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Detection and quantitation of sulfonylurea herbicides in soil at the ppb level by capillary electrophoresis

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Abstract

A multi-residue analytical method based on solid-phase extraction enrichment combined with capillary electrophoresis (CE), using micellar electrokinetic capillary chromatography, was developed to isolate, recover and quantitate three sulfonylurea herbicides (chlorsulfuron, chlorimuron and metsulfuron) from soil samples. Optimization for CE separation was achieved using an overlapping resolution map scheme. The recovery of each herbicide was >80% and the limit of detection was 10 ppb. The capability of CE in providing quantitative analysis of sulfonylureas in soil samples at the ppb level has been demonstrated.

1. Introduction

Sulfonylurea herbicides have extremely high biochemical activity, thus enabling extremely small dosages in field crops (2–60 g/ha). However, chlorsulfuron, metsulfuron and chlorimuron have the potential to persist in soil in quantities sufficient to injure susceptible crops [1–3]. Therefore, the monitoring of their residues in soil is essential to study the persistence and the environmental behaviour.

Analytical methods for the determination of sulfonylurea residues in soil use high-performance liquid chromatography (HPLC) [4,5], gas chromatography (GC) [6–8], immunoassay [9] and bioassay [10]. In spite of the impressive versatility of capillary electrophoresis (CE) [11], the low injectable volume (usually 1–60 nl) and the poor elution power of CE with respect to other chromatography techniques restricted the use of this analytical tool in environmental analysis of agrochemicals in soil at the ppb level. Recently, CE, using micellar electrokinetic capillary chromatography (MECC), has shown good potentiality to detect herbicides in water [12,13], but had never been applied to herbicide detection in soil samples. To apply CE in analyses of herbicides in soil, particular attention has to be devoted to the optimization of separation, in order to achieve the best selectivity and resolution in such a complex matrix where many potential compounds could interfere. Different approaches have been proposed for the tuning of CE separations, i.e. the Plackett–Burman statistical design implemented by Vindevogel and Sandra [14], a theoretical approach [15], a computer simulation [16] and the overlapping resolution mapping (ORM) procedure [17].

The objectives of this study were (a) to optimize CE separation using a three-dimensional ORM scheme for the "tuning" of three interactive components [concentrations of sodium

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dodecyl sulphate (SDS), methanol and isopropanol] of the electrolyte buffer and (b) to evaluate the potential applicability of CE, using MECC, combined with solid-phase extraction (SPE) enrichment for the simultaneous determination of three sulfonylureas in soil.

2. Experimental

2.1. Reagents

Reagents for SPE and CE analysis were supplied by Sigma (St. Louis, MO, USA). All solvents used were pesticide-free grade. The sample concentration column for SPE consisted in a Bakerbond (Phillipsburg, NJ, USA) C_{18} (1 g, 40-μm silica particles). Commercial formulations of chlorsulfuron [1-(2-chlorophenylsulfonyl)-3,4(methoxy-6-methyl-1,3,5-triazin-2-yl)-urea], metsulfuron [2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl) benzoic acid] and chlorimuron [2-(4-chloro-6-methoxypyrimidin-2-ylcarbamoylsulfamoyl) benzoic acid] were used. The parent herbicides were extracted from the commercial products with freshly redistilled dichloromethane in Soxhlet for 3 h. After dehydration with anhydrous sodium sulphate, dichloromethane was distilled off in rotary evaporator. The residual sulfonylureas were subjected to nuclear magnetic resonance, infrared and mass spectral analyses to confirm their identity and used for subsequent experiments without further purification, as reported by Galletti et al. [18].

