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On-line concentration in micellar electrokinetic chromatography for triazine determination in water samples: evaluation of three different stacking modes

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Three different stacking modes for the on-line concentration of seven selected triazines (desisopropylatrazine, desethylatrazine, simazine, hydroxyatrazine, atrazine, propazine and prometryn) by micellar electrokinetic chromatography (MEKC) were evaluated, namely the normal stacking mode, field enhancement sample injection and reverse electrode polarity stacking mode (REPSM). The best results were obtained using REPSM at –20 kV for 90 s. Concentration factors from 4 to 10 were achieved and the detection limits varied within the range 3.3–8.5 μg l^{-1}. This methodology was combined with a solid-phase extraction (SPE) procedure to allow the determination of these analytes at trace levels. The SPE-REPSM-MEKC method developed was validated with a certified reference material of pesticides in drinking water (CRM 606) and was applied to the determination of the selected triazines in drinking waters at the level required by the European Union (0.1 μg l^{-1}).

1. Introduction

The use of micellar electrokinetic chromatography (MEKC) for the determination of neutral compounds using capillary electrophoresis (CE) was introduced by Terabe *et al.*[1] in 1984. The interest in MEKC has accelerated over the past decade. This technique, the instrumental design of which has been simplified in recent years, provides separation efficiencies similar to that of gas chromatography (GC) and better than that of HPLC, with short analysis times.

The main drawback of MEKC, which is common to other CE separation techniques, lies in the high detection limits, usually one order of magnitude higher than in HPLC with the detectors commonly employed. This is due to the low sample volume injected into the capillary and the short optical pathlength for on-column detection. Special efforts have been made to solve this problem and various approaches have been developed,[2–5] some based on instrumental modification (powerful detectors or modified detection cells) and others on off-line and on-line preconcentration procedures.

Isotachophoresis and sample stacking are of great interest as on-line concentration techniques, because they are simple, economical and no additional instrumentation is necessary. Field amplified sample stacking (FASS) of charged analytes for capillary zone electrophoresis (CZE) was introduced by Chien and Burgi[6] after which Mikkers *et al.*[7] established the concentration principle. This technique allows injection volumes higher than in conventional CZE. The charged analytes are injected in a low conductivity matrix and, when the separation voltage is applied, their electrophoretic velocity is enhanced owing to the low conductivity of the sample zone (amplified field). When they reach the boundary between the sample matrix and the separation buffer, they slow down and stack, owing to the higher conductivity of the separation buffer.

Since the publication of Chien and Burgi's work, the interest in stacking techniques for charged compounds[8–19] has increased considerably. However, the problem of applying this phenomenon to the concentration of neutral analytes is that uncharged compounds do not experience velocity enhancement, since they have no electrophoretic mobility. To solve this problem, stacking has to be performed by providing them with effective electrophoretic velocity through interaction with anionic micelles.[20,21]

The different stacking modes for neutral analytes using MEKC and, more recently, a special concentration effect called sweeping,[22] have been described by Quirino and Terabe and reviewed by the same authors.[2,23] The stacking effect can be achieved under basic running buffer conditions, or by using reverse migrating micelles under acidic running buffer conditions. Other studies have been published using these methodologies for the analysis of hydrophobic compounds, aldehydes, carbamate insecticides and dioxin-related compounds.[24–27]

In previous work[28] we combined off-line solid-phase extraction (SPE) with MEKC for the determination of the triazines desisopropylatrazine (DIA), desethylatrazine (DEA), simazine (SIM), hydroxyatrazine (HA), atrazine (A), propazine (PPZ) and prometryn (PMT) in environmental waters. In this case a minimum preconcentration factor of 1000 by SPE on two poly(styrene–divinylbenzene) (PS–DVB) disks was needed to achieve detection limits low enough to determine these herbicides at the required concentration (<0.1 μg l^{-1}). Although the main objective was successfully achieved, the high sample volume employed made the method time consuming and not easily applicable for routine analysis of pesticides in which a large number of samples have to be processed.

In this work, three different stacking modes under basic conditions were evaluated in order to concentrate these analytes on-line and reduce the total analysis time, namely the normal stacking mode (NMS), reverse electrode polarity stacking mode

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(REPSM) and field enhancement sample injection (FESI). The SPE–on-line concentration–MEKC method developed was validated using a certified reference material of pesticides in drinking water (CRM 606) and applied to the determination of the selected triazines in mineral and tap water samples.

