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Determination of triazine herbicides in water by micellar electrokinetic capillary chromatography

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Abstract

Capillary electrophoresis was applied to the analysis of a mixture of triazine herbicides of environmental interest. Micellar electrokinetic capillary chromatography (MECC) proved to be satisfactory for the separation of triazines, providing high efficiency in short analysis times. Several electrophoretic parameters were investigated: injection conditions, pH and buffer concentration, surfactant concentration and applied voltage. The optimized MECC method was applied to the determination of these compounds in water samples; using a solid-phase concentration step, herbicide concentrations lower than 0.2 ng/ml can be measured, the relative standard deviations being about 20%.

Keywords: Micellar electrokinetic chromatography; Water analysis; Environmental analysis; Pesticides; Triazines

1. Introduction

Triazines are widely used as selective pre- and post-emergence herbicides for the control of broadleaf and grassy weeds in many agricultural crops. These compounds and their degradation products are relatively persistent and hence their determination is important. Several methods have been developed for the determination of triazines by gas chromatography [1,2] and high-performance liquid chromatography [3,4].

Capillary electrophoresis (CE) is particularly useful for separating aqueous mixtures of biomolecules [5] such as amino acids, peptides and proteins. Also, CE is an established separation technique within many industrial pharmaceutical analysis laboratories. Its applications include purity testing [6–8], determinations of active ingredients in formulations [9–11] and chiral analysis [12,13]. Other applications such as the determination of illicit drug substances have been reported [14,15]. The rapid expansion of research into both the instrumentation and applications of CE has demonstrated that capillary electrophoresis is more than a promising laboratory technique. However, the application of CE to the determination of toxic residues or contaminants in environmental samples is an underdeveloped field and literature concerning this aspect is still scarce, even though in recent years

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some studies have focused on the CE determination of some herbicides such as phenoxy acid herbicides [16], paraquat and diquat [17], sulfonylureas [18] and other solutes of environmental significance [19,20].

The use of CE for the separation of triazine herbicides has been also reported. Krivankova et al. [21] described a method for the determination of the herbicides prometryne, desmetryne, terbutryne, OH-atrazine and OH-simazine in an extract of milk by capillary isotachophoresis. Foret et al. [22] proposed a capillary zone electrophoretic (CZE) method for the separation of terbutryne, prometryne, desmetryne and some solvolytic products of atrazine and simazine using 0.02 *M* Tris buffer (pH 3.0); atrazine and simazine did not migrate under these conditions, even after 120 min of analysis. Cai and El-Rassi [23] proposed a method for the determination of prometon and prometryne by on-line preconcentration with octadecyl-bonded capillaries prior to CZE analysis.

Micellar electrokinetic capillary chromatography (MECC) was first developed in 1984 by Terabe et al. [24] in an effort to expand the applicability of CE to neutral molecules. MECC uses buffers to which surfactants have been added. Non-ionic solutes are partitioned into the micelles according to their hydrophobic nature, and separation is based on a combination of electrophoresis and partitioning of the analyte molecules between the aqueous mobile phase and the slower moving micellar phase. The technique of micellar electrokinetic capillary chromatography affords better separations for both charged and neutral small molecules (hydrophilic and hydrophobic).

Several theoretical works have proposed fundamental equations for MECC [25–28] and the effects of different modifiers on the control of the electroosmotic flow, the prevention of adsorption and improvements in separation [29,30]. Application of this technique to neutral molecules has increased considerably in recent years; concerning the analysis of contaminants, methods have been described for the determination of polycyclic aromatic hydrocarbons [31], phenols [32], pyrethrins [33], etc. MECC has been also evaluated for the determination of triazines such as propazine, prometryne, prometon and butachlor [34], atrazine and simazine in river water samples [35] and atrazine with some sulfonylurea herbicides [36].

However, the major drawback in MECC is the low system loadability. Accordingly, the technique must be coupled with a preconcentration step in order to determine trace level amounts.

This paper deals with the separation and determination of seven frequently used triazine herbicides by CE. Application to the determination of the triazines in natural waters, including a solid-phase (SPE) concentration step, is also reported.

2. Experimental

2.1. Apparatus

Capillary electrophoresis was performed with a P/ACE 2000 (Beckman, Fullerton, CA, USA) equipped with a UV detector. Standard P/ACE capillaries (57 cm long, 50 cm to the detector, 75 μm I.D.) were used.

Sep-Pak C_{18} cartridges (Waters, Milford, MA, USA) containing 360 mg of 40-μm C_{18}-bonded silica were used for the SPE of triazines from natural water samples. A Gilson Minipuls 2 HP 4 peristaltic pump with vinyl tubing was used to propel the water samples through the cartridge. A Rotavapor (Buchi, Flawil, Switzerland) was used for evaporating the eluent of the C_{18} cartridge to dryness.

