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Determination of triazine herbicides in natural waters by solid-phase extraction and non-aqueous capillary zone electrophoresis

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Abstract

Capillary zone electrophoresis (CZE) in an organic medium was used to analyse triazines at sub-ppb concentration levels in natural waters after a preconcentration step using conventional C_{18} cartridges and new Oasis HLB devices. With both sorbents, satisfactory results were obtained on analysing deionized water. However, on analysing natural waters, both sorbents showed very different types of behaviour. The different variables affecting the elution of both sorbents were studied, resulting in the choice of Oasis HLB as the most suitable for later separation by CZE in non-aqueous medium. Combination of a preconcentration step with electrokinetic injection revealed that capillary electrophoresis with simple UV detection can also be used satisfactorily for the quantification of micropollutants in natural waters. The detection limits obtained varied between 0.01 and 0.05 $\mu g\,l^{-1}$, depending on the type of matrix analysed. The day-to-day precision varied between 0.9% and 2.3%, expressed as the relative standard deviation.

Keywords: Water analysis; Environmental analysis; Non-aqueous capillary electrophoresis; Extraction methods; Triazines; Pesticides

1. Introduction

Capillary electrophoresis (CE) shows high efficiency in separating pollutants of environmental interest. However, the sensitivity achieved with this capillary technique is the main drawback in its application — which has been scant — to analysis at trace levels [1].

Attempts to circumvent this problem have focused on the use of on-column electrochemical detectors [2,3]. It is also possible to increase the sensitivity of CE analysis by using field-amplified injection techniques that allow the introduction of larger sample volumes: Chien et al. [4] took advantage of electroosmotic flow to remove the excess electrokinetically injected sample, at the same time producing analyte grouping through the sample stacking effect. Rodriguez et al. [5] have described a method for the determination of phenolic pollutants in drinking water using the sample stacking method to remove the matrix.

Another way of enhancing the sensitivity of CE in pollutant analysis is to perform on-column solid-phase extraction, either with a capillary column with a plug of inverse phase material at the start of the capillary, or by using material covalently bound to the inner wall of the capillary [1]. Cai et al. [6] have proposed a method for the determination of prometron and prometryne by on-line preconcentration

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with octadecyl-bonded capillaries prior to capillary zone electrophoresis (CZE).

Within the techniques or modes of CE, isotachophoresis (ITP) generates consecutive zones in which each of the analytes is located, which are not diluted by the background electrolyte as in CZE, thereby allowing an increase in the sensitivity of the technique. It is therefore of interest to inject enriched sample zones by ITP to perform a CZE separation (ITP–CZE coupling) [7].

Other procedures used to increase sensitivity have employed pre- and post-column derivatization reactions: Zhu [8] developed a simple post-column reaction system with minimal extra zone broadening and good reproducibility for CE with fluorescence detection.

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. Most of the reported methods for triazine determination in waters involves separation by HPLC or GC after liquid–liquid extraction [9,10], or solid-phase extraction (SPE) [11–13]. This is because of the low maximum permitted limits [14], i.e. 0.1 $\mu g\,l^{-1}$ per individual substance for pesticides in water destined for human consumption.

Several procedures employing CE have been proposed for the determination of triazine herbicides. Foret et al. [15] proposed a CZE method for the separation of terbutryne, prometryne, desmetryne and some solvolytic products of atrazine and simazine. Micellar electrokinetic capillary chromatography (MECC) has also been assessed for the determination of triazines such as propazine, prometryne, prometron and butachlor [16], atrazine and simazine in river water samples [17], and atrazine with some sulfonylurea herbicides [18]. Carabias-Martínez et al. [19] have proposed the determination of chloro- and methylthiotriazines by SPE prior to MECC separation. He and Lee [20] proposed a method based on the combination of SPE and field-amplified concentration with MECC, for the trace analysis of organo nitrogen pesticides in drainage water.

Here we propose a method in which the combination of a preconcentration step by SPE and later electrokinetic injection into the electrophoresis system allows the determination of chloro- and methylthio-derivatives in natural waters at sub-ppb levels.

2. Experimental section

2.1. Equipment

A P/ACE 2000 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA), equipped with a UV photometric detector. Standard capillaries of 57 cm (50 cm to the detector)×75 μm I.D. were used.

To convey the sample through the SPE cartridge a peristaltic pump was used. Drying and conditioning of the cartridges was accomplished in a vacuum pump (Afa, Barcelona, Spain) coupled to a 20-place manifold for sample preparation (Variant, Harbor City, USA). Evaporation of the different solvents was accomplished with a model 461 Rotavapor (Büchi, Flawil, Switzerland).

2.2. Chemicals

All triazine herbicides were obtained from Riedel-de Haën (Seelze-Hannover, Germany) and were used without further purification (minimum percentage purity greater than 98%). The chloro-triazines studied were as follows: atrazine (Az), 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine (pK_a 1.68); simazine (Sz), 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine (pK_a 1.65); and propazine (Pz), 2-chloro-4,6-bis(isopropylamino)-1,3,5-triazine (pK_a 1.85). The methylthiotriazines studied were: ametryne (Am), 2-methylthio-4-ethylamino-6-isopropylamino-1,3,5-triazine (pK_a 4.00); prometryne (Pm), 2-methylthio-4,6-bis(isopropylamino)-1,3,5-triazine (pK_a 4.05); and terbutryne (Tb), 2-methylthio-4-ethylamino-6-terbutylamino-1,3,5-triazine (pK_a 4.38); pK_a values correspond to aqueous medium [21]. Stock solutions of each triazine were prepared in methanol at 500 $\mu g\,ml^{-1}$.

