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Separation of sulfonylurea metabolites in water by capillary electrophoresis

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Abstract

The potential of capillary electrophoresis (CE) for the separation and detection of the metabolites of nine sulfonylurea herbicides in aqueous solution was evaluated. A relationship between the structure of the sulfonylureas tested and the metabolites formed was found: the non-*o*-benzene-substituted sulfonylurea rimsulfuron gave only one metabolite, whereas the other eight, *o*-benzene-substituted, sulfonylureas gave 4–6 metabolites. CE was confirmed to be a very efficient separation technique, suitable for the determination of sulfonylurea herbicides and their metabolites formed during hydrolysis.

1. Introduction

Sulfonylurea herbicides are widely used to control weeds in agricultural crops such as wheat, maize, soybean, sugarbeet and rice. The first sulfonylurea, chlorsulfuron, was marketed in USA in 1982. Worldwide, nineteen sulfonylurea herbicides had been commercialized by 1994, and five more are being developed. This rapid increase is due to their activity at low application rates (2–60 g ha^{-1}) and their low mammalian toxicity [1].

In recent years, because of the increased herbicide degradation product analysis requirements for pesticide registration, considerable interest has been generated concerning the detection and separation of herbicide metabolites. The most important degradation pathways of sulfonylureas are chemical hydrolysis and microbial breakdown [2,3]. Although several experiments have shown that sulfonylureas degrade to many compounds in water and soil [4–6], not many methods for the separation of sulfonylurea metabolites have been described. Sabadie and Bastide [5,6] and Harvey et al. [7], using HPLC, separated several hydrolytic metabolites of chlorsulfuron, metsulfuron and sulfometuron. Shalaby et al. [8] described the use of LC–MS thermospray for nicosulfuron and rimsulfuron and a major metabolite of each in soil. However, little information is available on the detection and separation of metabolites formed during the hydrolysis of most sulfonylureas. Because hydrolysis is a major pathway of degradation of sulfonylureas, investigations on metabolites formed during hydrolysis should provide basic information on their general behaviour.

A simple and reproducible method was needed for the determination of sulfonylurea metabolites in water samples. In a previous paper [9], we reported the detection and separation of the hydrolytic breakdown products of metsulfuron by capillary electrophoresis (CE) and their structural identification by GC–MS.

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The objectives of this study were to confirm the potential of CE for the detection and separation of the breakdown products of nine sulfonylureas in aqueous solution and to evaluate the dynamics of metabolite formation for each sulfonylurea.

2. Experimental

2.1. Reagents

Reagents for CE analysis were supplied by Sigma (St. Louis, MO, USA). All solvents used were of pesticide-free grade. The nine sulfonylureas studied were chlorsulfuron, metsulfuron, triasulfuron, ethametsulfuron, CGA 152'005, tribenuron, bensulfuron, chlorimuron and rimsulfuron. They were extracted from commercial products by Soxhlet extraction with freshly redistilled dichloromethane for 3 h. After drying with anhydrous sodium sulfate, dichloromethane was distilled off in a rotary evaporator. The residual sulfonylureas were subjected to nuclear NMR, IR and MS analyses to confirm their identity and were used in subsequent experiments without further purification, as reported by Galletti et al. [10].

2.2. Water samples fortification

The solutions were prepared using distilled water. Duplicate 50-ml water samples, each containing 25 mg l^{-1} of each sulfonylurea in 10 m*M* $NaHCO_3$, were buffered to pH 4 with 0.1 *M* HCl. The solutions were kept in the dark at 55°C in closed vials. Samples of 1 ml were taken from each vial at different times during a 10-h period and stored at −12°C until analysed.

2.3. Capillary electrophoresis

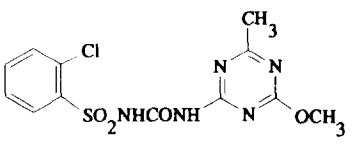
Aqueous samples were analysed directly by CE. Separation of herbicides and metabolites were performed using the micellar electrokinetic capillary chromatography with a P/ACE system from Beckman (Palo Alto, CA, USA). Separations were effected with a fused-silica capillary 50 cm long (from injection point 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 electrolyte buffer was 50 m*M* sodium borate–22 m*M* sodium dodecylsulfate–10% (v/v) methanol (pH 8.0). The separation efficiency was measured by the number of theoretical plates (N) according to the equation $N = 5.54\ (t_R/w)^2$, where t_R is the retention time of a compound and w is the peak width at half-height [11]. Peak area was used for residue quantification. The degradation rate of each sulfonylurea was determined by linear regression of the natural logarithm of percentage of parent herbicide remaining against time and the slope of each line was compared with analysis of variance.

