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PACKED CAPILLARY LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY USING BOTH DIRECT-COUPLING AND MOVING-BELT INTERFACES*

A. C. BAREFOOT and R. W. REISER*
E.I. du Pont de Nemours and Company, Inc., Agricultural Products Department, Building 402, Room 3110, Experimental Station, Wilmington, DE 19898 (U.S.A.)
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SUMMARY

Packed fused-silica columns of 0.25-mm I.D. have been interfaced to a quadrupole mass spectrometer by direct coupling and to a magnetic mass spectrometer with a moving-belt interface. Electron impact (EI) and chemical ionization (CI) spectra were obtained by both techniques. Using the direct-coupling interface, we have successfully analyzed and obtained molecular ions for a number of compounds that are too thermally labile for analysis by gas chromatography–mass spectrometry. However, extremely thermally labile compounds, such as sulfonylureas, did not give MH^+ ions in their CI spectra due to thermal degradation in the capillary interface line. With the moving-belt interface, MH^+ ions were obtained for sulfonylureas in the CI mode. Better quality chromatograms and spectra, and better EI sensitivity were obtained with the moving-belt interface than by direct coupling.

INTRODUCTION

Use of packed capillary liquid chromatography (LC) columns has much potential for LC–mass spectrometry (MS) analyses. The small amount of sample used with packed capillary columns is especially important in sample-limited analyses, such as metabolism studies where small quantities of metabolites are typically isolated. Use of a 0.25-mm I.D. column will increase the peak concentration in the mobile phase by two orders of magnitude over that obtained in a conventional 4.6-mm I.D. column of equal length when the same quantity of solute is injected into each column. In addition, the low flow-rates used for packed capillary columns simplify interfacing to a mass spectrometer and eliminate many of the restrictions placed on the mobile phase when conventional columns are used.

Packed capillary LC columns have been interfaced to a magnetic mass spec-

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trometer by direct coupling in the electron impact (EI) mode, and applications to some polar, thermolabile compounds were demonstrated[1]. EI spectra were obtained by direct coupling of packed microcapillary columns (40 μm I.D.) to a quadrupole mass spectrometer[2].

Our first approach was the use of direct coupling, since we felt that the best interface is no interface. However, we had two major problems with direct coupling: poor EI sensitivity and thermal degradation of thermally labile compounds in the transfer line.

Although direct coupling is highly successful in capillary gas chromatography (GC)–MS, there are major differences between introducing the GC carrier gas and the LC mobile phase. The GC carrier gas is rarely changed, and it is inert, low-molecular-weight and easily pumped away. In LC analyses, the mobile phase is often changed, and its presence in the mass spectrometer ion source often influences the spectra. We found that EI sensitivity was poor with direct coupling due to the presence of the mobile phase.

Another problem with direct coupling is that it is difficult to obtain evaporation of the liquid at the end of the capillary interface line due to the high vacuum in the mass spectrometer[3]. When vaporization takes place inside the capillary, extremely thermally labile compounds decompose prior to ionization. To obtain a liquid jet at flow-rates of 1–3 μl/min would require an extremely small orifice, which would be very subject to plugging.

Use of a moving belt interface offers a solution to these problems, and allows one to obtain EI spectra for structural information and library searching, and molecular weight information using the chemical ionization (CI) mode for many thermally labile compounds[4]. Another advantage of the moving belt interface with a high-resolution mass spectrometer is the ability to obtain accurate mass measurements. The moving belt has also been used for on-line LC–fast atom bombardment mass spectrometry (FAB-MS)[5].

EXPERIMENTAL

Column preparation and chromatographic conditions

Columns were prepared using a modification of published techniques for packing fused-silica columns[1,6,7]. A 30 cm × 0.050 mm I.D. fused-silica transfer line (SGE, Austin, TX, U.S.A.) was cemented with epoxy into a 30 cm × 0.25 mm I.D. fused-silica column (Supelco, Bellefonte, PA, U.S.A.). DuPont Permaphase ODS (30–40 μm) was added to the column to give a 1-mm layer above the end of the 0.050-mm tube, which acts as a frit. The column was filled with methanol and then a slurry of Nucleosil® ODS (3 μm, 86 mg/ml in isopropanol) or Zorbax® ODS (3 μm, 110 mg/ml in isopropanol) was pumped into the column at an initial rate of 5 ml/min using methanol as pumping solvent. The pressure rapidly reached 400 bar and was maintained at 400 bar as the column filled (can be followed visually) and for 30 min thereafter, then the pressure was released slowly.

