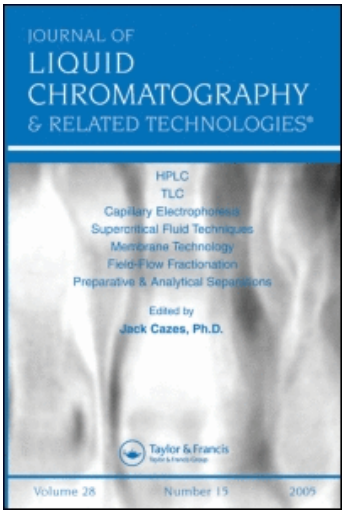

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MULTIRESIDUE ANALYSIS OF S-TRIAZINE HERBICIDES IN ENVIRONMENTAL WATER BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

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ABSTRACT

A micellar electrokinetic capillary chromatography (MEKC) method employing sodium dodecil sulfate (SDS) as the surfactant has been developed to separate and to quantificate simazine, aziprotryne, hexazinone, and diuron in environmental water samples.

Good linearity was obtained between normalized peak area and standard solution concentration for all studied compounds (concentration range: 0.1 to 5.0 μg/mL). The detection limit was lower than 6 pg (loaded into the capillary a 35 μL of sample volume) for all herbicides.

The optimized MEKC method after a 1000-fold concentration step by solid-phase extraction (SPE) was applied to determine these herbicides in environmental water samples. Herbicide concentrations lower than 0.1 ng/mL can be measured, and can have a relative standard deviation of about 4.0%. Simazine was detected at concentrations around 1.0 to 2.0 ng/mL in the Alberche and Tajuña river waters. The highest concentration appeared in the Tajuña river. The presence of simazine in both river waters was

contrasted with gas chromatography coupled to mass spectrometry analysis. The quantification of the samples was contrasted with immunochemical assays, obtaining good correlation between this and MEKC results.

INTRODUCTION

The intensive use of pesticides in the last years has increased the agricultural productivity. It has generated pesticide residues in natural waters at concentration levels which exceed the legal limits. Pesticides with different chemical structures can be found in ground and surface waters, e.g.: triazines and phenylurea compounds.

The European Union (EU) has issued directives and regulations, regarding the maximum residue levels (MRL) of pesticides in water, in accordance with the recommendations of the Codex Committee on Pesticide Residues (CCPR). In the case of drinkable water, the EU directive demands that the concentration should not exceed the level of 0.1 ng/mL for individual compounds and 0.5 ng/mL for total pesticides.[1] Essentially, this means the methods for water analysis must be around a thousand times more sensitive than those for foodstuffs.

S-triazine herbicides are among the most widely used pesticides to control broadleaf and grassy weeds in corn and other crops. Due to their extensive use and their relatively high persistence, chlorotriazines like simazine, contaminate the aquatic environment through agricultural runoff, direct applications, and leaching into ground water, in concentrations that are increasing. Many efforts have been devoted to develop rapid assays for the quantification of triazine herbicides at low levels in water.[2]

On the other hand, phenylureas are selective systemic herbicides commonly used in agriculture, alone or in combinations, for pre-emergence treatment of soil. Due to their polar nature, the increased possibility of leaching from the surface to the water supply and water reserves, together with the emergence of potential toxic degradation and metabolic products, may constitute a risk to human health. Several techniques have been reported for phenylurea determinations.[3,4]

Micellar electrokinetic capillary chromatography can be employed to separate and determine various pesticides. Despite its versatility, the major drawback of this technique is the very low injectable volume. Usually, the capillary dimensions are lower than 100 μm I.D. and 20–100 cm total length, resulting in a total column volume of only a few microliters. Consequently, the loadability of the system is limited to an injection volume lower than 100 μL.[5]

Although impressive MEKC detection limits in the subatomole range have been reported, this corresponding measurable sample preconcentration is still too high (0.1–0.5 μg/mL) to allow trace-level determination of pesticide analysis in water samples.[5] A decrease in the detection limit was accomplished by sample

preconcentration using solid-phase extraction (SPE). Applying SPE, the detection limit was enhanced about 1000-fold (0.1–0.5 ng/mL).[6]

The purpose of the present paper was to verify the viability of the preconcentration step and subsequent electrophoretical capillary determination for some herbicides using MEKC. Our attention was focused on the simultaneous identification and quantification of the three atrazine herbicides and diuron in different environmental water samples, from Comunidad Autónoma de Madrid (Madrid, Spain), by MEKC with previous SPE. The obtained results with the developed method were compared with an ELISA method, obtaining good correlation.

EXPERIMENTAL

Apparatus

Gas Chromatography

The experiments were carried out using a Carlo Erba MFC 500 gas chromatograph (ThermoQuest, Spain) equipped with a Mass Spectrometer detector MS VG Autospect (Micromass, Spain). An OV-1 fused silica capillary column (25 m x 0.25 mm), was employed. Helium was used as carrier gas at 6.5 psi. The injector port temperature was 250°C.

Samples were injected in splitless mode (1 μL), with the split valve closed, for 30 s. The samples, after preconcentration with SPE, were prepared in dichloromethane and were analyzed with the following temperature program: oven was held at 50°C for 5 min and then programmed at 15°C/min to 250°C, and held for 5 min. Spectra were acquired by scanning from m/z 50 to 500 every second.

