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Fast capillary electrophoresis–ion spray mass spectrometric determination of sulfonylureas

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ABSTRACT

This paper describes the use of a simple, laboratory-made capillary electrophoresis (CE) system equipped with relatively short (25–35 cm) capillaries that may be coupled to a pneumatically assisted electrospray (ion spray) interface for separations with on-line mass spectrometric (MS) detection. Sulfonylurea crop protection chemicals were used as model compounds to demonstrate the utility of the CE–MS system described. By using 35-cm fused-silica capillaries the CE–MS determination of an eight-component mixture of these compounds was accomplished within 5 min. In addition, on-line CE–MS under tandem mass spectrometric conditions (CE–MS–MS) was shown to give full-scan collision-induced mass spectra from 30-pmol levels of these compounds.

INTRODUCTION

Sulfonylurea crop protection chemicals play an important role in today's agricultural arena [1]. These compounds have use rates substantially less than those of previous herbicides and are rapidly and efficiently metabolized in plants, animals and soil [2]. Because of their polarity and chemical instability, their analytical determination is particularly challenging. They are very sensitive to thermal degradation caused by the elevated temperatures common to capillary gas chromatography (GC)–mass spectrometry (MS) and they cannot be analyzed intact by this technique. Liquid chromatography (LC)–thermospray MS typically does not give significant molecular ion abundances for the sulfonylurea compounds when the vaporizer temperature is optimized for maximum sensitivity. However, characteristic fragment ions may be obtained which can be monitored for the trace determination of sulfonylureas and their polar metabolites [3].

Recent developments in mild ionization conditions amenable to LC–MS include continuous-flow fast atom bombardment (CF-FAB) [4] and electrospray [5]. These techniques do not expose the analyte to excessive heat during either the separation or ionization process, and provide very mild ionization conditions that ensure molecular weight determination. It should be noted that FAB ionization usually produces some fragmentation along with molecular weight determination while the electrospray process produces essentially no fragmentation with the formation of abundant singly or multiply charged ions indicative of the molecular weight of the compound.

A recent report has described the packed capillary LC–MS determination of sulfonylureas using the CF-FAB technique [6]. Low nanogram levels of these compounds and their metabolites isolated from environmental samples could be identified. Analysis times were typically 30 min, and some use-

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ful fragment ions were observed that facilitated metabolite characterization studies of these compounds. These and related CF-FAB reports continue to demonstrate new advances in the utility of FAB-MS.

It is our view [7], however, that the alternative electrospray technique provides an easier, more analytically rugged approach to analytical problem solving via on-line separation using chromatography or electrophoresis [8–14]. When electrospray is coupled with on-line separation techniques such as high-performance and capillary electrophoresis (CE), its utility appears to be increased by assisting the "spray" aspect of the process by pneumatic assistance [15] or a coaxial sheath-flow of organic solvent such as 2-propanol or 2-methoxyethanol [16]. We prefer the former approach and have demonstrated the utility of the ion spray device coupled with a variety of modern separation techniques [7].

Following the lead of Reiser and co-workers' LC–CF-FAB-MS studies of sulfonylureas [2,3,6], we have investigated the analytical utility of open-tubular CE–MS via the liquid-junction–ion spray interface combination [11] for the determination of sulfonylurea compounds. Although CE–MS does not at present appear well suited for trace analysis studies, the speed of analysis, mild analysis and ionization conditions and the structural information available via CE–MS combined with tandem mass spectrometry (CE–MS–MS) suggest that this approach has come worthwhile analytical merits. In this paper we show that a mixture of eight important sulfonylurea compounds may be separated and identified within 5 min by on-line CE–MS with detection limits down to at least 600 fmol of injected components in the selected ion monitoring (SIM) mode of detection. Structural information is also available via tandem MS from on-line CE–MS–MS product ion scans of the $[M+H]^+$ precursor ions.

EXPERIMENTAL

Materials

The reference sulfonylurea compounds were kindly provided by R. W. Reiser of DuPont (Wilmington, DE, USA). All buffer and sample solutions were prepared fresh and filtered through nylon filter units of 0.2-μm pore size (Schleicher & Schuell, Keen, NH, USA). The buffer solutions were vacuum degassed by suction filtration and the samples were kept at 4°C when not in use. Optima-grade acetonitrile and methanol were obtained from Fisher Scientific (Fairlawn, NJ, USA). Water was purified in-house with a Barnstead Nanopure system; all other reagents were of electrophoresis grade and used without further purification.

Capillary electrophoresis instrumentation

The fused-silica capillary columns used in this work were made from polyimide-clad fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA). They were 75 μm I.D. and ranged in length from 1 m to 35 cm. No external cooling or temperature control of the capillary column was utilized.

The commercial CE system used during the early stages of this work was a P/ACE 2000 (Beckman, Palo Alto, CA, USA). This system was modified for CE–MS operation as described previously [12]. This included on-line UV detection 20 cm from the inlet of the separation capillary with the standard P/ACE 2000 UV detector [12,13], and passage of the capillary through the wall of the capillary cartridge holder with extension to the liquid junction coupling and ion spray interface [11,14].