3. Results and discussion

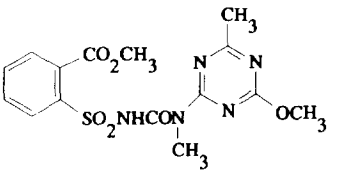
The structures and molecular masses of the sulfonylureas studied are reported in Fig. 1. Three moieties characterize the general structure: an aryl group, the sulfonylurea bridge and a nitrogen-containing heterocycle. Chlorsulfuron, metsulfuron, triasulfuron, ethametsulfuron, CGA 152'005 and tribenuron have a triazinic heterocycle group and are *o*-benzene-substituted; bensulfuron and chlorimuron have a diazinic heterocycle group and are *o*-benzene-substituted; and rimsulfuron has a diazinic heterocycle group but is not *o*-benzene-substituted.

The electropherograms of chlorsulfuron at three sampling times (0, 2 and 10 h after incubation) are shown in Fig. 2, as an example of sulfonylurea hydrolysis. Chlorsulfuron degradation in water led to the formation of six metabolites, numbered according to their increasing retention times. The electropherograms evidence the effectiveness of the separation, as suggested by the column efficiency, ranging between 50 000 and 163 000 theoretical plates.

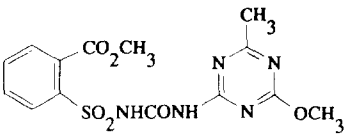
The retention times of each parent herbicide and their metabolites are shown in Table 1. The metabolites of each sulfonylurea were arbitrarily numbered according to their increasing retention



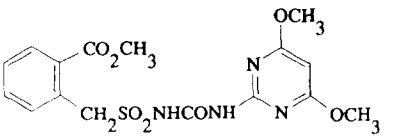
CHLORSULFURON M_r 357



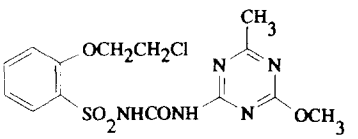
TRIBENURON M_r 395



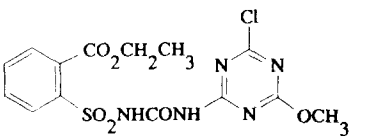
METSULFURON METHYL M_r 381



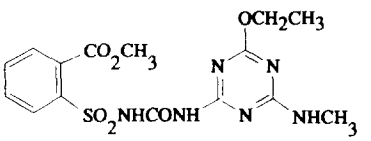
BENSULFURON M_r 410



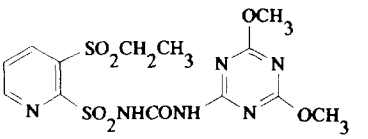
TRIASULFURON M_r 401



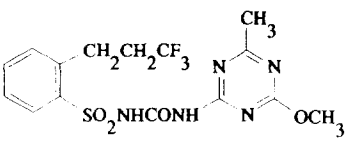
CHLORIMURON M_r 414



ETHAMETSULFURON M_r 410



RIMSULFURON M_r 431



CGA 152'005 M_r 419

Fig. 1. Structures and molecular mass of the sulfonylureas studied.

times. The number of metabolites formed during hydrolysis appeared to be related to the structure of the sulfonylurea. The sulfonylureas characterized by a triazinic heterocycle and *o*-benzene substitution decomposed in water, leading to at least six possible degradation products, with the exception of ethametsulfuron and tribenuron, which gave five metabolites. The concentration curves of the parent herbicides and their metabolites (Fig. 3) indicate that metabolites 2 and 4 of chlorsulfuron, metsulfuron, triasulfuron and CGA 152'005 appear with a delay of 1–3 h with respect to other metabolites, suggesting that they are secondary by-products of sulfonylurea hydrolysis. The same pattern was observed for the metabolite 3 of ethametsulfuron and metabolite 2 of tribenuron. Chlorimuron and bensulfuron, sulfonylureas characterized by a diazinic