Micellar Electrokinetic Capillary Chromatography

All experiments were performed with a SpectraPHORESIS 100 (Thermo Quest Corporation, Spain) equipped with a SC100 variable-wavelength UV/vis detector (Thermo Quest Corporation, Spain). Data acquisition and processing were accomplished using a PC/486 equipped with Chrom-Card software package (Thermo Quest Corporation, Spain).

Conditioning and Cleaning the Capillary

A start-up sequence was established to dispose of and to perform the daily operations. It consisted, to start with, of 3.0 min rinses of 1.0M NaOH, followed by 0.1M NaOH, followed by water, and finally with running buffer. Once this

procedure was complete, the capillary column was cleaned by flushing under pressure with 0.1M NaOH purified water, and the used buffer in the analysis, all during 2 min, after each injection. Unfused silica columns Supelco (Bellefonte, PA, USA) cat No.77500 (1 m x 75 μm I.D., 363 μm O.D. and 65 cm to detector) was used as separation capillary.

Electrophoretic Procedure

The running buffer consisted in a mixture of 20 mM borate buffer and 8.5 mM SDS at pH 8.30. Fresh buffer was prepared daily, sonicated for 5 min, and microfiltered through a 0.45 μm MFS-13 filter (Advantec MFS, Inc. California, USA). Buffer pH was checked daily using a pH meter. Its day-to-day reproducibility was 0.01 pH units.

Samples were introduced hydrodynamically, under pressure, in the cathodic vial (8.3 p.s.i.). Injection volume was calculated using the Poiseuille equation. Unless stated otherwise, analysis was performed with an applied voltage of +15kV, resulting in a current of approximately 5 μA. The system was thermostated at 23.0 ± 0.5°C. UV detection of the herbicides was performed at 215 nm for the previous studies. In order to optimize the detection limits in environmental water samples, two different wavelengths were used, 215 nm for simazine and diuron and 240 nm for hexazinone and aziprotryne.

The maximum absorption for these herbicides was obtained at these wavelengths. Samples were introduced in the capillary in 1.0% (v/v) methanol-water. A minimum of 3 runs for each sample was performed to calculate the average. After 10 injections the buffer was refreshed.

Reagents

All triazine herbicides and diuron were obtained from Riedel-de-Haen (Madrid, Spain) and were used without further purification. The triazines studied were simazine [2-chloro-4,6-bis(ethylamino)-1,3,5-triazine], aziprotryne [4-azido-N-isoproyl-6-methylthio-1,3,5-triazine-2-ylamine], and hexazinone [3-cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4-dione]. The studied phenylurea was diuron [3-(3,4-dichloro phenyl)-1,1-dimethylurea].

Sodium dodecyl sulfate (SDS) was obtained from Aldrich (Madrid, Spain). Methanol grade PESTANAL (Riedel-de-Haen, Spain) was used to prepare all stock solutions of each herbicide. All used chemicals for the preparation of the buffer electrolytes were of analytical-reagent grade.

The immunoassay studies were carried out with a Simazine Rapid Assay® Test Kit supplies for Strategic Diagnostic Inc (Gomensoro S.A., Spain).

Herbicide Standards

Stock solutions of each herbicide were prepared in methanol at 500 μg/mL and stored under refrigeration. The required dilutions were made using Milli-Q/Milli-RO plus water (Waters, Spain) until the desired final concentrations.

Sample Preparation

Fortified samples were prepared by addition of appropriate amounts of the standard solutions of herbicide to the water to yield the desired final concentrations. In all cases, a blank sample was submitted to the same procedure for comparison.

For sample preconcentration by SPE, a vacuum 20-place manifold (VARIAN, Spain) system was used, equipped with OASIS-HLB 6 cc (Waters, Spain) cartridges filled with 0.20 g of sorbent. The cartridges were activated and conditioned before use with 3 mL of methanol and 3 mL of water. Sample (500 mL) suction was performed at a rate of about 4 mL/min. After loading, the cartridges were washed with 1 mL of water. The herbicides were eluted with 4 mL of methanol. The organic solvent was evaporated to dryness under a gentle stream of dry nitrogen (99.999%) and the residue was dissolved in 500 μL of 1% (v/v) methanol-water. Samples were prepared in duplicate and 100 μL sample microvials were used to introduce the samples into the electrophoretic system.

The water samples were taken from different sites along the Alberche and Tajuña rivers. All the samples were collected following the EPA´s instructions on the 507 method. The analysis of the samples were carried out in the first 48 hours after the collection in the rivers.

The immunoassay studies were carried out following the instructions of the test kit.[7] To avoid the cross reactivity with some components present in the water samples, the immunoassay analyses were realized after preconcentration step.

RESULTS AND DISCUSSION

Triazines are basic species, so they are able to become protonated in acid media. The pK_a values of chlorotriazines and diuron are about 1.5, whereas those of methylthiotriazines are close to 4.[8,9]

Therefore, initially the possibility of performing the separation by capillary zone electrophoresis (CZE) in an acidic medium was entertained. Different acids were tested as the separation medium. The results obtained, show that in an acid medium in the absence of modifiers, were not possible to separate the chlorotriazines, hexazinone, and diuron owing to their low pK_a values. Since protonated triazines and diuron were unsuitable for separation by CZE, a MEKC was tried.

