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Solid-phase extraction and sample stacking–micellar electrokinetic capillary chromatography for the determination of multiresidues of herbicides and metabolites

R. Carabias-Martínez*, E. Rodríguez-Gonzalo, P. Revilla-Ruiz, J. Domínguez-Álvarez
Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Química, Universidad de Salamanca, Plaza de la Merced s/n, 37008 Salamanca, Spain

Abstract

Micellar electrokinetic capillary chromatography (MEKC) with diode array detection was used for the separation of 13 compounds (eight herbicides widely used in agriculture: metribuzin, lenacil, ethofumesate, atrazine, terbutryn, isoproturon, chlorotoluron and linuron, and five of their principal degradation products; namely, deethylatrazine, 2-hydroxyatrazine, deethyl-2-hydroxyatrazine, deisopropylatrazine and 3-chloro-4-methylphenylurea). Peak separation for the 13 analytes was not successful when a single surfactant system was employed, neither sodium dodecyl sulfate (SDS) nor dioctyl sulfosuccinate (DOSS) sodium salt. However, a mixture of these herbicides was successfully separated using a mixed micellar system involving SDS–DOSS in less than 14 min. An application study of an on-line concentration technique for MEKC was carried out to enhance sensitivity. The optimized on-line stacking procedure consisted simply of the addition of 50 m*M* of sodium chloride to the injection sample, the stacking effect being more intensive as analyte polarity increased. When this stacking procedure was combined with an off-line sample preconcentration step, based on solid-phase extraction, analytes could be detected in the ppb range. The whole method was applied to ultra-high-quality and natural waters. Linear relationships between the analytical signal and the initial analyte concentration were found to be independent of the type of water, except for the more polar analytes for which small differences were observed.

Keywords: Sample stacking; Micelles, mixed; Pesticides

1. Introduction

In recent years, many works have emphasised the ecological damage caused by the widespread use of agricultural pesticides. The residue levels of such compounds may represent an important source of pollution, especially if they have been used for long periods of time.

The pollution of surface and ground waters by pesticides is governed by the physicochemical characteristics of the compounds, by the properties of the medium in which they are applied and by other external factors. Among the most important physicochemical properties of pesticides are their water solubility, their capacity to be retained by the organic part of the soil (characterised by the solid organic carbon sorption coefficient, K_{oc}, which is closely related to the octanol/water partition coefficient, K_{ow}) and their half-life in soils (DT_{50}). The groundwater ubiquity score (GUS) is a simple

*Corresponding author. Tel.: +34-923-294-483; fax: +34-923-294-483.
E-mail address: rcm@gugu.usal.es (R. Carabias-Martínez).

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parameter that can be used to assess the leachability of pesticides. The GUS index can be written as: $GUS = \log(DT_{50})[4 - \log(K_{oc})]$.

Persistence (expressed as DT_{50}) and mobility (expressed as K_{oc}) are key parameters that seem to be particularly representative of the overall leaching potential of non-ionic compounds. The trends as regards the contamination of groundwaters are (a) non-leacher ($GUS < 1.8$), (b) transition ($1.8 < GUS < 2.8$), (c) leacher ($GUS > 2.8$). The GUS values for a set of herbicides whose physicochemical parameters are available are shown in Fig. 1. It can be seen that except for terbutryn, the rest of the herbicides are able to reach the ground water system.

In each agricultural area there is a tendency to combine a given number of chemically different herbicides for use as plague controllers. The number of active compounds used as herbicides is very high, and hence, as established by EC Directive 98/83 [1], it is only necessary to analyse the pesticides likely to be present in the area of interest. On the other hand, until recently research has tended to focus on active herbicide compounds. However, biotic and abiotic processes in water and soil may transform the herbicides and thus the inclusion of metabolites is crucial if an understanding of the fate of herbicides is to be gained.

These reasons have led to the development of methods for the determination of multiresidues and their metabolites as a proper way for practical pesticide analysis.

In the present study, eight of the herbicides most frequently used in the area of Salamanca, Spain (atrazine, metribuzin, lenacil, isoproturon, chlorotoluron, ethofumesate, linuron, terbutryn) and five of their main degradation products (deethylatrazine (DEA), 2-hydroxyatrazine (HA), deethyl-2-hydroxyatrazine (DEHA), deisopropyl-2-hydroxyatrazine (DIHA) and 3-chloro-4-methylphenylurea (CMPU)) were selected on the basis of data provided by the Territorial Service of Agriculture and Livestock Raising of The Junta of Castilla-León (Spain).

At present, high-performance liquid chromatography (HPLC) is one of the techniques most commonly used in the residue analysis of herbicides, particularly in water samples [2–7]. Capillary electrophoresis has also been used for pesticide analysis [8–13], although most research has focused on the analysis of pesticides belonging to the same chemical group: triazines, phenoxy acids and carbamates. In this work we studied the possibilities offered by the use of micellar electrokinetic chromatography (MEKC) with diode array detection (DAD) for the separation, quantification and identification of mul-

