is the first paper describing the simultaneous analysis of the five pesticides—naphthalene acetamide (NAD), carbaryl (CAR), 1-naphthol (NAF), thiabendazole (TBZ), and carbendazime (CAB)—that are used extensively in an agricultural zone in southern Spain. All common names and structures are summarized in **Table 1**.

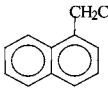
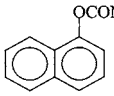
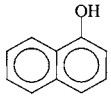
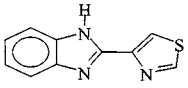
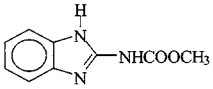
EXPERIMENTAL PROCEDURES

Reagents and Materials. All solutions were prepared with water purified by a Milli-Q^{uv} Plus system. Acetone and sodium phosphate were from Merck (Darmstadt, Germany). Ammonium chloride was from

* Address correspondence to this author at the Department of Analytical Chemistry, Faculty of Sciences, University of Granada, C/Fuentenueva s/n, 18071 Granada, Spain (e-mail ansegura@ugr.es; fax +34-58-249510).

10.1021/jf040074k CCC: $27.50
Published on Web 08/28/2004

Table 1. Names and Molecular Structures for the Five Analytes under Study

Name	Estructure
Naphthalene acetamide (NAD)	
Carbaryl (CAR)	
1-naphthol (NAF)	
Thiabendazole (TBZ)	
Carbendazime (CAB)	

Panreac (Montcada I Reixac, Barcelona, Spain). Sodium dodecyl sulfate was from Sigma (St. Louis, MO), and sodium hydroxide was from Riedel-de-Häen (Seelze, Germany).

The pesticides 1-NAF and NAD were purchased from Sigma and CAB and TBZ from Aldrich (Madrid, Spain), whereas CAR was acquired from Labor Dr. Ehrenstorfer.

Apparatus. Experiments were carried out on a Beckman P/ACE System MDQ capillary electrophoresis instrument. The system consisted of a 0−30 kV high-voltage built-in power supply, equipped with a diode array UV−vis detector and the P/ACE System MDQ software for system controlling and data handling. The separation capillary was made from bare fused silica obtained from Beckman Instruments, Inc. (Fullerton, CA), and had an internal diameter (i.d.) of 75 μm and an external diameter (e.d.) of 375 μm, with 57 cm of total length (L_T), 50 cm of effective length (L_D), and a detector window of 100 × 800 μm.

Electrophoretic Conditions. The capillary was conditioned prior to its first use by flushing with 0.5 M NaOH for 10 min, followed by 5 min of deionized water, and finally with the background electrolyte solution for 5 min. It is important to maintain a consistent electrophoretic flow (EOF) from run-to-run because any variation results in poor migration time precision. To prevent difficulties owing to column wall adsorption and to ensure a consistent EOF, the capillary was regenerated by rinsing with 0.5 M NaOH for 2 min, with ultrapure water for 2 min, and with fresh buffer for 2 min between runs.

Samples were injected by hydrodynamic injection for 10 s (0.5 psi). The electrolyte and operating voltage were varied according to the experiments. Separations were carried out at a temperature of 25 °C with 29 kV and at a wavelength detection of 228 nm. UV detection was performed at 228 and 200 nm simultaneously. Diode array detection was used over the range of 190−600 nm to achieve spectral data. Peak identification was done by comparing both migration time and spectral data obtained from standards. Peak areas at 228 nm were used for quantification of the analytes.

Stock solutions of each analyte were prepared in acetone, except CAB was prepared in *N*,*N*-dimethylformamide (DMF), at a concentration of 1000 μg mL^{-1}. Working standard solutions were prepared by diluting the residue of the stock standard solutions after evaporation by nitrogen in 30 mM ammonium chloride/ammonia and 15 mM SDS solution (pH 9). The background electrolyte NH_4Cl/NH_3 and the SDS solutions were prepared by dissolving the required amount of the substances in Milli-Q water to obtain the final concentrations indicated.

Preparation of Samples. Samples of cucumbers were purchased at the local supermarket. A liquid−liquid extraction methodology (LLE) (*16*) was applied to extract the pesticides from the samples.

