Journal of Chromatography, 488 (1989) 73-85
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4554

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY COUPLING AND ITS APPLICATION IN PHARMACEUTICAL RESEARCH

K. VEKEY, D. EDWARDS and L.F. ZERILLI*

Lepetit Research Centre, Via R Lepetit 34, 21040 Gerenzano (Varese) (Italy)

SUMMARY

The advantages and technical problems related to the on-line connection of liquid chromatography and mass spectrometry are described, with special emphasis on applications in pharmaceutical chemistry. The most important characteristics of various interfaces, such as direct liquid introduction, Magic, continous flow fast atom bombardment and thermospray, are discussed. Special applications to the study of thermally very labile compounds are considered.

INTRODUCTION

Interfacing gas chromatography (GC) with mass spectrometry (MS) is inherently easier than that of liquid chromatography (LC) with MS, as the mass flow is ca. 10 000 times smaller in the former instance. Mainly owing to this problem, LC–MS is a much less widespread and routine technique than GC–MS. The first GC–MS connection was reported in 1957 [1], only 5 years after GC was developed, and by the late 1960s and early 1970s GC–MS was already an established routine method. An indication of the technical difficulties in coupling LC and MS is that the first paper on the possibility of LC–MS interfacing appeared only in 1968 [2]. Technical development was very slow, and in spite of enormous interest LC–MS is only now starting to become a more or less routine analytical method.

The connection of a liquid chromatograph with a mass spectrometer presents formidable technical problems, mainly resulting from the usually high flow-rates used in high-performance LC (HPLC) systems (ca. 1 ml/min) and the necessity to maintain a high vacuum in the mass spectrometer system. Typical operating pressures in the analyser part of the mass spectrometer should be better than 10^{-5} Pa (ca. 10^{-10} atm). The pressure in the source housing in the electron impact mode should be about 10^{-4} Pa and in the chemical ionization mode about 10^{-2} Pa (ca. 100 Pa inside the source block). Even the largest pumping systems

0378-4347/89/$03.50 © 1989 Elsevier Science Publishers B.V.

cannot cope with the total effluent from a conventional HPLC packed column and keep the necessary high vacuum in the mass spectrometer. Possible solutions are as follows:

(i) splitting the effluent from a conventional HPLC column and introducing only a few percent (i.e., 10–50 μl/min of liquid) into the mass spectrometer; even this usually requires an increase in the pumping efficiency of the mass spectrometer vacuum system, usually by the addition of a cryopump cooled by liquid nitrogen;

(ii) using small-bore packed or open-tubular columns, eliminating the necessity for splitting; this solution has shown promising results, but it is still in an experimental stage;

(iii) separation or partial separation of the solute and the eluent prior to introduction to the mass spectrometer ion source or mass analyser.

In addition there are other, also important, considerations, as follows.

(a) The possibility of using various ionization techniques in mass spectrometry. Depending on the compounds to be studied and the information sought, different ionization methods might be suitable. Electron impact (EI) is the oldest and still most often used ionization method in mass spectrometry. It provides abundant fragmentation, therefore supplying important structural information. Furthermore, large computer libraries of EI spectra are available, which facilitate the rapid identification of many compounds. Its disadvantage is that some compounds do not give a molecular ion (i.e., molecular mass) and the sample needs to be evaporated prior to ionization (a vapour pressure of at least 10^{-4} Pa is required), and therefore many ionic, high-mass (>1000 a.m.u.) or thermally labile compounds cannot be studied.

Chemical ionization (CI) is a so-called "soft" ionization method, indicating that mainly a molecular ion is formed with little fragmentation. The term "chemical ionization" in LC–MS work is, however, often used loosely to encompass a variety of techniques that give spectra comparable to those obtained with classical CI. The classical ionization mechanism involves the reaction of sample molecules with reagent ions in the gas phase. In LC–MS, the finally observed gas-phase ions may instead be produced by evaporation of or ejection from liquid droplets, or via an aerosol, or may even involve ion–molecule reactions in a near-critical fluid; their production may be assisted by heating, by electric fields or electric discharges; the source pressure may be increased to atmospheric for some interfaces.

CI methods are well suited to molecular mass determination, quantitative studies and trace analysis, but give limited structural information. This disadvantage can be overcome by using tandem mass spectrometry (MS–MS) with, however, a correspondingly lower sensitivity, where ion formation and fragmentation occur in separate steps. Fast atom bombardment (FAB), and also field, laser and plasma desorption, are used mainly to study non-volatile compounds.