Two types of sorbent were used to perform the solid-phase extraction, some were C_{18} ($+C_{18}$ Sep-Pak Plus) and others were polymeric (Oasis HLB); both types were from Waters (MA, USA). The Oasis HLB is a hydrophilic–lipophilic balanced copol-

ymer of poly(divinylbenzene-co-*N*-vinylpyrrolidone) which exhibits both hydrophilic and lipophilic retention characteristics allowing retention of a wide spectrum of both polar and non-polar compounds.

The organic solvents, acetonitrile (MeCN), methanol (MeOH) and ethyl acetate (AcOEt), were of HPLC grade and were used as received.

All chemicals used for the preparation of the buffer electrolytes were of analytical reagent grade.

2.3. Procedure

2.3.1. Non-aqueous CZE separation of triazines

Uncoated capillaries were used throughout. Prior to use they were washed for 15 min with water and then for a further 10 min with the separation buffer.

Before each injection, the capillaries were washed for 2 min with the separation buffer.

The applied potential was 22 kV; the capillaries were thermostatted at 25±1°C and detection was performed at 214 nm. Unless indicated, injections were electrokinetic.

The separation medium, optimized in a previous work [22] was acetonitrile–methanol 50:50 (v/v), $7.5 \cdot 10^{-3}$ mol l^{-1} perchloric acid and $17 \cdot 10^{-3}$ mol l^{-1} sodium dodecyl sulfate (SDS). Electrolyte solutions of perchloric acid in acetonitrile–methanol 50:50 (v/v), were prepared freshly and not used after long storage periods.

2.3.2. SPE procedure

The cartridges were conditioned with 10 ml of methanol, followed by air-drying at 7 mm Hg over 2 min.

Sample passage, at a volume of 500 ml, was accomplished at a flow-rate of 7 ml min^{-1} with the peristaltic pump. Once the retention step had been completed, the cartridges were dried under vacuum for 10 min at 20 mm Hg (1 mm Hg=133.322 Pa). Elution of the retained components was achieved with 10 ml of methanol, acetonitrile or ethyl acetate, depending on the case, and the organic phase thus obtained was brought to complete dryness in a Rotavapor at 45°C.

The dry residue was dissolved in 100 μl of a solution of acetonitrile–methanol 70:30 (v/v), $1 \cdot 10^{-3}$ mol l^{-1} perchloric acid. A 70 μl aliquot of this solution was then placed in a 100 μl injection microvial.

Quantification was performed by evaluating the normalized area of each triazine vs. that of an internal standard.

2.3.3. Analysis of triazine herbicides in water samples

Samples of river water were taken from the River Tormes in the city of Salamanca (Spain). They were collected directly in 1-l glass containers, stored at 4°C in the dark and analysed within 24 h after collection. All river water samples were filtered through sintered glass filters (No. 5) to remove suspended particulate matter before use. Deionized and tap water samples were used without any further treatment.

3. Results and discussion

Capillary zone electrophoresis (CZE) in non-aqueous media and in the presence of ionic additives has been applied with success to the determination of triazine herbicides (chloro- and methylthio-derivatives) [22].

CZE separation of these analytes (especially chlorotriazines) in aqueous solution is difficult because of the low pH required for their conversion into protonated cationic form (HA^+). However, in mixed non-aqueous solvents, acetonitrile–methanol 50:50 (v/v), the acid–base characteristics of these compounds are modified, yielding protonated ionic species that are susceptible to migration when subjected to an electric field. A noteworthy increase in the selectivity and resolution of separations can be achieved using ionic additives. For the separation of chloro- and methylthiotriazines, $7.5 \cdot 10^{-3}$ mol l^{-1} perchloric acid in acetonitrile–methanol 50:50 (v/v), and $17 \cdot 10^{-3}$ mol l^{-1} SDS proved to be satisfactory, providing high resolution in short analysis times.

3.1. Separation of triazines in non-aqueous medium

To perform herbicide separation, a previously described CZE mode [22,23] in non-aqueous medium was used, in which there is almost no

electroosmotic flow, that takes advantage of the change in the acid–base properties undergone by triazines in organic medium and that provides good sensitivity and signal resolution. The number of theoretical plates per meter for the triazines studied ranged between 200 000 for prometryne and 111 000 for propazine, at a concentration level of 25 $\mu g\,l^{-1}$.

The main problem of the method is the poor reproducibility of the electrokinetic injection (Table 1). This aspect is especially important when one wishes to quantify analytes at trace levels. As a result of this, the signals of the area normalized vs. the migration time (NPA=peak area/migration time) of each triazine were normalized vs. an internal standard. The internal standard used for terbutryne and prometryne was ametryne while propazine was used as the internal standard for simazine and atrazine. In turn, terbutryne was used as the internal standard for ametryne and atrazine was used as the internal standard for propazine. The new signals (NPAis) were normalized areas vs. the internal standard:

$$\text{NPAis} = \left(\frac{\text{NPA}}{\text{NPAi}}\right)\text{Ci}$$

where NPA is the normalized peak area vs. the migration time of the analyte in question, and NPAi and Ci are, respectively, the area normalized vs. time, and the concentration of the internal standard.