2.2. Reagents

All triazine herbicides were obtained from Riedel-de Haën (Seelze, Hannover, Germany) and were used without further purification (minimum purity >98%). The chlorotriazines studied were atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), cyanazine [2-chloro-4-ethylamino-6-(1-cyano-1-methylethylamino)-1,3,5-triazine], simazine [2-chloro-4,6-bis(ethylamino)-1,3,5-triazine] and propazine [2-chloro-4,6-bis(isopropylamino)-1,3,5-triazine]

and the methylthiotriazines were ametryne (2-methylthio - 4 - ethylamino - 6 - isopropylamino - 1,3,5-triazine), prometryne [2-methylthio-4,6-bis(isopropylamino) - 1,3,5 - triazine] and terbutryne (2 - methylthio - 4 - ethylamino - 6 - terbutylamino-1,3,5-triazine).

Sodium dodecyl sulfate (SDS) was obtained from Fluka (Buchs, Switzerland). HPLC-grade methanol (Carlo Erba, Milan, Italy) was used for preparing stock solutions of each triazine. All chemicals used for the preparation of the buffer electrolytes were of analytical-reagent grade.

2.3. Samples

Stock solutions of each triazine were prepared in methanol at 500 μg/ml. A standard mixture of all seven triazines at 10 μg/ml each was prepared by diluting the stock solutions with the separation buffer. This solution was further diluted to obtain working standard solutions.

2.4. Procedure for CZE separation of triazines

Uncoated capillaries were used throughout the study. Before each run, the capillaries were pretreated for 5 min with 0.5 *M* sodium hydroxide and 10 min with the most concentrated acid (100 m*M*), after which separation was carried out. After each separation, the capillaries were rinsed with water for 10 min and then for 5 min with sodium hydroxide. Before carrying out the separation in a more dilute acid medium, the capillaries were treated for 5 min with the concentrated acid, followed by a further 10 min with the acid in which separation was to be performed.

2.5. Procedure for MECC separation of triazines

Uncoated capillaries were used throughout the study. All new capillaries were conditioned before use. They were pretreated sequentially for 10 min with 0.1 *M* hydrochloric acid, 5 min with water, 5 min with 0.5 *M* sodium hydroxide and 10 min with the separation buffer. This conditioning procedure was also applied as a daily start-up procedure. No changes in migration times were observed on a run-to-run basis.

Before each run, the capillary was rinsed for 10 min with distilled water and 5 min with 0.5 *M* sodium hydroxide prior to the passage of the separation buffer. Samples were introduced into the capillary under pressure (85 p.s.i.) for a fixed period of time (10 s). Unless stated otherwise, analysis was performed with an applied voltage of 22 kV with the capillary thermostated at 25 ± 0.1°C and with UV detection at 214 nm. The separation medium was 60 m*M* borate buffer (pH adjusted to 9.2)–50 m*M* SDS. Samples were introduced in the capillary in 3.5% (v/v) methanol–borate buffer. Standard 4.5-ml sample vials were used throughout the study, except for water sample analysis.

2.6. MECC determination of triazines in water samples

Spiked water samples were prepared by adding 50 μl of triazine mixture in methanol, at the appropriate concentration, to 100 ml of water sample. Spiked samples were passed through the SPE cartridge at a flow-rate of about 3 ml/min. Before use, the C_{18} cartridge was equilibrated with 5.0 ml of methanol and 5.0 ml of distilled water. Desorption was carried out by elution with 10 ml of pure methanol; this solution was evaporated to dryness at 30°C and the dry extract was dissolved in 200 μl of 10% (v/v) methanol–separation buffer using an ultrasonic bath; the sample was then ready for analysis. Samples were prepared in duplicate and 100-μl sample microvials were used to introduce the samples into the electrophoretic system.

Additionally, non-spiked water samples were first analysed following the same procedure to check for the presence of the triazines under study.

The recoveries in deionized water samples were determined using the external standard method and taking normalized peak areas [37] obtained by dividing the observed peak area values (valley-to-valley) by their corresponding migration times. The standards were prepared using 100 ml of deionized water, preconcentrated

in a C_{18} cartridge as described above; after the elution of the cartridge with 10 ml of pure methanol, this solvent was evaporated and the dry extract was spiked with 200 μl of 10% (v/v) methanol–separation buffer containing the triazine mixture at the appropriate concentration.

Owing to the baseline drift found with preconcentrated samples, quantification of triazines in natural water samples was carried out using the method of standard additions.

3. Results and discussion

3.1. CZE separation of triazines

Triazines are basic species able to become protonated in acid media. The pK_a values of chlorotriazines are about 1.5 whereas those of methylthiotriazines are close to 4 (Table 1). In view of this, initially the possibility of performing the separation by CZE in an acidic medium was entertained.

Different acids were tested as the separation medium: citric acid at concentrations ranging between 100 and 20 m*M*, phosphoric acid between 100 and 3 m*M* and perchloric acid between 100 and 1 m*M*. The study was conducted by modifying the concentration of the respective acids in decreasing order.

