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MICROCOLUMN LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY USING MOVING BELT AND CONTINUOUS FLOW FAST ATOM BOMBARDMENT INTERFACES

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SUMMARY

Packed fused-silica liquid chromatographic columns were interfaced to a magnetic mass spectrometer using a commercially available moving belt interface to obtain electron ionization and chemical ionization mass spectra, and a continuous flow fast atom bombardment interface built in our laboratories to obtain fast atom bombardment mass spectra. Good quality spectra were obtained on 0.1-μg samples injected into the liquid chromatographic column with all three ionization techniques. Examples showing the utility of these techniques for the identification of herbicides and their plant metabolites are presented.

INTRODUCTION

In our work on the identification of pesticide metabolites isolated from plants, animals, soil or water, we frequently have very small quantities of sample to work with. Typically, the total amount of metabolite isolated is 1 μg or less. These samples often contain impurities which preclude their analyses by mass spectral probe techniques. Most of these metabolites are too polar or thermally labile for direct gas chromatographic–mass spectrometric (GC–MS) analysis, and many cannot be derivatized for GC–MS, therefore liquid chromatography (LC)–MS is the method of choice for their identification.

Miniaturization of the LC column has allowed us to obtain improved performance of the moving belt interface with reversed-phase columns using aqueous mobile phases[1,2]. The moving belt interface allows one to obtain electron ionization (EI) spectra for structural information and library searching, and chemical ionization (CI) spectra with a choice of reagent gases[3,4]. LC-fast atom bombardment (FAB)–MS with a moving belt interface has been reported in the literature[3,5], but the sensitivity is poor, possibly due to the short residence time of the sample in the ion source. With the

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continuous flow FAB interface[6], the sample remains on the FAB target until it is ionized, vaporized or sputtered away. Continuous flow FAB has been interfaced to a conventional bore LC column[7]; however the column effluent was split and only about 1% was introduced into the mass spectrometer. Recent work in several laboratories[8,9] has shown the potential of interfacing packed fused-silica capillary LC columns with continuous flow FAB, where the entire sample is introduced to the mass spectrometer.

We have designed and constructed a continuous flow FAB (CFF) interface which is interchangeable with the moving belt interface. This paper reviews the performance of the CFF interface, and demonstrates the utility of the LC–MS in the EI, CI and FAB modes for the identification of herbicides and their plant metabolites.

EXPERIMENTAL

The 0.25-mm I.D. fused-silica columns (J&W Scientific, Folsom, CA, U.S.A.) were slurry packed with 3-μm Nucleosil ODS (Macherey-Nagel, Duren, F.R.G.), as described in ref. 1. A 0.05-mm I.D. fused-silica (SGE, Austin, TX, U.S.A.) transfer line of approximately 70 cm length was epoxy bonded into the column.

A Beckman Model 114M LC pump was used for both packing columns and LC–MS analyses. For LC–MS, the constant pressure mode was used. Typical pressures for a 30-cm column were 140–190 bar to obtain flow-rates from 1 to 3 μl/min.

A Valco (Houston, TX, U.S.A.) Model C14W submicroliter injection valve with a 0.1-μl rotor was used. The analysis of dilute solutions was accomplished by removing the rotor and slowly depositing 1–5 μl of the sample dissolved in a volatile solvent, such as methanol or acetonitrile, while allowing the solvent to evaporate[1,2]. The rotor was replaced, flow restored, and the sample was injected by turning the valve to the "inject" position for 10 s.

A Finnigan MAT Model 8230 magnetic-sector mass spectrometer equipped with a moving belt interface[3,4] was used. A schematic diagram of this interface is

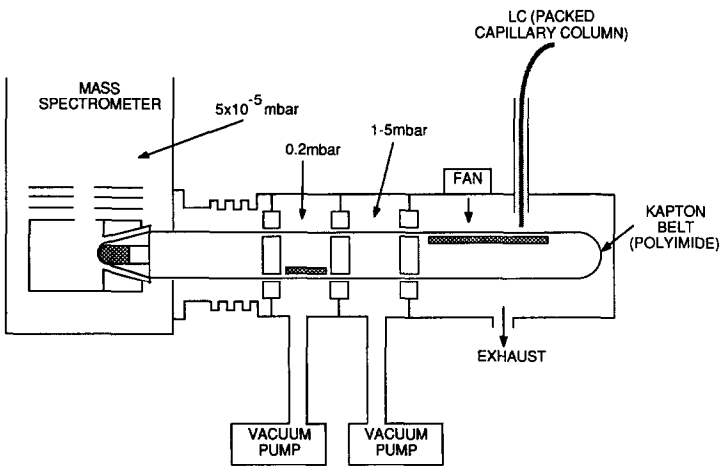

Fig. 1. Schematic diagram of moving belt interface.

