A new thermospray (TSP) LC/MS interface design offers advantages over existing systems and an alternative ionization method. The cover photo shows the thermospray interface recently developed at Finnigan MAT. TSP overcomes at least two major difficulties found in the operation of existing systems: the inability to handle a large flow of water solutions and the risk of thermal decomposition of low volatility compounds. Chemical ionization occurs when the salt ions in the buffer solution react with the sample. An article in this issue by Dr. William McFadden describes the advantages of the TSP interface.
Contents

Letter to the Reader .................................................. PAGE 2

Combined Liquid Chromatography/Mass Spectrometry (LC/MS)
D. E. Games ........................................................... PAGE 3

The Moving Belt as an Interface for HPLC/MS
P. Vouros and B. L. Karger ........................................... PAGE 9

First Steps in LC/MS With Simple Interfaces for the Finnigan MAT 44
N. Evans ..................................................................... PAGE 14

Experiments With the Coupling of a Jasco Micro LC to a Finnigan MAT
3300 Quadrupole Mass Spectrometer
A. P. Bruins and B. F. H. Drenth ................................ PAGE 18

Thermospray LC/MS: Supplement or Substitute for Existing Techniques
W. H. McFadden ........................................................ PAGE 23

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community.
Letter to the Reader

Mass spectrometrists in many laboratories are often faced with two problems which are difficult to resolve. One is the decision as to which of the myriad of relatively new mass spectral techniques they require in their laboratory, and if they have access to them, which to use when. The other involves the place of mass spectrometry in the solution of analytical problems. Involvement in mass spectrometry is very expensive for any organization, and it is important that the instrumentation is used in the most efficient and cost effective way, i.e. to solve those problems which the technique is uniquely suited to solving.

There is an increasing tendency for various groups within any organization to become highly specialized in their own area. Since there is only a relatively small pot of gold available they tend to push their own technique, often with the result that the problem would have been better solved by a combination of techniques or by a technique available in another section. While this is the type of situation that arises because of rivalries between chromatographers and spectroscopists of various kinds, other problems arise in the mass spectrometry area itself. In the euphoria of discovering a new ionization technique or another way of putting a mass spectrometer together, the advantages are typically highlighted, and drawbacks which can cause considerable problems in using the instrumentation in a routine manner tend to be overlooked or de-emphasized.

Journals such as this one, can go a long way towards assisting the practitioner of mass spectrometry as to the relative merits of new techniques in problem solving. Attendance at conferences can also be very fruitful in this respect, usually in the more informal of workshop situations where people tend to be more forthcoming as to the samples that didn’t work and why. It is a pity that in the more formal situation people are inhibited from admitting that maybe there is no utopia. If there was, one of our major stimuli would be missing, i.e. the solution of the problem we can’t currently solve. Academics tend to be the worst offenders in this context, which is a pity since it is often on their advice that industrial and government laboratories venture into new techniques. One can fully understand the initial euphoria of finding the new technique, but one must also try to be objective as to its true place in the scheme of things.

With regard to the position of mass spectrometry in the solution of problems, improvements in one’s general knowledge can only come by talking to the chromatographers and spectroscopists and finding out what they have to offer. Major advances in terms of the capabilities of these other techniques continue to be made. We should be aware of them if we are going to keep mass spectrometry as a front line technique. There are excellent ways of keeping abreast with changes in other areas; regular reading of Analytical Chemistry is one which immediately springs to mind. However, perhaps the various national organizations involved in mass spectrometry also have a part to play in this, and keynote lectures in various areas other than mass spectrometry could be incorporated in their meetings to give members a wider appreciation of their position in the role in science. The American Society for Mass Spectrometry has already gone some way toward fulfilling this function in that one of their plenary lectures is often devoted to an area outside mass spectrometry.

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Combined Liquid Chromatography/Mass Spectrometry (LC/MS)

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Why does one get involved in new techniques? For the person working in applied scientific areas, the answer is usually that the problems being addressed either cannot be solved or are very difficult to solve with existing techniques. This was our reason, nearly six years ago, for obtaining a prototype liquid chromatographic/mass spectrometric interface for our Finnigan MAT 4000. We had found that many classes of organic compounds which we were studying were not directly amenable to gas chromatography, due to their thermal instability, and/or low volatility. However, they could be readily analyzed by liquid chromatography (LC), and we felt that the prospect of obtaining on-line mass spectral data from the liquid chromatograph was an exciting possibility, which became a reality.

This issue of SPECTRA is devoted to LC/MS. In this article the various approaches to LC/MS will be briefly reviewed and the applicability of the technique to problem solving will be illustrated with some examples from our current studies. Finally, a bit of crystal ball gazing will be indulged in, in an attempt to define future developments. The other four articles in this issue illustrate other groups' experiences in LC/MS. Nigel Evans and Andries Bruins have built interfaces of the direct liquid introduction type for their Finnigan MAT 44 and Finnigan MAT 3300 instruments respectively and discuss their experiences with this approach. Paul Vouro has had extensive experience in using a moving belt interface and describes improvements to the system which have been developed in his laboratory and discusses the theoretical implications of a moving belt interface as a liquid chromatographic detector. William McFadden reports in this issue on a new direct thermospray technique being investigated and developed by Finnigan MAT. I hope that this issue results in a reasonably balanced view of the current state of LC/MS. I am indebted to Nigel, Andries, Paul and Bill for their willingness to assist me in this task.

Combining Liquid Chromatographic and Mass Spectrometric Systems

Combining a liquid chromatograph with a mass spectrometer is not easy, since the two techniques are mutually incompatible. Three problems have to be addressed. One is that the mass spectrometer is not capable of handling the high gas flow volumes generated when conventional LC is used. Secondly, useful mass spectra are required from compounds which in many cases are thermally labile and/or have low volatility. Finally, one wishes to maintain the chromatographic performance.

A variety of different methods for overcoming these problems have been described, and the reader is referred to recent reviews (1 through 6) for a detailed discussion. Primarily these methods fall into two major categories: a) Direct Liquid Introduction (DLI) methods; and b) Liquid Transport methods. Here the general approaches will be briefly discussed.

A Look at Several Direct Liquid Introduction Methods

The use of atmospheric pressure ionization (API) to facilitate DLI offers the advantage of allowing all the effluent from the liquid chromatograph to be handled by the mass spectrometer (7). This early system was limited, in that only relatively volatile compounds could be studied, and the obtained spectra lacked structurally significant fragment ions. Recent developments where a nebulizer is used for sample introduction (8) and fragmentation can be induced (9) suggest the approach may, in fact, be a useful one. Field induced ion evaporation (10) also has potential in this area, and recently it has been shown that collision induced dissociation can be performed with LC/MS using an API instrument (11).

Membrane (12) and jet (13) separators have been used to effect enrichment of solute relative to solvent for LC/MS, with unimpressive results. Better results were obtained...
using a vacuum nebulizer (14), but the system has not yet been demonstrated with more difficult compounds and requires use of microbore LC for its most effective utilization. Use of molecular beam techniques (15) has been a more effective approach and has recently resulted in the discovery of thermospray ionization (16). Rapid heating of the effluent from the liquid chromatograph produces a jet of vapor and aerosol which undergoes adiabatic expansion under vacuum. A portion of the jet passes through a skimmer where it impinges on a heated probe. The net effect is formation of ions from mass spectrometrically difficult compounds. LC/MS has been effectively demonstrated with this technique, and although the on-line spectra are not as good as those obtained off-line, the approach appears to have potential for the LC/MS of compounds which cannot be handled with existing systems.

One of the most widely used interfaces for LC/MS involves introduction of a portion of the liquid chromatographic effluent into the mass spectrometer ion source where the solvent is used as the CI reagent gas (17). Early studies used a magnetic instrument, but because of the lower ion source potentials, interfacing to a quadrupole instrument is much easier (18). These early studies encountered two problems. One was that since only a portion of the effluent could be handled, sensitivity was poor, however this can be overcome by the use of microbore LC (19). The second was an inability to handle polar low volatility compounds, due to their blocking of the capillary interface. Use of a small diameter diaphragm to effect nebulization and a water-cooled probe has improved the range of compounds which can be handled (20).

There is no doubt that this relatively simple approach to LC/MS is a practical one, as can be seen from a perusal of the literature (e.g., 21, 22) and articles in this volume. Recent studies (23, 24) in which a desolvation chamber is incorporated between the DLI interface and the CI source block indicate that the range of molecules amenable to study by this approach can be considerably extended, but, as yet, no real LC/MS studies have been reported with this improved interface.