The other CE system used in this work was constructed in-house from readily available materials. The system design goals included (a) small CE instrument "foot print", (b) pressure injection capability and (c) robotic operation of the capillary inlet for sample loading and conditioning of the inside capillary surface, and utilization of 35-cm separation capillaries. The simple CE system constructed for this work is shown in Fig. 1. It consists of a plastic desiccator cabinet equipped with a rubber o-ring seal between the lid and the cabinet (Item H42053-0000; Bel-Art Products, Pequannock, NJ, USA), a security switch (J) that shuts off the high-voltage power supply if the lid is opened while the high voltage is on (A), a gas inlet fitting, for pressurizing the device with nitrogen (0.5 p.s.i) for pressure injection during free solution CE techniques, and a remote battery-operated (C) sample vial–running buffer vial lifting and switching mechanism for transferring the capillary inlet from running buffer to the sample vial. The latter was obtained from the "dump" and "steering" mechanisms of a toy dump truck (Model 60-2309, Radio Shack, Fort Worth, USA). After timed pressure sample injection, the

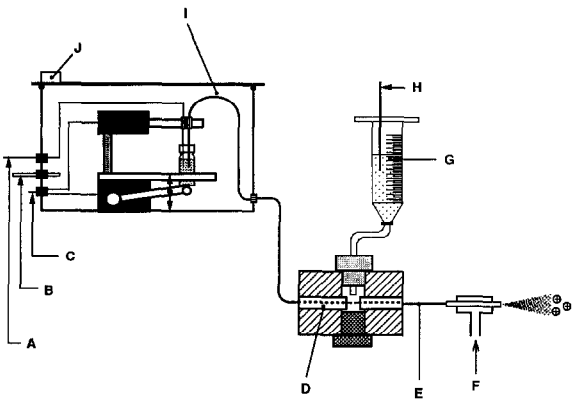

Fig. 1. Capillary electrophoresis–liquid junction–ion spray device. A = Capillary electrophoresis high-voltage supply lead running to anode buffer vial; B = pressurization inlet for pressurized injection and flushing of capillary column; C = battery-powered supply to activate lifting actuator mechanism for sample vial–running buffer vial placement of capillary inlet; D = exit end of capillary electrophoresis column placed in the liquid junction device; careful alignment of a 10-μm grap is required between the capillary exit and the corresponding 75 μm *i.d.* capillary transfer line leading to the ion spray interface; E = 6 cm × 75 μm I.D. transfer capillary connecting the liquid junction and ion spray devices; F = nitrogen inlet of ion spray interface; G = liquid junction reservoir; H = high-voltage lead for ion spray interface (2.5–4.0 kV); I = capillary electrophoresis column, typically 35–50 cm × 75 μm I.D.; J = safety switch that opens the capillary electrophoresis high-voltage supply when the pressurized box device is opened.

capillary inlet was moved to the running buffer by battery-powered remote control. A positive high-voltage potential (A) (30–38 kV or 300–1000 V/cm) was then applied across the capillary column to commence the CE separation. The capillary column exit could either be connected directly to a modified Waters Model 440 micro-UV detector (Waters Chromatography Division, Milford, MA, USA), or to the liquid junction coupling–ion spray devices (D–H) for mass spectrometric detection. The UV detector was not used in series with MS detection in order to minimize the effective size of the combined CE–MS system.

The positive polarity high voltage for CE was supplied by a Model RHR60P30 high-voltage reversible polarity 60-kV power supply (Spellman, Plainview, NY, USA). The capillary column (I) passes through an adaptor fitting installed in the wall of the plastic dessiccator cabinet and the capillary inlet immersed into the running buffer vial while the exit is connected to the liquid junction (D) coupling (see below). The CE system is placed on a Lab-Jack to facilitate adjustment of the liquid level of the inlet buffer vial to the same height as the level of the make-up buffer in the liquid junction reservoir. This simple, inexpensive device provided a desirable feature not incorporated in our commercial CE system. This pertains to its reduced size so it can be placed close to the ion sampling region of the mass spectrometer. This allows the use of short (35–50-cm) capillaries for CE–MS which can provide a five fold decrease in CE–MS analysis times compared with separations using 100-cm capillaries.

Mass spectrometry

The mass spectrometer used in this work was a Sciex TAGA 6000E tandem triple quadrupole system upgraded to an API III (Sciex I., Thornhill, Ontario, Canada). The capillary end from the CE system was coupled to the atmospheric pressure sampling region of the mass spectrometer via a liquid junction coupling and a pneumatically assisted electrospray (ion spray) interface [14]. This interface has been described previously [11,14]. The only modification in this work was that the inner capillary in the ion spray interface consisted 5 cm × 75 μm I.D. polyimide-clad fused silica instead of stainless steel [14]. During CE–MS operation the liquid junction along with the ion spray interface may be floated at +3 kV and the mass spectrometer operated in the positive-ion detection mode. When the high voltage is maintained at 30–38 kV, the 3 kV potential maintained on the ion spray interface produces a potential difference across the separation capillary of 27–35 kV. Using this configuration, organic cations in solution are introduced into the gas phase at atmospheric pressure by the ion evaporation mechanism prevailing in the electrospray process [17].