Table 1 shows the values for reproducibility obtained for a working day together with the day-to-day reproducibility. It may be seen that use of normalization with an internal standard considerably improves the precision of the method.

The analytical characteristics of the method employing non-aqueous CZE are shown in Table 2. Calculation of the detection limit was performed by quantifying the peak heights of the triazines in the recordings corresponding to a concentration of 25 $\mu g\,l^{-1}$. The standard deviation of the noise of the blank sample and the initial concentration corresponding to a peak height 3-fold that of the standard deviation were calculated. In organic medium, the CZE method is some 25-fold more sensitive than the usual determination using MECC [21] (detection limits of 3 $\mu g\,l^{-1}$ as compared with 75 $\mu g\,l^{-1}$, respectively).

3.2. SPE with C_{18} sorbents

In a previous work [19] it was reported that preconcentration of water samples using C_{18} sorbents, if these are eluted with 10 ml of methanol, provides suitable recoveries in the analysis of triazines. In that method, the sample obtained was injected under pressure into the electrophoresis system and separation was performed by MECC. Under these conditions, and at preconcentrating volumes of 100 ml, different signals appeared in the electropherograms that interfered in the quantification of the triazines; these must correspond to other analytes that are preconcentrated and later become associated

Table 1
Relative standard deviation [S_{n-1} (%)], without SPE step, of normalized peak area with time (NPA) and normalized peak area with internal standard (NPAis) values for each triazine[a,b]

	S_{n-1} (%)			
	1 day[c] (7 samples)		Day-to-day[d] (9 days)	
	NPA	NPAis	NPA	NPAis
Am	8.2	1.0	17.7	0.9
Tb	8.0	1.0	18.3	0.9
Pm	7.4	2.4	19.4	2.3
Sz	8.0	2.7	19.5	2.3
Az	8.7	1.8	21.2	0.9
Pz	8.7	1.8	21.6	0.9

[a] Injected sample: $2\cdot10^{-3}$ mol l^{-1} $HClO_4$ in acetonitrile.
[b] Separation buffer: MeOH–MeCN (50:50, v/v), $7.5\cdot10^{-3}$ mol l^{-1} $HClO_4$, $17\cdot10^{-3}$ mol l^{-1} SDS.
[c] Concentration level: 200 $\mu g\,l^{-1}$.
[d] Concentration level: 5 mg l^{-1}.

Table 2
Analytical characteristics of CZE method without SPE step[a,b,c]

	NPAis (mol l^{-1}) vs. $C_{triazine}$ (mol l^{-1})			
	Slope	Intercept$\times10^8$	r^2	D.L.[d] ($\mu g\,l^{-1}$)
Am	1.028±0.005	−2±1	1.000	2.3
Tb	0.973±0.005	2±1	1.000	2.3
Pm	0.984±0.006	0±1	0.999	2.5
Sz	1.39±0.02	−2±4	0.997	2.1
Az	1.10±0.01	1±3	0.998	2.8
Pz	0.911± 0.009	−1±2	0.998	3.4

[a] Concentration range: 25 $\mu g\,l^{-1}$–1 mg l^{-1}. Confidence intervals for 95%.
[b] Injected sample: $2\cdot10^{-3}$ mol l^{-1} $HClO_4$ in acetonitrile.
[c] Separation buffer: MeOH–MeCN (50:50, v/v), 7.5×10^{-3} mol l^{-1} $HClO_4$, $17\cdot10^{-3}$ mol l^{-1} SDS.
[d] Detection limit: signal/noise ratio of 3.

