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Determination of pesticides in waters by automatic on-line solid-phase extraction–capillary electrophoresis

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Abstract

The separation of seven pesticides by micellar electrokinetic capillary chromatography in spiked water samples is described, allowing the analysis of pesticides mixtures down to a concentration of 50 $\mu g\ l^{-1}$ in less than 13 min. Calibration, pre-concentration, elution and injection into the sample vial was carried out automatically by a continuous flow system (CFS) coupled to a capillary electrophoresis system via a programmable arm. The whole system was electronically coupled by a micro-processor and completely controlled by a computer. A C_{18} solid-phase mini-column was used for the pre-concentration, allowing a 12-fold enrichment (as an average value) of the pesticides from fortified water samples. Under the optimal extraction conditions, recoveries between 90 and 114% for most of the pesticides were obtained.

Keywords: Environmental analysis; Water analysis; Automation; Pesticides

1. Introduction

Pesticides are important and diverse environmental and agricultural species. Their determination in pesticides formulations, in feed and food, and in complex environmental matrices (e.g., water, soil, sludge, sediments, etc.) often requires a separation method of high efficiency, unique selectivity and high sensitivity [1]. Capillary electrophoresis (CE) meets these requirement and has proved to be a suitable microseparation technique for the analysis of a wide variety of chiral and achiral pesticides. Although gas chromatography (GC) and high-performance liquid chromatography (HPLC) are the two most commonly used approaches for the analysis of pesticides [2,3], CE is capable of determining pesticides at trace levels as those usually encountered in environmental samples by combining selective pre-column derivatisation schemes, sensitive detection methods (e.g., laser-induced fluorescence detection) and trace enrichment techniques [4]. With the exception of CE–laser-induced fluorescence detection, the concentration limit of detection of CE is relatively high, a fact that hinders its use in the determination of pesticides at trace levels. However, the combination of on-line and off-line concentration procedures with selective precolumn derivatisation schemes has solved the problem of detectability and allowed CE to become one of the most suitable techniques for the

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separation and determination of pesticides in water and soil samples.

Due to the neutrality of the investigated pesticides CE has to be modified. Adding an ionic surfactant to the buffer system provides the possibility to separate both, neutral and charged analytes simultaneously. In these micellar electrokinetic capillary chromatography (MEKC) methods, the uncharged compounds are separated by different distributions between the aqueous and micellar phase. Different buffers have been described to separate several pesticides present in liquid sample. The key problem of CE separations was the selection of a suitable background electrolyte. As the solubility of triazine in water is less than 0.001 mol l^{-1}, the use of mixed methanol–water solvents for the preparation of the electrolyte was necessary to avoid precipitation and sorption of sample components onto the capillary wall [5]. Foret et al. [5] proposed a method to separate some triazine herbicides by using an electrolyte consisting of 0.02 *M* Tris, adjusted to pH 3.0 with trichloroacetic in ethanol–water (30:70, v/v). In an initial study concerning MEKC with in situ charged micelles, Cai and El Rassi [6] developed a method for the separation of various neutral and charged species including three *s*-triazine herbicides, namely prometon, prometryne, propazine and an acetamide herbicide (butachlor). Wu et al. developed a method for the separation of a group of herbicides (chlorophenoxy acids). In this case, sodium dodecyl sulphate (SDS), Brij 35, cetyltrimethylammonium bromide (CTAB) and methanol were introduced in the buffer to investigate their effects on the separation of these herbicides [7]. Galcerán et al. [8] developed a method to separate paraquat and diquat by using an acetic–acetate buffer solution at pH 4.0 with 100 m*M* sodium chloride. Schmitt et al. [9] used a 50 m*M* acetate buffer, pH 4.65 to separate *s*-atrazine herbicides. Nelson et al. separated a group of four triazine herbicides (atrazine, propazine, ametryne and prometryne) by using 25 m*M* SDS in 20 m*M* ammonium acetate, pH 6.8 as a running buffer [10]. Carabias-Martínez et al. used for the separation of chloro- and methylthiotriazines a mixture of 10 m*M* perchloric acid in acetonitrile–methanol (50:50, v/v) and 20 m*M* SDS, providing high resolution in short analysis times [11]. Most of these buffers present some shortcomings and they do not allow the simultaneous determination of the seven pesticides of interest in this work. In the presented method a buffer system was optimised for the simultaneous separation of fenuron, simazine, atrazine, carbaryl, ametryn, prometryn and terbutryn.

