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Optimization of a supercritical fluid chromatograph–atmospheric pressure chemical ionization mass spectrometer interface using an ion trap and two quadrupole mass spectrometers

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Abstract

The optimization of an interface for coupling supercritical fluid chromatography (SFC) with atmospheric pressure chemical ionization mass spectrometry (APCI-MS) is described. Data presented demonstrate that the internal diameter and length of the transfer line between the SFC unit and the APCI source are *not* critical in maintaining peak shape or retention time under the set of conditions tested. The inlet capillary of the APCI source optimizes at 75 μm I.D., thus maintaining peak shape while enabling the use of a full range of pressure gradients (8–37 MPa). A comparison of responses from an in-line UV detector, two quadrupole mass spectrometers and an ion trap is presented to demonstrate limits of detection and linear ranges for the SFC separation of a six-compound test mix. © 1998 Elsevier Science B.V.

Keywords: Interfaces, SFC–MS; Instrumentation; Supercritical fluid chromatography–mass spectrometry; Aromatic compounds

1. Introduction

Over the past few years, there has been an increasing interest in coupling supercritical fluid chromatography (SFC) and mass spectrometry (MS) [1–19]. The use of LC–MS interfaces and ionization sources has carried over into the area of SFC–MS including the use of the following: particle beam [4,5], thermospray [6–9], electrospray [10], ionspray [11], and atmospheric pressure chemical ionization (APCI) [12–19].

When coupling SFC–MS using APCI, it has been assumed that a source of protons (usually methanol [12,13] or water [14–16]) needs to be added, for analyte protonation to occur. In fact, it has been stressed that an unstable ion beam occurs without the addition of such a proton source [14] and that charge exchange ionization, $M^{+\cdot}$, occurs over protonation $[M+H]^+$, especially for small molecules with low proton affinities [15,17]. To this end, methanol or water has been supplied by either bubbling the APCI source gas through the appropriate solvent or by addition of methanol to the supercritical fluid mobile phase. Another common theme is having the restrictor of the SFC unit incorporated as part of the APCI source, typically at the very end of the APCI source capillary. Thus, an APCI source had to be modified and restrictors of 20 μm or less were incorporated into the source [12–16].

Previously [19], we have demonstrated that protonated molecular ions can be produced from compounds separated using SFC coupled to APCI-MS without the addition of a modifier and/or the addition of a solvent to the APCI source. It was also shown that the SFC can be connected directly to the APCI source without major modifications.

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The current work presented here expands upon the theme of directly connecting the SFC unit to an APCI-MS instrument and generating protonated molecular ions. The optimization of an SFC–APCI interface is described. The design of the interface uses the internal variable restrictor of the SFC unit and a standard APCI source coupled with standard polyether ether ketone (PEEK) tubing and allows for independent operation of both instruments. Data are presented showing the effects of transfer line length and internal diameter (I.D.) on mass spectral peak shape. Also the effect of the APCI source capillary I.D. has been studied.

We have investigated the responses produced from an in-line UV detector along with the responses from an ion trap and two quadrupole mass spectrometers. The data comparison includes: limits of detection, dynamic linear range, and the magnitude of linear range for the SFC separation of a six-compound mixture.

When the SFC instrument was connected to the ion trap, MS–MS and MS–MS–MS (MS^3) data were generated for 4-fluorophenyl sulfone. For comparison, MS–MS data and source collisionally induced dissociation (CID) followed by MS–MS (pseudo-MS^3) data were generated for the same compound using a triple-stage quadrupole mass spectrometer.

2. Experimental

2.1. Supercritical fluid chromatograph

The SFC unit used was a Hewlett-Packard SFC Model G1205A which included a supercritical fluid pump, a separate modifier pump, and a variable restrictor. The separation of a six-compound test mix was carried out using a LiChrospher 100 DIOL cartridge column, 250×4.0 mm using 100% CO_2. The initial post-column pressure of 26.9 MPa (265 atm) was held for 1 min, followed by a linear gradient to 37.0 MPa (365 atm) at 5 MPa/min. The final pressure of 37.0 MPa was held for 1 min. The complete separation time was 4 min.

The test mix was prepared in hexane and included: nitrobenzene, azobenzene, 1-naphthyl isothiocyanate, 1-cyanonaphthalene, diphenylamine, and 4-fluorophenyl sulfone. The oven temperature was 70°C, the variable restrictor temperature was 75°C, and the flow-rate was 2 ml/min. SFC/SFE-grade CO_2 (Air Products, Hometown, PA, USA) was used which had a label claim of 99.9999% purity with <250 ppb of water. A transfer line was connected from the outlet of the SFC unit, at the exit of the variable restrictor, to the inlet of the APCI sources of the various mass spectrometers.

2.2. Mass spectrometers

Three different mass spectrometers were used for the comparison and included: a VG Quattro 1 triple-stage quadrupole, a Finnigan TSQ7000 triple-stage quadrupole, and a Finnigan LCQ ion trap. The VG Quattro (Manchester, UK) was used with the APCI source under the following conditions: probe temperature 200°C, cone voltage at 20 V, and the corona at 4.5 kV. The first quadrupole was used for full-scan analyses from m/z 90 to 1000 at 0.5 s/scan. The Finnigan TSQ7000 triple quadrupole (San Jose, CA, USA) was operated in APCI mode under the following conditions: vaporizer temperature at 150°C, corona at 5.0 kV, capillary temperature at 150°C and capillary voltage at 26 V. Full-scan mass spectra were collected scanning from m/z 50 to 1000 at 0.5 s/scan. The Finnigan LCQ ion trap was operated under the following conditions: vaporizer temperature at 200°C, corona at 5.0 kV, capillary at 40 V, and capillary temperature at 125°C. Ion collection was for three total microscans with a maximum ion injection time of 100 ms. The instrument was scanned from m/z 90 to 1000. All of the mass spectrometer conditions were optimized for all six compounds of the test mix.