And Liquid Transport Methods

Removal of the solvent from the LC effluent can be effected using a transport system. Based on Scott's early work (25) using a wire transport, McFadden (26) produced a system which used a belt and hence effected better transfer efficiencies. Modification of the system by incorporation of an infrared heater (27) enabled more polar mobile phases to be used. After the solvent is thermally separated from the solute and evacuated, the solute is flash vaporized from the belt into the mass spectrometer where EI or CI spectra can be obtained. Initially a stainless steel belt was used, but Kapton® was later found to cause less sample decomposition. Incorporation of the interface into the mass spectrometer ion source should result in enhanced sensitivity and better handling of difficult compounds (28, 29). For example, excellent spectra of compounds, which do not provide relative molecular mass data on the other belt interfaces, are obtained from the new Finnigan MAT interface. This is illustrated in Figure 1, which shows the water CI spectrum obtained from 2'-deoxyadenosine-5-phosphate using this interface.

One aspect of the transport approach which augurs well for future developments is that they can be used with surface ionization techniques. The possibility of SIMS (30), $^{125}$Cf plasma desorption (31), laser desorption (32) and FAB (33, 34) studies by LC/MS have been demonstrated. FAB LC/MS would be a particularly attractive possibility, and Figure 2 shows the FAB spectrum of raffinose obtained from the Finnigan MAT interface without use of a glycerol matrix.

Interfaces of the transport type have also been shown to be practically useful, and the wide range of areas where they have been applied have recently been reviewed (35).

An alternative approach to LC/MS, whereby solvent is removed by use of a heated wire of decreasing diameter has been reported (36). Data presented to date are with relatively undemanding compounds, and evaluation with more difficult compounds is necessary before the potential of this approach can be assessed.

![Figure 1. Water CI mass spectrum of 2'-deoxyadenosine-5-phosphate obtained using a Finnigan MAT moving belt LC/MS interface.](image1)

![Figure 2. FAB mass spectrum of raffinose obtained without glycerol matrix using a Finnigan MAT moving belt LC/MS interface.](image2)
Application of the Moving Belt Interface

In this section we describe some of our experiences using interfaces of the moving belt type for LC/MS. There is now a considerable volume of literature on LC/MS, and many papers show either data from a single compound or from relatively simple mixtures. While such data is useful for evaluation purposes, the former type of data where an LC column is not used can hardly be described as LC/MS, and in the latter case, the problem could be equally effectively solved by preparative LC and direct probe studies. A true test is to examine complex mixtures and see if good chromatographic and mass spectral data can be obtained.

Figure 3 shows the reconstructed ion chromatogram (RIC) obtained by EI LC/MS of an extract from an ergot fermentation broth, which contains ergot alkaloids of the clavine type (37). Similar quality data was obtained using isobutane CI, and the traces compared well with the trace obtained by LC using a UV detector. Figures 4 and 5 show the EI and isobutane CI mass spectra of one of the components. Comparison of the mass spectral and retention time data with that obtained from known compounds enables these compounds to be readily identified and new compounds to be readily located. New compounds can subsequently be isolated by preparative LC for full characterization. This type of approach has been applied by us to studies of a wide range of natural products including alkaloids of the Amaryllidaceae and Cinchona types; gibberellins, griseofulvin, and its co-metabolites, pseudomonic acids, coumarins and plant phenolics from Dalbergia species.

While the belt systems cannot handle all the classes of compounds one would like to study by LC/MS, a very wide range of compounds are covered which are not directly amenable to GC/MS study or whose analysis is preferentially performed by LC. Limitations in the range of compounds which can be handled can be conveniently

defined in terms of sugars. Molecular weight data can be obtained using ammonia CI from underivatized mono- and di-saccharides, but we have failed to obtain such data from trisaccharides.

It should be noted that the new Finnigan MAT interface does provide such data from trisaccharides with ammonia CI, and its upper limitation in this context has not yet been defined. An alternative way of defining the situation is that previous interfaces of the moving belt type readily handle compounds which give EI and/or CI spectra from a good direct insertion probe, while the new Finnigan MAT interface (29) appears to extend the range to compounds which can only provide molecular weight data by use of desorption chemical ionization. Perusal of the literature indicates that current commercially available interfaces of the DLI type do not have any particular advantages in their ability to handle more difficult compounds, although they may have advantages when small amounts of sample are being studied, since there appears to be less evidence of thermal degradation of sample at low levels (38).

![Figure 3. Reconstructed ion chromatogram (RIC) obtained by EI LC/MS of ergot fermentation broth extract. The column was packed with Spherisorb 5W. Methylene chloride and methanol and ammonium hydroxide (95:5:0.1) at a flow rate of 1 ml/min. was used as the solvent system. Reprinted from Reference 37 by permission of John Wiley & Sons Ltd. ©Heyden & Son Ltd. 1982.](image-url)

![Figure 4. EI mass spectrum of component O, chanoclavine-I.](image-url)

![Figure 5. Isobutane CI mass spectrum of component O, chanoclavine-I.](image-url)
Few quantitative studies using LC/MS have been reported. We (39) and others (40) (see article by P. Vouros in this issue), have shown that the belt system is viable for studies of this type; however, care should be exercised if data is required at low levels with thermally labile compounds, since thermal decomposition may occur.

The belt system is relatively easy to use but does have some problems, the range of compounds which can be handled and the possibility of sample decomposition at low levels are two which have already been discussed. Sensitivity is a further area where improvements would be welcome. With conventional LC columns full spectra are obtainable in the high ng/low µg range, and for selected ion monitoring, detection limits are in the low ng range. These figures refer to average levels which can be expected on-line when the real problems are being investigated. A further problem is encountered in handling mobile phases containing a high percentage of water. There have been a number of solutions to the latter problem (34) (see P. Vouros' article). We have recently studied the use of microbore LC to obtain improvements in both these contexts (41, 42).

Microbore LC uses columns of smaller internal diameter (0.5–1 mm) than conventional LC. This results in much lower flow rates of the mobile phase, hence making LC/MS easier. Our initial studies (41) utilized a JASCO system with PTFE columns of 0.5 mm i.d. Although we were able to effect considerable improvements in the performance of these columns, their efficiencies did not match those obtained with conventional LC columns. However, because of lower flow rates, splitting of column effluent was not necessary and background due to solvent impurities was lower. Hence sensitivity was improved and full scan EI spectra were obtainable which gave good matches with the library in our data system. In addition, aqueous mobile phases containing up to 80% water were readily handled.

More recently we have extended these studies to glass-lined stainless steel columns (1 mm i.d.) which are produced by Whatman. Using a Waters pump modified for low flow rates, and a 0.2 or 0.5 µl volume valve loop injector, we have been able to obtain excellent LC/MS data. Chromatographic performance is comparable with conventional columns, and the advantages gained with the PTFE microbore columns are also obtainable. Full scan spectra of carbamate pesticides have been obtained at the 5 ng injected on-column level, together with excellent quantitative data. More difficult compounds e.g., gibberellins give full spectra at the 20 ng level as opposed to 3 µg with conventional columns.

Advantages of the belt system over the DLI systems in this area are that flow rates in excess of 20 µl/min can be handled without the necessity of splitting the column effluent. This is an advantage if flow programming or fast analysis is required. The latter is illustrated in Figure 6, which shows the RIC obtained from a mixture of the carbamate pesticides 1PC and C1PC at a flow rate of 200 µl/min. A further advantage is that the column can be directly interfaced to the belt thus minimizing dead volumes and ensuring good chromatographic performance.

For studies of complex mixtures of varying polarity the use of a gradient system is an advantage. We have used this technique with conventional columns and have recently developed its use with microbore columns. Figure 7 shows the RIC obtained under El conditions from an extract of a test-well sample taken from a land-fill site. This study was performed in collaboration with Glenys Foster of the Ontario Ministry of the Environment, Toronto (43). The components marked on the chromatogram were readily identified on the basis of their El mass spectra and liquid chromatographic retention times. A number of

![Figure 6](image1.png)

**Figure 6.** RIC obtained by El LC/MS of a mixture of 1PC and C1PC. A Whatman microbore LC column packed with Partisil 10 ODS-3, 250×1 mm, was used, with methanol and water and acetic acid (75:22:3) at a flow rate of 20 µl/min as the mobile phase.