Gas-phase ions generated from the ion spray interface via the ion evaporation mechanism are sampled into the mass spectrometer by a potential difference of about 2.5 kV set between the ion spray interface and the ion sample orifice. The sampling orifice is a 100-μm diameter hole in the end of a conical skimmer extended towards the atmosphere. To minimize solvent cluster formation, this system utilizes a curtain of ultrapure nitrogen applied to the atmospheric side of the skinner. For full-scan

CE–MS work, the first quadrupole (Q1) was scanned repetitively from m/z 300 to 700 in 5 s with a scan step of 1 u. For CE–MS–MS work, ultrapure argon (AIRCO, BOC Group, Murray Hill, NJ, USA) was used as the collision gas and introduced into the collision cell (Q2) at a target gas thickness of $200 \cdot 10^{12}$ atoms/cm^2. The collision energy used for the MS–MS experiments was 50 V (laboratory frame). Both the first and third quadrupole analyzers were operated at unit mass resolution in this work. The mass spectrometer data system was a standard Macintosh IIcx-based data acquisition and software package provided by Sciex for the API III system.

Methods

Sequential rinsing and conditioning of the capillary between analyses was typically performed by pressurizing the corresponding inlet vial to 20 p.s.i. for 2 min with distilled water and then with the electrophoretic buffer. This procedure is beneficial not only for conditioning the capillary wall surface, but also for cooling it from the Joule heating produced by the applied 1000 V/cm and for refilling the capillary with fresh running buffer. The running buffer used for all the separations described in this work consisted of ammonium acetate–acetonitrile (75:25) adjusted to pH 5 with 17% acetic acid. Although methanol behaves similarly to acetonitrile as an organic modifier in these applications, the latter was chosen because it produces slightly improved peak shapes and separation efficiencies, as we have reported previously [12]. For full-scan and SIM CE–MS determinations, 7 and 1 pmol per component were injected in 15 and 5 nl of running buffer respectively. Injection was accomplished by pressurizing the sample vial at 0.5 in.Hg for 10–15 s followed by transfer of the capillary inlet into a vial containing the running buffer. The high-voltage potential applied across the separation capillary was typically 300 V/cm, but for the fastest separations 1000 V/cm was used. For those CE–MS analyses accomplished with the commercial CE system, the separation capillary was 100 cm × 75 μm I.D., whereas for those CE–MS analyses performed with the laboratory-made CE system the separation capillary was 35 cm × 75 μm I.D.

RESULTS AND DISCUSSION

Our initial observations focused on the relatively short analysis times observed resulting from CE–UV method development for the separation of a synthetic mixture of the eight sulfonylurea compounds available in this study (Fig. 2). Preliminary CE studies were initiated without sulfometuron methyl (compound **2**) because this compound was not initially available to us. As shown in Fig. 3A, the seven-component mixture containing *ca.* 7 pmol of each compound could be separated and detected by UV at 254 nm with the commercial P/ACE 2000 system, within about 9 min using a running buffer of 5 m*M* ammonium acetate–acetonitrile (75:25), pH adjusted to 5 with acetic acid, with 300 V/cm across a 100 cm × 75 μm I.D. capillary column. However, as shown in Fig. 3B, on-line detection by SIM mass spectrometry where the analytes migrated through an additional 80 cm of the capillary column and the liquid junction–ion spray coupling required *ca.* a 40-min total analysis time. When the

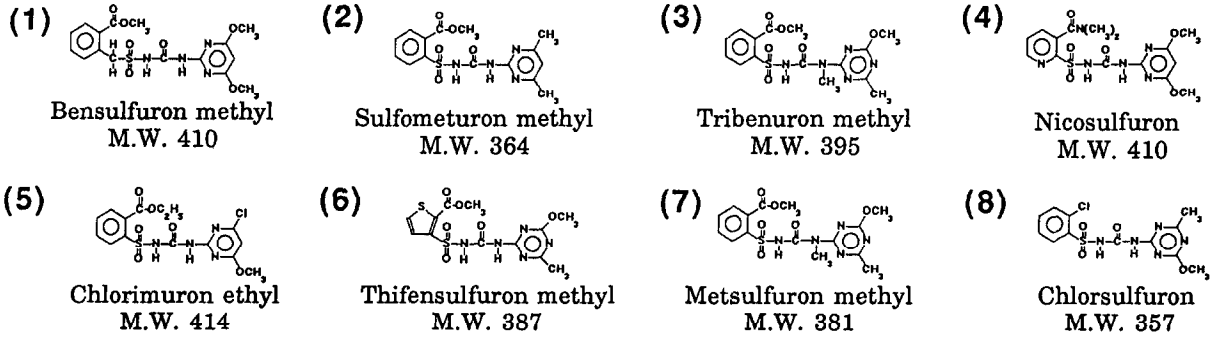

Fig. 2. Structures, names and molecular weights of sulfonylurea compounds studied.