2.3. Supplies

Six compounds were used for all testing as supplied by the various vendors. Azobenzene (96%), 1-cyanonaphthalene (98%), diphenylamine (99%), and 4-fluorophenyl sulfone (99%) were purchased from Aldrich (Milwaukee, WI, USA). Nitrobenzene (99%) was obtained from Eastman-Kodak (Rochester, NY, USA) and 1-naphthyl isothiocyanate (98%) was obtained from Lancaster Synthesis (Windham, NH, USA). All of these compounds share structural

features with Occupational Safety and Health Administration (OSHA)-regulated carcinogens and as such should be handled with proper laboratory safety procedures.

PEEK tubing was purchased from Upchurch Scientific (Oak Harbor, WA, USA) and ranged in internal diameter from 125 μm to 1 mm. Capillary tubing was supplied by J&W Scientific (Folsom, CA, USA) and ranged in internal diameter from 50 to 180 μm.

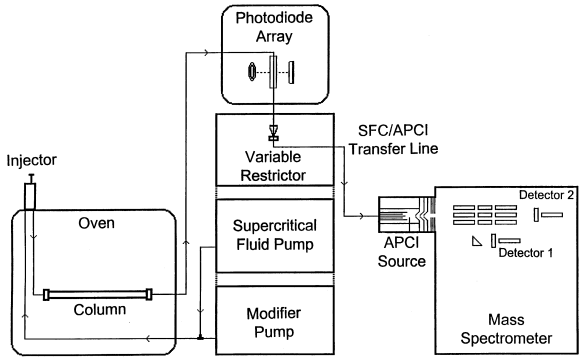

Fig. 1. Schematic diagram of the SFC–APCI-MS system.

3. Results and Discussion

3.1. Optimization of the SFC–APCI interface

The initial goal was to couple a packed column SFC to the APCI source of a mass spectrometer without making major modifications to the source. The choice of packed column over capillary was to be able in the future to increase sample loading and thus be able to decrease the volumetric limit of detection when looking at low levels of impurities [20]. Fig. 1 shows a schematic diagram of the SFC coupled to an APCI–MS system. The design of this system enables the coupling of an SFC unit to the

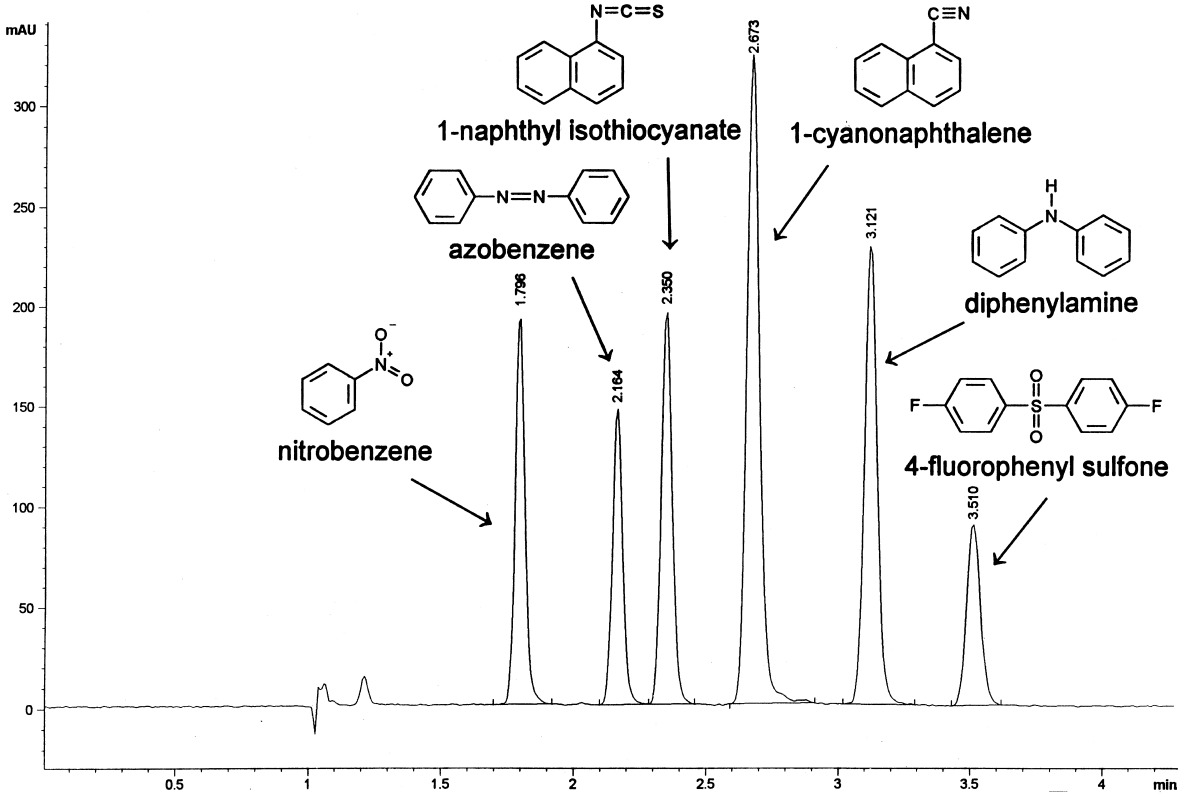

Fig. 2. SFC separation monitored by UV at 210 nm.

