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Trace-level determination of triazines and several degradation products in environmental waters by disk solid-phase extraction and micellar electrokinetic chromatography

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Abstract

An analytical method combining disk solid-phase extraction with micellar electrokinetic chromatography has been developed for the determination of atrazine, simazine, hydroxyatrazine, deisopropylatrazine, deethylatrazine, propazine and prometryn in water samples. The influence of the buffer and sodium dodecyl sulfate (SDS) concentration, pH and organic modifier on the separation has been studied. Baseline separation of the seven triazines was achieved under the following conditions: 10 m*M* borate buffer, 60 m*M* SDS, 20% methanol and pH 9.2. C_{18}-bonded silica and poly(styrene–divinylbenzene) (PS–DVB) disks were evaluated for solid-phase extraction of the selected pesticides (1 l of water sample). Using two PS–DVB disks, quantitative recoveries were obtained for all pesticides tested. The method was successfully applied for the determination of the seven triazines in drinking and well water at the 0.1 $\mu g\ l^{-1}$ and 0.5 $\mu g\ l^{-1}$ concentration levels, respectively. The detection limits for these analytes using the proposed analytical method were within the 0.02–0.06 $\mu g\ l^{-1}$ range in drinking water and the 0.06–0.30 $\mu g\ l^{-1}$ range in well water.

Keywords: Water analysis; Environmental analysis; Pesticides; Triazines

1. Introduction

Triazinic herbicides have been extensively used for weed control in recent years, making their determination necessary in environmental samples.

After application, several degradation processes take place leading to dealkylation of amine groups (positions 4 and 6 of the triazinic structure) or hydrolysis of the substituent in position 2. The most common degradation products of atrazine are deethylatrazine (DEA), deisopropylatrazine (DIA) and hydroxyatrazine (HA), and HA is a common contaminant of environmental waters [1].

In general triazinic herbicides are mainly determined by chromatographic techniques such as high-performance liquid chromatography (HPLC) or gas chromatography (GC), and there is extensive information available on them in published reviews [2,3].

Capillary electrophoresis (CE) is becoming extremely popular in analytical practice. CE has important advantages over HPLC or GC, such as highly efficient separations in relatively short times, the use

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of very small sample volumes (nanoliters), and very low consumption, when necessary, of organic solvents. In addition, polar compounds cannot be directly determined by GC due to their low volatility, so a prior analyte derivatization step is required [4].

Capillary zone electrophoresis (CZE) [5], isotacophoresis (ITP) [6] and capillary isoelectric focusing (cIEF) [7] have been used for the determination of triazines. However, these techniques do not allow the separation of neutral compounds, so they are only useful for the analysis of ionic triazines. Non-ionic triazines can only be determined by micellar electrokinetic chromatography (MEKC). In this technique, a surfactant is added to the buffer, and the formed micelles act as a pseudostationary phase in the chromatographic process. The separation is based on the different distribution of the analytes between the buffer and the micelles. Organic modifiers can be added to the buffer to improve the resolution.

The maximum levels of pesticides in drinking water allowed by the European Union are extremely low, so it is necessary to combine solid-phase extraction (SPE) preconcentration with electrophoretic analysis. Several works have been published on the separation of triazines by MEKC, with a focus on the performance of the separation [8–13], and using a preconcentration process [14,15], but not low enough detection limits were reported to allow determination of these analytes in environmental waters at the real concentration level. Recently Loos and Niessner reported the application of MEKC to the analysis of hydroxy metabolites of atrazine in tap and river water [16].

The main aims of the work described in this paper were as follows:

(i) The separation of four herbicides (atrazine, simazine, propazine and prometryn) and three degradation products of atrazine (DEA, DIA and HA).

(ii) The development of a SPE method for preconcentration of the afore mentioned analytes prior to MEKC analysis.

(iii) The application of the developed SPE–MEKC method to the determination of these herbicides in environmental waters at the real concentration level.

2. Experimental

2.1. Apparatus and materials

All measurements were performed with a capillary electrophoresis system HP ^{3D}CE (Hewlett-Packard Española) equipped with a diode array detector and controlled by a HP Vectra VL computer and a HP Chemstation Program Manager. Separations were carried out in a fused-silica extended light path capillary of 48.5 cm (40 cm effective length)×75 μm I.D. and 200 μm optical path length from Hewlett-Packard Española.

