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Comparative study of high-performance liquid chromatography and micellar electrokinetic capillary chromatography applied to the analysis of different mixtures of pesticides

Adriana Farran*, Santiago Ruiz, Carme Serra, Manuel Aguilar

Department of Chemical Engineering (ETSEIB-UPC), Diagonal 647, 08028 Barcelona, Spain

Abstract

High-performance liquid chromatography (HPLC) and micellar electrokinetic capillary chromatography (MECC) methods for the determination of different pesticide mixtures have been developed. These mixtures include triazine, phenylurea, phenoxyalkyl acid, carbamate and organophosphorous pesticides that have been selected because their use in different combinations in commercial formulations. The best results have been obtained by using mobile phase gradients in HPLC and by working with *n*-alcohols as mobile phase modifiers in MECC. When the quality parameters of both methods have been compared, it was observed that they are not sensitive enough for environmental analysis. In this sense two different concentration methodologies, off-line solid extraction by using Carbopack columns and a special injection MECC method, have been studied to enhance sensitivity. Off-line solid extraction can be used for both HPLC and MECC methodologies and permits to detect low ppb levels of individual pesticides. Special injection methods applied to the MECC system provides a 200-fold sensitivity improvement for the ionic pesticides.

Keywords: Mobile phase composition; Organic modifiers; Environmental analysis; Pesticides; Alcohols

1. Introduction

Several hundreds of pesticides of different chemical nature are widely used throughout the world. Because of their widespread use, pesticides are currently analyzed by determination of their residues in various environmental matrices, such as soil, water and air [1]. In most cases commercial formulations consist of mixtures of compounds of different chemical nature [2]. So, it is of great importance to develop analytical methods for the simultaneous determination of pesticide mixtures. At present, gas chromatography (GC) is the primary method used for pesticide multiresidue analysis, but in some cases advantages are claimed for liquid chromatography (LC), which is suitable for high-molecular-mass, polar and thermally-labile pesticides [3].

The recent development of capillary electrophoresis (CE) presented the technique as complementary and in some instances a substitute for LC. Some works [4–7] have demonstrated that CE is a feasible method for the determination of acidic herbicides, and in a recent work [8] the capacity of micellar electrokinetic capillary chromatography (MECC) for the separation of neutral and ionic herbicides was demonstrated.

Depending on the chemical nature of the solute, different problems arise when using MECC. On the one hand, there are only few interactions between the ionic compounds and the micelles; on the other hand,

*Corresponding author.

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the highly hydrophobic solutes interact strongly with the micelles. In both cases the addition of a surfactant to the CE system does not improve its separation efficiency.

One factor that can improve the separation in MECC is the addition of mobile phase modifiers [9–11]. In this sense, the influence of the addition of aliphatic alcohols to the MECC buffer has been studied recently [12].

In the present work, the capability of *n*-alcohols to enhance separation in MECC has been proved by studying two different pesticide mixtures that include triazine, phenylurea, phenoxyalkyl acid, carbamate and organophosphorous compounds (see Table 1).

Mixture A includes two anionic herbicides (2,4-D and 2,4,5-T), and mixture B includes two highly hydrophobic compounds (ethyl- and methyl-chlorpyriphos), which are not well resolved without addition of modifiers.

In order to compare the new MECC method with the known HPLC methodology, HPLC methods for the analysis of the same pesticide mixtures have been developed.

When the quality parameters of both methods were compared, it has been observed that they are not sensitive enough for environmental analysis. In this sense, two different concentration methodologies, off-line solid extraction by using Carbopack columns and special CE injection methods have been studied to enhance sensitivity. Off-line solid extraction can be used for both HPLC and MECC methodologies and allows to detect low ppb levels of individual species. Special injection methods have been applied to the MECC system, with a 200-fold sensitivity improvement for the ionic compounds.

2. Experimental

2.1. Chemicals

Atrazine, azinphos-methyl, dimethoate, diuron, 2,4-D and 2,4,5-T were purchased from Riedel-de Haën (Seelze, Germany), chlorpyriphos, methylchlorpyriphos and Simazine from Dr. Ehrenstorfer (Augsburg, Germany), and carbaryl and terbutryn from Promochem (Wesel, Germany).