The columns were evaluated using a mobile phase of 100% acetonitrile (Nucleosil column) or 100% methanol (Zorbax column) with anthracene as the test compound. At a flow-rate of 1.8 μl/min the Nucleosil column was calculated to have 22 000 theoretical plates and at 1.7 μl/min the Zorbax column gave 17 000 plates.

The mobile phase was changed to acetonitrile–water (60:40) in both columns, and a terbacil solution was injected. At a flow-rate of 1 μl/min the column gave 28 000 plates, while the Zorbax column (1.5 μl/min) gave 23 000 plates.

A Beckman Model 114 M pump operated in the constant pressure mode delivered solvent to a Valco (Houston, TX, U.S.A.) injection valve fitted with a 0.1-μl internal loop. The column was connected directly to the valve using a Kel-F® or Vespel™/graphite ferrule. Dead volume in the connection was minimized through use of a spacer made of Teflon™ tubing. Column pressures were 40–120 bar, and flow-rates were 1–3 μl/min.

LC–MS interfacing and mass spectrometer conditions

Direct coupling. Direct coupling experiments were conducted with a Finnigan MAT 4500 quadrupole mass spectrometer. The 0.05-mm I.D. transfer line was introduced through the GC inlet into the ion source, which was held at 170°C. At a flow-rate of 2 μl/min, the source pressure was 0.2 Torr using the GC–CI ion volume. Addition of methane to bring the source pressure to 0.5 Torr (the optimum pressure for GC–MS–CI) resulted in lower sensitivity without an increase in the intensity of molecular ions. The probe EI ion volume was used to obtain EI spectra. Attempts to operate with no ion volume in the EI mode resulted in very poor sensitivity. The mass spectrometer was scanned from 130–500 in 6 s in CI, and 110–500 in EI.

Several attempts to interface through the direct probe inlet resulted in frequent pressure surges that caused the high vacuum interlock to shut down the mass spectrometer. Pressure surges were considerably less frequent when the GC inlet was used.

Moving-belt interface. A Finnigan MAT Model 8230 mass spectrometer equipped with a moving-belt interface was used for this work. A Kapton™ polyimide belt was used at a speed of 3 cm/s. The solvent heater and tip heater temperatures were found to be critical for sulfonylureas. The solvent heater was held at 80°C and the minimum tip heater setting was used. The 50 μm transfer line was adjusted so it just touched the moving belt, and deposition of the LC effluent onto the belt was checked visually.

In the CI mode, the belt tip was inserted into the CI ionization chamber, and the spectra obtained were similar to those obtained by desorption chemical ionization (DCI) probe. Methane, isobutane and ammonia were used as CI reagent gases. The mass spectrometer resolution was 1000, and the scan rate was 2 s per decade over a mass range of 44–550 in the EI mode and 78–700 in the CI mode. The source ion gauge reading was $4 \cdot 10^{-5}$ mm in the EI mode and $4 \cdot 10^{-4}$ in the CI mode. Ionizing voltage was 70 eV in EI and 200 eV in CI, and the source temperature was 200°C.

RESULTS AND DISCUSSION

Fig. 1 shows a separation obtained on the herbicide terbacil and its metabolites B and C using direct coupling in the CI mode, and the CI mass spectrum of metabolite C is shown on Fig. 2. The LC mobile phase acted as the CI reagent gas. MH^+ ions were obtained for all three components in this sample; however, when the extremely thermally labile sulfonylurea herbicides were analyzed by this same technique, MH^+ ions were not obtained due to degradation in the interface line. Spectra of the sul-

