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Recent developments in electrospray mass spectrometry including implementation on an ion trap*

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Abstract: Mass spectrometry (MS) may be the ultimate detection technique when combined with modern condensed phase separation sciences. The technique combines sensitivity with excellent specificity, so the pharmaceutical analyst can obtain definitive information regarding components separated in a mixture. Thus, mass spectrometric detection not only provides evidence of a chromatographic peak, but it also provides important information including molecular weight and structural information enabling identification of the components. The coupling of an atmospheric pressure ionization (API) mass spectrometer to most of the separation science techniques offers a simpler alternative from earlier non-routine, less sensitive systems where the vacuum systems struggled to handle the liquid effluent from these systems. Contemporary sensitive and analytically rugged API systems can be operated unattended for extended periods of time thus reducing the cost per sample to a reasonable value especially given the wealth of information provided. Although the mass spectrometer is more complicated than conventional spectroscopic detectors, present day API systems effectively decouple the liquid-phase separation inlet from the high-vacuum system where mass analysis occurs. The ability to form gas-phase ions at atmospheric pressure and sample primarily the analyte ions into the mass spectrometer promises a bright future for combining on-line condensed phase separation science techniques with mass spectrometry. The increasing ease of performing these experiments offers new analytical opportunities for pharmaceutical laboratories.

Keywords: *Electrospray mass spectrometry; ion trap; tandem mass spectrometry (MS/MS); LC/MS.*

Introduction

The success and general acceptance of capillary gas chromatography–mass spectrometry (GC/MS) in the pharmaceutical industry attests to the importance and need for on-line separation–mass spectrometric detection of organic compounds. Unfortunately, many compounds are not amenable to GC conditions even with modern derivatization techniques and column technology due to their thermal instability, highly polar nature, or high molecular weight. When this is the case many practising analysts resort to LC techniques using UV, fluorescence or other detectors. More recently, ion pairing techniques [1] and capillary electrophoresis (CE) [2] have been used to separate mixtures containing a wide variety of organic compounds. Many of these mixtures include compounds that are not amenable to GC analysis because they contain peptides, proteins, or ionic species in solution.

LC techniques are now ubiquitous in academic and industrial laboratories and CE is attracting considerable interest as instrumentation becomes commercially available. Unfortunately, however, the practising LC or CE analyst soon returns to the frustration and uncertainty experienced in the gas chromatography–flame ionization detection (GC/FID) in the days before GC/MS was available where a peak's retention time is the only information available. At this stage one must either co-inject a sample of the suspect compound after guessing what it is, or preparatively collect the peak and perform further characterization. Clearly it is more definitive to have a mass spectrometer as a detector for LC and CE.

Although a variety of combined LC/MS systems have been reported [3] and [4], we suggest that atmospheric pressure ionization (API) mass spectrometry is a preferred way to simplify the coupling of liquid inlet systems such as LC, CE and ion pair chromatography

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to MS [5]. A key feature of this approach is that liquid effluent from a particular separation science is not directly introduced into the mass spectrometer vacuum system. As a result the mass spectrometer high vacuum pumps do not have to deal with removing the solvent and buffer additives while enriching the analytes of interest. As shown in Fig. 1 the effluent is 'sprayed' in the vicinity of an ion sampling orifice that effectively separates high vacuum from atmospheric pressure. Gas-phase ions are formed in this region by either electrospray [6] or atmospheric pressure chemical ionization [5], and then these ions are sampled through the ion sampling orifice into the vacuum system for mass analysis. Large excesses of solvent from the effluent do not enter the vacuum system, thus making life much easier for the mass spectrometer hardware and pumping system.

Many different classes of compounds may be detected via this approach provided the analyst has a few different 'tools' in his or her toolbox. For example, the mass spectrometer should be capable of detecting both positive and negative ions. Thus, when the proton affinity of an analyte is sufficient to allow protonation, a positively charged ion is produced requiring positive ion detection. When negatively charged ions are the more stable species such as sulphonate, carboxylate, or phenolate ions, then negative ion detection is preferred. Sometimes either mode of detection may be possible, but usually one will provide better sensitivity than the other.

In addition to being able to detect either cations or anions it is useful to have more than one 'interface' for coupling a separation technique to an API mass spectrometer. An interface is typically a simple, relatively inexpensive probe-type device that may be placed in front of the ion sampling orifice of the mass spectrometer. It usually is designed to provide a particular type of gas-phase ionization and/or handle different effluent flows from the condensed phase separation system. For example, a pure electrospray interface coupled to an API mass spectrometer is optimized with efluent flows in the 1–5 μl min^{-1} range. This confines one to using either open tubular LC, packed capillaries, or a significant post-column split from larger bore LC systems. In contrast, the pneumatically-assisted electrospray or 'ion spray' LC/MS interface [7] increases the usable flow rate range of electrospray to 40–50 μl min^{-1} or, with some reduction in sensitivity, to 100–200 μl min^{-1}. This allows routine LC/MS operation using 1–2 mm i.d. microbore columns with total effluent introduction, or conventional 4.6 mm i.d. LC columns with some post-column split of the total effluent.

In contrast to problems dealing with too much effluent from LC columns the technique of CE with electro-osmotic or bulk flow ranging from zero to a few nanolitres per minute poses different challenges. In this instance a sufficient make-up flow of buffer must be provided to sustain a stable spray from either electrospray or ion spray. Smith *et al.* [8] have described a sheath-flow device that delivers a make-up flow co-axially at the CE column terminus. In contrast, a liquid junction device has been described that is placed between the CE column exit and the ion spray interface [9]. Both devices provide the opportunity for 'decoupling' to some extent the running buffer conditions necessary for the CE process from the preferred and oftentimes different buffer

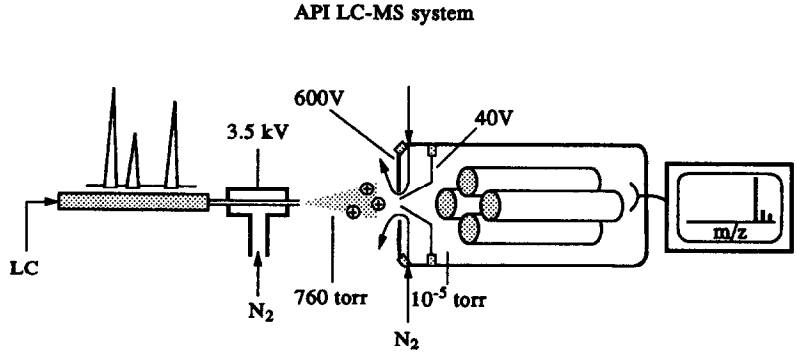

Figure 1
Schematic of a generic liquid inlet APIMS system. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

conditions for optimum performance from the electrospray ionization process.