APCI source of a mass spectrometer along with in-line monitoring by a photodiode array detector. The SFC separation of the test mix was carried out using 100% CO_2 without the addition of an organic modifier. All testing was performed without the addition of methanol or water into the APCI source. The UV response at 210 nm for the SFC separation of the six-compound test mix is presented in Fig. 2. These compounds were chosen to represent small molecule impurities or degradants with a varying range of proton affinities which may be present during a sample analysis. The proton affinities ranged from 194 kcal/mol for nitrobenzene to 222 kcal/mol for diphenylamine (1 cal=4.184 J) [21]. Fig. 3 is the reconstructed ion chromatogram (RIC) for the SFC separation using the VG Quattro. Table 1 is a comparison of the peak pair resolution as obtained with the in-line UV detector and the mass spectrometer. While the peak pair resolution was slightly degraded between the UV data and the MS data, adequate resolution was maintained when coupling the SFC and the MS instrument.

Table 1
Comparison of peak resolution

Peak pair	UV	MS
1–2	3.4	2.6
2–3	1.8	1.4
3–4	2.6	1.9
4–5	3.3	2.5
5–6	3.1	2.7

It must be noted that there was no organic modifier in the mobile phase and that the SFE/SFC-grade CO_2 claims <250 ppb water content, yet protonated molecular ions were adequately generated for the test compounds as noted from the mass spectra presented in Fig. 4. Since the separation was carried out using 100% CO_2, the source of protons

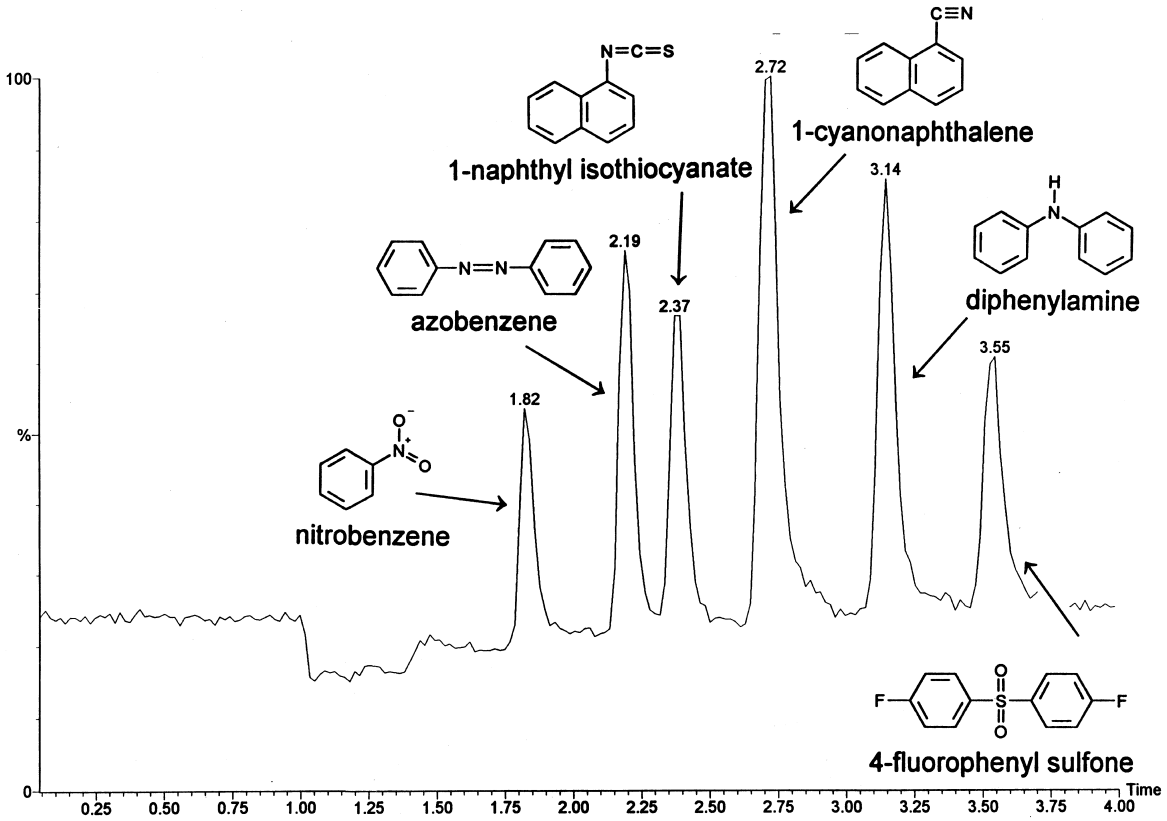

Fig. 3. Reconstructured ion chromatogram for the SFC–APCI-MS separation as monitored using the VG Quattro.