Electrophoretic method development was based on the application of MEKC, used for the separation of neutral compounds. Sodium dodecil sulfate (SDS) was chosen as the micelle forming agent because it forms a pseudophase into which the analyte molecules are partitioned. Increasing concentrations of the surfactant were tested with borate running buffer. Separation was achieved only when the concentration of SDS was higher than 7 mM. At a 8.5 mM SDS concentration, the electropherogram showed good separation and efficiency of approximately 200.000 theoretical plates for each compound. When the SDS concentration increased (higher than 10 mM) the efficiency decreased because the peak height response did not increase and the peaks became broader.

Figure 1 shows the evolution of the migration time corresponding to the same sample solution run with buffer solutions, differing only in the SDS concentration. At 8.5 mM SDS concentration was found to be the optimum running electrolyte composition.

Different pH values, between 7.0 and 9.0, were tested for the separation of the herbicides. A borate buffer, pH 8.3, proved to be the best value for the separations.

The effect of the concentration of the separation buffer, modifying the total concentration of the buffer between 10 and 100 mM, was studied using borate buffer (pH 8.3). An increase in the buffer concentration caused a considerable

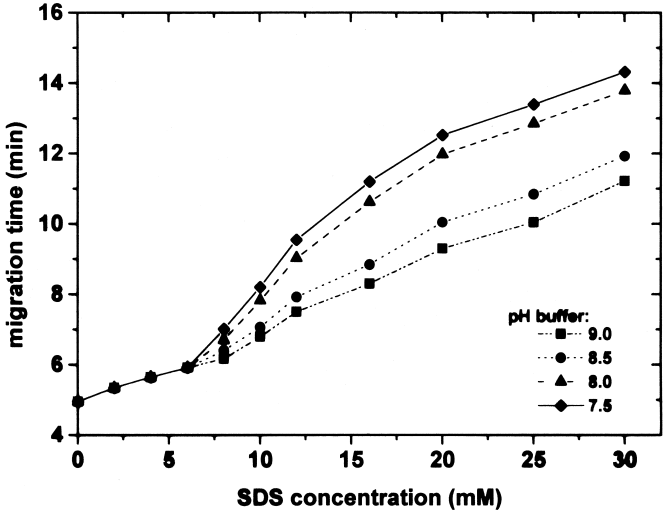


Figure 1. Evolution of the migration time with the SDS concentration. Conditions: 20 mM borate buffer at different pH, indicated in the figure, volume injection 45 nL, voltage + 20 kV, sample concentration 2.0 μg/mL of each herbicide, wavelength detection 215 nm.

increase in the migration time of all the herbicides; the mobility is inversely dependent on the square root of the buffer concentration. A concentration of 20 mM proved to be suitable.

A mixture of 20 mM borate buffer and 8.5 mM SDS adjusted pH 8.3, was found to be the optimum running electrolyte composition and was used for the subsequent analysis. Figure 2 shows the typical electrophoretic separations for the four herbicides.

Once the optimum composition of the separation buffer had been determined, a study was made of the most suitable applied voltage for herbicide separation. The applied voltage was modified between 5 and 30 kV. Resolution improved with increasing the voltage from 5 to 25 kV, selecting a running voltage of 15 kV (5 μA) for the subsequent studies in order to avoid the possibility of Joule's heating, and allowing that the time of analysis be sufficiently short (10 min). The values of the obtained resolution under these conditions were 2.5 between hexazinone and simazine, and 1.7 between diuron and aziprotryne.

The representative electrophoretic parameters of these herbicides were calculated following the deduced expressions by Jorgenson and Lukacs.[10] The number of theoretical plates calculated for + 15 kV were 125.000 for hexazinone, 325.000 for simazine, 280.000 for diuron, and 175.000 for aziprotryne. The apparent mobilities were estimated: 7.75×10^{-4} for hexazinone, 7.51×10^{-4} for simazine, 6.95×10^{-4} for diuron, and 6.74×10^{-4} cm^2/Vs for aziprotryne.

Hydrodynamic injection was chosen to introduce the samples into the capillary column. The stock solution of the herbicide was prepared in methanol; subsequently, we studied the effect of the sample composition on hydrodynamic injection. Different aliquots containing the herbicides were mixed with water to obtain the same concentration of herbicides and different proportions of methanol/water, between 0.5 and 5.0% (v/v). The variation on the resolution for the herbicides was critical when the methanol proportion was higher than 2.5%.

The migration time was almost constant for hexazinone and simazine, but very different in the case of diuron and aziprotryne. The normalized peak area decreased considerably on increasing the proportion of methanol. 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 containing SDS, leading to a widening of the size of the injected sample.[11]

The hydrodynamic injection volume was optimized with respect to peak height and normalized peak area at two different concentration levels: 0.5 and 2.0 μg/mL, containing 1% (v/v) methanol-water. Although, in some cases, peak area was linear with injection volumes over 58 nL and resolution was maintained, peak height response did not increase and peaks became broader with a decrease in the number of theoretical plates. An injection volume of 35 nL provided the best results at the herbicides concentration studies.