![Figure 7](image2.png)

**Figure 7.** RIC obtained by El LC/MS of an extract from a test-well sample taken from a land-fill site. A Whatman microbore LC column packed with Partisil 10 C8, 250×1 mm, was used, with a solvent program (slope 6, linear) consisting of: A) acetonitrile and water (2:80), and B) acetonitrile going from 0 to 100% B in 15 min. Reprinted from Reference 43 by permission of John Wiley & Sons Ltd. © Wiley Heyden Ltd. 1983.
compounds were found to be present which had not been found in capillary GC/MS studies.

Many liquid chromatographic studies require additives for optimal LC. With belt systems addition of volatile buffers, various acids and bases present no problems. Involatile materials (e.g., many ion pair reagents) can be handled by use of a scrubber unit; however, use of an online extraction system is preferable (44).

In this section we have given a brief description of some of the areas where current LC/MS systems can assist in the solution of problems. Our experience has been that interfaces of the belt type are remarkably reliable and can be used relatively routinely for LC/MS in a wide range of investigations. Some of the problems encountered with these interfaces have been discussed and most can be overcome. There is no doubt that the availability of both EI and CI data is a considerable advantage in the solution of many problems.

What Does the Future Hold for LC/MS?

In this author’s opinion, we may well be using several types of LC/MS interfaces in the future depending on the types of problems being addressed. Undoubtedly for problems which require compound characterization, and where the compounds are not amenable to gas chromatographic study but are amenable to desorption chemical ionization study, the availability of both EI and CI mass spectral data will be advantageous. The new Finnigan MAT interface would appear to be the system of choice. For quantitative studies at low levels, interfaces of the direct liquid introduction type might have advantages if they are used with microbore LC. However, as yet, quantitation has not been shown to be a viable proposition with interfaces of this type. For compounds whose volatility is outside this range and hence are unlikely to yield useful EI data, an alternative approach is necessary. FAB LC/MS is one attractive possibility, particularly if the use of solvent matrices can be avoided, sensitivity is reasonable, and if methods for the removal of nonionized sample are devised. Currently use of the thermospray approach looks viable for handling compounds of this type, and recent advances in the DLI approach where desolvation chambers have been incorporated show promise. In this latter case, on-line LC/MS must be demonstrated. It may well be that the handling of more difficult samples must be completely reviewed and that supercritical fluid chromatography rather than liquid chromatography is the answer to obtaining on-line mass spectral data.

In the words of one of the pioneers in this area, Jack Henion, “come on in and join us; the water’s fine.”

Acknowledgments

Figures 1 and 2 were presented at the 9th International Mass Spectrometry Conference in Vienna 1982 by P. Dobberstein, E. Korte, G. Meyerhoff and R. Pesch of the Finnigan MAT Research and Development Laboratory in Bremen, W. Germany. I am grateful to them for allowing me to include this data in this article.

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Biography

David E. Games received his Ph.D. from King’s College, London in 1962 and his D.Sc. from the University of Wales in 1982. After spending a year working with Professor D. MacLean at McMaster University, Hamilton, Ontario, on structural studies of alkaloids he went to University College, Cardiff where he spent a year studying natural coumarins with Professor L. Crombie and was appointed to the staff there in 1965 as a lecturer. In 1974 he was made a Senior Lecturer and in 1980 was promoted to Reader. In addition to being Joint Editor in Chief of Biomedical Mass Spectrometry with Catherine Fenselau, he is the current Chairman of the British Mass Spectrometry Society.
The Moving Belt as an Interface for HPLC/MS

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For the past three years our laboratory has been conducting research in the area of combined high performance liquid chromatography/mass spectrometry (HPLC/MS) using the Finnigan MAT moving belt interface. Our interest and involvement in this program has been prompted to a large extent by the recognized need to improve the compatibility between the two techniques and, in particular, between reversed phase HPLC and mass spectrometry. Specifically, we have been interested in establishing the conditions for the use of HPLC solvents containing non-volatile buffers or other such modifiers and/or a high percentage of water without compromising chromatographic fidelity or the operational characteristics of the mass spectrometer. We felt that a successful resolution of this problem would improve the overall utility of on-line HPLC/MS, since a very large fraction of HPLC is conducted in the reversed phase mode.

In the course of these studies we have evaluated the conditions of the belt interface necessary for the use of aqueous solvents. In addition, we have developed an on-line extraction technique which can be coupled to the belt and which permits the use of HPLC mobile phases containing non-volatile buffers. This approach has introduced opportunities for conducting post-column reactions which can further improve the on-line compatibility between HPLC and mass spectrometry. In this report we present some of the highlights of this work.

The Moving Belt Interface

The moving belt interface may be generally subdivided into four zones as shown in Figure 1. These are:

1. Region of deposition of the HPLC effluent along with its solute contents onto the belt.
2. IR heater region where the bulk of the solvent is vaporized while the non-volatile solutes remain behind as a thin film on the belt surface.
3. Vacuum locks region through which the belt passes as the solute is transferred from atmospheric pressure to the high vacuum of the mass spectrometer.
4. Vaporization region, where the solute is desorbed from the belt and flash vaporized into the ion source of the mass spectrometer.

The Sample Deposition Process

In the original design of the Finnigan MAT moving belt interface (1), the solute was deposited onto the belt via a capillary tube connected to the outlet of the HPLC. The outlet of the capillary was placed very close to the belt surface (≤1 mm), and the solution was allowed to drip or flow onto the belt. As we showed recently (2), this approach can result in poorly defined chromatographic profiles with the occurrence of spikes on the chromatographic peaks. It appears that as the solvent evaporates, the film of liquid can break into droplets and this results in localized concentrations of the solute/solvent and the subsequent irregularities. This is illustrated in Figure 2a which shows the LC profiles of two polynuclear aromatic hydrocarbons. In addition to these irregularities, the original method of sample transfer onto the belt introduces significant band broadening as a result of liquid bead formation at the point of contact of the glass capillary tip and the belt interface.

Previous publications (3 and 4) have recommended the use of an aerosol spray for deposition of the HPLC effluent on the belt. We have adopted an improved design of this approach. The solvent is converted into a fine mist which facilitates vaporization of the liquid, resulting in a thin film of solvent on the surface of the belt. The improvement realized by the use of the aerosol is illustrated in Figure 2b which shows the HPLC/MS profile of the same two PNAS of Figure 2a under identical HPLC conditions. Notable is the clear definition of the chromatographic peaks and the improvement in resolution. In recent months we have examined more closely the process of sample deposition via the aerosol spray and have found that by
placing the nebulizer at an angle of approximately 60° to the belt surface (Figure 1) it is possible to match the chromatographic qualities obtained with a UV detector. It is reasoned that application of the cone of the aerosol spray at an angle to the belt minimizes beading at the site of sample deposition. These modifications significantly reduce chromatographic band broadening as shown in the following results.

![Figure 1. Schematic of moving belt interface.](image)

**Quantitative Assessment of Band Broadening**

Much of the information available regarding the chromatographic behavior of the moving belt and other types of HPLC/MS interfaces has been qualitative in nature. In order to facilitate comparison between different types of systems and also to improve our understanding of the factors which contribute to band broadening, we undertook a quantitative study of the chromatographic data generated from our own Finnigan MAT HPLC-moving-belt-MS combination. At the heart of this study was the determination of peak area, variance and skew (asymmetry). These quantities were determined as a function of belt speed, HPLC mobile phase flow rate and spray conditions — three parameters deemed critical as far as their contribution to band broadening was concerned.

Based on the principle of additivity of variances and comparison of the HPLC/MS peaks to those obtained by HPLC/UV using the same chromatographic system, it was possible to determine experimentally the variance contributed by the mass spectrometer interface (i.e., the moving belt) as a function of the three principal variables noted above. We found, for example, that there was an optimum in belt speed for which both peak variance and skew were at a minimum. It is reasoned that at very low belt speeds the HPLC effluent is deposited over a small cross sectional area of the belt, resulting in the formation of a thick layer of liquid. This liquid accumulation causes backmixing of the solute and the observed band broadening. On the other hand, at very high belt speeds the HPLC effluent is transported very rapidly to the mass spectrometer, before the solvent has an opportunity to vaporize completely. Freezing and beading of the liquid as it enters the higher vacuum region may occur, a condition which introduces irregularities to the chromatographic profile and loss of resolution. It thus appears that at some intermediate belt speed an optimum in chromatographic efficiency is reached.