2. Experimental

2.1. Apparatus and materials

All measurements were performed with an HP 3D CE capillary electrophoresis system equipped with a diode array detector and controlled by an HP Vectra VL computer and an HP Chemstation program manager (Hewlett-Packard Española, Madrid, Spain). Separations were carried out in a fused silica extended light path capillary of 40 cm effective length (total length 48.5 cm), 75 μm id and 200 μm optical pathlength from Hewlett Packard Española.

Water samples were pre-filtered through 0.45 μm, 47 mm 100/Pk nylon filters (Micron Separations, USA). The pesticides were extracted in a vacuum filtration system on PS–DVB 3M Empore extraction disks obtained from Scharlab (Barcelona, Spain), and the extracts were cleaned in 1 g of Dionex Power 1-X8 anion-exchange resin (Fluka, Buchs, Switzerland) packed in a polyethylene column (11.0 × 1.0 cm, J. T. Baker, Deventer, Netherlands).

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.

The buffer, standard solutions and redissolved samples were filtered prior to electrophoretic analysis through a 0.45 μm PTFE syringe filter obtained from Scharlab (Barcelona, Spain).

2.2. Reagents

Atrazine, desethylatrazine, desisopropylatrazine, 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.

The certified reference material (CRM 606) was supplied by the Institute for Reference Materials and Measurements (Geel, Belgium).

Analytical-reagent grade sodium tetraborate ($Na_2B_4O_7$, anhydrous), sodium dodecyl sulfate (SDS, $C_{12}H_{25}SNa$), sodium chloride and sodium phosphate (Na_3PO_4) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide solutions for HPCE of 1 and 0.1 M were supplied by Sigma-Aldrich Química (Madrid, Spain). Methanol (HPLC grade) was obtained from Scharlab (Madrid, Spain).

Purified water from Millipore Ibérica (Madrid, Spain) was used to prepare the reagent solutions.

2.3. Conditioning, REPSM and MEKC separation

Before starting a sequence 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) and running buffer (5 min). The running buffer (background solution) consisted of 10 mM borate buffer solution containing 125 mM SDS and 20% methanol. Between runs, the conditioning step consisted of only 2 min of 0.1 M NaOH and 2 min of running buffer in order to avoid memory effects.

To achieve the selected optimum stacking conditions (REPSM), samples were prepared in 10 mM phosphate buffer (pH 7) medium, and injected in the hydrodynamic mode by applying 50 mbar for 90 s (0.45 μl). A constant voltage of 20 kV at negative polarity was applied until the current reached 99% of the predetermined value, provided by the running buffer at this negative voltage, followed by MEKC separation runs at a constant voltage of 30 kV at positive polarity. The background solution was previously filtered through a 0.45 μm PFTE syringe filter.

The temperature of the capillary was adjusted to 25 °C and the triazinic herbicides were monitored at 220 nm.

2.4. Sample preparation

Certified reference material. In accordance with the technical specifications,[29] 0.5 g of freeze-dried tap water residue was dissolved in 500 ml of 10^{-3} M HCl solution at ambient temperature and this solution was stirred for 10 min, avoiding direct sunlight.

The pH of the reconstituted sample was adjusted to 7 with 0.1 M NaOH.

Spiked water samples. Mineral and tap water samples (collected in Madrid) were spiked with each of the above-mentioned pesticides at the 0.1 μg l^{-1} concentration level.

All water samples were pre-filtered through a 0.45 μm filter to remove the suspended matter.

2.5. Solid-phase extraction

A PS–DVB solid-phase extraction disk was placed in the filtration system, activated and conditioned with 10 ml of methanol and 10 ml of water. After percolating the water sample (spiked samples, 250 ml; reconstituted CRM, 100 ml), the disk was washed with 5 × 2 ml of water and vacuum-dried in order to remove all remaining water. Subsequently, the analytes were eluted with 3 × 2 ml of methanol. This fraction was collected for further clean-up on an anion exchange resin.

The anion exchange resin was washed with 25 ml of 1% HCl and water at 0.5 ml min^{-1} until the effluent became neutral and conditioned with 2 × 5 ml of methanol. The sample extract (from the SPE) was then loaded into the column and the effluent was collected. Finally, the column was rinsed with 3 × 1 ml of methanol. The effluent and washing extracts were mixed in the same vial and vacuum-evaporated to dryness. The residue obtained was subsequently dissolved in 1 ml of 10 mM phosphate buffer (pH 7) and filtered through a 0.45 μm PTFE filter before injection.

