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Evaluation of the particle beam interface for packed-column supercritical fluid chromatography–mass spectrometry with pure and modified CO_2

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Abstract

The particle beam interface was evaluated for microbore packed-column supercritical fluid chromatography–mass spectrometry coupling. The experiments were conducted with mobile phase of pure CO_2 and methanol-modified CO_2 under flow injection and chromatographic conditions. The effects of various operational parameters were characterized. The electron impact limits of detection (LODs) of the system for single ion monitoring and scan modes were determined to be 5 and 40 ng for caffeine, respectively. These LODs are 2.5 to 25 times better than previous reported estimates. Sensitivity was found to be highly dependent on the modifier percentage with 4% methanol-modified CO_2 yielding optimal results. Separations of phenylurea pesticides and steroids are shown as demonstrations of system utility. Background subtracted electron impact spectra were artifact-free and comparable with on-line library spectra.

1. Introduction

Mass spectrometric (MS) detection for chromatography is most desirable considering the plethora of detectors. Besides Fourier transform IR and NMR, no other chromatographic detector can provide both quantitative and structural information. Specifically, electron impact (EI) MS is most useful because of the extensive fragmentation resulting in unique compound spectra. These spectra maybe subjected to a comparison with computerized library spectra thereby making analyte identification easier. The specificity of EI spectra is in contrast to other "softer" methods of ionization (thermospray, chemical ionization) which yield spectra characterized by little or no fragmentation. Because of their speed and simplicity, on-line detection (chromatography–MS) is most desirable. Although GC–EI-MS and LC–EI-MS are well established analytical tools, this is not true for other forms of chromatography such as supercritical fluid chromatography (SFC). The goal of a robust and sensitive interface for packed column SFC–EI-MS has been especially elusive. A certain degree of success has been achieved for capillary SFC–EI-MS [1–5]. Even in those experiments, however, some of the resulting spectra were not true EI spectra but were composites of charge-exchange and EI spectra [1,3,4]. The

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sensitivity for these capillary systems was in the pg range for scan data which were comparable to GC–EI-MS. A similar robust and practical solution for packed-column SFC has not been demonstrated. To date two interfaces have been used for packed-column SFC–EI-MS: the moving belt interface and the particle beam (PB) interface. Games and co-workers [6–10] have shown successful coupling via the moving belt interface with applications ranging form steroids and bile acids to antibiotics. The sensitivity for scan EI was reported in the low nanograms. This particular solution is not very desirable because of the inherited non-robustness of the introduction method, the mechanical complexity of the interface, decomposition of thermally labile analytes, and problems with quantitative transfer of non-volatile analytes [11–14]. None of these difficulties hampered the PB interface to the same extent since this interface is mechanically simpler. However, the PB interface does present two important disadvantages: complex optimization and poor mass transfer to the ion source which results in a limited sensitivity.

Two research groups have investigated the PB interface for packed column SFC–EI-MS coupling. The work of Edlund and Henion [15] was performed on a laboratory-made prototype PB interface coupled to a Hewlett-Packard 5985 mass spectrometer. In these experiments no optimization was performed, thus the authors estimated sensitivities in the low micrograms for full scan EI. Separations on analytical-scale (4.6 mm I.D.) and narrow-bore (2.0 mm I.D.) columns with methanol-modified fluids were presented. The utility of the system was demonstrated with applications for the following classes of compounds: pesticides, polymer additives, steroids and xanthines. These separations were accomplished under isobaric conditions at liquid CO_2 mobile phase flow-rates of 0.5 to 1.5 ml/min. Except for the polymer additive separation, the mobile phases were isoconfertic. The mass spectra were artifact-free and comparable to those obtained under GC–EI-MS conditions. In order to facilitate aerosol formation and transmission of analyte through the interface, all data were collected with methanol introduced into the mobile phase post-column and pre-nebulizer.

Browner and co-workers [16,17] demonstrated PB application in SFC–MS with both packed and open tubular columns. This work was performed employing a laboratory-constructed interface coupled to a VG ZAB-E double focusing mass spectrometer. All data were acquired with methanol or acetonitrile introduced prior to nebulization of the mobile phase. Extract of marine sediment was separated on an open tubular column (50 μm I.D.) with pressure programming and a mobile phase flow-rate of 1–5 μl/min of pure liquid CO_2. Packed-column separations of ω3 fatty acids and isomers of pyrrolizidine alkaloids were reported. EI spectra of the compounds were presented with no indication of solvent interference. The detection limit of the system was estimated to be between 100 ng to 1 μg for cholesterol (full scan).

In this paper a performance evaluation of a commercial PB-MS system is presented. The goal of these studies was to evaluate the performance of the PB interface with pure CO_2 and methanol-modified CO_2 as the mobile phase. All experiments were performed without the addition of particle forming solvents prior to nebulization as was done in the referenced studies. Elimination of the particle-forming solvent greatly simplifies the system by eliminating the need for a secondary pump and other hardware. Furthermore, pressure or density programming maybe used without the need for synchronization of CO_2 and particle forming solvent pumps during pressure/density ramps. Parameters that affect the performance of the PB were evaluated. Optimization of the nebulization process was investigated by examining the effects of restrictor position and nebulizing gas pressure on sensitivity. The effect of CO_2 flow-rate and percent of modifier were assessed for their impact on sensitivity. Quantitative EI data are also presented for scan and single ion monitoring. Separations of steroids and phenyl urea pesticides are used to demonstrate the utility of this system for compound identification through artifact-free, library searchable EI spectra.