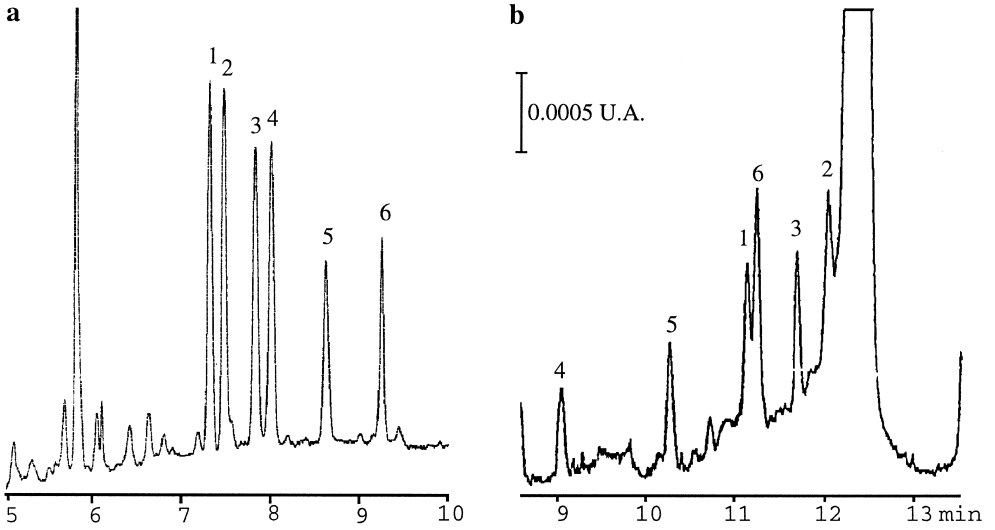

Fig. 1. Electropherograms of deionized water samples spiked with triazines and preconcentrated by SPE with C_{18} as sorbent. (a) 500 ml spiked at 0.1 $\mu g\,l^{-1}$ of each triazine, preconcentrated and redissolved on 100 μl. Separated with non-aqueous CZE method. (b) 100 ml spiked at 0.5 $\mu g\,l^{-1}$ of each triazine, preconcentrated and redissolved on 100 μl. Separated using the MECC method. 1, ametryne; 2, terbutryne; 3, prometryne; 4, simazine; 5, atrazine; 6, propazine. Injected sample: (a) MeOH–MeCN (30:70, v/v), $2\cdot10^{-3}$ mol l^{-1} $HClO_4$. (b) $60\cdot10^{-3}$ mol l^{-1} borate buffer, pH 9.2, 10% (v/v) MeOH. Separation buffer: (a) MeOH–MeCN (50:50, v/v), $7.5\cdot10^{-3}$ mol l^{-1} $HClO_4$, $17\cdot10^{-3}$ mol l^{-1} SDS. (b) $60\cdot10^{-3}$ mol l^{-1} borate buffer, pH 9.2, $50\cdot10^{-3}$ mol l^{-1} SDS. U.A. = AU.

with the micelles in the electrokinetic separation step [Fig. 1(b)].

Using the CZE separation mode in organic medium and electrokinetic injection, the electropherograms obtained, after preconcentrating a volume up to even 5-fold greater (500 ml), were clean and very well resolved and quantification of the triazines did not point to any interference [Fig. 1(a)]. This kind of behaviour is due to the fact that in this mode only positively charged analytes are able to enter the capillary and migrate to the detector.

However, on analysing samples of surface and drinking water, spiked with a concentration of 0.5 $\mu g\,l^{-1}$, no signals possibly corresponding to the analytes of interest were observed between 6 and 8 min [Fig. 2(a) and (b)]. When sorbent elution was performed with 10 ml of acetonitrile instead of methanol, a similar electropherogram was obtained. When ethyl acetate was used as eluent, an electropherogram was obtained in which, at the migration times of the analytes, a series of poorly defined signals appeared, possibly corresponding to the triazines studied [Fig. 2(c)]. Careful scrutiny of the eluate achieved with ethyl acetate revealed that in this case two liquid phases were obtained: one corresponding to the ethyl acetate and another smaller one, about 200 μl, corresponding to the aqueous phase.

In order to remove the water from the eluate, we performed a step of sorbent drying, keeping it in a stream of nitrogen for 50 min after 10 min under vacuum at 20 mm Hg. In this case, later elution with ethyl acetate generated two new phases, although the volume of the aqueous phase was smaller (some 100 μl). On applying the remaining steps of the preconcentration and separation stage only to the organic phase, an electropherogram with good resolution for the triazines studied was obtained [Fig. 2(d)]. This electropherogram was similar to that obtained on preconcentrating deionized water.

The above results show that the C_{18} sorbent should not be eluted with solvents as polar as methanol or acetonitrile, since these displace the water retained in the sorbent and, moreover, must elute certain components that prevent electrokinetic introduction of the triazines. Like methanol and acetonitrile, ethyl acetate displaces the water retained in the C_{18} cartridge but in this case it is possible to