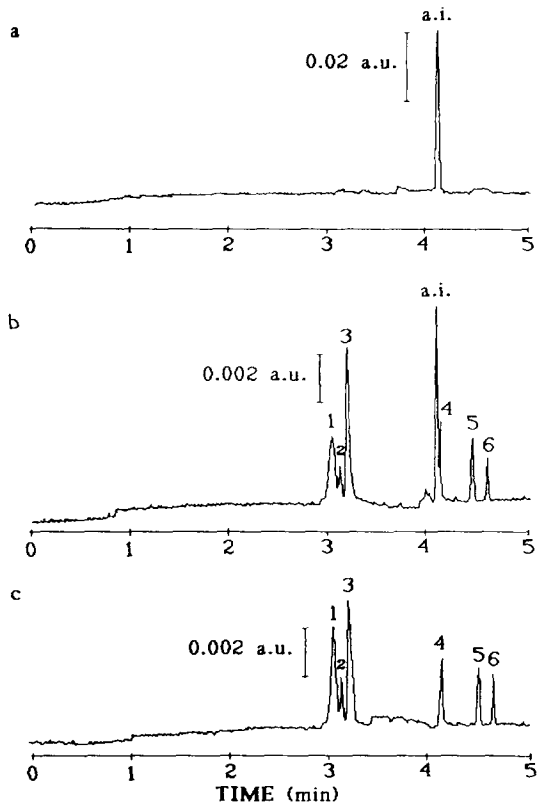


Fig. 2. Electropherograms of chlorsulfuron samples in aqueous solution. Samples in (a), (b) and (c) correspond to sampling times of 0, 2 and 10 h after incubation, respectively. The parent herbicide is indicated by a.i. and metabolites are arbitrarily numbered according to their increasing retention times. Separation conditions as reported under Experimental.

heterocycle and *o*-benzene substitution, degraded in water to form four metabolites (Fig. 3). The non-*o*-benzene-substituted sulfonylurea rimsulfuron gave only one metabolite (Fig. 3).

Hydrolysis of all the sulfonylureas followed first-order kinetics, as regression analysis of the natural logarithm of herbicide remaining yielded significant linear determination coefficients for all nine sulfonylureas (Table 2). Analysis of variance showed differences in the degradation rate constants (k) among the sulfonylureas. Tribenuron, the sulfonylurea most susceptible to hydrolysis, had a half-life of 0.37 h, whereas those for triasulfuron, ethametsulfuron and chlorimuron, the least susceptible, were >2 h. The seven times longer half-life of chlorimuron than tribenuron confirms the large differences in degradation rate within this class of herbicides, as observed in other work [1,12]. It is also clear that many sulfonylureas are extremely labile in aqueous solution and most of them undergo various transformation reactions to generate a complex mixture of decomposition products.

This study revealed that sulfonylureas and their degradation products may be simultaneously detected and separated by CE. The ease and efficiency of the method make it suitable for the analysis of large numbers of water samples for sulfonylurea metabolites and parent herbicides. However, the nature of the degradation products was not investigated in this work. Further studies are necessary to identify their structures and to

Table 1
Retention times (min ±S.D., $n = 16$) of sulfonylureas and their metabolites

Sulfonylurea	Parent herbicide	Metabolite No.					
		1	2	3	4	5	6
Chlorsulfuron	4.26 ± 0.11	3.19 ± 0.09	3.32 ± 0.08	3.55 ± 0.09	4.33 ± 0.10	4.62 ± 0.12	4.78 ± 0.11
Metsulfuron	4.45 ± 0.07	3.36 ± 0.06	3.50 ± 0.04	3.54 ± 0.06	4.50 ± 0.08	4.64 ± 0.09	4.92 ± 0.05
Triasulfuron	4.51 ± 0.09	3.36 ± 0.06	3.51 ± 0.03	3.61 ± 0.04	4.73 ± 0.06	5.01 ± 0.08	5.37 ± 0.06
CGA 512'005	4.57 ± 0.10	3.38 ± 0.06	3.55 ± 0.07	4.14 ± 0.09	4.67 ± 0.08	4.77 ± 0.11	4.95 ± 0.09
Ethametsulfuron	4.62 ± 0.05	3.26 ± 0.08	3.62 ± 0.03	4.70 ± 0.05	4.93 ± 0.06	5.20 ± 0.05	–
Tribenuron	4.31 ± 0.03	3.19 ± 0.02	3.39 ± 0.04	3.49 ± 0.05	4.68 ± 0.03	4.81 ± 0.04	–
Bensulfuron	4.39 ± 0.08	3.47 ± 0.09	3.63 ± 0.07	4.56 ± 0.08	4.82 ± 0.09	–	–
Chlorimuron	4.96 ± 0.05	3.99 ± 0.08	4.31 ± 0.02	5.53 ± 0.04	5.87 ± 0.03	–	–
Rimsulfuron	4.59 ± 0.03	6.10 ± 0.07	–	–	–	–	–