Water samples were pre-filtered onto 0.45-μm Microm-47 mm-100/Pk nylon filters (Micron Separations, USA). Extraction of pesticides were performed in a vacuum filtration system into C_{18} and poly(styrene–divinylbenzene) (PS–DVB) 3M Empore Extraction Disks obtained from Scharlau (Barcelona, Spain).

A Univapo Concentrator Centrifuge coupled to a Unijet II Refrigerated Aspirator (supplied by Biogen Científica, Madrid, Spain) was used for evaporation of the sample extracts.

Buffer, standard solutions and extracted samples were filtered through a 0.45-μm PTFE syringe filter obtained from Scharlau.

2.2. Reagents

Atrazine, deethylatrazine, deisopropylatrazine, hydroxyatrazine, simazine, propazine and prometryn were obtained from Riedel-de Häen (Seelze, Germany). Stock standard solutions (1 g l^{-1}) were prepared in acetonitrile and stored at −18°C.

Analytical-grade sodium tetraborate ($Na_2B_4O_7$ anhydrous), sodium dodecyl sulfate (SDS) ($C_{12}H_{25}SNa$) and sodium chloride were purchased from Merck (Darmstadt, Germany). One *M* and 0.1 *M* sodium hydroxide solutions for high-performance capillary electrophoresis (HPCE) were supplied by Fluka. Methanol (HPLC grade) was obtained from Scharlau.

Purified water (Milli-Q) from Millipore Ibérica was used to prepare reagent solutions.

2.3. Procedures

2.3.1. Conditioning and MEKC separation

MEKC separations of triazines were performed with a 10 m*M* sodium borate buffer containing 60 m*M* SDS and 20% methanol at pH 9.2. The buffer was filtered though a 0.45-μm PFTE syringe filter. Before starting a sequences of runs, the capillary was conditioned by rinsing in the high-pressure mode, in the following order: 0.1 *M* NaOH (5 min), purified water (2 min), run buffer (5 min). Between each run, the conditioning step only consisted of 2 min of NaOH and 2 min of run buffer in order to avoid memory effects.

Samples were injected in the running buffer medium in hydrodynamic mode by applying 50 mbar for 10 s (52 nl), and the separation runs were carried out at a constant voltage of 30 kV. The temperature of the capillary can be controlled and it was adjusted to 25°C. Triazinic herbicides were monitored at 220 nm.

2.3.2. Solid-phase extraction

Two PS–DVB SPE disks were placed in the filtration system and activated and conditioned with 2×10 ml of methanol and 2×10 ml of water. After percolating the spiked sample (1 l), the adsorbent was washed with 5×2 ml of water and vacuum dried in order to remove all remaining water. Subsequently, analytes were eluted with 6×2 ml of methanol.

Extracts were vacuum evaporated to dryness and the residues were subsequently redissolved in 1 ml of running buffer and filtered through a 0.45-μm PTFE filter before injection (enrichment factor 1000).

2.3.3. Sample preparation

Mineral water and tap water (Madrid, Spain) were spiked at the 0.1 μg l^{-1} level of each pesticide. Natural well water (Toledo, Spain) was spiked at the 0.5 μg l^{-1} level. Tap water and well water samples were adjusted to pH 12.

Water samples were pre-filtered through a 0.45-μm filter to remove the suspended matter, and then triazinic herbicides were extracted by following the double PS–DVB disk SPE process described above.

3. Results and discussion

3.1. MEKC separation performance

To investigate the performance of the separation, variables which have a direct influence on both the electroosmotic flow (EOF) and the micellar phase were evaluated. Due to the polarity of the selected herbicides, 20% of methanol in the running buffer was needed to achieve good resolution. The main problem was co-elution of DIA and DEA (the most polar) with the injection peak. Therefore, optimization of the process focused on achieving the baseline separation of the two compounds.