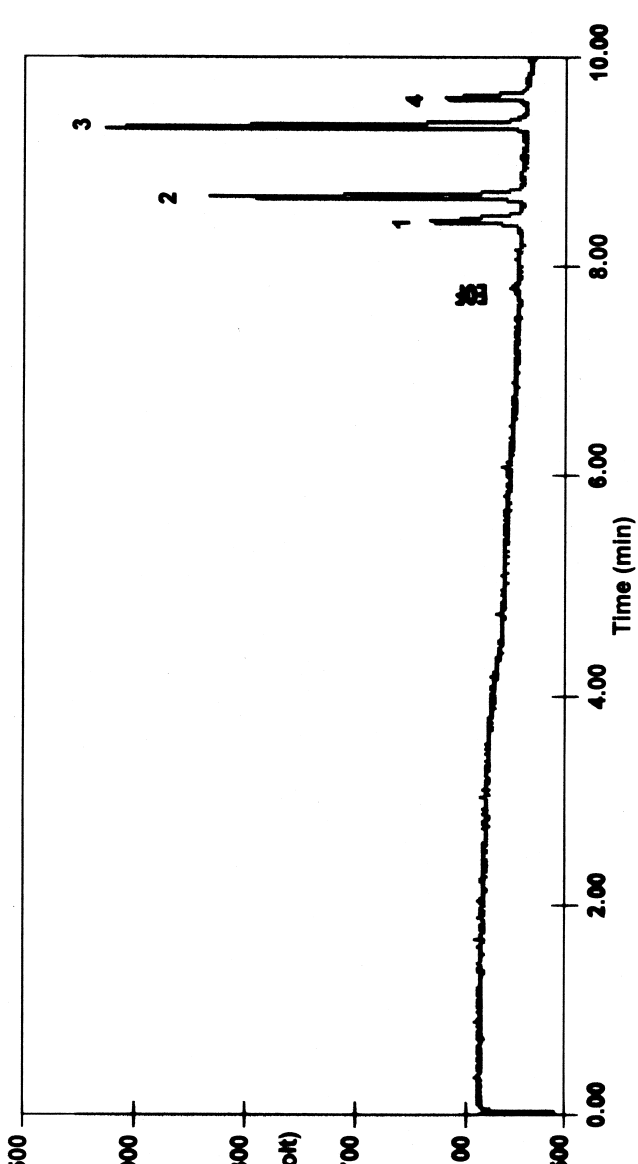


Figure 2. Electropherogram for the MEKC separation of hexazinone (1), simazine (2), diuron (3), and aziprotryne (4) under optimal conditions. Conditions: 20 mM borate buffer containing 8.5 mM SDS adjusted at pH 8.3, volume injection 35 nL, voltage + 15 kV (current 5 μA), temperature 23.0 ± 0.5°C, wavelength detection 215 nm, sample concentration 0.8 μg/mL of each herbicide containing 1.0% (v/v) methanol-water.

In the subsequent studies, injection was carried out under pressure and the sample was introduced into the capillary column dissolved in 1.0% (v/v) methanol-water.

Base calibration curves on normalized peak area were prepared with introduced volumes of 35 nL and were used to quantify of the four herbicides. In Table 1, the regression equations, correlation coefficients, and detection limits for all the compounds are listed. Each point was reported as the average of five analyses. The UV detector response at 215 nm was linear in the range of sample concentration between 0.1 and 5.0 μg/mL. Also, linearity was maintained at higher concentrations, but it was not considered of practical use, taking into account the expected concentration levels for these compounds in the environmental water samples. The detection limits, calculated at a signal to noise ratio for 3, were lower than 170 ng/mL for hexazinone and aziprotryne and lower than 40 ng/mL for simazine and diuron.

The migration time and peak response reproducibility were evaluated at a concentration of 1.0 μg/mL of each herbicide to check the performance of the MEKC system for these four herbicides. The relative standard deviations are given in Table 1; the RSD values obtained were below 3.0% for the normalized peak areas and below 0.3% for the migration times.

After method development, our efforts were focused on the quantitative determination of these herbicides in environmental water samples. It is well known, that the sensitivity of capillary electrophoresis is lower compared to other separation methods using UV detectors, due to the short path length and partial reflection of the incident light falling onto curved capillary surface. The allowed low minimum residue level (MRL is 0.1 ng/mL) in water samples by the European Community Drinking Water Directive, necessitated concentration of the samples to achieve a fit in the linear range of the method. For this reason, most reported methods for herbicide determinations in water samples involve liquid-liquid extraction[12] or SPE.[13,14]

In our work, a solid-phase trace enrichment step was optimized to determine herbicide residues in environmental water samples. Commercially available OASIS-HLB cartridges were used.[15] For the study of recovery, samples of deionized water were used. The spiked volume of the herbicide mixture at a concentration of 0.5 ng/mL of each herbicide was 500 mL. The eluate (4 mL of methanol grade Pestanal) was evaporated to dryness and the residue was dissolved in 500 μL of 1% (v/v) methanol-water. These samples were introduced directly into the capillary using 100 μL sample microvials.