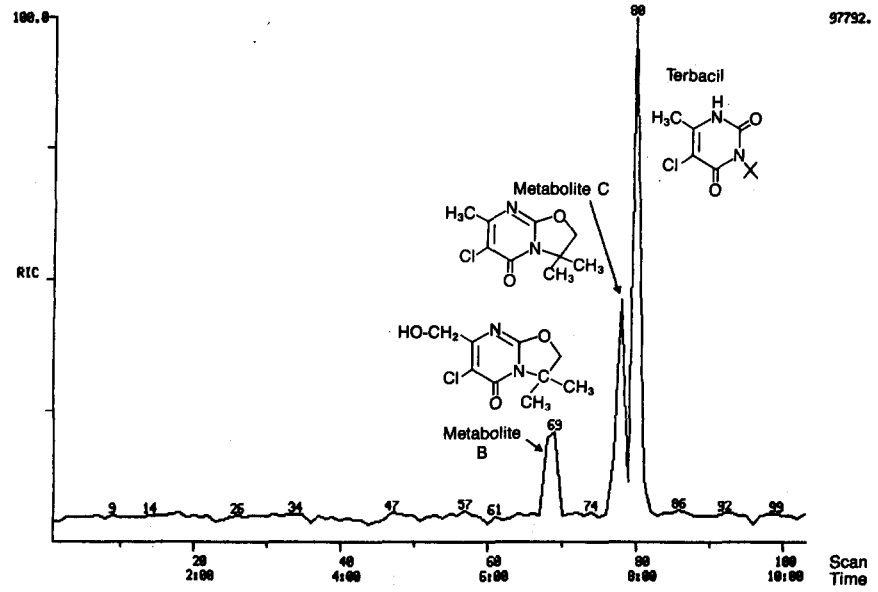

Fig. 1. Separation of terbacil and metabolites using direct-coupling CI. Sample size: 0.5 μg of each component. Column: Nucleosil ODS. Mobile phase: acetonitrile–water (80:20). RIC = reconstructed ion current.

fonamide and heterocycle amine thermal degradation products of the sulfonylureas were obtained. Lowering the ion source temperature in an attempt to reduce decomposition caused pressure surges due to freezing of the capillary tip.

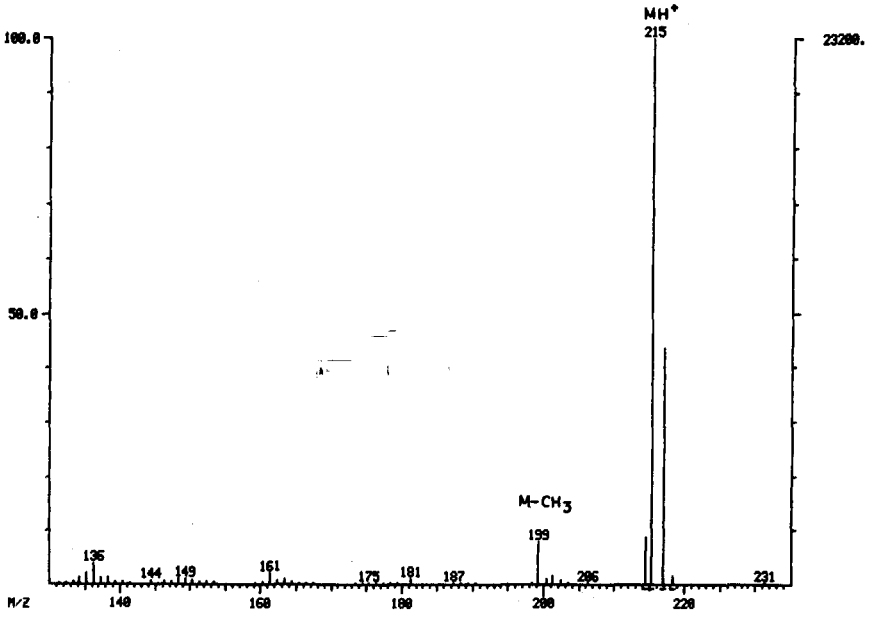

Fig. 2. CI mass spectrum of terbacil metabolite C.