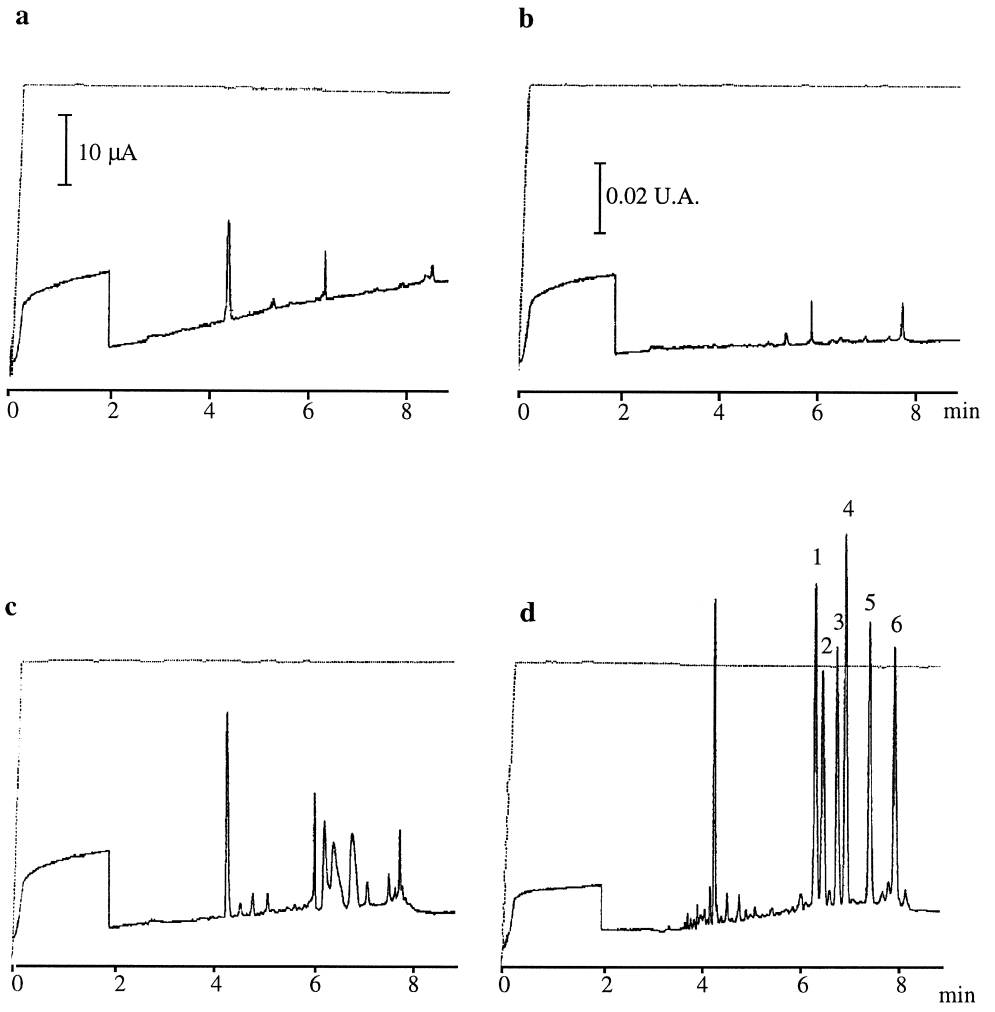

Fig. 2. C_{18} SPE preconcentration. (a) Surface water–MeOH as eluent. (b) Drinking water–MeOH as eluent. (c) Surface water–AcOEt as eluent. (d) Surface water–AcOEt as eluent without the eluted aqueous phase. Samples spiked at 0.5 $\mu g\,l^{-1}$ of each triazine. 1, ametryne; 2, terbutryne; 3, prometryne; 4, simazine; 5, atrazine; 6, propazine. Injected sample: MeOH–MeCN (30:70, v/v), $2\cdot10^{-3}$ $mol\,l^{-1}$ $HClO_4$. Separation buffer: MeOH–MeCN (50:50, v/v), $7.5\cdot10^{-3}$ $mol\,l^{-1}$ $HClO_4$, $17\cdot10^{-3}$ $mol\,l^{-1}$ SDS.

see the water because ethyl acetate and water are immiscible. Additionally, ethyl acetate does not elute ionic organic compounds since the counterions, which are usually inorganic, are not soluble in it.

Accordingly, different studies were undertaken using another type of sorbent (Oasis HLB), of polymeric nature, whose capacity to retain the analytes of interest was equal to or even greater than that of C_{18}.

Three samples of surface waters spiked with the triazines at individual concentrations of 0.5 $\mu g\,l^{-1}$ were preconcentrated with Oasis HLB and were then eluted with 10 ml of ethanol, acetonitrile and ethyl acetate, respectively. It should be mentioned that in the elution with ethyl acetate only one phase was obtained. Fig. 3 shows the electropherograms obtained. These results show that with the Oasis HLB sorbents methanol elutes the triazines together with other organic compounds that perturb the electrokinetic introduction of the triazines. This effect was