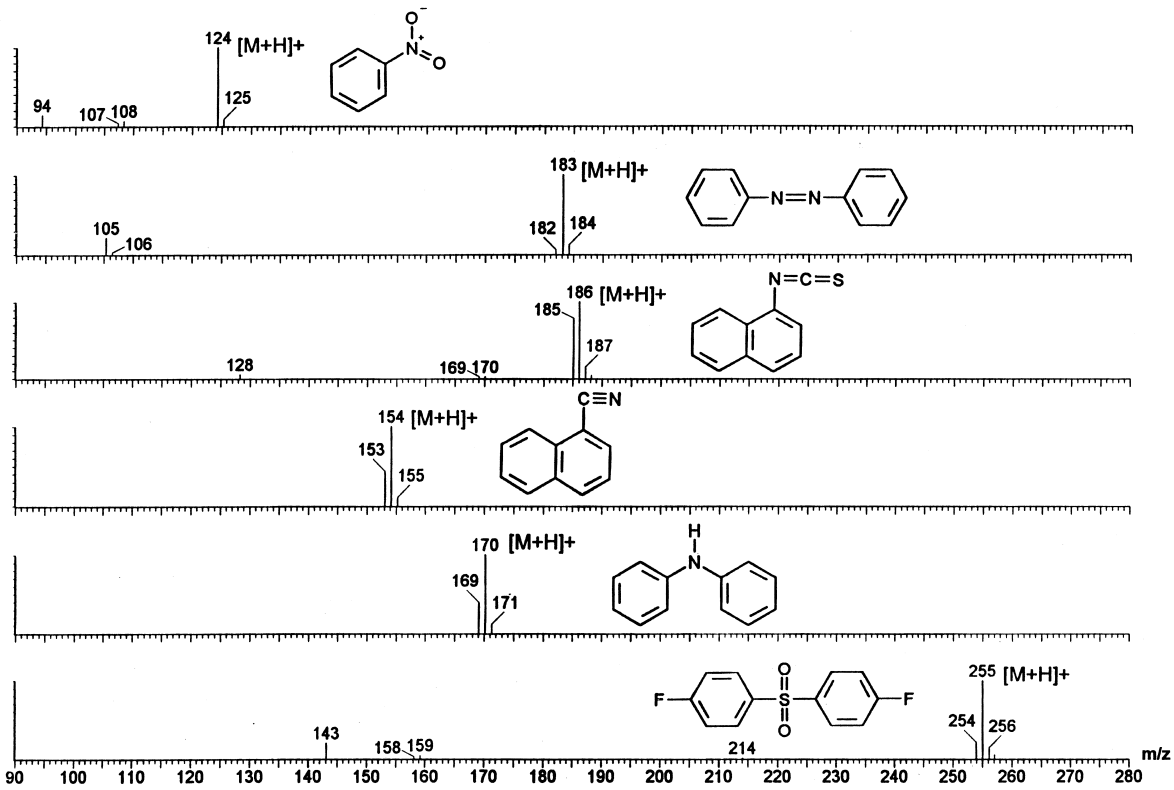

Fig. 4. Mass spectra of the test compounds produced using the VG Quattro.

available for analyte protonation in the APCI source was limited to residual water present in the CO_2 (<250 ppb label claim) or most likely due to residual moisture in the APCI source and possibly humidity from the laboratory air. With the VG Quattro, a strong signal at m/z 19, $[H_3O]^+$, was observed during the tuning process. As noted in Fig. 4, protonated molecular ions were generated for each of the test compounds without the addition of an organic modifier and suggested that there was another source of protons present in the ion source other than that supplied by the mobile phase. It has been proposed [15,17] that residual moisture is present in a typical APCI source, but that the limited amount of moisture leads to enhancing charge transfer, $M^{+\cdot}$, in lieu of analyte protonation, especially for small molecules with low proton affinities. For azobenzene, 1-cyanonaphthalene, 1-naphthyl isothiocyanate, and 4-fluorophenyl sulfone, the charge transfer $M^{+\cdot}$ was noted, but the corresponding protonated molecular ions were the most abundant ion signal. This was contrary to what has been previously noted [17] with polycyclic aromatic hydrocarbons (PAHs), using SFC–APCI-MS without the addition of water or methanol into the source, where only the $M^{+\cdot}$ was noted; whereas, the protonated molecular ion was formed and became the dominating species based on the amount of water added into the source.

With the design of the current SFC–APCI interface, the transfer line does not need to be heated as proposed [13,14,16,18] and works for both stainless steel as well as PEEK tubing. This point can be demonstrated by the data obtained from the SFC–MS shown in Fig. 3 for the test compounds investigated. However, heating of the transfer line may be necessary to facilitate mass transport for larger compounds or when an organic modifier is used. This is the subject of future work in this laboratory. Initial tests of the SFC–APCI interface were per-

formed using 250-μm I.D. tubing of 75 cm in length. Fig. 5 shows the effect of changing the tubing I.D. on peak shape and retention time for 4-fluorophenyl sulfone. This compound was chosen since it elutes latest in the pressure gradient and thus should be the most sensitive to changes in decreasing pressure, i.e. sufficient pressure not being maintained to the APCI source. When going from 125 μm to 1 mm I.D., there was roughly a 0.3-min increase in retention time with minimal increase in peak width. Fig. 6 shows the effect on the same compound with varying the transfer line length while keeping the I.D. constant at 250 μm. As noted in Fig. 6, there was minimal increase in retention time and peak shape when going from 75 to 300 cm in length (almost 10 ft. of transfer line)! Therefore, the transfer line I.D. and length were not considered crucial for interfacing the SFC to the APCI source as it would be for conventional LC–MS.

The effect of the APCI inlet source capillary was also investigated. Here many other researchers [12–18] have placed the restrictor end of the SFC unit at the outlet end of the APCI source capillary that were 20 μm or less [12–16]. In the present configuration, the APCI source capillary acts as a secondary flow restrictor, instead of as the primary SFC restrictor. Therefore, the SFC and the APCI-MS instruments can be operated independently. Fig. 7 shows the effect of changing the capillary I.D. with respect to peak shape and retention time. It was noted that varying the capillary I.D. from 50 to 75 μm had little effect on peak shape and retention, while the peak was greatly distorted with 100 and 180 μm I.D. capillary tubing. There appeared to be no difference between using activated versus deactivated capillary tubing when comparing the data for the 50-μm I.D. tubing. From our experience, the 75-μm I.D. capillary was easiest to work with and was most efficient