shown in Fig. 1. The 0.05-mm I.D. fused-silica transfer line was fed through stainless-steel capillary tubing and adjusted so it just touched the center of the moving belt. The following interface settings were used: belt speed, 3 cm/s; solvent evaporator temperature, 100°C; vaporizer tip set at 5 W; cleanup heater set at 80% (approximately 250°C). Typical MS conditions were: ion source temperature, 200°C; ionizing voltage, 70 eV in EI and 200 eV in CI; CI source pressure using ammonia as reagent gas, 0.5 Torr (source ion gauge reading $5 \cdot 10^{-4}$); resolution, 1000; scan rate, 2 s per decade.

A CFF probe was designed and constructed to be interchangeable with the moving belt interface, since the alignment of the moving belt with the hole in the ion source is very critical, and it is desirable not to dismount the hardware once it is aligned. Fig. 2 shows a schematic diagram of the CFF probe. The internal moving belt unit was removed, then the first chamber (atmospheric pressure chamber) was removed prior to inserting the CFF probe.

For CFF, the LC parameters were the same as described above. An Ion Tech FAB gun was used with xenon gas, at 8 kV anode voltage, 1–2 A discharge current and 0.4 mA monitor current. The ion source temperature was held at 200°C to provide heat to the capillary tip to prevent freezing of the LC solvent as it evaporates. The source ion gauge reading was $3 \cdot 10^{-4}$–$5 \cdot 10^{-4}$ mm during operation.

RESULTS AND DISCUSSION

CFF interface

The FAB target is heated by radiation from the ion source. With the source at 200°C, the target temperature is 45–50°C, which is about optimum for CFF[6]. A benefit of keeping the source at 200°C, rather than the normal low temperature commonly used for conventional FAB (used to prevent the matrix compound from evaporating from the target too fast), is less contamination/less frequent source cleaning.

The 0.05-mm I.D. fused-silica transfer line was fed through the center of the probe and adjusted so the end was flush with the stainless-steel FAB target. The hole in the target was obtained by wire electric discharge machining drilling, and was made slightly larger than the O.D. of the fused-silica tubing to obtain a snug fit. The snug fit

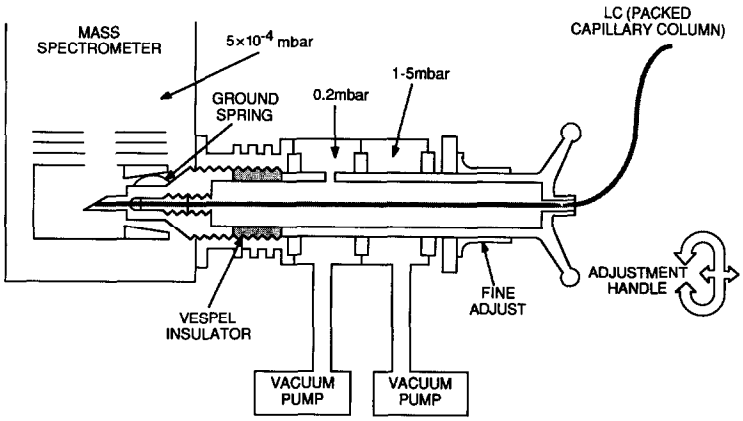

Fig. 2. Schematic diagram of continuous flow FAB interface.

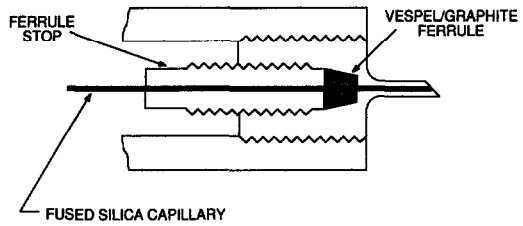

Fig. 3. Schematic diagram of threaded FAB target.

and a Vespel/graphite ferrule incorporated into the threaded FAB target, as shown on Fig. 3, prevent backflow of liquid into the probe.

Glycerol was added to the LC mobile phase at the 10% level. With acetonitrile–water mobile phases, addition of 10% glycerol caused the formation of two phases if the acetonitrile concentration was increased above 50%. We found acetonitrile–water–glycerol (50:40:10) to be a good general purpose mobile phase. If a stronger mobile phase is needed, methanol–water can be used, such as: methanol–water–glycerol (80:10:10). The mobile phase was degassed in an ultrasonic bath for 10 min to eliminate air bubbles which give an unstable vacuum. Mobile phase was prepared fresh weekly since aged mobile phase appeared to cause an unsteady vacuum, possibly due to bacterial action generating carbon dioxide. Typically a steady vacuum and matrix ion current was obtained about 30 min after start of operation.