3.1.1. Effect of buffer concentration and pH

As mentioned above, there is a distribution equilibrium of pesticides between the polar running buffer and the non-polar micelles, and therefore, they appear in order of decreasing polarity. The higher the ionic strength of the buffer, the lower the EOF, resulting in longer migration times and therefore in a better separation of analytes. In contrast, an increase in buffer concentration leads to an increase in the Joule heating effect and, in addition, to band broadening and an increase in analysis time.

In this sense, buffer concentration was varied within the 5–30 m*M* range, and the obtained results shown that at borate buffer concentrations equal to or higher than 10 m*M*, DIA and DEA did not co-elute with the injection peak, although increases in buffer concentration did not improve the resolution of these analytes. Therefore 10 m*M* borate buffer was selected for further experiments.

No pH adjustment in the useful range of the borate buffer (pH 8–10) was necessary due to its negligible effect on resolution. Ten m*M* borate buffer gives a pH of 9.2.

3.1.2. Effect of SDS concentration

An important property of micelles is their ability to enhance the solubility of hydrophobic organic compounds in aqueous media. It is known that micellar solubilization of compounds controls the separation process. The phase ratio of the system, the elution window and the efficiency of separation are influenced by the concentration of micelles [17].

The SDS concentrations tested were within the 20–60 m*M* range. Increases in the SDS concentration leads to an increase in retention time, and baseline resolution of the seven herbicides was achieved with 60 m*M* SDS, so this concentration was chosen as optimum.

3.1.3. Effect of organic modifier

The addition of an organic component to the running buffer usually improves the separation. It affects the distribution equilibrium of analytes between the buffer and the micelles, the EOF, and sample solubility in the buffer by changing the viscosity.

The influence of methanol as organic modifier was tested between the 5–30% range. Using 10% methanol DIA and DEA still co-eluted, while at or above 20% methanol, the two peaks could be baseline resolved.

Fig. 1 shows the electropherogram obtained in the optimal conditions, at the 1.25 mg l^{-1} concentration level for each pesticide.

3.2. Calibration

Calibration was carried out by injection of standard solutions containing herbicide concentrations within the 50 μg l^{-1}–2 mg l^{-1} range. Linear calibration curves were found in the tested range and the correlation factors obtained ranged from 0.996 to 0.999 depending on the pesticide.

The detection limits obtained were within the 20 μg l^{-1}–40 μg l^{-1} range, calculated as three-times the signal/noise ratio. Thus, under these experimental conditions, a preconcentration factor of 1000 is required to analyze these pesticides at a real environmental level.

3.3. Solid-phase extraction

Due to the extremely low concentrations of pesticides in environmental waters, a SPE and preconcentration procedure is necessary. SPE using Empore extraction disks has proved to be an appropriate method for handling water samples when high preconcentration factors are required. Empore ex-

