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Ion spray mass spectrometric detection for liquid chromatography: a concentration- or a mass-flow-sensitive device?

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ABSTRACT

As the mass spectrometer becomes more accepted as a detector for HPLC, its characteristics should become better understood by those performing routine LC–MS experiments. In particular, the ion current response for quantitative analysis studies involving significant dynamic range in concentration for target analytes must be determined as well as other factors that affect MS response. This work describes the concentration-sensitive response for the ion spray (pneumatically-assisted electrospray) LC–MS interface from the chromatographer's perspective. A comparison of LC–MS ion current response in the isocratic mode resulting from studies of a synthetic mixture containing alkyl benzoates is presented. LC–MS total ion current chromatograms from three different column sizes (1 mm I.D., 2.1 mm I.D. and 4.6 mm I.D.) with and without a post-column split, and high-flow ion spray LC–MS without a post-column split illustrates that the former behaves as a concentration-sensitive detector whereas the latter behaves as a mass-flow-sensitive detector. The flexibility of ion spray to high-flow applications allows the use of HPLC eluent flow ranging from 0.001–2.0 ml/min. The use of solvent–buffer post-column addition also allows optimization for improved analyte ion current response.

INTRODUCTION

Liquid chromatography (LC) is largely used for the determination of polar or thermolabile compounds with UV, fluorescence, or electrochemical detection. More recently mass spectrometry (MS) has been introduced as a highly specific and sensitive detector for HPLC for quantitative or qualitative studies. Several approaches have been used to combine LC and MS including direct liquid introduction (DLI) [1], thermospray (TS) [2], particle beam (PB) [3] and more recently electrospray [4] and ion spray (IS) [5].

Unfortunately, LC–MS combinations often place restrictions on LC flow range or eluent composition [6,7]. For thermospray LC–MS applications, an eluent flow or 1 ml/min using reversed-phase conditions and volatile buffers are typical. Particle beam LC–MS applications allow similar HPLC conditions, but with effluent flow restricted to between 0.2–0.5 ml/min. Both of these LC–MS approaches sometimes provide disappointing limitations regarding detection limits for trace analyses. In contrast, pure electrospray can only handle eluent flow in the 0.002–0.005 ml/min range, yet provides very high sensitivity for certain polar analytes. Re-

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cently, an ultrasonic nebulizer device has increased electrospray flow capability to 0.4 ml/min [8], but little is known presently about on-line LC–MS capabilities with this approach. Intermediate between thermospray and pure electrospray flow ranges is pneumatically assisted electrospray or ion spray [5]. This simple modification of electrospray performs best with flow rates from 1–100 μl/min, and many applications of LC–MS have been reported [1,5]. Ion spray LC–MS is thus compatible with reduced diameter micro-bore columns without post-column splitting while larger bore columns require a post-column split that confines the interface flow to this range. Recently, we have extended the application of ion spray to flow-rates up to 2 ml/min using a liquid shield [9], which avoids the need for conventional post-column splitting with HPLC columns up to 4.6 mm I.D.

The analyst is faced with the challenge of coupling different HPLC column diameters and their corresponding optimum flow-rates to the LC–MS interfaces mentioned above, each of which has its own limitations regarding effluent flows. Electrospray, for example, has required a substantial post-column split with 4.6 mm I.D. columns in order to restrict the 1 ml/min column effluent flow at the electrospray interface to less than 5 μl/min. The question that often arises is does diverting 95% of the HPLC effluent away from the mass spectrometer reduce LC–MS sensitivity? Similarly, if 1 mm I.D. HPLC columns are used with electrospray, does one obtain improved sensitivity? An understanding of these factors affecting these questions is important for optimizing an experimental set-up for LC–MS experiments.

In general a mass spectrometer equipped with an electron ionization (EI) or chemical ionization (CI) ion source behaves as a mass-flow-sensitive detector [7]. In contrast, both electrospray [4] and ion spray [5] LC–MS techniques behave as a concentration-sensitive detector [10], where the ion current response is directly proportional to the analyte concentration in a chromatographic peak. The latter is dependent on the column inside diameter. The first part of this work re-investigates the benefits and the limitations of HPLC–IS-MS with different column I.D.s including 1 mm (micro-bore), 2 mm (narrow-bore), and 4.6 mm (standard-bore). Alkyl benzoate esters are used as model compounds for low-molecular-mass analytes to compare with LC–UV performance in the isocratic mode. The isocratic mode was chosen relative to the gradient mode to minimize instrumental variables.

The second part of this report describes an approach that significantly increases the analyte ion current response in IS-MS. Since the ion spray ion current is dependant upon the nature of the analyte as well as the composition of the mobile phase, we have studied various means for improving the analyte response without affecting the chromatographic conditions. In some cases, the analyst must compromise MS response to achieve desired HPLC separation. Voyksner *et al.* [11] have demonstrated for thermospray that post-column addition or buffer addition can sometimes significantly improve the overall response of the analytes. The limited flow capabilities of pure electrospray have precluded this ploy for LC–MS applications using larger-bore HPLC columns. Using high-flow ion spray [9], where the detector behaves like a mass-flow-sensitive device, we have investigated the potential of post-column addition using two pesticides as model compounds to improve IS-MS response.