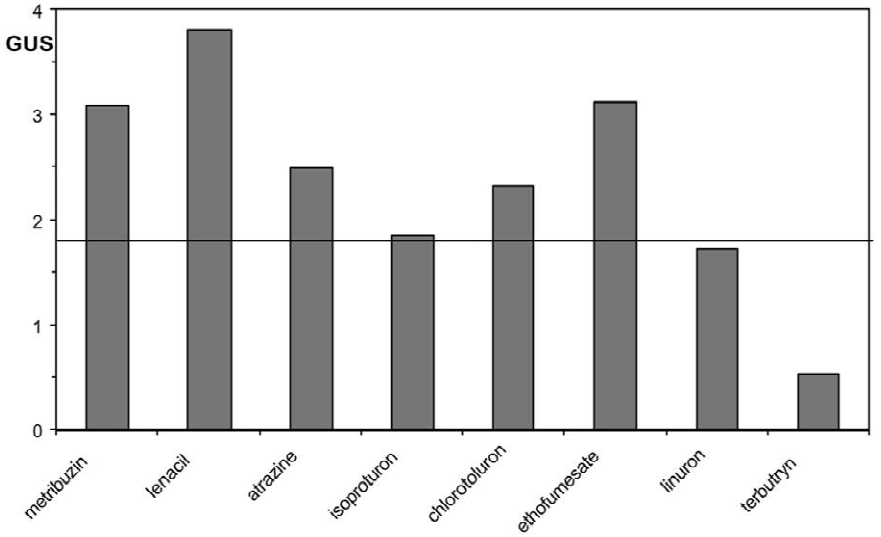

Fig. 1. Groundwater ubiquity score (GUS) values for some of the analytes studied. Solid line indicates the limit for a pesticide to accede to the groundwater. DT_{50} and K_{oc} values from Ref. [30].

tiresidues of herbicides and metabolites. Nevertheless, the very limited optical path length, due to small inner diameter of the capillary (25–100 μm), and low sample volume injected (nl), mean that the detection of low-concentration samples with a UV detector is difficult or even impossible without sample preconcentration.

With respect to the problem of sensitivity, several on-line or on-capillary focusing methods have been developed [14–24] for the preconcentration of neutral analytes inside the capillary before separation. The concentration effect is based on the sudden change in micelle electrophoretic velocity due to the difference in the magnitude of the electric field between the sample region and the separation region. For the focalisation of analytes with different chemical structures and polarity, in this work two sample-stacking procedures were evaluated; namely, the reversed electrode polarity stacking mode (REPSM) [25] and the high-conductivity sample stacking mode (HCSSM) [26–28].

REPSM involves field polarity reversal after the capillary has been filled with a large volume of sample of lower conductivity than is used for separation. HCSSM uses a high-conductivity sample matrix to transfer field amplification from the sample zone to the separation buffer. This causes the micellar carrier in the separation buffer to stack before it enters the sample zone. Neutral analytes moving out of the sample zone with electroosmotic flow are efficiently concentrated at the micelle front.

The combination of the on-line focusing procedure with an off-line preconcentration step, based on solid-phase extraction (SPE) solved the problem of detection and allowed MEKC to be considered a suitable technique for the separation and determination of multiresidues of herbicides in natural waters.

2. Experimental

2.1. Chemicals

The herbicides were obtained from Riedel-de Haën (Seelze-Hannover, Germany) and were used without further purification (minimum percent purity greater than 98%). The herbicides studied were as follows: chlorotoluron, 3-(3-chloro-*p*-tolyl)-1,1-dimethylurea, CAS RN [15545-48-9]; atrazine, 6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine, CAS RN [1912-24-9]; terbutryn, N^2-*tert*.-butyl-N^4-ethyl-6-methylthio-1,3,5-triazine-2,4-diamine, CAS RN [886-50-0]; metribuzin, 4-amino-6-*tert*.-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one, CAS RN [21087-64-9]; lenacil, 3-cyclohexyl-1,5,6,7-tetrahydrocyclopentapyrimidine-2,4(3H)-dione, CAS RN [2164-08-1]; linuron, 3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea, CAS RN [330-55-2]; isoproturon, 3-(4-isopropylphenyl)-1,1-dimethylurea, CAS RN [34123-59-6], ethofumesate, (±)-2-ethoxy-2,3-dihydro-3,3-dimethylbenzofuran-5-yl methanesulfonate, CAS RN [26225-79-6]. Stock solutions of each herbicide were prepared in methanol at 200 μg ml^{-1}.

The metabolites were obtained from Dr. Ehrenstorfer (Augsburg, Germany) and were as follows: DIHA, 2-hydroxy-*N*-ethyl-1,3,5-triazine-4,6-diamine; DEHA, 2-hydroxy-*N*-isopropyl-1,3,5-triazine-4,6-diamine; DEA, 6-chloro-*N*-isopropyl-1,3,5-triazine-2,4-diamine; HA, 2-hydroxy-N^4-ethyl-N^6-isopropyl-1,3,5-triazine-4,6-diamine; and CMPU, *N*-(3-chloro-4-methylphenyl)urea. Stock solutions of DEA and CMPU were prepared in acetonitrile at 200 μg ml^{-1} and solutions of the rest of the metabolites were prepared in acetonitrile–0.1 *M* hydrochloric acid (80:20, v/v), at a concentration of 200 μg ml^{-1}.

The sorbents used for solid-phase extraction were: silica-based bonded C_{18} cartridges (Sep-Pak Plus, Waters) and polymeric cartridges (Oasis HLB, Waters and LiChrolut EN, Merck).

Sodium dodecyl sulfate (SDS) was obtained from Fluka (Buchs, Switzerland) and dioctyl sulfosuccinate (DOSS) sodium salt from Aldrich (Steinheim, Germany). The organic solvents–acetonitrile (ACN), methanol (MeOH) and ethyl acetate (AcOEt)–were of HPLC grade (Merck) and were used as received. Ultra-high-quality water was obtained with an Elgastat UHQ water purification system. All chemicals used for the preparation of the buffer electrolytes were of analytical reagent grade.