With CFF we obtained a ten-fold increase in sensitivity over that obtained with the conventional FAB probe, likely due to increased sample concentration in the glycerol matrix since a much thinner film of glycerol is obtained with the continuous flow probe. Good quality FAB spectra were obtained on 0.1-μg quantities injected into the LC.

Fig. 4 shows a separation obtained on a mixture of four sulfonylurea herbicides

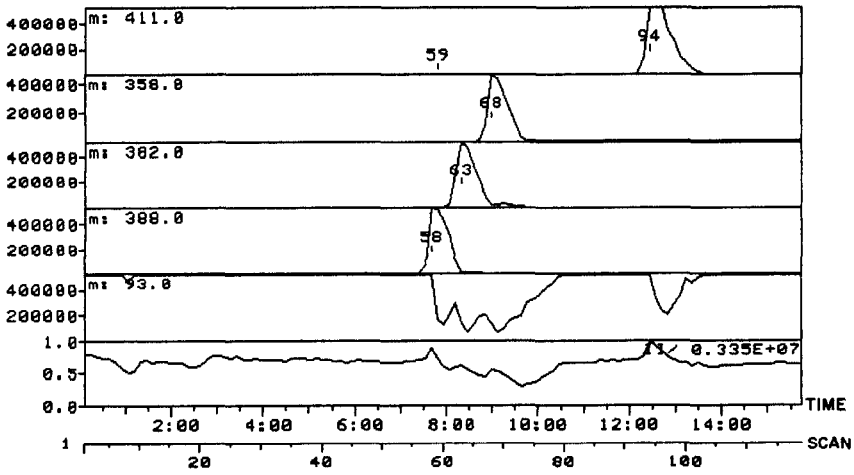

Fig. 4. Separation of four sulfonylurea herbicides, the active ingredients in: Harmony® (M + 1 at 388), Ally® (M + 1 at 382), Glean® (M + 1 at 358) and Londax® (M + 1 at 411), using LC–FAB-MS. Column: 30 cm × 0.25 mm I.D. Nucleosil ODS. Mobile phase: acetonitrile–glycerol–water (pH 3, formic acid) (50:10:40).

at the 0.1 μg/component level. The mass range scanned was 45–650, therefore the glycerol matrix ions were acquired by the data system. Since the glycerol ions were more intense than the sample ions, the LC peaks were not observed in the total ion plot (bottom trace). We have used two techniques to locate the LC peaks: (1) use the data system to re-plot the chromatogram using the high mass ions, *e.g.* *m/z* 300–650, (2) examine the plot of the protonated glycerol ion, *m/z* 93, which is often suppressed during elution of the LC peak as shown on Fig. 4. This suppression suggests a CI process is occurring, where the sample molecule is protonated by the glycerol ion.

Analysis of sulfometuron methyl and plant metabolites

An example showing the complementary data obtained on sulfometuron methyl, a sulfonylurea herbicide, and two plant metabolites using LC–MS in the EI, CI and FAB modes is presented. Fig. 5 shows the LC–EI-MS chromatogram obtained using the moving belt interface. The three components were readily separated by the reversed-phase LC column. The EI mass spectrum of the parent compound (sulfometuron methyl, the active ingredient in Oust® herbicide) is shown in Fig. 6. As with most sulfonylurea herbicides, a molecular ion is not obtained in the EI mass spectrum. However, a lot of structural information is obtained from the fragment ions, as shown in Fig. 6. Major fragment ions are obtained by cleavage on both sides of the carbonyl, which can allow one to deduce the molecular weight and assign a structure. The identity of the parent compound was confirmed by library search. The EI spectrum of the hydroxylated metabolite (Fig. 7) also gives a lot of structural information. The pyrimidine amine and isocyanate fragment ion are shifted sixteen mass units higher, showing that hydroxylation occurred on this side of the molecule. The EI mass spectrum of the glucose conjugate (Fig. 8) shows fragment ions from the sulfonyl isocyanate portion of the molecule, but is of minimum value for structure determination.