GENERAL CONSIDERATIONS

Chromatographic separation

A chromatographic separation is based on two principles: resolution between two compounds and analyte dilution in the mobile phase [12]. The latter dilution can be described by a Gaussian distribution [13], where the maximum peak concentration (C_{max}) of the eluting compound is a function of injected analyte (m), the efficiency of the column (N), the capacity factor (k') of the analyte and the dead volume of the column (V_0) which is also dependent on the length (l) and internal diameter (d) of the column. The relationship between these parameters is shown in eqn. 1 [13].

$$C_{max} = \frac{mN^{1/2}}{(2\pi)^{1/2}V_0(1+k')} \quad (1)$$

The C_{max} is directly proportional to the mass of analyte and the efficiency of the column and retention time also affects C_{max}. High plate numbers and short retention time will provide the best sensitivity for a concentration-sensitive device. One of the most important parameters which affects C_{max} is the column internal diameter. For two columns with the same packing material and the same length, but different internal diameter (d_{c1} and d_{c2}) the C_{max} ratio can be described by eqn. 2

$$C_{max}\text{ ratio} = \frac{d_{c1}^2}{d_{c2}^2} \quad (2)$$

Table I shows the relative increase in C_{max} *versus* different column inside diameters based on a ratio of cross-sectional areas. The theoretical increase in sensitivity using a column with 1 mm I.D. instead of a 4.6 mm I.D. is about a factor of 21 due to smaller V_0 and less sample dilution.

Mass-flow-sensitive detector

A mass-flow-sensitive detector is defined as one where the response is directly proportional to the amount of analyte reaching the detector *per unit time* [7]. Typical mass-flow-sensitive devices are the flame ionization detector and the classical EI mass spectrometer. Both of these are also destructive detectors [14]. The response (R) can be described by eqn. 3 with (m) the analyte mass, (F) the flux, (S) the splitting ratio, (a) response factor, and (t) time. C_{max} is the maximum concentration of the analyte.

$$R = a \cdot \frac{\partial m}{\partial t} = aC_{max}FS \quad (3)$$

The response R with a mass-flow-sensitive detector is directly proportional to the flux and a decrease of flow-rate, as with post-column splitting, will produce a decrease in response. When the flow-rate is maintained constant, the response is directly proportional to C_{max}. In the case of an "infusion" experiment with a constant analyte concentration, an increase in flow will result in a linear increase in signal. This means that for flow injection analysis a reduction in flow for example, will lower the peak height but the peak area will remain the same when the same quantity of material is injected.

TABLE I

THEORETICAL INCREASE IN C_{max} FOR DIFFERENT COLUMN I.D.s

	d_c (mm)		
	4.6	2.0	1
Increase in C_{max}	1	5	21

Concentration-sensitive detector

For a concentration-sensitive device the detector signal depends on the concentration of the sample in the carrier flow. Most of the detectors used in liquid chromatography, such as UV and fluorescence devices, are concentration-sensitive detectors where the response R is directly proportional to C_{max} and can be described by eqn. 4 where a is a response factor [15].

$$R = aC_{max} \quad (4)$$

Concentration-sensitive detectors are generally non-destructive and the flow-rate does not affect the response because the physical measurement of the analyte is much faster than the sample flow-rate [14]. For an infusion experiment with a constant analyte concentration the response stays constant with an increase of flow-rate. This means that for flow injection analysis a decrease in flow will increase the peak area but the peak height will remain the same. This report describes a series of experiments designed to demonstrate and compare the results obtained from on-line ion spray LC–MS experiments, in the isocratic mode, with a view of those factors important to consider for optimizing conditions for LC–MS analyses.

EXPERIMENTAL

Chemicals

The water used was obtained from a Barnstead Nanopure cartridge system (Boston, MA, USA). Acetonitrile and methanol were of Fischer Optima grade and ammonium acetate was of Fischer HPLC grade (Fair Lawn, NY, USA).

High-purity trifluoroacetic acid (TFA), and tetradecyl ammonium bromide (TDAB) were obtained from Sigma (St Louis, MO, USA), Tetrabutyl ammonium hydroxide (TBAH) was purchased from Southwestern Analytical Chemicals (Austin, TX, USA). Ethyl, propyl and butyl benzoate esters were purchased from Aldrich (Milwaukee, WI, USA). Monuron and carbofuran were obtained from the US Environmental Protection Agency Repository (Research Triangle Park, NC, USA).

Liquid chromatography

The synthetic compound mixture was analyzed on 100 mm length Keystone BDS-Hypersil 5 μm C_8 columns (Keystone Scientific, Bellefonte, PA, USA) with internal diameters of 1.0, 2.0 and 4.6 mm. The packing material for each of these columns was from the same lot. Mobile phase was delivered by an Hitachi L-6200A pump (Hitachi Instruments, Danbury, CT, USA). Isocratic separation of the alkyl benzoate esters was achieved with an 0.15% TFA in acetonitrile–water (60:40) mobile phase. The use of TFA does not affect the chromatographic separation of the alkyl benzoate esters, but does improve the IS-MS response of these neutral organic compounds. Isocratic separation of the two pesticides used in this study was achieved with a 5 m*M* ammonium acetate in acetonitrile–water (40:60) solution. HPLC mobile phases were filtered and degassed prior to use by sparging with helium. The compounds were loaded on the HPLC columns using an external loop Model 7125 injector with a 5-μl loop or a Model 7520 injector with a 0.5-μl internal loop (Rheodyne, Cotati, CA, USA). The splitting and post-column experiments were performed with a low-dead-volume tee (Upchurch Scientific, Oak Harbor, WA, USA).