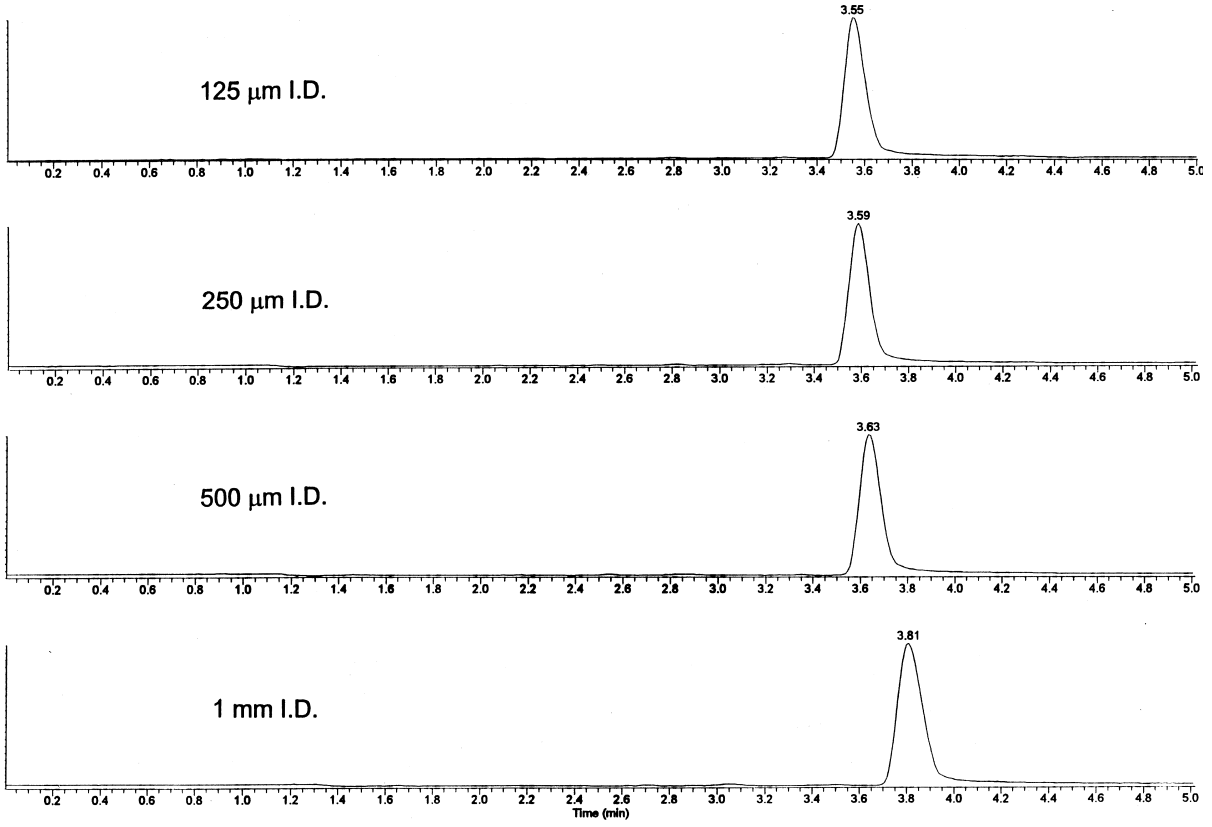

Fig. 5. Effect of transfer line internal diameter on peak shape and retention time.

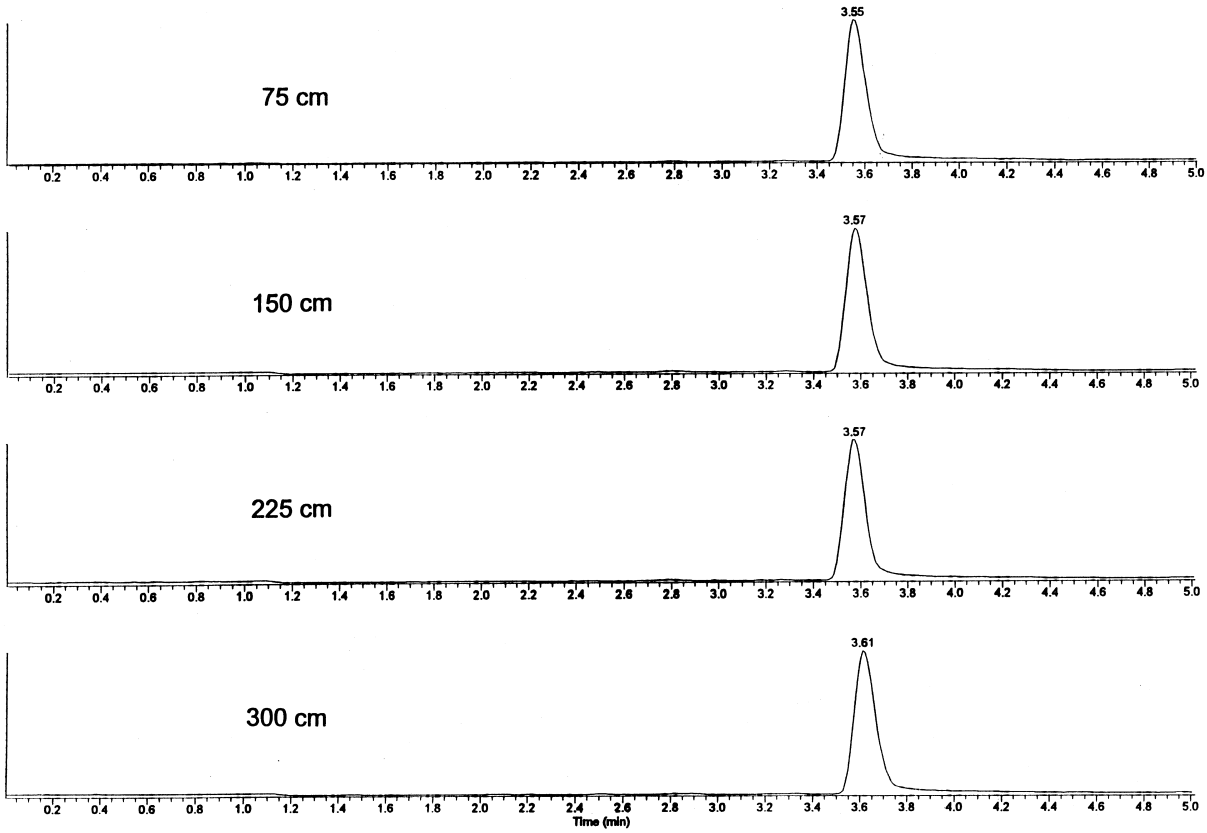

Fig. 6. Effect of transfer line length on peak shape and retention time.