A representative portion of the vegetable samples was chopped and homogenized in a food chopper (Ultra Turrax). Then, a 150 g portion was weighed and placed into a centrifuge tube and homogenized with 30 mL of acetone for 30 s using mechanical agitation by Ultra Turrax. Then, 30 mL of dichloromethane and 30 mL of petroleum ether were added to the mixture, and the homogenization was carried out for another 30 s. The tubes were centrifugated at 2000 rpm for 2 min. The resulting upper organic layer was brought to dryness in a rotary evaporator under reduced pressure and temperature of 40 °C, and the residue was redissolved in 2 mL of 30 mM ammonium chloride/ammonia and 15 mM SDS solution (pH 9)and filtered through a 0.45 μm filter before the capillary electrophoresis analysis.

Extraction recoveries were determined by fortifying fresh samples after being chopped and homogenized (150 g) with 0.01 mg of each analyte, so that 100% recovery would provide a concentration of 5 μg mL^{-1} in the final 2 mL of sample. The fortifying samples were left to rest during 1 h before application of the extraction methodology.

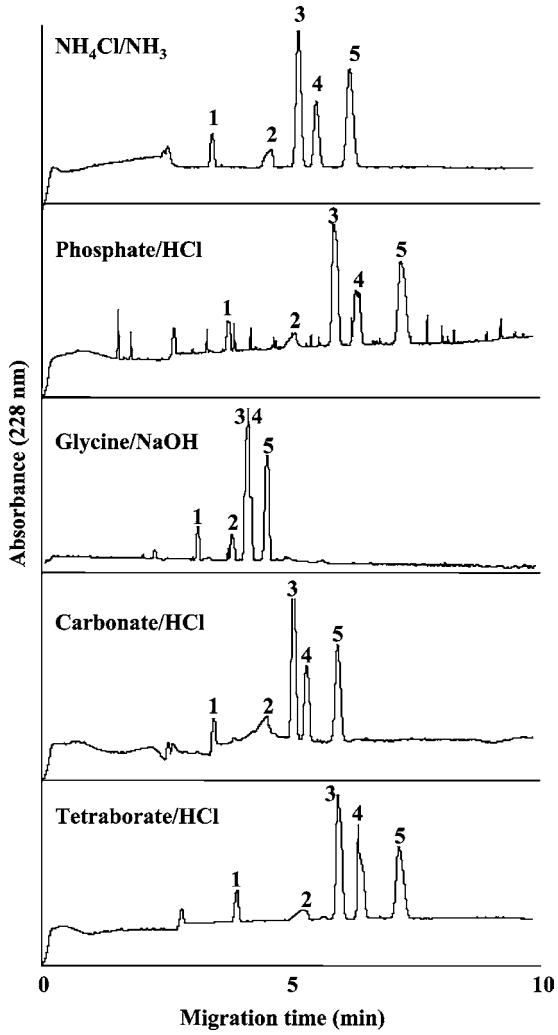


Figure 1. Electropherograms of the five pesticides (5 μg mL^{-1}) in different buffers. Operating conditions: 29 kV, 25 °C, 10 s injection, and 15 mM SDS. Peaks: (1) CAB; (2) TBZ; (3) NAD; (4) NAF; and (5) CAR.

RESULTS AND DISCUSSION

The separation of the five uncharged pesticides was carried out using the MEKC methodology. When MEKC is employed, it is necessary to optimize the pH because it is important that the analytes are in neutral form. In this case, the pH is also an important variable because if the pH is >9, carbaryl is hydrolyzed to its major metabolite, 1-naphthol.