Reagents for the preparation of the mobile phases and buffer solutions were: analytical reagent-grade Na_2HPO_4, NaH_2PO_4 and sodium dodecyl sulphate (SDS) from Merck (Darmstadt, Germany), 1-butanol, ammonium acetate and sodium tetraborate from Panreac (Barcelona, Spain), HPLC-grade acetonitrile from Romil (Leicester, UK) and HPLC-grade methanol from Promochem.

Cartridges with 250 mg of Carbopack B from Shandon and 500 mg of C_{18} from J and W Scientific were used for concentration experiments.

2.2. Apparatus and conditions

The HPLC system consisted of a Spectra-Physics (San José, CA, USA) model SP-8700 solvent delivery unit, an injection valve Rheodyne (Cotati, CA, USA) with a 10 μl sample loop, an UV–Vis detector (model SP-8440) and a reversed-phase Brownlee Labs ODS-224 column (220×4.6 mm I.D.).

Different ratios of aqueous solutions of 0.1 *M* ammonium acetate at pH 6 and acetonitrile for mixture A and of acetonitrile and water for mixture B were used as mobile phases. All solutions were

Table 1
Studied compounds

Mixture A		Mixture B	
Phenoxyalkyl acids	(1) 2,4 D	Organophosphorous	(7) Dimethoate
	(2) 2,4,5-T		(8) Azinphos-methyl
Phenylureas	(3) Diuron		(9) Chlorpyriphos-methyl
Triazines	(4) Atrazine		(10) Chlorpyriphos-ethyl
	(5) Simazine		
	(6) Terbutryn	Carbamates	(11) Carbaryl

degassed with helium. The flow-rate was 2 ml min^{-1}.

CE analyses were performed with an integrated system ISCO (Lincoln, NE, USA) model 3850, equipped with a fused-silica capillary column (540× 0.05 mm I.D.) and an on-column UV detector at a placed position of 40 cm from the anode. The sample was introduced into the system by vacuum injection (0.5 p.s.i.; 1 p.s.i.=6894.76 Pa) for different injection times (between 20 and 3 s, that means injection volumes of 20 and 3 nl). Voltages of 27.5 and 25 kV were applied for the separation of mixtures A and B compounds. For mixture A, the MECC experiments were performed by using phosphate buffer at pH 7 containing SDS so that, after addition of 1-butanol, the concentrations were 0.02 *M* for phosphate and 0.05 *M* for SDS. 1-butanol was added to achieve concentrations of 4 to 10% (v/v). For mixture B, an 0.05 *M* SDS–0.02 *M* borate buffer at pH 9 was used with the addition of 10–40% (v/v) methanol.

In all cases buffer solutions, mobile phases and samples were passed through a 0.45 μm filter (Millipore, Bedford, MA, USA).

The detection in HPLC and MECC experiments was performed at 225 and 200 nm for mixtures A and B, respectively.

For data processing, a Spectra-Physics integrator (SP-4270) and a Varian Star Work Station (Sunnyvale, CA, USA) were used.

3. Results and discussion

3.1. Micellar electrokinetic capillary chromatography

The first experiments with a MECC system without mobile phase modifiers resulted in an acceptable separation for all compounds with the exception of the two acidic herbicides in the case of mixture A and the two highly hydrophobic compounds in the case of mixture B. As it has been mentioned previously, the separation in MECC can be improved by the addition of aliphatic alcohols to the mobile phase [12]. Which alcohol to use depends on the problem that has to be solved. In the case of mixture A the problem are the anionic pesticides. It has been observed [12,13] that the most important effect encountered when a medium-chain alcohol is added to the mobile phase is the reduction of the micelle surface charge density, so the probability of interaction between the SDS micelle and the anionic solutes increases and their separation can be enhanced. So, the chosen alcohol for mixture A was *n*-butanol. In the case of mixture B, the problem arises with the high hydrophobic solutes. It has been observed that the most important effect obtained when a short-chain alcohol is added to the micelle is the decrease of the aqueous phase polarity. This fact facilitates the dissolution of neutral compounds in that phase and their separation can be increased. For that reason, the chosen alcohol for mixture B was methanol.