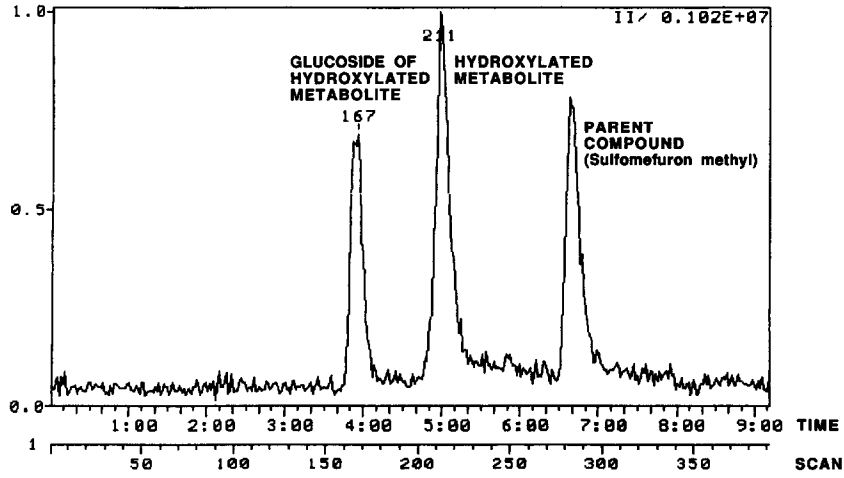

Fig. 5. Separation of sulfometuron methyl and two plant metabolites using LC–EI-MS. Column as in Fig. 1. Mobile phase: acetonitrile–water (60:40) (pH 3, formic acid).

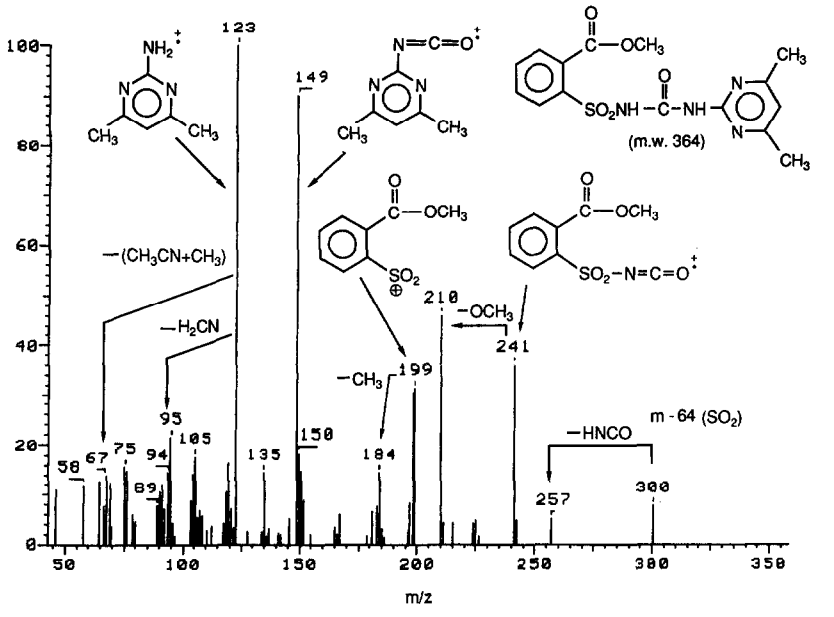

Fig. 6. EI mass spectrum of sulfometuron methyl.

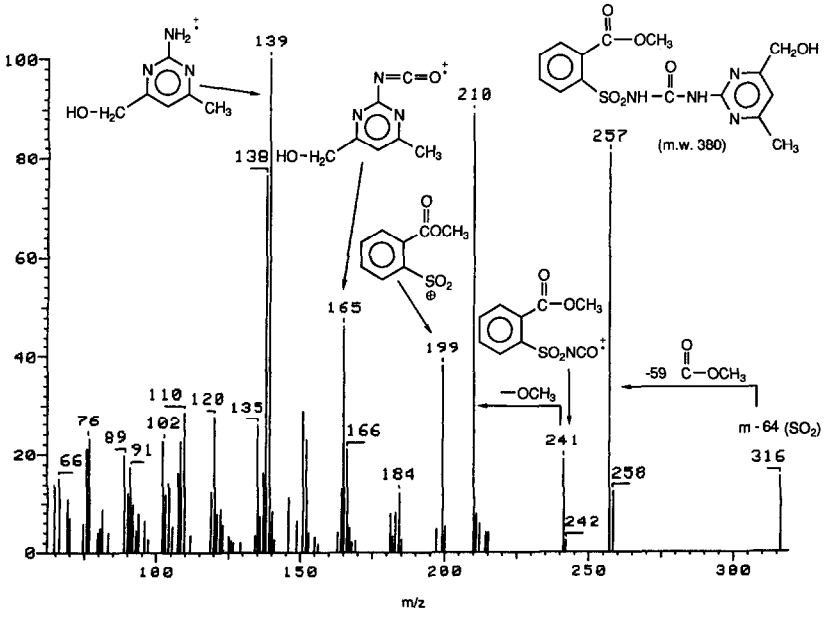

Fig. 7. EI mass spectrum of hydroxylated metabolite of sulfometuron methyl.