The flexibility afforded by the API mass spectrometric approach stems in part from its ability to accommodate a variety of different LC/MS-type interfaces to one mass spectrometer. One can couple several different separation science techniques to the same mass spectrometer simply by utilizing the appropriate 'interface'. This requires no modification of the chromatographic or CE hardware or the mass spectrometer. It may, however, necessitate some compromise of the chromatographic eluent or CE buffer which basically requires the avoidance of inorganic modifiers and buffer concentrations >20 mM. The analyst should appreciate that any ionized species in the effluent will likely be detected by the mass spectrometer. Depending upon their molecular weights these components may contribute high chemical noise or interfere with detection of either target or unknown analytes.

Aspects and examples of combining condensed phase separation techniques with API mass spectrometric detection will be described in this paper. The strengths and limitations of the techniques will be highlighted from the viewpoint of both the separation science and mass spectrometry. We will address the current status of API LC/MS and related techniques, describe where the technology is going in the future, and discuss the potential limitations of the techniques from the perspective of the separation scientist and the mass spectroscopist.

Instrumentation

Three different quadrupole mass spectrometer systems were used to obtain the results described in this paper. Mass spectrometer I is a TAGA 6000E upgraded to an API III tandem triple quadrupole (Sciex, Thornhill, Ontario), mass spectrometer II is a modified single quadrupole 5970 mass selective detector (Hewlett–Packard Co., Palo Alto, CA), and mass spectrometer III is a modified Saturn II benchtop ion trap (Varian Instruments, Palo Alto, CA). The first system is a commercially available standard API instrument with a nitrogen gas curtain feature and a mass range to 2400 Da. The second instrument does not require a curtain- or bath gas [10] feature and has a mass range to 800 Da. However, it is a significantly modified benchtop GC/MS system that accommodates sampling gas-phase ions formed at atmospheric pressure, and is not yet commercially available. The third system has a mass range extending only to 650. The ion spray and related interfaces may be used with either of these systems. System I can provide pre-mass selection followed by collision-induced dissociation (CID) [11] in the central quadrupole collision cell, while the single quadrupole system II and the ion trap system provide CID in the free-jet expansion region in the first pumping region prior to mass analysis. During CID experiments mass spectrometer I maintains an analyser vacuum of 2.5×10^{-5} torr. Argon is used for CID in the collision region (second quadrupole) with 2×10^{14} atm cm^{-2} target gas thickness. The precursor ions are formed following their field-induced desorption from the condensed phase, transmitted to the second quadrupole for CID, and the resulting product ions mass-analysed in the third quadrupole to provide a CID mass spectrum. Both mass spectrometer systems are operated with unit mass resolution in either the full-scan acquisition or selected ion monitoring (SIM) modes with either positive or negative ion detection.

The condensed phase separations described on-line with MS in this report include HPLC and CE. An example of each will be described in sequence using the commercial API mass spectrometer I system followed by an example of LC/MS performed on the modified MSD and ion trap benchtop mass spectrometer systems II and III. It is the view of the authors that this last example offers considerable potential to the practising separation scientist in the future because of its simplicity, potential low cost, and analytical flexibility. However, the tremendous analytical potential of the more sophisticated tandem mass spectrometry (MS/MS) system will continue to be of considerable value for more challenging problems.

Micro LC/MS analysis of a peptide digest using a commercial API mass spectrometer

The HPLC systems for LC/MS included a 1 mm i.d. × 100 mm C-18 bonded, 5 μm particle-size packed column coupled to a ABI Model 140 dual-syringe micropump (Applied Biosystems Inc., Santa Clara, CA) equipped with a Model 9125 biocompatible syringe-loading sample injector (Rheodyne, Cotati, CA). The micro HPLC pumping system main-

tained an eluent flow of 40 μl min^{-1}, while the gradient began at 0% B and was programmed directly to 10% B over the first minute followed by a slow gradient program to 90% B over 24 min following the injection of a 20 μl sample (A = 0.05% TFA in water; B = 0.05% TFA in 50:50 acetonitrile–water). The column exit was connected directly to a Model 757 micro UV detector set at 215 nm (Applied Biosystems, Inc.) followed by the ion spray interface that accepted the total micro HPLC effluent. The Sciex API III mass spectrometer was operated in the full-scan, single MS mode with positive ion detection.

The sample of sulphonated RNase B was prepared according to the procedure of Thannhauser *et al.* [12]. The crude sample was desalted with 50 mM ammonium bicarbonate with a PD-10 (Pharmacia, Uppsala, Sweden) column, and digested with TPCK-treated trypsin (Sigma Chemical Co., St Louis, MO) using a 50:1 substrate–enzyme ratio at 37 degrees for 12 h. Aliquots of this sample (20 μl) were injected on-column for LC/UV/MS analysis.

Micro LC/MS techniques are helpful because of the need for increased sensitivity for detection of low level analytes in complex mixtures. Since there is some thought that peptides and related compounds may be among the drugs of the future, we have explored new methods for the characterization of these polar, non-volatile compounds. It is generally accepted that capillary GC/MS techniques are not well suited for the analysis of samples containing peptides and glycopeptides, so we have evaluated the analytical potential of micro LC/MS techniques for this purpose. As an example we are interested in the characterization of the carbohydrate composition of glycoproteins such as RNase B, and have studied the products resulting from the sulphonation of the disulphide bonds followed by tryptic digestion. These tryptic peptides are representative of larger biomolecules that may be important in the pharmaceutical arena in the future. The LC/UV chromatogram (215 nm) from the analysis of the tryptic digest of S-SO_3-RNase B is shown in Fig. 2(A). This shows the chromatographic separation of a complex mixture over a period of about 25 min under the reversed-phase micro HPLC conditions described. The injection of approximately 1 nmol of the digest material provides evidence for even weakly absorbing components in the mixture. LC/UV identification of any peaks of interest would require laborious experiments designed to compare retention times between a known compound and a peak of interest in Fig. 2(A). The correct choice of the 'known compound' can be very problematic since complex mixtures such as this can contain many surprises.