3.2. Effect of the addition of n-butanol on the separation of mixture A

n-Butanol has been added in different proportions to the mobile phase to enhance separation conditions. It has been observed that the variation in the alcohol percentage practically does not affect the elution time of neutral compounds, if they are compared to the elution time of the acidic 2,4-D and 2,4,5-T herbicides (see Fig. 1). For alcohol percentages up to 4% the retention times of 2,4-D and 2,4,5-T are lower than those of neutral compounds. This is due to the non-interaction between the anionic analytes and the micelles, so 2,4-D and 2,4,5-T move with a total velocity that is a combination of the electrophoretic velocity of the analyte and the electroosmotic effect. For alcohol percentages higher than 4% their retention times increase because the change on the properties of the system increase their probability of interaction with the micelles.

So, by controlling the percentage of added *n*-butanol to the system, the position of the anionic pesticides on the chromatogram can be selected. This behaviour is interesting because it can be used to enhance separation and to avoid interferences.

The resolution values (R_s) between both compounds, and between them and the closer phenylurea or triazine peaks have been calculated (Table 2). The

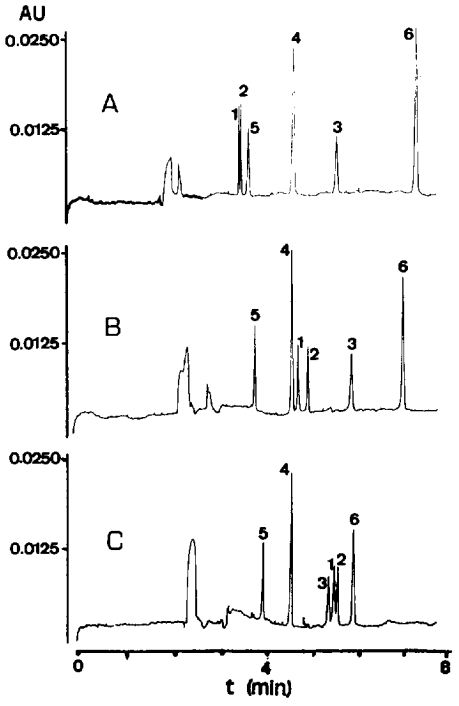

Fig. 1. MECC chromatogram of a standard solution of mixture A. Applied voltage 27.5 kV. UV detection at 225 nm. Buffer: 0.02 *M* phosphate (pH 7)–0.05 *M* SDS, with different ratios of added *n*-butanol. (A) 4% butanol; (B) 8% butanol; (C) 10% Butanol. For compound assignment, see Table 1.

best separation has been achieved with 8.8% butanol. Percentages of butanol greater than 10 were not tested because the obtained micelle breakdown [12].

Table 2
Calculated R_s values of mixture A compounds, for different percentages of *n*-butanol added to the buffer system

	% *n*-Butanol			
	4	8	8.8	10
R_{1-2}	0.77	1.80	2.00	0.6
R_{2-5}	2.13	–	–	–
R_{4-1}	–	0.90	5.00	–
R_{2-3}	–	6.23	1.80	–
R_{3-1}	–	–	–	0.7
R_{2-6}	–	–	–	4.6

For numbers assignment, see Table 1.

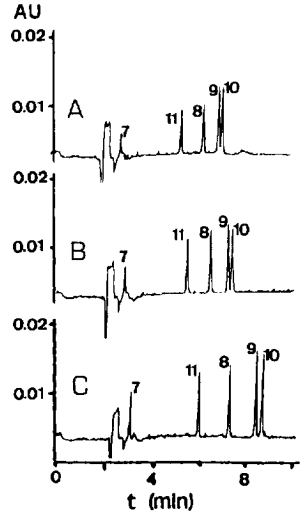

Fig. 2. MECC chromatogram of a standard solution of mixture B. Applied voltage 25 kV. UV detection at 200 nm. Buffer: 0.02 *M* borate (pH 9)–0.05 *M* SDS, with different ratios of added methanol. (A) 0% methanol; (B) 10% methanol; (C) 40% methanol. For compound assignment, see Table 1.