2. Experimental

2.1. Instrumentation

A Model 5988A MS (Hewlett-Packard, Palo Alto, CA, USA) was coupled via a Model 59980A PB interface (Hewlett-Packard) to the SFC system. For basic studies the SFC system was the Model 200A (Suprex, Pittsburgh, PA, USA) consisting of a syringe pump, oven, injector and dedicated controller. The injection loop was 0.5 μl in volume. For the CO_2 flow-rate study, a Model 100D syringe pump (Isco, Lincoln, NE, USA) was used which consisted of a pump with a controller and an injector (Valco, Houston, TX, USA). The separations of steroids and phenylurea pesticides were generated using a Model 600 Dionex SFC system (Salt Lake City, UT, USA). This system consisted of a syringe pump, oven, 0.5-μl injector and personal computer. All chromatography was performed on a Deltabond CN packed column (100 × 1 mm, 5 μm particle size) (Keystone Scientific, Bellefonte, PA, USA). Pressure in these systems was maintained by a 40 cm linear (25 μm I.D.) capillary restrictor constructed of deactivated fused silica (SGE, Austin, TX, USA). The restrictor was installed in the nebulizer of the PB so that the restrictor tip was even with the orifice of the stainless-steel tube nebulizer (Fig. 1).

2.2. Chemicals

Caffeine and steroids (Sigma, St. Louis, MO, USA) were used as received. Standard solutions of caffeine and steroids were prepared in HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA, USA) and filtered through a 0.2-μm membrane PTFE filter (Supelco, Bellefonte, PA, USA). Solutions of the phenyl urea pesticides (Aldrich, Milwaukee, WI, USA) were prepared in HPLC-grade methylene chloride (Fisher Scientific). SFC-grade CO_2 and methanol-modified CO_2 were obtained from Scott Specialty Gases (Plumsteadville, PA, USA).

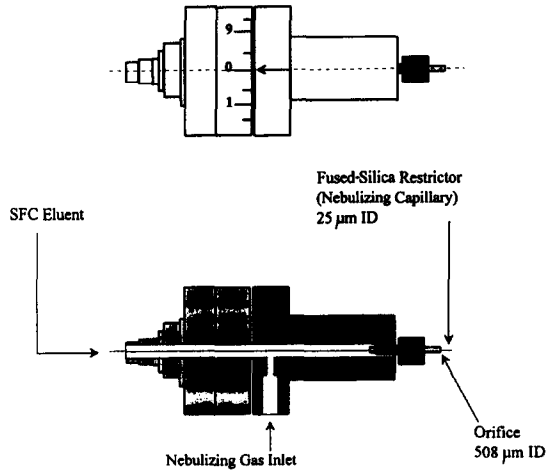

Fig. 1. Schematic of the nebulizer.

3. Results and discussion

3.1. Principle of operation

The PB interface traditionally allows for coupling of the SFC or LC chromatograph to a mass spectrometer by (a) forming an aerosol, (b) separating the analyte from the solvent, (c) concentrating the analyte into a beam and (d) transporting the analyte to the ion source of the mass spectrometer. Following the exit from the column, the effluent is introduced into a pneumatic nebulizer. The nebulizer's function is to generate a fine aerosol of droplets. This aerosol traverses the desolvation chamber, allowing for volatile solvent to evaporate thereby forming optimum size droplets of about 6–15 μm in diameter [18]. This mixture of solvent vapor and analyte droplets encounters the momentum separator. The function of the momentum separator is to separate droplets and vapor based on momentum. Taking advantage of the difference in momenta, the higher-momentum analyte particles are transmitted while the low-momentum vapor is pumped away. From the momentum separator, analyte particles travel through the transfer tube into the ion source where analyte is vaporized, ionized, and focused into the analyzer where detection of ions occurs. For

optimum performance of the interface, a number of parameters must be considered.

Pneumatic nebulization is employed (Fig. 1) to generate an aerosol of droplets. Nebulization occurs due to coaxial flow of helium gas that is introduced before the exit of the nebulizing capillary and that flows concentrically the length of the capillary. Droplets are formed as the nebulizing gas encounters the effluent at the point of exit from the capillary causing shearing of droplets. The effect of the nebulizing gas on sensitivity may be studied by changing the helium gas pressure which in turn affects the size and distribution of the droplets [18–22]. Changing the pressure of the nebulizing gas alters the pressure in the desolvation chamber and consequently alters the efficiency of the desolvation process. Another parameter important in sensitivity optimization is the position of the nebulizing capillary relative to the orifice of the nebulizer. The position of the nebulizing capillary determines the aerosol characteristics of the resulting spray.

The aerosol formed by the nebulizer is sprayed into the desolvation chamber which is a hollow, thermostatically controlled cylinder. The desolvation chamber allows time and effective thermal conduction to occur. Since the desolvation chamber is maintained at a pressure of about 200 Torr (1 Torr = 133.322 Pa), thermal conduction is maintained between the walls of the desolvation chamber and the aerosol droplets. The desolvation chamber temperature influences the size of the droplets and consequently the efficiency of the analyte transport.