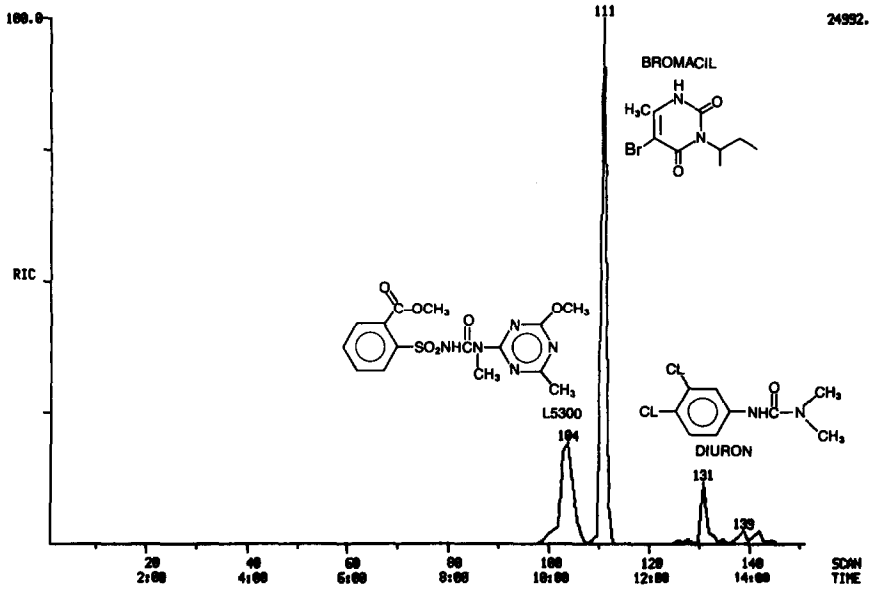

Fig. 3. Separation using direct-coupling EI. LC conditions as in Fig. 1. Sample size: 0.5 μg of each component.

A separation of three herbicides using direct coupling in the EI mode is shown on Fig. 3. Fig. 4 shows the library match (DuPont Agricultural Products Mass Spectral Library) obtained with the bromacil spectrum, which shows good agreement with the library spectrum above mass 110, the starting mass for this experiment. The

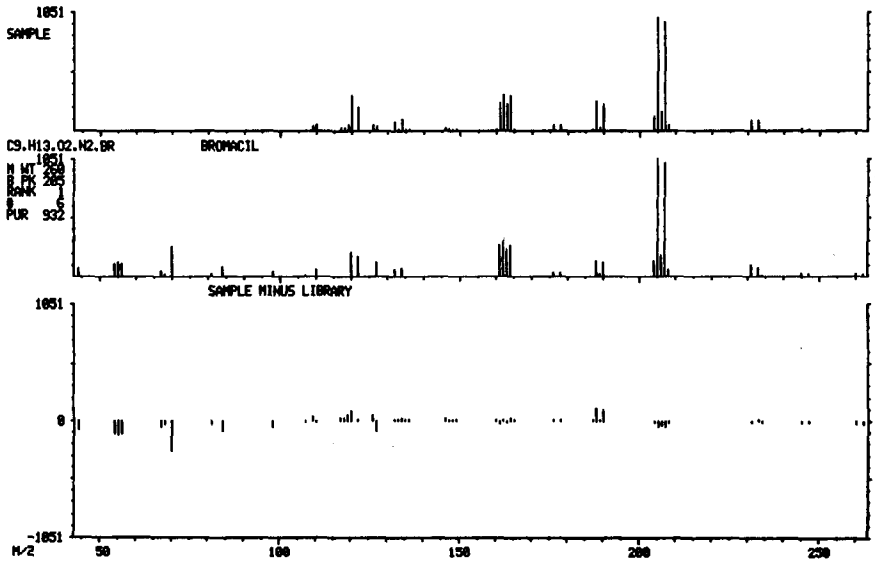

Fig. 4. Library search of bromacil EI mass spectrum.

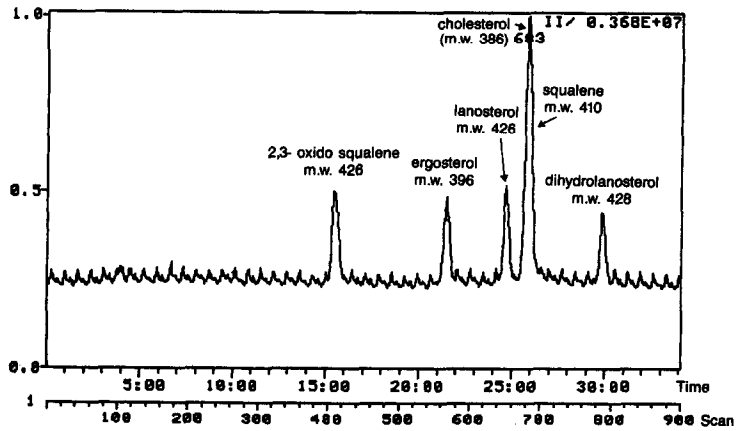

Fig. 5. Separation of a steroid mixture using the moving-belt interface EI. Column: Nucleosil ODS. Mobile phase: acetonitrile–methanol (50:50). Sample size: 0.1 μg of each compound.

scan was started at 110 to eliminate interference from mobile phase ions. Note that a molecular ion was not obtained using direct coupling, while it is present in the library spectrum obtained by direct probe. This is likely due to thermal degradation in the capillary interface.