Fig. 1 shows the electropherogram obtained in 11.2 m*M* perchloric acid, but similar results were obtained in citric acid and phosphoric acid media. The results obtained show that in an acidic medium, in the absence of modifiers, it is not possible to separate the chlorotriazines owing to their low pK_a values, and the separation of the methylthiotriazines is also deficient.

Table 1
Solubility in water and pK_a values of triazine herbicides

Herbicide	Water solubility (μg/ml)	pK_a
Simazine	5	1.65
Cyanazine	171	1.30
Atrazine	33	1.68
Ametryne	193	4.00
Propazine	8.6	1.85
Prometryne	48	4.05
Terbutryne	58	4.38

Data from Ref. [38].

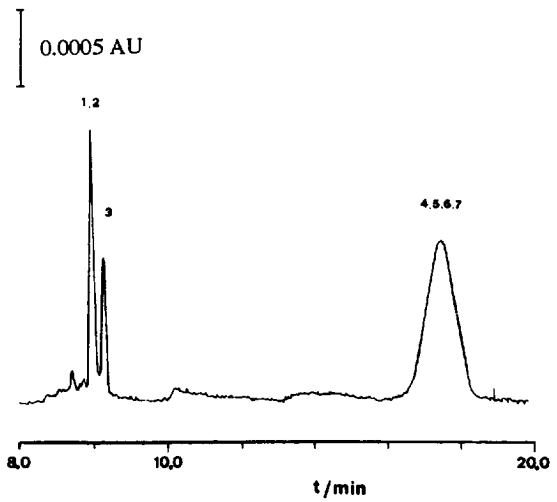

Fig. 1. CZE separation of triazine herbicides in 11 m*M* perchloric acid (pH 1.9). Conditions: sample concentration, 2.2 μg/ml of each triazine; hydrodynamic injection, 10 s; applied voltage 25 kV. Peaks: 1 = ametryne; 2 = terbutryne; 3 = prometryne; 4 = simazine; 5 = cyanazine; 6 = atrazine; 7 = propazine.

3.2. MECC separation of triazines

Since protonated triazines were unsuitable for separation by CZE, MECC was tried. To find suitable conditions for triazine separation, a detailed study was conducted on all the variables influencing the system, both those affecting the introduction of the samples into the capillary and those affecting separation.

Optimization of variables affecting sample introduction

The mode employed for sample injection and the medium in which the samples were injected into the capillary affect the sensitivity and resolution of the triazines studied. Separation was performed using 60 m*M* borate buffer (pH 9.2) in the presence of (100 or 50 m*M*) SDS, applying a potential of 22 kV.

Initially, hydrodynamic injection was used, varying the medium in which the sample to be separated was introduced. Three aliquiots containing the herbicides dissolved in methanol were brought to dryness; one the residues was dissolved in an aqueous solution of borate buffer, the second was dissolved in 3.5% (v/v) methanol–borate buffer and the third was dissolved in pure hexane. The electropherograms obtained reflect the different sensitivies in the three media studied (Figs. 2 and 3). The sample dissolved in borate buffer had a very low sensitivity for the triazines simazine, atrazine and propazine. However, all the triazines showed very similar sensitivities when the sample was dissolved in 3.5% (v/v) methanol–borate buffer. This kind of behaviour can be explained in terms of the low water solubility of simazine, atrazine and propazine (Table 1). These data are confirmed by the electropherogram of the sample dissolved in hexane (Fig. 3). More polar triazines (i.e. simazine, atrazine and propazine) showed a higher sensitivity than the other triazines, which remained partially solubilized in the hexane and only a fraction of them is solubilized in the separation medium. Moreover, the sample dissolved in hexane permitted injection times of only a maximum of 5 s to be used.

The proportion of methanol in the sample was varied between 3.5% and 12%. The variation in the resolution (R_s) for the herbicides ametryne and propazine (peaks 4 and 5), which were the critical pair for complete resolution, is shown in Table 2. For the other herbicides no significant variations were seen when the percentage of methanol was increased. The migration time was almost constant and the normalized area decreased slightly on increasing the percentage of methanol. The loss of resolution cannot be attributed to the greater amount injected on increasing the percentage of methanol. In this case, an increase in the percentage of methanol led to an increase in the viscosity of the sample and hence the amount injected was smaller [39]. The loss of resolution must be due to the greater longitudinal diffusion of the sample in the capillary owing to the different thermophysical properties of the sample and the separation buffer,

