ELSEVIER

Journal of Chromatography B, 656 (1994) 275–280

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Separation and detection of herbicides in water by micellar electrokinetic capillary chromatography

Giovanni Dinelli[a,*], Alessandra Bonetti[a], Pietro Catizone[a], Guido C. Galletti[b]

[a]*Institute of Agronomy, University of Bologna, Via Filippo Re 6/8, 40126 Bologna, Italy*
[b]*Institute of Microbiology, Agricoltural and Forestry Technology, University of Reggio Calabria, P.zza San Francesco 4, 89061 Gallina (RC), Italy*

Abstract

The herbicides linuron, metolachlor, atrazine and metsulfuron were analysed using micellar electrokinetic capillary chromatography (MECC) after a 1000-fold concentration step by solid-phase extraction (SPE). Recoveries ranged from 80 to 92%, depending on the concentration and the number of active substances in the sample. Furthermore, the hydrolysis products of metsulfuron were analysed by MECC and by gas chromatography–mass spectrometry (GC–MS). Representative MECC and GC–MS profiles are shown and the structures of the hydrolysis products are proposed on the basis of their chromatographic and mass spectra features. A tentative pathway for the degradation of metsulfuron is proposed.

1. Introduction

Capillary electrophoresis (CE) is a very efficient separation technique with a high resolution power. The development of CE in the 1980s made the technique complementary to and, in some instances, an even better substitute for HPLC [1]. The most remarkable feature of CE is its versatility. As reported in the literature, a broad range of structurally similar compounds or complex pharmaceutical samples can be separated by CE [2]. Micellar electrokinetic capillary chromatography (MECC) was introduced by Terabe *et al.* [3], and is based primarily on partitioning phenomena rather than electrophoretic effects, thus improving the overall performance of CE and extending its applications.

Despite its versatility, the major drawback of CE is the very low injectable volume. Usually, the capillary dimensions are between 20 and 100 μm I.D. and 20–100 cm long, resulting in a total column volume of only a few microliters. Consequently, the loadability of the system is limited to an injection volume of 1–60 nl [4]. Although impressive CE detection limits in the subattomole range have been reported [5,6], the corresponding measurable sample concentrations are still too high (mg/l) to allow trace-level determination of *e.g.* drugs in plasma or residue analysis in water and soil [4]. Thus preconcentration is an essential step in trace analysis. Because of its poor sample capacity, the only applications of CE in herbicide studies today are the detection of glyphosate in animal serum [7] and sulphonylurea herbicides in water [8].

The aim of this paper was to verify the

* Corresponding author.

0378-4347/94/$07.00
SSDI 0378-4347(94)00058-D

viability of the preconcentration step and subsequent CE determination in herbicide studies, using MECC. Our attention was focused on two features of herbicide studies: (a) multiresidue determination at the μg/l level of linuron [3-(3, 4 - dichlorophenyl)-1-methoxy-1-methylurea], atrazine (2-chloro-4-ethylamino-6-isopropilamino-*s*-triazine), metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide] and metsulfuron (methoxycarbonyl-2-N-[(methoxy-4-methyl-6-triazine-1, 3,5-yl-2) aminocarbonyl] benzene sulfonamide) in tap water, and (b) detection and separation of the breakdown products of metsulfuron by CE and their structural identification by gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Reagents

Reagents for CE analysis were supplied by Sigma (St. Louis, MO, USA). All solvents used in the extraction procedure, supplied by Bakerbond (Phillisburg, NJ, USA), were pesticide-free grade. The herbicides under study (metsulfuron, metolachlor, linuron and atrazine) were purchased from Lab Service (Bologna, Italy) at certified purities of 99%. Solid-phase extraction columns for sample concentration were reversed-phase C_{18} (500 mg) supplied by Bakerbond.

2.2. Residue determination

Fortified samples were prepared by dissolving pure standards in separate flasks. Appropriate amounts of these stock solutions were added to tap-water samples to produce the desired final concentrations. One-liter samples, containing the single herbicide (monoresidue determination samples) and the pool of the four herbicides (multiresidue determination samples), were prepared. The final concentrations tested either for mono- and multiresidue determinations were 1, 10 and 50 μg/l. For each concentration and for mono- and multiresidue determinations three replicates were prepared. Drinking water from the Municipal Water System of Bologna was used for sample fortification. Before use, the water was tested for the absence of the active compounds employed.

Extraction of the active ingredient (a.i.) from the aqueous solutions by the C_{18} SPE columns was performed according to Junk and Richard [9]. The 1 μg/l samples were concentrated 1000-fold, while the 10 and 50 μg/l samples were concentrated 100-fold.