2.2. Apparatus

All experiments were performed with a Hewlett-Packard ^{3D} Capillary Electrophoresis System (Wald-

bronn, Germany) equipped with fused-silica capillaries of 58.5 cm, (50 cm to the detector)×75 μm I.D×363 μm O.D. obtained from Supelco. The temperature of the capillary was maintained at 20±1 °C by the instrument thermostatting system. An optimum detection wavelength was selected for each analyte based upon the spectra recorded by the diode-array detector. The detection was performed at 200, 220 and 240 nm.

To convey the sample through the SPE cartridge, a Gilson minipuls 2 HP 4 peristaltic pump was used. Drying and conditioning of the cartridges were 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).

2.3. Procedures

2.3.1. MEKC separation

Uncoated capillaries—58.5 cm (50 cm effective length)×75 μm I.D.—were used throughout the study. All new capillaries were conditioned before use. They were pretreated sequentially for 20 min with 0.1 *M* sodium hydroxide, 10 min with 60 m*M* borate buffer and 10 min with separation buffer. This was also applied as a daily start-up procedure reducing the time length of the steps in a half. Before each run, the capillary was rinsed for 3 min with 60 m*M* borate buffer prior to the passage of the separation buffer.

Samples were introduced into the capillary under pressure (12 mbar) for a fixed period of time (5 s). Analysis was performed applying 20 kV during the first 11 min of analysis followed by a voltage step to 30 kV at this time.

The separation medium was: 60 m*M* borate buffer, pH 9.2, 20 m*M* SDS, 10 m*M* DOSS and 8% (v/v) methanol.

The injection solution was: 0.5 m*M* borate buffer, pH 9.2, 20% (v/v) methanol and 50 m*M* sodium chloride to produce the staking phenomenon.

2.3.2. Stacking

Two stacking modes have been evaluated. The stacking procedure for REPSM was as follows: the silica capillary was filled with the background solution and then a long plug of sample, prepared in a low-conductivity matrix, was introduced under hydrodynamic pressure (12 mbar). A high voltage at negative polarity (−20 kV) was then applied to remove the sample matrix from the capillary. Current decreases due to the slight electrical resistance caused by the lower conductivity of the sample, but rises again as the sample is removed from the capillary. The polarity was switched to normal mode (20 kV) when the current reached 95–99% of the original value.

The stacking with high-conductivity sample matrix was induced simply by adding sodium chloride to the sample matrix.

2.3.3. Solid-phase extraction procedure

Extraction and preconcentration of the analytes from water samples were achieved with LiChrolut EN (200 mg) styrene–divinylbenzene polymeric sorbents.

Solid sorbents were conditioned with 5 ml of methanol followed by 5 ml of ethyl acetate and 5 ml of ultra-high-quality (UHQ) water. The passage of the samples (volume, 250 ml) through the cartridges was carried out at a flow-rate of 7 ml min^{-1} by means of a peristaltic pump. Once the retention step had been completed, the cartridges were dried for 15 min under a vacuum of −15 mmHg (1 mmHg= 133.322 Pa). The components retained were eluted with 5 ml of methanol and 5 ml of ethyl acetate. The organic phase thus obtained was evaporated to complete dryness in a rotary evaporator (Büchi) at a temperature of 45–50 °C. The dry residue was dissolved in 500 μl of the injection solution.

3. Results and discussion

3.1. MEKC herbicide multiresidue separation

Micellar electrokinetic capillary chromatography, MEKC, was initially applied to 11 of the 13 herbicides, and the *N*-dealkylated hydroxy metabolites of atrazine, DIHA and DEHA, were incorporated later. A single surfactant system, SDS in borate buffer, was first tested, but in the range of SDS concentrations assayed, 10–100 m*M*, it was not possible to reach the complete separation of the 11

analytes studied, due to their wide polarity range. To improve the electrophoretic separation, the addition of organic solvents (methanol and acetonitrile) to this separation medium, was also assayed. The presence of methanol, at 8% (v/v) in the separation buffer, resulted in a better resolution of several analytes (for instance, atrazine and isoproturon) but complete peak separation was still not achieved. When acetonitrile was employed as organic modifier, the electropherograms displayed an unstable baseline that disturbed the quantification.

Another possibility for selectivity manipulation is to change the surfactant system. In this case, DOSS was used. It has been reported [29] that this surfactant appears as a vesicle system at a concentration above 1 m*M* and, in order to obtain reproducible conditions, the size of these aggregates must be small because of the risk of obtaining perturbing electropherograms. Thus, the use of DOSS concentrations of up to 40 m*M* is appropriate.

Initially, an attempt was made to use DOSS as an individual surfactant system to check its separation possibilities. Table 1 shows the results obtained. It was observed that the DOSS surfactant interacts stronger than SDS with the less polar analytes (CMPU, linuron and terbutryn). However, this interaction is weaker with the analytes of intermediate polarity (chlorotoluron, isoproturon, atrazine, lenacil, HA) and similar for the more polar ones (DEA, metribuzin). On employing DOSS or SDS micellar systems individually, it was observed that, whereas some analytes were separated with one of the surfactants, they were not well resolved with the other one and vice-versa.

The results suggested the use of a mixed SDS–DOSS system for the separation of this mixture of herbicides of such different polarities. To check this, a separation medium was prepared containing a constant 20 m*M* SDS concentration and the DOSS concentration was varied in the 5–20 m*M* range. Fig. 2 shows the electropherograms for different DOSS concentrations. It can be observed that the mixed SDS–DOSS system containing 20 m*M* SDS and

Table 1
Influence on migration time for the herbicides and metabolites, employing SDS or DOSS as an individual surfactant system

	Migration time (min)	
	SDS[a]	DOSS[b]
DEA	5.00	4.60
Metribuzin	5.12	4.96
HA	7.29	5.18
Lenacil	7.24	6.04
Atrazine	7.78	5.95
Isoproturon	7.78	6.04
Chlorotoluron	7.58	6.47
CMPU	8.07	9.00
Ethofumesate	9.83	9.70
Linuron	9.02	11.80
Terbutryn	10.74	11.87

[a] Separation buffer: 60 m*M* borate, 3% (v/v) MeOH, 20 m*M* SDS.