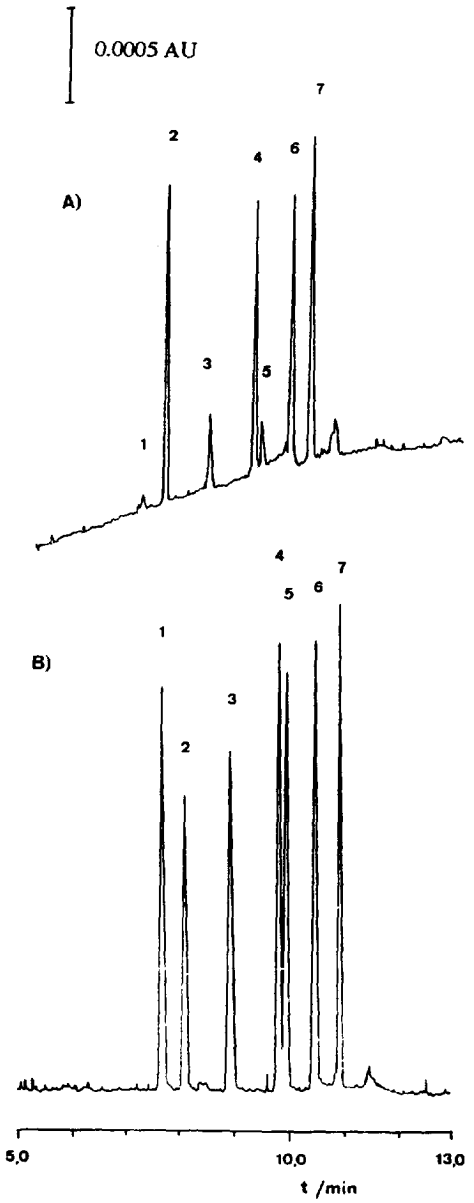

Fig. 2. MECC separation of triazine herbicides. Influence of the medium in which the sample was dissolved. Triazines were dissolved in (A) 60 m*M* borate buffer (pH 9.2) or (B) 3.5% (v/v) methanol–60 m*M* borate buffer (pH 9.2). Conditions: sample concentration, 2.5 μg/ml of each triazine; separation buffer, 60 m*M* borate buffer (pH 9.2)–50 m*M* SDS; hydrodynamic injection, 10 s; applied voltage 22 kV. Peaks: 1 = simazine; 2 = cyanazine; 3 = atrazine; 4 = ametryne; 5 = propazine; 6 = prometryne; 7 = terbutryne.

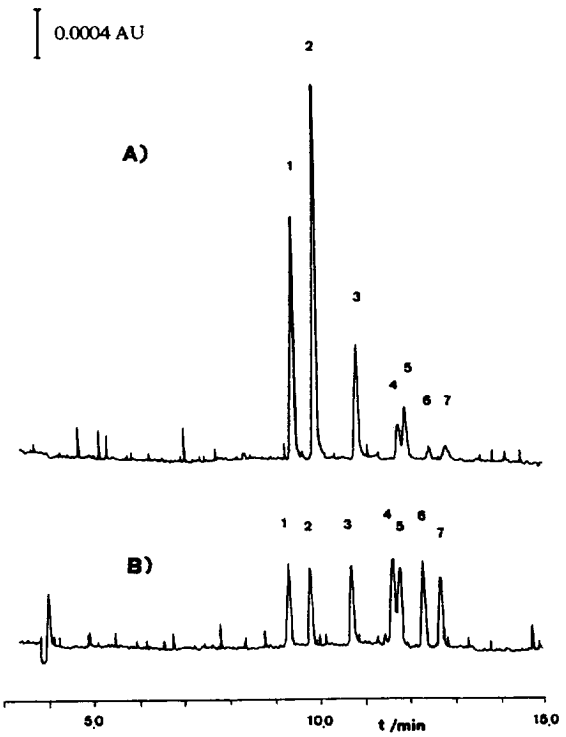

Fig. 3. MECC separation of triazine herbicides. Influence of the medium in which the sample was dissolved. Triazines were dissolved in (A) pure *n*-hexane or (B) 3.5% (v/v) methanol–60 m*M* borate buffer (pH 9.2). Conditions: hydrodynamic injection, 2 s; separation buffer, 60 m*M* borate buffer (pH 9.2)–100 m*M* SDS. Other conditions and peak assignments as in Fig. 2.

Table 2
Resolution (R_s) of ametryne and propazine as a function of the methanol content in the sample and the injection time

Methanol (%, v/v)	R_s [a]	R_s [b]	$t_{inj.}$ (s)	R_s [a]
3.5	1.04	1.58	2	1.20
5.0	0.97	1.11	5	1.18
6.5	0.83	0.40	10	1.00
8.0	0.23	0.33	12	0.65
10.0	0.18	0.20	15	0.53
12.0	0.10	0.17	20	0.30

Sample concentration: 2.5 μg/ml of each triazine.
[a] Separation medium: 60 m*M* borate buffer (pH 9.2)–100 m*M* SDS.
[b] Separation medium: 60 m*M* borate buffer (pH 9.2)–50 m*M* SDS.

leading to a broadening of the size of the sample injected [40].

The injection time was varied between 2 and 20 s. The normalized area increases with increase in the injection time but poor resolution between ametryne and propazine was found for times longer than 10 s (Table 2). A compromise between resolution and sensitivity must be made. Because of this, a 10-s injection time was considered to be the most suitable.

Electrokinetic injection was also tested. When injection was carried out by applying a potential of 10 kV over 10 s, the electropherogram obtained displayed lower sensitivity and resolution than that obtained on performing injection under pressure over 10 s.