UV detection

For UV detection an Applied Biosystems (San José, CA, USA) Model 757 variable-wavelength UV detector was used with interchangable 0.5-μl (1 mm path length) and 2.4-μl cells (6 mm path length). The LC–UV experiments were performed using the 4.6, 2 and 1 mm I.D. columns with the 0.5-μl cell. For the post-column experiments with the 1 mm column the 2.4-μl cell was

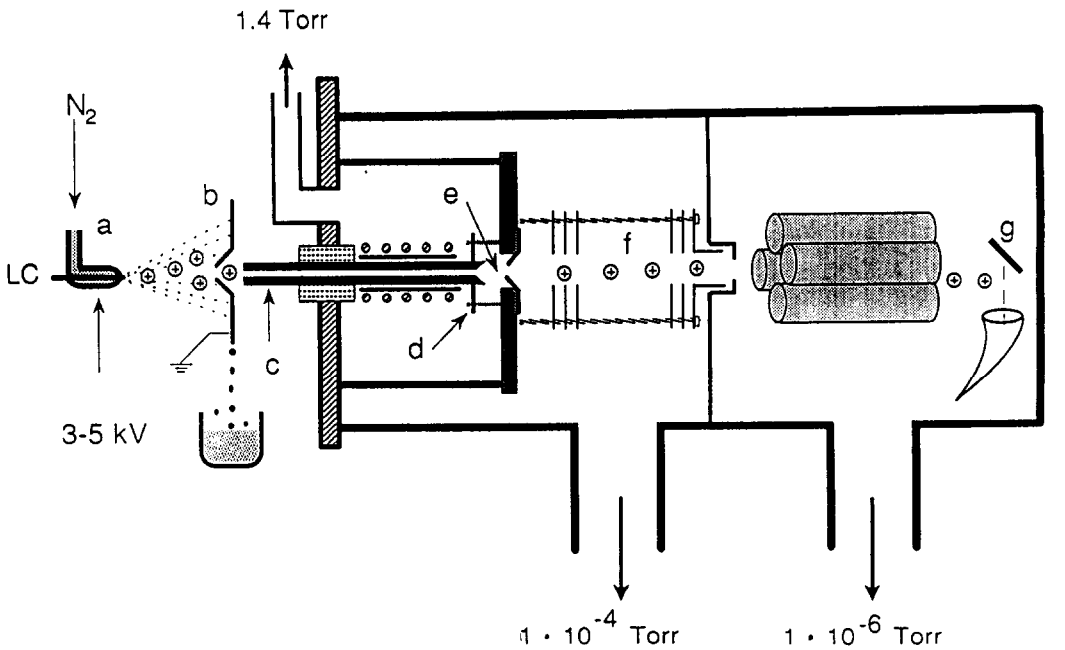

Fig. 1. General schematic of the ion spray LC–MS system incorporated on an HP 5985B mass spectrometer. a = Ion spray: b = liquid shield; c = heated ion capillary; d = centering device; e = skimmer; f = Einzel lenses; g = high-energy dynode detector. 1 Torr = 133.322 Pa.

used. Every effort was made to minimize extra-column dead volume in these experiments, in particular minimum bore tubing (I.D. = 100 μm) and zero-dead-volume fittings were used. The wavelength was set at 254 nm for the benzoate esters and at 214 nm for the pesticides. UV chromatograms were recorded by a Hewlett-Packard 3390A integrator (Avondale, PA, USA).

Ion spray mass spectrometric detection

All LC–MS measurements were performed under selected ion monitoring (SIM) conditions on a Hewlett-Packard Model 5985B mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) with an in-house constructed interface capable of sampling ions formed at atmospheric pressure [9]. Fig. 1 shows a schematic drawing of the system. The standard EI source and its associated Einzel lens system were replaced by a two-stage vacuum region designed to sample ions formed at atmospheric pressure. The system is patterned after that reported by Chait *et al.* [16], and is composed of the following key features (Fig. 1): (a) ion spray LC–MS interface, (b) grounded liquid shield, (c) heated ion sampling capillary in the first vacuum region, (d) centering device, (e) ion sampling skimmer, (f) second vacuum region housing the ion optics lens, (g) high-vacuum region housing the quadrupole mass analyzer and high-energy dynode electron multiplier. A complete description of this system is given elsewhere [9].

The setup for high-flow ion spray is similar to conventional ion spray which has been described previously. The LC column effluent (1–2000 μl/min) travels through a connecting fused-silica capillary (100 mm length × 0.1 mm I.D. × 0.25 mm O.D.) that protrudes from a stainless-steel capillary (0.004 in I.D. × 0.009 in O.D.) housed in the ion spray LC–MS tee fitting [4]. High voltage (3–5 kV) is applied to the effluent near the sprayer tip via an electrical contact on a stainless steel tee fitting that houses the two concentric capillaries positioned by graphite ferrules. Nitrogen gas (50–80 p.s.i.; 1 p.s.i. = 6894.76 Pa) is passed through the annular space between the inner and the outer capillaries to affect pneumatically-assisted electrospray (ion spray) ionization. For HPLC experiments the sprayer is positioned off-center (5 mm) and about 10 mm distant from the orifice in the liquid shield. The excess eluent is allowed to drip from the liquid shield into a beaker (Fig. 1). Some safety precautions should be taken to vent the excess solvent vapor.