in achieving initial conditions in pressure gradients, especially when going as low as 8 MPa, thus re-equilibration times could be greatly reduced and a full range of pressure gradients could be employed. In this design, the APCI inlet capillary was acting as a secondary flow restrictor in the sense that sufficient pressures were maintained while going from the variable restrictor of the SFC to the APCI source. It must be stressed that the pressure and density in the transfer line under these experimental parameters were not under control. For these studies, the low-molecular-mass, non-polar compounds were not effected by the pressure drops within the transfer line until the APCI capillary was 100 μm I.D. or greater, which can be noted from Fig. 7. Concerns have been raised as to the mass transfer ability of larger compounds or the possibility of phase separation in the transfer line when an organic modifier is employed. This is area of future studies in this laboratory. Using the 75 μm I.D. capillary has made it possible to do SFC–MS as well as conventional LC–MS using the same commercially available APCI source without modifications.

3.2. Linearity and limits of detection

Limits of detection (LODs) and linear ranges were generated for the six test compounds as analyzed by the three different mass spectrometers and the in-line UV detector at 210 nm. Five injections were analyzed for each concentration of calibration standard analyzed. All of the linearity data were generated without the addition of methanol or water into the APCI source. The mass spectral data were derived from the full-scan analyses using reconstructed ion chromatograms (RICs) for the protonated molecular ions of interest.

Table 2 presents the LODs as determined by the

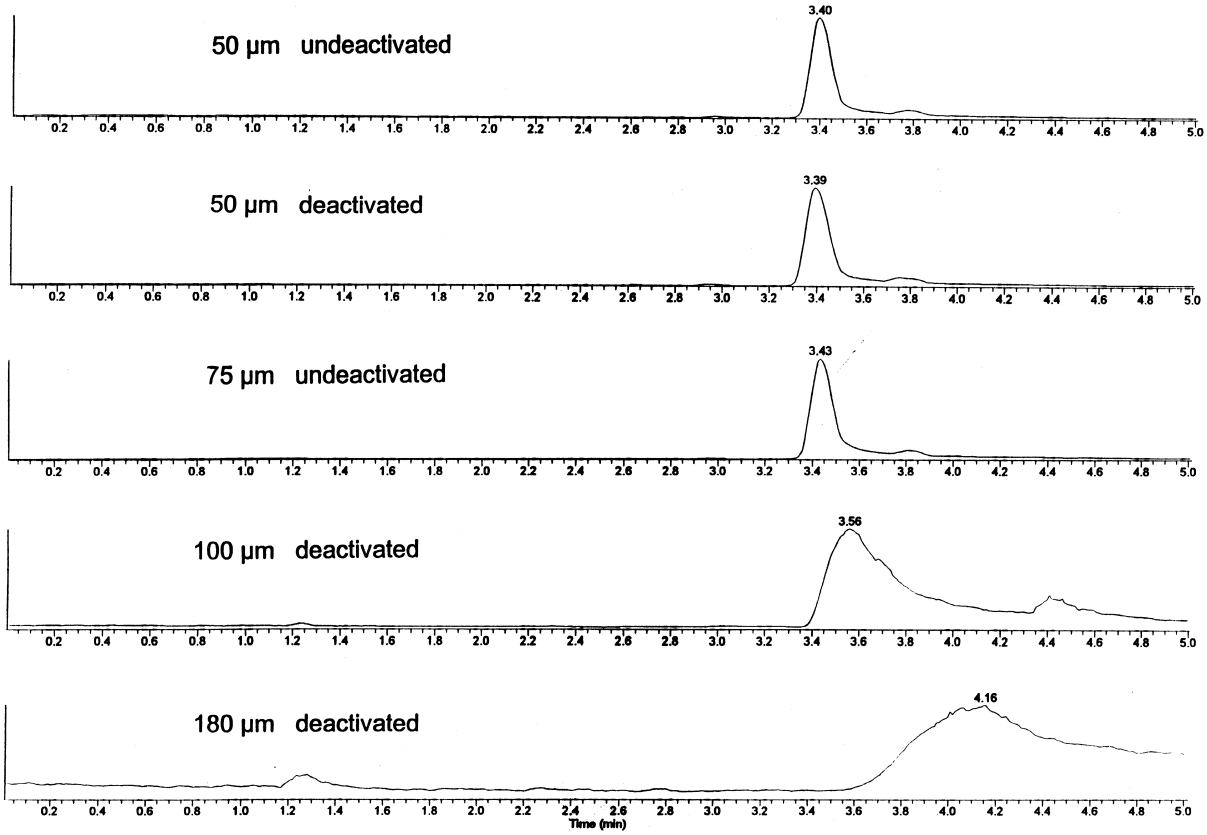

Fig. 7. Effect of APCI inlet capillary internal diameter on peak shape.

four instruments. It was noted that nitrobenzene had the highest LOD when analyzed using the Finnigan instruments, especially when compared to that of the Quattro. This LOD variation has to do with differences in APCI source designs between the Quattro and the Finnigan instruments and may be related to relative amounts of moisture present in the sources. Since nitrobenzene has the lowest proton affinity of the six compounds tested, one would expect a higher LOD if the source of protons was reduced. Interestingly, the VG Quattro provided uniform LODs of 8 ng on column for all six test compounds. For the majority of the compounds tested, the UV detector provided the lowest LODs, but the mass spectrometers could easily produce lower LODs when operated in selected ion monitoring (SIM) mode, an area of further investigation.