3.3. Effect of the addition of methanol on the separation mixture B

When methanol is added to the MECC mobile phase, it can be observed that the retention times of the neutral compounds increase with the alcohol percentage (Fig. 2). Table 3 shows the calculated values of resolution. It can be seen that the R_s values increase with the alcohol percentage for all compounds, but specially for the two most hydrophobic,

Table 3
Calculated R_s values of mixture B compounds, for different percentages of methanol added to the buffer system

	% Methanol				
	0	10	20	30	40
R_{7-11}	13	13.5	14.5	15	15.5
R_{11-8}	5	5.5	6	6.5	7
R_{8-9}	3.25	3.75	4.5	5	6
R_{9-10}	0.75	1	1.25	1.5	2

For numbers assignment, see Table 1.

methyl- and ethylchlorpyriphos, and that the best results have been obtained with 40% methanol.

So the observed results confirm that short-chain alcohols increase the dissolution of the neutral compounds in the aqueous phase, and so their separation is enhanced.

3.4. Comparison between HPLC and MECC

In order to compare the studied MECC method with the known HPLC methodology, HPLC methods for the analysis of mixtures A and B have been developed. In both cases, a C_{18} column and gradient elution have been used.

For mixture A, the mobile phase consists of a mixture of an aqueous solution of 0.1 *M* ammonium acetate (pH 6) and acetonitrile. The best results have been obtained with a 10-min gradient elution from 28 to 60% acetonitrile.

For mixture B, a water–acetonitrile mixture has been used as mobile phase. In this case, the best results have been obtained using a 10-min gradient elution from 42 to 92% acetonitrile.

The number of theoretical plates (N) and the limit of detection (LOD) have been calculated for both MECC and HPLC (Table 4).

As known, the efficiency of a system is attributable to its flow profile. Since the flow of the mobile phases are controlled differently, by a pump in HPLC and electrically in CE, the flow profiles are different. In HPLC the profile is parabolic and, as a result of its contribution to peak broadening, the separation efficiency theoretically achievable is limited. In CE, where mainly longitudinal diffusion is present, the flow profile is flat resulting in narrower peaks. When observing the N values for both techniques, it can be seen that while for the anionic compounds the value of N for MECC is between 300 and 400-times higher than for HPLC, the difference is not so important for the neutral pesticides. It is known that the addition of micelles to a CE system increase the band-broadening in the separation column, mainly due to sorption–desorption kinetics [14], so the observed differences on efficiency can be attributable to the different capacity of interaction with the micellar phase. The experimental results support this hypothesis. On the one hand there is a decrease on the efficiency of the neutral compounds that strongly interact with the micelles. On the other hand, there is a decrease on the efficiency of the anions as a result of the increase of the alcohol percentage that increases the anion–micelle interaction (see Table 5).

It is known that, when optical detectors are used, the concentration sensitivity for HPLC tends to be better than that of CE as it can be observed in Table

Table 4
Number of theoretical plates (N) and limit of detection (LOD) for HPLC and MECC techniques

	$N/10^3$		LOD (mg/l)	
	HPLC	MECC	HPLC	MECC
Mixture A compound				
2,4-D	0.3	117.0	0.33	1.04
2,4,5-T	0.4	127.0	0.24	1.04
Simazine	4.0	21.0	0.15	0.57
Atrazine	5.0	22.0	0.08	0.24
Diuron	14.0	26.0	0.37	1.25
Terbutryn	22.0	13.0	0.10	0.48
Mixture B compound				
Dimethoate	3.6	4.6	0.44	2.5
Carbaryl	3.7	16.4	0.33	0.48
Azinphos-methyl	4.6	23.7	0.50	1.13
Chlorpyriphos-methyl	12.2	31.7	0.21	1.05
Chlorpyriphos-ethyl	25.1	33.8	0.25	1.19

Table 5
MECC efficiency, calculated as number of theoretical plates ($N/10^3$), for the anionic pesticides as a function of the percentage of alcohol (*n*-butanol) added to the buffer

Anionic compund	% *n*-Butanol			
	4.0	8.0	8.8	10
2,4-D	179	154	117	64
2,4,5-T	184	166	127	83

4 where the sensitivity (LOD, calculated for a signal-to-noise ratio of 2) in HPLC is 3–5-times higher than that of MECC.