With direct coupling we often obtained noisy LC peaks, likely due to uneven vaporization out of the capillary line. A relatively slow scan speed (6 s) was used to compensate for this, and the LC peaks shown on Figs. 1 and 3 represent only one or two mass spectra *versus* a more conventional 5–6 spectra/peak. This was not the case with the moving belt interface, and scan rates of 1–2 s per decade were used. The data obtained with packed capillary LC–MS with the moving-belt interface is similar to that obtained in GC–MS analyses.

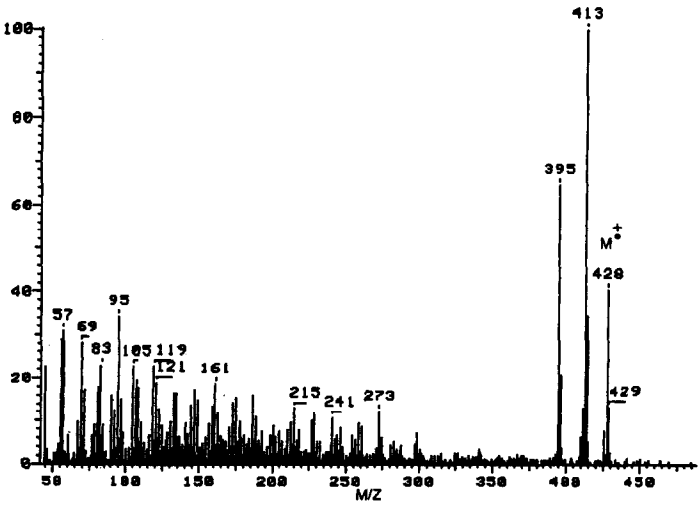

Fig. 6. EI mass spectrum of dihydrolanosterol peak.

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DM88:[300,301]M86128C.ENH;7          Scan:  788
STEROID MIXTURE,0.1UL ON-COL.              ENH#:781-795
    Library Search Results Ordered by  Purity

 1  NBS:34769  LANOST-8-EN-3-OL, (3.BETA.)-
    Purity: 837  Fit: 895  Rfit: 917  mw: 428
    CAS# 79-62-9  C30.H52.O

 2  NBS:34659  LANOSTA-8,24-DIEN-3-OL, (3.BETA.)-
    Purity: 641  Fit: 738  Rfit: 761  mw: 426
    CAS# 79-63-0  C30.H50.O

 3  NBS:34784  LANOSTANE, 11,18-EPOXY-, (11.BETA.)-
    Purity: 577  Fit: 737  Rfit: 697  mw: 428
    CAS# 52474-94-9  C30.H52.O

 4  NBS:34766  CHOLEST-14-EN-3-OL, ACETATE, (3.BETA.,17.ALPHA.)-

    Purity: 505  Fit: 784  Rfit: 685  mw: 428
    CAS# 58072-58-5  C29.H48.O2
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Fig. 7. Library search of mass spectrum shown in Fig. 6.

Fig. 5 shows a separation of a steroid mixture obtained with the moving-belt interface in the EI mode. Good sensitivity (0.1 μg of each component injected) and good quality EI spectra were obtained. The mass spectrum of the last peak in the chromatogram, dihydrolanosterol, and the library search results are shown on Figs. 6 and 7. Two of the components, cholesterol and squalene, were not separated by the LC column, but the mass chromatograms of their molecular ions showed a very slight separation, and use of background subtraction allowed us to obtain fairly good spectra of both components.