Different buffers, namely, tetraborate/HCl, carbonate/HCl, glycine/NaOH, phosphate/HCl, and NH_4Cl/NH_3 at pH 9, were tested (see **Figure 1**). The optimum buffer was ammonium

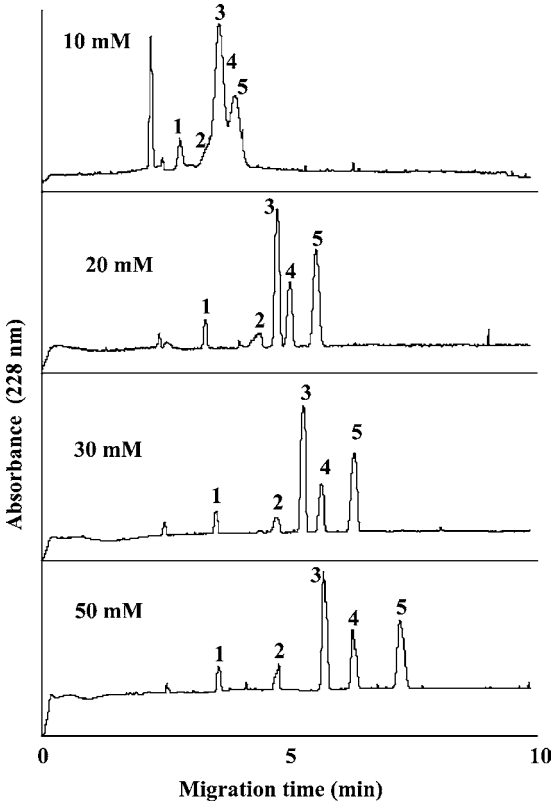


Figure 2. Electropherograms of the five pesticides (5 μg mL^{-1}) at different NH_4Cl/NH_3 concentrations. Operating conditions: 29 kV, 25 °C, and 10 s injection. Experimental variables: pH 9 and 15 mM SDS. Peak numbers are as in **Figure 1**.

chloride/ammonia because its electropherogram has a good baseline and migration times of the analytes are short.

The ionic strength was also an important parameter for the efficiency and resolution of the separation system. There are many publications concerning the influence of the buffer concentration on both electroosmotic and electrophoretic mobility in CE (*17*−*19*).

The effect of the buffer solution concentration from 10 to 50 mM on the migration time and resolution of the pesticides is shown in **Figure 2**. As can be seen, although the resolution improved with increasing buffer concentration, the operation time became longer. Higher electrical currents would result in high concentrations, and these should be avoided to suppress Joule heat generation (*20*, *21*). Hence, a NH_4Cl/NH_3 buffer concentration of 30 mM was adopted for the measurements.

The five pesticides used in this study are uncharged at pH 9.0. Therefore, it was not possible to separate them by employing CZE. MEKC using SDS as micelles was investigated for separating the five pesticides. The effect of the SDS concentration from 0 to 30 mM on the migration time and resolution of the pesticides is shown in the **Figure 3**. When a 30 mM NH_4Cl/NH_3 buffer with 15 mM SDS (pH 9) was used, baseline separation of the uncharged pesticides was optimized.

The electric field applied across the capillary had to be chosen carefully to minimize band broadening effects. The electric field is defined as the applied voltage divided by the total capillary length. Both the electrophoretic migration velocity and electroosmotic flow velocity are directly proportional to the electric field. Highest field strengths would bring about the shortest analysis times, but Joule heat production could limit the application of high field strengths. The influence of the applied voltage on the separation is summarized in **Figure 4**. As

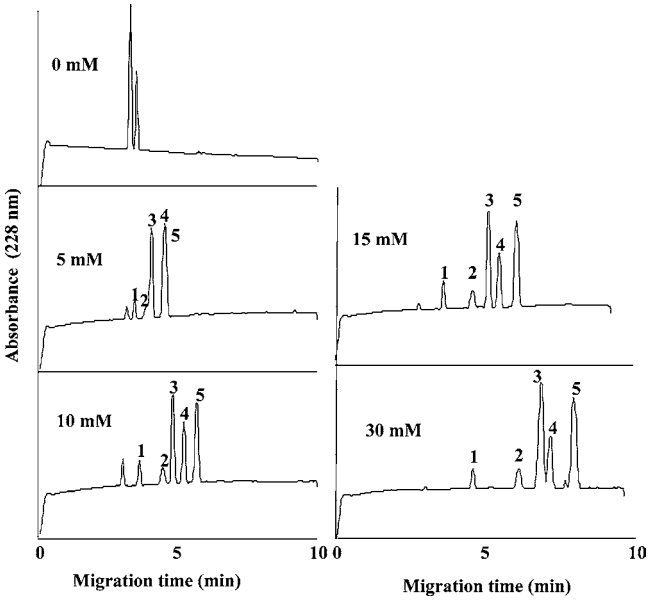


Figure 3. Electropherograms of the five pesticides (5 μg mL^{-1}) at different SDS concentrations. Operating conditions: 29 kV, 25 °C, and 10 s injection. Experimental variables: pH 9 and 30 mM NH_4Cl/NH_3. Peak numbers are as in **Figure 1**.