In any case, the concentration levels attainable by both methodologies (several hundred ppb) are far from the requirements of environmental analysis. Two different approaches are studied to increase concentration sensitivity, an off-line extraction and concentration technique using solid-phase extraction (SPE) for both MECC and HPLC methods, and a special injection technique such as field-amplified injection for the MECC method.

Some phenylurea and phenoxyacid herbicides have been studied with respect that two different preconcentration methods. The studies were done in both aqueous standards and spiked real water samples.

3.5. Solid-phase extraction

The ability of two sorbents for SPE of acidic and neutral pesticides, a non-specific material such as graphitized carbon black (Carbopack B), and an apolar material such as C_{18} has been investigated. The experimental set-up has been summarized in Table 6 and the obtained recovery results are shown in Table 7. Some conclusions, that agree with other published results [15–19], can be drawn. When using the C_{18} cartridge the pH of water has to be adjusted to 2, as this sorbent is unable to retain the acidic pesticides at the pH value of the water. The Carbopack B cartridges are able to extract simultaneously acidic and neutral pesticides with good recoveries, both for aqueous standards and for spiked real water samples. The recoveries are practically the same when the volume sampled was varied from 0.25 to 1 l. Fig. 3 shows the MECC chromatogram obtained for a real water sample spiked with 0.4 ppb of some phenoxyacid and phenylurea herbicides, after the

Table 6
Experimental set-up for the SPE of mixtures of pesticides from water samples

	C_{18}	Carbopack B
Conditioning	10 ml methanol+10 ml distilled water	5 ml CH_2Cl_2–water (80:20)+2 ml methanol+15 ml HCl (pH 2)
Water sample flow	20 ml/min	33.3 ml/min
Washing	7 ml distilled water	7 ml distilled water
Water removal	Centrifuge 2000 rpm+flow N_2 (1 min)	Centrifuge 2000 rpm+flow N_2 (1 min)
Elution	5 ml methanol	8 ml CH_2Cl_2–methanol (60:40) +0.016 *M* KOH
Concentration	Evaporation to dryness at 35°C under N_2 stream	Evaporation to dryness at 35°C under N_2 stream
Redissolution	100 μl 0.05 *M* SDS	100 μl 0.05 *M* SDS

When using C_{18} cartridges, water samples without and with previous acidification (pH 2) have been studied

Table 7
Recovery percentages for a mixture of phenylurea and phenoxyalkyl acid herbicides when using different SPE cartridges for two different solid phase sample volumes (250 and 1000 ml)

	Aqueous standards						Real water Carbopack
	C_{18}		C_{18} (pH 2)		Carbopack		
	250	1000	250	1000	250	1000	1000
Phenylureas	90–95	90–95	90–95	88–93	95–100	90–95	90–95
Phenoxyacids	<10	<10	85–90	75–85	95–100	90–95	90–95

Concentration of the samples: 1.6 ppb and 0.4 ppb for sample volumes of 250 and 1000 ml, respectively. Recovery results are the interval of percentage values obtained for five phenylurea and four phenoxyacid herbicides.

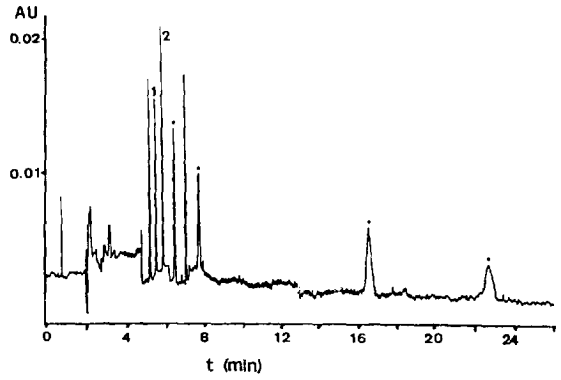

Fig. 3. MECC chromatogram obtained for a real water sample spiked with 0.4 ppb of some phenoxyacid and phenylurea (*) herbicides, after the extraction of 1 l of the sample by the Carbopack cartridge. The experimental conditions are the same as in Fig. 1, with a fixed butanol percentage of 8.8. For compound assignment, see Table 1.

extraction of 1 l of the sample by the Carbopack cartridge.