An alternative to the trial and error approach mentioned above is the acquisition of on-line LC/MS results while scanning the mass spectrometer across its available mass range so the molecular weights of all components detected may be determined. The total ion current (TIC) profile shown in Fig. 2(B) was obtained for this sample by monitoring the total micro HPLC effluent while scanning the first quadrupole from m/z 300–2400. Each chromatographic peak represents one or more tryptic peptides or glycopeptides. Inspection of the full-scan mass spectrum for any of these peaks provides the molecular weight for each component in the form of its doubly protonated molecule ion. The mass spectrometer may be tuned to provide little or no fragmentation so that only the molecular weight species are observed, or alternatively, sufficient energy may be imparted to the ions as they pass through the ion sampling orifice so that some fragmentation occurs. In the latter case simple structural information may be available in addition to the molecular weight information. In almost all cases, however, the molecular weight may be determined from these experiments. This information is very useful for the characterization of the composition of a complex mixture such as that shown in Fig. 2(A).

Figure 3 shows a representative mass spectrum obtained from the indicated 8.7 min retention time region shown in Figs 2(A) and (B). These LC/MS data reveal the co-elution of several doubly and triply protonated glycopeptides consisting of differing numbers of mannose linkages eluting in the same retention time window. For example, if x represents the number of mannose linkages on the doubly protonated, doubly charged glycopeptide shown in Fig. 3 these data suggest the presence of compounds that differ by 5–9 mannose linkages on the NLTK-chitobiose core glycopeptide. Similarly, if y represents the number of mannose linkages on the triply protonated, triply charged glycopeptide we see that the number of glycopeptides with different numbers of mannose linkages ranges from 7 to

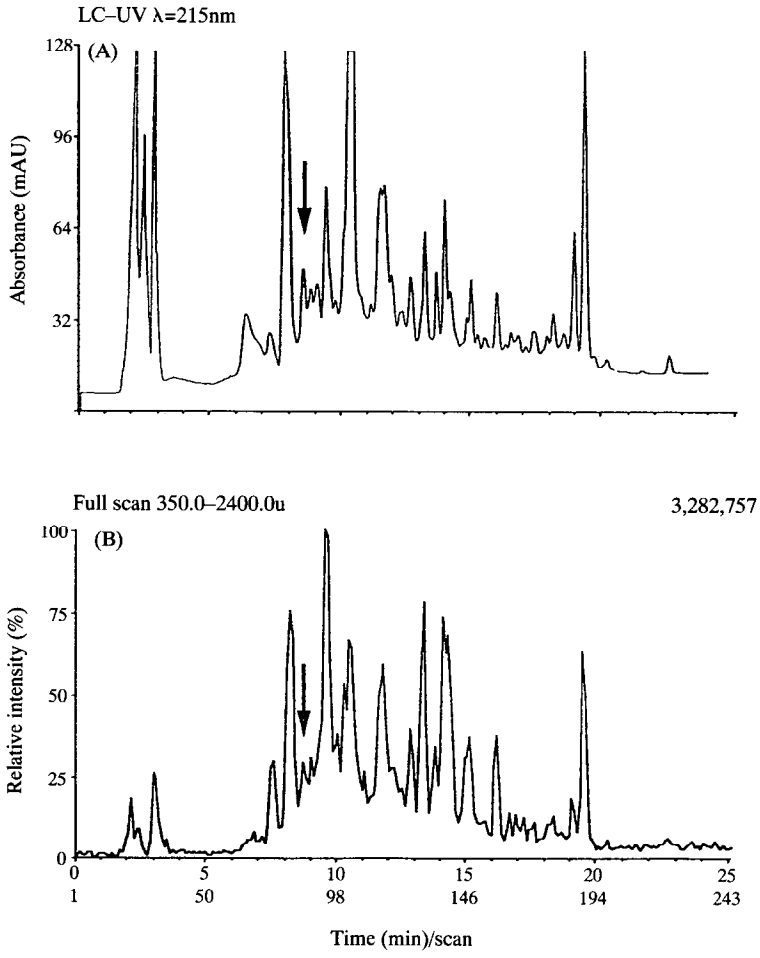

Figure 2
(A) LC/UV and (B) LC/MS TIC from the analysis of 1 nmol from a tryptic digest of S-SO_3 RNase B. Full-scan LC/MS data were acquired from *m/z* 300–2400 in 1 Da steps with a 2.86 ms dwell time per step. The TIC is plotted from 350 to 2400 Da to give an improved S/N ratio. See text for HPLC and gradient conditions. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

9. The absence of ions where $y = 5$ and 6 in this ion series presumably is due to their weak abundance in the triply protonated, triply charged ion series.

Although these LC/MS data do not reveal directly the identity of all the components observed, they do provide considerably more information than can be obtained from the LC/UV chromatogram shown in Fig. 2(A). For example, several minor components are observed in Fig. 2(B) that would not have been suspected from LC/UV results. Additional protocol utilizing tandem mass spectrometry (LC/MS/MS) can be used to obtain more structural information such as the product ion fragmentation behaviour of the multiply charged precursor ions. Since CID of doubly charged precursor ions of tryptic peptides give primarily singly charged product ions, the interpretation of these data can be quite informative. These and related LC/MS/MS experiments may be easily performed when one has a reliable LC/MS interface and a tandem mass spectrometer.