Following determination of the belt speed optimum, the effect of spray deposition conditions and mobile phase flow rate were also evaluated. It was observed that selection of the proper flow rate and temperature of the gas used in the nebulizer was critical for retention of chromatographic integrity. At high gas flow rates and temperatures, it is easier to create a fine spray mist which simplifies the final step of removal of the solvent by the IR heater. However, it should be noted that excessive gas flow rates and temperatures may also be detrimental because they may result in significant sample losses. It is thus important to maintain a fine spray which yields a thin uniform liquid film that adheres to the belt. For extreme variations in mobile phase polarity, adjustments have to be made depending on the mobile phase used and, under conditions which result in minimum chromatographic band broadening, sample recoveries of 70–75% have been typical. It is thus possible to select the optimum conditions for operation of the HPLC/MS system and, as shown in Figure 3, an almost perfect match between HPLC/MS and HPLC/UV can be obtained. Significantly, analogous results were also obtained with aqueous solvents containing as much as 40–50% water in CH₂CN at flow rates as high as 1 ml/min using a hot (approximately 80°C) nitrogen gas for nebulization. Detection limits of 40 pg injected into the HPLC were also obtained for certain PNA's with the mass spectrometer operated in the electron ionization mode. While much work still needs to be done, it appears that one of the major problem areas associated with the use of the moving belt interface, i.e., the process of sample deposition, can be effectively solved.

![Figure 2. HPLC/MS profiles of PNAs obtained using: a) direct solvent deposition method; and b) spray deposition method.](image)
Despite the fact that the problem of sample deposition on the belt has been largely overcome, the comparison of the HPLC/UV and HPLC/MS chromatograms in Figure 3 also illustrates one of the potential pitfalls of the moving belt interface. It may be noted that the next to the last peak in the HPLC/UV chromatogram is not observed in its HPLC/MS counterpart. In that specific analysis, the temperature of the flash vaporizer was below the vaporization temperature of the indicated compound. In a subsequent chromatogram, the vaporizer temperature was increased sufficiently to vaporize this less volatile component and all the missing information was recovered in the HPLC/MS analysis. This example demonstrates that caution should be exercised in the final vaporization step in order to maintain complete compatibility between HPLC and MS when using moving belt.

Post Column Techniques

On-Line Extraction

Use of hyphenated analytical techniques invariably requires compromising the effectiveness of either or both of the associated methodologies. In the case of combined HPLC/MS via the moving belt interface, retention of chromatographic fidelity is influenced significantly by the mode of sample deposition onto the belt and subsequent transfer to the mass spectrometer. While, as stated above, this aspect of the combination can be dealt with even with mobile phases comprising a net aqueous flow rate of up to 0.4 ml/min, the use of non-volatile salts or buffers still presents a major problem. It is generally the case that introduction of such materials into the mass spectrometer ion source on a continuous basis, even for a short period of time, will result in rapid loss of sensitivity and instrument shutdown.

Our approach to the solution of this problem has relied on the introduction of an additional interface between the HPLC column and the moving belt. This interface performs a post column extraction of the solutes from the aqueous mobile phase into an organic solvent (e.g., CH₂Cl₂), immiscible with the HPLC effluent (5). Salts and other ionic compounds remain in the aqueous phase and are carried to waste (Figure 4). Post column continuous extraction techniques based on a phase separator and air or liquid segmentation have been used in other applications (6). The effectiveness of the method for a specific analysis hinges, to a large extent, on the efficiency with which a given analyte is extracted from the aqueous to the non-aqueous phase. In our initial experiments we conducted analyses of compounds of varying polarity in order to prove the feasibility of the method. For a series of test solutes such as benzyl alcohol, benzoic acid, metimethoxybenzoic acid, para-methoxybenzoic acid and meta-hydroxybenzoic acid, the extraction efficiency, from 60%—40% H₂O: CH₃OH into CH₂Cl₂, varied from a low of ~11% for hydroxybenzoic acid to a high of 52% for benzyl alcohol. Ion suppression to facilitate the extraction of benzoic acid was based on selection of the proper pH of the mobile phase using 0.10 M NaH₂PO₄ buffer. Significantly, even at these high salt concentrations, the mass spectrometer could be operated continuously for several days without deterioration of performance. This result was indeed important in that it demonstrated the practicality of conducting reversed phase HPLC/MS despite the presence of non-volatile buffers.

![Figure 4. Schematic block diagram of continuous extraction interface.](image-url)
counters typically employed in HPLC analysis, i.e., long chain alkylsulfates and alkylsulfonates. Examination of the mass spectra of ion pairs formed between such counterions and amine solutes revealed that the mass spectrum of the ion pair was virtually the exact sum of the spectra of its individual components. This result is illustrated in Figure 5 which shows the electron ionization mass spectrum of the ion pair formed from the combination of α-Me-phenylcyclopropylamine and the sodium salt of n-decylsulfate as the counterion. Marked with an asterisk are the principal ions contributed by the counterion. These ions correspond to the spectrum of the olefin formed upon thermal elimination of the sulfate group during flash vaporization. The spectrum in Figure 5 is representative of the type obtained using the continuous extraction interface during HPLC/MS (7).

Our initial decision to select counterions of the Cα-alkylsulfate or sulfonate variety was based on the prior use of these species for HPLC using UV detection because of their non-chronomophoric characteristics. For detection and analysis by mass spectrometry, however, the absence of UV absorbivity is not a requirement. Indeed, the selection of counterions bearing aromatic groups can be advantageous for HPLC/MS, particularly if the mass spectrometer is operated in the EI mode. This mode of operation may often be necessary if detailed structural information or spectral fingerprint matching for identification is desirable. In such a case, contributions from the fragmentation of the Cα-alkyl chain of the counterion may cover a wide mass range of the spectrum thereby masking that of the solute. This problem may be readily addressed by selection of counterions upon consideration of the factors which control the fragmentation of organic molecules upon ionization. Aromatic counterions were, in fact, found to be very suitable for HPLC/MS since their EI fragmentation is minimal.

Figure 6a and 6b compare the EI mass spectra of the ion pairs of α-methylparnate with C10-alkylsulfate and picrate, respectively. As indicated, contribution from the counterion in the case of the picrate ion pairs is limited to the ion peaks at m/z 199 and 229, respectively. These ion peaks are completely removed from the mass regions encompassing the spectrum of the solute. Analogous results were obtained with other counterions such as 2,4,5-trimethylbenzenesulfonate and 2-naphthalene-sulfonate. These results demonstrate that considerable flexibility is available when conducting HPLC/MS using ion pairs. In principle, an ion pairing reagent can be selected on the basis of its potential contribution to the mass spectrum of the solute following a simple consideration of its structural features and their effect on its fragmentation pattern.

Implicit in our demonstration of the compatibility of ion-pair HPLC with MS is the feasibility of analyzing ionic compounds via the combined HPLC-continuous-extraction-moving-belt-MS approach. It should be recognized that the roles of the counterion and the amine solute are interchangeable and a variety of simple amines can be used as counterions for the analysis of organic salts of alkylsulfonates. The latter compounds are extremely difficult to introduce into the ion source of the mass spectrometer using conventional vaporization techniques. However, we have found that ion-pair formation with an amine and vaporization into the mass spectrometer yields a volatile sulfonic acid during the thermal desorption step, and this species can be analyzed by conventional EI or CI mass spectrometry (8). This effect is, indeed, very significant in that it demonstrates that it should be possible with ion-pair derivatization to analyze ionic compounds and organic salts by HPLC/MS using conventional mass spectrometry detection techniques. The ultimate usefulness of HPLC/MS will be judged in terms of its ability to handle satisfactorily non-volatile materials such as organic salts and high molecular weight compounds. In this respect, it appears that ion-pair derivatization procedures may provide at least a partial solution towards attaining that goal.
Conclusion

Our experiences thus far with the Finnigan MAT moving belt interface have enabled us to identify the following potential operation problem areas: the process of sample deposition onto the belt; the inability to handle large flow rates (e.g., > 0.5 ml/min) of water; the inability to handle mobile phases containing non-volatile modifiers; and difficulties associated with the introduction of non-volatile solutes into the mass spectrometer ion source at the thermal desorption step.