3.6. Field-amplified injection in MECC

On column sample concentration in CE, known as field-amplified injection (in the case of electrokinetic injection) or as sample stacking (in the case of hydrodynamic injection) has been extensively discussed in a series of recent papers [7,20–22]. MECC was originally conceived for the analysis of non-ionic compounds and therefore mobile phase focusing, which relies on the electrophoretic mobility of the sample ions is not applicable. However, MECC has also been shown to be useful for the analysis of ionic compounds. Some works [23,24] have investigated the possibility to perform sample stacking of ionic compounds by MECC. In the present work, the possibility to perform polarity-switching field-amplified injection in a MECC system to concentrate on-column a mixture of phenoxyacid herbicides has been studied. The following experimental procedure has been used: A very large volume of sample dissolved in water was injected electrokinetically. The voltage was switched on and the original sample matrix was pushed back from the column. At the same time, anionic compounds were focused in the boundary with the MECC buffer. The polarity was switched back to its original position when the current reached approximately 95% of its normal value at the applied voltage, and the MECC analysis was performed. Different injection times, from 35 to 480 s, were used and their effect on the peak efficiency and peak height has been investigated. The obtained results are shown in Fig. 4, where it can be observed that the peak height increases with the injection time, while the peak efficiency decreases. From the observed results, we concluded that an injection time of 120 s can be used without losing much efficiency. In Fig. 5, where the MECC chromatogram obtained for a mixture of 50 ppb of 2,4-D and 2,4,5-T injected during 120 s is shown, it can be seen that both compounds can be detected with a good resolution. When comparing these results with that obtained when working with a 5 ppm sample using normal injection conditions (20 s), it can be deduced that an enrichment factor between 100 and 200 can be easily reached. This value is higher than that obtained by sample stacking in MECC [24] and

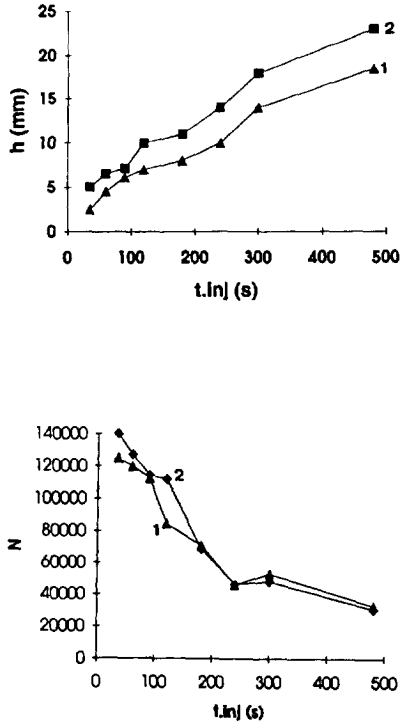

Fig. 4. Influence of injection times (t) on the peak efficiency (N) and the peak height (h) for a mixture of 100 ppb of 2,4-D and 2,4,5-T in water injected by field-amplification in the MECC system. Other experimental conditions as in Fig. 3.

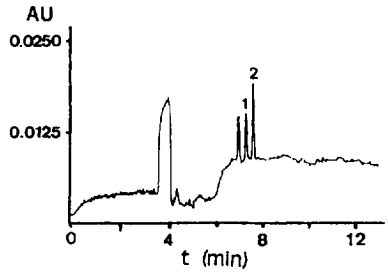

Fig. 5. MECC chromatogram for a mixture of 50 ppb of 2,4-D and 2,4,5-T obtained by field amplification. Injection time 120 s. Other experimental conditions as in Fig. 3. For compound assignment, see Table 1.

is comparable to the reported by Nielen [7] for a field-amplification injection in CZE.

Starting from these data it can be concluded that the separation of the two pesticide mixtures can be achieved with both HPLC and MECC techniques. When mixtures of neutral and ionic pesticides have to be analyzed the MECC system offers some advantages, while when samples contain mixtures of neutral pesticides HPLC will be the best election. In both cases, sample preconcentration is necessary for environmental analysis. Solid-phase extraction with Carbopack is a good choice to preconcentrate ionic and non-ionic compounds for both HPLC and MECC techniques. Field-amplification injection is a rapid and inexpensive method only applicable to the preconcentration of ionic compounds in the MECC system, but with a lower enrichment factor than SPE.

Acknowledgements

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