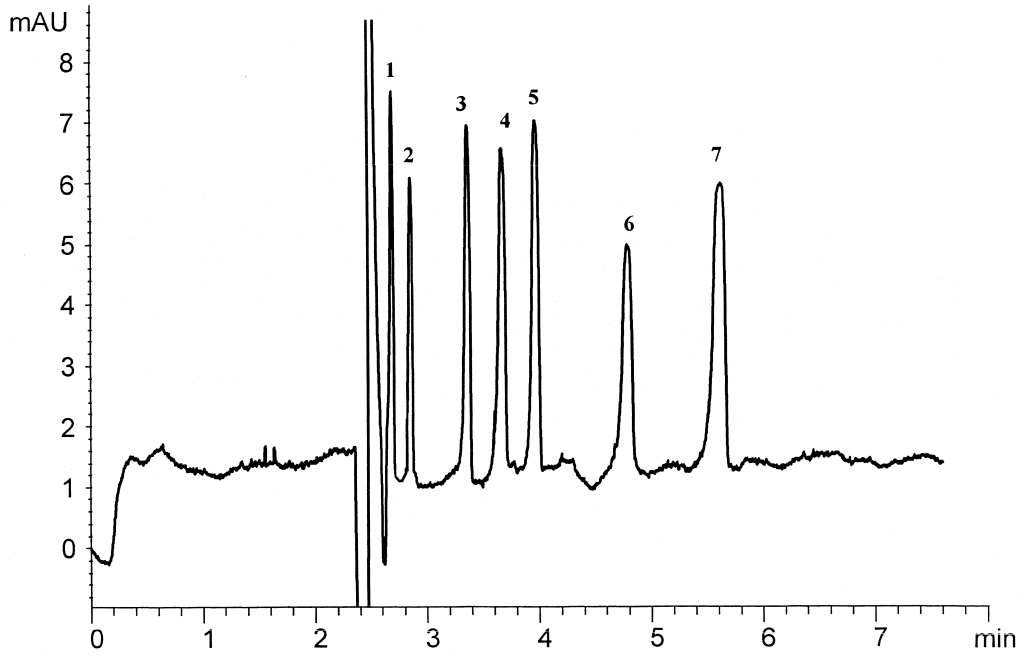

Fig. 1. Electropherogram obtained under the optimum conditions: 10 m*M* borate buffer, 60 m*M* SDS, 20% methanol and pH 9.2. 1=DIA, 2=DEA, 3=simazine, 4=hydroxyatrazine, 5=atrazine, 6=propazine, 7=prometryn, at the 1.25 mg l^{-1} concentration level for each pesticide.

traction disks allow a high sample flow-rate and a fast mass transfer because of the smaller particle sizes [18].

C_{18}-bonded silica and PS–DVB Empore extraction disks were evaluated for SPE of the seven pesticides. A breakthrough volume study was realized using one C_{18} disk by calculating the recoveries obtained after percolating 100, 250, 500 and 1000 ml of purified water solutions containing 1 μg of each pesticide. The results shown that 100 ml and 250 ml are the breakthrough volumes for the more polar analytes DIA and DEA, respectively. For the other pesticides, breakthrough volume was not achieved in the range tested.

As can be observed in Table 1, recoveries obtained for the 1 l solution using one C_{18} disk were quantitative for all pesticides except for DIA and DEA due to its low breakthrough volume. In order to improve them, the addition of 10% NaCl to the sample solution (''salting out effect'') was evaluated. The recovery was quantitative for all pesticides except for DIA.

In SPE, the amount of adsorbent employed has a direct influence on analytes recovery. Therefore, a second approach using two C_{18} disks was evaluated, with and without addition of salt. Only the first procedure yielded quantitative recoveries for all pesticides.

However, the addition of NaCl, using one or two C_{18} disks, has negative effects on MEKC analysis: (a) an increase in the ionic strength of the samples, which decreases the EOF and therefore produces changes in retention times, and (b) serious interference in the electropherogram. Using one C_{18} disk two humps appeared making quantification of hydroxyatrazine and prometryn almost impossible, and, when two C_{18} disks were used, the hump also involved the propazine peak.

Consequently, SPE on PS–DVB disks was evaluated. The PS–DVB polymer offers a higher affinity for analytes because aromatic rings in the polymeric matrix produce intense $\pi-\pi^*$ interactions and so that permits the utilization of higher sample volume without exceeding the breakthrough volume of the more polar analytes.

As can be observed in Table 1, the recoveries obtained with PS–DVB were higher than with C_{18} in all cases, although, when only one PS–DVB disk was used, the recovery for deisopropylatrazine was still very low (49%). However, two PS–DVB disks yielded quantitative recoveries for all analytes tested, except for DIA (73.5%), and thus obviated the need for salt addition. The RSDs were lower than 8% for all triazines.

3.4. Analysis of water samples

The developed procedure was applied to the determination of the selected pesticides in drinking and well water samples.

European Union legislation on pesticides control in environmental waters establishes a maximum pesticide level in drinking water of 0.1 μg l^{-1} [19]. For environmental water (ground, river, etc.), 1 μg l^{-1} is the so-called alert level for pesticides [20,21].