In the subsequent studies, injection was carried out under pressure for 10 s and the sample was introduced into the capillary dissolved in 3.5% (v/v) methanol–separation buffer.

Optimization of variables affecting separation

Using borate buffer (pH 9.2), the effect of the concentration of the separation buffer, modifying the total concentration of the buffer between 20 and 130 m*M*, was studied. An increase in buffer concentration caused a considerable increase in the migration time of all the triazines, since mobility is inversely dependent on the square root of the buffer concentration. A concentration of 60 m*M* was used in subsequent experiments since this was found to give good resolution and a reasonable analysis time.

Different pH values, ranging from 8.5 to 9.7, were tested for the separation of the triazine herbicides. For pH values above 9.2, the migration times increased considerably, producing a loss of resolution between ametryne and propazine. A buffer pH of 9.2 proved to be the best value for the separations.

The SDS concentration was studied in the range 50–150 m*M*. The migration times decreased with a decrease in SDS concentration. A value of 50 m*M* proved to be suitable.

Once the optimum composition of the separation buffer had been determined, a study was made of the most suitable applied voltage for triazine separation. The applied voltage (V) was

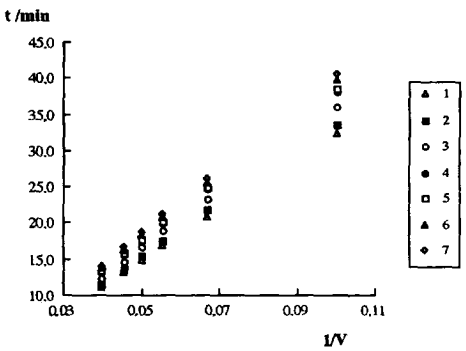

Fig. 4. Dependence of the migration time on the reciprocal of applied voltage. Conditions: sample concentration, 2.5 μg/ml of each triazine in 3.5% (v/v) methanol–60 m*M* borate buffer (pH 9.2); separation buffer, 60 m*M* borate buffer (pH 9.2)–100 m*M* SDS. Other conditions and peak assignments as in Fig. 2. *t* vs. $1/V$ slopes: (1) 351.88; (2) 368.80; (3) 392.03; (4) 414.68; (5) 418.40; (6) 429.87; (7) 439.47. $R^2 = 0.999$.

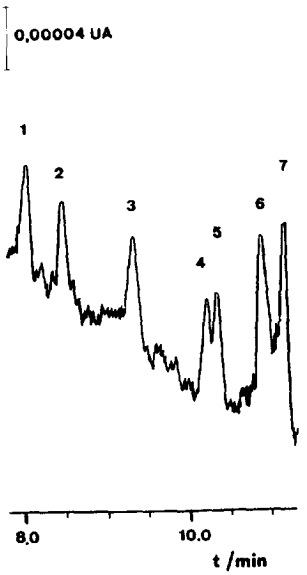

Fig. 5. Electropherogram obtained for a standard solution at the concentration level of the estimated detection limit. Conditions: sample concentration, 0.05 μg/ml of each triazine in 3.5% (v/v) methanol–6.0 m*M* borate buffer (pH 9.2). Other conditions and peak assignments as in Fig. 2.

modified between 10 and 25 kV. The dependence of the migration time for each triazine on $1/V$ is shown in Fig. 4. As expected, shorter analysis times were obtained at higher applied voltages, although ametryne and propazine were poorly resolved at voltages higher than 22 kV. Additionally, extremely high currents were obtained at voltages above 22 kV and frequent drops in current were observed.

Analytical data

The experimental relationship between normalized peak area and herbicide concentration in the samples (Table 3) was found to be linear over the whole range tested, i.e., $2 \cdot 10^{-7}$–$8 \cdot 10^{-5}$ *M* (0.05–20 μg/ml). The detection limits, calculated at a signal-to-noise ratio of 2, were close to 0.05 μg/ml. An electropherogram corresponding to a standard solution near the detection limit is shown in Fig. 5. The relative standard deviations for six replicates at a concentration of 2 μg/ml

Table 3
Analytical characteristics of the MECC method for the determination of triazines

Herbicide	Intercept	Slope (area units/mol l^{-1})	Correlation coefficient	DL[a] (μg/ml)
Simazine[b]	$(3.1 \pm 2.9) \cdot 10^{-3}$	$(1.81 \pm 0.07) \cdot 10^{3}$	0.9907	0.051
Cyanazine	$(-4.7 \pm 1.1) \cdot 10^{-4}$	$(2.50 \pm 0.03) \cdot 10^{3}$	0.9990	0.048
Atrazine	$(2.0 \pm 2.5) \cdot 10^{-3}$	$(2.12 \pm 0.07) \cdot 10^{3}$	0.9939	0.050
Ametryne	$(-1.5 \pm 5.2) \cdot 10^{-3}$	$(3.05 \pm 0.04) \cdot 10^{3}$	0.9982	0.045
Propazine[b]	$(3.0 \pm 4.6) \cdot 10^{-4}$	$(2.23 \pm 0.04) \cdot 10^{3}$	0.9975	0.050
Prometryne	$(3.5 \pm 2.8) \cdot 10^{-4}$	$(2.62 \pm 0.03) \cdot 10^{3}$	0.9991	0.042
Terbutryne	$(4.3 \pm 0.4) \cdot 10^{-4}$	$(2.33 \pm 0.06) \cdot 10^{3}$	0.9970	0.045