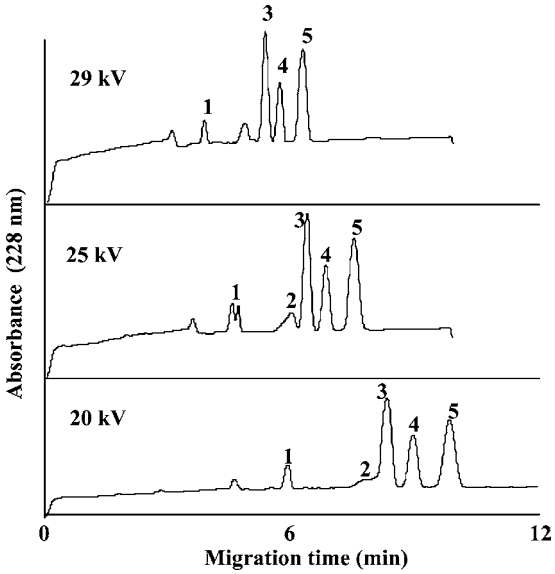


Figure 4. Electropherograms of the five pesticides (5 μg mL^{-1}) at different voltages. Operating conditions: 25 °C and 10 s injection. Experimental variables: pH 9, 30 mM NH_4Cl/NH_3, and 15 mM SDS. Peak numbers are as in **Figure 1**.

expected, when the voltage was increased from 20 to 29 kV, in general, the migration time of all the analytes decreased as well as the resolution between the peaks. However, the highest value used, 29 kV, provided a very good analysis time and reasonable resolution between peaks of the analytes under study. Therefore, this value that corresponds to a current value of 96 μA was employed in all of these studies.

Calibration graphs were obtained using liquid standards containing 2−10 μg mL^{-1} of the pesticides, to check the reliability of the method. Each point of the calibration graph corresponds to the mean value obtained from three independent area measurements. These calibration graphs showed a good correlation between peak areas and pesticide concentrations, with regression coefficients of 0.99 in all cases.

Table 2. Analytical Parameters of the Proposed Method

	CAB	TBZ	NAD	NAF	CAR
linear range (μg mL^{-1})	2.4–10	0.7–10	2.7–10	3.8–10	0.7–10
detection limit (μg mL^{-1})	0.7	0.2	0.8	1.1	0.2
quantification limit (μg mL^{-1})	2.4	0.7	2.7	3.8	0.7
RSD (%)					
2 μg mL^{-1}	11.2	3.3	11.8	15.1	3.5
4 μg mL^{-1}	4.7	1.6	6.0	8.1	1.6
6 μg mL^{-1}	3.4	1.0	3.8	5.5	1.0
8 μg mL^{-1}	2.6	0.8	3.0	4.2	0.8
10 μg mL^{-1}	2.2	0.7	2.6	3.5	0.7

The limits of detection (LOD) and quantitation (LOQ) were calculated from the experiments carried out by Cuadros et al. (*22*) and were below 1.1 and 3.8 μg mL^{-1}, respectively, for the different analytes under study. Linear ranges of the calibration curves were studied by a series of injections of standard mixtures containing different concentrations of pesticides. The different performance parameters for the five pesticides under study are summarized in **Table 2**.

APPLICATIONS

CAB, TBZ, NAD, NAF, and CAR were selected to measure their presence in vegetable samples of cucumbers. The samples were fortified at a 0.066 mg kg^{-1} level of each pesticide by adding 10 μL of a solution of 1000 mg L^{-1} of each pesticide over 150 g of cucumber, and the extraction procedure was applied. One hundred percent recovery would result in a concentration of 5 μg mL^{-1} in the final 2 mL of prepared sample.