Capillary electrophoresis/mass spectrometry (CE/MS)

A homemade capillary electrophoresis system was used in this work. The column was untreated fused-silica (75 μm i.d. × 35 cm) that was connected directly to a liquid junction interface. The latter was directly connected to an ion spray interface that was operated as described above. These combined units are shown in Fig. 4 and described in more detail elsewhere [9]. The liquid junction along with the ion spray interface floats at 3 kV. The

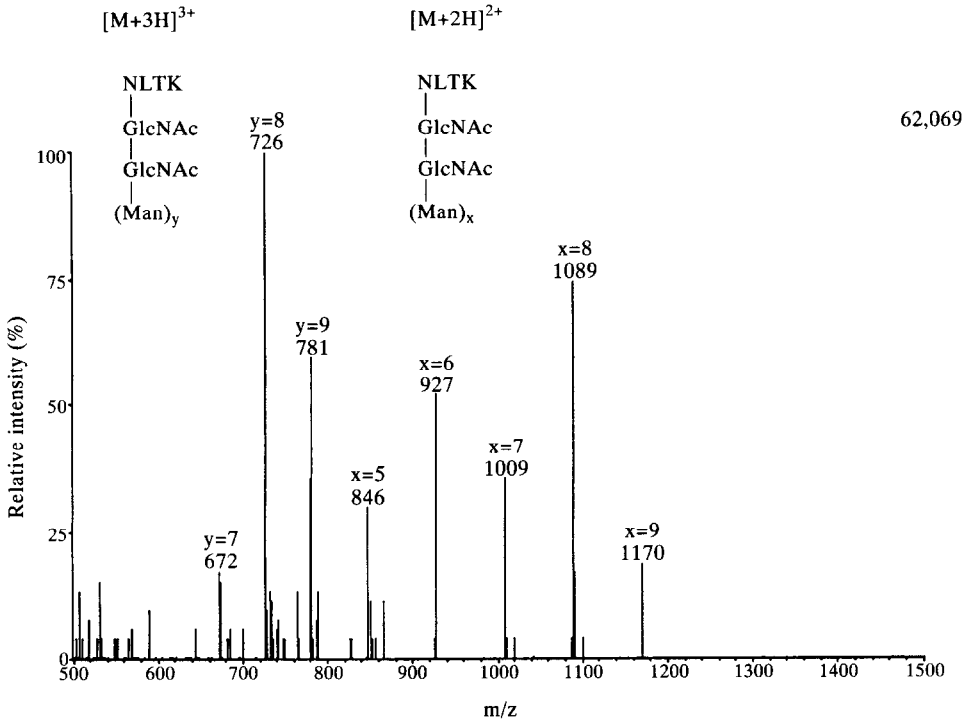

Figure 3
Full-scan LC/MS spectrum of glycopeptides with varying mannose compositions observed at a retention time of 8.7 min in Fig. 2(B). The doubly protonated, doubly charged (*x*-series) ions and the triply protonated, triply charged (*y*-series) ions show the distribution of co-eluting glycopeptides present in the digest mixture. See text for more detailed discussion. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

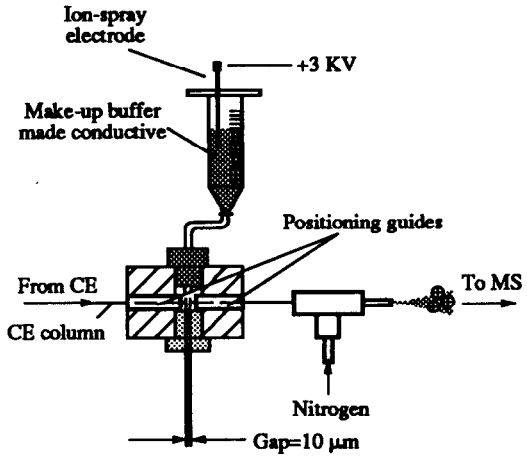

Figure 4
Combined liquid junction–ion spray interface for CE/MS applications. The potential across the capillary column is the difference between that applied at the inlet end and the potential applied in the make-up reservoir of the liquid junction. The ion spray interface floats at the same potential as the liquid junction by conduction through the buffer electrolyte. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

potential difference across the CE column is the difference between the applied potential at the capillary inlet end and the voltage applied to the liquid junction–ion spray interface combination. The key features of the liquid junction coupling device include a means of decoupling the separaration requirements of the running buffer from the buffer requirements necessary for optimal ion spray operation. In addition, the liquid junction provides a make-up flow for the very low electroosmotic or bulk flow of the running buffer. This device is used instead of the sheath-flow approach described by others. The running buffer used in this work was 5 mM ammonium acetate in 25% acetonitrile adjusted to pH 5 with acetic acid. The make-up buffer in the liquid junction reservoir was 25% acetonitrile in 5% formic acid, and an applied voltage of 38 kV was placed at the inlet (anode) end using a Model 230-30R series reversible polarity high voltage power supply (Bertan Assoc., Inc., Hicksville, NY) while the exit (cathode) end was maintained at the ion spray interface voltage of +3 kV. This produced a potential

difference across the capillary column of 35 kV. Samples were loaded into the inlet of the capillary via hydrostatic injection by elevating the inlet 6 cm for 5 s. The 6 cm distance was measured between the liquid surface of the sample vial and the surface of the liquid junction buffer reservoir. The API mass spectrometer used was the same as described above, and was operated in the SIM mode for these experiments.

Although a variety of capillary electrophoresis applications have appeared in the recent past using UV [13] fluorescence [14], and indirect fluorescence detection [15], there have been relatively few reports of on-line CE/MS. The two approaches to CE/MS to-date include those performed using continuous flow fast atom bombardment (CFAB) [16] and electrospray [6]. CE/MS via CFAB has been reported using two different approaches [16, 17], and CE/MS via electrospray has been reported using the sheath-flow [8] and the liquid junction devices [9]. Neither approach to CE/MS is trivial to accomplish, but there is a developing opinion that the sheath-flow approach is more practical experimentally. Our recent experience with a commercial P/ACE 2050 CE system (Beckman Instruments, Inc., Palo Alto, CA) and a homemade system using the above-described liquid junction–ion spray interface suggests that CE/MS techniques may play a significant rôle in the future of analytical chemistry. The recent commercial availability of CE instruments that provide an automated, instrumental format for electrophoresis promises to complement the long, favourable history of slab gel electrophoresis.