Concentration range between ca. $2 \cdot 10^{-7}$ and $5 \cdot 10^{-5}$ *M* (0.05–20 μg/ml) of each triazine.
[a] DL = detection limit for a signal-to-noise ratio of 2.
[b] $C < 3 \cdot 10^{-5}$ *M* (5 μg/ml).

Table 4
Relative standard deviation (%) for MECC determination of triazines

Herbicide	(a)		(b)		(c)	
	t_M	Normalized peak area	t_M	Normalized peak area	t_M	Normalized peak area
Simazine	0.5	2.3	1.1	4.5	0.6	12.0
Cyanazine	0.5	1.8	1.1	11.3	0.6	7.0
Atrazine	0.6	2.1	1.1	8.3	0.6	11.8
Ametryne	0.6	2.3	1.2	20.8	0.7	6.7
Propazine	0.6	2.7	1.2	21.9	0.7	2.2
Prometryne	0.6	1.8	1.2	12.0	0.7	11.2
Terbutryne	0.6	1.4	1.3	13.8	0.7	9.8

(a) $n = 6$, same capillary, conventional 4.5-ml sample vial (2.5 μg/ml of each triazine).
(b) $n = 6$, three different capillaries, conventional 4.5-ml sample vial (2.5 μg/ml of each triazine) (day-to-day precision).
(c) Ten injections, same capillary, 100-μl sample microvial (0.1 μg/ml of each triazine).

are given in Table 4(a); the R.S.D. values obtained were below 3% for the normalized peak areas and below 1% for the migration times (t_M).

The day-to day precision with different capillaries, expressed as R.S.D., was also evaluated. Table 4(b) shows the R.S.D. values obtained for six determinations carried out in three different capillaries. Good reproducibility in the migration times (t_M) of all the triazines was found, with an R.S.D. of about 1%. In contrast, the values of the normalized peak areas had R.S.D. values lower than 15% for all the triazines except ametryne and propazine, for which the R.S.D.s were 20%.

3.3. Determination of triazine herbicides in water

Most reported methods for triazine determinations in waters involve liquid–liquid extraction [41] or SPE [42,43]. In this work, a solid-phase trace enrichment step was optimized, to determine herbicide residues in natural water samples. Commercially available C_{18} cartridges were used.

For the determination of triazines in water samples, the samples were injected into the capillary using 100-μl sample microvials. A prior study was therefore conducted on the reproducibility of microvial injection. Table 4 (c) shows the R.S.D.s obtained for ten repeated injections of the same sample distributed in different microvials. As can be seen, the R.S.D. values for the normalized areas were higher than those obtained when conventional 4.5-ml vials were employed.

For the study of recovery, samples of deionized water were used. The volume spiked with the triazine mixture at a concentration of 20 ng/ml of each triazine was 100 ml. The eluate (10.0 ml of pure methanol) was evaporated to dryness and the residue was dissolved in 1.0, 2.0 and 5.0 ml of 3.5% (v/v) methanol–borate buffer. These samples were injected directly into the capillary using 100-μl sample microvials.

The recoveries and their standard deviations are shown in Table 5 (a)–(c). The values obtained range between 81 and 105% when 5.0 ml of 3.5% (v/v) methanol–borate buffer were used [Table 5 (c)].

As stated before, the detection limit of the triazines using the MECC method described here is about 50 ng/ml (Table 3). By using this SPE procedure, a preconcentration factor of 20 is achieved; hence the minimum detectable concentration would now be close to 2.0 ng/ml. In order to optimize the method for lower concentrations than that, further experiments were carried out, preconcentrating a volume of water of 100 ml containing 1 ng/ml of each triazine and dissolving the residue in 200 μl of 10% (v/v) methanol–borate buffer. In this case, the con-

Table 5
Triazine recoveries from deionized water samples as a function of the composition and volume of the solvent used for dissolving the dry extract after the SPE procedure

Herbicide	Recovery ±S.D.[a] (%)			
	3.5% (v/v) methanol–borate buffer			10% (v/v) methanol–borate buffer
	(a) 1 ml	(b) 2 ml	(c) 5 ml	(d) 0.2 ml
Simazine	44 ± 3	93 ± 5	98 ± 8	89 ± 6
Cyanazine	34 ± 2	64 ± 3	83 ± 3	84 ± 8
Atrazine	42 ± 3	78 ± 9	100 ± 3	84 ± 6
Ametryne	40 ± 4	89 ± 5	97 ± 7	101 ± 8
Propazine	48 ± 4	82 ± 7	105 ± 7	104 ± 7
Prometryne	32 ± 2	75 ± 5	81 ± 6	82 ± 7
Terbutryne	28 ± 3	64 ± 6	88 ± 8	95 ± 11