A new detection wavelength was used to optimize the detection limits of the hexazinone and aziprotryne. The new value was 240 nm, at this wavelength the absorption for aziprotryne and hexazinone was higher than at 215 nm. In the first part of the work, we used 215 nm because simazine presented a very low absorption signal at 240 nm and then only one wavelength was used. For this reason, and

Table 1. Analytical Characteristics of the MEKC Method for the Herbicide Determination

Herbicide	Regresion Equation[a]	Correlation Coefficient (n = 10)	Detection Limit (μg/mL)[b]	% RSD[c]	
				Normalized Area	Migration Time
Hexazinone	y= –0.42 + 20.64 x	0.9994	0.17	2.0	0.3
Simazine	y= –0.43 + 54.42 x	0.9998	0.04	3.0	0.3
Diuron	y= 1.08 + 72.03 x	0.9991	0.02	2.6	0.3
Aziprotryne	y= –0.04 + 16.06 x	0.9993	0.12	2.8	0.3

[a]Where y is the normalized peak area and x is the analyte concentration in μg/mL
[b]Detection limit for a signal-to-noise ratio of 3
[c]Ten injection, in the same capillary (1.0 μg/mL of each herbicide).

for the subsequent studies in environmental water, a 215 nm wavelength for diuron and simazine and a 240 nm for aziprotryne and hexazinone were used.

Recovery of the extraction procedure for each herbicide in spiked deionized water samples at 5 fold concentrations of the MRL (0.5 ng/mL) were 47 ± 3, 99 ± 2, 109 ± 5, and 41 ± 3 for hexazinone, simazine, diuron, and aziprotryne, respectively (n=3).

As stated before, the detection limit of the herbicides using the MEKC (described method here) is lower than 170 ng/mL for hexazinone (for other herbicides see Table 1). By using the method in environmental water, with the SPE developed procedure and the UV detection at 215 nm (simazine and diuron) and 240 nm (aziprotryne and hexazinone), the minimum detectable concentration would be lower than 0.1 ng/mL. Table 2 shows the comparison of the detected obtained limits with and without solid phase extraction procedure.

Application of the method to water samples of different natures revealed, that in some cases, certain matrix compounds were also preconcentrated. This effect could be observed in a rise of the electropherograms baseline, although, not to a poorer resolution of the herbicides. Figure 3 shows the obtained electropherograms for a well water sample and the same sample spiked with the all herbicide studies, preconcentrated 1000 fold.

In order to check the applicability of the proposed method, environmental water samples of different origins (river, well, tap, etc.) were analyzed. A non-spiked 500 mL aliquot of each sample was first analyzed, following the sample procedure to check the presence of these compounds. Qualitative analysis of the concentrated extract of Alberche and Tajuña rivers water samples (Madrid, Spain) showed the presence of simazine.

A GC/MS studies was performed to obtain additional information about the presence of simazine and other compounds also preconcentrated in the SPE process. The GC/MS results of the samples, also analyzed by MEKC, revealed

Table 2. Comparison of Detection Limits with and without Solid-Phase Extraction[a]

Herbicide	MEKC without SPE at 215 nm (ng/mL)	MEKC with SPE at 215 nm (ng/mL)
Hexazinone	170.0	0.22
Simazine	38.0	0.04
Diuron 22.0	0.03	
Aziprotryne	125.0	0.17

[a]Detection limit for a signal/noise ratio of 3.
Preconcentration factor, 1000 fold.

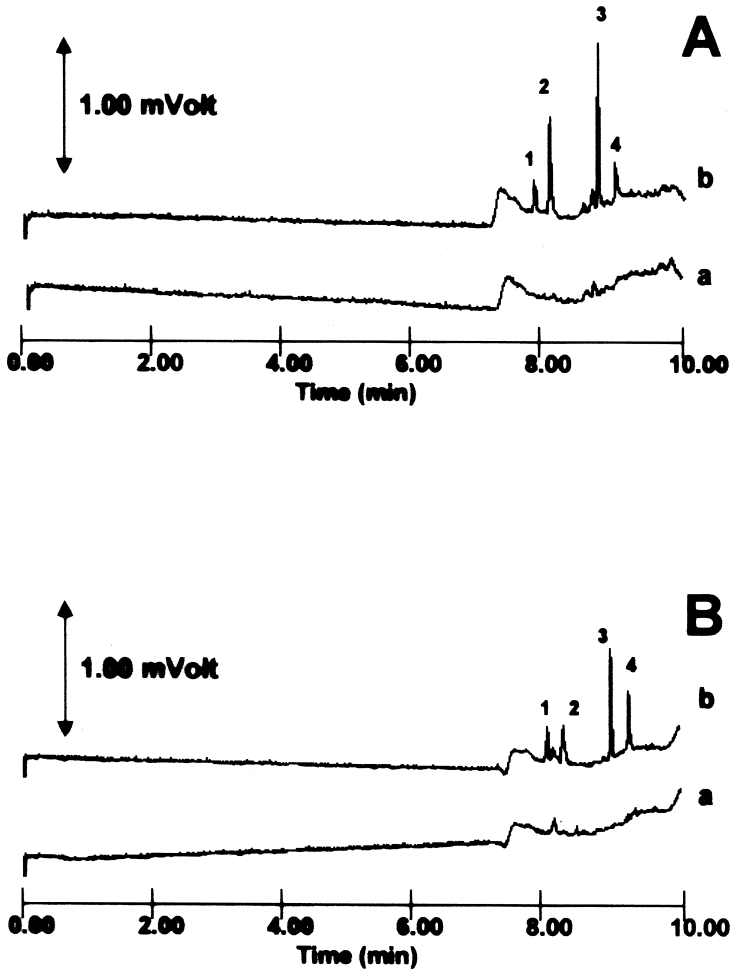


Figure 3. Electropherograms of a preconcentrated 1000 fold well water sample. Detection at: 215 nm (A) and 240 nm (B). In both cases: (a) well water sample, (b) well water sample spiked with 0.25 ng/mL of each herbicide. Hexazinone (1), simazine (2), diuron (3), and aziprotryne (4). Same conditions as Figure 2.