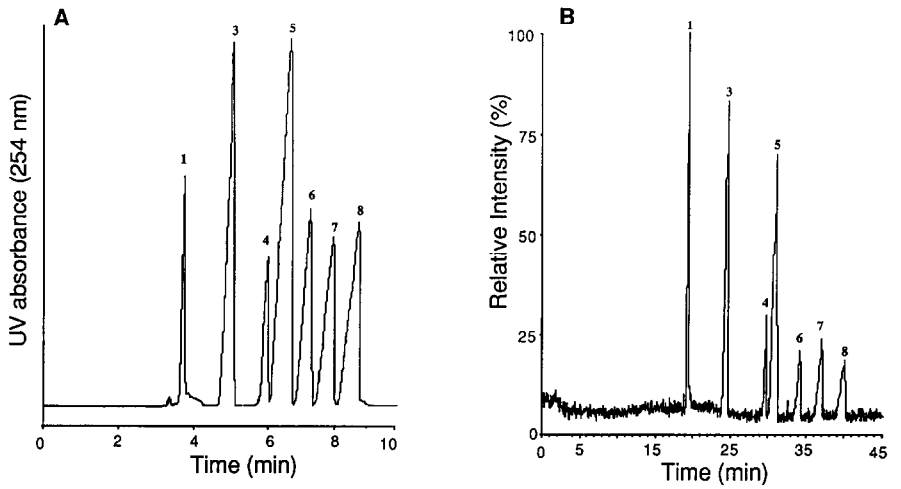

Fig. 3. Capillary electrophoresis analysis of a seven-component synthetic mixture containing sulfonylurea crop protection chemicals (compound **2**, sulfometuron methyl, was not present in this mixture). (A) CE–UV electropherogram containing *ca.* 7 pmol of each compound. The running buffer consisted of 5 m*M* ammonium acetate–acetonitrile (75:25), pH adjusted to 5 with acetic acid, with 300 V/cm across a 100 cm × 75 μm I.D. capillary column that produced 15 μA current. (B) SIM CE–MS total selected ion current electropherogram after the analytes migrated through an additional 80 cm of the capillary column and through the liquid junction–ion spray coupling.

data in Fig. 3A and B are compared, it is clear that a better separation is achieved, for example, between the last three components in the mixture under these conditions with MS detection than with UV detection. This is easily explained, however, by the fact that the analytes are detected in Fig. 3B after traversing a much longer (80 cm) portion of the capillary compared with Fig. 3A. Therefore, if one is interested in simply achieving baseline separation for these compounds, the full 100 cm of capillary column is not necessary.

One limitation of many commercial CE instruments is their large physical size, which restricts their placement very close to the ion sampling source region of a mass spectrometer. Therefore, in most instances a longer than necessary capillary column is used not only to achieve the required separation efficiency for a particular application, but rather simply to "reach" the mass spectrometer. The results shown in Fig. 3 are representative of this situation. We have constructed a small, simple CE device that allows the use of shorter capillary columns for close coupling to the ion sampling region of an atmospheric pressure ionization mass spectrometer [18]. The system is depicted in Fig. 1. It incorporates a pressurization feature that allows both "conditioning" and cooling of the capillary with running buffer and solvents between analyses and the loading of sample, or injection, by pressurization. These features are useful for the CE–MS system described here because it obviates dismantling the capillary exit from the carefully aligned liquid junction coupling as would be required if suction techniques were used to serve these purposes. Robotic placement of the capillary inlet into either a sample or a running buffer vial is provided by the electronic actuator system described in the Experimental section. The clear plastic desiccator box facilitates observation of the robotic mechanism relative to the vials inside and its small size (length 22.5 × width 16 × height 20 cm) allows its placement immediately in front of the mass spectrometer ion sampling region. On-line UV and MS detection is not utilized with this simple laboratory-made system because of the extra column length that would be required to accomplish this task. However, a modified Waters Model 440 UV detector may be used in place of the mass spectrometer when only UV detection is needed.

Fig. 4 shows the on-line full-scan CE–MS total ion current profile (TIC) obtained using the laboratory-made CE system described above. This

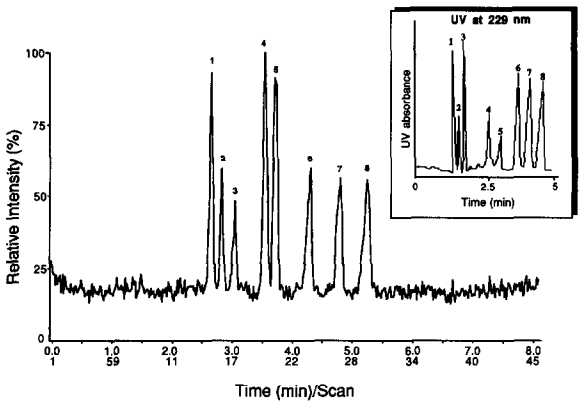

Fig. 4. On-line full-scan CE–MS total ion current profile (TIC) obtained using the laboratory-made CE system and a 35-cm capillary column described in Fig. 1. The running buffer consisted of 5 m*M* ammonium acetate–acetonitrile (75:25), pH adjusted to 5 with acetic acid, with 1000 V/cm across a 35 cm × 75 μm I.D. capillary column. This mixture contained the eight sulfonylurea compounds shown in Fig. 2 with *ca.* 7 pmol of each compound loaded into the capillary column and detected by the mass spectrometer operated in the positive-ion mode of detection while scanning repetitively from *m/z* 300 to 700. The inset is the UV electropherogram obtained with the same system when the mass spectrometer and the liquid junction–ion spray system were replaced with the micro-UV detector.