2.2. ORM scheme

A three-dimensional ORM scheme to optimize the concentration of surfactant (SDS) and organic modifiers (methanol and isopropanol) was employed according to Li [17]. Briefly, eleven pre-planned experiments were carried out at strategic positions on a cubic diagram. The eleven electrolyte buffers used are reported in Table 1. Maximum and minimum concentrations of SDS tested were 80 and 30 mM, respectively, while those of methanol and isopropanol were 40–0% and 20–0% (v/v), respectively. From the experimental results of retention times, the resolution (R_s) between each pair of adjacent peaks of the electropherogram was calculated according to the following equation: $R_s = [2(t_{R1} - t_{R2})/w_2 + w_1]p$, where t_{R1} and t_{R2} are the retention times of two adjacent peaks, w_1 and w_2 are the widths of the two pairs of peaks and p is the value of penalty. In order to avoid unacceptable retention times (too short or too long), the penalty value (p) was introduced in the resolution equation. The penalty value was set at

Table 1
Pre-planned experiment conditions carried out to obtain the ORM scheme

Experiment	SDS (x_1, mM)	Methanol (x_2, %)	Isopropanol (x_3, %)	Percentage (x_1, x_2, x_3)
1	30	0	0	0, 0, 0
2	80	0	0	1, 0, 0
3	30	40	0	0, 1, 0
4	30	0	20	0, 0, 1
5	80	40	0	1, 1, 0
6	80	0	20	1, 0, 1
7	30	40	20	0, 1, 1
8	80	40	20	1, 1, 1
9	55	0	0	0.5, 0, 0
10	30	20	0	0, 0.5, 0
11	30	0	10	0, 0, 0.5

The eleven experiments were performed with 30 mM borate buffer at pH 7.0.

0.25 for retention times of metsulfuron (first peak eluted) in the range 0–5 min and 25–30 min, at 0.75 for retention times in the range 10–15 min and 20–25 min, at 1 for retention times ranging from 15 to 20 min. The calculated R_s values were then fitted in the following polynomial equation [17]:

$$R_s = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3 + a_{123}x_1x_2x_3$$

where a_i values are coefficients and x_1, x_2 and x_3 correspond to the percentage fractions at the respective axes of concentration of SDS, methanol and isopropanol in the electrolyte buffer.

2.3. Preparation of standards

A stock solution at the concentration of 100 ppm was prepared dissolving 10 mg each of the three sulfonylureas in 100 ml of methanol–water (50:50). Appropriate dilutions of this stock solution were made in methanol–water (50:50) to obtain final concentrations of 0.63, 1.25, 2.5, 3.5, 5 and 10 ppm.

2.4. Soil sample fortification and extraction

A sandy loam soil obtained from Cadriano, near Bologna, Italy, was used to prepare the soil samples for this study. The average soil composition was 27% clay, 15% silt, 58% sand, 1.3% organic matter and pH of 6.5. A stock solution containing 1 ppm of the three sulfonylureas was obtained dissolving 10 mg of each compound in 10 l of bidistilled water. From this stock solution, appropriate quantities were added to 500 g (oven-dry basis) of soil, screened through a 3-mm sieve, to give a final herbicide concentration of 10, 20 or 50 ppb. After the fortification, soil samples were mixed for 5 min in a blender and frozen at −12°C.

SPE was performed in duplicate as described by Dinelli et al. [13]. Briefly, 200 ml of sodium hydrogencarbonate solution (0.1 *M*, pH 7.8) was added to 100 g of soil (50 and 20 ppb fortifications) and to 200 g of soil for the 10 ppb level. The suspension was shaken for 1 h. The slurry was centrifuged at 13 100 *g* for 5 min. The extraction procedure was repeated twice and the liquid extracts were combined. The extracts were brought to pH 2.5 with 0.1 *M* HCl and passed, under vacuum, through a C_{18} solid-phase column. Dry residues of the 50 and 20 ppb samples were reconstituted with 1 ml of methanol–water (50:50, v/v) solution, thus obtaining an enrichment factor of 100. Dry residues of the 10 ppb samples were reconstituted with 1.5 ml of the methanol–water solution, thus obtaining an enrichment factor of 150.

2.5. CE analyses

Analyses of soil extracts were performed using the MECC technique, with the CE apparatus P/ACE System from Beckman (Palo Alto, CA, USA). Separations were made with a fused-silica capillary of 50 cm (from injection point to detector) × 75 μm I.D., thermostated at a constant temperature of 35°C. The applied voltage was 25 kV, with an injection pressure of $3.44 \cdot 10^3$ Pa for 10 s, corresponding to an injection volume of 60 nl. The electrolyte buffer, chosen according to the ORM optimization described in the Results and discussion section, was 30 m*M* sodium borate, 80 m*M* SDS, 14% methanol and 20% isopropanol (v/v), pH 7.0. The detection wavelength was set at 214 nm. The separation efficiency was measured by the number of theoretical plates (N) according to: $N = 5.54(t_R/w)^2$, where t_R is the retention time of a compound and w is the peak width at half-peak height [19]. Peak area was used for calibration curve and residue quantification of chlorsulfuron, metsulfuron and chlorimuron.