Accordingly, recovery studies were carried out on

Table 1
Recovery[a] (*R*) of triazines after solid-phase extraction of 1 l of purified water spiked at 1 μg l^{-1}

Triazine	C_{18}, one disk				C_{18}, two disks				PS–DVB, one disk		PS–DVB, two disks	
	Without NaCl		10% NaCl		Without NaCl		10% NaCl		Without NaCl		Without NaCl	
	R (%)[a]	RSD (%)	*R* (%)[a]	RSD (%)	*R* (%)[a]	RSD (%)	*R* (%)[a]	RSD (%)	*R* (%)[a]	RSD (%)	*R* (%)[a]	RSD (%)
DIA	21.5	18.6	42.3	13.6	35.8	18.2	89.2	8.7	49	9.3	73.5	3.3
DEA	50.4	9.3	98.4	6.2	60.5	13.1	95.4	6.3	100.3	3.5	97.7	4.2
Simazine	100.2	6.1	93.5	7.8	96.4	8.7	91.7	6.2	104.1	6.5	96.3	5.6
HA	103.7	4.7	n.d.[b]	–	93.6	7.5	n.d.[b]	–	101.2	4.3	93.5	3.5
Atrazine	94.6	8.7	98.3	4.9	104.3	4.6	97	4.1	96.5	3.2	98.0	7.2
Propazine	96.8	6.5	102.5	5.8	92.1	9.1	n.d.[b]	–	102.0	6.3	102.4	4.7
Prometryn	97.5	7.3	n.d.[b]	–	89.7	6.3	n.d.[b]	–	97.2	5.1	96.5	4.4

[a] Average of three independent determinations.
[b] Not detected.

the selected pesticides in drinking water (mineral and tap water) and well water spiked at 0.1 μg l^{-1} and 0.5 μg l^{-1}, respectively. A parallel water blank in each case was also analyzed following the same procedure. None of the selected triazines was found in these matrices.

Fig. 2 shows the electropherogram obtained in the analysis of mineral water spiked at 0.1 μg l^{-1}. It clearly shows that all the selected pesticides can be determined at this low concentration.

However, a hump appeared in the middle of the electropherogram when tap and well water samples were analyzed, making it difficult to quantify several triazinic herbicides. In addition, it is important to point out that the presence of cationic metals (especially Ca^{2+} and Mg^{2+}) in samples to be analyzed induces formation of the corresponding dodecyl sulfate precipitates inside the capillary [22] which causes total loss of EOF and a drop in current, making it impossible to work with MEKC.

To solve the above-mentioned problems, the pH of the samples was increased to 12. In this way, the cationic metals were eliminated by formation of their corresponding hydroxides and subsequent filtration through a 0.45-μm filter. Also, at basic pH values a high number of phenolic groups of humic and fulvic acids are in their ionic form, and thus, their extraction and elution with the selected pesticides is avoided.

Figs. 3 and 4 show the electropherograms obtained after SPE of 1 l of tap and well water, respectively, at pH 12, spiked with the different pesticides. Although humic and fulvic acids were not completely removed, the analytes can be quantitatively determined with the proposed method, except deethylatrazine in tap water (peak 2) and propazine (peak 6) in well water, due to the presence of unknown peaks at their retention times.

In short, Table 2 shows the recoveries obtained for the selected pesticides in the analysis of water samples with the developed procedure. These values are acceptable and demonstrate the repeatability of the method. The detection limits, also shown in Table 2, calculated as three-times the signal/noise ratio, were within the range of 0.02–0.06 μg l^{-1} in drinking water and 0.06–0.30 μg l^{-1} in well water.