mixture contains the eight sulfonylurea compounds shown in Fig. 2 with *ca.* 7 pmol of each compound loaded into the capillary column and detected by the mass spectrometer operated in the positive-ion mode of detection while scanning repetitively from *m/z* 300 to 700. Scanning below *m/z* 300 was not done as no analyte ions were observed in this region. The running buffer consisted of 5 m*M* ammonium acetate–acetonitrile (75:25) adjusted to pH 5 with acetic acid, with 1000 V/cm across a 35 × 75 μm I.D. capillary column. Thus the shorter analysis time is accomplished both by shortening the length of the capillary column and increasing the voltage per unit length (300 *vs.* 1000 V/cm). The inset in Fig. 4 is the UV electropherogram obtained with this same system when the mass spectrometer and the liquid junction–ion spray system were replaced with the micro-UV detector. These data reveal the similarity of the sulfonylurea peak shapes and separation efficiency. The slightly longer analysis time observed in the CE–MS TIC compared to the CE–UV electropherogram is believed to be due to the *ca.* 6 cm greater distance that the separated components must travel through the liquid junction–ion spray system in contrast to on-column UV detection in the CE–UV experiment.

The poor electrophoretic peak shape for slower migrating sulfonylurea components (in particular peaks 6–8) observed throughout this work remains a problem. The last three peaks seen in both the CE–UV and CE–MS electropherograms in Figs. 3 and 4 show considerable "fronting". Several attempts to eliminate this behavior were largly unsuccessful. Although conditioning and treatment of the inside surface of the fused-silica capillary occasionally seemed to reduce the problem, it is possible that the injected levels used in this work (1–15 pmol) were sufficiently high to cause overloading of the capillary by localized electrical effects in the fused-silica capillary that produces the observed fronting. Another possible problem is the Joule heating caused by the 1000 V/cm potential (15 μA) used in this work in the absence of any capillary column cooling. These conditions caused considerable variation in migrating times from one run to the next, and would benefit from external cooling of the capillary. However, this feature is not easy to incorporate during CE–MS experiments because of the need to extend the column from the CE system to the mass spectrometer. In any case, these compounds appear to be particularly susceptible to this behavior and elimination of this problem may require either more efficient surface treatment of the fused silica or more sensitive MS detection, or both. As both the UV and MS detectors appeared to give the same behavior, it is unlikely this is a detector phenomenon.

In contrast to LC–FAB-MS results [6], the mass spectra obtained from the electrospray process used in this work display only molecular weight information with no fragmentation. Fig. 5 shows the full-scan CE–MS mass spectra for nicosulfuron (peak 4) and chlorsulfuron (peak 8) obtained from the corresponding CE–MS TIC observed in Fig. 4. From these data it is evident that the predominant ion observed for each compound is the $[M+H]^+$ ion with some evidence for an ammoniated molecule such as $[M+NH_4]^+$. Hence the ion spray CE–MS system described here appears to provide sufficiently mild separation and ionization conditions to produce no detectable breakdown of these labile compounds.

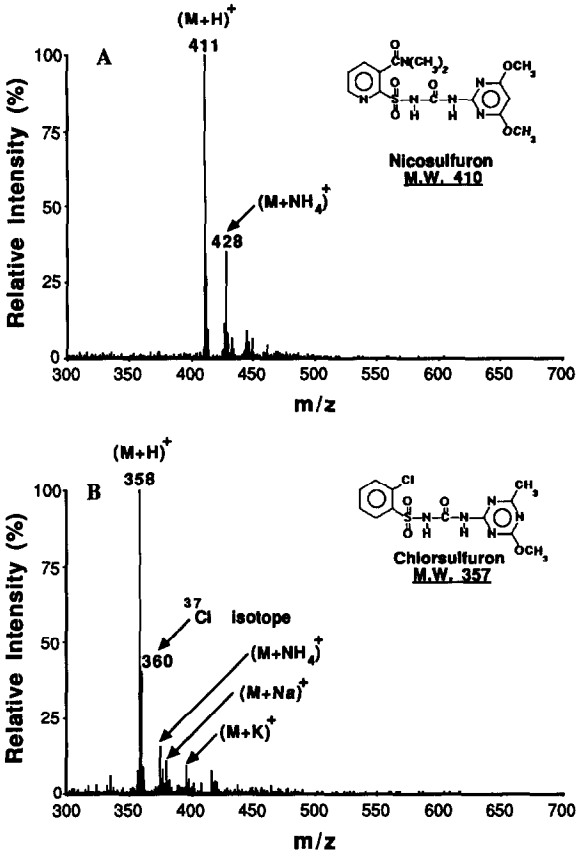

Fig. 5. Representative full-scan CE–MS mass spectra for peaks 4 and 8 in Fig. 4.