At the end of the desolvation chamber the solvent vapor and analyte droplets are separated and concentrated by the momentum separator. The momentum separator consists of a nozzle and a pair of skimmers. The function of the nozzle is to focus the mixture of vapor and droplets formed in the desolvation chamber. Because of the difference (a 40-fold reduction) in pressure, a supersonic jet is created at the exit of the nozzle. As a consequence of the adiabatic cooling by the supersonic jet, the droplets become particles, hence the name particle beam. Some distance from the nozzle orifice a mach

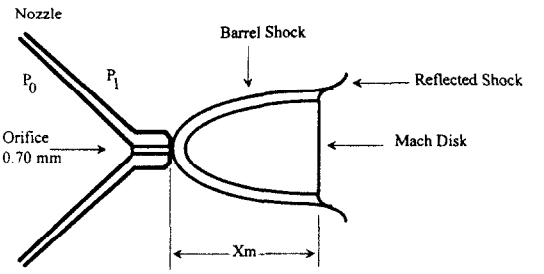

Fig. 2. Diagram of the supersonic jet expansion formed at the nozzle.

disk is formed where the flow makes a transition from supersonic to subsonic velocity. A diagram depicting the supersonic jet is shown in Fig. 2. The distance from the nozzle to the mach disk (X_m) may be predicted by Eq. 1

$$X_m = 0.67 D_n \left(\frac{P_0}{P_1}\right)^{0.5} \quad (1)$$

where D_n is the diameter of the nozzle (0.70 mm), P_0 is the upstream pressure of the gas (200 Torr) and P_1 is the downstream pressure in the expansion region (5 Torr) [23]. The first skimmer is positioned to be just within the distance to the mach disk. At this distance sampling of the particle beam is maximized and the vapor is skimmed off. The volume between the nozzle and the first skimmer is referred to as the first stage of this differentially pumped interface. The second stage of the particle beam exists between the first and second skimmer. At the second stage, there is a reduction in pressure (about 20-fold), resulting in supersonic jet formation. The second skimmer, like the first, samples most of the beam excluding the vapor which gets pumped away by the second-stage mechanical pump. Following the momentum separator, the beam of particles is transported by the transfer tube into the ion source where vaporization and ionization occur. The ions are focused into the quadrupole and detected.

3.2. Optimization under SFC conditions

In the following sections the results of studies of each of the parameters discussed above are

evaluated under SFC conditions. Although the PB interface has been under scientific evaluation for a number of years in different variations (i.e. Thermabeam, MAGIC, Universal interface), the operation of PB at first principles has not been yet achieved. Much of the available data is in qualitative form. However, some areas of the PB have received more attention (i.e. supersonic jet formation and sampling) therefore we hope to use the available principles to give a more concrete description of the operating mechanism(s). Qualitative interpretation of the data is given for the most part. Furthermore, since detection with methanol-modified CO_2 mobile phase was only possible, liquid-like nebulization is assumed. The data were acquired under flow injection conditions (FIA). The mass spectrometer was operated in single-ion monitoring mode; monitoring the 1941 u molecular ion of caffeine. Each data point is the average of at least three replicates. The relative standard deviation (R.S.D.) for these points ranged from 4 to 8%.

Position of the restrictor relative to the end of the nebulizer

At positions far outside of the nebulizer orifice (position >5), a single liquid jet with little divergence was visually observed. The sensitivity decreased in this range due to large droplets formation. Large droplet formation has been shown to be favored by increasing the distance between nebulizer and nebulizing capillary due to a decrease in the interaction between the nebulizing gas and mobile phase flow [18,20–22]. Large droplets are, however, inefficiently transported due to impaction within the interface and gravitational settling. Just outside the nebulizer orifice (position 5), the nebulizing gas and the emerging mobile phase interacted most favorably to yield maximum signal intensity. With the nebulizing capillary within the nebulizer assembly, another optimum setting was found where the flow of the helium gas was least turbulent and formed an efficient aerosol. Beyond this optimum, sensitivity decreased because the analyte was sprayed on the inside of the nebulizer and resulted in inefficient transport of the analyte. This decrease in transport efficiency was observed due to the buildup of caffeine on the inside of the nebulizer. Direct comparison of these data with those obtained under LC conditions is difficult because of the difference in nebulization conditions (nebulizing capillary I.D., mobile phase flow-rate and composition). Nonetheless, under LC conditions the response surface of nebulizing capillary position versus signal exhibits a difference between the peaks and the valleys from 30 to 50% [24,25], whereas these data under SFC indicate less of a range (15 to 20%) between the peaks and the valleys. These results indicate, as might be expected, that the decompressing mobile phase participates in self nebulization. However, this self nebulization alone is not sufficient for efficient transport of analyte through the PB as indicated by the data in the following section.

Pressure of the nebulizer gas

The explanation of the effect of nebulizing gas pressure on signal intensity maybe attributed to changes in the following parameters: size of droplets, desolvation of droplets and sampling efficiency of skimmer 1. It has been shown that with increasing nebulizing gas pressure droplets size decreases [18,20–22]. In this experiment optimum conditions were attained at 45 p.s.i. (1 p.s.i. = 6894.76 Pa) resulting in production of droplets which were effectively transported. Above 45 p.s.i., the produced droplets were too small to be effectively transported through the interface and were pumped away with the vapor.