As we have described in the preceding discussion, our experiments and modifications of the system have enabled us to deal with many of these problems with reasonable effectiveness. For example, the use of an aerosol spray for the deposition of the HPLC effluent on the belt, with the nebulizer placed at a ~60° angle to the belt, minimizes beading of the liquid and irregularities of the chromatographic peak shape. Upon optimization of the belt speed and spray conditions, chromatographic resolution comparable to that obtained with HPLC/UV is also attainable with HPLC/MS. Moreover, the use of hot N₂ gas for nebulization permits effective operation of the HPLC/MS system even at mobile phase flow rates corresponding to a net aqueous flow rate of ~0.4–0.5 ml/min.

Quantitation using the moving belt interface is perfectly feasible under either EI or CI conditions. Detection limits in the picogram range with a linear dynamic range extending at least over four orders of magnitude have been obtained. For certain polynuclear aromatic hydrocarbons the detection limits observed corresponded to ~40 pg injected into the HPLC, while the mass spectrometer was operated in the EI mode using SIM.

When dealing with mobile phases containing non-volatile buffers or other additives, use of a continuous extraction interface can overcome many of the associated effects which can be detrimental to the operation of the mass spectrometer. Derivatization techniques such as ion-pair formation, alkylation reactions, etc. can be conducted using this interface, thus further increasing the compatibility between the HPLC and the mass spectrometer. Post-column reactions provide the added feature that, in principle, it should be possible to optimize the HPLC separation procedures independent of the operation of the mass spectrometer. Chemical modifications after the column can then provide for the formation of volatile derivatives to increase the compatibility of the moving belt with the mass spectrometer. Work along these lines is currently in progress in our laboratory.

Our work to this date has focused on the regions of the interface which deal with the transfer of the solute to the moving belt. While this issue can be dealt with, a crucial problem still remains to be solved, namely, the introduction of highly non-volatile solutes into the mass spectrometer via the flash vaporizer.

The apparent difficulty to introduce highly non-volatile solutes into the ion source may be attributed, in part, to the design of the system which places the belt outside the ion source. Nevertheless, recent advances in mass spectrometric ionization techniques hold considerable promise for adaptation with HPLC/MS.

Desorption chemical ionization (DCI) (9) in which the solute molecules are directly exposed to the CI reagent gas ions should lend itself well for adaptation with a moving belt interface which passes through the ion source chamber. Finally, the development of fast atom bombardment (FAB) mass spectrometry is another logical approach for conducting HPLC/MS via the moving belt (10). Encouraging results have already been obtained with secondary ion mass spectrometry (SIMS) on a continuous basis (11) or using a ribbon storage technique (4). Coupled with near satisfactory resolution of several of the problems associated with the sample deposition process and the demonstration that satisfactory chromatography can be obtained using the moving belt (12), it is not unrealistic to look towards the future of HPLC/MS with a degree of optimism.

Acknowledgment

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References

First Steps in LC/MS With Simple Interfaces for the Finnigan MAT 44

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f the five research establishments maintained by the British Gas Corporation, London Research Station is the one most closely involved with developments in the field of analytical chemistry. The Station acts as the Corporate Laboratory for the industry and encounters a wide spectrum of problems that arise from the corporation's activities. Major applications of mass spectrometry include support for projects investigating the production of Substitute Natural Gas (SNG) from coal and petroleum, and involve the analysis of crude oil fractions, coal derived liquids and other gasification byproducts. Other applications include the analysis of synthetic polymers, and samples associated with industrial hygiene or of environmental significance.

In addition to providing a routine service, opportunities arise for developing new methods or investigating novel techniques. We were initially attracted to on-line liquid chromatography/mass spectrometry (LC/MS) by the enormous potential of the combined method, and could identify several applications where the separating power of the chromatograph in combination with specificity and sensitivity of the mass spectrometer could provide unique information on sample composition.

The potential applications for LC/MS fell into two broad categories. First, there were applications where LC is used routinely, but problems of peak identity and detector sensitivity/specificity were encountered. Second, we wished to use LC/MS in the same way as GC/MS, i.e., to investigate samples of unknown source and composition or detect specific components in complex matrices. To achieve these aims we envisage using the spectrometer in both electron ionization and chemical ionization modes, while the chromatograph would be operated with either isocratic or gradient elution, employing solvents having a wide range of polarities. An example of LC separation where the availability of LC/MS would be of advantage is shown in Figure 1. It can be seen from the chromatogram that off-line coupling would be extremely time consuming. What is not immediately obvious from the chromatographic data is that the majority of the peaks arise from two or more components, thus making identification from retention time alone extremely difficult, if not impossible.

At the start of our investigations, we encountered a fundamental difficulty — commercial interfaces for the Finnigan MAT Model 44 were unavailable. It was thus necessary to consider building interfaces of our own design. The remainder of this paper will briefly review the interfaces we have constructed and discuss how far they have been able to satisfy our requirements for LC/MS.

Construction and Operation of the Interfaces

Although commercial interfaces are becoming available for most spectrometers, novel methods of interfacing and ways of optimising interface performance continue to be investigated. Of the various techniques, direct liquid introduction (DLI) (1, 2), where a small flow of eluent passes directly into the ion source of a mass spectrometer operating in the chemical ionization (CI) mode, seemed to us to be the method which required least mass spectrometer modification. In spite of the fact that CI spectra alone can be obtained with this type of interface, our initial investigations were in this direction.

Figure 2 shows a schematic representation of such an interface. Full details of dimensions and construction have been presented elsewhere (3). The heart of the interface is a glass capillary which is installed in a modified line-of-sight probe; the capillary (20–70 micron i.d.) transfers a portion of the eluent from conventional or microbore columns directly to the ion source. In our original design, the capillary was hand drawn from Pyrex tubing and was supported by Teflon ferrules. In later versions, we have used fused silica capillaries and Vespel ferrules. For the larger internal diameter capillaries, the tip at the source end is drawn out into a point. To maintain chromatographic resolution, the positioning of the LC end of the capillary is critical. For conventional columns, the
The capillary must pass through the T-piece splitter and enter the Teflon tubing connecting the column to the interface. With microbore columns, the capillary butts against the glass plug or frit at the end of the column. Dead volume in both cases is then kept to a minimum.

A development of the simple interface is shown in Figure 3. In this adaptation, a capillary tube is again used to transfer a portion of the LC eluent, but at the ion source end of the interface, vacuum nebulisation with helium is employed in an attempt to increase the range of compounds that can be transferred by the interface. This interface is also constructed as a removable probe, and the distance from capillary tip to nebulisation orifice and from capillary tip to counter orifice can be varied when the interface is in position. The connection of the interface to the chromatograph is identical to that for the simple split interface.

In practice, both interfaces are easy to operate and are inserted into the mass spectrometer in the same manner as a normal solids sample probe. A needle valve on the exit side of the interface is used to control the pressure drop across the capillary, and hence the amount of liquid entering the mass spectrometer. Depending on the nature of the solvent, we estimate for our mass spectrometer that between 3–8 µl/min enter the ion source. This represents less than 1% of the eluent from a conventional column and approximately 30–50% of the eluent from a microbore system.

A problem encountered with all DLI interfaces, whether using a pinhole orifice or capillary tube to split the LC eluent, is that of blockage. Solvent purification and in-line filters can help to minimize this difficulty. For the two interfaces we have designed, if blockage occurs the capillary tube can be quickly and easily replaced within 10 to 15 min. Typical lifetimes for each capillary are 2 to 8 hr. In sharp contrast to this, all attempts to modify the interfaces along lines reported in the literature so as to incorporate a disc with a 2 to 5 micron pinhole, met with very little success. Time and again the pinhole blocked within a few minutes.

**Application of LC/MS With DLI Interfaces**

One area where we envisaged using LC/MS is the characterization of aqueous solutions of phenols and related compounds (4). In our laboratories, samples of this nature are routinely analyzed by reversed phase LC; no attempt is made to extract or derivatize the phenolic compounds because previous experience has shown that additional problems caused by severe oxidation of some components can occur during these procedures. Figure 4 shows the total ion current trace for a standard mixture of phenolic compounds separated on a microbore LC column. Good resolution and sensitivity was obtained. The associated spectra showed intense M+H+ ions with little fragmentation. Unfortunately we experienced difficulty in analyzing a real sample with this system as the capillary tube rapidly blocked. The samples also contain high levels of inorganic salts, and we believe that these are the cause of blockage. Certainly no difficulty was encountered with the standard mixtures.