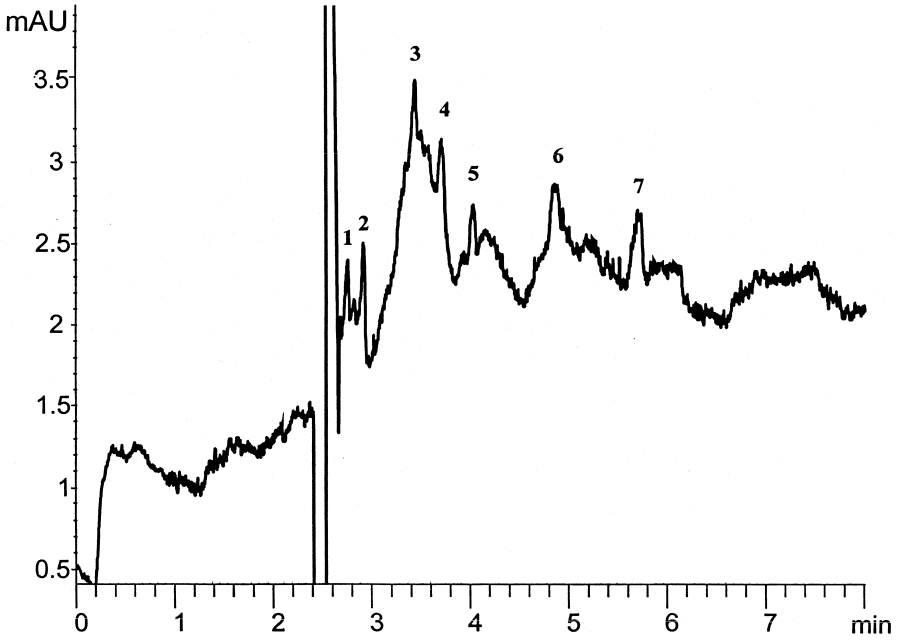

Fig. 2. Electropherogram obtained for mineral water spiked at 0.1 μg l^{-1} of each triazine after SPE onto two PS–DVB disks (enrichment factor 1000). 1=DIA, 2=DEA, 3=simazine, 4=hydroxyatrazine, 5=atrazine, 6=propazine, 7=prometryn. Electrophoretic conditions as in Fig. 1.

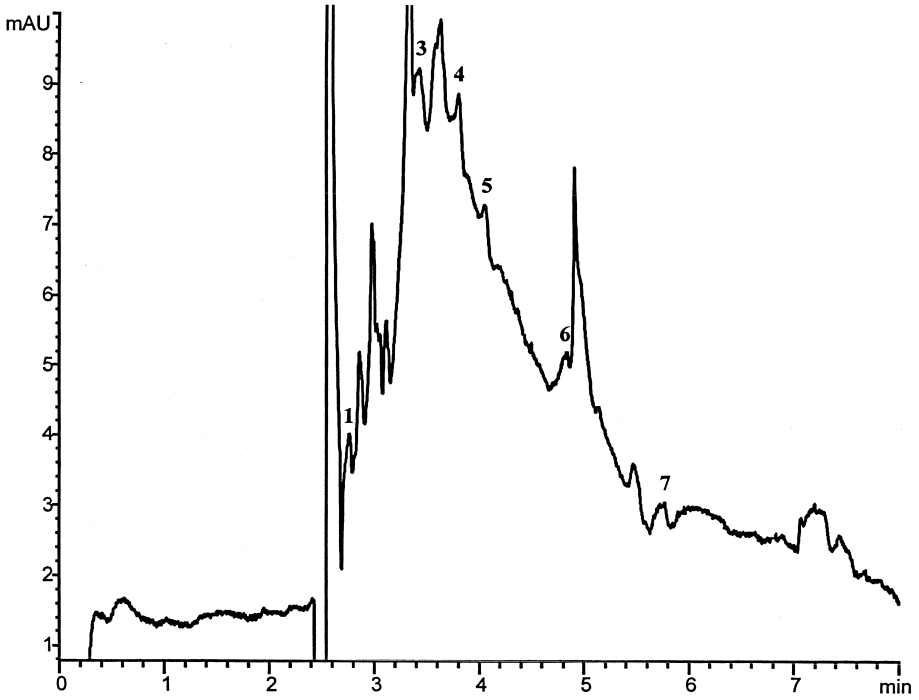

Fig. 3. Electropherogram obtained for tap water spiked at 0.1 μg l^{-1} of each triazine after SPE onto two PS–DVB disks (enrichment factor 1000). 1=DIA, 3=simazine, 4=hydroxyatrazine, 5=atrazine, 6=propazine, 7=prometryn. Electrophoretic conditions as in Fig. 1.