An example of on-line CE/MS using the combined liquid junction–ion spray apparatus is shown in Fig. 4. In this example, 8 pmol per component of a synthetic mixture containing sulphonylurea herbicides were loaded onto the inlet end of the capillary. The mass spectrometer was operated in the SIM mode with positive ion detection. The protonated molecule ions for each component were monitored at *m/z* 365, 396, 411 and 415. The molecular weights of these compounds in their order of elution in Fig. 5 are 410, 364, 395, 410 and 414. These data reveal the quality of separation efficiency provided, and relatively high speed of separation of the CE/MS technique described here. In contrast to our earlier report [5] where it took over 24 min to separate five sulphonylurea compounds, the shorter CE column used for the data shown in Fig. 5

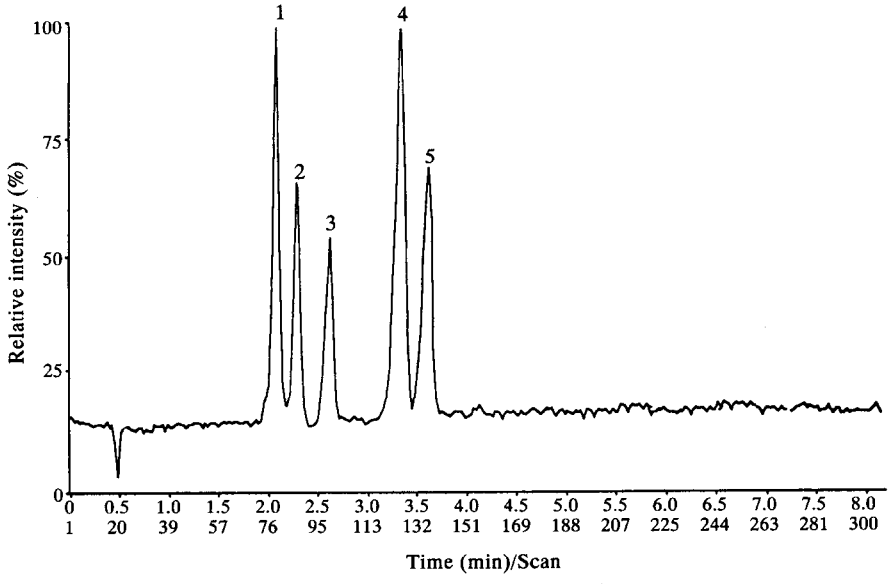

Figure 5
SIM CE/MS total selected ion current electropherogram for 2 pmol per component of a synthetic mixture containing sulphonylurea herbicides. The separation conditions were: 75:25 CH_3CN–5 mM NH_4OAc, pH = 5. The sample was loaded into the capillary column hydrostatically by elevating the inlet 6 cm for 5 s while the column inlet was immersed in the sample. The capillary was uncoated fused-silica, 75 μm i.d. × 35 cm. The anode was held at 38 kV while the liquid junction was held at 3 kV to give a potential across the column of 35 kV. The peak elution order is (1) bensulphuron methyl, mol wt = 410; (2) sulphomethuron methyl, mol. wt = 364; (3) tribenuron methyl, mol. wt = 395; (4) nicosulphuron, mol. wt = 410; and (5) chlorimuron ethyl, mol. wt = 414. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

demonstrate the separation in <4 min. Additional structural information is available using tandem mass spectrometry techniques such as CID of the precursor ions while scanning the third quadrupole through the full mass range to observe all the product ions formed. This capability is a useful adjunct to the ion spray CE/MS technique which by itself only provides molecular weight information.

Because the CE liquid inlet system is physically separate from the APIMS vacuum system, coupling these two analytical techniques is straightforward. In contrast, the CFAB approach to CE/MS requires that the exit of the CE capillary or a connecting capillary from 0.5–1.0 m long be directed inside the vacuum system of the mass spectrometer. The vacuum system thus 'pulls' on the exit of the capillary and can contribute to band broadening and other adverse effects unless the latter is decoupled from the separation capillary [16, 17]. In addition, the CFAB approach requires a matrix of glycerol or related material to facilitate the FAB ionization process. This material must be continuously introduced into the mass spectrometer either coaxially [17], or via a make-up junction along with the running buffer to provide continuous ionization [16]. Sometimes additional material must be added to the matrix to provide uniform response for all analytes of interest [18]. These requirements for CFAB combined with CE place stringent practical demands for accomplishing routine CE/MS. Although impressive CFAB CE/MS results have been reported, it appears that the electrospray ionization approach to CE/MS may be more widely used in the future.

A limitation remains, however, for CE/MS and CE/MS/MS due to the low sample capacity of the CE capillary column, and the limited sensitivity afforded by today's mass spectrometers. In addition, sometimes it is necessary to compromise on the buffer used to achieve adequate sensitivity from the electrospray ionization process. Although SIM techniques provide better sensitivity than full-scan CE/MS experiments, our best performance has struggled to detect low fmol quantities of well-behaved small peptides [9], and typically we are limited to high fmol to low pmol CE/MS detection capability. In contrast, others have demonstrated much better detection limits using, for example, indirect fluorescence detection [15]. Even UV detection typically has better detection limits for well-behaved compounds than present MS capability. This situation may change, however, as ion trap mass spectrometers are adapted to CE/MS capability (*vide infra*).

Ion spray LC/MS on a benchtop mass spectrometer

The micro HPLC system used for LC/MS was a Model 600 MS (Millipore) operated under isocratic conditions at 40 μl min^{-1}. The eluent was 5 mM ammonium formate in 70:30 acetonitrile–water. The column used for the elution of the indole alkaloid, reserpine, was a 1 mm i.d. × 100 mm Zorbax RX-C-8, 5 μm particle-size custom-packed by Keystone Scientific (Bellefonte, PA). A fixed-loop (1 μl) Rheodyne Model 7520 syringe-loading sample injector was used to inject the sample onto the microbore HPLC column. The total effluent was directed through the ion spray interface and the spray region of the interface was positioned near the ion sampling capillary of the modified MSD mass spectrometer (Fig. 6).

The modification and performance of the Hewlett-Packard 5970 MSD single quadrupole mass spectrometer has been described elsewhere [10]. Basically, the GC and the entire standard electron ionization (EI) ion source-lens system were removed from the MSD. The analyser housing was replaced with one fitted with a 4 inch flange that accommodates a larger (330 l s^{-1}) turbomolecular pump. A two-stage API sampling inlet for the high vacuum system was fabricated with an ion sampling capillary similar to that reported by Chair *et al.* [19]. A heated ion sampling capillary (500 μm i.d. × 14 cm) provides differential pumping between atmospheric pressure and an intermediate 1.4 torr region (Fig. 6). Ions formed at atmospheric pressure via the ion spray interface [Fig. 6(A)] are sampled through the ion sampling capillary [Fig. 6(B)] and enriched relative to gas molecules in the region between the capillary tube exit and the ion sampling conical orifice [Fig. 6(E)]. The latter [Fig. 6(D)] separates the intermediate 1.4 torr region from the high vacuum (1×10^{-4} torr) quadrupole mass analyser region. By applying appropriate voltage potentials on the ion sampling capillary and the ion sampling conical orifice, ions can be focused into an Rf-only quadrupole lens that is capacitivity coupled to the standard MSD mass analyser (not shown in Fig. 6).