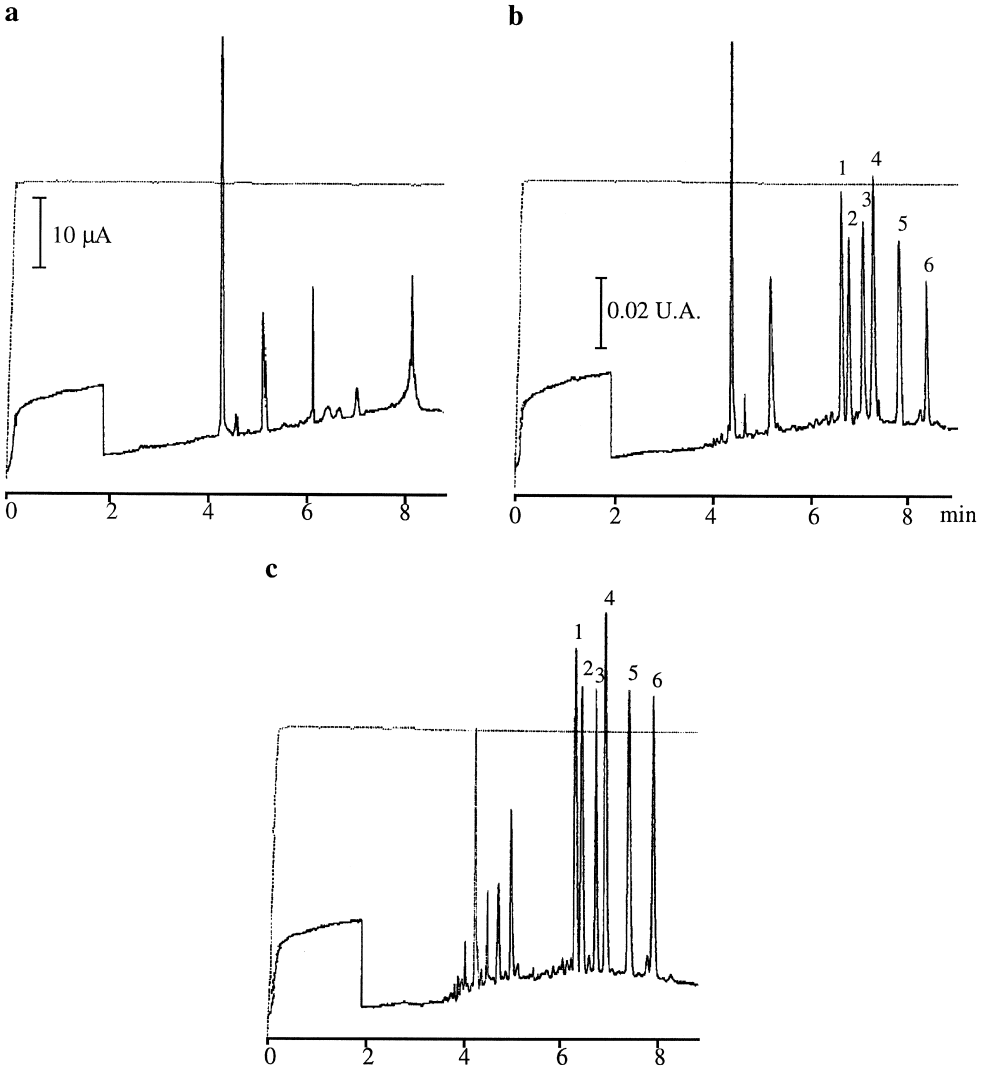

Fig. 3. Oasis HLB SPE preconcentration of surface water samples. Eluents: (a) MeOH; (b) MeCN; and (c) AcOEt. Samples spiked at 0.5 $\mu g\,l^{-1}$ of each triazine. 1, ametryne; 2, terbutryne; 3, prometryne; 4, simazine; 5, atrazine; 6, propazine. Injected sample: MeOH–MeCN (30:70, v/v), $2\cdot10^{-3}$ $mol\,l^{-1}$ $HClO_4$. Separation buffer: MeOH–MeCN (50:50, v/v), $7.5\cdot10^{-3}$ $mol\,l^{-1}$ $HClO_4$, $17\cdot10^{-3}$ $mol\,l^{-1}$ SDS.

scarcely visible when acetonitrile was used as eluent and was not observed at all when ethyl acetate was used as eluent.

3.3. SPE with polymeric sorbents (Oasis HLB)

3.3.1. Recoveries in the extraction process

To assess the recoveries obtained using polymeric sorbents, two 500-ml samples of deionized water spiked with terbutryne, prometryne, simazine and atrazine at a concentration of 1 $\mu g\,l^{-1}$ of each (0.2%, v/v, of methanol) were prepared. The described preconcentration procedure was applied to these samples using ethyl acetate as eluent. The dry residue obtained was dissolved in 100 μl of a solution of optimum composition for injection and also containing ametryne and propazine at a con-

Table 3
Recovery with the Oasis HLB SPE procedure[a]

	C_{theor}[b] (mg l^{-1})	C_{calc}[c] (mg l^{-1})	Recovery (%)
Am	5.24	5.1±0.3	97±6
Tb	5.04	4.5±0.3	89±6
Pm	5.00	4.74±0.05	95±1
Sz	5.08	4.2±0.1	83±2
Az	5.28	4.8±0.3	91±6
Pz	5.20	5.9±0.3	114±6

[a] Confidence intervals for 95%.
[b] Injected sample concentration for a 100% theoretical recovery.
[c] Mean of two replicates.

centration of 5.0 μg l^{-1} of each, which in this case acted as internal standards for the others.

At the same time, a further two 500-ml samples containing ametryne and propazine at the same concentration as before (1 μg l^{-1}) were prepared, after which the same preconcentration procedure was applied. This time, the sample solution for injection contained the triazines terbutryne and atrazine, which served as internal standards for the other triazines.

Electrokinetic injection was performed over 15 s at 5 kV. The values of the normalized area with an internal standard allowed the determination of the triazine concentrations of the samples injected. The results are shown in Table 3. The recovery values obtained were in the 97–114% range.

3.3.2. Analytical data

The analytical characteristics of the method using polymeric sorbents are shown in Table 4. Samples of deionized water, surface water and drinking water were analysed for the presence of triazines and in no case were signals observed at the migration times of the triazines studied. The fortified concentration range was between 0.1 and 2 μg l^{-1}.

The electropherograms shown in Fig. 4 correspond to samples of natural waters spiked with triazines at a concentration of 0.1 μg l^{-1} of each triazine and preconcentrated using polymer-based cartridges. In the electropherogram corresponding to surface water, a stronger perturbation of the baseline was seen.

In order to check that 'NPAis vs. $C_{initial\ of\ triazine}$' obtained after SPE preconcentration with polymeric sorbent were not significantly different when passing from one type of matrix to another (deionized water, surface water, drinking water), the responses obtained for each type of water were compared.

To do so, linear regression between the values of NPAis of the calibration straight line in one type of matrix and the NPAis values of the calibration line in another type of matrix was performed. If in this regression, for a 95% significance level the intercept obtained did not differ significantly from zero and the slope was not significantly different from unity and the correlation coefficient was close to one, then both matrices could be said to show little difference in their behaviour, and hence it would be possible to use one calibration or the other to determine the concentration of the triazines in both types of water. Table 5 shows the results of these comparisons.