In contrast to the problems encountered with the DLI interface, the belt type of interface can easily cope with these samples. The TIC trace for such a sample obtained on a Finnigan MAT 4000 fitted with a belt interface is shown in Figure 5. There is some loss of resolution at the front end of the trace compared with LC analysis. This arises from the limitation on the amounts of H2O that can be handled by the belt interface. However, good EI and Cl spectra were obtained.
Figure 4. TIC trace obtained during microbore LC/MS using DLI interface shown in Figure 2. A 250 x 0.5 mm column packed with Spherisorb S5-ODS was used with methanol/water (1:1) as mobile phase at 10 μl/min. The mixture contained N-heterocyclic compound (A), resorcinol (B), p-cresol (C), 2,5-xylene (D), 1-naphthol (E), and 2,4,6-trimethylphenol (F). Reprinted from Reference 4 by permission of John Wiley & Sons, Ltd. © Heyden & Son Ltd. 1982.

Figure 5. Computer reconstructed TIC trace obtained during LC/MS under CI (methane) conditions of an aqueous effluent, using a Finnigan MAT 4000 and belt interface. A 100 x 0.5 mm column packed with 5μ OD was used with methanol/water (1:1) as mobile phase. Reprinted from Reference 4 by permission of John Wiley & Sons, Ltd. © Heyden & Son Ltd. 1981.

A very successful application (5) of LC/MS with the DLI interfaces has been the determination of herbicides in complex matrices such as soils. The normal method of analysis for many of the herbicides we encounter involves a lengthy extraction procedure followed by GC with a selective detector or GC/MS determination (6). We have found that LC/MS is a viable alternative to these procedures, the main advantage being that a simple soxhlet extraction is all that is required to isolate the herbicide. Figure 6 shows data for the triazine herbicide, atrazine. The intense response from the UV detector arises from other coeluting components of the soil matrix, but the selected ion chromatogram from m/z 216 and 218, the M + 1 ions for the herbicide enable the compound to be determined to sub mg/kg levels in the original soils.

Recoveries for the LC/MS method are typically better than 90%. Table I shows a comparison of results obtained by LC/MS and GC/MS for bromacil. As can be seen there is good agreement between the two sets of data. Although the detection limit for LC/MS with a DLI interface is normally an order of magnitude or more above that for GC/MS, at least for conventional LC columns, it is the substantial saving in time that makes LC/MS the method of choice.

Table I

<table>
<thead>
<tr>
<th>Sample</th>
<th>GC/MS (mg/kg)</th>
<th>LC/MS (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.3</td>
<td>4.2</td>
</tr>
<tr>
<td>B</td>
<td>2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>C</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>D</td>
<td>0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Other applications using DLI interfaces have included the analysis of polycyclic aromatic hydrocarbons with up to five rings in coal derived liquids and gasification byproducts, and determination of nicotine in tobacco smoke condensate and air monitoring samples.

DLI Interfaces — Where Next?

From the above discussions, a fair assessment of current DLI interfaces can be made. Some of the comments apply to all DLI interfaces including more sophisticated systems based on pinhole orifices and employing cryocooling; others are specific to capillary based interfaces.
The major advantages of the simple interfaces described above is that they are inexpensive, simple to operate and construct, and require no modification to an existing quadrupole mass spectrometer equipped with a CI source. The major disadvantage is the limited range of compounds that can be handled. In this respect, the performance of the interface which incorporates vacuum nebulisation proved disappointing. We found little difference between this interface and our simple version, and concluded that efficient nebulisation was not occurring. This is probably due to problems of alignment between nebulising tip and counter orifice.

Other general limitations of DLI interfaces include the fact that only CI spectra are produced. In our experience using methanol/water or pentane/dichloromethane solvent mixtures, very little fragmentation occurs, and it is then very difficult to identify unknown components. On the other hand, the simplicity of the spectra make the system ideal for analyses involving selected ion monitoring. To date all our applications have adopted this approach.

Finally, it is worthwhile considering gradient elution which has proved difficult to implement with a capillary based interface. In particular, if solvents of very different viscosities are used, as the eluent composition changes, the pressure drop across the capillary and hence the flow rate into the ion source varies. The overall effect is changes in the reagent gas pressures and hence ion source focusing, which affect mass spectrometer sensitivity.

Conclusions

Although limited in the range of compounds that can be handled, DLI interfaces based on glass capillaries are simple and easily constructed. They provide an inexpensive means of performing LC/MS on a quadrupole mass spectrometer. As only CI spectra can be produced, applications involving the use of selected ion monitoring are most successful.

References


Biography

Nigel Evans received his B.Sc (1970) and Ph.D (1973) degrees at the University of Exeter. The Ph.D thesis work involved the development of photochemical routes to the synthesis of polycyclic aromatic hydrocarbons and heterocyclic compounds related to indole alkaloids. A two year postdoctoral fellowship at University College, Cardiff followed. Since 1975, he has worked for the British Gas Corporation in their research laboratories at Fulham, and is currently in charge of the mass spectrometry and NMR laboratories.
Experiments With the Coupling of a Jasco Micro LC To a Finnigan MAT 3300 Quadrupole Mass Spectrometer

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When we investigated the possibility of on-line LC/MS, the commercial Finnigan MAT transport system could not be accommodated in our budget. Also a practical consideration of the combination of the 3300 with the belt system is that the LC interface uses the solids probe inlet, which is used routinely in our laboratory for the introduction of the Desorption Chemical Ionization probe (1).

A direct liquid introduction system for the 3300, using a glass capillary and a splitter has been published (2). Our first attempts were along these lines. The prime advantage is of course the very low cost of the interface. The major disadvantage lies in the use of only 1 to 2% of the eluate for mass spectrometric identification.

If the standard liquid chromatograph is replaced by micro HPLC equipment operating at a much lower flow rate (5-15 µl/min), the total effluent can be fed into the CI source, giving a dramatically increased sensitivity. This approach has been pioneered by S. Rottschaefer at Smith, Kline and French and by J. Henion at Cornell University (3, 4).

The use of a pneumatic nebulizer (5) was not considered because of the constraints imposed by the small diameter of the solids probe introduction port of the 3300.

The various types of small bore HPLC columns may be classified as open tubular (capillary), packed capillary and microbore columns (6). At present, the last category is beyond the development stage and commercially available. In view of the results published by Henion and Maylin (3), the Jasco micro LC was chosen for coupling to the mass spectrometer.

The Finnigan MAT 3300 CI source has been designed to accept 20 atm ml methane per minute from a packed column and reach a pressure reading of 1.0 Torr at this flow rate (0.5 Torr on a McLeod gauge). A flow of 10 µl/min of water will produce 12 atm ml water vapor per minute. Mixtures of water with acetonitrile or methanol will present a lower gas load to the vacuum system. So the CI source can easily accept the total effluent from the micro LC at 10 µl/min and there is enough pumping capacity to modify the reactant ion spectrum by bleeding ammonia gas into the source (7).

Experimental

A Finnigan MAT 3300 GC/MS equipped with the standard CI source was used. Data were processed by a Finnigan MAT 6110 computer system. Full scan spectra were recorded under the following conditions: mass range, 150-350; integration time, 12 ms; seconds per scan, 3. The source temperature was kept constant by a CRL 405 digital temperature controller (CRL, Worthing, England) using the original Finnigan MAT thermocouple as sensor. The temperature readout was calibrated against a Pt resistance thermometer, inserted into the source via the solids probe inlet port. The calibrated source temperature was varied between 200 and 265°C.

The original Varian thermocouple gauge is not well suited for recording the source pressure. It is located upstream in the flow of vapors and gases towards the ion volume, and it has a fairly slow response. We have used an IM 10 high pressure ion gauge (Leybold-Heraeus, Köln, W. Germany), which has a measuring range of 1 × 10⁻⁶ to 1.0 mBar, to monitor the pressure in the pumping line to the source diffusion pump. A value of approximately 2 × 10⁻³ mBar (1 mBar = 0.75 Torr) was measured if 10 µl/min of acetonitrile-water 70:30 were introduced. The magnitude of pressure fluctuations can be recorded conveniently by connecting the output of the IM 10 to a pen recorder. The reactant ion spectrum was also recorded, using the hardware ion current integrator of the Quadrupole Electronics Module (mass range approximately m/z 10-100, fast response, electron multiplier 1000 V) connected to the second channel of the two-pen recorder. When ammonia gas was added, it was admitted via the solids probe valve, to make sure that solvent vapors were swept efficiently towards the ion volume.