2.3. Identification of metabolites

Three 1-l bi-distilled water samples, containing 50 mg/l of metsulfuron were buffered to pH 4.0 with 0.1 *M* HCl . The samples were kept at 45°C for four days to obtain complete degradation of the a.i. by hydrolysis, as reported by Sabadie [10] and Sabadie and Bastide [11].

2.4. Basic extraction

In order to obtain the extraction of the breakdown products, the aqueous solutions, containing the metabolites of metsulfuron, were brought to pH 9.0 with 1.0 *M* NaOH. The samples were extracted with methylene chloride (3×50 ml). The combined organic extracts were dried over anhydrous sodium sulphate. The solvent was evaporated under a stream of nitrogen at a temperature of 50°C. The residue was dissolved in 2 ml of methanol for CE and GC–MS analysis.

2.5. Acid extraction

The aqueous phase of the "basic extraction" was collected and adjusted to pH 2.0 with HCl. The sample was extracted with methylene chloride and treated as described above, to obtain a residue which was dissolved in 2 ml of methanol for CE and GC–MS analysis.

2.6. CE analysis

Separation of herbicides and metabolites was performed, using the MECC technique, with the CE apparatus P/ACE System 2000 from Beck-

man (Palo Alto, CA, USA). Separations were made with a fused-silica capillary 50 cm long (from injection point to detector), 75 μm I.D., at a constant temperature of 25°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 detection wavelength was set at 214 nm. The electrolyte buffer for residue determination was 50 m*M* sodium borate–35 m*M* sodium dodecyl sulphate (SDS)–10% methanol (v/v), pH 8.0. The electrolyte buffer for metabolite studies was 50 m*M* sodium borate–22 m*M* SDS–10% methanol (v/v), pH 8.0. The separation efficiency was measured by the number of theoretical plates (N) according to the formula: $N = 5.54\ (t_R/w)^2$, where t_R is the retention time of a compound and w is the peak width at half-peak height [12].

2.7. GC–MS analysis

The GC–MS system was a Varian (Walnut Creek, CA, USA) 3400 gas chromatograph equipped with a Supelco (Bellefonte, PA, USA) SPB-5 column (30 m × 0.32 mm I.D., film thickness 0.25 μm) heated from 50 to 300°C at 10°/min. The injector was at 220°C and the injection mode was split (ratio *ca.* 1/100). The gas chromatograph was coupled to a mass spectrometer Finnigan (San Jose, CA, USA) MAT with an ion-trap detector (ITD) Model 800 through a transfer line heated to 220°C. Mass spectra were recorded in the electron-impact mode at 70 eV.

3. Results and discussion

3.1. Residue determination

The separation of the four standard active ingredients (a.i.s) by MECC is shown in Fig. 1a). The electropherogram shows a good separation and a column efficiency with a plate number of 136 500. The calibration curves used for quantitation of the four a.i.s were linear in the range of sample concentrations between 0.5 and 10 mg/l. The regression equation was for metsulfuron $y =$

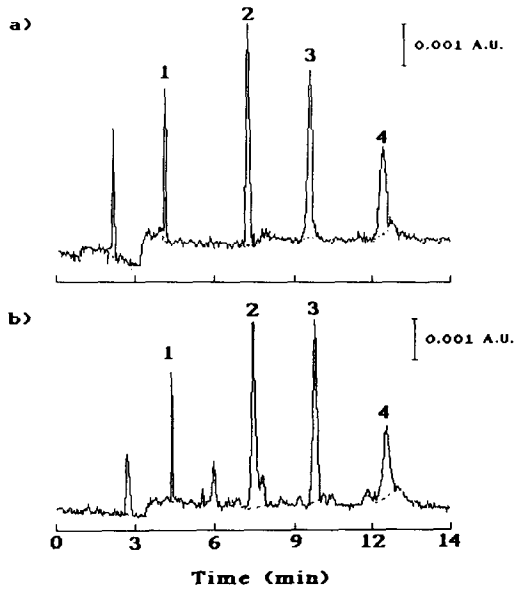

Fig. 1. (a) CE electropherogram of four standard herbicides: (1) metsulfuron, (2) atrazine, (3) linuron, and (4) metolachlor, at a concentration of 1 mg/ml. (b) CE electropherogram of the four herbicides extracted by means of a C_{18} SPE column at 1 μg/l and concentrated 1000-fold. Elution order as in (a). Capillary: 50 cm × 75 μm I.D.; operating voltage: 25 kV at 25°C; UV detection: 214 nm; separation buffer: 50 m*M* sodium borate–35 m*M* SDS–10% methanol (v/v), pH 8.0.