A separation of a mixture of sulfonylurea herbicides obtained with the moving belt interface in the CI mode is shown on Fig. 8 (most sulfonylureas do not have molecular ions in their EI mass spectra, but significant MH^+ ions are obtained by DCI). Each peak represents 0.1 μg of the compound injected into the LC. MH^+ ions

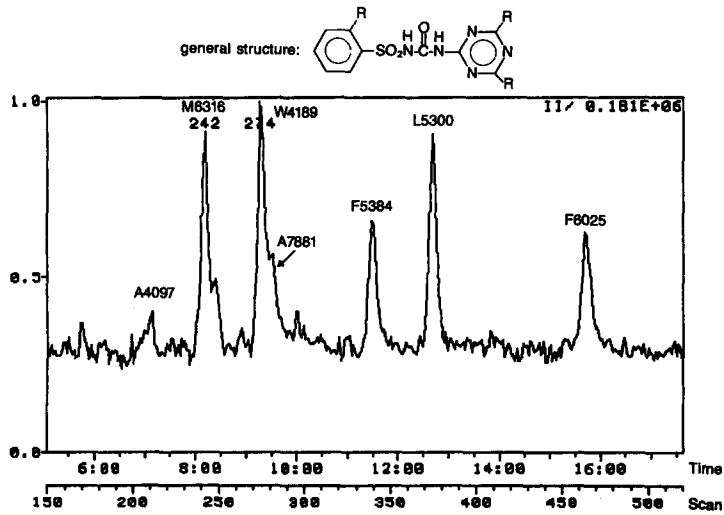

Fig. 8. Separation obtained on a mixture of sulfonylureas using the moving-belt interface, CI/CH_4. Column: Nucleosil ODS. Mobile phase: acetonitrile–water (60:40) (pH 3). Sample size: 0.1 μg of each compound.

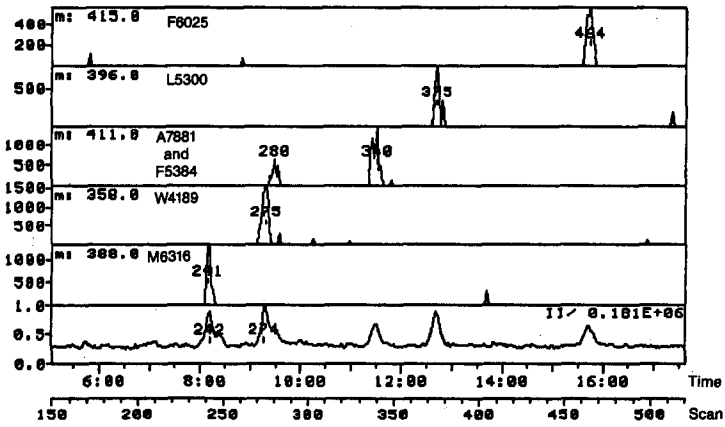

Fig. 9. Mass chromatograms of the MH^+ ions of the sulfonylurea components.

were obtained for each component, as shown on Fig. 9. The CI mass spectrum obtained for W4189 (chlorsulfuron) is shown on Fig. 10. Structurally useful fragment ions were obtained in addition to the MH^+ ion. With the very low flow-rates used with packed capillary columns, the solvent evaporator temperature on the moving-belt interface can be kept low (80°C was used), which minimizes decomposition of thermally sensitive compounds.

Figs. 11 and 12 show a comparison made between a packed fused-silica capillary column and a commercially available 1-mm I.D. stainless-steel column. A mixture of terbacil and three metabolites was injected using a 0.1-μl loop with the fused-silica column, and a 1.0-μl loop with the stainless-steel column. The sample was

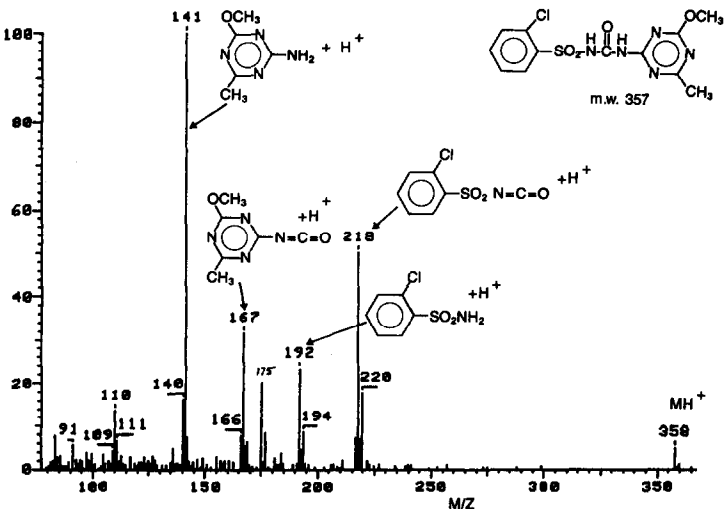

Fig. 10. CI mass spectrum of W4189 (chlorsulfuron).