Although some variation in electrophoretic peak shape and migration time persisted through this work, we were able to obtain the shortest analysis times with reduced variation in migration time for the last three components (peaks 6–8) by utilizing the pressurization feature of the laboratory-made CE system to implement a form of "flow programming" during the CE–MS analysis. Fig. 6A shows the total selected ion current profile from the SIM CE–MS analysis of 1 pmol per sulfonylurea component under conditions optimized for the shortest analysis time. These include a running buffer consisting of 5 m*M* ammonium acetate–acetonitrile (75:25), pH adjusted to 5 with acetic acid, and 1000 V/cm across 35 × 75 μm I.D. capillary column. The $[M+H]^+$ ion for each sulfonylurea was monitored sequentially using a dwell time of 200 ms for each ion with mass spectral acquisition commencing with application of high voltage across the CE capillary column.

The total selected ion current electropherogram shown in Fig. 6A displays relatively good peak shape for peaks 1–5, but broad, well separated peaks for peaks 6–8. To compensate for this behavior, the results shown in Fig. 6B were obtained by repeating the same experiment, but applying a 0.5 p.s.i. pressurization of the desiccator box immediately following the elution of the fifth component at *ca.* 3.8 min. This procedure essentially increases the bulk, electroosmotic flow by *ca.* 7 nl/min, and "pushes" the remaining three components through

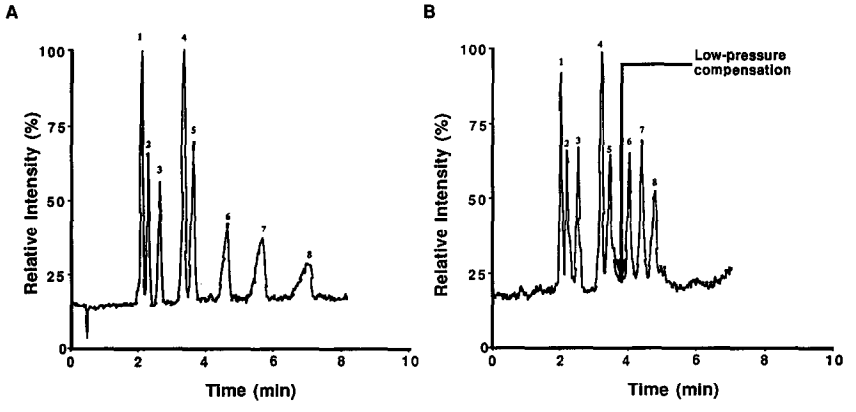

Fig. 6. SIM CE–MS total selected ion current electropherograms for a synthetic mixture containing the eight sulfonylurea compounds, (A) without pressure compensation and (B) with pressure compensation. The latter is accomplished by applying a 0.5 p.s.i. pressurization of the desiccator box immediately following the elution of the fifth component at *ca.* 3.8 min. See text for experimental details.

the capillary to the mass spectrometer. Although this procedure certainly decreases the separation efficiency for these components, it does reduce the analysis time by more than 2 min and improves the "apparent" peak shape for these components. This procedure may not be of general use, but could be useful in those instances where interfering components are not observed and rapid analyses are required.

Tandem mass spectrometry

It was noted above that CE–MS analysis of the target sulfonylurea compounds using the ion spray interface provides abundant molecular weight information for these compounds, but no fragment ions to facilitate their structural characterization. The mild ionization conditions common to electrospray provide an ideal scenerio for tandem mass spectrometry (MS–MS) [19]. Thus, on-line CE–MS–MS analysis of the sulfonylurea mixture can produce a full-scan collision-induced dissociation (CID) spectrum for each component in the mixture that contains fragment ions indicative of their molecular structures. This experiment may be conducted in a manner similar to those described above, but with some important exceptions. First, we chose to slow down the CE separation to provide wider electrophoretic peaks to allow scanning the full mass range (m/z 50–700) at a rate sufficient to provide good ion statistics for each scan. The same 35 cm × 75 μm I.D. capillary column and 5 mM ammonium acetate–acetonitrile (75:25) adjusted to pH 5 with 17% acetic acid, but an 800 V/cm potential on the capillary were used in this example. Pressure compensation following, in this case, peak 6, was utilized to minimize the otherwise slow migration of the last two sulfonylurea compounds (peaks 7 and 8).

The tandem triple quadrupole mass spectrometer was operated in the product ion scan mode [19] by focusing the protonated molecule precursor ion in the first quadrupole mass analyzer (Q1) and scanning the third quadrupole (Q3) through the mass range inclusive of the precursor ion and m/z 50. This is done for each component in sequence according to its migration time, while the collision quadrupole (Q2) is pressurized with argon at a target gas thickness of $200 \cdot 10^{12}$ atoms/cm^2.

Fig. 7 shows an overlay of the TICs for each of the eight target sulfonylurea compounds obtained from the on-line CE–MS–MS analysis of a synthetic mixture containing *ca.* 30 pmol per component dissolved in 15 nl of the running buffer that was loaded onto the capillary by pressure injection. These relatively high levels were required owing to the inherent ion transmission losses in the MS–MS mode. Although it has been reported that MS–MS techniques significantly reduce both the chemical and electrical noise [20], the absolute level of analyte ion current is also significantly reduced relative to a single MS scan such that the available ion current signal may be too weak to produce a good CID mass spectrum.