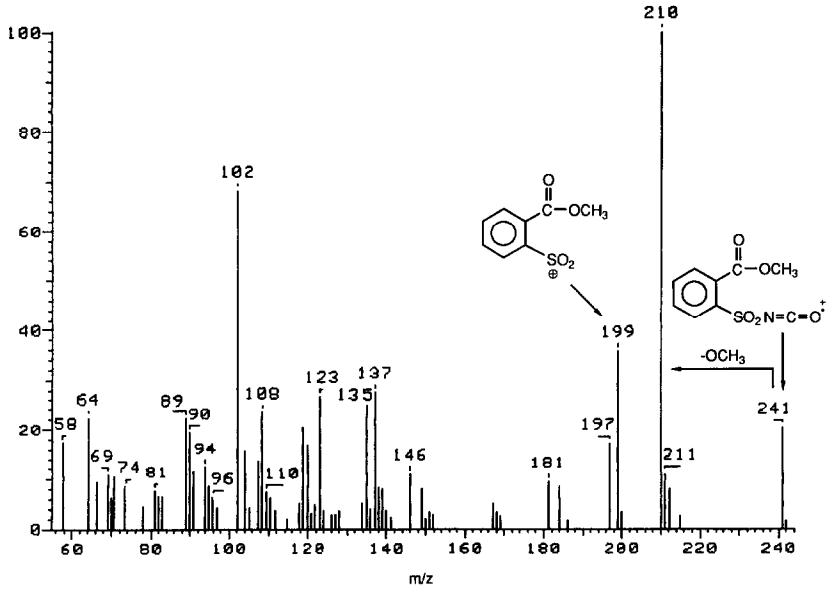

Fig. 8. EI mass spectrum of glucoside of hydroxylated sulfometuron methyl.

LC–MS using ammonia CI gave protonated molecular ions in the mass spectra of the parent compound and the hydroxylated metabolite, and structurally useful fragment ions due to cleavage on both sides of the carbonyl. The ammonia CI mass spectrum of the glucoside does not include molecular weight related ions, but does give structural information, as shown in Fig. 9. The thermospray mass spectrum of the

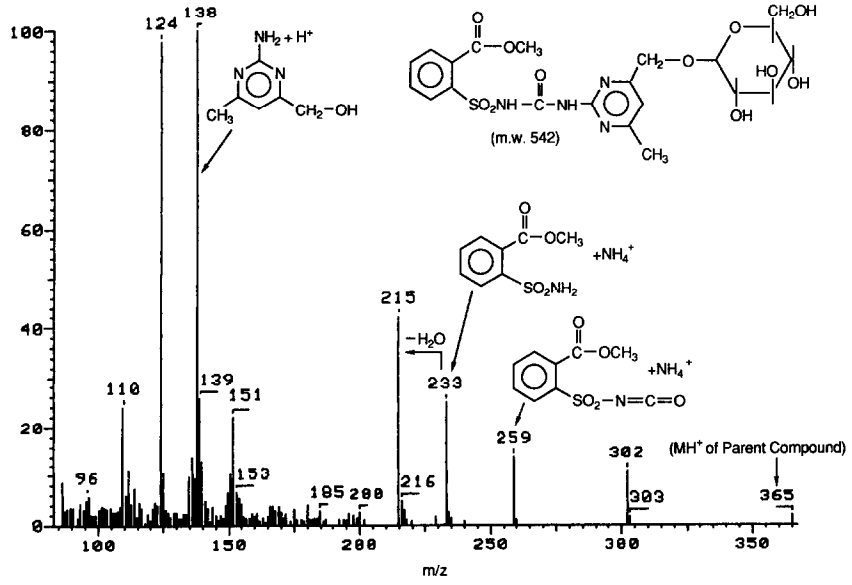

Fig. 9. Ammonia CI mass spectrum of glucoside of hydroxylated sulfometuron methyl.