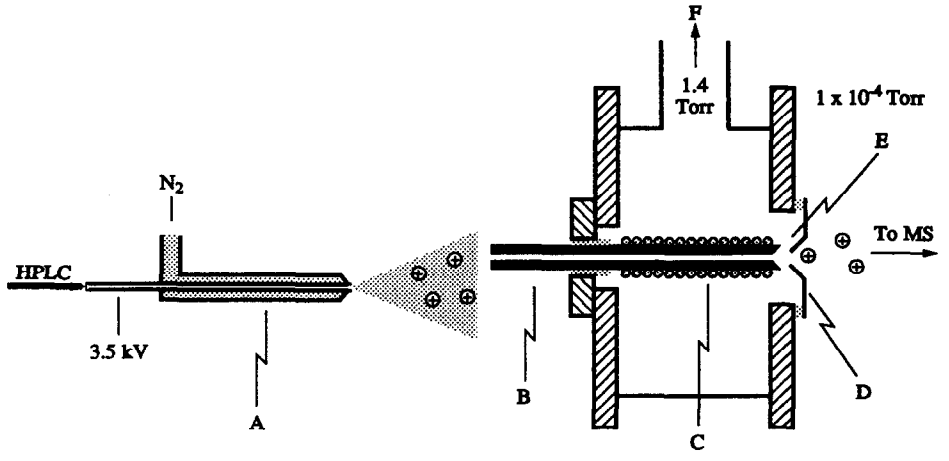

Figure 6
Ion-spray–API MSD combination inlet system on a modified Hewlett–Packard 5970 mass selective detector. (A) Ion spray interface; (B) ion sampling capillary; (C) heated region (80 degrees) of ion sampling capillary; (D) conical ion sampling orifice separating the first vacuum stages from the high vacuum mass analyser region of the single quadrupole mass spectrometer; (E) CID region where ion–molecule interactions can be utilized to produce fragmentation of protonated molecule ions; (F) rotary pump outlet to evacuate the first state of pumping to 1.4 torr. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

Minor adjustment of the standard Rf-scan coils allows the combined Rf-only mass analyser quadrupole system to function in tandem.

The system described above has been used to analyse samples containing environmentally important pesticides, benzodiazepines and their metabolites in human urine, LSD, and tetraalkyl ammonium compounds [10]. Recent results in the negative ion mode of detection have shown feasibility for the detection of inorganic anions such as nitrate, sulphate and halides with this system. The LC/MS data shown in Figs 7 and 8 were obtained from the analysis of the potent tranquilizer drug, reserpine. This thermally unstable basic drug is not amenable to GC/MS characterization yet very sensitive analytical capability is required to detect its presence in biological fluids such as equine plasma [20]. In an effort to determine whether the benchtop LC/MS system could be used to regularly monitor reserpine in such biological fluids, we have begun preliminary ion spray LC/MS investigations with this system. Fig. 7 shows a full scan CID mass spectrum obtained from the on-line LC/MS analysis of a 600 ng sample of reserpine injected onto the 1 mm i.d. micro HPLC column. This rather high level of sample was used initially for a preliminary determination of the optimum tuning parameters for this analyte. The CID mass spectrum shown in Fig. 7 is not a classical MS/MS mass spectrum. Instead, the mass spectrum in Fig. 7 was obtained by increasing the potential difference between the ion sampling capillary [Fig. 6(B)] and the ion sampling cone [Fig. 6(D)] in the first vacuum region. This increases the energy of ion–molecule collisions in this region thus causing fragmentation of the protonated molecule ion for reserpine at *m/z* 609. This process differs from the true MS/MS situation where there is mass analysis prior to the CID process. Thus this 'poor mans' CID does not provide mixture analysis as does MS/MS, but it does provide structural information by producing a full-scan mass spectrum characteristic of the compound's structure. Figure 7 is not an EI mass spectrum, but there are similarities between it and a conventional EI mass spectrum from reserpine [21]. For example, the *m/z* 448, 397, 365, 195 and 174 ions observed in Fig. 7 are also present in the EI mass spectrum of reserpine although the latter has additional ions not observed in our CID mass spectrum.

After optimizing the ion spray benchtop CID LC/MS conditions for reserpine, characteristic fragment ions from Fig. 7 may be selected and monitored in the SIM mode in subsequent CID LC/MS experiments. By monitoring only three abundant, structurally important ions, e.g. *m/z* 609 $(M + H)^+$, 195 and 174, the mass spectrometer does not waste time scanning regions in the mass scale where there is no significant information. These SIM experiments can provide significantly im-