[b] Separation buffer: 60 m*M* borate, 3% (v/v) MeOH, 20 m*M* DOSS.

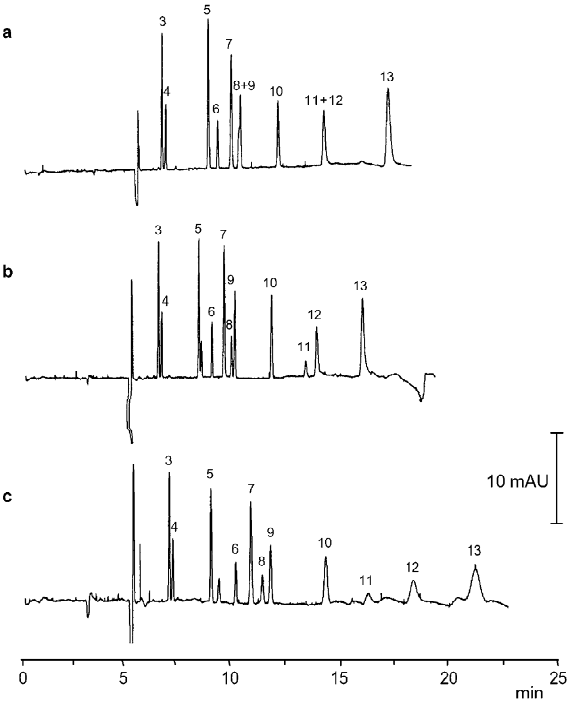

Fig. 2. Electropherograms using a mixed SDS–DOSS system. Separation buffer: 60 m*M* borate (pH 9.2), 8% (v/v) MeOH, 20 m*M* SDS and (a) 5 m*M* DOSS, (b) 10 m*M* DOSS, (c) 20 m*M* DOSS. Sample: 10 mg l^{-1} each compound in 60 m*M* borate (pH 9.2) and 3% (v/v) MeOH. Instrumental conditions: pressure injection (12 mbar, 5 s); applied voltage, 22 kV; detection wavelength, 220 nm. Peaks: (3) DEA, (4) metribuzin, (5) HA, (6) lenacil, (7) atrazine, (8) isoproturon, (9) chlorotoluron, (10) CMPU, (11) ethofumesate, (12) linuron, (13) terbutryn.

10 m*M* DOSS permits the separation of the 11 pesticides in less than 16 min. Lower DOSS concentrations do not allow the separation of isoproturon–chlorotoluron and ethofumesate–linuron peaks, while higher DOSS concentrations produce a loss of peak efficiency for the less polar analytes.

Because the mixed SDS–DOSS system had afforded a fairly wide separation window, it was decided to incorporate the hydroxylated atrazine metabolites (DIHA and DEHA) to the sample, obtaining complete separation for the 13 herbicides studied (Fig. 3). A voltage step was set up in order to decrease the analysis time.

3.2. Focusing improvement by MEKC stacking procedures

On-line stacking procedures were evaluated with the aim of improving sensibility. In this work, two stacking procedures were tested: the reversed electrode polarity stacking mode (REPSM) and the high-conductivity sample stacking mode (HCSSM).

3.2.1. Reversed electrode polarity stacking mode (REPSM)

In this modality [25] a large sample volume with low conductivity is injected into the capillary. Application of a reverse voltage permits the micelles, located in the cathodic vial, to be electrokinetically injected into the sample area and transport neutral analytes towards the anode. Compounds with high retention factor (*k*) travel faster than compounds with low *k*. Simultaneous to this, the sample is pumped out from the capillary to the cathodic vial by the electroosmotic flow and is replaced by the background solution coming from the anodic vial. An adequate polarity change permits the separation of the analytes from this focalised situation.

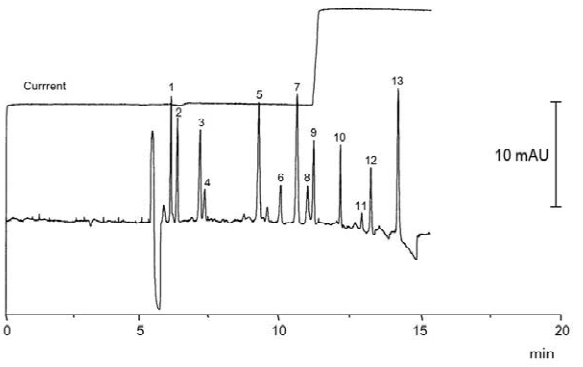

Fig. 3. Electropherogram obtained for the 13 herbicides and metabolites under the optimized conditions. Separation buffer: 60 m*M* borate (pH 9.2), 8% (v/v) MeOH, 20 m*M* SDS, 10 m*M* DOSS. Sample: 10 mg l^{-1} each compound in 60 m*M* borate (pH 9.2) and 10% (v/v) MeOH. Instrumental conditions: hydrodynamic injection (12 mbar, 5 s); voltage step from 20 to 30 kV at 11 min; detection wavelength, 220 nm. Peaks: (1) DIHA, (2) DEHA, (3) DEA, (4) metribuzin, (5) HA, (6) lenacil, (7) atrazine, (8) isoproturon, (9) chlorotoluron, (10) CMPU, (11) ethofumesate, (12) linuron, (13) terbutryn.