the presence of different peaks (see Figure 4). The peak appearing at 13.29 min was characterized like simazine, other characteristic peaks for triazines were not detected in the studied samples. Other compounds present in the chromatogram, and characterized in the samples, were: 1H-indane, 2,4-diphenyl-4-methyl-2pentene, 1H 2,3-dihydroindene, and bis(2-methylpropyl) phthalate ester.

Figure 5 shows the electropherograms for the Alberche river water samples after preconcentration 1000 fold at 215 (5A) and 240 nm (5B). Figure 5 shows the electropherograms of the river water samples without the herbicides spike, note with *a*, in these electropherograms we can see the presence of simazine. In the same figure, note with *b*, the spiked sample with a standard solution containing 0.25 ng/mL of each herbicide.

The obtained results with the MEKC developed method showed that the level of simazine concentration present in the river samples was 1.5 ng/mL in the Tajuña river and 1.8 ng/mL in the Alberche river. This level of simazine was contrasted with an immunoassay test. Different immunochemical assays to detect and to determinate simazine and other triazine have been reported in literature.[16] Immunoassay analysis has been shown to be a useful screening method, as long as the positive sample can be validated by other methods such as HPLC and GC. The analysis results have been found to correlate well with HPLC[17] and GC-MS.[18]

The studies realized with the used immunoassay test, showed a cross reactivity in the direct analysis of the water samples. The obtained results with these characteristics, showed levels of simazine lower than the real concentrations of the herbicide. The obtained results with immunoassay tests for the samples previously preconcentrated by SPE, showed good results and corroborated the obtained data by MEKC conditions.

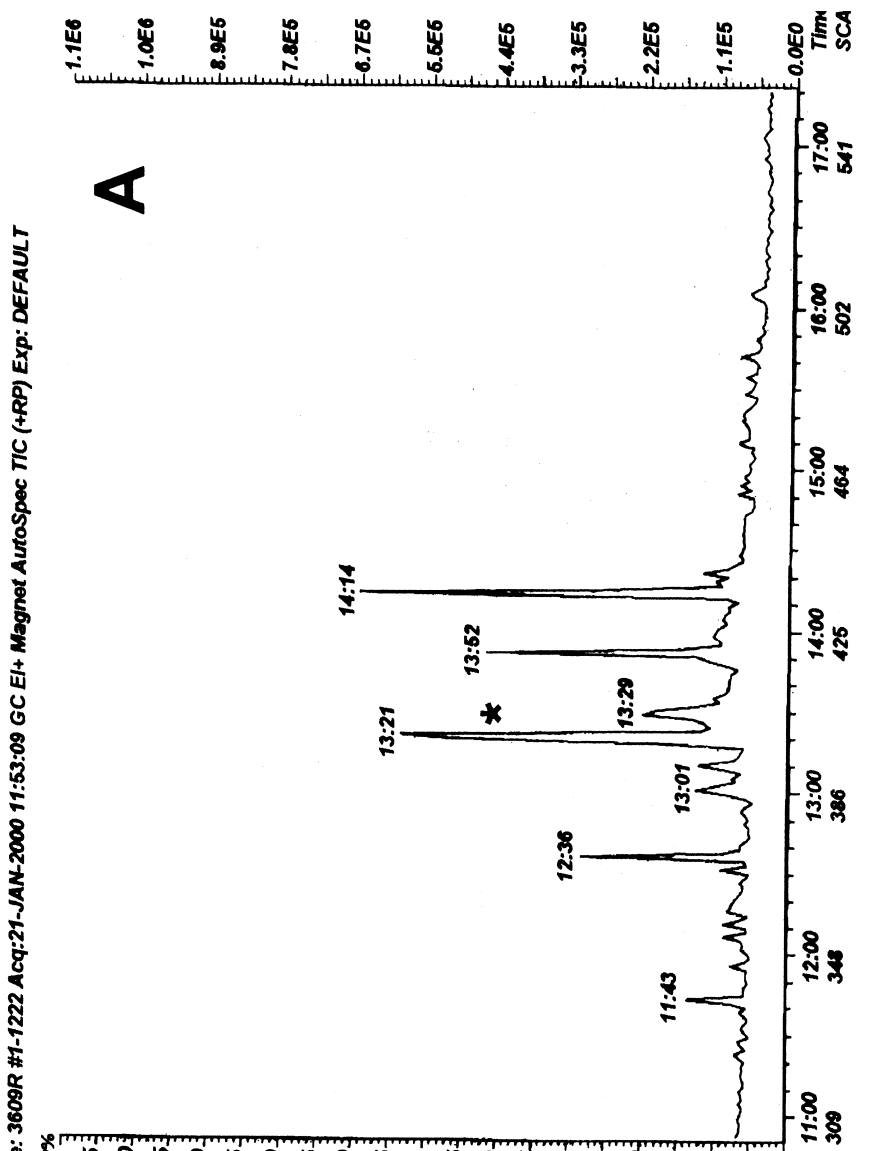
CONCLUSIONS

The reported data show that MEKC is suitable for mono and multiresidue analysis of hexazinone, simazine, diuron, and aziprotryne in environmental water at the ng/mL level, using an appropriate concentration procedure. Thus far, the presented SPE method can be used for the analysis of drinking water by MEKC. The detection limits of the method, without any preconcentration step, are lower than 0.2 μg/mL in all cases (see Table 1). With a sample and fast preconcentration step, assays at the MRL can be carried out without interference from other substance present in the water.