3. Results and discussion

3.1. On-line concentration of triazinic herbicides

As reported previously,[28] atrazine, simazine, hydroxyatrazine, DIA, DEA, propazine and prometryn can be separated at 30 kV with a 10 mM borate buffer, 60 mM SDS and 20% of methanol (pH 9.2). It was necessary to preconcentrate 1 l of the water sample off-line on two PS–DVB disks to a final volume of 1 ml. This percolation step takes around 45 min–1 h depending on the sample. In order to reduce the sample volume needed, three stacking techniques were evaluated.

Normal stacking mode (NSM). This stacking mode is based on the assumption that when analytes in a low-conductivity matrix are injected into the capillary filled with the background solution (BGS) containing anionic micelles, and a positive voltage is applied, micelles from the BGS migrate towards the anode, enter the sample zone, enhance their migration velocity in this low-conductivity matrix and stack as described above for

charged compounds. If the analytes interact with the incoming micelles, they also will be stacked. The NSM is illustrated in Fig. 1(a).

It is well known that the stacking effect increases in proportion to the retention factor (k), which means that hydrophobic compounds stack better than polar compounds which have less interaction with the micelles. Hence the ability of micelles to stack effectively the relatively polar selected triazines was evaluated. This study was made in terms of recovery, by comparing the areas obtained injecting the pesticides in pure water with those in the running buffer (60 mM SDS), at a constant injection time (10 s, 50 mbar). These studies were made with the corrected areas, calculated by dividing the areas obtained by the retention time in each case. The results are given in Table 1. As was expected, low recoveries were obtained for all the pesticides except for the most hydrophobic,

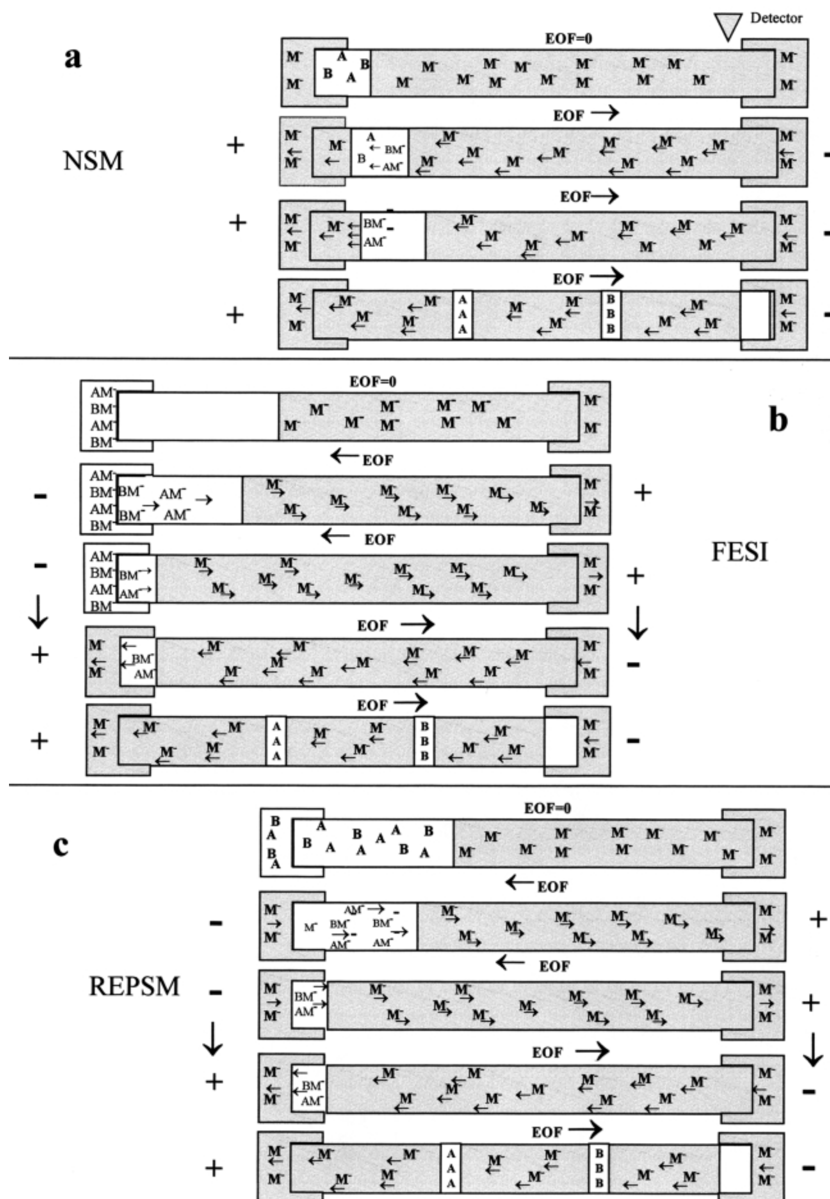

Fig. 1 Behaviour of micelles and neutral analytes during the stacking processes. (a) Normal stacking mode; (b) field enhancement sample injection; (c) reverse electrode polarity stacking mode.