In order to increase the conductivity differences between the sample and buffer solutions, the sample was prepared with only 0.5 m*M* borate buffer, pH 9.2.

It was observed experimentally that, in this case, the maximum length of sample introduced must be 10% of the effective capillary length. Larger sample volumes resulted in a peak broadening, with a serious loss of peak resolution.

Fig. 4 shows the electropherograms obtained when the REPSM mode was applied. Fig. 4b corresponds to a polarity change at the moment at which the current had reached 99% of its final value, meaning that almost the whole sample volume had already been removed from the capillary. Fig. 4c–d shows the electropherograms obtained on switching polarity from negative to positive at different current values (97 and 95%, respectively). When polarity was switched later (Fig. 4b), a loss of the more polar analytes was observed. This is due to the fact that these compounds are pumped out from the capillary by the electroosmotic flow when the reversed voltage has been applied because they are not sufficiently well retained by the micelles (low retention factor). In the other cases, 97 and 95% of the final current, these polar analytes are present but they are not focalised. Only the less polar analytes undergo an improvement in focalisation.

3.2.2. High conductivity sample stacking mode (HCSSM)

In this stacking technique, the sample is prepared in a high conductivity medium in order to focalise the micelles in the sample-separation buffer interface [26]; consequently, neutral analytes are swept [28] by the grouped micelles at the interface.

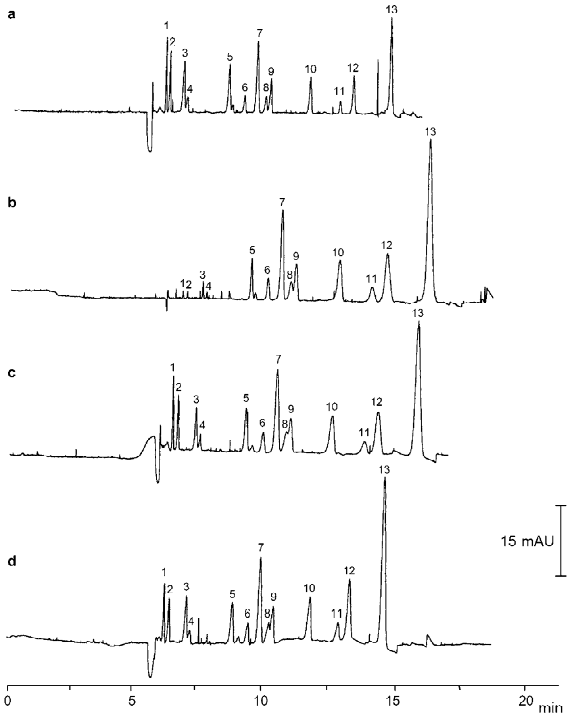

Fig. 4. REPSM focalisation for a 10% of the effective capillary length filled with sample. Separation buffer: 60 m*M* borate pH 9.2, 8% (v/v) MeOH, 20 m*M* SDS, 10 m*M* DOSS. (λ=220 nm). Sample: 10 mg l^{-1} each compound in 0.5 m*M* borate (pH 9.2) and 10% (v/v) MeOH. (a) Without stacking, hydrodynamic injection (12 mbar, 5 s), (b) 99%, (c) 97% and (d) 95% of the final current reached. Peaks: (1) DIHA, (2) DEHA, (3) DEA, (4) metribuzin, (5) HA, (6) lenacil, (7) atrazine, (8) isoproturon, (9) chlorotoluron, (10) CMPU, (11) ethofumesate, (12) linuron, (13) terbutryn.

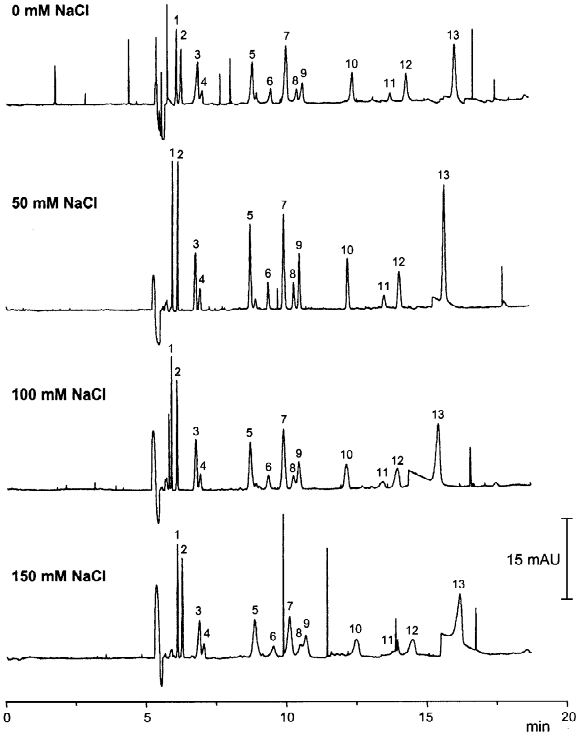

Fig. 5. HCSSM focalisation. Separation buffer: 60 m*M* borate (pH 9.2), 8% (v/v) MeOH, 20 m*M* SDS, 10 m*M* DOSS. Sample: 10 mg l^{-1} each compound in 0.5 m*M* borate (pH 9.2), 10% (v/v) MeOH and different concentrations of NaCl (0–150 m*M*). Instrumental conditions: hydrodynamic injection (12 mbar, 5 s), wavelength 220 nm. Peaks: (1) DIHA, (2) DEHA, (3) DEA, (4) metribuzin, (5) HA, (6) lenacil, (7) atrazine, (8) isoproturon, (9) chlorotoluron, (10) CMPU, (11) ethofumesate, (12) linuron, (13) terbutryn.