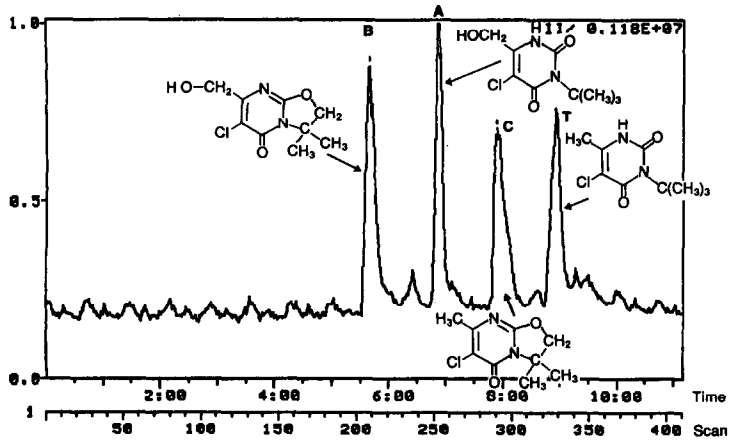

Fig. 11. Separation of terbacil and metabolites using the moving-belt interface CI. Column: 30 cm × 0.25 mm fused-silica packed with Zorbax ODS. Mobile phase: acetonitrile–water (60:40). Flow-rate: 2 μl/min. Injection volume: 0.1 μl. Sample size: 0.5 μg of each compound.

diluted ten-fold for analysis on the 1-mm I.D. column, so the same quantities were injected into each column. The response of the more polar metabolites A and B was low on the 1-mm I.D. column. These results were reproducible in both the CI and EI modes. The low response of metabolites A and B may be due to adsorptive loss on the stainless-steel wall and frits in the column. There has recently been concern about the use of stainless-steel in high-performance liquid chromatography columns due to chemical interactions with the sample or mobile phase, and it has been demonstrated that proteins are strongly adsorbed to stainless-steel frits[8].

A limitation to use of packed capillary columns is the small injection volume required. We were able to increase the amount of analyte injected by removing the

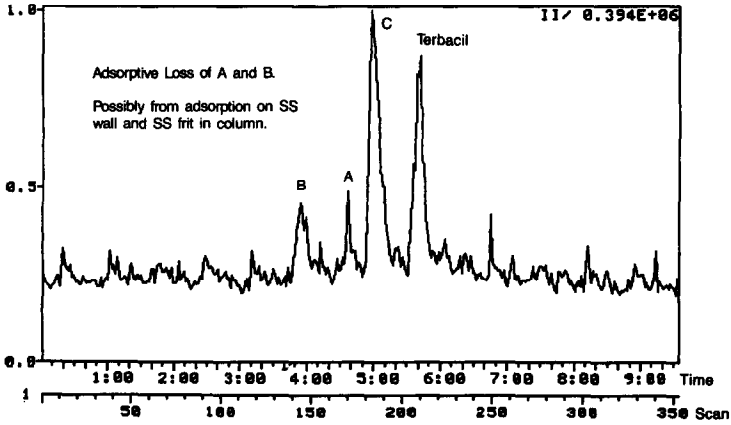

Fig. 12. Separation of terbacil and metabolites using the moving-belt interface CI. Column: 15 cm × 1 mm stainless-steel packed with Phase SEP ODS 2. Mobile phase: acetonitrile–water (60:40). Flow-rate: 20 μl/min. Injection volume: 1.0 μl. Sample size: 0.5 μg of each compound.

0.1 μl valve insert and slowly depositing 1–10 μl of sample on the loop while allowing the solvent to evaporate. The valve insert was replaced, flow restored, and the sample injected. The valve was returned to the load position after 3 s to minimize peak tailing from slow desorption of sample from the loop. This procedure allowed us to maintain the chromatographic resolution through injection of a small volume while effectively increasing sample size by a factor of 10–100.

CONCLUSIONS

Use of packed capillary LC columns with the moving-belt interface allowed us to obtain good quality EI and CI mass spectra at high sensitivity. We found the moving belt to be more trouble free and reliable than direct coupling. The ability to obtain good quality EI and CI spectra allows more conclusive identification of unknowns.

The sensitivity and ease of use obtained with packed capillary LC–MS moving-belt interface is approaching that currently obtained with capillary GC–MS.

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