In the comparison of drinking water vs. deionized

Table 4
Analytical characteristics for Oasis HLB SPE preconcentration[a,b]

	Deionized water				Drinking water				River water			
	Slope	Intercept ×10^{-11}	r^2	D.L.[c]	Slope	Intercept ×10^{-11}	r^2	D.L.[c]	Slope	Intercept ×10^{-10}	r^2	D.L.[c]
Am	1.17±0.07	8±33	0.994	0.02	0.82±0.02	8±8	0.999	0.01	1.16±0.05	−3±3	0.997	0.05
Tb	0.87±0.05	4±22	0.994	0.02	1.22±0.02	−9±9	0.999	0.01	0.85±0.04	3±2	0.996	0.04
Pm	0.91±0.04	13±22	0.996	0.02	1.12±0.02	−9±9	0.999	0.01	0.9±0.1	4±9	0.955	0.04
Sz	1.54±0.05	−9±30	0.998	0.01	1.48±0.08	−44±44	0.996	0.01	1.67±0.09	−4±4	0.996	0.03
Az	1.06±0.03	0±17	0.999	0.02	1.07±0.04	4±17	0.998	0.01	1.12±0.07	2±3	0.995	0.03
Pz	0.95±0.02	0±14	0.999	0.02	0.94±0.03	−5±14	0.999	0.01	0.88±0.04	−1±2	0.996	0.05

[a] Confidence intervals for 95%.
[b] Regression parameters of NPAis (mol l^{-1}) vs. $C_{triazine}$ prior to preconcentration step (mol l^{-1}).
[c] Detection limit (μg l^{-1}) for a signal/noise ratio of 3.

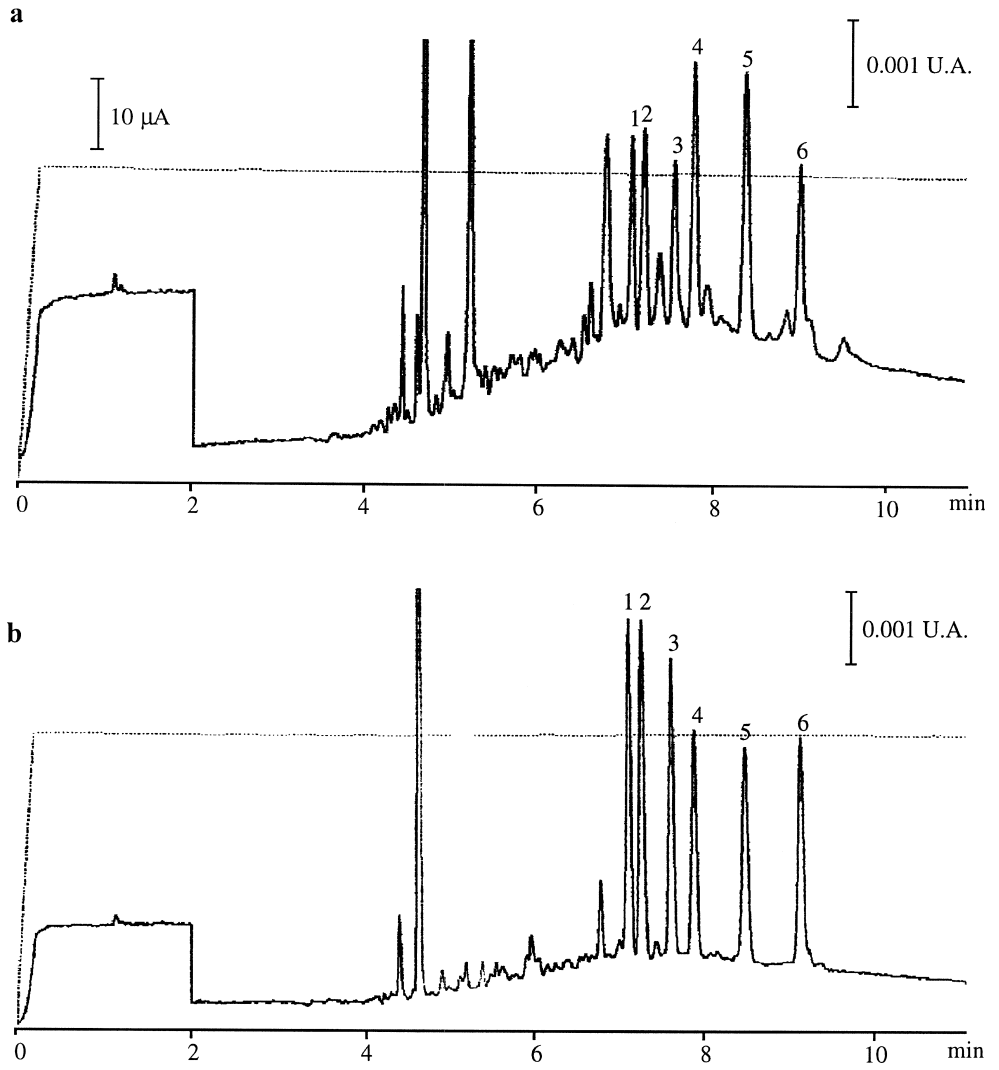

Fig. 4. Oasis HLB SPE preconcentration of natural water samples spiked at 0.1 μg l^{-1} of each triazine. (a) River water. (b) Drinking water. 1, ametryne; 2, terbutryne; 3, prometryne; 4, simazine; 5, atrazine; 6, propazine. Injected sample MeOH–MeCN (30:70, v/v), $2 \cdot 10^{-3}$ mol l^{-1} $HClO_4$. Separation buffer: MeOH–MeCN (50:50, v/v), $7.5 \cdot 10^{-3}$ mol l^{-1} $HClO_4$, $17 \cdot 10^{-3}$ mol l^{-1} SDS.