From an experimental point of view, this stacking procedure is very simple because only the addition of a high salt concentration to the sample is required. In this work we studied the effect that the addition of sodium chloride to the sample produces on the stacking of the analytes. Samples with sodium chloride concentrations in the 0–200 m*M* range were prepared. The results are shown in Figs. 5 and 6. An increase in stacking up to 50 m*M* of sodium chloride is observed, decreasing at higher concentrations.

Palmer et al. [26] and Quirino et al. [28] reported curve shapes similar to those obtained in Fig. 6. A possible explanation for the decrease in stacking at higher sodium chloride concentrations could be that high sodium ion concentrations produce a co-ion layer around the surfactant micelle that is less diffuse than with lower sodium chloride concentrations. This effect can hinder the interaction between the analyte and the micelle and, consequently, tends to reduce its stacking.

On the other hand, unlike Palmer et al. [26], we failed to find any clear relationship between analyte polarity and stacking. That directs to think in analyte chemical structure jointly with its polarity in focusing mechanisms. Accordingly, further studies are essential to find out more about the sweeping mechanism.

From the results obtained employing the two stacking modalities (REPSM and HCSSM), it was decided to use the latter one because a focusing

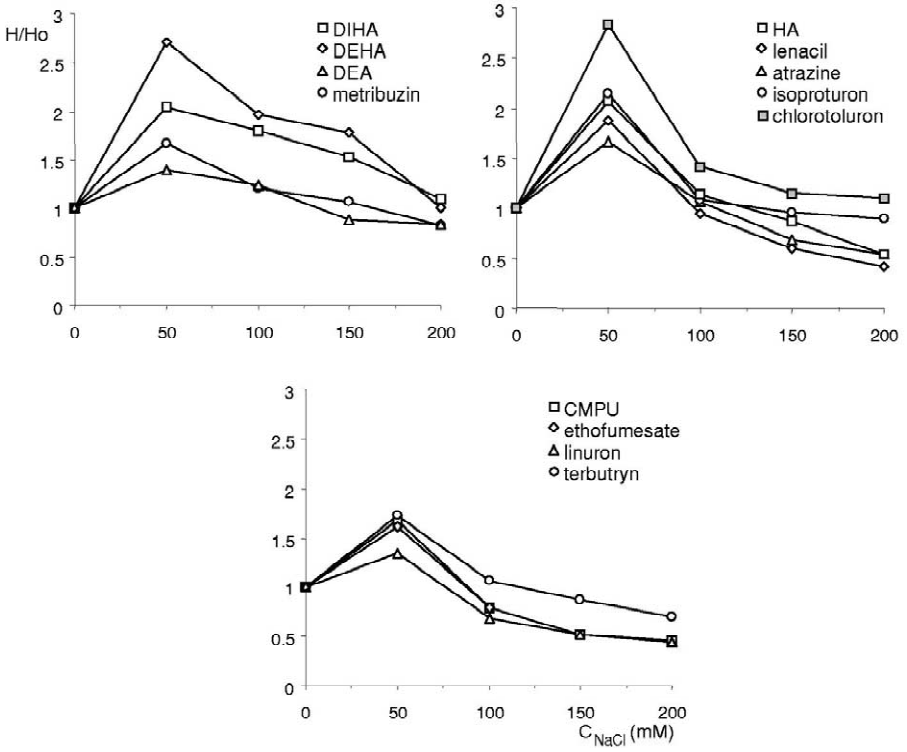

Fig. 6. HCSSM focalisation. Peak height (H) for different amounts of NaCl added to the sample related to peak height without NaCl in the sample (H_o).

effect was obtained for the 13 herbicides, this being greater for the more polar compounds (DEHA and DIHA), which are assumed to be less retained in a potential off-line preconcentration. Additionally, to accomplish the HCSSM mode it is only necessary to add a definite salt concentration in the injection sample, while with the REPSM mode a salt-dilute sample is necessary, which in practice is more difficult to obtain when natural waters are employed. Moreover, an exhaustive current control is necessary in order to minimise the loss of the more polar analytes.

The above method of analysis shows linearity in the range of concentration studied (0.5–4.5 mg l^{-1}) for both peak areas and peak heights, with a peak height relative standard deviation ($n=8$ at the 1 mg l^{-1} level) ranging from 3.8% for DEA to 15.5% for ethofumesate. Detection limits ($S/N=2$) varied between 0.07 mg l^{-1} for DEHA to 0.53 mg l^{-1} for lenacil.

3.3. Solid-phase extraction and recovery studies

With a view to obtaining a more sensitive method for the quantification of the herbicides and their metabolites, a study was performed using SPE as a preconcentration step prior to electrophoretic determination. Different types of sorbents were used to accomplish this step: silica-based bonded C_{18} cartridges and polymeric sorbents, Oasis HLB, a copolymer of poly(divinilbenzene–co-*N*-vinylpyrrolidone, and LiChrolut EN, a polymeric cartridge of styrene–diviniybenzene. To do so, samples of river water spiked with the 13 analytes at a concentration of 12.5 μg l^{-1} were used to evaluate the capacity of the three sorbents to retain these compounds. Methanol and ethyl acetate were used to elute the analytes from the cartridges. The results showed that the more polar analytes (DIHA, DEHA) were not retained either in the C_{18} or in Oasis HLB cartridges, whereas they were retained in the LiChrolut EN

polymeric sorbent because this sorbent displays a very high accessible surface area (1200 m^2 g^{-1}). LiChrolut EN was therefore chosen as the sorbent for the SPE in further studies.