The general aim of this work was the automation of sample-treatment process carried out in a continuous flow system (CFS) coupled to a commercial CE system, as well as the automatic calibration in the CE instrument. This particular coupling has been described in a previous work by the authors [12]. It was achieved by a mechanic and an electronic interface. The mechanic interface was a laboratory-made programmable arm for direct injection of the sample into the sample vial placed in the autosampler of the CE equipment. The electronic interface was a D/A converter board that allowed to control all components of the hyphenated system [13]. A solid-phase extraction process was incorporated in the CFS for the automatic pre-concentration, elution, injection and determination of pesticides in spiked river water sample by CE.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and deionised water with a resistivity above 18 MΩ cm^{-1} was used. Seven pesticides were determined. Fenuron, simazine, atrazine and carbaryl were supplied by Chemserv; prometryn and terbutryn by Riedel-de Haën, and ametrin by Ciba. Stock standard solutions of 200 μg ml^{-1} of each compound were prepared in methanol and stored in the refrigerator. Working standard solutions were prepared daily by diluting with purified water. SDS (Aldrich), disodium hydrogenphosphate (Merck) and HPLC-grade acetonitrile (Riedel-de Haën) were used for preparing the buffer. C_{18} micro-columns (100 mg) for pre-concentration of the pesticides were supplied by Varian and the nylon filters (0.45 μm) for river water sample pretreatment by Cameo.

2.2. Apparatus

A Beckman P/ACE 5500 capillary electrophoresis

system provided with a diode array detector and a fused-silica capillary was used for the separation of the analytes. System control and data processing was carried out with Gold software. Gilson Minipuls-3 peristaltic pumps, PTFE tubings of 0.5 mm I.D., pump tubings of 1.02 mm I.D. and an automatic 10-port injection valve (Omnifit, Cambridge, UK) were used to set up the CF manifold. The mechanical interface for coupling the CFS to the CE instrument was a laboratory-made programmable arm [12]. The whole system (pumps, valve, programmable arm and CE instrument) was electronically coupled using a D/A converter board (CIO-DDA06/Jr, ComputerBoards, USA). The CE equipment was one that controlled the whole hyphenated system by sending transistor-transistor logic (TTL) signals to the secondary computer which controls the CFS by a program written in GW-BASIC [13].

2.3. Operating conditions

The MEKC separation was performed in a fused-silica capillary (47 cm×75 μm I.D.) with a positive power supply of 25 kV (average current 135 μA) at a temperature of 20°C. The carrier electrolyte was 60 m*M* SDS, 10 m*M* Na_2HPO_4, 8% acetonitrile adjusted to pH 9.5 with 0.05 m*M* NaOH. Electropherograms were recorded at 226 nm. Sample injection was carried out hydrodynamically for 5 s. The capillary was conditioned daily by flushing it with ultrapure water, 0.1 *M* NaOH and buffer, 5 min each. Between separations the capillary was rinsed with water (1 min), 0.1 *M* NaOH (0.5 min) and buffer (2 min).

2.4. Continuous flow system

The CFS used for the pre-treatment (dilution and pre-concentration) of the standards and the samples is shown in Fig. 1. The manifold consisted of three peristaltic pumps, an automatic 10-port switching valve (used in the two-position mode) and a programmable arm for sample injection. The whole system was controlled automatically via an electronic interface as previously described by us [13]. The first peristaltic pump (PP1) was used for water, the second (PP2) for the standard pesticide solution or the sample respectively and the third (PP3) for the eluent (30% acetonitrile). Pre-concentration, elution and injection worked in the following way. Firstly, the laboratory-made programmable arm for injection moved down to the waste vial of the autosampler of the CE instrument. PP1 and PP3 ran at the maximum velocity (10 rpm) to clean the system with water and to condition the micro-column with eluent. In this step the switching valve remained in the inject position. PP3 stopped and the valve changed to the load position to clean the micro-column with water. Next, the valve changed to the inject position and PP1 and PP2 ran at the appropriate velocity to prepare the desired dilution. When analysing real samples only PP2 ran, as no dilutions were necessary in these cases. After 1 min the switching valve changed to the load position and the sample is pre-concentrated for 10 min. In order to clean the column form matrix compounds, water is passed through the column for 1 min after the loading step. Afterwards, the valve changed to the inject position and PP1 ran at the maximum velocity to clean the system with water. PP3 also started to run at maximum velocity to elute the first fraction of the sample (the time, which was necessary to pass the sample from the end of the micro-column to the end of the arm for injection). PP1 and PP3 stopped, the arm moved up, the autosampler of the CE equipment changed to the sample vial and the arm moved down again. PP1 started to run to clean with water again. PP3 started to run to elute the sample and to inject it into the sample vial. PP1 and PP3 stopped, the arm moved up, the autosampler changed to the waste vial and while the separation of the sample was carried out, the next sample was prepared in the manifold.