As the pressure in the desolvation chamber rose due to increasing nebulizing gas pressure, the efficiency of the desolvation process changed. With increasing desolvation chamber pressure, thermal conductivity between chamber walls and the droplets increased. Increases in thermal conductivity resulted in more efficient desolvation of droplets. This relationship was favorable until optimum conditions at a pressure of 45 p.s.i. were reached. Beyond the optimum, signal intensity decreased because of excessive desolvation of droplets. Sampling efficiency of skimmer 1 is dependent on the position of the mach disk. The position of the mach disk (X_m) is determined by Eq. 1. With the rise in desolvation

chamber pressure, the distance from the nozzle to the mach disk increases. An increase in X_m translated into changes in the sampling efficiency of skimmer 1. As the pressure in the desolvation chamber increased, sampling efficiency of skimmer 1 increased and eventually became optimum. Beyond the optimum nebulizing gas pressure, signal intensity decreased due to the influence of an other factor: droplet size reduction as the result of increased desolvation efficiency.

Temperature of the desolvation chamber

The effect of desolvation chamber temperature has been shown to result in decreasing droplet size with increasing temperature [18,21,22]. For our work, the optimum temperature for the desolvation chamber was between 30 to 45°C. In this range the droplets were not excessively desolvated, and droplet distribution was optimum. At temperatures above 45°C, the efficiency of the desolvation process increased resulting in a decrease in the size of the produced droplets. The reduction in droplet size translated into reduction in the efficiency of the momentum separator to transmit smaller and smaller droplets.

Distance of the nebulizer relative to the opening of the nozzle

Since the data on the effect of the desolvation chamber temperature have shown that no extra energy was required to increase the effectiveness of the desolvation process, the effect of the distance of the nebulizer to the nozzle was investigated. The distance between the nebulizer and the opening of the nozzle was changed by increasing the length of the nebulizer. As a consequence of the change in the length of the nebulizer, the time that the droplets spent in the desolvation chamber also was altered. Increasing the length of the nebulizer effectively decreased the time the aerosol was resident in the desolvation chamber. The reduction in time resulted in decreased effectiveness of the desolvation process [21,22]. Large droplets were formed that were too large to be transported to the nozzle orifice. The loss in sensitivity occurred because these droplets impacted in the desolvation chamber in the region in front of the nozzle orifice. Losses occurred also to the preferential loss of these larger droplets to gravitational settling. Also it has been calculated that the Reynolds number (*Re*) at the nebulizer (14 000) exceeds the critical value (2000–3000), thus indicating turbulent flow [22]. Laminar flow is promoted by lengthening the distance for the aerosol to travel. For these reasons, the most effective position of the nebulizer was, therefore, found to be at the maximum distance from the nozzle.

3.3. Mobile phase flow-rate

Since the separation in SFC is achieved (assuming a fixed restrictor) by increasing the pressure (or density) of supercritical fluid while allowing the flow-rate to increase, the effect of mobile phase flow-rate on sensitivity is an important consideration. Furthermore, column selection (I.D.) is dependent on the operating flow-rate of a detector. As the liquid flow-rate of the CO_2 was increased beyond 0.64 ml/min, the efficiency of the interface decreased and so did the sensitivity (Fig. 3). The reasons for the decrease in efficiency and sensitivity were due to inefficient nebulization and increased aerosol impact at high mobile phase flow-rates. As the CO_2 flow-rate increased, the contribution of CO_2

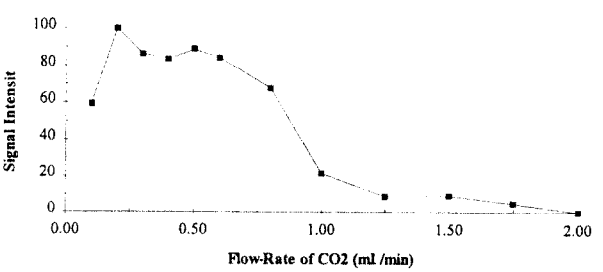

Fig. 3. Effect of liquid CO_2 flow-rate on signal intensity. Conditions: Isco SFC system; 100% CO_2; PB conditions: restrictor position, 5; desolvation chamber temperature, 40°C; nebulizing gas pressure, 45 p.s.i.. FIA of 0.5-μl injection of 100 ng/μl caffeine in methanol. Other conditions given in Experimental section.

to nebulization increased in an adverse manner. With increased flow-rate, a decrease in nebulization efficiency was observed due to the formation of larger droplets. At mobile phase liquid flow-rates greater than 1.0 ml/min, droplets on the underside of the nebulizer and scattering of droplets perpendicular to the nebulizing gas flow were observed. Furthermore, with increasing CO_2 flow-rate, divergence of the spray was observed that resulted in the impact of aerosol on the walls of the desolvation chamber as well as on the nozzle. For these reasons, a reduction in signal intensity was observed as the analyte transport through the PB and into the mass spectrometer decreased. At 0.10 ml/min, the decrease in sensitivity was due to formation of very small droplets. These smaller droplets were inefficiently transported and resulted in a signal decrease. For liquid flow-rates between 0.1 ml/min to 0.64 ml/min, the response was nearly constant. This range is sufficient for optimum operation of 1.0 and 2.0 mm I.D. packed columns.