The mass spectrometer data acquisition was performed using the standard Pascal Workstation with release 3.1.1. HP software (Hewlett-Packard). The tuning and mass calibration of the HP-5985B were achieved via a 4 μl/min infusion of a 10 pmol/μl methanol solution of either TBAH while monitoring $m/z = 242.24$, 142.16, 100.01, 57.07 or tetradecyl ammonium bromide (TDAB) while monitoring $m/z = 578.66$, 310.35, 184.21 using the manual tune software. Fragment ions were obtained by increasing the potential difference between the ion sampling capillary (Fig. 1, c) and the skimmer (Fig. 1, e) which produces collision-induced dissociation using a potential difference of 85 V for TBAH and 160 V for TDAB [4]. The mass resolution was adjusted to provide peak widths of 0.6 u. at half-height across the mass range (50–1000 u).

RESULTS AND DISCUSSION

Conventional ion spray

Fig. 2 shows the LC–UV traces of three alkyl benzoate (ethyl, butyl, propyl) esters using three different column I.D.s: (A) 1, (B) 2 and (C) 4.6 mm. The flow-rates were optimized to obtain similar analysis times for all three columns with the same mobile phase composition. The same hardware (pump, injector, detector equipped with a micro cell) was used for all three experiments and special care was taken to minimize extracolumn variance for the micro-bore separation, in particular tubing (100 μm I.D.) and connections. The injection volume (5 μl) and the injected amount was indentical on all three columns. When the column I.D. is reduced from 4.6 to 1 mm the increased peak height (butyl benzoate ester) is only 7.4 *versus* 21 which is theoretically expected. All our efforts failed to produce the theoretical 21-fold increase presumably because it is not practical to eliminate all extra-column dead volume in the micro-bore system. A smaller injection volume of 0.5 μl

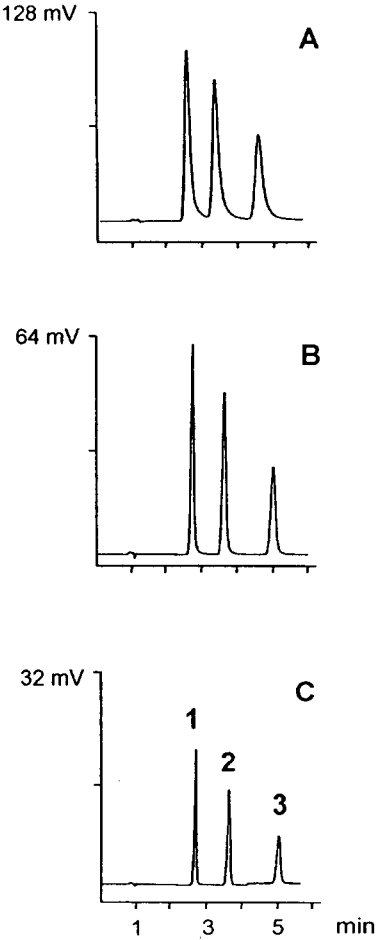

Fig. 2. LC–UV traces of alkyl benzoate esters on three different column I.D.s: (A) 1 mm I.D., flow-rate = 65 μl/min; (B) 2.0 mm I.D., flow-rate = 210 μl/min; (C) 4.6 mm I.D., flow-rate = 1000 μl/min. UV Detector with a 0.5-μl cell, 254 nm, 5-μl injection. Peaks: 1 = ethyl benzoate; 2 = propyl benzoate; 3 = butyl benzoate.

with an internal loop injector did not provide improved resolution. It is also well-known that packing techniques for micro-bore columns are more critical so these 1 mm I.D. columns often do not provide comparable separation efficiencies to 4.6 mm I.D. columns. The separation efficiency achieved on the butyl benzoate ester ($N = 1588$) for the 1 mm I.D. column, as illustrated in Fig. 2A is not as good as for the 4.6 mm column ($N = 6774$) result shown in Fig. 2C. Similarly, for the 2 mm I.D. column (Fig. 2B) the loss of chromatographic performance is about 35% resulting in a 3.8-fold increase in response rather than 5 which is theoretically expected.

The same experiments were repeated with HPLC–IS-MS conditions using a post-column split for the larger I.D. columns to give the results shown in Fig. 3 where the flow to the mass spectrometer was maintained at 65 μl/min. Similar detector response behavior is observed for HPLC–IS-MS as for LC–UV. In this case the increase in peak height for the butyl benzoate ester is about 8.5 using the 1 mm I.D. column compared to the 4.6 mm I.D. column as shown