18 Spring 1983
A Jasco Familic 100 N micro liquid chromatograph with a 500 µl syringe pump was used. It was equipped with a Jasco ML 422 micro loop injector (0.3 µl internal loop), a Jasco pressure monitor and the Jasco Uvidec 100-III spectrophotometer detector (cell volume 0.3 µl). The homemade PTFE columns (about 150 mm long × 0.5 mm i.d. × 1.8 mm o.d.) were packed with Nucleosil 5 C 18 (Machery and Nagel) using a tetrabromoethane-n-butanol (1 + 1) slurry. The columns had a plate count of 2000 to 2500 for the compounds shown below. It is expected that better columns can be made by following the directions given by M. Lant and S. Westwood of D. E. Games' group (8).

The LC/MS interface probe was inserted such that the copper cylinder (Figure 1) was located approximately halfway inside the inlet port of the source block. Narrow bore fused silica tubing was purchased from S.G.E. (Melbourne, Australia).

Results and Discussion

During preliminary experiments using standard HPLC equipment only a very small portion of the eluate (1-2%) was actually used for mass spectrometry. The interface was a 0.1 mm i.d. × 4.0 mm o.d. glass capillary tube with a restriction on the MS side (2). Attempts to create a jet of droplets were successful, but a fairly high liquid pressure was required, and a stable source pressure could not be obtained. To investigate the cause of this problem, we observed the jet inside a glass envelope. It appeared then, that a jet, which was straight in the air, started to bend when vacuum was applied. Our explanation is that very small droplets are also formed due to the somewhat irregular shape of the orifice. These small droplets evaporate more rapidly than the larger droplets in the center of the jet. If the small droplets are predominantly formed on one side of the main jet, the expanding vapors from the small droplets push the main jet away from the center, when vacuum is applied. The occasional deviation of the jet emitted from a pinhole orifice has also been reported (7).

Because we had no rigid control over the shape and diameter of the restriction in the glass capillary, we abandoned the principle of jetting the liquid into the source. It was also questionable if the Jasco micro LC could deliver the necessary high pressure.

The next series of experiments used the Jasco micro LC and the same glass capillary, but the restriction on the MS side was not as narrow. The solvent and solute now have to evaporate just inside the restricted end of the capillary. This of course limits the LC/MS system to samples that can be run off the normal solids probe at temperatures below 250°C. Nevertheless, there are enough problems that can be solved this way, as shown by Rottschaefer, Henion, and more recently by Evans and Williamson (9) and by Schaefer and Levensen (10).

In our hands, success was variable, but the time spent on this approach was rather short. Sometimes the source pressure was entirely stable for a whole day, and good chromatographic peak shapes were observed, but the next day the source pressure might fluctuate strongly. We also found the direct connection of the column with the glass capillary, or the use of a short transfer line from the UV detector to the glass capillary (made of 0.1 mm i.d. stainless steel tubing) rather inconvenient.

A major step forward was the advent of narrow bore flexible fused silica capillary tubing. A length of 70 cm was sufficient to achieve transfer from the UV detector into the ion source without making further connections, and still allowed adequate flexibility in the positioning of the LC relative to the mass spectrometer. Capillaries having internal diameters of 25 µm and 50 µm have been tried so far. The 25 µm capillary requires a pressure drop of 20 Bars at a flow rate of 10 µl/min (acetonitrile-water 70:30), while the 50 µm capillary showed a pressure drop of only a few Bars. All further experiments were done with the 50 µm i.d. capillary, which we considered more suitable in view of the pressure that can be delivered by the Jasco.

The main problem with a simple DLI interface is how to obtain a stable ion source pressure. Because no restriction is made to the side of the capillary which is located in the source, the liquid is under reduced pressure. Gases dissolved in the liquid phase may easily form small bubbles that expand rapidly and make the liquid being transferred into the source a series of short plugs. Severe pressure fluctuations are the result. We have overcome this problem by thoroughly degassing the liquid phase using an ultrasonic bath, followed by extensive flushing of all connecting tubing, the column and the detector cell.

A second problem is that the water in the liquid phase may freeze during the evaporation process, if insufficient heat is transferred to the tip of the fused silica capillary. This phenomenon can easily be recognized if the fused silica capillary is connected directly to the micro loop injector valve, omitting the column and UV detector. As soon as a plug of ice is formed inside the capillary, the Jasco pressure monitor indicates a pressure buildup, which is accompanied by a pressure drop in the source and source pumping line. After some time, the plug of ice is forced out of the capillary, the source pressure shows a peak, while the liquid pressure drops.

A nude fused silica capillary very clearly showed this effect. The thermal mass of the tip and the transfer of heat to the tip are insufficient to support a steady evaporation. The situation was improved by sliding a 50 mm long piece of 0.5 mm o.d. × 0.25 mm i.d. stainless steel tubing over the fused silica tubing. To have better control over the temperature, the interface was further modified as shown in Figure 1. A 5 mm long × 4.9 mm o.d. copper cylinder was soldered onto the end of the stainless steel tube. As the internal diameter of the source inlet port is 5.1 mm, the copper cylinder will almost certainly touch the source, and heat will be transferred efficiently. The fused silica capillary is fed through so far that its end is just observed with a magnifying glass. All further experiments were performed with this interface.

![Figure 1. Schematic representation (not drawn to scale) of the interface probe: (1) copper, 4.9 mm o.d.; (2) stainless steel, 0.5 mm o.d. × 0.25 mm i.d.; (3) teflon insulator; (4) stainless steel, 6.4 mm o.d. × 4.6 mm i.d. and (5) fused silica capillary.](image-url)
Figure 2 shows the reactant ion spectrum. Sample molecules are ionized by proton transfer from the \((M + H)^+\) and \((2M + H)^+\) ions of acetonitrile at \(m/z\) 42 and 83. The reactant ion spectrum can be modified by bleeding \(\text{NH}_3\) gas into the source at such a rate that \(m/z\) 42 and 83 have just disappeared. \(\text{NH}_3\) has the higher proton affinity, and the set of reactant ions is presented in Figure 3. It may be advantageous to use this modified reactant gas, because it will result in a softer proton transfer to the sample, or in the formation of \((M + \text{NH}_4)^+\) ions (7). The background ion spectrum up to \(m/z\) 200 is also changed, which may be useful in view of the possibility of interference by certain background ions.

Because of our limited experience, we cannot yet judge whether the performance of the interface is also influenced by the addition of \(\text{NH}_3\) gas.

Figure 4 gives the liquid chromatogram of a mixture of four components. Full details regarding the analytical chemical aspects of the samples have been published elsewhere (11). Figure 5 presents the reconstructed (total ion current) liquid chromatogram for the same run, and indicates that 10 ng per component is just sufficient in the scanning mode (\(m/z\) 150 to 350). Figure 6 demonstrates that the signal-to-noise ratio of the total ion current profile can be improved by using only \(m/z\) 200 to 350 from the same data file for calculation of the reconstructed ion chromatogram. The extracted ion current profiles of \(m/z\) 266, 280 and 300 show that the separation efficiency has deteriorated, probably due to peak broadening in the UV detector. Injection of 50 ng per component resulted in the total ion current profile (\(m/z\) 150 to 350) of Figure 7. \(\text{NH}_3\) was used to modify the reactant ion spectrum, but it did not cause a change in the observed mass spectra of the compounds under investigation. The extracted ion current
profile of m/z 266 is also given. Both profiles are remarkably free of noise. Figure 8 presents the mass spectrum of the last component.

The results obtained thus far show that the simple interface is quite effective. The next series of experiments will be directed towards the analysis of samples of biological origin, in support of drug metabolism studies.

In conclusion, the advantages of the home-made DLI system, combined with a micro LC are low cost, simple construction and good sensitivity. The major disadvantage is that the so called nonvolatile samples cannot be handled.