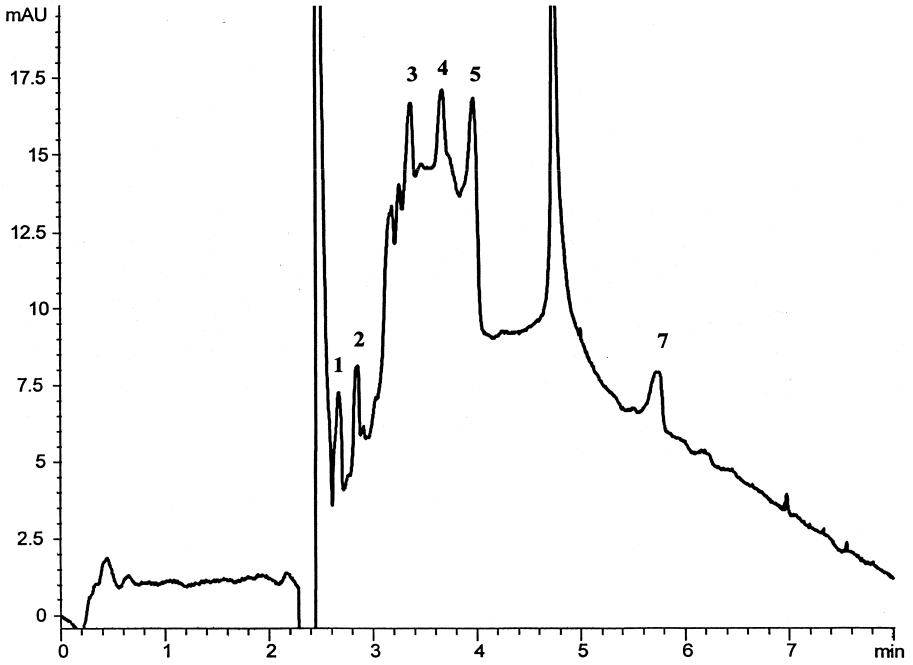

Fig. 4. Electropherogram obtained for well water spiked at 0.5 μg l^{-1} of each triazine after SPE onto two PS–DVB disks (enrichment factor 1000). 1=DIA, 2=DEA, 3=simazine, 4=hydroxyatrazine, 5=atrazine, 7=prometryn. Electrophoretic conditions as in Fig. 1.

Table 2
Recovery[a] (R) after solid-phase extraction of 1 l spiked water samples onto two PS–DVB disks

Triazines	Mineral water (0.1 μg l^{-1})			Tap water (0.1 μg l^{-1})			Well water (0.5 μg l^{-1})		
	R (%)[a]	RSD (%)	Detection limit (μg l^{-1})	R (%)[a]	RSD (%)	Detection limit (μg l^{-1})	R (%)[a]	RSD (%)	Detection limit (μg l^{-1})
DIA	75.6	3.2	0.03	76.7	6.5	0.04	71.8	4.1	0.06
DEA	88.2	4.5	0.02	n.d.[b]	–	–	81.7	6.2	0.07
Simazine	87.7	9.3	0.03	78.5	12.3	0.03	104.1	8.7	0.15
HA	97.1	8.5	0.03	96.3	10.2	0.04	85.3	7.2	0.21
Atrazine	89.3	6.7	0.03	81.3	13.5	0.05	93.2	9.1	0.30
Propazine	95.4	8.2	0.02	90.7	7.8	0.05	n.d.[b]	–	–
Prometryn	102.1	6.3	0.03	93.2	8.5	0.06	85.8	4.6	0.07

[a] Average of five independent determinations.
[b] Not detected.

These detection limits are in agreement with current European regulations.

4. Conclusions

It has been demonstrated that MEKC allows the baseline separation of triazines and several degradation products in less than 6 min avoiding, almost completely, the use of organic solvents (around 20 μl in each run). Therefore this technique is highly appropriate for the analysis of pesticides and is comparable to established separation techniques such as HPLC or GC with common detectors.

The use of two PS–DVB disks for SPE of water samples was shown to be an easy, fast and efficient preconcentration system for all the pesticides tested, including the most polar (DIA and DEA). The combination of this SPE method with MEKC allows the determination of these triazines at the required level for the control of water in Europe.

If hard water samples with high humic and fulvic acid contents have to be analyzed, it is recommended to increase the sample pH to 12 in order to remove the aforementioned interferents. But even in this case, this procedure has some limitation because DEA and propazine could not be determined in tap and well water, respectively.

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