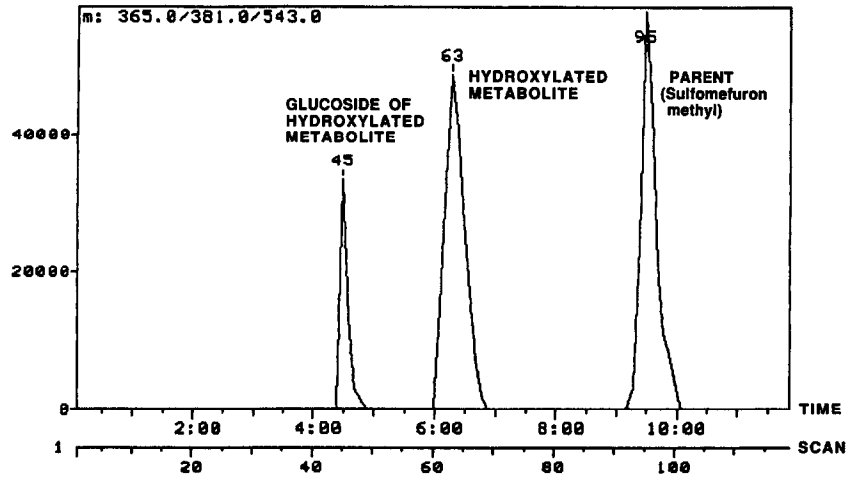

Fig. 10. Separation of sulfometuron methyl and two plant metabolites using LC–FAB-MS. Column and mobile phase as in Fig. 4.

glucoside was similar to the ammonia CI spectrum obtained with the moving belt interface, and also did not show molecular weight related ions[10].

The LC–FAB-MS chromatogram obtained by plotting the sum of the protonated molecular ion intensity for each component is shown on Fig. 10, and the background subtracted FAB spectra obtained are shown in Figs. 11–13. The glycerol matrix ions can easily be subtracted out of the spectra. The protonated molecular ion is the base peak in the FAB spectra of all three components. This is a particularly

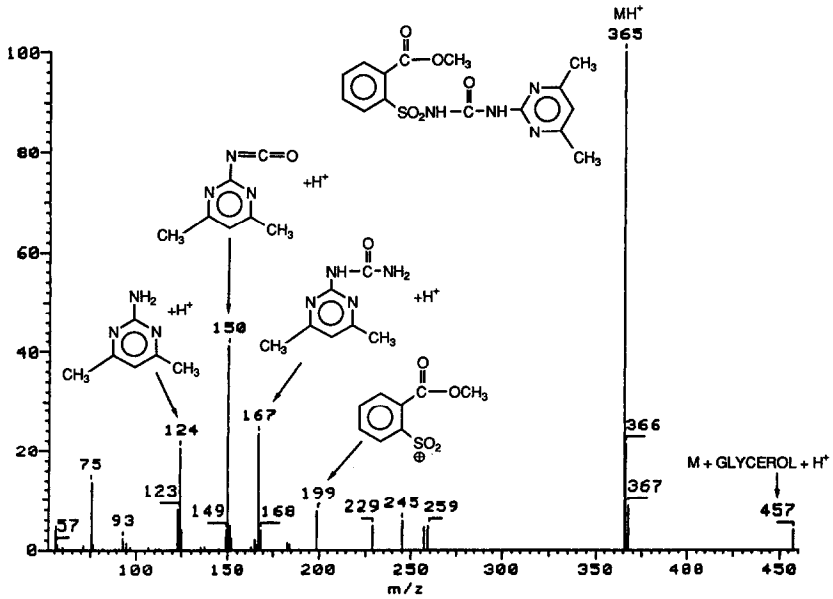

Fig. 11. FAB mass spectrum of sulfometuron methyl.

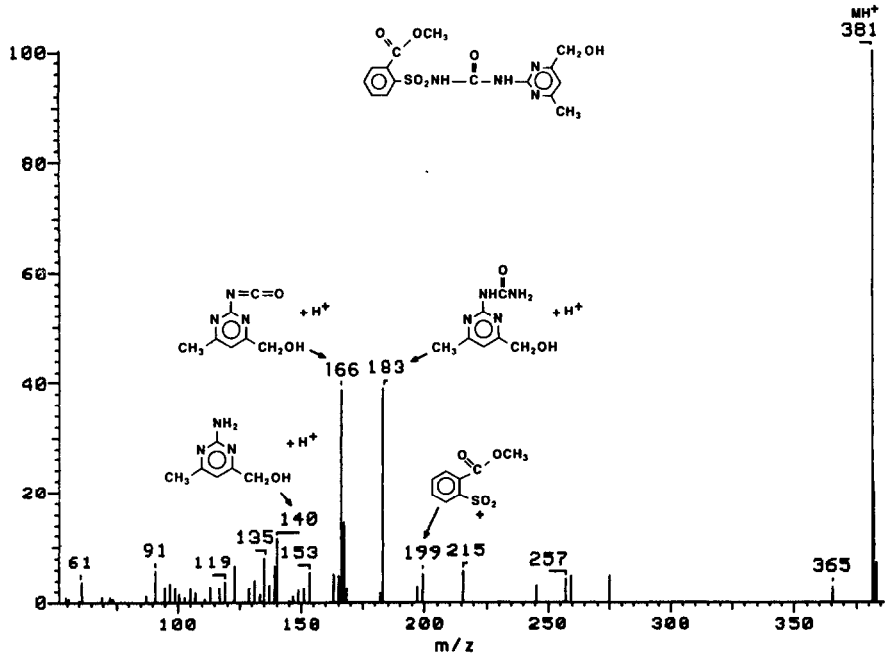

Fig. 12. FAB mass spectrum of hydroxylated sulfometuron methyl.