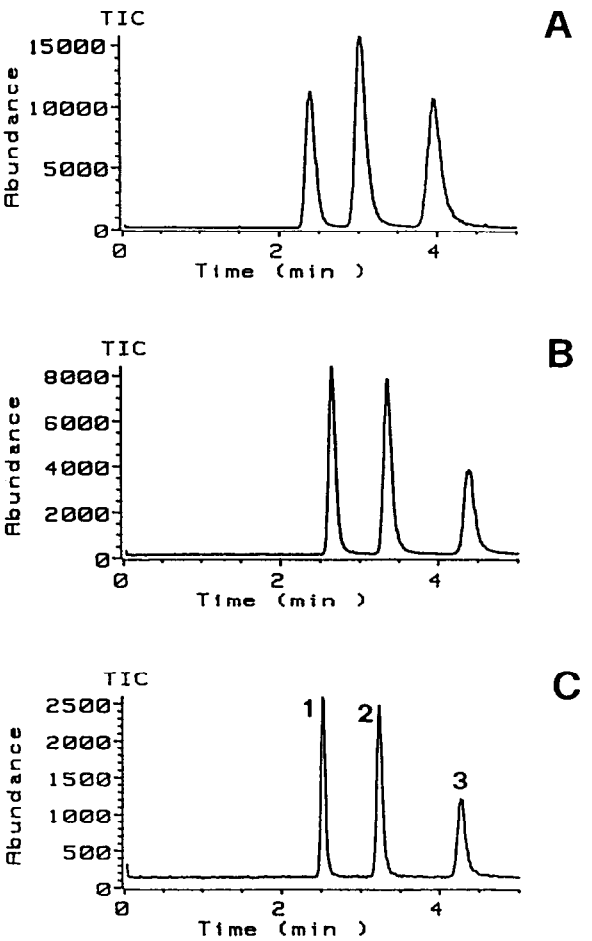

Fig. 3. LC–IS-MS traces of alkyl benzoate esters on three different column I.D.s with post-column splitting. (A) 1 mm I.D., flow-rate = 65 μl/min; (B) 2.0 mm I.D., flow-rate = 210 μl/min, splitting flow-rate to mass spectrometer 65 μl/min; (C) 4.6 mm I.D., flow-rate = 1000 μl/min, splitting flow-rate to mass spectrometer 65 μl/min, injection volume 5 μl. TIC = Total ion current.

TABLE II

OBSERVED RELATIVE INCREASE IN RESPONSE AS A FUNCTION OF COLUMN I.D.

Butyl benzoate ester, $n = 3$.

	1/4.6	2.0/4.6	4.6/4.6
LC–UV (peak height)	7.4	3.8	1
LC–UV (peak area)	13.8	4.6	1
LC–MS (peak height)	8.5	3.5	1
LC–MS (peak area)	15.5	4.5	1

in Fig. 3A and C. For the narrow-bore column the increase in response is about 3.5-fold as shown in Fig. 3B. Under these conditions where the flow to the mass spectrometer is constant, the analyte response is directly proportional to the analyte concentration. Table II summarizes the results for all three columns for peak area and peak height for LC–UV and HPLC–IS-MS. Since the detection limit may be defined as the ratio between the signal and the baseline noise (S/N) [14], then the peak height relative to the baseline noise is an important factor for determining sensitivity. For these reasons, peak areas are given only for reference. Table III shows the plate numbers obtained for butyl benzoate ester with the different columns and the two detectors. Table III also shows that the current design of the LC–MS ion spray interface does not affect chromatographic performance when eluent flow from the 4.6 mm column is split to 65 μl/min.

Post-column splitting does not affect the concentration of the analyte, but it does affect the quantity of analyte delivered per unit time. The effect of post-column splitting for a concentration-sensitive detector is illustrated in Fig. 4 where the split delivers reduced flow to the UV detector with no significant decrease in signal while good chromatographic performance is maintained. For Fig. 4C a slightly lower signal is observed which is due to some extra-column dispersion. As expected from eqn. 3 a post-column split for a mass-flow-sensitive device will result in a significant decrease in response, and direct coupling with micro-bore columns will provide the best overall sensitivity. This has been described previously for micro-LC–MS with DLI [1] under CI conditions. The analyte response from ion spray and electrospray as a function of flow rate has been studied by Ikonomou *et al.* [18]. The authors observed that an increase in flow of a given solution by infusion does not correspond to a significant increase in total ion current. In the flow range 1 to 100 μl/min conventional ion spray behaves like a UV detector and post-column splitting does not affect the analyte response in terms of peak height or total ion current. These facts suggest that post-column splitting using larger-bore HPLC columns coupled with electrospray or ion spray will not result in dramatic LC–MS sensitivity loss as would be expected in the case of mass-flow sensitive device from eqn. 3. The split effluent can of course be directed to, for example, a diode array for complementary spectroscopic information. However, HPLC plumbing problems associated with maintaining precisely controlled flow to the interface may occur which detracts from the apparent ease of these experiments and in particular for quantitative work.

Micro-bore columns provide better response than standard-bore columns for the same injected amount with ion spray, but chromatographic

TABLE III

OBSERVED LOSS OF EFFICIENCY UPON SCALE DOWN TO 1 mm I.D. COLUMN

$n = 5.54(t_R/W_{0.5})^2$.