3. Results and discussion

3.1. Trace enrichment

The determination of pesticides in environmental samples may not be achieved directly without a pre-concentration step and/or sample cleanup, because most of them are extremely diluted in the environmental media (e.g., water, soil, sediments, etc.). Moreover, they are complex mixtures of several compounds [14]. Therefore a solid-phase con-

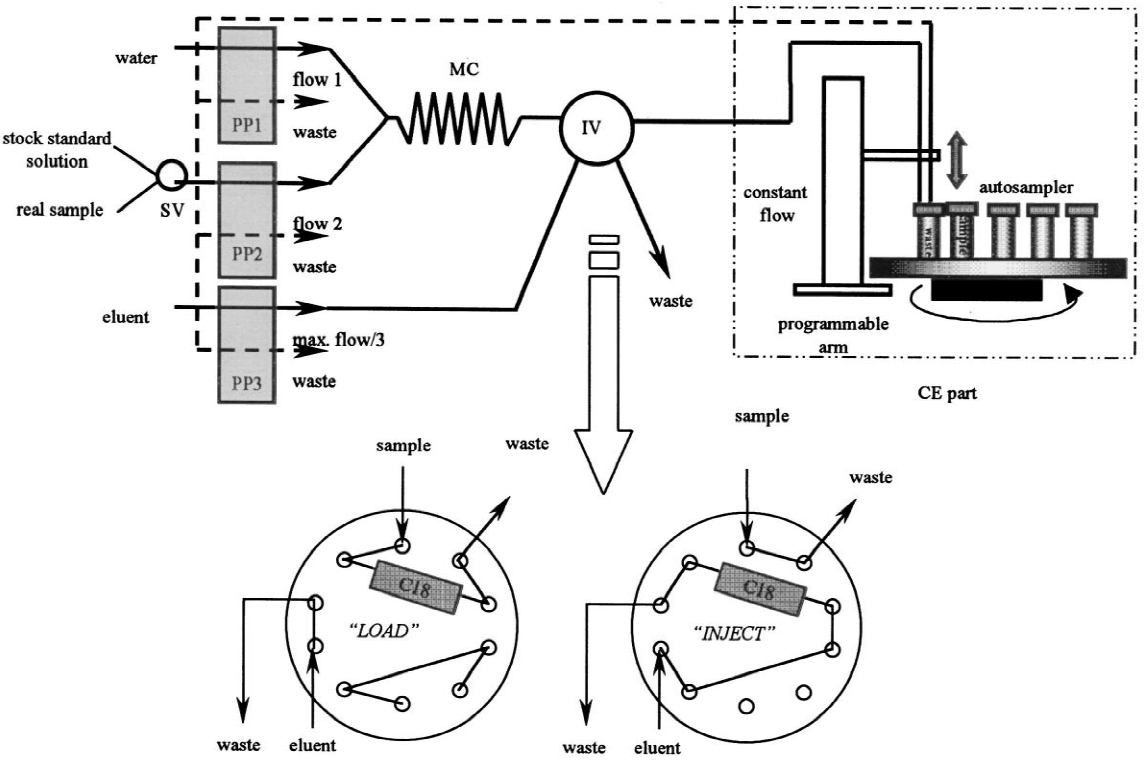

Fig. 1. CFS manifold for the pre-concentration of the pesticides; MC: mixing coil; IV: injection valve and SV: selection valve.

centration step was included into the sample preparation process.

Commercially available C_{18} columns (100 mg) were used for pre-concentration and put directly into the CFS manifold after preconditioning with methanol, water and elution solvent. The concentration of acetonitrile for elution and the loading time were optimised to provide best efficiency of pre-concentration. A solution of 30% of acetonitrile proved as the best eluent (Fig. 2A). Higher concentrations of acetonitrile did not significantly increase the amount of eluted sample and negatively influenced the separation in the following two way. The higher the amount of acetonitrile in the sample matrix, the higher the signal of the electroosmotic flow (EOF), which makes the analysis of fenuron nearly impossible as its migration time is only slightly higher than that of the EOF. The current for separation needs more time to reach its steady state resulting in an instability of the baseline.