Table 1 Recovery (R) and RSD of triazines in different sample matrices in NMS (n = 5)

SDS in the running buffer/mM	Matrix	DIA		DEA		SIM		HA		A		PPZ		PMT	
		R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)	R (%)	RSD (%)
60	Purified water	22	6	45	6	62	6	54	6	73	5	101	4	99	3
125	Purified water	46	5	70	5	102	3	93	4	97	4	98	5	103	4
125	10% Methanol	60	5	98	4	97	4	102	4	103	4	102	4	97	3
125	15 mM SDS	58	4	95	4	95	4	97	3	101	3	95	4	105	3
125	10 mM NaCl	85	4	102	3	98	3	96	4	98	4	104	4	99	3
125	10 mM Na_2SO_4	87	4	100	4	97	3	103	3	99	4	98	4	94	4
125	Phosphate buffer	91	3	97	4	103	4	98	4	105	3	99	5	101	3

propazine and prometryn. In order to increase the retention factors of triazines and improve the recoveries, the effect of increasing the SDS concentration in the running buffer to 125 mM was evaluated. Recoveries were still low for the most polar analytes, DIA and DEA, whereas for the other triazines it was close to 100% (see Table 1).

In a second approach, other variables that might favour the micelle–analyte interaction were evaluated (presence of methanol, micelles and salts in the sample solution), and the results are also summarized in Table 1. The presence of methanol in the sample matrix was tested in the range 2–10% and the results show that, although the recoveries increased with increasing amount of methanol, there was also significant band broadening. The presence of micelles in the sample plug up to 15 mM SDS was also evaluated, showing an increase in the recovery of DIA and DEA; however, the higher micelle concentration distorted the peak shape.

The most promising results were achieved with samples prepared in 10 mM NaCl, 10 mM Na_2SO_4 or 10 mM sodium phosphate (pH 7). It could be expected that the stacking effect would decrease with the addition of salts, but their positive effect can be considered to be due to the 'salting-out effect' in the extraction processes. A special sharpening effect was observed when the sample was prepared in 10 mM sodium phosphate (pH 7), and this sample matrix was selected as the most appropriate to facilitate the analyte–micelle interaction.

The injection time was increased to 40 s, but injections longer than 20 s resulted in broader peaks. The stacking efficiencies obtained in terms of area (SE_{area}) for a 20 s injection, calculated by dividing the corrected areas by those obtained in the 10 s injection non-stacking mode, were close to 2, showing that these analytes can be concentrated only twofold using this stacking mode.

The band broadening observed for injection times longer than 20 s is due to the dispersive effect on the focused bands brought about by the local electroosmotic velocity mismatch between the sample zone and the running buffer.[23] This restricts the sample volume injected and therefore the concentration factor. This dispersive effect can be minimized with FESI and REPSM.

Field-enhanced sample injection (FESI). In this stacking mode, a water plug is introduced at the inlet end of the capillary filled with the BGS. The sample is prepared in a low-conductivity micellar matrix, and electrokinetically injected at negative polarity. During injection, micelles from the sample vial and the analytes solubilized in them enter the capillary owing to the enhanced field in the water plug that generates electrophoretic velocities greater than the bulk electroosmotic flow (EOF). When the current reaches 90% of the predetermined current at negative polarity (−20 kV), which means that the water plug has moved far away from the capillary, the voltage is shut off and the sample vial is replaced by another BGS vial, to perform the separation step at positive polarity. As the water zone is removed, the aforementioned dispersive effect is minimized. FESI is illustrated in Fig. 1(b).

As described previously,[30] the maximum length of the water plug (x^*_{max}) that allows migration of neutral analytes (solubilized on the micelles) into the capillary is dependent on k. In the hydrodynamic injection mode the length of the water plug can be expressed as the injection time at 50 mbar, which is an instrumental parameter easy to control [$t_{(s)} = 1.18x_{(mm)}$ under the instrumental conditions used]. In order to find the corresponding t^*_{max} for each analyte, the injection time of the water plug was varied from 10 to 100 s. This study was carried out with a sample solution containing 1 mg l^{-1} of each triazine and 15 mM SDS in water. The results are shown in Fig. 2. The stacking efficiencies increase with increasing water plug length (x), but only to a maximum value (x^*_{max}), achieved when the water plug injection time is t_{max}. Water plug lengths greater than x^*_{max} do not allow the migration of analytes and, therefore, the stacking process does not start until x^*_{max} is reached. For this reason, the time of effective concentration and, consequently, the SE_{area} achieved, are the same for plug lengths greater than x^*_{max}.