Table 3 presents a comparison of the linear ranges

Table 2
Limits of detection in nanograms on column

	UV, 210 nm	VG Quattro	Finnigan LCQ	Finnigan TSQ7000
Nitrobenzene	2	8	125	125
Azobenzene	1	8	2	2
1-Naphthyl isothiocyanate	1	8	4	4
1-Cyanonaphthalene	0.5	8	1	0.5
Diphenylamine	0.3	8	0.5	4
4-Fluorophenyl sulfone	2	8	1	0.3

Table 3
Linear ranges in nanograms on column

	UV, 210 nm	VG Quattro	Finnigan LCQ	Finnigan TSQ7000
Nitrobenzene	16–2000 (2.1)	31–500 (1.2)		
Azobenzene	8–2000 (2.4)	31–1000 (1.5)	16–250 (1.2)	16–500 (1.5)
1-Naphthyl isothiocyanate	8–500 (1.8)	31–1000 (1.5)	31–250 (0.9)	16–1000 (1.8)
1-Cyanonaphthalene	4–500 (2.1)	31–500 (1.2)	8–500 (1.8)	4–1000 (2.4)
Diphenylamine	2–2000 (3.0)	31–1000 (1.5)	4–500 (2.1)	6–1000 (1.8)
4-Fluorophenyl sulfone	16–2000 (2.1)	31–1000 (1.5)	8–500 (1.8)	2–250 (2.1)

Orders of magnitude in parentheses.

for the six compounds as determined by the four instruments. The orders of magnitude for the linear ranges are in parentheses. Linear ranges were determined by analyzing several concentration standards with five replicate injections each. All sets of replicate injections were within 5% relative standard deviation or less. Due to the high LOD for nitrobenzene with the Finnigan instruments, only three data points were available for linearity and this data was not presented. The data indicate that the linearities are clearly instrument dependent, with no single instrument prevailing for all six test compounds.

3.3. SFC–MS–MS and SFC–MS^3

Fig. 8 shows averaged spectra generated using SFC–MS with the LCQ ion trap. The top spectrum is

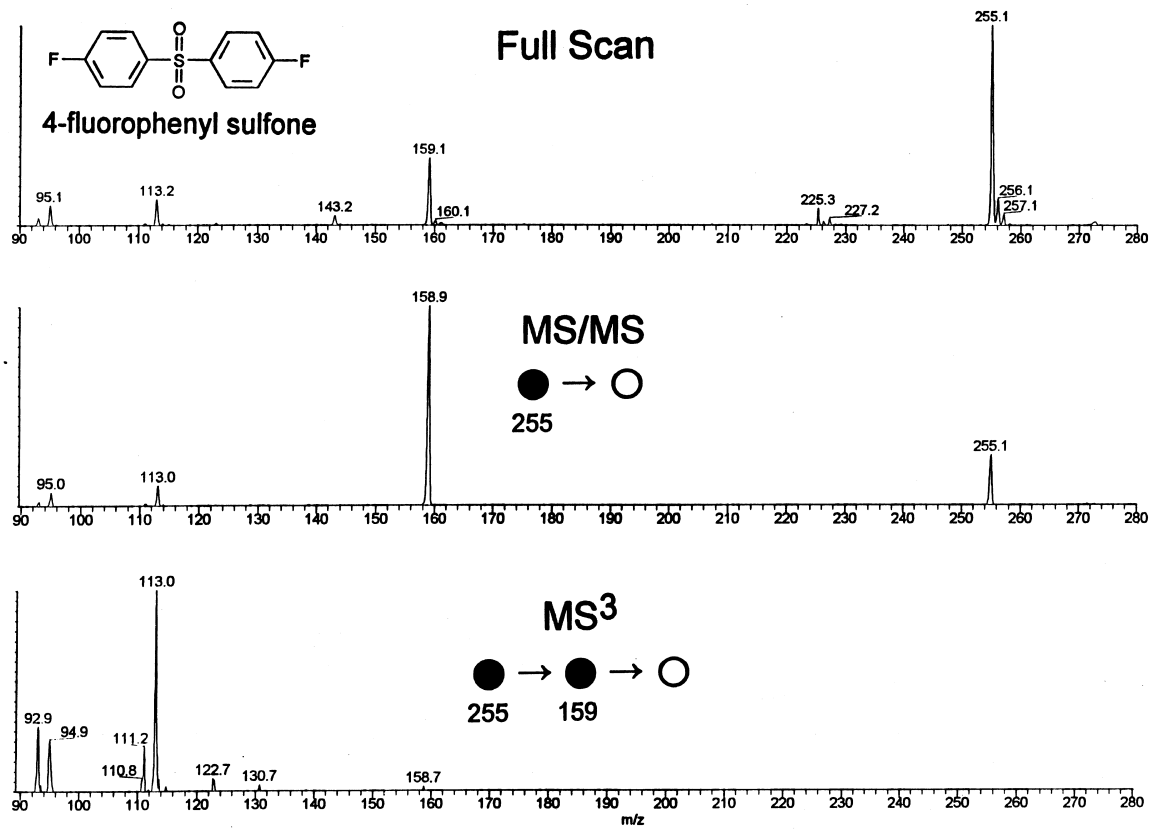

Fig. 8. SFC–MS, MS–MS and MS^3 produced on a Finnigan ion trap.

the full scan of 4-fluorophenyl sulfone, $[M+H]^+$ of m/z 255. The middle spectrum is the collisionally induced dissociation (CID) product ion spectrum of m/z 255 and the bottom spectrum is the CID product ion spectrum of m/z 159, a product of m/z 255. All of the spectra were collected during a single analysis and demonstrated that MS^3 data generation was possible. Further MS^n spectra were not obtained due to the low m/z product ions and the limited low mass range of the current ion trap.