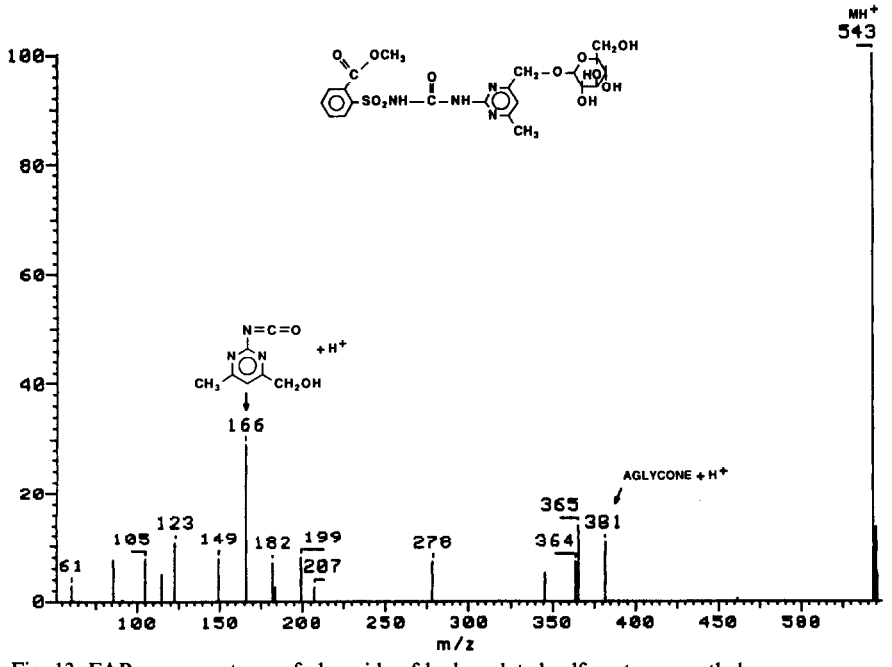

Fig. 13. FAB mass spectrum of glucoside of hydroxylated sulfometuron methyl.

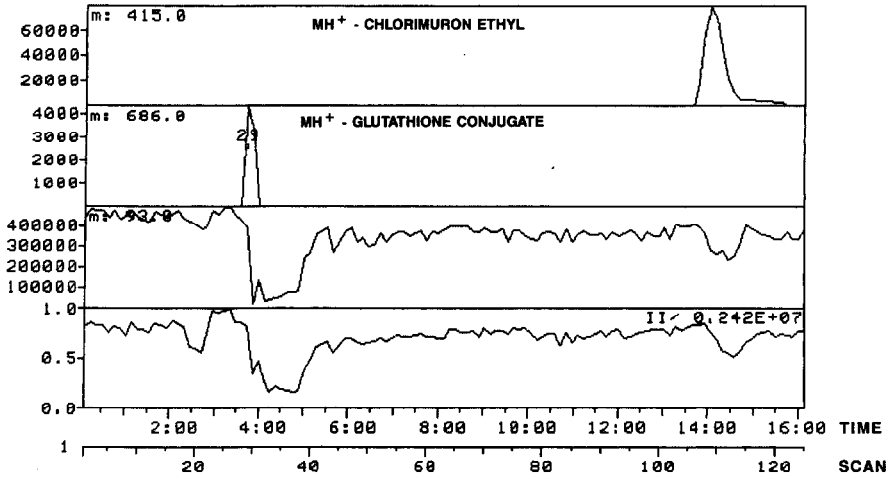

Fig. 14. Separation of chlorimuron ethyl and its glutathione conjugate using LC–FAB-MS. Column: 15 cm × 0.25 mm I.D. Nucleosil ODS. Mobile phase as in Fig. 4.

impressive result for the glucoside metabolite, since this is very polar and extremely thermally labile, and molecular ions were not obtained with ammonia CI or thermospray ionization. The FAB spectra also give some structural information, with fragment ions due to the protonated pyrimidine amine, isocyanate and urea and a weak fragment at *m/z* 199 due to the methyl benzoate sulfonyl ion, as shown in Figs. 11–13.