As can be observed in Fig. 2, the t^*_{max} found for all analytes tested was very low, indicating low concentration factors and, in addition, poor peak shapes were obtained for water plug injections longer than 25 s, even when the sample was prepared in 10 mM sodium phosphate–15 mM SDS. Although increasing the micelle concentration on the sample matrix up to 100 mM increased the SE_{area}, the peak shapes did not improve. This problem could be explained by the dispersion of the micelles in the corresponding monomers when they enter the water plug, due to the high dilution.[30] Therefore, another surfactant with a lower critical micelle concentration than SDS (c.m.c. = 8.27 mM) was also tested. The influence on the SE_{area} and peak shapes of the presence of glycodeoxycholic acid (c.m.c. = 2 mM) was evaluated the 0.1–1% range in the running buffer (10 mM sodium tetraborate–100 mM SDS–20% methanol) and in the sample matrix. The best results were obtained when the sample matrix consisted of a twofold dilution of the running buffer containing 0.2% glycodeoxycholic acid. Although the peak shapes improved significantly, allowing water plug injections up to 70 s, the SE achieved were not higher than those obtained with SDS and a water plug injection of 25 s.

These results suggest that the retention factor has a dual influence on the final stacking efficiency in FESI: it controls the amount of analyte electrokinetically injected and influences the stacking efficiency inside the capillary (much the same as in NSM). Because of this dual effect, FESI is not an appropriate means of concentrating the selected triazines, since they will be poorly introduced into the capillary by the electrokinetic injection.

Reverse electrode polarity stacking mode (REPSM). As in FESI, the dispersive effect can be minimized in REPSM by pumping out the low-conductivity sample matrix by applying voltage at negative polarity. Initially, the capillary is filled with the BGS and the analytes in the low-conductivity matrix are introduced hydrodynamically. Next, the sample vial is replaced by a BGS vial and voltage is applied at negative polarity. In this step, micelles from the BGS vial enter the sample zone and stack the analytes, and the low-conductivity matrix is pumped out from the capillary by the EOF. When the current reaches the predetermined value, the voltage is switched to positive polarity and the separation step takes place [Fig. 1(c)].

In this case, x^*_{max} is the maximum fraction of the capillary filled with the sample matrix that allows movement of the analytes towards the concentration boundary. If the length of the capillary filled with the sample solution exceeds x^*_{max}, a fraction of the analytes will be lost to the cathodic vial until the

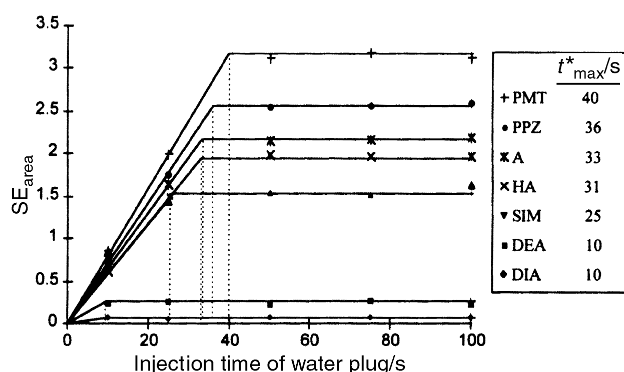

Fig. 2 Experimental determination of t^*_{max} of triazines in field enhancement sample injection.

corresponding x^*_{max} is reached. This fraction will increase as the corresponding retention factor decreases.

Standard solutions containing 1 mg l^{-1} of each triazine in pure water and in 10 mM sodium phosphate (pH 7) were prepared and analysed and, as in NSM, the best results in terms of SE_{area} and sharp peaks were obtained in the buffer medium. This effect can be observed in Fig. 3. Further experiments were therefore performed in this sample matrix.

In order to find the corresponding t^*_{max} under these conditions for each triazine, the effect of the sample injection time at 50 mbar on SE_{area} and SE_{height} (peak height obtained in each case divided by that of the 10 s injection) was evaluated. The influence of the percentage of predetermined current achieved when the polarity is switched was also studied. Different experiments for each sample injection time (from 10 to 100 s) at different percentages of the predetermined current (from 80 to 99%) were performed. The t^*_{max} obtained were in the 30 s (DEA) to 90 s (prometryn) range, and the stacking