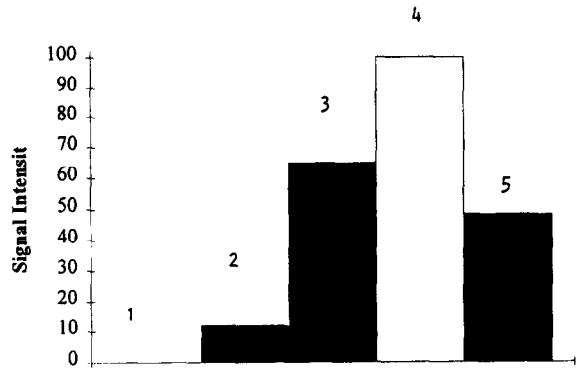

Fig. 4. Effect of percent modified CO_2 on signal intensity. Conditions: Dionex SFC system; PB conditions: restrictor position, −4; desolvation chamber temperature, 40°C; nebulizing gas pressure, 45 p.s.i.. Column: Deltabond CN 100 × 1 mm; isobaric, 75°C; 0.5-μl injection of 400 ng/μl caffeine in methanol. Other conditions given in Experimental section. 1 = Pure CO_2; 2–5 = methanol-modified CO_2: 2 = 1%, 3 = 2%, 4 = 4% and 5 = 8% methanol.

3.4. Mobile phase composition

The effect of percent modified CO_2 was evaluated at four modifier concentrations (mol/mol). Methanol-modified CO_2 was delivered from premixed CO_2 cylinders. The caffeine sample was chromatographed isobarically on a Deltabond CN column with a liquid flow-rate of 0.2 ml/min. As shown in Fig. 4, with increasing modifier percentage (0 to 8%), the signal intensity increased to an optimum at 4% methanol-modified CO_2. At 8% the signal intensity decreased to less than half of the signal intensity at 4%. With pure CO_2 as the mobile phase, no response was observed which is consistent with the available data concerning droplet formation on decompression with pure CO_2. Randall and Wahrhaftig [23] have derived an empirical relationship which allows for estimation of the average number of molecules in a particle (N)

$$N = 6 \cdot 10^{11} P_{0,SF}^{1.44} D_n^{0.86} T_0^{-0.54} \quad (2)$$

where $P_{0,SF}$ is the pressure of the supercritical fluid in Torr, D_n is the diameter of the restrictor orifice in mm and T_0 is the temperature of the supercritical fluid in K [23]. For typical conditions $P_{0,SF} = 304\,117$ Torr (or 395 bar), $D_n = 0.025$ mm and $T_0 = 313$ K, the average cluster size is about $6.6 \cdot 10^4$ molecules which results in a particle size of 0.12 μm in diameter. The typical droplet size distribution under LC conditions was found to range from 6 to 15 μm [18,19]. Therefore, the resulting decrease in the size of the particles (50- to 125-fold reduction) explains the lack of sensitivity when using pure CO_2. The data (Fig. 4) suggest that the introduction of modifier fosters improved aerosol generation and an increase in particle size as indicated by the increase in signal from 1 to 4% modified CO_2. However, at 8% modified CO_2, the decrease in signal may be due to losses of analyte in the nebulization and desolvation processes as a consequence of further increase in particle size as well as dilution of the analyte with the increased amount of modifier. Further studies characterizing the aerosol (particle size and distribution) as a function of modifier concentration are needed to ascertain the exact influence of modifier on sensitivity.

3.5. Sensitivity

Following particle beam optimization, the sensitivity of the SFC–PB-MS system was evaluated. Caffeine was chromatographed isobarically and isoconfertically with 4% methanol-modified CO_2. Data for both single ion monitoring (SIM) and full scan (150 u wide) were acquired with an ion source temperature at 250°C. The mass spectrometer was calibrated with perfluorotributylamine (PFTBA) and tuned to maximize the 219 u peak of PFTBA. The data for SIM are shown in Fig. 5 for the 194 u molecular ion of caffeine. The calibration curve was non-linear ($r = 0.986$) which is consistent with the reported observations by other workers under LC–MS conditions [25–28]. The experimentally evaluated limit of detection (LOD) was 5 ng caffeine injected at S/N 3. The correlation coefficient for scan (150 u wide) acquired data was 0.995 and the LOD was experimentally found to be 40 ng injected at S/N 3. A chromatogram of three replicate injections of 40 ng caffeine is shown in Fig. 6. The reproducibilities for integrated signal ranged from 2 to 10% R.S.D. at the high and low ends of the calibration plots. These obtained detection limits are 2.5 to 25 times lower than the best reported estimates of LOD by other researchers [15,16].

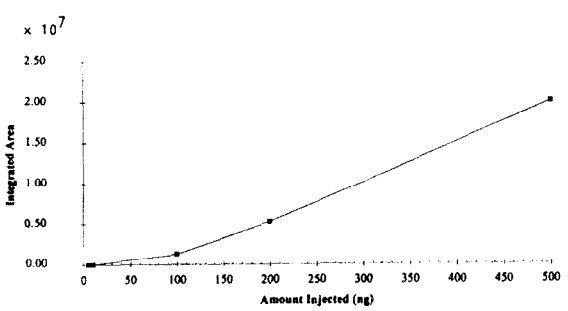

Fig. 5. Calibration curve for caffeine with SIM. Conditions: Dionex SFC system; column 100 × 1 mm I.D. Deltabond CN maintained at 60°C; mobile phase 4% methanol-modified CO_2, 197 bar, and 0.20 ml/min liquid flow; PB conditions: nebulizing gas pressure, 40 p.s.i.; desolvation chamber temperature, 40°C; restrictor position, −3. Other conditions given in Experimental section.