3. Results and discussion

3.1. ORM optimization

The values of R_s for the eleven pre-planned experiments (Table 1) were calculated and fitted in the polynomial equation, according to Li [17]. Resolution values for all possible combinations

of x_1 (SDS), x_2 (methanol) and x_3 (isopropanol) were calculated and represented on a three-dimensional resolution diagram, using a basic computer program. The region defining the optimum conditions for the separation of the three sulfonylureas was identified with $x_1 = 100\%$, corresponding to a concentration of SDS in the electrolyte buffer of 80 m*M*. The final overlapped resolution diagram is presented in Fig. 1. Two different zones with high response ($R_s > 1.5$) can be seen. The first partial optimum is placed at low methanol content, whereas the second is placed at low isopropanol content. It is important to note that buffers of differing compositions were able to separate the three sulfonylureas with a $R_s > 1.5$. Arbitrarily, the electrolyte buffer marked by "A" in the optimum zone at low methanol content (Fig. 1) was chosen for the subsequent analysis of soil samples, as described in the Experimental section. An electropherogram obtained with this electrolyte buffer is shown in Fig. 2a. A complete separation of the sulfonylurea peaks was achieved, with an observed R_s of 1.99 against a predicted R_s of 1.73, calculated from the polynomial equation. The column mean efficiency in the separation of the three sulfonylureas was 152 000 theoretical plates, while the asymmetry was 0.89, 0.90 and 0.78 for metsulfuron, chlorimuron and chlorsulfuron peaks, respectively. The composition of the electrolyte buffer marked by "B" in Fig. 1 was chosen from a region expected to produce poor separation. The results (Fig. 2b) confirmed the validity of the ORM scheme, as suggested by the low value of the observed R_s (0.45), against a predicted value of 0.37.

3.2. Calibration curves and sulfonylurea separation

The calibration curves demonstrate the linear response of the method with the injection of standard with concentration in the 0.6–10 ppm range. The chlorsulfuron regression equation is $y = -0.013 + 0.103x$ ($r^2 = 0.997$), that for metsulfuron $y = -0.013 + 0.171x$ ($r^2 = 0.986$) and that for chlorimuron $y = 0.008 + 0.065x$ ($r^2 = 0.993$), where y is the peak area and x is the herbicide concentration in ppm. Detection limits were calculated for a signal-to-noise ratio of 3 and were 0.63 ppm for chlorimuron and 0.5 ppm for metsulfuron and chlorsulfuron, corresponding to an injection on-column of about 10 pg of each sulfonylurea. The values of intra-day and inter-day retention times and of their relative standard deviations (R.S.D.s) for the runs made 10 times on the same day in 10 different

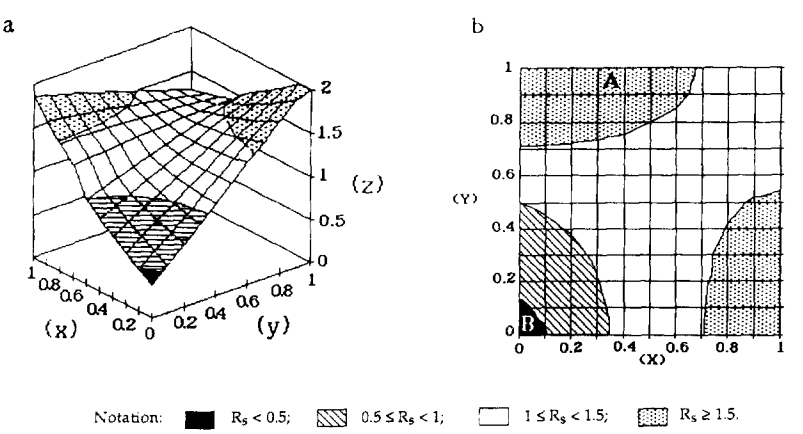

Fig. 1. Final overlapped diagrams for the separation of sulfonylureas with 80 m*M* SDS. (a) Three-dimensional view; the x-axis is the percentage proportion of methanol (1 = 40% methanol) in the electrolyte buffer; the y-axis is the percentage proportion of isopropanol (1 = 20% isopropanol) in the electrolyte buffer; the z-axis represents the resolution (R_s). (b) Two-dimensional projection; axes as in (a).