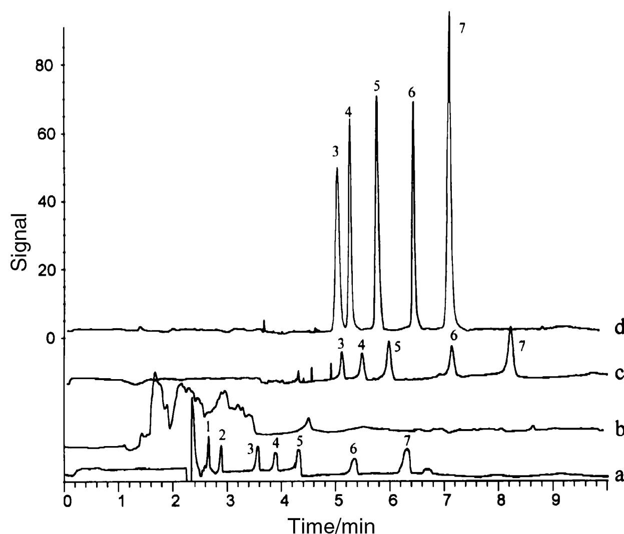

Fig. 3 Electropherograms obtained for triazines (1 mg l^{-1}) by MEKC. Running buffer: 10 mM borate buffer, 125 mM SDS, 20% methanol. 1 = DIA; 2 = DEA; 3 = simazine; 4 = hydroxyatrazine; 5 = atrazine; 6 = propazine; 7 = prometryn. (a) and (b) without REPSM. Sample matrix, running buffer; injection time, (a) 10 and (b) 90 s. (c) and (d) with REPSM (injection time 90 s). Sample matrix: (c) purified water and (d) 10 mM phosphate buffer (pH 7).

efficiencies were higher than in NSM and FESI. The response surfaces obtained for DIA and DEA were very similar but different to those obtained for the other triazines (which were similar among themselves). Fig. 4 shows the results obtained for DIA and prometryn and the t^*_{max} found for all triazines. As can be observed, a current higher than 90% gave rise to a decrease in SE_{area} and SE_{height} in the case of DIA (and DEA), probably due to losses of this analyte to the cathodic vial. For the other triazines, although SE_{area} remains constant, an increase in SE_{height} is observed, especially for prometryn which has a higher retention factor. It may be that, as prometryn is the first analyte to start the stacking process, it is the first to reach the BGS–sample matrix boundary, and the rest of the time a negative polarity is employed to shorten the zone of this analyte. The final stacked zone width of each analyte will increase as the corresponding retention factor decreases. Therefore, if the DIA and DEA stacked zones are still broad when the current reaches 90%, part of these zones will be lost to the cathodic vial during the time required to achieve 99%.

The maximum concentration factors achieved for DIA and DEA were 2 and 2.5, these values being comparable to those obtained using NSM; however, the concentration of the other triazines was considerably improved using REPSM (from 5 to 14). Therefore, REPSM was selected to perform the reversed polarity step up to 99% of the predetermined current, because it provides the best signal-to-noise ratio for these analytes. The t^*_{max} obtained for prometryn (90 s) was selected as optimum. Under these conditions, REPSM was chosen for on-line concentration of the selected triazines.

3.2. Analytical performance

Table 2 shows the SE_{area}, SE_{height} and detection limits obtained using REPSM, calculated as three times the noise signal, compared with those obtained without on-line concentration. As can be observed, good stacking efficiencies were achieved in all cases, indicating a major improvement in the detection limit, up to one order of magnitude in the case of prometryn.

Under the optimum conditions (90 s injection and 99% of predetermined current), calibration curves were prepared by injecting a standard solution containing herbicide concentrations within the 10 μg l^{-1}–1 mg l^{-1} range. Linear calibration curves were found within the tested range and the correlation coefficients obtained ranged from 0.997 to 0.999. The relative

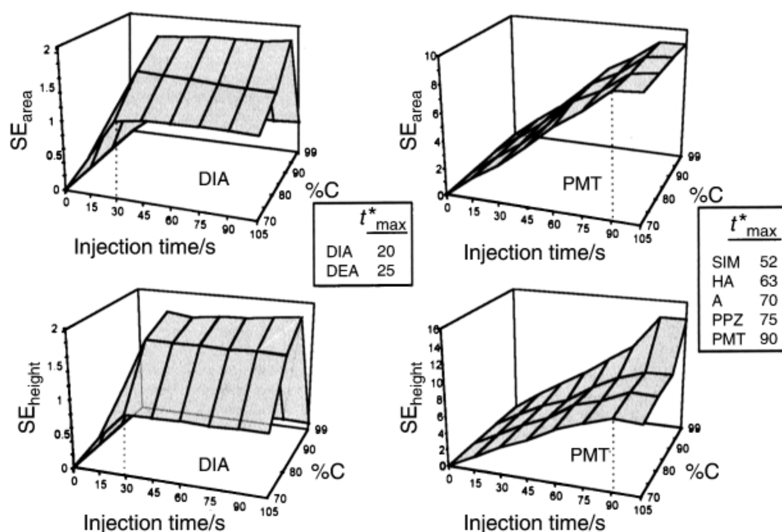

Fig. 4 Variation of SE_{area} and SE_{height} with injection time and percentage of current at negative polarity in REPSM. Response surfaces obtained for DIA and prometryn.