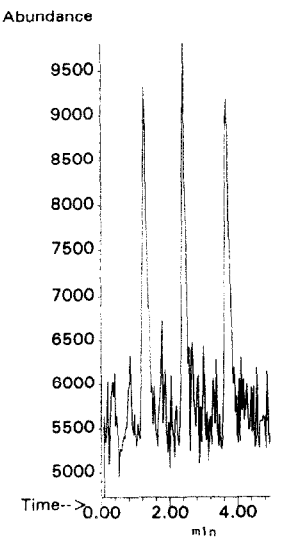

Fig. 6. Chromatogram of three replicate injections of caffeine at 40 ng.

3.6. Applications

The ability to positively identify unknown compounds by virtue of unique EI spectra is the biggest advantage of SFC–PB-MS. An additional advantage of packed-column SFC is that very fast and efficient separations may be generated. Also, due to the mild conditions of SFC, thermally labile compounds maybe separated. Because of their thermal decomposition phenylurea pesticides may not be separated and analyzed by GC–MS directly. Thus, the analysis of these pesticides demonstrates the capabilities of the present system. Fig. 7 shows a separation of six phenylurea pesticides in less than 5 min. The separation was accomplished using a 10 cm × 1.0 mm I.D. microbore column with 4% methanol-modified mobile phase under isobaric conditions. Figs. 8 and 9 show background-subtracted and library spectra of monuron and siduron, respectively. Characteristic of phenylurea pesticides is fragmentation yielding a base peak at 61, 72 or 93 u and a molecular ion peak at less than 50% ion intensity. These base peak fragments are generated by the cleavage of the carbonyl carbon–nitrogen bond. In the case

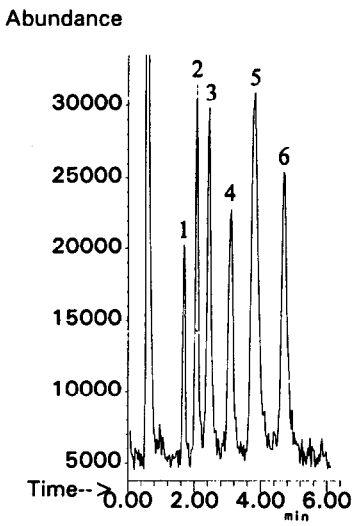

Fig. 7. Separation of phenylurea pesticides. Peaks: 1 = metobromuron; 2 = linuron; 3 = chlorbromuron; 4 = monuron; 5 = siduron; 6 = diuron. Conditions: Dionex SFC system; column 100 × 1 mm I.D. Deltabond CN maintained at 85°C; 4% methanol-modified CO_2; 197 bar; flow-rate, 0.20 ml/min liquid flow; injection 0.5 μl of 1.0 μg/μl per component; PB conditions: restrictor (linear, 25 μm I.D.) position, −4; desolvation chamber temperature, 40°C; nebulizing gas pressure, 45 p.s.i.. Other conditions given in Experimental section.

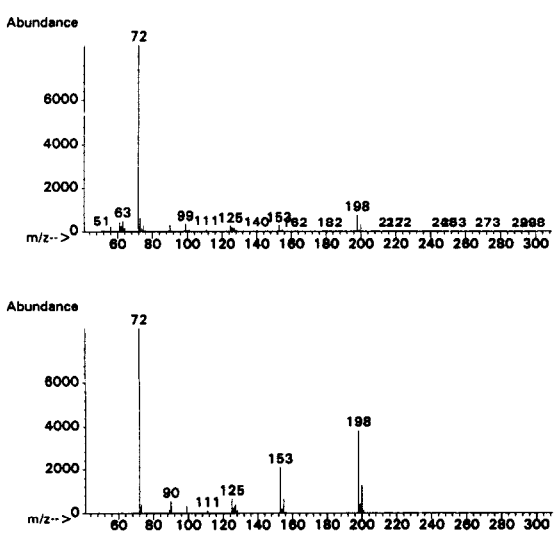

Fig. 8. EI spectrum of monuron (top) and library spectrum (bottom).

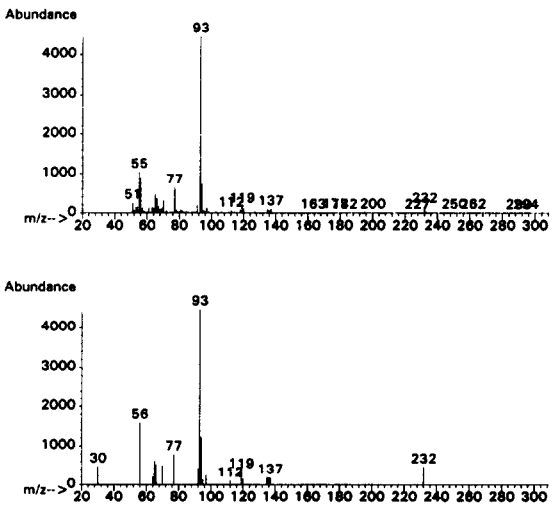

Fig. 9. EI spectrum of siduron (top) and library spectrum (bottom).