Column I.D. (mm)	N (LC–UV)	N (LC–IS-MS)
1.0	1588	2547
2.0	4422	6936
4.6	6774	8948

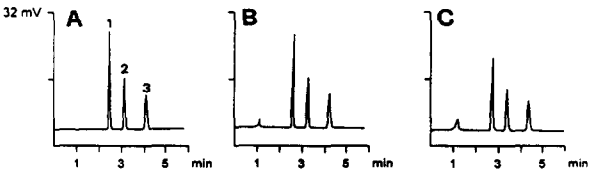

Fig. 4. LC–UV response with post-column splitting. The separation of the alkyl benzoates was performed on a 100 × 4.6 mm column, UV detector with a 2.8-μl cell, 254 nm. (A) No split, 1000 μl/min to the detector: (B) split 5:1, 200 μl/min to the detector; (C) split 20:1, 50 μl/min to the detector.

performance is often compromised. This results not from problems with MS, but practical limitations with minimizing extra-column volume resulting from connecting tubing and HPLC fittings. The increase in response is mainly related to increased C_{max} of the analyte with the smaller column internal diameter. Micro-bore columns with smaller I.D. than 1 mm or even open tubular columns have shown increased separation efficiencies and resolution because the latter have greater permeability and minimized diffusion which allows the use of longer packed HPLC columns. However, in the case of packed columns the diameter of the particles is still an important parameter for chromatographic resolution. Optimizing the design of small diameter columns and packing as well as automated HPLC systems will certainly be of benefit to similarly improve LC–MS sensitivity.

The available injection volume is also an important factor to consider when evaluating the overall sensitivity of an LC–MS system. Micro-bore columns will tolerate a limited injection volume of about 5 μl in the isocratic mode under most common HPLC conditions. On standard-bore columns injection volumes of 50 μl or more of biological fluid extracts are common. When large volumes of sample must be introduced for trace investigation, the highest efficiency is obtained with sample solvents weaker than the mobile phase. "Peak compression" may be used to inject larger amounts of samples on micro-bore columns. However, these columns are very sensitive to pressure changes and injection of large amounts of plasma extracts, for example, will require special care for sample work-up. On the other hand the mass spectrometer is a very specific detector and quantitative analysis with high through-put in the isocratic mode is preferred for speed, simplicity and analytical ruggedness.

When minimal sample consumption is required micro-bore columns are the best choice for maximum ion spray LC–MS sensitivity. Another important advantage of micro-bore columns is reduced solvent consumption where about 15 times less solvent is used compared to 4.6 mm I.D. columns. However, when sufficient sample is available to allow larger injection volumes, maximum overall response, best chromatographic performance and analytical ruggedness will be obtained with a 4.6 mm I.D. HPLC column. An intriguing alternative to 4.6 mm I.D. columns for HPLC–IS-MS are short 2 mm I.D. columns packed with 3-μm particles. These columns offer reduced solvent consumption and increased in sensitivity without compromizing chromatographic performance or analytical ruggedness.

High-flow ion spray

Recently we extended the application of ion spray techniques to HPLC flows up to 2 ml/min by adding a simple grounded liquid shield between the sprayer and the ion sampling capillary of a heated capillary-type atmospheric pressure interface (Fig. 1). Using this interface LC–MS experiments can be carried out over a large range of flow-rates which provides benefits for conventional LC–MS applications where previously the post-column split was required for standard HPLC columns [9]. We have shown that under high-flow ion spray conditions the analyte ion current signal increases with flow-rate for a solution with a fixed concentration. This behavior is in contrast to conventional ion spray described above and is characteristic of a mass-

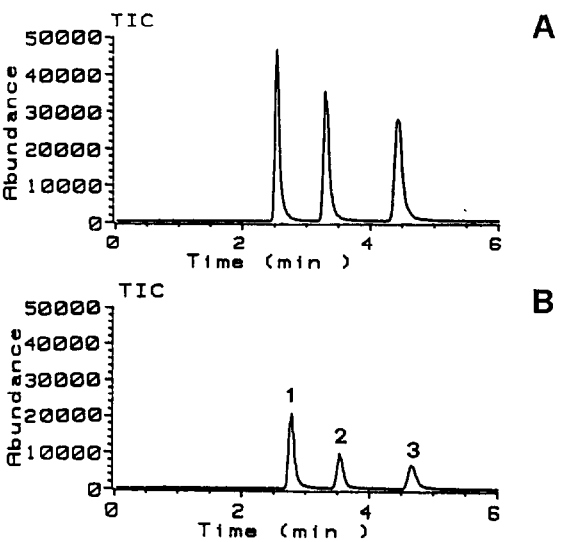

Fig. 5. High-flow ion spray *versus* conventional ion spray of alkyl benzoate esters on a 100 × 4.6 mm column SIM of m/z = 151, 165, 179 (MH^+), dwell time = 300 ms. (A) High-flow ion spray without post-column split and liquid shield; (B) conventional ion spray with post-column split and no liquid shield.

flow-sensitive detector [7]. Higher ion current response is obtained at 1 ml/min than with lower flow due presumably to a mass-flow effect as might expected for a mass spectrometer [7]. Fig. 5A shows the SIM LC–MS comparison of the alkyl benzoate esters using a 4.6 I.D. Column without post-column splitting using the liquid shield (1 ml/min to mass spectrometer) and Fig. 5B with conventional post-column splitting (40 μl/min to mass spectrometer). The position of the sprayer was optimized for each experiment. In both cases chromatographic performance is conserved and for the higher flow a 3-fold increase in analyte ion current signal is observed. Most chromatographic separations are still based on standard-bore columns so high-flow ion spray provides HPLC–IS-MS capability without post-column splitting while retaining good sensitivity. It also allows simple transfer of established conventional (using 4.6 mm I.D. columns) LC–UV separations to LC–MS experiments. In electrospray and ion spray we have at least two important steps. The first is the formation of the spray while the second is the generation of gas-phase ions at atmospheric pressure. These ions must then be sampled from atmospheric pressure into the high-vacuum region of the mass spectrometer. The mass flow or the concentration behavior of ion spray may depend upon these two steps and the particular design of the atmospheric pressure ionization interface.