standard deviations (RSDs) found for each triazine at the 20 and 200 μg l^{-1} concentration levels were calculated (n = 5) and are also given in Table 2.

Although this method did not allow the determination of these analytes in environmental waters without a previous SPE, the time and sample volume required in this step are considerably reduced.

3.3. Analysis of water samples

In order to demonstrate the applicability of the SPE-RESPM-MEKC method, mineral and tap water samples, spiked at concentration levels permitted by current regulations, were analysed. A certified reference material (CRM 606) containing atrazine, simazine, linuron, propanil, carbaryl and fenamiphos was also analysed for validation purposes.

Method validation. The freeze-dried blank and water sample reference material residues had been reconstituted following the technical specification[29] described above and, after the analytes had been preconcentrated on a PS–DVB disk, the extracts obtained were analysed by REPSM-MEKC.

Unfortunately, it was impossible to detect atrazine and simazine using this procedure, owing to the large amount of fulvic and humic acids appearing as a hump in the middle of the electropherogram. Peaks corresponding to atrazine and simazine were completely overlapped by the broad peak corresponding to the sample matrix, making a further clean-up step necessary.

At neutral pH, humic and fulvic acids are mainly in their anionic form;[28] consequently, they should be retained on an anion exchange resin (on which non-ionic compounds should not be retained). Accordingly, the methanol extracts obtained from the SPE were run through a cartridge containing an anion exchange resin, following the procedure described above, and the final extracts were vacuum-dried, redissolved and analysed by REPSM-MEKC. Fig. 5 shows the electropherogram obtained by the complete method employed, allowing the determination of atrazine and simazine in the certified reference water sample.

Table 3 gives the results obtained by the proposed method for the determination of these analytes, together with their corresponding certified values. No significant differences were found at the 95% confidence level, verifying the validity, in terms of accuracy and precision, of the method developed for triazine determination in drinking water samples. Also, the presence of other commonly used pesticides such as linuron, propanil, carbaryl and phenamiphos in the CRM allowed the assessment of the selectivity of the method. No interferences in the determination of atrazine and simazine were observed.

Analysis of drinking water. Although the proposed method was successfully validated, the pesticide concentration level present in CRM 606 was higher than that established by the current EU regulation[31] (0.1 μg l^{-1}). The method developed was therefore applied to the determination of the selected triazines in mineral and tap water at this concentration level.

Table 2 Summary of analytical parameters for triazines after REPSM under the optimum conditions

Triazine	SE_{area}	SE_{height}	LOD/μg l^{-1} RESPM-MEKC	LOD/μg l^{-1} MEKC	RSD (%) 20 μg l^{-1}	RSD (%) 200 μg l^{-1}
SIM	5.0	5.1	8.5	30	6.4	5.1
HA	6.5	9.1	7.1	32	6.0	5.0
A	6.9	9.3	5.5	29	5.8	4.6
PPZ	7.4	12.8	6.2	38	6.3	5.2
PMT	8.5	14.0	3.3	40	5.1	4.0

Fig. 6 shows the electropherograms obtained after preconcentration of 250 ml of mineral and tap water samples spiked at the 0.1 μg l^{-1} concentration level. Peak areas were integrated manually and the recoveries obtained varied within the range 95–110% (Table 3) with an RSD of < 10% (n = 5 on two different days). These values are acceptable, confirming the good reproducibility of the method. No interferences due to substances that could be present in the water samples were observed.

The detection limits, also reported in Table 3, varied from 15 to 30 ng l^{-1} in mineral water and from 20 to 35 ng l^{-1} in tap water, depending on the pesticide. As can be observed, the five triazines can be detected and quantified at this trace level and the detection limits obtained are in agreement with current EU regulations.