$(5.9 \cdot 10^{-3}) + (2.3 \cdot 10^{-2})x$, $(r^2 = 0.98)$, for atrazine $y = (6.3 \cdot 10^{-2}) + (9.1 \cdot 10^{-2})x$, $(r^2 = 0.97)$, for linuron $y = (4.6 \cdot 10^{-3}) + (8.4 \cdot 10^{-2})x$, $(r^2 = 0.96)$, and for metolachlor $y = (2.4 \cdot 10^{-2}) + (6.1 \cdot 10^{-2})x$, $(r^2 = 0.96)$, where y is the peak area and x is the herbicide concentration in mg/l. The minimum detectable concentration for the herbicides was 0.5 mg/l.

The herbicide extraction and concentration procedure tested permitted the detection of the four a.i. at the μg/l level. The electropherogram of a 1000-fold concentrated sample with a concentration of 1 μg/l (Fig. 2a) showed the efficiency of multiresidue analysis by CE. Furthermore, SPE sample preconcentration did not alter the efficiency of the system, the mean column efficiency being 132 500 plates. Herbicide recoveries as determined by CE are reported in Table 1. The average overall recovery for the four herbicides, in the concentration range of 1–50 μg/l was 87.2 ± 11.6%. The mean recovery

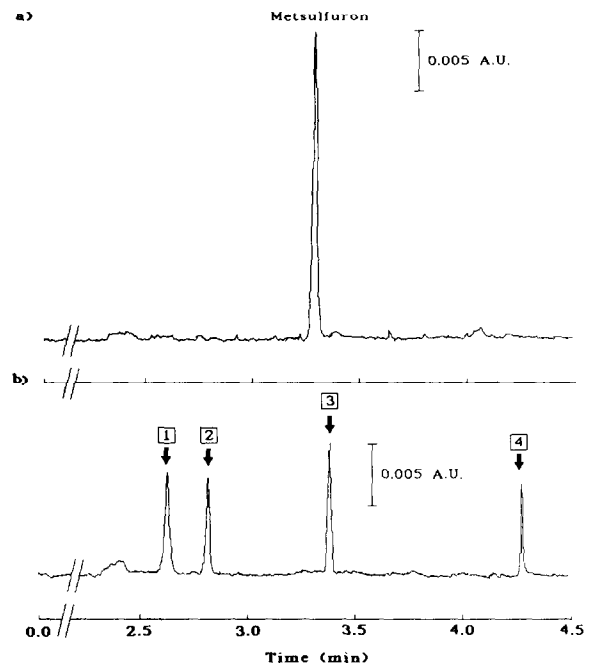

Fig. 2. Metsulfuron degraded in aqueous solution at pH 4.0 at 45°C analysed by CE. (a) Day 0, and (b) day 4 of degradation. Separation conditions as in Fig. 1. Separation buffer: 50 m*M* sodium borate–22 m*M* SDS–10% methanol (v/v), pH 8.0.

of the herbicides was not affected by the sample concentration in the 1–50 μg/l range, except for metsulfuron at a concentration of 50 μg/l, which showed a very low recovery. In order to explain the low recovery of metsulfuron at 50 μg/l in the multiresidue determination, monoresidue determination of the four herbicides was performed. The results (Table 1) showed that the average overall recovery of the four herbicides over the range 1–50 μg/l, was 90 ± 4.8%.

Metsulfuron was recovered in high yields when analysed separately. This observation suggested that a multiresidue determination of a sample containing the four herbicides in a concentration of 50 μg/l each (*i.e.* a total a.i. concentration of 200 μg/l) may overload the SPE column, thus resulting in a decreased recovery of the more polar compound, *i.e.* metsulfuron. In contrast, for the more hydrophobic a.i.s linuron, atrazine and metolachlor herbicides this problem did not occur.