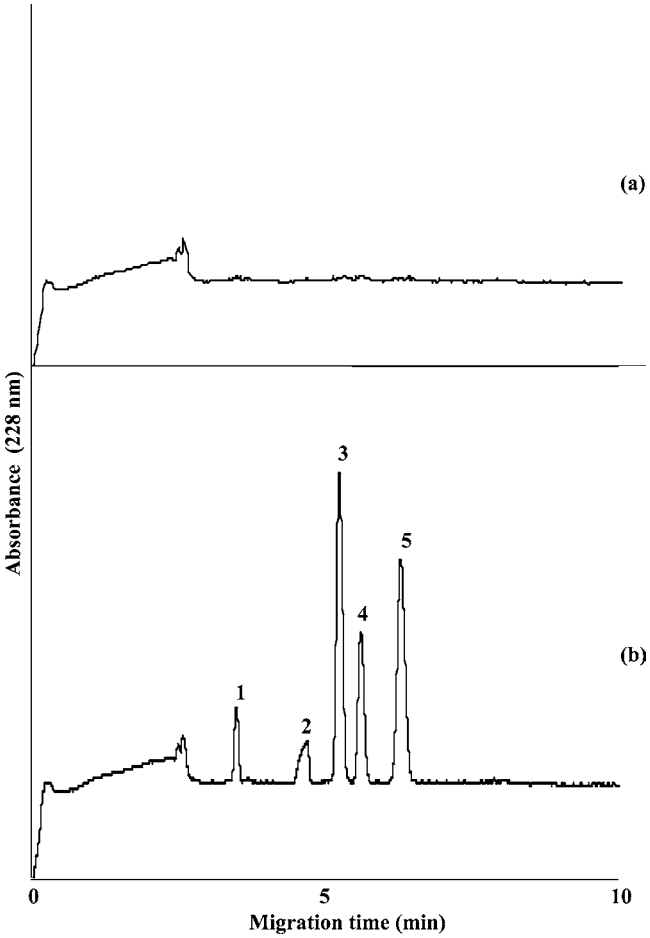


Figure 5. Electropherogram of (**a**) unfortified cucumber sample and (**b**) cucumber fortified with the five pesticides at 0.066 mg kg^{-1} each. Experimental conditions: 29 kV, 25 °C, 10 s injection, pH 9, 30 mM NH_4Cl/NH_3, and 15 mM SDS. Peak numbers are as in **Figure 1**.

Table 3. Recovery Values (*R*) and Relative Standard Deviations (RSD) of Pesticides in Cucumber Samples

pesticide	*R* (%)	RSD (%)
CAB	110.2	6.7
TBZ	91.7	7.0
NAD	98.9	6.3
NAF	90.1	9.5
CAR	103.0	6.5

An electropherogram of a fortified cucumber sample is shown in **Figure 5**. An electropherogram of the cucumber sample before fortification was recorded, but no peaks were obtained. The recovery values obtained for seven samples can be observed in **Table 3**.