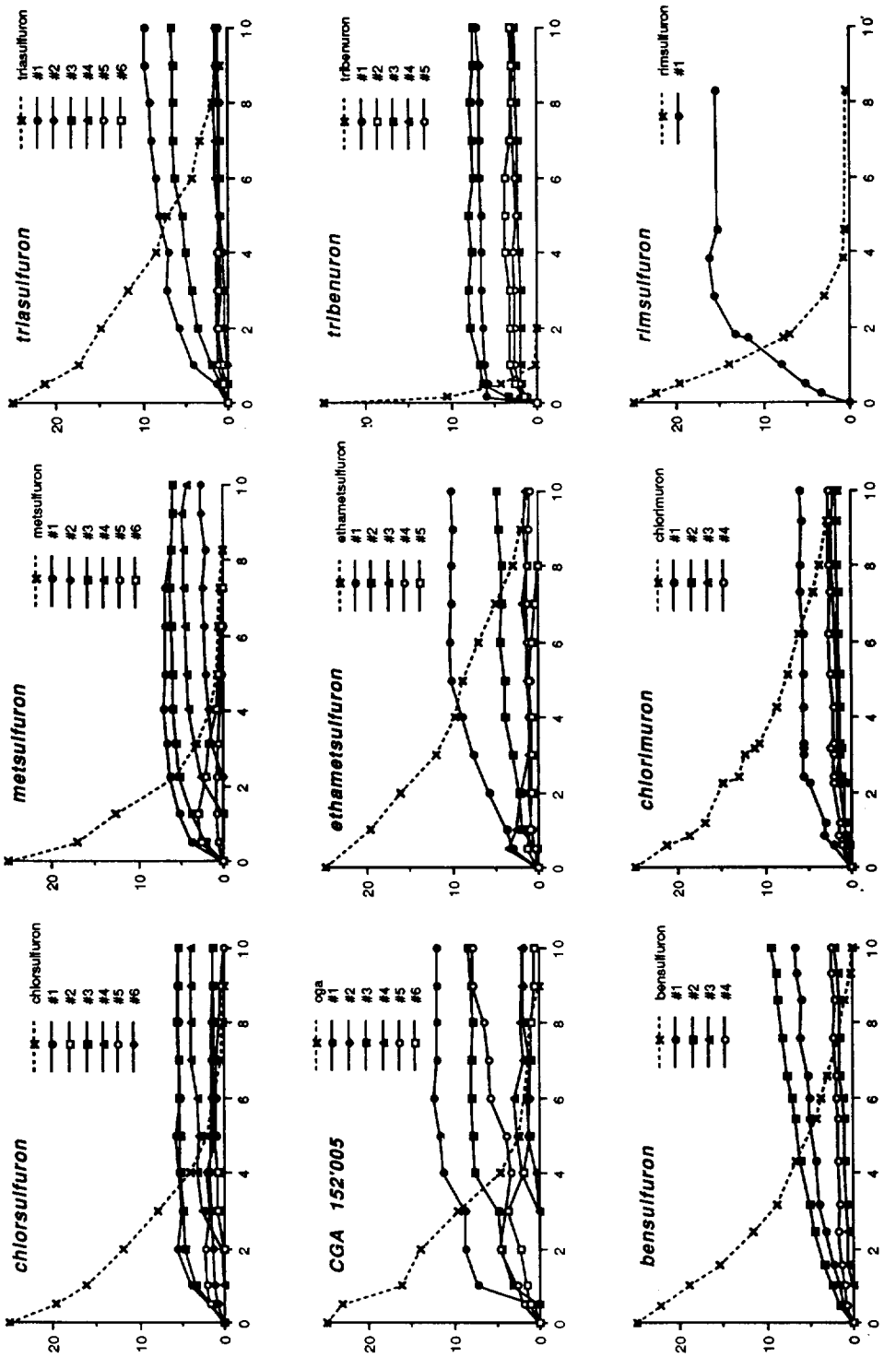


Fig. 3. Hydrolysis of the nine sulfonylureas and formation of degradation products.

Table 2
First-order rate constants (k), half-lives ($t_{1/2}$) and determination coefficients (r^2) for the sulfonylureas at pH 4 and 55°C in aqueous solution

Sulfonylurea	Rate constant[a], k	$t_{1/2}$ (h)	r^2 value[b]
Tribenuron	1.855 (a)	0.37	0.996
Rimsulfuron	0.602 (b)	1.15	0.962
Metsulfuron	0.540 (c)	1.28	0.988
CGA 152'005	0.498 (c)	1.39	0.991
Chlorsulfuron	0.463 (c)	1.50	0.993
Bensulfuron	0.356 (d)	1.95	0.975
Triasulfuron	0.299 (d)	2.32	0.987
Ethametsulfuron	0.275 (d)	2.52	0.984
Chlorimuron	0.259 (d)	2.68	0.988

[a] Means within the column followed by the same letter are not significantly different at $p \leq 0.05$ according to Fischer's protected LSD test.
[b] Linear determination coefficient describing the regression of the natural logarithm of herbicide remaining in aqueous solution over time. All values of r^2 are significant at $P \leq 0.01$.

define more accurately the degradation scheme of each sulfonylurea.

Acknowledgements

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