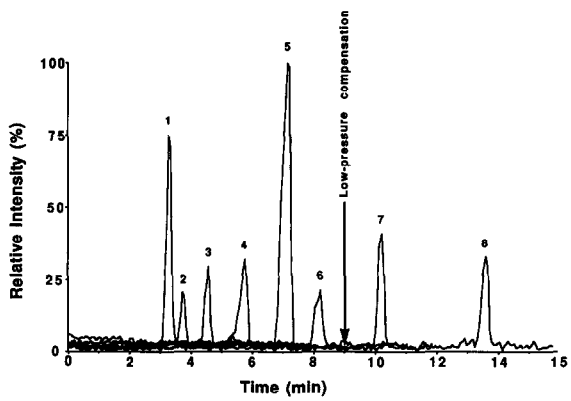

Fig. 7. An overlay of the full-scan TICs for each of the eight target sulfonylurea compounds obtained from the on-line CE–MS–MS analysis of the synthetic mixture containing *ca.* 30 pmol per component dissolved in 15 nl of the running buffer that was loaded on to the capillary by pressure injection. A 35 cm × 75 μm I.D. capillary column containing 5 mM ammonium acetate–acetonitrile (75:25) adjusted to pH 5 with 17% acetic acid, but 800 V/cm potential on the capillary, was used in this example to slow the separation and allow better ion statistics during this CE–MS–MS analysis.

Representative full-scan CID mass spectra are shown in Fig. 8 that display important structural features common to the target analytes. For example, Fig. 8A shows the base peak at m/z 149 indicative of cleavage of the benzylic–sulfonamide bond in benzsulfuron methyl (peak 1). The other major fragmentation process observed for benzsulfuron methyl appears to be dictated by the tendency for carbonyl carbon α-cleavage of the urea portion of the compound to give an ion of m/z 182. Related fragmentation processes appears in Fig. 8B and C to give their corresponding base peaks at m/z 155

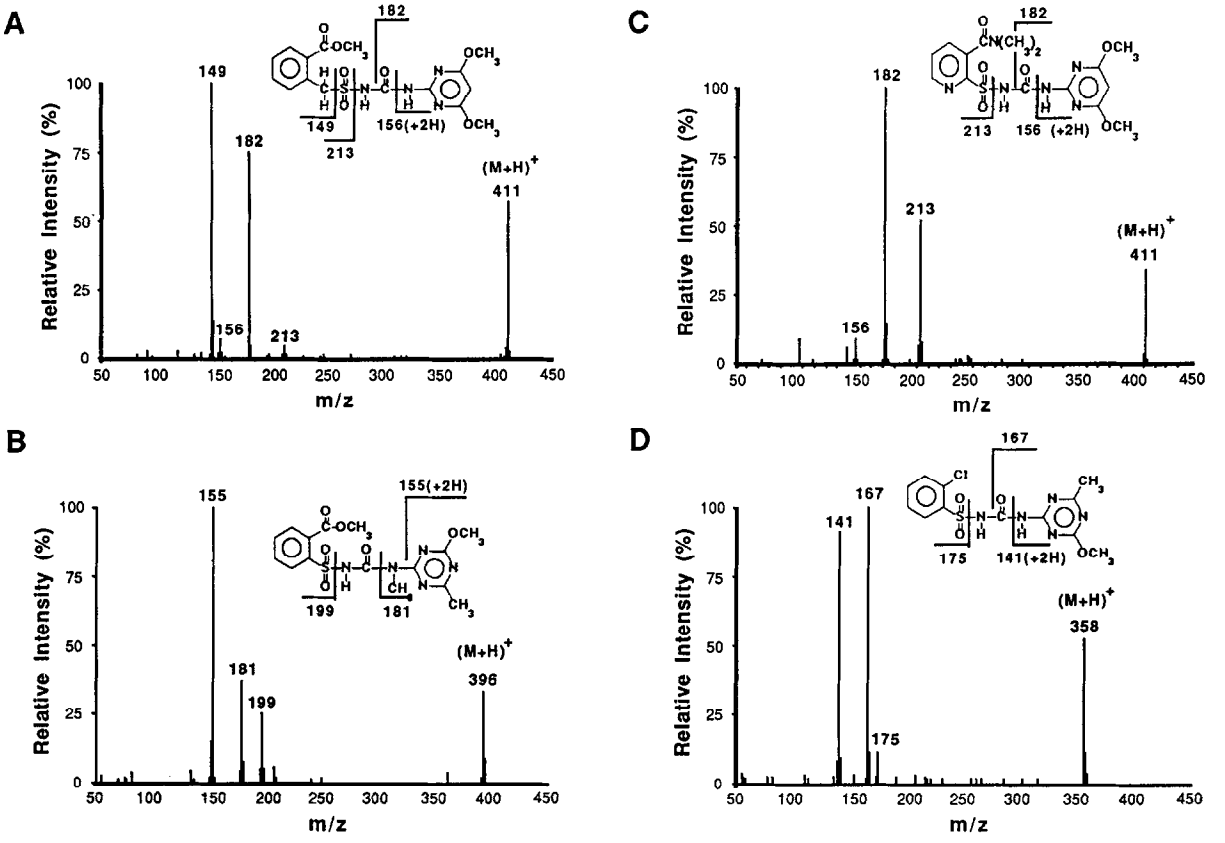

Fig. 8. CID mass spectra obtained from the CE–MS–MS results shown in Fig. 7. (A) Peak 1, bensulfuron methyl, M.W. 410; (B) peak 3, tribenuron methyl, M.W. 395; (C) Peak 4, nicosulfuron, M.W. 410; (D) chlorsulfuron, M.W. 357.

and 182, respectively. This prevalent cleavage α to the sulfonyl group is common to this class of compounds and generally is indicative of this functional group. Chlorsulfuron (Fig. 8D, peak 8) displays its base peak at *m/z* 167 as a result of the competing process due to cleavage α to the carbonyl carbon in the "bridge" [2,3] urea portion of this compound. The *m/z* 141 ion again appears to originate from α cleavage of the urea carbonyl carbon in this compound.