**Figure 5.** Total ion current profile of the liquid chromatogram of Figure 4 (m/z 150–350).

**Figure 6.** (a) Same total ion current profile as in Figure 5, but calculated from m/z 200 to 350. (b) Extracted ion current profile of m/z 266, 280 and 300.
References


Biographies

Andries P. Bruins studied organic chemistry in Amsterdam and earned his Ph.D. on a project on functional group interactions in EI spectra and ion-molecule reactions in ICR, under the supervision of N.M.M. Nibbering. In 1975/76 he spent one year as postdoctoral fellow with K. R. Jennings, and worked on negative ion Cl and B/E linked scanning. Since 1976 he has operated the mass spectrometry service facility in Groningen.

Ben F. H. Drenth studied pharmacy at the State University in Groningen. He specialized in analytical chemistry, with emphasis on HPLC. In 1977 he became involved in drug metabolism research. In 1982 he earned his Ph.D. on a thesis dealing with analytical chemistry in the field of drug metabolism.
Thermospray LC/MS: Supplement or Substitute for Existing Techniques

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The beginning of combined liquid chromatography/mass spectrometry (LC/MS) can be marked circa 1970 (1-4). Yet even in the second decade of LC/MS, its use seems limited to no more than a few hundred laboratories, many of which are classified more as research laboratories than as analytical laboratories. Throughout the preceding articles, there is an underlying sub-theme suggesting that good LC/MS requires careful selection of the interface method, adaptation of ancillary techniques, and compromise of the LC conditions. Apparently, no one technique is the answer to all analytical problems, and it is quite possible that the natural incompatibilities of LC and MS (primarily large solvent flow especially polar solvents, involatility of sample, presence of buffer salts and flow programming) will necessitate that there always be several alternative procedures. But we will keep trying for the universal system.

Thermospray provides yet another LC/MS interface system. This unique process has evolved from several years of research by Professor Marvin Vestal into the nature of LC/MS interface phenomena (5-8). As a first appeal, thermospray (TSP) provides a method to handle a large flow of water solutions (e.g., 1-2 cc/min of 80-90% H₂O/MeOH with buffer salts) and also to put low volatility compounds into an ionic vapor form without excessive risk of thermal decomposition. Since these two problems have posed the maximum difficulty for LC/MS during the past decade, it is obvious that TSP will be another important technique for LC/MS interfacing.

Thermospray is more than just another LC/MS interface; it is also another ionization method. Unlike most mass spectral ionization methods used in organic chemistry, TSP does not use particle bombardment as the primary mode of creating the ionic species. In contrast, the source of ions for TSP is the salt ions in the buffer solution and organic ions are formed by a chemical ionization reaction with the salt ions during the TSP evaporation process. This unique mode of organic ionization does not subject the sample to energetic bombardment and does not permit the sample to have significant high temperature exposure to hot metal surfaces. Thus decomposition is minimized.

Figure 1 gives a schematic diagram of the thermospray LC/MS interface. Flow from the LC column (a) enters a heated tube of 0.015 cm ID (b). The temperature in this region is carefully controlled so that the solvent emerges as a very fine spray which enters the jet chamber (c). In the jet chamber, the fine droplets evaporate and during this process, charge exchange occurs between salt ions and the organic sample. Both positive and negative ions are formed, and the jet stream is sampled into the mass spectrometer by means of a sampling cone (d) with approximately a 0.05 cm diameter hole. The ability to pump the large volume of solvent (around 2000 cc/min of vapor at 1 atmosphere) results from the fact that the jet emerging from the tip of the vaporizer acts as its own ejector pump and provides a strong unidirectional motion toward the mechanical pump (e).

Figure 2 presents typical background spectra for ammonium acetate in (a) pure H₂O, (b) H₂O/methanol, and (c) H₂O/acetonitrile. The total primary beam (sum of all ions) is in the range of 10⁻¹⁰ amps which, at a multiplier gain of 10⁵, gives a rather excessive background signal so that most organic analyses are restricted to above mass 100. The exact pattern of the solvent/water/ammonium adduct ions depends upon the solvent concentration and the temperature of the jet chamber.

Although thermospray is still in a very early development period, applicability has been demonstrated by a small number of laboratories for a relatively large number of difficult mass spectral samples. Many data have not yet been published but a partial list of compounds would include saccharides, peptides, vitamins, alkaloids, glucuronides, nucleosides, nucleotides, antibiotics, etc. (9-11). Sensitivity has been shown to be compound dependent and is related to proton/electron affinity. Samples such as aliphatic hydrocarbons and polycyclic aromatic hydrocarbons are very nearly opaque to the TSP ionization process and an auxiliary electron bombardment is used, thus providing more conventional chemical ionization reactions. When conditions are ideal, a detection limit as low as 30-40 picograms may be anticipated. However, a full scan analysis of higher molecular weight compounds may need at
least 100–200 ng depending upon compound compatibility and molecular weight. It appears that at this early stage of development, TSP spans the same sensitivity range as other LC/MS methods but there may be a potential through further research to extend this level lower at least 1 or 2 orders of magnitude.

Perhaps the most important feature of TSP is that it extends LC/MS analyses to the more difficult classes of compounds usually ionized by particle bombardment (FAB, DISIMS, Cf252) or intense radiation or electric fields (laser radiation, field desorption). Since TSP is a natural relative of liquid chromatography, acquisition of LC/MS data on difficult samples such as small peptide mixtures is facilitated.

An example of TSP on a thermally delicate sample is shown in Figure 3 (12). Ranitidine has been used as a test sample for LC/MS methods in previous studies (6) and results were shown to a detection limit of ca 10 ng, depending on the method. The TSP process easily gave detection at a low picogram level as seen in Figure 5.

Figure 1. Schematic of thermospray LC/MS interface. a) LC flow, 0.5–2 cc/min, solution containing 0.1M ammonium acetate. b) Thermospray evaporation chamber, 0.015 cm ID SS tubing. c) Jet chamber d) Source block with ion sampling cone. e) Pumping lead to mechanical forepump. f) Differentially pumped quadrupole assembly.

Figure 2a. Background ions, pure H2O, 0.1 MNH4Ac, observed in the thermospray process.

Figure 4 shows an LC/MS analysis by TSP of two common drugs, tegretal and Primidone. The mass spectra (Figures 4b, 4c) show typical TSP behavior in which ions M + H, M + NH4, and M + Na are observed. In Multiple Ion Detection (MID) mode, the detection limit for these drugs was in the range of 100 pg.

It is clear from the results obtained to date that TSP will be an additional LC/MS interface method. Even in its embryonic state, TSP shows potential to handle a full flow of LC solvent (up to 2 cc/min), provide gentle ionization to thermally labile samples (including high molecular weight compounds), and to give good sensitivity under favorable circumstances. Some of the existing limitations are: 1) the necessity to have salt ions, preferably a volatile salt, which eliminates low polarity solvents; 2) careful control of the vaporization temperature; 3) with existing designs, solvent flow must be greater than 0.5 cc/min or the TSP vaporization conditions are not realized; and 4) for some samples, careful selection of flow and temperature may be necessary to prevent thermal destruction.

In theory, at least, solutions to these problems can be conceived and all of the above are expected to be of minimal concern as TSP is further developed. Whether or not TSP proves to be the universal LC/MS interface is yet to be demonstrated but that it will be a very useful technique extending present capabilities in LC/MS is a certainty.
Figures 2b and 2c. Background ions, a) H$_2$O/MeOH, 0.1 MNH$_4$Ac and b) H$_2$O/CH$_3$CN, 0.1 MNH$_4$Ac, observed in the thermospray process.
Figures 3a and 3b. Thermospray analysis of Ranitidine. a) TSP mass spectrum, 2.5 ng. b) Ion chromatogram showing detection at 0.5 ng.
Figure 4a. Chromatogram of Tegretal and Primidone by thermospray.

Figure 4b. Mass spectrum of Tegretal

Volume 9, Number 1
Figure 4c. Mass spectrum of Primidone

References


Biography

W. H. McFadden is employed in the Engineering Department of Finnigan Corporation, San Jose, CA. His responsibilities involve development of new accessories for the 4000 line mass spectrometers, and recently he has been involved in design and construction of a new Thermospray Interface for LC/MS.

Since receiving his Ph.D. from the University of Utah, Dr. McFadden has been involved in a wide variety of mass spectral activities. He is well known for his pioneering developments in both GC/MS and LC/MS interface methods. His recent activities in Thermospray represent another stage of this interest.

He has published over 100 journal articles and is author of the book “Techniques of Combined Gas Chromatography/Mass Spectrometry.”