Fig. 9 is the complementary data generated when coupling the SFC instrument to the Finnigan TSQ7000. The top spectrum is the full-scan MS data. The middle spectrum shows the CID product ions (MS–MS) of m/z 255, and the bottom spectrum shows the product ions formed using source-induced CID coupled with CID in Q2 of m/z 159. These data demonstrated results (pseudo-MS^3) comparable to those achieved when using the SFC–LCQ system.

4. Conclusions

SFC–MS is an under-utilized analytical technique deserving much attention. As demonstrated, an SFC unit can easily be coupled to an APCI mass spectrometer without major modifications to the commercially available APCI source or to the SFC unit. With this arrangement, protonated molecular ions were sufficiently generated from the SFC components using residual moisture within the APCI source. Analyte protonation was obtained without the pres-

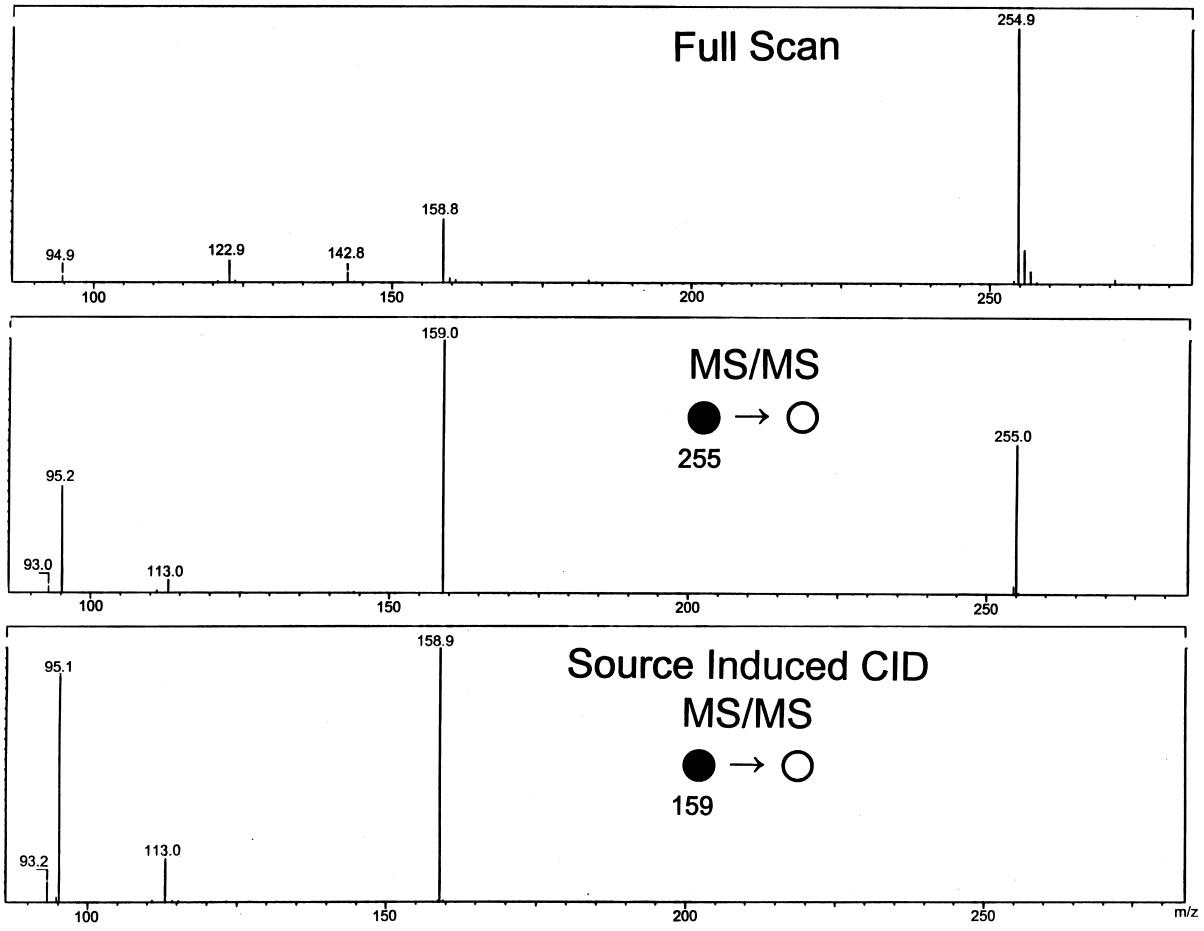

Fig. 9. SFC–MS, MS–MS, and source CID MS–MS produced on a Finnigan TSQ7000.

ence of an organic modifier in the SFC mobile phase and without the addition of water or methanol directly into the APCI source.

The SFC–APCI transfer line length, internal diameter, and heating were not crucial parameters for maintaining optimum conditions leading to generation of narrow peaks in the APCI source as noted for the types of compounds used during these analyses. Whether this statement will hold true for larger compounds or when an organic modifier is incorporated into the separation scheme is the subject of future work. The internal diameter of the inlet capillary of the APCI source plays a major role in maintaining peak shape and is optimum at 75 μm, leading to the ability to use the full range of pressures available with the current SFC unit.

Coupling the SFC to an ion trap, MS–MS and MS^3 data can be generated during a single analysis. Complementary MS–MS and pseudo-MS^3 data can be generated on a triple-stage quadrupole mass spectrometer using source-induced CID followed by ion selection and MS–MS analysis, but MS–MS and pseudo-MS^3 must be performed and acquired in consecutive analyses.

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