Post-column addition

A convenient feature of the ion spray interface compared to, for example, electrospray or particle beam LC–MS approaches, is its ability to handle a wider range of flow-rates including those common to micro- and standard-bore eluent flows. Since ion spray analyte response is dependent on the nature of the analyte and the eluent composition such as the organic modifier and ionic salt content, post-column addition is an alternative for improving sensitivity and has also been demonstrated for thermospray LC–MS applications [11]. However, the addition of a post-column liquid essentially dilutes the analyte concentration and hence the analyte response if detection is based upon concentration. This effect is illustrated in Fig. 6A–C for the LC–UV determination of monuron and carbofuran and

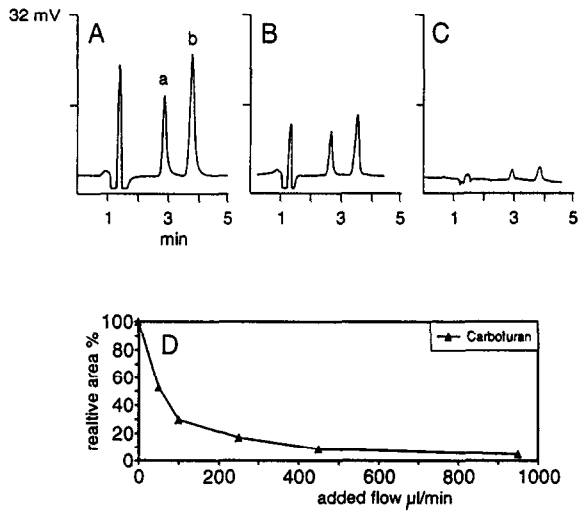

Fig. 6. LC–UV response of monuron (a) and carbofuran (b) with post-column addition on a 100 × 1 mm I.D. column (see Experimental for conditions). (A) No post-column addition; (B) 50 μl/min post-column addition; (C) 450 μl/min post-column addition; (D) summary of LC–UV response for carbofuran with various post-column additions of column eluent.

summarized for carbofuran in Fig. 6D. Fig. 6A shows the separation of the two pesticides on a 1 mm I.D. column with a 5 m*M* ammonium acetate in water–acetonitrile (60:40) mobile phase maintained at 50 μl/min. The addition of 50 μl/min of the same eluent via a post-column tee located between the column exit and the UV detector reduces the analyte response by a factor of two as illustrated in Fig. 6B. A 9-fold dilution (Fig. 6C) results in a loss of about 84% of the UV signal. Fig. 7A shows the SIM trace of monuron and carbofuran (50 ng each) under conventional ion spray LC–MS conditions separated on the 1 mm I.D. column with no post-column addition and with the same mobile phase as for LC–UV at 50 μl/min (see Fig. 6). If the liquid shield is placed in the spray region of the ion spray interface so that high-flow ion spray conditions are used [9], post-column eluent addition may be readily implemented. When 450 μl/min mobile phase is added to the effluent of the HPLC column an approximatively 70% decrease in analyte response is also observed (Fig. 7B). This LC–MS behavior parallels that of the LC–UV detector response described above (Fig. 6A–D) and suggests that the dilution effect is a

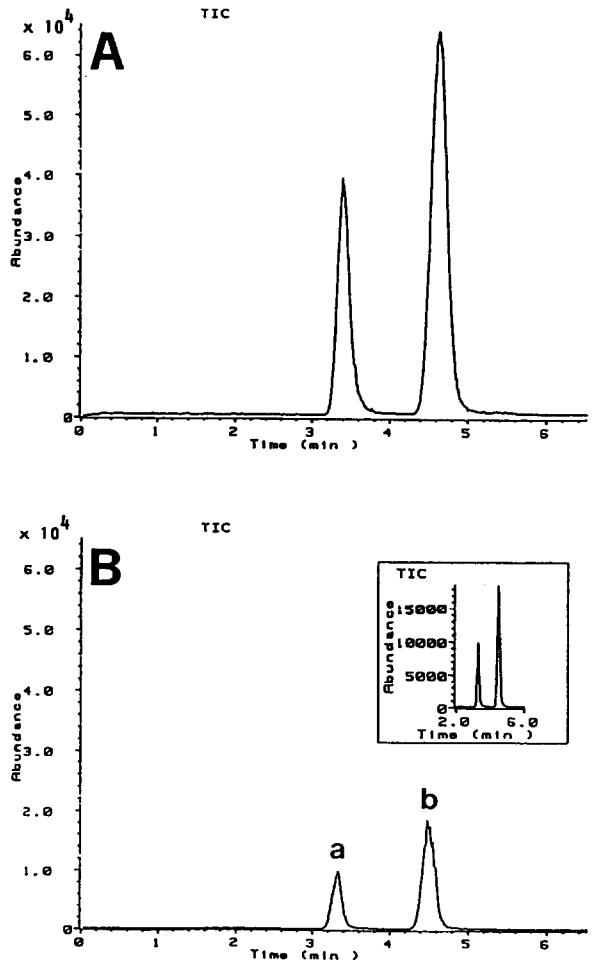

Fig. 7. SIM LC–MS response of monuron (a) and carbofuran (b) (50 ng on-column) on a 100 × 1 mm I.D. column at 50 μl/min (m/z = 199, 222, dwell time = 300 ms). (A) Conventional ion spray with no post-column addition; (B) high-flow ion spray post-column addition of 450 μl/min using the liquid shield.