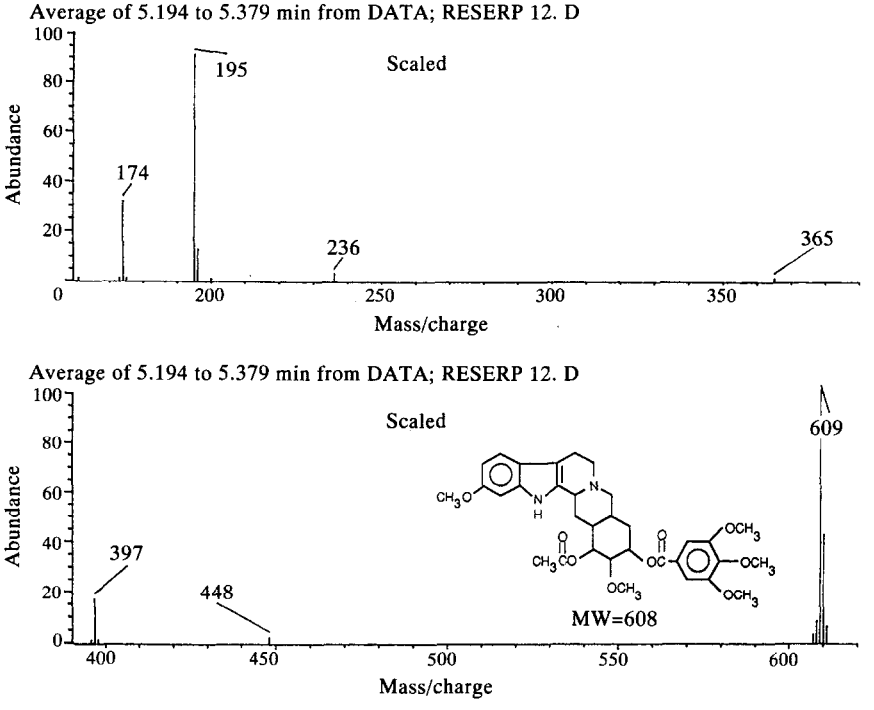

Figure 7
Unsubstracted full-scan CID mass spectrum from the on-column LC/MS analysis of a 600 ng sample of reserpine. A potential difference of 100 V was applied between the ion sampling capillary [Fig. 8(B)] and the conical ion sampling orifice [Fig. 8(D)]. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

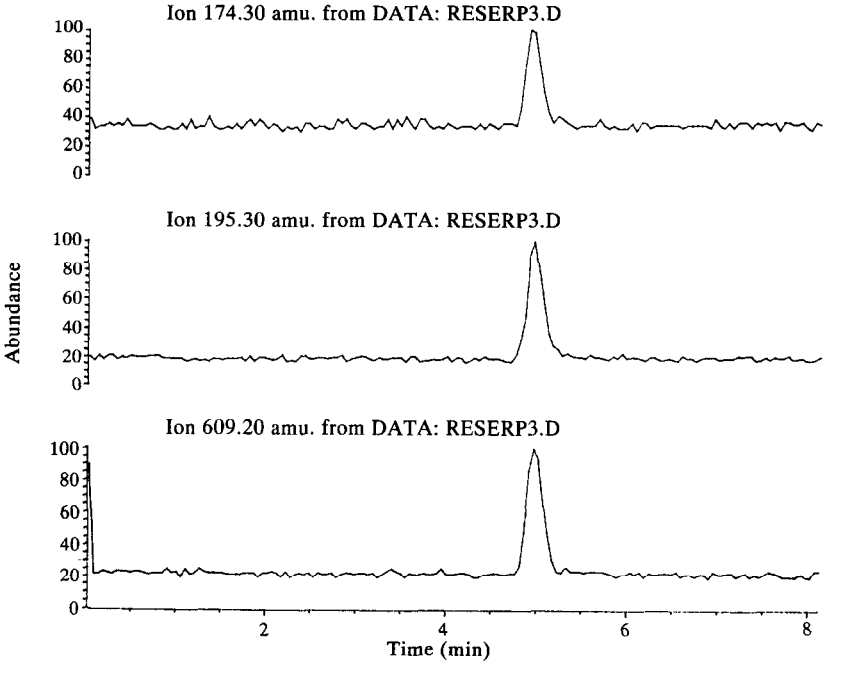

Figure 8
SIM CID LC/MS selected ion current profiles for *m/z* 174.3, 195.3 and 609.2 $[(M + H)^+]$ following on-column injection of 1.5 ng reserpine. See text for HPLC conditions. (Reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a division of Preston Industries, Inc.)

proved sensitivity. Figure 8 shows the results from such a SIM CID LC/MS analysis of a standard reserpine sample when 1.5 ng of the compound are injected on-column. The three panels in Fig. 8 show the ion current profiles (from top to bottom) for *m/z* 174.3, 195.3 and 609.2. The signal-to-noise ratio for these data is very good suggesting that subnanogram detection limits may be possible for this compound with the current system. We are currently working to improve the sensitivity of this system further and believe that another factor of 10 improvement should be possible.

In many laboratories and areas of analytical chemistry high sensitivity and trace analysis capability remains an elusive goal. The techniques described in this paper do not currently satisfy all requirements of an ultra-sensitive technique, but progress is being made towards this goal. For example, ion trap technology originally available for GC/MS applications continues to look very promising. When one considers recent developments with the quadrupole ion trap mass spectrometer (ITMS) [22] [Finnigan MAT (San Jose, CA)] it becomes apparent that this detector could add significantly improved capability over what we now have from more conventional mass spectrometers. The recent report by Cooks *et al.* has demonstrated high sensitivity, high mass resolution, and multiple stages of MS/MS [23]. The dramatically increased sensitivity shown here may be a significant benefit towards matching the low sample capacity demands of CE/MS described above. When this capability is combined with electrospray [24] and becomes routinely available on commercial systems, on-line LC/MS analyses using ion trap technology [25] may be able to provide much better analytical capability than is available today. In addition, the quadrupole ion trap hardware is inherently more simple, and presumably could be significantly less expensive than today's benchtop systems. This could of course make it even easier to implement this analytical capability into more laboratories. Progress is being made in our laboratory as well as in other laboratories to implement electrospray ionization on simple, inexpensive ion trap systems.

To test the ruggedness and utility of our first-generation or 'phase I' benchtop ion trap instrument as an on-line detector a series of experiments were conducted coupling HPLC