LITERATURE CITED

(1) Deyl, Z.; Miksik, I.; Tagliaro, F. Advances in capillary electrophoresis. *Forensic Sci. Int.* **1998**, *92*, 89−124.
(2) Tagliaro, F.; Manetto, G.; Crivellente, F.; Smith, F. P. A brief introduction to capillary electrophoresis. *Forensic Sci. Int.* **1998**, *92*, 75−88.
(3) Valcárcel, M.; Arce, L.; Ríos A Coupling continuous separation techiniques to capillary electrophoresis. *J. Chromatogr. A* **2001**, *924*, 3−30.
(4) Dinelli, G.; Bonetti, A.; Catizone, P.; Galleti, G. C. Separation and detection of herbicides in water by micellar electrokinetic capillary chromatography. *J. Chromatogr. B* **1994**, *656*, 275−280.
(5) Jorgenson, J. W.; Lukacs, K. D. Zone electrophoresis in open-tubular glass-capillaries. *Anal. Chem.* **1981**, *53*, 1298−1302.
(6) Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. Electrokinetic separations with micellar solutions and open-tubular capillaries. *Anal. Chem.* **1984**, *56*, 111−113.
(7) http://www.mcx.es/plaguicidas/ingles.asp.
(8) Van der Hoft, G. R.: van Zoonen, P. *J. Chromatogr. A* **1999**, *843*, 301−322.
(9) Hogendoorn, E.; van Zoonen, P. *J. Chromatogr. A* **2000**, *892*, 435−453.
(10) Lin, C. E.; Lin, W. C.; Chiou, W. C. Migration behaviour and selectivity of dichlorophenols in micellar electrokinetic capillary chromatography—Influence of micelle concentration and buffer pH. *J. Chromatogr. A* **1996**, *722*, 333−343.
(11) Carabias-Martínez, R.; Rodríguez-Gonzalo, E.; Revilla-Ruiz, P.; Domínguez-Álvarez, J. Solid-phase extraction and sample stacking-micellar electrokinetic capillary chromatography for the determination of multiresidues of herbicides and metabolites. *J. Chromatogr. A* **2003**, *990*, 291−302.
(12) Quirino, J. P.; Inoue, N.; Terabe, S. Reversed migration micellar electrokinetic chromatography with off-line and on-line concentration analysis of phenylurea herbicides. *J. Chromatogr. A* **2000**, *892*, 187−194.
(13) Otsuka, K.; Terabe, S. Enanatiomer separation of drugs by micellar electrokinetic chromatography using chiral surfactants. *J. Chromatogr. A* **2000**, *875*, 163−178.
(14) Schmitt, P.; Garrison, A. W.; Freitag, D.; Kettrup, A. Application of cyclodextrin-modified miclellar electrokinetic chromatography to the separation of selected neutral pesticides and their enantiomers. *J. Chromatogr. A* **1997**, *792*, 419−429.
(15) Molina, M.; Silva, M. Rapid determination of fungicides in fruit juices by micellar electrokinetic chromatography: Use of organic

modifiers to enhance selectivity and on-column high-salt stacking to improve sensitivity. *Electrophoresis* **2000**, *21*, 3625−3633.
(16) Van Zoonen, P., and working group "Ontwikkeling van Residuanalysemethoden" ("Development and Improvement of Residue-analytical Methods"). *Analytical Methods for Pesticide Residues in Foodstuffs*, 6th ed.; Van Zoonen, P., Ed.; General Inspectorate for Health Protection, Ministry of Public Health, Welfare and Sport: Bilthoven, The Netherlands, 1996.
(17) Wright, P. B.; Dorsey, J. G. Silver(I) mediated separations by capillary zone electrophoresis and micellar electrokinetic chromatography: Argentation electrophoresis. *Anal. Chem.* **1996**, *68*, 415−424.
(18) Masselter, S. M.; Zemann, A. J. Influence of organic-solvents in coelectroosmotic capillary electrophoresis of phenols. *Anal. Chem*. **1995**, *67*, 1047−1053.
(19) Williams, B. A.; Vigh, G. Effect of initial potential ramp of the accuracy of electrophoretic mobilities in capillary electrophoresis. *Anal. Chem.* **1995**, *67*, 3079−3081.
(20) Knox, J. H.; McCormark, K. A. Temperature effects in capillary electrophoresis. 1. Internal capillary temperature and effect upon performance. *Chromatographia* **1994**, *38*, 207−214.
(21) Knox, J. H.; McCormark, K. A. Temperature effects in capillary electrophoresis. 2. Some theoretical calculations and predictions. *Chromatographia* **1994**, *38*, 215−221.
(22) Cuadros Rodríguez, L.; García Campaña, A. M.; Jiménez Linares, C.; Román Ceba, M. Estimation of performance-characteristics of an analytical method using the data set of the calibration experiment. *Anal. Lett*. **1993**, *26*, 1243−1258.

Received for review February 17, 2004. Accepted June 25, 2004. We greatly appreciate the financial support of the Acciones Coordinadas de los Grupos de Investigación y Desarrollo Tecnológico de Andalucía and the Project of the Ministerio de Ciencia y Tecnología (BQU2002-03418) and Cajamar.

JF040074K