Experiments were carried out for optimising the time of pre-concentration of the sample with loading times between 4 and 12 min (Fig. 2B). A time of 10 min was finally used as increasing the time of pre-concentration did not significantly increase the amount of recovered sample.

3.2. Effect of buffer system

By using a simple borate or phosphate buffer the pesticides cannot be separated by capillary zone electrophoresis (CZE) since all substance migrates with the same velocity (that of the EOF). The anionic surfactant SDS was used to form micelles to obtain different migration behaviour due to the different interactions between the pesticides and the micelles.

3.2.1. Effect of surfactant concentration

The key aspect for an efficient separation of the pesticides is the amount of surfactant added to the buffer. Conditions as previously reported by Pen-

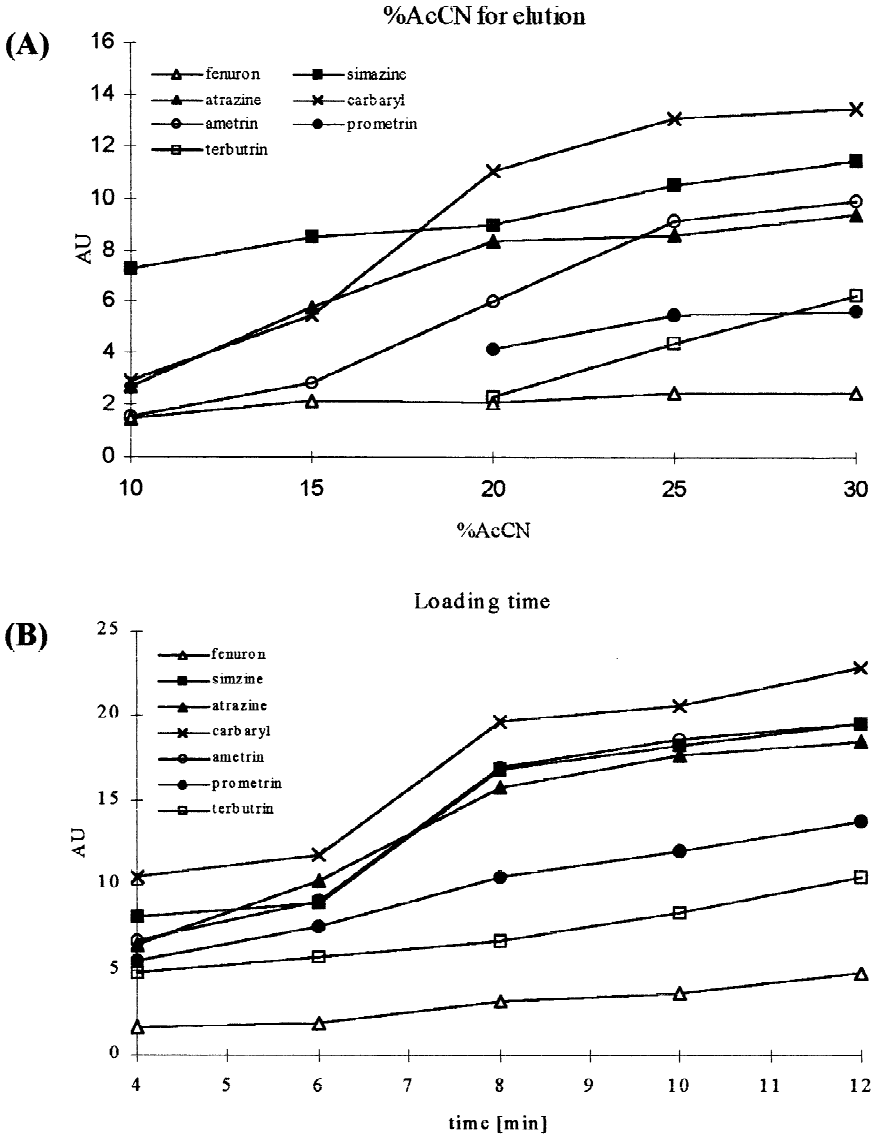

Fig. 2. (A) Influence of the concentration of acetonitrile on the elution. (B) Influence of the loading time.

metsa et al. [15] can efficiently separate the investigated pesticides when dissolved in aqueous media using 30 m*M* of SDS. When working with on-line pre-concentration it is inevitable that one must use a certain amount of organic solvent to elute the sample from the micro-column. In this case the optimised amount of eluent was 30% acetonitrile. It was found that this matrix did strongly affect the resolution of separation and led to peak broadening and overlap. To resolve this problem the amount of surfactant had to be increased to 60 m*M* for best separation and peak shape. Higher concentrations of SDS did not improve the separation and resulted in increasing analysis time.