3.2. Identification of metabolites

The pattern of metsulfuron degradation in water is shown in Fig. 2. After four days of exposition to pH 4.0 at 45°C, the a.i. was completely chemically degraded. This result is in perfect agreement with those of Sabadie [10] and Sabadie and Bastide [11], who reported a half-life for metsulfuron in water of 5 h (pH 4.0 and 45°C). Peaks marked by arrows (Fig. 2b) repre-

Table 1
Recovery of variable amounts of metsulfuron, atrazine, linuron and metolachlor from fortified water samples[a]

Fortification (μg/l)	Average recovery (%)			
	Metsulfuron	Atrazine	Linuron	Metolachlor
Multiresidue determination				
1	95 ± 4.6	91 ± 6.6	86 ± 6.0	87 ± 4.5
10	92 ± 5.6	90 ± 3.2	87 ± 5.8	90 ± 4.8
50	54 ± 8.8	92 ± 3.3	90 ± 4.2	93 ± 3.2
Overall[b]	80.3 ± 19.7	91 ± 4.7	87.6 ± 5.6	90 ± 4.8
Monoresidue determination				
1	93 ± 2.2	89 ± 6.0	90 ± 3.0	89 ± 4.8
10	90 ± 2.4	93 ± 5.4	88 ± 3.0	90 ± 3.2
50	90 ± 5.1	94 ± 6.1	86 ± 3.6	88 ± 4.2
Overall[b]	91 ± 4.1	92 ± 6.8	88 ± 4.9	89 ± 4.6

[a]One-liter samples; 500 mg C_{18} adsorbent and 2 ml ethyl acetate eluting solvent were used. Reconstitution was with methanol.
[b]There was no statistical difference among these means ($P < 0.05$).

sent degradation products, formed during the hydrolysis process.

The basic and the acid extracts were analysed by CE in order to verify their contents (Fig. 3). The basic extract contained mainly compound 1 (89%) and compound 4 (11%) (Fig. 3a). The acid extract contained the breakdown compounds 2, 3, and 4 (18%, 52% and 30% relative amounts, respectively) (Fig. 3b).

The total-ion chromatograms (TIC) of the basic and acid extracts are shown in Fig. 4, which also shows the structures of the metsulfuron degradation products. Peaks were identified on the basis of their mass spectra and chromatographic retention times [13]. Retention times and the five most abundant ions of the metsulfuron breakdown products are reported in Table 2.

GC–MS identification and relative percentages were consistent with those found in the literature [13]. However, no evidence of breakdown product 3 was found. Such a compound was tentatively identified with the structure reported in Fig. 5. Such a structure would be consistent with chemical degradation data [10,11] and CE properties

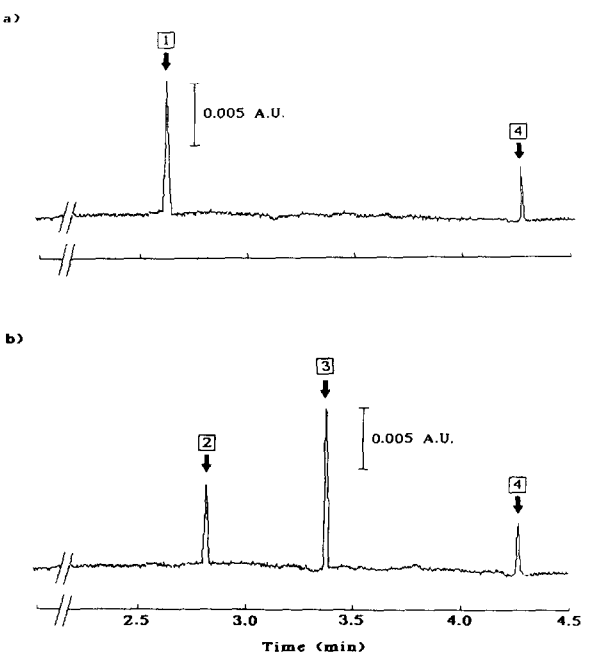

Fig. 3. CE separation of (a) the basic extract, and (b) the acid extract. Separation conditions as in Fig. 2.

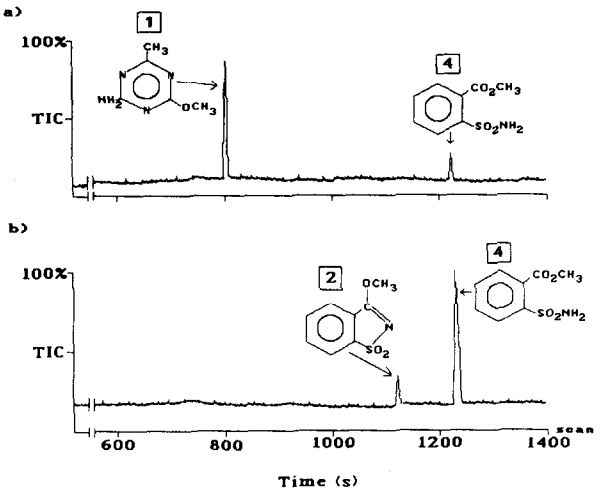

Fig. 4. Total-ion chromatogram of metsulfuron breakdown products. (a) Basic extract, and (b) acid extract. Supelco SPB-5 column 30 m × 0.32 mm I.D., heated from 50 to 300°C at 10°/min. Split injection at 220°C.

and would be difficult to determine by GC–MS due to its high polarity.