water, the responses corresponding to the more polar triazines (chlorotriazines) were not significantly different for either type of water. By contrast, the analytical data for methyltriazines differed considerably. This indicates that some matrix effect exists for this group. On comparing the analytical data from the river water vs. that of the deionized water, the resulting graph was a straight line of unit slope, zero intercept for all the triazines. Thus, no significantly different results were obtained for those two matrices.

3.4. Determination of triazines in natural waters

To check the applicability of the proposed method, natural water samples of different origins were analysed. A non-spiked 500 ml aliquot of each sample was first analysed following the same pro-

Table 5
Oasis HLB SPE preconcentration[a]

	Drinking vs. deionized water[b]			River vs. deionized water[c]		
	Slope	Intercept	r^2	Slope	Intercept	r^2
Am	0.71±0.03	0.03±0.03	0.999	0.99±0.07	−0.04±0.08	0.998
Tb	1.42±0.05	−0.03±0.04	0.999	0.99±0.05	0.05±0.05	0.998
Pm	1.20±0.09	−0.05±0.09	0.995	1.0±0.2	0.0±0.1	0.982
Sz	1.0±0.1	−0.0±0.2	0.992	1.1±0.1	−0.1±0.2	0.992
Az	1.01±0.06	0.01±0.08	0.997	1.06±0.09	0.1±0.1	0.995
Pz	0.99±0.06	−0.01±0.06	0.998	0.93±0.07	−0.02±0.07	0.996

[a] Comparative plots among different aqueous matrices. Confidence intervals for 95%.
[b] NPAis from drinking water vs. NPAis from deionized water.
[c] NPAis from river water vs. NPAis from deionized water.

cedure to check the presence of these compounds. For quantification purposes, the calibration straight lines obtained in each type of aqueous matrix were used.

In three of the samples analysed, the presence of terbutryne was detected at a level close to the detection limit. In another sample, the presence of atrazine at a concentration of 0.15 $\mu g\,l^{-1}$ was detected. To determine the value of the method, this sample was analysed using HPLC following a procedure previously proposed by our team [24]. The results obtained (Table 6) were in good agreement with those found using CZE analysis.

Another two samples not containing triazines were spiked at 0.5 $\mu g\,l^{-1}$ and used in recovery studies. The results obtained (Table 6) indicated the reliability of the proposed method.

4. Conclusions

Using separation by CZE in non-aqueous medium it is possible to quantify ametryne, terbutryne, prometryne, simazine, atrazine and propazine at a concentration level 25-fold lower than when using MECC separation. However, to determine these analytes at sub-ppb levels, a preconcentration step must first be implemented.

Using electrokinetic injection and separation by CZE in organic medium, the C_{18} sorbents eluted with methanol, acetonitrile or ethyl acetate do not provide satisfactory results as regards the analysis of triazines in natural waters. It is recommended that preconcentration using polymeric sorbents (Oasis HLB) be performed, in which case the drying step is not very critical, using ethyl acetate as eluent.

Table 6
Determination of triazine herbicides in natural waters after Oasis HLB SPE preconcentration

	River water				Drinking water	
	Sample 1[d]		Sample 2[d]		Sample 3[d]	
	C_{added}[a]	C_{found}[b]	C_{HPLC}[c]	C_{found}	C_{added}	C_{found}
Am	0.52	0.51±0.03	ND[e]	ND[e]	0.52	0.53±0.01
Tb	0.50	0.51±0.03	ND[e]	ND[e]	0.50	0.50±0.01
Pm	0.50	0.56±0.10	ND[e]	ND[e]	0.50	0.49±0.01
Sz	0.51	0.48±0.03	ND[e]	ND[e]	0.51	0.55±0.04
Az	0.53	0.51±0.04	0.14	0.15±0.04	0.53	0.55±0.03
Pz	0.52	0.53±0.03	ND[e]	ND[e]	0.52	0.50±0.03

[a] Spiked concentration ($\mu g\,l^{-1}$).
[b] Calculated concentration ($\mu g\,l^{-1}$) with appropriate regression line.
[c] Concentration found ($\mu g\,l^{-1}$) with a SPE–HPLC procedure.
[d] Mean of two replicates.
[e] ND: Not detected.

Although electrokinetic injection is less precise than injection under pressure, use of an internal standard does allow suitable precision. Additionally, on using this type of injection in a medium without electroosmotic flow, it is possible to obtain greater selectivity, with no interferences in the electropherograms.

The results obtained here indicate that capillary electrophoresis can be satisfactorily used to quantify micropollutants in environmental samples at levels in consonance with the maximum levels permitted by current legislation.

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