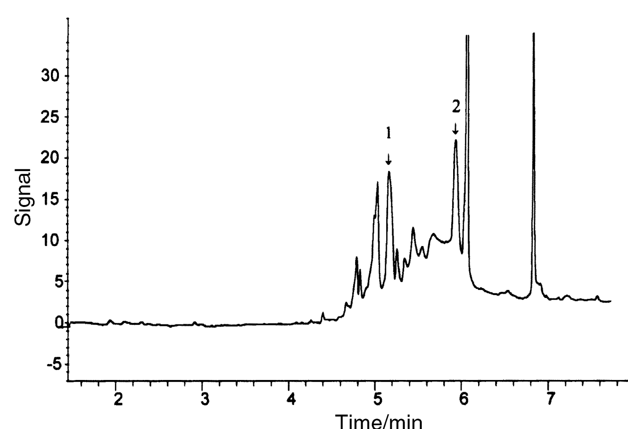

Fig. 5 Electropherogram obtained for the reconstituted freeze-dried water sample (CRM 606) after SPE-REPSM-MEKC using an anion exchange resin for clean-up. 1 = Simazine; 2 = atrazine. Electrophoretic conditions as in Fig. 3.

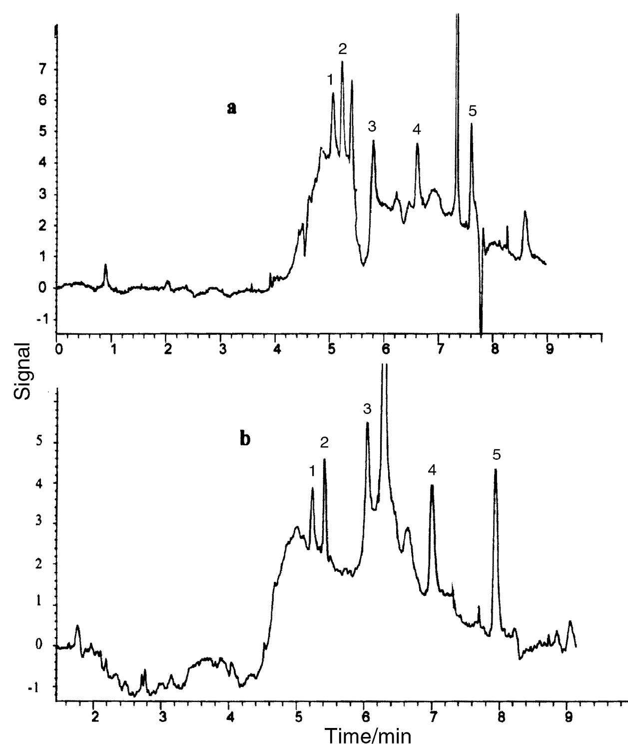

Fig. 6 Electropherograms obtained for (a) tap and (b) mineral water samples spiked with 0.1 μg l^{-1} of each triazine after SPE-REPSM-MEKC. 1 = Simazine; 2 = hydroxyatrazine; 3 = atrazine; 4 = propazine; 5 = prometryn. Electrophoretic conditions as in Fig. 3.

Table 3 Recovery[a] (R) of water samples by SPE-REPSM-MEKC. Validation with the certified reference material (CRM 606)

	Mineral water (0.1 μg l^{-1})			Tap water (0.1 μg l^{-1})			CRM 606	
Triazine	*R* (%)[a]	RSD (%)	LOD/ng l^{-1}	*R* (%)[a]	RSD (%)	LOD/ng l^{-1}	MEKC value[a]/μg l^{-1})	Certified value[b]/μg l^{-1}
Simazine	102	6.6	30	110	7.3	35	4.4 ± 0.3	4.7 ± 0.6
HA	95	7.2	22	98	8.1	25	—	—
Atrazine	108	7.8	25	105	8.0	30	6.6 ± 0.4	6.7 ± 0.8
Propazine	110	6.5	20	99	7.1	22	—	—
Prometryn	102	5.8	15	95	6.3	20	—	—

[a] Average of five independent determinations. [b] Confidence interval at 95% confidence level.[29]

4. Conclusions

On-line concentration of triazines by REPSM has proved to be an easy and fast methodology for improving the detection of these analytes in MEKC, except for highly polar compounds, such as DIA and DEA. In this case, the low concentration factor obtained by NSM cannot be improved by REPSM, and further research on stacking procedures is necessary to increase these values.

The combination of REPSM-MEKC with the proposed SPE procedure allows the determination of these analytes in drinking water at the concentration level required by current regulations, while reducing the sample volume and total analysis time. The proposed method has been validated, and the detection limits reached for the selected triazines using on-line concentration were comparable to those commonly obtained by HPLC. The greater sensitivity achieved by on-line sample concentration techniques represents an important advance in the suitability of MEKC for routine environmental analysis and in the control of these analytes in drinking water.

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