On the other hand, the reported data show that the sample preconcentration technique does not alter physicochemical characteristics of the four herbicides.

Due to the small sample and required electrolyte volume, MEKC appears to be less expensive and environmentally safer than other analytical methods. The technique shows high separation efficiency (theoretical plates number higher than 170.000) and constitutes a good alternative to HPLC.

The obtained results for simazine, in Alberche and Tajuña river water samples with MEKC, were comparable to those obtained by the immunoassay methods. The level of simazine in these river waters were 1.8 and 1.5 ng/mL, respectively.



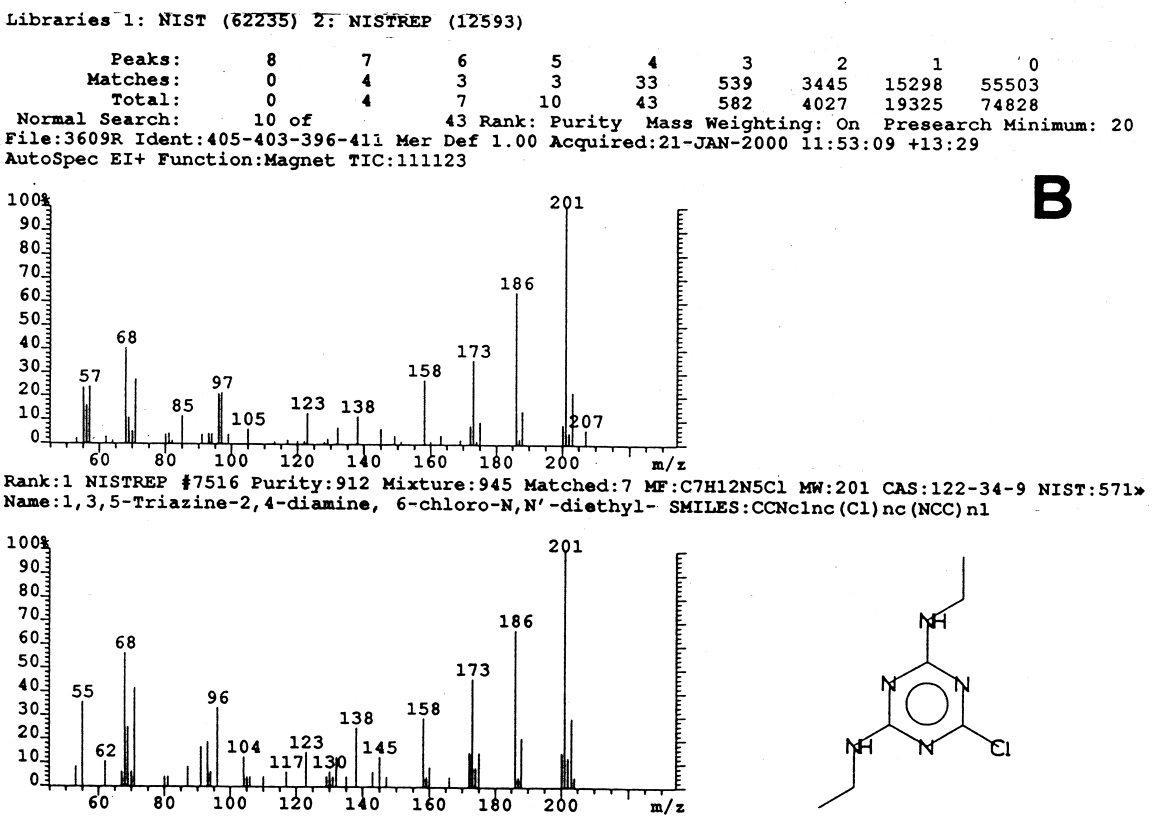


Figure 4. Gas chromatogram of the Alberche river water samples (A) and the mass spectra from GC-MS results for the appeared peak on 13.29 min (B). The sample was previously extracted on SPE and the injection into the chromatograph by described conditions in Experimental.