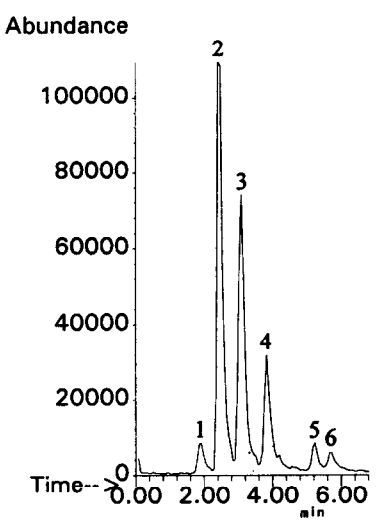

Fig. 10. Separation of steroids. Peaks: 1 = cholesterol; 2 = progesterone; 3 = testosterone; 4 = 17a-hydroxyprogesterone; 5 = 11-deoxycortisol; 6 = corticosterone. Conditions: Dionex SFC system; column 100 × 1 mm I.D. Deltabond CN maintained at 75°C; 4% methanol-modified CO_2; 148 bar; flow-rate, 0.20 ml/min liquid flow; injection 0.5 μl of 2.0 μg/μl per component; PB conditions: restrictor (linear, 25 μm I.D.) position, −2; desolvation chamber temperature, 40°C; nebulizing gas pressure, 40 p.s.i.. Other conditions given in Experimental section.

of siduron, the base peak (93 u) is due to the anilino ion. Whereas for the other five pesticides, the base peak is due to the dimethylisocyanate ion (72 u) or the methylmethoxyisocyanate ion (61 u). The library search results gave search quality results from 70 to 90 which indicate an excellent agreement with the library spectrum. Also, during method development the variation of mobile phase composition (from 0 to 8% methanol-modified CO_2) had no effect on the quality of the spectra. The quality of the spectra is also dependent on the complexity of the chromatogram. Thus, when dealing with real samples where interferences are prevalent, identification of compounds with library searching (or manually) would not be as facile as with these model mixtures.

Another application is the separation of steroids all of which may not be analyzed by GC–MS directly because of their polarity. A fast separation of six steroids under 6 min is shown in Fig. 10. The background-subtracted spectra along with library spectra of progesterone and testosterone are shown in Figs. 11 and 12. Typical of this group of compounds was the extensive fragmentation with the molecular ion present at less than 20% or not at all. In both chromatograms the peaks are symmetrical in-

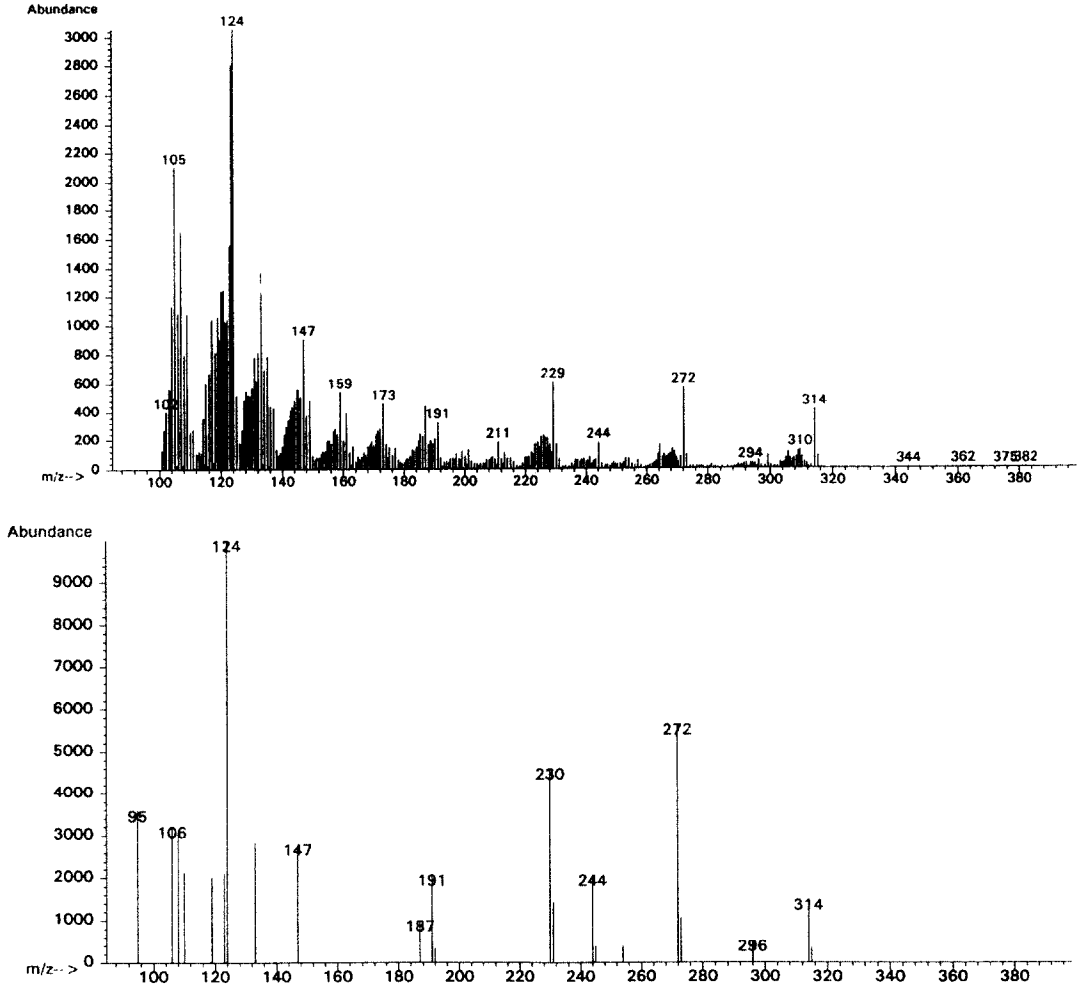

Fig. 11. EI spectrum of progesterone (top) and library spectrum (bottom).