3.2.2. Effect of organic modifier

The presence of organic modifiers like methanol or acetonitrile changes the viscosity in the buffer

system and manipulates the interaction between analytes and micelles [16]. In some cases small percentages can improve the efficiency of separation, which has been the topic of various studies [17–19]. In the context of this work, methanol and acetonitrile were studied as modifiers, both of them leading to best separation efficiency at a concentration of 8%. Higher and lower concentrations than 8% of these modifiers produced worse resolution between peaks. When working with methanol as a modifier, baseline instabilities occurred making quantification difficult, especially at trace analysis level. Finally, an amount of 8% of acetonitrile was chosen.

3.2.3. Effect of pH

Significant changes in the rate of EOF can be obtained by altering pH of the buffer. The EOF is determined by the surface charge on the capillary wall. Therefore the electroosmotic mobility was low in the acidic range and increased strongly at higher pH values of the buffer. The optimised pH for separation was 9.5 (adjusted with 0.05 *M* NaOH), resulting in satisfactory separations and short analysis time.

Fig. 3 shows an electropherogram of the seven pesticides (5 μg ml^{-1} each) obtained under the optimised operating conditions.

3.3. Performance of the CE method

In order to check the performance of the electrophoretic method a calibration graph was constructed without any pre-concentration of the sample in the range between 1 and 5 μg ml^{-1}. The calibration was carried out automatically with the continuous flow system described in the previous section from a stock solution of 5 μg ml^{-1}. The results are summarised in Table 1. The calculated limits of quantification were between 0.5 and 0.9 μg ml^{-1} for all pesticides.

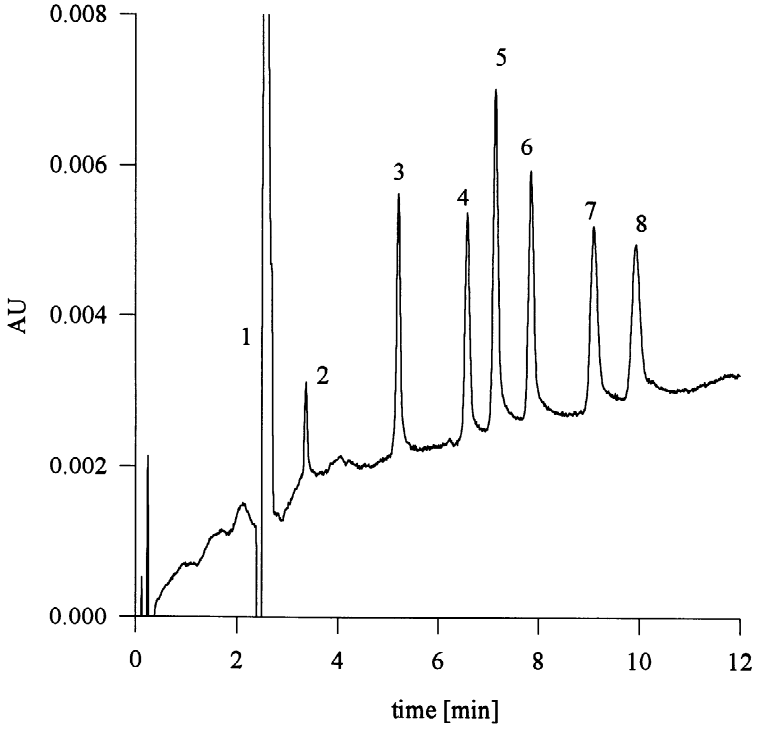

Fig. 3. Electropherogram of a mixture of the seven investigated pesticides (5 μg ml^{-1}). 1=EOF; 2=fenuron; 3=simazine; 4=atrazine; 5=carbaryl; 6=ametryn; 7=prometryn; 8=terbutryn.