As a final example, the implementation of CE–MS–MS for the determination of sulfonylurea compounds in a *Fargo* soil extract was investigated. The TIC for the full-scan CE–MS–MS analysis of a spiked soil extract (*Fargo*) is shown in Fig. 9A. The soil extraction procedure has been described previously [6]. In this instance the extract of *Fargo* soil (5 g) was fortified with Bensulfuron methyl at a level of 411 ng/μl and 15 nl (15 pmol) were injected into the capillary column by pressure injection. This concentration of sulfonylurea in soil is much higher than the low nanograms per gram (ppb) level usually observed in soil samples. However, an elevated level was used in this case owing to the low injection volume required by CE and the limited detection limit of the CE–MS–MS technique described here. The TIC shown in Fig. 9A was obtained by tuning Q1 to transmit the $[M+H]^+$ ion for bensulfuron methyl at *m/z* 411 into the argon-pressurized Q2 region whereupon CID occurred to produce the full-scan CID spectrum shown in Fig. 9B. From these data it is evident that the migration time for bensulfuron methyl is 2.9 min under these conditions, and no other significant *m/z* 411 species are detected in the soil extract. Inspection of the full-scan CID spectrum obtained from the electropho-

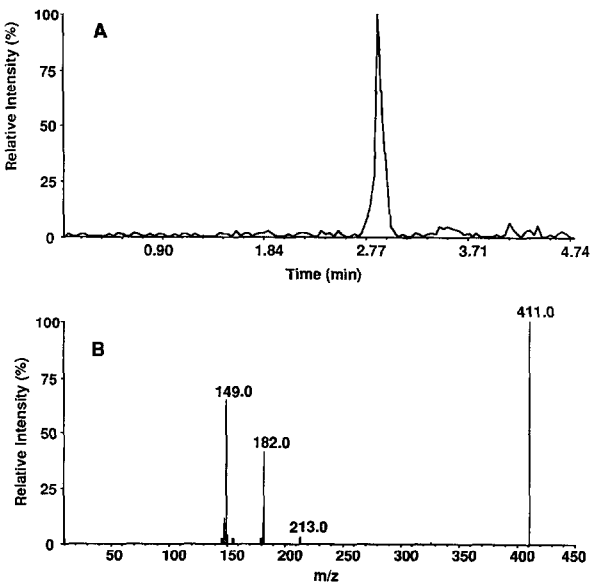

Fig. 9. CE–MS–MS analysis of a *Fargo* soil extract fortified with bensulfuron methyl (compound **1**, Fig. 2). The extract of soil contained this analyte at a level of 411 ng/μl, and 15 nl (15 pmol) of sample were injected into the capillary column by pressure injection. Experimental conditions as in Fig. 8. See text for details.

retic peak maximum shown in Fig. 9A gives the mass spectrum shown in Fig. 9B. These combined data provide unique selectivity and good analytical support for the identification of bensulfuron methyl in this fortified sample. In those instances where high levels of sulfonylurea compounds are present, this CE–MS–MS approach offers unique analytical utility. However, significant improvement in MS detection limits will be required to use this combination of techniques routinely for the determination of the low ppb levels of these compounds in environmental samples. We plan to investigate the feasibility of coupling an ion spray–ion trap system with on-line CE in an effort to achieve improved capability in this regard [2].

CONCLUSIONS

The CE–MS and CE–MS–MS results described here suggest that relatively rapid analysis times may be obtained for the separation and identification of sulfonylurea crop protection chemicals using shortened capillary columns and high-voltage potentials across the separation capillary. It is not straightforward to implement the use of short capillaries (less than 50 cm) for CE–MS with many commercial CE instruments because of the relatively large size and configuration of these instruments. The simple, laboratory-made system described here does not have the versatility of commercial units, but does provide the basic requirements for the work described. It should be noted that the use of high-voltage potentials (800–1000 V/cm) does create considerable Joule heating, which can generate excess heat in the separation capillary. The use of external cooling of the capillary (not implemented in this work) would facilitate this approach, but is difficult to implement in practice while coupled to the mass spectrometer. When the liquid junction coupling and the ion spray interface described in this work are properly adjusted, the system is relatively routine to use.

It should also be noted that CE–MS detection limits are not yet entirely satisfactory. The very low levels of analytes that are typically loaded into a CE column often require SIM CE–MS techniques and sometimes even then there is insufficient sensitivity. Efforts are being made to improve this situation using a benchtop ion trap mass spectrometer.

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