Analysis of chlorimuron ethyl and plant metabolite

Chlorimuron ethyl, the active ingredient in Classic® herbicide, forms a glutathione conjugate in plants through displacement of the chlorine atom. The LC–FAB-MS chromatographic data obtained on a mixture of chlorimuron ethyl and its glutathione conjugate are shown in Fig. 14, and the background subtracted FAB spectra are shown in Figs. 15 and 16. The spectra show prominent protonated molecular ions and [M + Na^+] adducts, and characteristic fragment ions. The glutathione conjugate did not show molecular ion adducts by LC–CI-MS or thermospray ionization[10], again showing the value of LC–FAB-MS for identification of polar conjugates.

CONCLUSIONS

The LC–EI-MS, LC–CI-MS and LC–FAB-MS techniques described in this paper have provided the high sensitivity and specificity needed for the identification of unknown metabolites obtained in environmental studies of agricultural chemicals. LC–FAB-MS is particularly valuable for obtaining molecular weights of highly polar metabolites that cannot be obtained by EI, CI or thermospray ionization techniques. We are using these techniques daily to identify unknown metabolites obtained in environmental studies of agricultural chemicals.

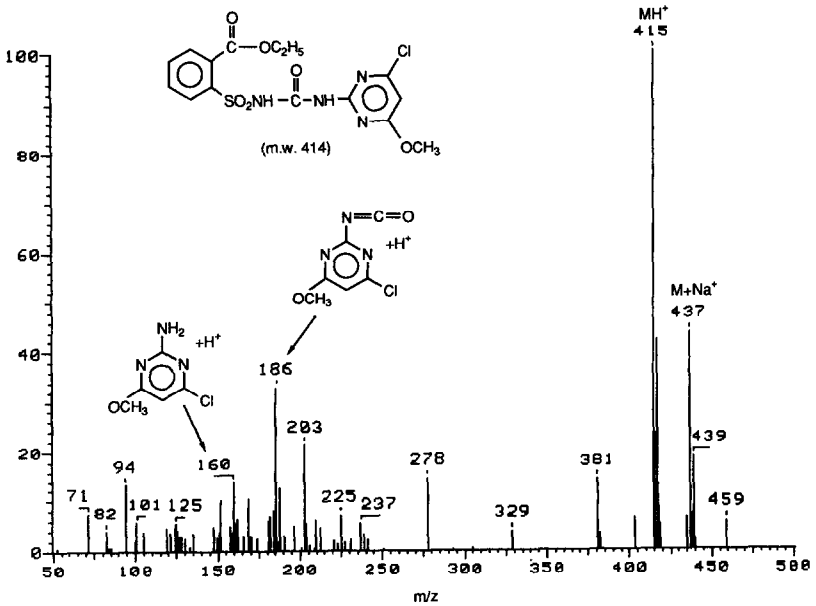

Fig. 15. FAB mass spectrum of chlorimuron ethyl.

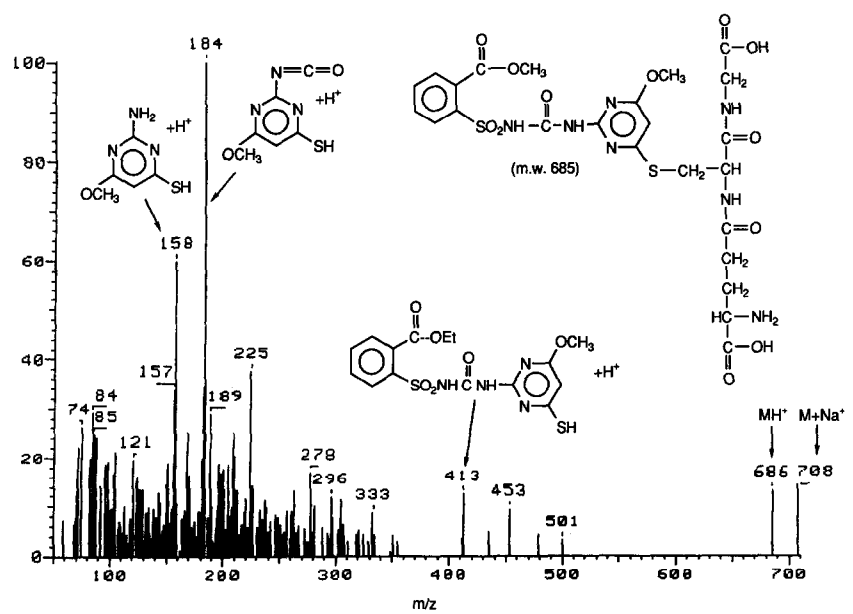

Fig. 16. FAB mass spectrum of glutathione conjugate of chlorimuron ethyl.

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