Fortification level: (a), (b) and (c) 20 ng/ml and (d) 1 ng/ml of each triazine. Sample concentration: (a) 2 μg/ml [=20 ng/ml × (100 ml/1 ml)] of each triazine; (b) 1 μg/ml [=20 ng/ml × (100 ml/2 ml)] of each triazine; (c) 0.4 μg/ml [=20 ng/ml × (100 ml/5 ml)] of each triazine; (d) 0.5 μg/ml [=1 ng/ml × (100 ml/0.2 ml)] of each triazine.
[a] $n = 3$.

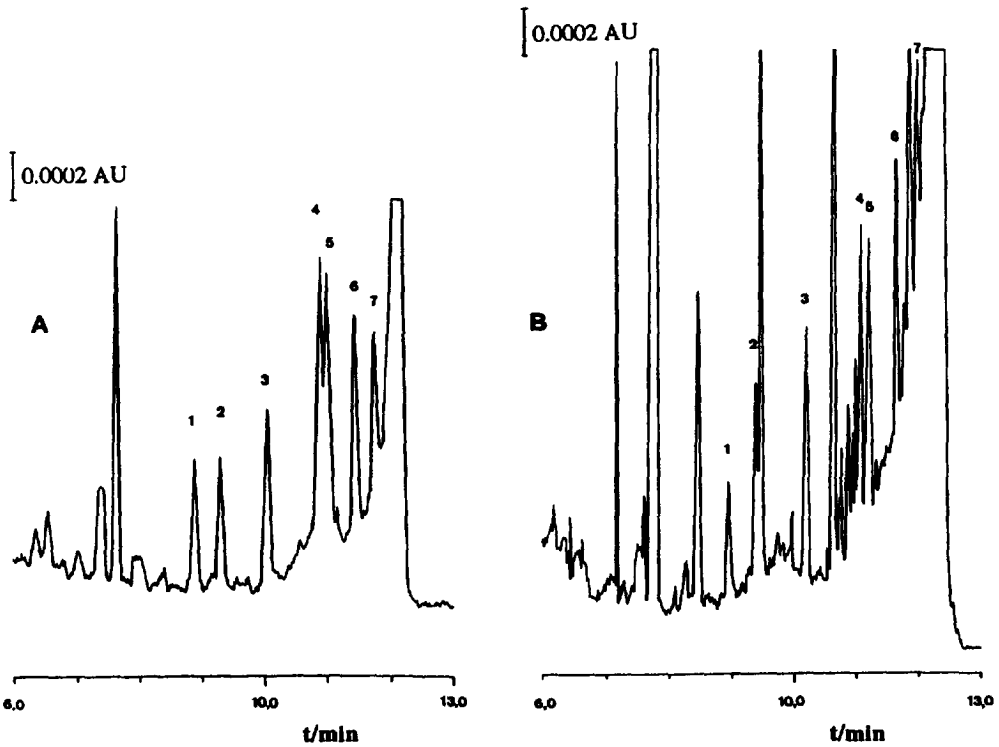

Fig. 6. Influence of preconcentrated sample volume. (A) 100 ml of deionized water spiked at 1 ng/ml and (B) 1000 ml of deionized water spiked at 0.1 ng/ml. Injected sample concentration, 500 ng/ml of each triazine. Preconcentration procedure as described under Experimental. Peak assignments as in Fig. 2.

Table 6
Analytical characteristics of the MECC method with solid-phase preconcentration

Herbicide	Intercept	Slope (area units/mol l^{-1})	Correlation coefficient	RSD[a] (%)	DL[b] (ng/ml)
Simazine	$(8.4 \pm 5.0) \cdot 10^{-4}$	$(8.5 \pm 0.4) \cdot 10^{5}$	0.9956	17	0.23
Cyanazine	$(-0.6 \pm 4.5) \cdot 10^{-4}$	$(1.41 \pm 0.04) \cdot 10^{6}$	0.9982	11	0.21
Atrazine	$(2.5 \pm 4.3) \cdot 10^{-4}$	$(1.23 \pm 0.03) \cdot 10^{6}$	0.9981	16	0.20
Ametryne	$(9.4 \pm 7.4) \cdot 10^{-4}$	$(1.18 \pm 0.06) \cdot 10^{6}$	0.9939	13	0.20
Propazine	$(2.3 \pm 0.4) \cdot 10^{-3}$	$(1.30 \pm 0.03) \cdot 10^{6}$	0.9944	21	0.18
Prometryne	$(8.4 \pm 3.9) \cdot 10^{-4}$	$(1.02 \pm 0.04) \cdot 10^{6}$	0.9967	15	0.18
Terbutryne	$(-1.6 \pm 7.1) \cdot 10^{-4}$	$(9.0 \pm 0.6) \cdot 10^{5}$	0.9890	23	0.10