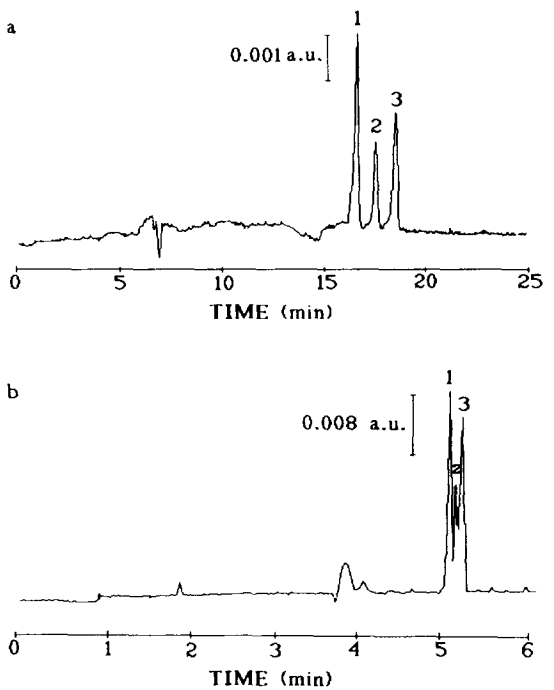

Fig. 2. Separation of sulfonylureas by CE. (a) Conditions corresponding to point "A" in Fig. 1b: 30 m*M* borate buffer pH 7; 80 m*M* SDS, 14% methanol, 20% isopropanol. Separation voltage 25 kV, pressure injection for 10 s, capillary 50 cm × 75 μm I.D., thermostated at 35°C, detection wavelenght 214 nm. (b) Conditions corresponding to point "B" in Fig. 1b: 30 m*M* borate buffer pH 7, 80 m*M* SDS, 0% methanol, 0% isopropanol. Separation parameters as in (a). Peaks: 1 = metsulfuron; 2 = chlorimuron; 3 = chlorsulfuron.

days (Table 2) indicate good repeatability. The area mean values of the peaks corresponding to injections of 3.5 ppm sulfonylurea solution and their respective R.S.D.s (Table 3) suggest good quantitative accuracy for CE.

3.3. Sulfonylurea recovery and quantitation

The electropherogram presented in Fig. 3a provides a typical profile of the soil without herbicides and shows no interferences at the retention times for the three compounds of interest. Fig. 3b displays the electropherogram of a soil sample spiked at 20 ppb with the three sulfonylureas, after SPE and a 100-fold enrichment. The identification of the sulfonylurea peaks was based on the retention time. To confirm the identification, single subsequent fortifications of the soil extract were made with each sulfonylurea at 5 ppm and reanalyzed (data not shown). The electropherogram of the soil extract after the three further fortifications corroborates the identification of peaks 1, 2 and 3 as metsulfuron, chlorimuron and chlorsulfuron, respectively (Fig. 3c). It is interesting to note that there was a surprisingly low interference caused by other constituents present in the soil samples. In fact, the peak area of the three sulfonylureas represent about the 30% of the total area of the electropherogram (Fig. 3b). In a previous research [18], samples of the same soil containing four sulfonylureas at 10 ppb were extracted by SPE and analyzed by RP-HPLC. The percent area of the sulfonylureas was about the 5% of the

Table 2
Intra-day and inter-day retention time (t_R) repeatibility (±S.D.) and R.S.D.

	Intra-day		Inter-day	
	t_R (min)	R.S.D. (%)	t_R (min)	R.S.D. (%)
Metsulfuron	16.7 ± 0.3	1.9	16.8 ± 0.4	2.5
Chlorimuron	17.5 ± 0.4	2.4	17.8 ± 0.7	4.0
Chlorsulfuron	18.2 ± 0.4	2.1	18.5 ± 0.7	3.8

Average of 10 injections of 3.5 ppm sulfonylurea solutions in the same day (intra-day), in 10 different days over one month.