Table 1
Figures of merit for the electrophoretic method[a]

Analyte	Equation	r	R^2	$S_{y/x}$	LOD	LOQ
Fenuron	$a=-0.0004\pm0.0007$ $b=0.0129\pm0.0002$	0.998	99.64	0.0012	0.16	0.54
Simazine	$a=-0.0006\pm0.0055$ $b=0.0637\pm0.0016$	0.996	99.14	0.0090	0.25	0.86
Atrazine	$a=-0.0141\pm0.0049$ $b=0.0692\pm0.0014$	0.997	99.42	0.0080	0.21	0.71
Carbaryl	$a=-0.0349\pm0.0091$ $b=0.1151\pm0.0027$	0.996	99.27	0.0149	0.24	0.79
Ametrin	$a=-0.0304\pm0.0054$ $b=0.0887\pm0.0016$	0.998	99.56	0.0090	0.18	0.61
Prometryn	$a=-0.0360\pm0.0057$ $b=0.0839\pm0.0017$	0.997	99.45	0.0095	0.20	0.68
Terbutryn	$a=-0.0272\pm0.0049$ $b=0.0787\pm0.0015$	0.998	99.55	0.0081	0.19	0.62

[a] a: Intercept; b: slope; $S_{y/x}$: standard deviation of residuals; r: regression coefficient; R^2: curve fitting level (in %) obtained by analysis of variance (ANOVA) for the validation of the model; LOD: limit of detection; LOQ: limit of quantification (LOD and LOQ in μg ml^{-1}).

Furthermore a calibration was carried out including the pre-concentration step on the C_{18} columns with concentrations of the analytes between 0.05 and 0.25 μg ml^{-1} (see Table 2). In this case the calculated limit of quantification was equal to or below 0.05 μg ml^{-1} for five of the pesticides, except for prometryn and terbutryn. In this case a value of 0.09 μg ml^{-1} was achieved.

Table 2
Figures of merit for the calibration with pre-concentration[a]

Analyte	Equation	r	R^2	$S_{y/x}$	LOD	LOQ
Fenuron	$a=-0.0039\pm0.0008$ $b=0.2326\pm0.0050$	0.997	99.43	0.0014	0.01	0.03
Simazine	$a=0.0121\pm0.0049$ $b=0.9699\pm0.0296$	0.994	98.80	0.0081	0.02	0.05
Atrazine	$a=0.0115\pm0.0025$ $b=0.7611\pm0.0148$	0.998	99.51	0.0004	0.01	0.03
Carbaryl	$a=0.0428\pm0.0058$ $b=1.1280\pm0.0348$	0.994	98.77	0.0095	0.02	0.05
Ametrin	$a=0.0184\pm0.0030$ $b=0.6799\pm0.0178$	0.996	99.11	0.0049	0.01	0.04
Prometryn	$a=0.0288\pm0.0030$ $b=0.3162\pm0.0178$	0.980	96.03	0.0048	0.03	0.09
Terbutryn	$a=0.0159\pm0.0025$ $b=0.2883\pm0.0153$	0.982	96.45	0.0042	0.03	0.09

[a] Symbols as in Table 1.

3.4. Analytical application on real samples

Four independent river water samples (Guadalquivir) were analysed in the context of this work. Prior to the analysis the samples were filtered through a 0.45-μm nylon filter and directly preconcentrated in the continuous flow system. As expected, amounts of all the investigated pesticides were found. Therefore – in order to validate the proposed analytical method – the water samples were spiked. A 25-ml sample was spiked at three different concentration levels: 0.1, 0.2 and 0.3 μg ml^{-1} for fenuron, simazine, atrazine, carbaryl and ametryn and 0.2, 0.3 and 0.4 μg ml^{-1} for prometryn and terbutryn. The results are summarised in Table 3, showing recoveries between 90 and 114%. These results suggest that there is no significant evidence of systematic errors in the proposed method. It should be noted that the standard addition method was applied to each individual sample in order to ensure the absence of significant matrix differences and then it was used for the direct determination of a mixture of pesticides in real samples. Fig. 4 shows the electropherorgrams of a spiked river water sample and an unspiked sample.