On the basis of the CE and GC–MS data on the metsulfuron breakdown products, a hypothetical degradation scheme for metsulfuron in water was formulated (Fig. 5). Such a degradation pathway is in agreement with the litera-

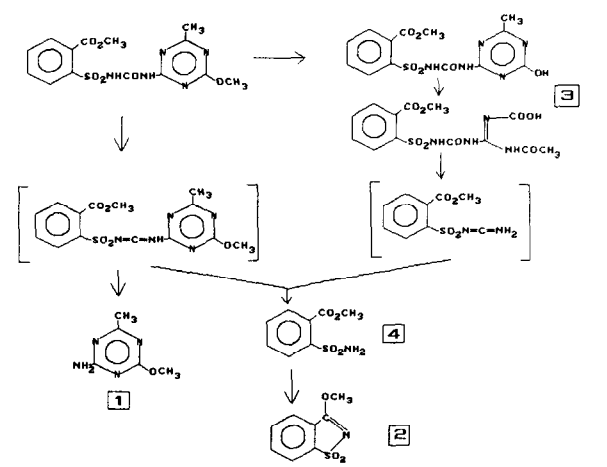

Fig. 5. Proposed degradation scheme of metsulfuron in water based on CE and GC–MS results.

Table 2
Five-peak mass spectra of metsulfuron breakdown products[a]

Compounds	t_R	M_w	m/z (%)				
1	13.34	140(34)	69(100)	42(82)	110(43)	140(34)	43(32)
2	18.71	197(7)	76(100)	50(85)	77(81)	104(62)	133(50)
3	20.57	215(0)	77(100)	105(71)	92(54)	79(49)	135(48)

[a]Numbers and retention time (t_R, min) refer to Fig. 3.

ture [10,11], except for compound 2 which was not previously reported as a hydrolytic metsulfuron degradation product.

4. Conclusion

The reported data show that CE is suitable for mono- and multiresidue analysis of herbicides in tap water at the μg/l level, using an appropriate concentration procedure. Thus far, the SPE method presented can be used for the analysis of drinking and tap water by CE. Further studies to verify the feasability of CE for the detection of herbicides in more complex matrices such as soil, drainage water and groundwater are necessary.

Moreover, CE is an important tool for other types of studies related to the environmental behaviour of herbicides, such as chemical degradation of metsulfuron in water. The potential of using both CE and GC–MS for the studies of herbicides has been demonstrated. The breakdown product analysis by CE and GC–MS permitted the postulation of a metsulfuron degradation pathway in water. However, further investigations are needed to positively identify the nature of some of the metsulfuron degradation products.

5. Acknowledgement

This work was financed by EEC within the project "Application and evaluation in agriculture of a new method for the monitoring of pesticide pollution in water", Contract No. AIR 3-ST92-002.

6. References

[1] H.J. Issaq, G.M. Janini, I.Z. Atamna and G.M. Muschik, *J. Liq. Chromatogr.*, 14 (1991) 817.
[2] W.G. Kuhr, *Anal. Chem.*, 62 (1990) 403R.
[3] S. Terabe, K. Otsuka, A. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
[4] D.S. Stegehuis, H. Irth, U.R. Tjaden and J. Van der Greef, *Anal. Chem.*, 538 (1991) 393.
[5] B.W. Wright, G.A. Ross and R.D. Smith, *J. Microcol. Sep.*, 1 (1989) 85.
[6] J. Lui, F. Banks, Jr. and M. Novotny, *J. Microcol. Sep.*, 1 (1989) 136.
[7] M. Tomita, T. Okuyama, Y. Nigo, B. Uno and S. Kawai, *J. Chromatogr.*, 571 (1991) 324.
[8] G. Dinelli, A. Vicari and P. Catizone, *J. Agric. Food Chem.*, 41(5) (1993) 742.
[9] G.A. Junk and J.J. Richard, *Anal. Chem.*, 60 (1988) 451.
[10] J. Sabadie, *Weed Res.*, 30 (1990) 413.
[11] J. Sabadie and J. Bastide, *Weed Res.*, 30 (1990) 1.
[12] S. Fujiwara, S. Iwase and S. Honda, *J. Chromatogr.*, 447 (1988) 133.
[13] G.C. Galletti, G. Chiavari, F.A. Mellon and K. Parsley, *J. Anal. Appl. Pyrolysis*, 21 (1991) 239.