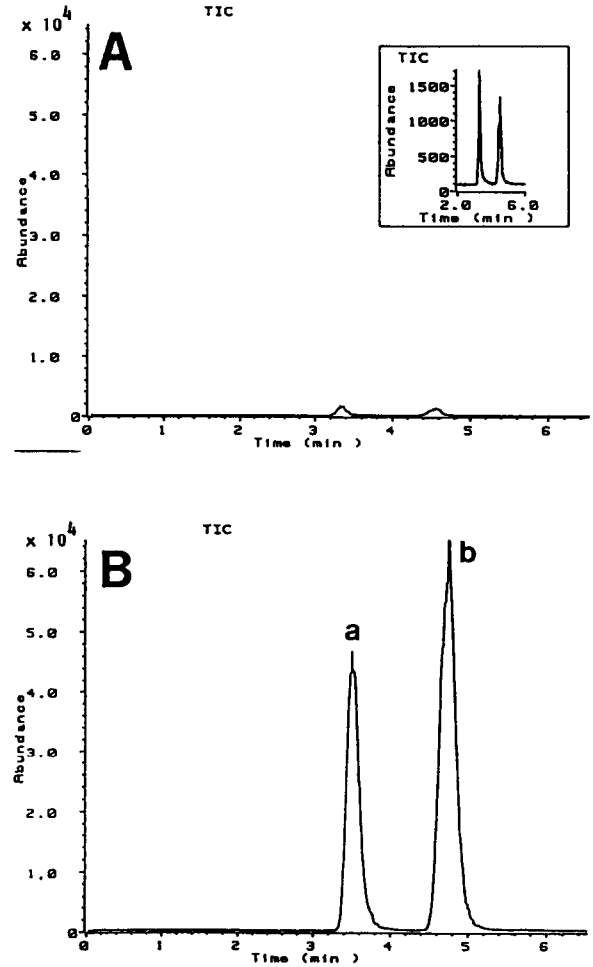

Fig. 8. SIM LC–MS response of monuron (a) and carbofuran (b) (see Fig. 7 for conditions). (A) High-flow ion spray with post-column addition of 450 μl/min 100% CH_3CN; (B) high-flow ion spray post-column addition of 450 μl/min 100% MeOH using the liquid shield.

more important parameter than the increase in flow, and negatively affects the overall analyte ion current response in this case.

Acetonitrile and methanol are well known to enhance analyte response under ion spray conditions [18]. However, the post-column addition of 100% acetonitrile under high-flow ion spray LC–MS conditions significantly suppresses the ion current response for the two pesticides as illustrated in Fig. 8A. Addition of 0.1% formic acid (not shown) produces some improvement in the analyte ion current response. In contrast, when 100% methanol is used we observe a considerable enhancement of the signal (Fig. 8B) which appears to compensate for the dilution effect. Since under ion spray conditions it is generally accepted that high organic content improves the analyte response, the observed difference between acetonitrile and methanol may be related to the nature of the solvent and in particular to ion solvation where acetonitrile is aprotic compared to the protic nature of methanol. Raffaelli and Bruins [19] investigated the response of quaternary ammonium salts, which are preformed ions, *versus* acetonitrile and methanol

and observed the opposite effect. Thus they observed a better analyte signal using acetonitrile than for methanol. In contrast to quaternary ammonium salts, the two pesticides studied here do not carry their own positive charge. In the case of acetonitrile the weak signal may be partially due to the fact that the compounds are not preformed ions in solution and this may result in an inefficient ion release from the condensed phase. The improved response by the addition of formic acid is consistent with this explanation. However, gas-phase processes involving proton transfer cannot be excluded.

Post-column addition is very useful for enhancing MS response without compromising the HPLC separation. We illustrate here the feasibility of post-column addition with simple solvents and with a high dilution factor. More appropriate solvent ratios and even post-column chemistry may be an appropriate way to extend the application of IS-MS to molecules which under normal conditions show a poor IS-MS ion current signal. Under these conditions the behavior of the detector may be difficult to predict, because the MS response is primarily dependent on the mobile phase composition and the analyte.

CONCLUSIONS

In contrast to typical EI and CI mass spectrometric performance conventional ion spray behaves as a concentration-sensitive detector so that post-column splitting under LC–MS conditions does not affect the LC–MS response. The practical increase in sensitivity with 1 mm I.D. *versus* 4.6 I.D. columns with the same length and the same packing material in the isocratic mode while injecting the same quantity of sample is about 8-fold, but chromatographic performance and analytical ruggedness are compromised. Micro-bore columns are the best choice when minimum consumption of the sample is required. Better 1 mm I.D. columns in the future will certainly improve these results. For LC–MS applications 2 mm I.D. columns may be a practical alternative for lower solvent consumption, increased analyte response, and good chromatographic performance. High-flow ion spray with the liquid shield behaves more like a mass-flow-sensitive detector. Higher liquid flow allows post-column addition to micro-bore separations which is a useful approach for improving MS response without compromising HPLC performance.

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