4. Conclusions

The proposed combination of a CFS (for sample pre-concentration) and commercial CE system (to provide the required selectivity) proved to be a powerful tool for the automatic determination of pesticides in river water samples. This system allows

Table 3
Analysis of pesticides in real samples

Analyte	Concentration added (μg ml^{-1})	River water sample I		River water sample II		River water sample III		River water sample IV	
		Concentration found (μg ml^{-1})	Recovery (%)	Concentration found (μg ml^{-1})	Recovery (%)	Concentration found (μg ml^{-1})	Recovery (%)	Concentration found (μg ml^{-1})	Recovery (%)
Fenuron	0.10	0.112	112	0.100	100	0.092	92	0.114	114
	0.20	0.215	107.5	0.206	103	0.198	99	0.222	111
	0.30	0.282	94	0.294	98	0.287	95.6	0.294	98
Simzine	0.10	0.096	96	0.097	97	0.098	98	0.107	107
	0.20	0.210	105	0.198	99	0.221	110.5	0.208	104
	0.30	0.312	104	0.291	97	0.298	99.3	0.299	99.6
Atrazine	0.10	0.095	95	0.096	96	0.110	110	0.103	103
	0.20	0.199	99.5	0.196	98	0.209	104.5	0.213	106.5
	0.30	0.277	92.3	0.296	98.6	0.285	95	0.310	103.3
Carbaryl	0.10	0.093	93	0.106	106	0.097	97	0.094	97
	0.20	0.227	113.5	0.204	102	0.213	106.5	0.212	106
	0.30	0.271	90.3	0.290	96.6	0.287	95.6	0.297	99
Ametryn	0.10	0.099	99	0.103	103	0.097	97	0.110	110
	0.20	0.217	108.5	0.208	104	0.208	104	0.202	101
	0.30	0.285	95	0.297	99	0.312	104	0.294	98
Prometryn	0.20	0.206	103	0.192	96	0.214	107	0.211	105.5
	0.30	0.321	107	0.300	100	0.313	104.3	0.298	99.3
	0.40	0.419	104.7	0.398	99.5	0.409	102.2	0.397	99.25
Terbutryn	0.20	0.179	89.5	0.200	100	0.192	96	0.198	99
	0.30	0.300	100	0.310	103.3	0.317	105.6	0.307	102.3
	0.40	0.368	92	0.397	99.2	0.397	99.25	0.412	103

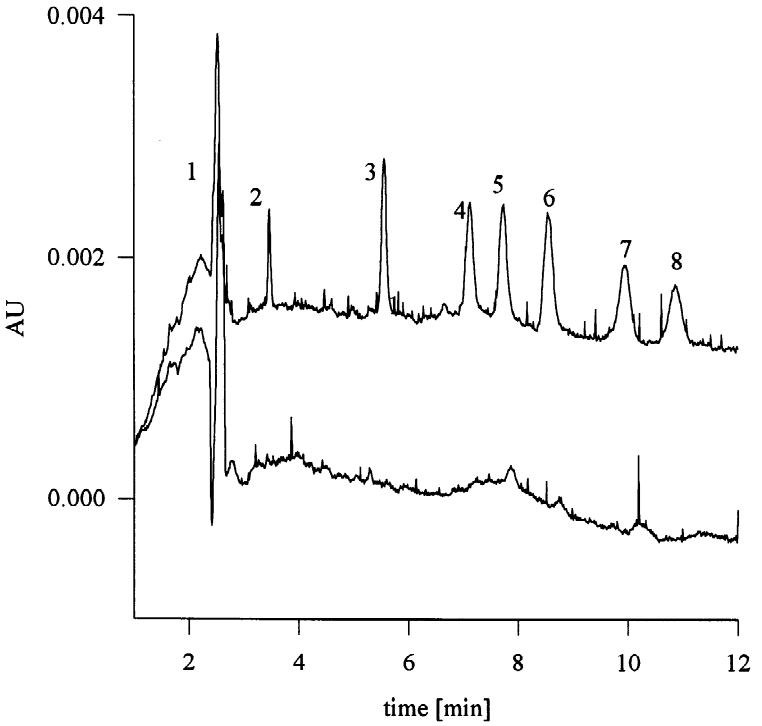

Fig. 4. Electropherograms of an unspiked and spiked river water sample (0.25 μg ml^{-1}). 1=EOF; 2=fenuron; 3=simazine; 4=atrazine; 5=carbaryl; 6=ametryn; 7=prometryn; 8=terbutryn.

the completely automated calibration, pre-concentration and determination of analytes present in real samples at trace levels. Moreover the proposed method is very useful for application to any kind of toxic analytes, as it avoids hazardous manual sample preparations.

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