Deionized water samples spiked at concentration levels between ca. 0.2 and 5 ng/ml of each triazine.
[a] R.S.D. for a concentration of 0.5 ng/ml ($n = 6$).
[b] DL = detection limit for a signal-to-noise ratio of 2.

centration injected was 500 ng/ml and the resolution of ametryne and propazine was greater than unity. The recoveries thus obtained ranged between 82 and 104% [Table 5 (d)]. However, these recoveries varied considerably when the preconcentrated volume was 1000 ml. Fig. 6 shows the electropherograms obtained on preconcentrating 100 ml of a solution containing ca. 1.0 ng/ml of each triazine and the electropherogram obtained on preconcentrating 1000 ml, with 0.1 ng/ml of each of the triazines. In these electropherograms, apart from the signals corresponding to the preconcentrated triazines, a strong signal is seen at a time close to the migration time of the micellar phase (t_{mc}); this signal must correspond to compounds of very low polarity that are strongly associated with the micelle. The normalized area of this signal is a linear function of the volume of the preconcentrated sample.

To test the performance of the SPE method over a wide range of concentrations, a linearity study was performed in deionized water, using seven concentrations: 0.2, 0.3, 0.5, 1.0, 2.0, 4.0 and 5.0 ng/ml. The experimental relationships between the normalized peak areas and triazine concentrations in the water sample were found to be linear over the range test (Table 6).

To determine the detection limit of the triazines using this method, two blank samples of water were used and the average noise was determined. The detection limits, calculated at a signal-to-noise ratio of 2, were 0.1 ng/ml for terbutryne and 0.2 ng/ml for all the other triazines investigated (Table 6). The R.S.D.s for six replicates spiked at a concentration of 0.5 ng/ml range between 11 and 23%.

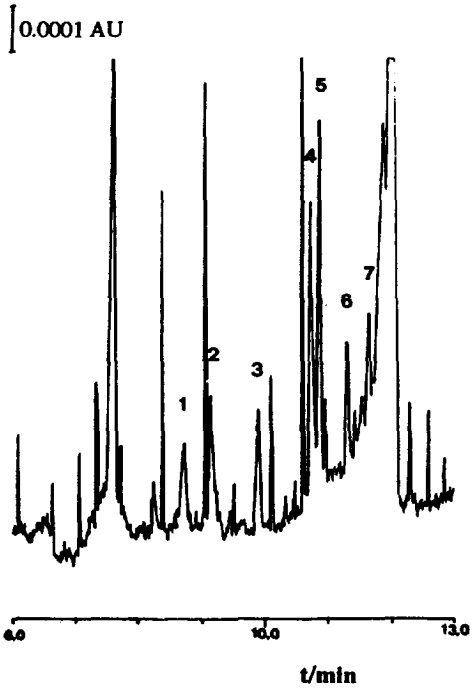

Fig. 7. Representative electropherogram of a bottled water sample obtained after SPE preconcentration of 100 ml (ca. 0.3 ng/ml of each triazine). Preconcentration procedure as described under Experimental. Peak assignments as in Fig. 2.

Table 7
Triazine recoveries from natural water samples after SPE preconcentration

Herbicide	Recovery ±S.D.[a] (%)			
	Bottled water		Tap water	
	(a)	(b)	(a)	(b)
Simazine	111 ± 16	105 ± 16	81 ± 11	96 ± 16
Cyanazine	106 ± 15	94 ± 14	112 ± 12	115 ± 13
Atrazine	92 ± 9	130 ± 15	117 ± 19	114 ± 18
Ametryne	110 ± 16	98 ± 16	114 ± 16	91 ± 11
Propazine	109 ± 18	118 ± 12	97 ± 20	116 ± 23
Prometryne	98 ± 14	98 ± 16	97 ± 16	116 ± 15
Terbutryne	112 ± 21	94 ± 19	79 ± 18	100 ± 20

Fortification level: (a) 0.3 ng/ml and (b) 0.5 ng/ml of each triazine.
[a] $n = 4$.

In order to check the applicability of the proposed method, natural water samples of different origins were analysed. A non-spiked 100-ml aliquot of each sample was first analysed following the sample procedure to check for the presence of these compounds. The samples were then spiked at two concentration levels, 0.3 and 0.5 ng/ml of each triazine (Fig. 7). The analyte recoveries found ranged from 79 to 130% (Table 7).

4. Conclusions

Conditions have been established for the MECC determination of trace levels of simazine, cyanazine, atrazine, ametryne, propazine, prometryne and terbutryne. The detection limits of the method, without any preconcentration step, are 50 ng/ml, only twice those obtained using HPLC [4].

An SPE method for the extraction and preconcentration of these analytes in water samples was developed. The proposed method is relatively simple and allows detection limits suitable for the MECC determination of these compounds in environmental samples to be achieved. The present findings show that MECC, coupled to a preconcentration step, is a valid alternative for the control of micro-contaminants.

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