Table 3
Intra-day and inter-day peak area (arbitrary units, a.u.) repeatibility (±S.D.) and R.S.D.

	Intra-day		Inter-day	
	Area (a.u.)	R.S.D. (%)	Area (a.u.)	R.S.D. (%)
Metsulfuron	0.70 ± 0.01	1.6	0.71 ± 0.04	5.0
Chlorimuron	0.19 ± 0.00	2.4	0.19 ± 0.01	6.8
Chlorsulfuron	0.37 ± 0.02	4.9	0.37 ± 0.03	7.3

Average of 10 injections of 3.5 ppm sulfonylurea solutions in the same day (intra-day), in 10 different days over one month.

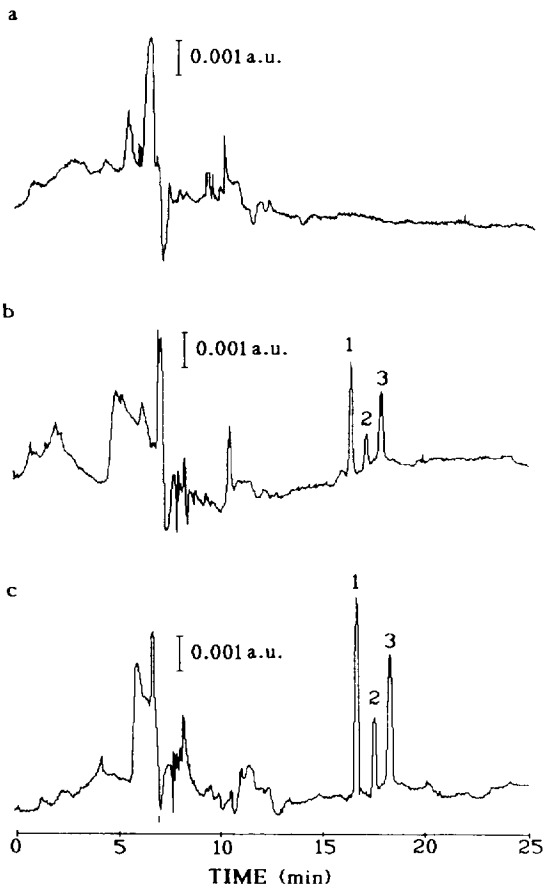

Fig. 3. Electropherograms of (a) soil sample extract without fortification; (b) soil sample extract fortified at 20 ppb; (c) soil sample extract fortified at 20 ppb with an additional fortification of each sulfonylurea at 5 ppm. Separation conditions as in Fig. 1a.

total area of the chromatogram. This suggests an on column exclusion of some soil matrix interferences during the CE separation, as shown also by Dinelli et al. [20].

The recoveries of the sulfonylureas after duplicate SPE are reported in Table 4. The mean overall recovery for the three herbicides was 95.4 ± 16.1% and the recovery was not affected by the sample concentration in the range from 10 to 50 ppb. These results confirm that SPE does not alter physicochemical traits of the three sulfonylureas and is a valid method for multiresidue analysis of sulfonylureas in CE.

Table 4
Sulfonylurea recoveries (±S.D.) after duplicate SPE from soil samples at different herbicide concentrations

	Recovery (%)		
	10 ppb	20 ppb	50 ppb
Metsulfuron	101.9 ± 16.1	91.4 ± 1.1	91.4 ± 5.7
Chlorimuron	83.0 ± 0.6	81.9 ± 3.5	82.9 ± 2.6
Chlorsulfuron	130.0 ± 6.0	104.9 ± 8.7	91.7 ± 7.9

4. Conclusions

The reported data show that CE is suitable for multiresidue detection and quantitation of sulfonylureas at the ppb level in soil. The major drawback of CE is the low system loadability, which is in the range of nanoliters. However, this drawback has been overcome by an appropriate SPE sample enrichment and an effective tuning of the separation conditions, using an ORM scheme. For soil samples with concentrations of sulfonylureas less than 10 ppb, the concentration became a limiting factor. At these concentrations, other chromatographic techniques, such as HPLC, are easier and more accurate than CE.

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