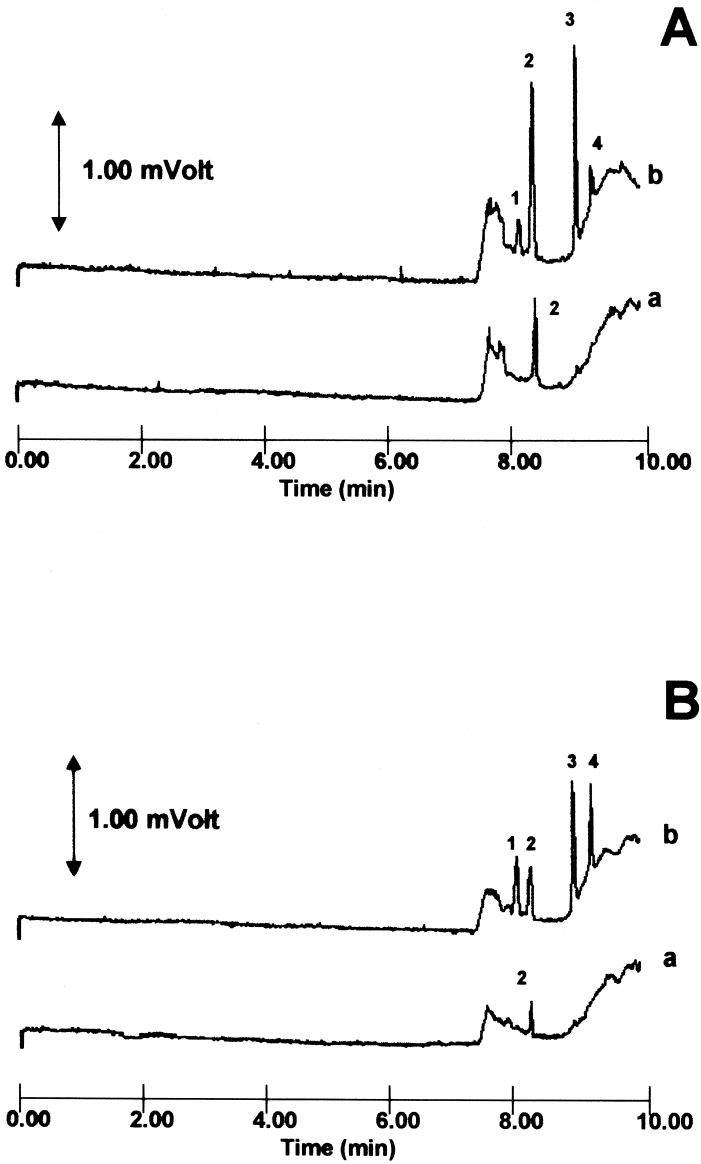


Figure 5. Electropherograms of Alberche river water after preconcentrated 1000 fold. Detection was performed at 215 nm (A) and 240 nm (B). In both cases (a) river water sample and (b) river water spiked with 0.25 ng/mL of each herbicide. Hexazinone (1), simazine (2), diuron (3), and aziprotryne (4). Same conditions as Figure 2.

Further studies will be undertaken to verify the feasibility of the developed method for the quantification of these herbicides in complex matrices such as soils. In these matrices, the allowed maximum residue levels are higher, but more interference can be expected.

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REFERENCES

1. ECC. Directive Relating to the Quality of Water Intended for Human Consumption 80/778/ECC. Off. J. Eur. Comm. **1980**, *23* (Aug), L229/1130.
2. Dean, J.R.; Wade, G.; Barnabas, I.J. J. Chromatogr. A **1996**, *733*, 295–335.
3. Süsse, H.; Müller, H. J. Chromatogr. A **1996**, *730*, 337–343.
4. Sherma, J. J. Assoc. Off. Anal. Chem. **1997**, *80*, 283–287.
5. Stegehnis, D.S.; Irth, H.; Tjaden, U.R.; Vander Greef, J. Anal. Chem. **1991**, *538*, 393–398.
6. Barroso, M.B.; Konda, L.N.; Morovjan, G. J. High. Resol. Chromatogr. **1999**, *22* (3), 171–176.
7. *Applications Notes for Simazine Rapid Assay Test Kit. Ref. A00245*; Strategic Diagnostic, Inc.: **1999**.
8. Weber, J.B. Residue Rev. **1970**, *32*, 93.
9. Anon. *The Agrochemicals Handbook*, 3rd Ed.; The Royal Society of Chemistry: Cambridge, **1991**.
10. Jorgenson, J.W.; Lukacs, K.D. Anal. Chem. **1981**, *53*, 1298–1302.
11. Grushka, E.; McCormick, R. J. Chromatogr. **1989**, *471*, 421–428.
12. Muir, D.C.G.; Baker, B.E. J. Agric. Food. Chem. **1978**, *26*, 420–427.
13. Carabias, R.; Rodriguez, E.; Muñoz, A.I.; Dominguez, J.; Hernández, J. J. Chromatogr. A. **1996**, *733*, 349–360.
14. Barroso, M.B.; Konda, I.N.; Morovjan, G. J. High Resol. Chromatogr. **1999**, *22* (3), 171–176.
15. *Oasis® HLB Sample Extraction Applications for Agrochemical and Environmental Analysis*; **1998**.
16. Wortberg, M.; Kreissig, S.B.; Jones, G.; Rocke, D.M.; Hammock, B.D. Anal. Chim. Acta. **1995**, *304*, 339–346.
17. Denkwardt, A.; Pullen, S.; Rauchalles, S.; Kramer, K.; Just, F.; Hock, B.; Hofmann, R.; Shewes, R.; Maidl, F.X. Anal. Lett. **1995**, *28*, 621–632.

18. Thurman, E.M.; Meyer, M.; Powes, M.; Perry, C.A.; Schwab, A.P. Anal. Chem. **1990**, *62*, 2043–2048.

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