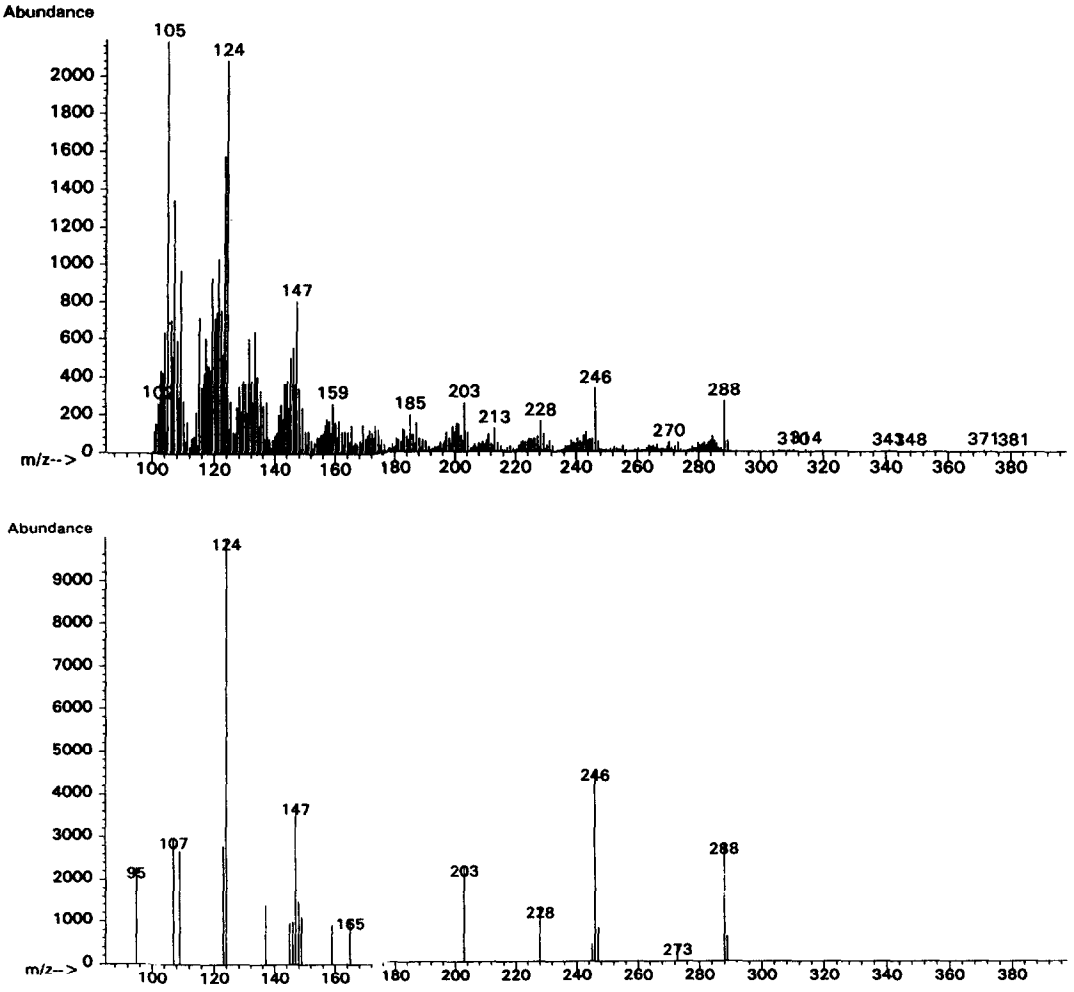

Fig. 12. EI spectrum of testosterone (top) and library spectrum (bottom).

dicating chromatographic fidelity was maintained.

4. Conclusions

Packed-column SFC–PB-MS has been found successful in analyzing phenylurea pesticides and steroids. These compounds because of their thermal lability or polarity may not be easily analyzed by other methods directly (GC–MS). With the current system, the resulting EI spectra were of sufficient quality for identification of these compounds. The effects of various operational parameters were characterized with mobile phase of pure CO_2 and methanol-modified CO_2 under FIA and chromatographic conditions. The position of the restrictor, nebulizing gas pressure, and temperature of the desolvation chamber were found to have a significant effect on the performance of the system. These parameters were optimized separately. However due to an interrelation of these parameters, a sophisticated optimization scheme (i.e. simplex optimization) would have resulted in a better overall sensitivity. For example, such an optimization would have lessened the effect of modifier concentration on sensitivity. Following optimization

of parameters, the EI LODs of the system for SIM and scan were determined to be 5 and 40 ng for caffeine, respectively. These LODs are 2.5 to 25 times better than the previous estimates [15,16]. However, the sensitivity was found to be highly dependent on the modifier percentage with 4% methanol-modified CO_2 yielding optimal sensitivity. Method development in SFC is achieved (in order of most effectiveness) by addition of various amounts of modifier, pressure/density programming and temperature variation [29,30]. Since sensitivity was shown to be highly dependent on modifier concentration, the goals of maximum sensitivity and efficient separations are in direct conflict. Currently studies are underway to address this and other concerns.

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