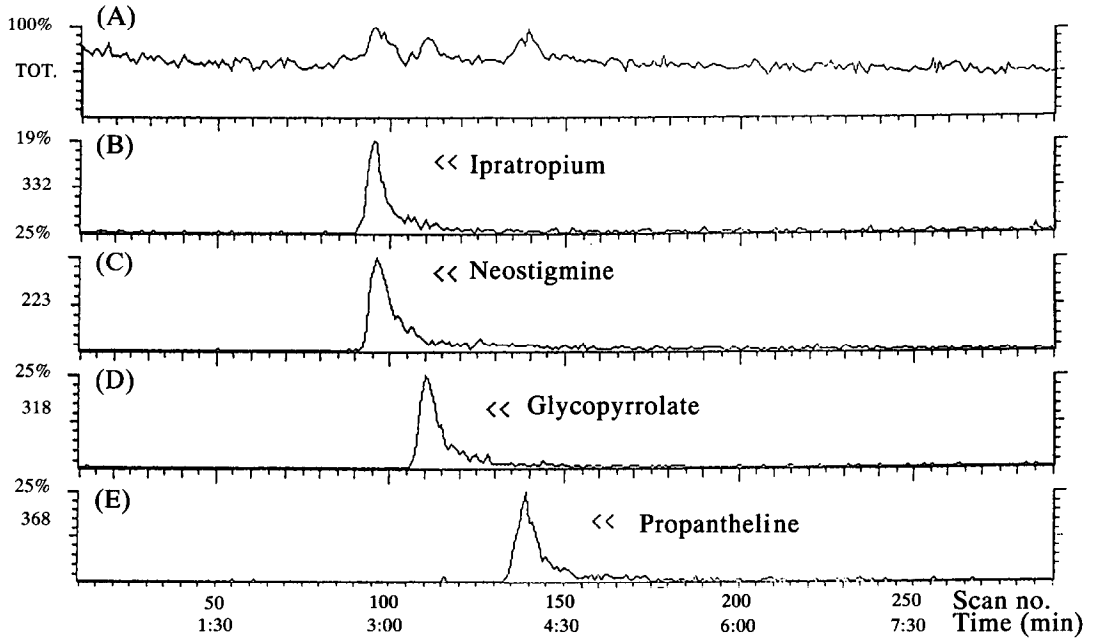

Figure 9
Ion spray HPLC/ITMS analysis of a synthetic mixture containing 5 ng each of four quaternary ammonium drugs. Conditions: 1 mm i.d. × 150 mm C-8 HPLC column operated in the isocratic mode with 40 μl min^{-1} of 80:20 acetonitrile–water containing 20 mM ammonium acetate and 0.1% trifluoroacetic acid (TFA). Potential difference across first vacuum region was 70 V. (A) TIC from *m/z* 100–400; (B) EICP for *m/z* 332, ipratropium; (C) EICP for *m/z* 223, neostigmine; (D) EICP for *m/z* 318, glycopyrroloate; (E) EICP for *m/z* 368, propantheline. (Reproduced from the *Rapid Communications in Mass Spectrometry* by permission of John Wiley and Sons, Inc.)

and CE to our API-ion trap system [26]. The compounds chosen for these studies were small quaternary ammonium drugs. These charged compounds were chosen because they are not amenable to GC without pyrolysis, so the combination of LC or CE with MS may provide a preferred tool for their identification at the low nanogram level. Results of the on-line coupling of HPLC/ITMS are shown in Fig. 9. A synthetic mixture containing 5 ng of each drug was injected on-column under micro HPLC conditions. The total column effluent (40 μl min^{-1}) was directed to the mass spectrometer in this experiment. The potential drop across the first vacuum region was 70 V. Panel A is the total ion current (TIC) with a scan range from 100 to 400 amu, while panels B–E are the extracted ion current profiles (EICP) of the molecular ions of ipratropium, neostigmine, glycopyrrolate and propantheline, respectively. Figure 10 shows the full-scan mass spectrum of propantheline (peak at scan No. 138, Fig. 9) as an example of a typical mass spectrum acquired from 5 ng of drug. The base peak is the molecular ion, m/z = 368, while the mild declustering conditions used also lead to the formation of two minor fragment ions at m/z = 181 and 326. The on-line analysis of this same mixture by CE/MS on the benchtop ion trap system has also been described [26] where the injected quantities of these compounds was only 150 pg per component. A full-scan mass spectrum for these levels of materials was reported.

Summary and Conclusions

This report has presented results demonstrating that an atmospheric pressure ionization mass spectrometer is an effective and useful detector for HPLC and CE. Both conventional HPLC and micro HPLC flow rates have been described as well as submicrolitre per minute flow in the case of CE. In addition, three different mass spectrometers and three different 'liquid inlet' systems housed in the same laboratory were used by at least three different people to obtain the results shown. Another related but different LC/MS interface, the heated pneumatic nebulizer device, is also routinely used, although results from this interface are not described in this work [5, 27]. It is usually implemented for applications involving less polar analytes, and has been used with either of the first two mass spectrometers described [5, 10]. The routine use of these systems occurs in this labortory for basic and applied research as well as specialized service. Both qualitative [28] and quantitative [29] results are possible in addition to coupled-column HPLC combined with MS/MS [30]. These techniques have all been demonstrated and may be implemented by the appropriately initiated and qualified personnel.

The future appears bright for dramatic improvements in the ease of use and performance of the techniques described in this report as well as the newer benchtop technol-

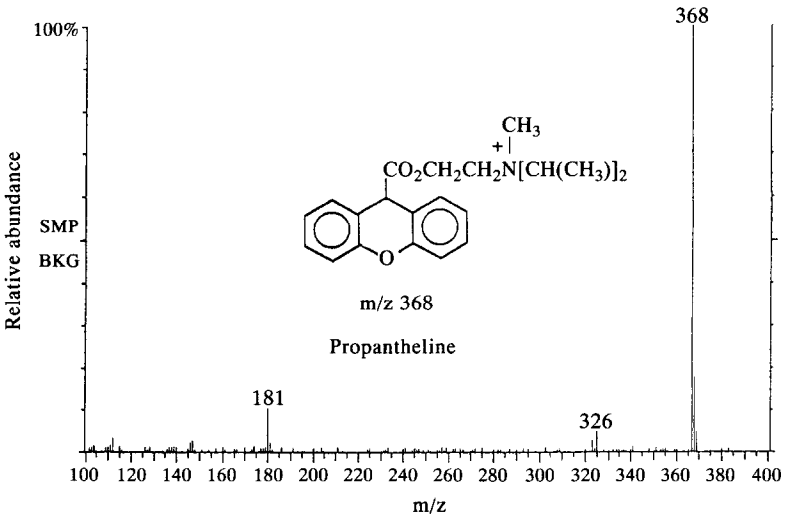

Figure 10
Full mass spectrum of 5 ng propantheline from Fig. 9. Peaks at m/z 368, 326 and 181 are suggested to be M^+; (M^+ – C_3H_6) and $(C_6H_4CHOC_6H_4)^+$, respectively. (Reproduced from the *Rapid Communications in Mass Spectrometry* by permission of John Wiley and Sons, Inc.)

ogies. These improvements will make pharmaceutical LC/MS techniques more available and thus better prepare laboratories for the challenging analytical demands of the future.

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