3.3.1. Recoveries in LiChrolut EN sorbents

In SPE, one way of decreasing the detection limit consist in increasing the volume of water to be preconcentrated. However, in the case of highly polar analytes, breakthrough may occur when the volume to be preconcentrated is increased. The breakthrough in UHQ and non-polluted natural water (mineral and river) was evaluated. Fig. 7 shows the recoveries for the LiChrolut EN sorbent as a function of the preconcentrated sample volume and of water matrix (UHQ, mineral and river water). For every sample, the SPE step was performed by triplicate and the extracts were injected by duplicate, thus obtaining six electropherograms per sample. Relative standard deviations in the range 6–17% were achieved in all cases.

It can be concluded that the recoveries for the less polar analytes are independent of the preconcentrated sample volume while the polar analytes exhibit an important breakthrough effect. On comparing the three different matrices, natural waters are seen to exhibit a lower degree of retention in the solid-phase for the more polar analytes, probably due to the presence of salts and other polar compounds in the matrix. A 250-ml water sample volume was selected as the preconcentration volume.

3.4. Analytical data for preconcentration on-line and off-line

The whole method, in which a stacking procedure (HCSSM) together with an off-line SPE are carried out, was applied to a set of UHQ and non-polluted natural water samples in order to determine its analytical possibilities. Table 2 shows the analytical characteristics of the method.

It can be concluded that there is a good linear relationship between the analytical signal selected (peak height) and analyte concentration prior to preconcentration. Moreover, sensitivity (slope) in all water matrices appears to be lacking significance differences, except for DIHA, DEA and metribuzin, for which sensitivity is higher in UHQ than in natural water matrices. This can be justified bearing in mind that these polar compounds exhibit a lower degree of retention in the LiChrolut EN sorbent when they are in the presence of other matrix compounds belonging to the natural water matrix, as already explained.

The 1-day precision, expressed as the relative standard deviation, is in general slightly poorer in the natural water samples than in UHQ water, and never up to 20%, except for metribuzin (23% in natural water matrices) because of its lower sensitivity. These values are acceptable if it is taking into account that the whole procedure is composed of an off-line preconcentration step followed by a redissolution of the evaporated extract.

The detection limits in natural waters ranged from 0.13 $\mu g\ l^{-1}$ for DEHA in river water to 2.73 $\mu g\ l^{-1}$ for metribuzin.

4. Conclusions

Separation of the 13 herbicides and metabolites of interest can be achieved in less than 14 min by using a mixed micellar SDS–DOSS system and applying a voltage step during the analysis.

For the focalisation in the capillary of analytes with different chemical structures and polarities, the REPSM approach does not seem to be very appropriate. In this modality, the focusing of lower polarity compounds can be achieved but not that of the more polar analytes due to their loss during the focusing step. Additionally, this modality involves the use of low-conductivity samples that, in practice, are difficult to obtain when working with natural samples.

With the HCSSM stacking modality, focusing of the 13 compounds can be obtained, being higher for the two most polar analytes, DIHA and DEHA. This is important bearing in mind that the more polar analytes are usually less retained in a potential SPE step. This stacking modality is simply to use as an on-line stacking procedure because it only requires the addition of sodium chloride, or a similar salt, to the injection sample to enhance conductivity. The maximum focusing effect is observed at a sodium chloride concentration of 50 m*M*.

The use of an off-line SPE procedure is necessary

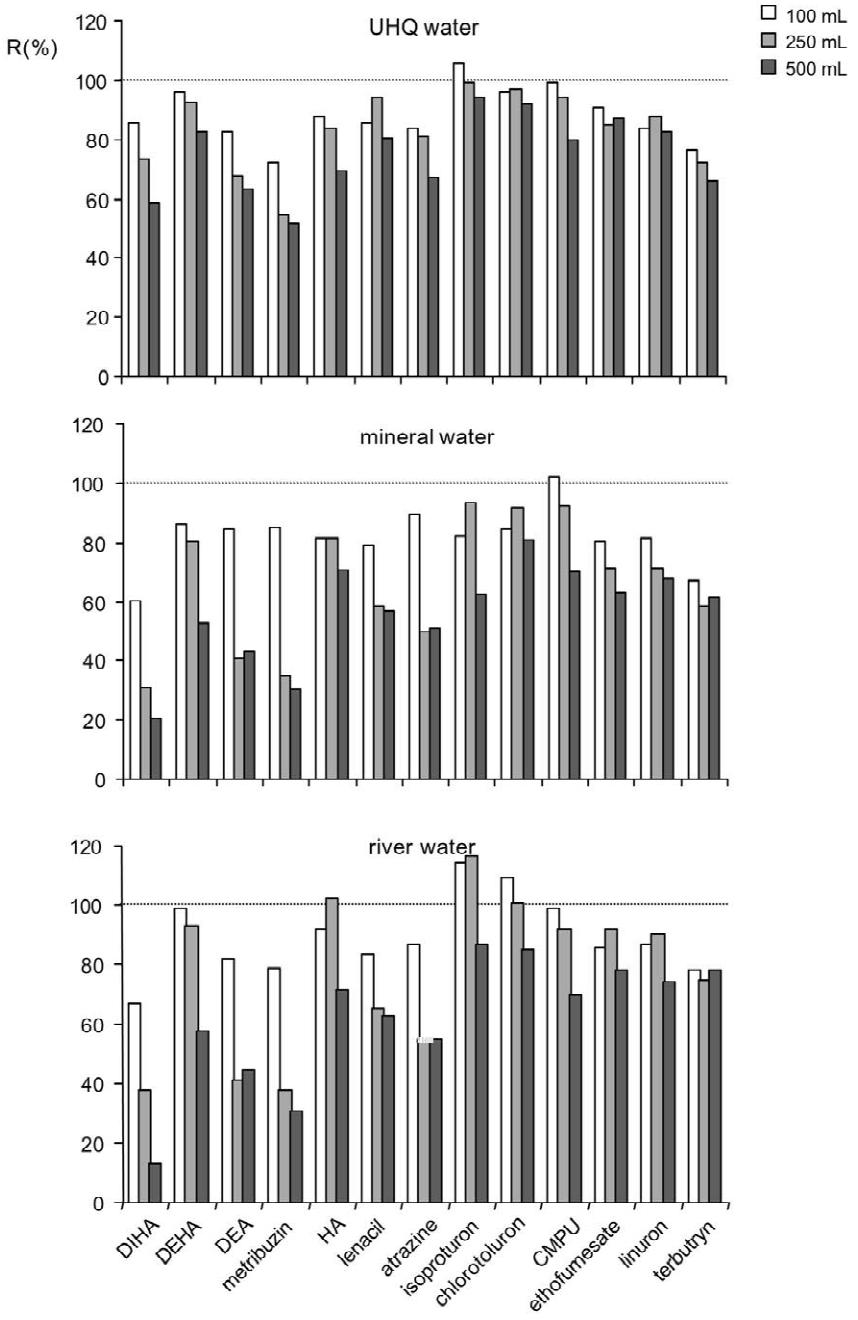

Fig. 7. Influence of the sample volume and sample matrix on the recoveries obtained with LiChrolut EN. All sample aliquots were spiked with 1.25 μg of each analyte.

to reach the concentration levels demanded by current legislation for pesticides in potential drinking water samples. Among the sorbents tested, choice of the LiChrolut EN polymeric cartridges was based on the fact that this sorbent exhibits retention of the more polar analytes (DIHA, DEHA).

Table 2
Analytical characteristics for the proposed SPE–HCSSM–MEKC method[a]

	λ	UHQ water				Mineral water				River water			
	(nm)	Slope (U.A. μg^{-1} l)	Intercept (U.A.)	RSD[b] (%)	D.L.[d] ($\mu g\ l^{-1}$)	Slope (U.A. μg^{-1} l)	Intercept (U.A.)	RSD[c] (%)	D.L.[d] ($\mu g\ l^{-1}$)	Slope (U.A. μg^{-1} l)	Intercept (U.A.)	RSD[c] (%)	D.L.[d] ($\mu g\ l^{-1}$)
DIHA	240	0.50±0.03	0.05±0.15	4.3	0.16	0.24±0.03	0.04±0.1	9.6	0.33	0.26±0.03	0.05±0.1	7.5	0.31
DEHA	240	0.50±0.05	0.1±0.2	5.5	0.16	0.53±0.05	−0.03±0.2	6.7	0.15	0.60±0.04	−0.02±0.2	7.3	0.13
DEA	220	0.24±0.02	−0.006±0.09	5.6	0.42	0.16±0.01	0.04±0.05	13.5	0.56	0.14±0.01	0.10±0.05	12.5	0.64
Metribuzin	220	0.07±0.01	0.04±0.04	18.8	1.51	0.04±0.01	0.05±0.03	22.8	2.14	0.033±0.004	0.09±0.03	17.7	2.73
HA	240	0.34±0.4	−0.04±0.2	11.5	0.24	0.34±0.03	−0.01±0.1	15.4	0.24	0.40±0.02	−0.02±0.1	5.1	0.20
Lenacil	220	0.21±0.02	−0.007±0.1	5.2	0.48	0.17±0.04	0.1±0.2	19.5	0.59	0.23±0.03	0.4±0.3	15.3	0.43
Atrazine	220	0.43±0.04	0.2±0.2	13.5	0.23	0.37±0.04	0.2±0.1	9.9	0.27	0.38±0.03	0.2±0.2	8.6	0.26
Isoproturon	240	0.45±0.03	0.01±0.1	6.5	0.18	0.44±0.06	0.06±0.2	8.0	0.18	0.47±0.02	0.09±0.1	2.5	0.17
Chlorotoluron	240	0.37±0.02	0.08±0.08	6.7	0.22	0.36±0.03	0.06±0.1	11.5	0.22	0.39±0.02	0.09±0.1	2.5	0.21
CMPU	240	0.48±0.03	−0.09±0.1	4.6	0.17	0.45±0.03	−0.0001±0.1	4.6	0.18	0.51±0.05	−0.08±0.2	7.2	0.16
Ethofumesate	200	0.37±0.02	0.2±0.1	9.9	0.29	0.39±0.05	0.1±0.2	13.8	0.31	0.43±0.05	0.2±0.3	21.5	0.28
Linuron	240	0.19±0.02	0.1±0.1	15.1	0.42	0.22±0.02	0.07±0.1	10.8	0.36	0.24±0.03	0.09±0.1	14.3	0.33
Terbutryn	240	0.25±0.03	0.02±0.1	5.1	0.32	0.25±0.05	0.2±0.2	10.3	0.32	0.27±0.03	0.03±0.2	21.6	0.30

[a] 95% significance level.
[b] RSD, relative standard deviation for a concentration of 2.0 $\mu g\ l^{-1}$ (n=6).
[c] RSD, relative standard deviation for a concentration of 2.0 $\mu g\ l^{-1}$ (n=5).
[d] D.L., detection limit for a S/N ratio of 2.

The proposed combination of SPE and on-column sample stacking can be used for the multiresidue determination of 13 compounds in natural water samples. Detection limits, between 0.13 and 2.73 μg l^{-1} depending on the compound in question, were obtained, allowing the analysis of these compounds in potential drinking water samples at the levels established by the current legislation.

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