(b) Depending on the LC–MS interface used, there are various restrictions on the chromatographic conditions. Usually only volatile buffers can be used, the water content in the eluent might be restricted, there may be limitations on the flow-rate, etc. The maintenance of high or very high chromatographic resolution is not always possible, partly owing to the restrictions on the chromatographic

conditions, partly to peak broadening in the interface and partly to "memory" effects in the mass spectrometer ion source. This effect is usually more than offset by the specificity of the mass spectrometer as a detector: two compounds exhibiting different mass spectra can be separated by deconvolution or by monitoring the abundance of selected ions in the mass spectra even if the respective peaks in the total ion chromatogram are not resolved.

(c) The sensitivity of an LC–MS system may be limited either by the available amount of the sample mixture to be analysed or the concentration of the component to be detected (be it relative to other compounds present, relative to the maximum solubility in the mobile phase or to limits of column capacity). Small-bore columns offer a significant advantage when the sample amount is limited, but there is no significant gain when concentration is the limiting factor. Although the sensitivity of mass spectrometers is at least 10^3–10^9 times higher than that of NMR or infrared spectrometers, it is still less than that of other LC detectors if full mass spectra are required. The sensitivity can be significantly improved when using selected ion monitoring. Detection limits in favourable instances are in the low picogram range. More important, the high selectivity of the mass spectrometer reduces problems concerning the background, interferences and artifacts.

LC-MS INTERFACES

Various types of interfaces have been developed for LC–MS, and there is still a lot of activity in this area. Although thermospray [3] was developed only about 5 years ago, now most application-oriented studies use this technique, described below [4]. The moving belt interface [5] is probably the most flexible type, meaning that the mass spectrometer side and the HPLC side are nearly completely separated, so in principle any ionization mode (EI, CI, FAB laser or particle desorption, etc.) can be used. However, it is a complicated system with moving parts, which has lost ground recently, and will not be described in detail. Direct liquid introduction (DLI) is the oldest and probably the simplest, but still popular LC–MS interface. There is renewed interest in this technique, partly owing to its suitability for micro-HPLC techniques and partly to a new variation suitable for FAB analysis (continuous-flow FAB) and another suitable for both electron impact and chemical ionization ("Magic" [6,7]). Both have recently been developed commercially, and promise to be of importance for some chromatographic applications. These interfaces will be described in detail. Atmospheric pressure ionization with the electrospray (ion spray, ion evaporation) technique also has interesting possibilities, and is now commercially available. It has a high sensitivity, a very high effective mass range due to creation of multiply charged ions, and can be coupled to capillary zone electrophoresis. However, there are only to date few applications in the pharmaceutical field, so this and some other less commonly used interfaces will not be described here, but the reader is referred to other reviews on LC–MS [4].

Supercritical fluid chromatography (SFC) is becoming a popular method in its own right, and is less difficult to interface to mass spectrometers than liquid chromatography. Most interfaces used for LC–MS can also be used for SFC–MS, e.g., thermospray, moving belt and some versions of DLI. However, we consider

SFC–MS outside the scope of the present paper, so this technique will not be discussed further.

Direct liquid introduction

The basic variations of this type of LC–MS interface have been discussed recently in two excellent reviews [8,9]. In DLI the column effluent flows directly into the mass spectrometer through some kind of restriction. The simplest and first idea was to use a capillary as a restriction device. It was shown later that in all practical instances the solvent evaporates inside the capillary tube prior to entering the mass spectrometer system. This creates several problems (e.g., frequent clogging in the capillary), and this approach was later abandoned.

In contemporary DLI methods (Fig. 1) a small diaphragm of about 5 μm diameter is used as a restrictor. Provided that there is a certain minimum liquid velocity and a minimum pressure drop, a liquid jet will form which immediately afterwards break into small droplets. These have a diameter about twice as large as that of the diaphragm. The next step is desolvation, in which the eluent evaporates. This requires heating and occurs in a so-called desolvation chamber. After this the effluent vapour enters the ion source of the mass spectrometer.

As discussed above, mass spectrometer vacuum systems cannot cope with the total effluent from a conventional packed HPLC column (ca. 1 ml/min). The effluent has to be split so that only 10–50 μl of liquid enters the mass spectrometer, resulting in a loss of sensitivity. A significant improvement in DLI performance came with the development of micro-HPLC techniques, and the realization that these eliminate the need for splitting. Flow-rates using 1 mm I.D. columns are typically 10–50 μl/min, suitable for introduction into a mass spectrometer

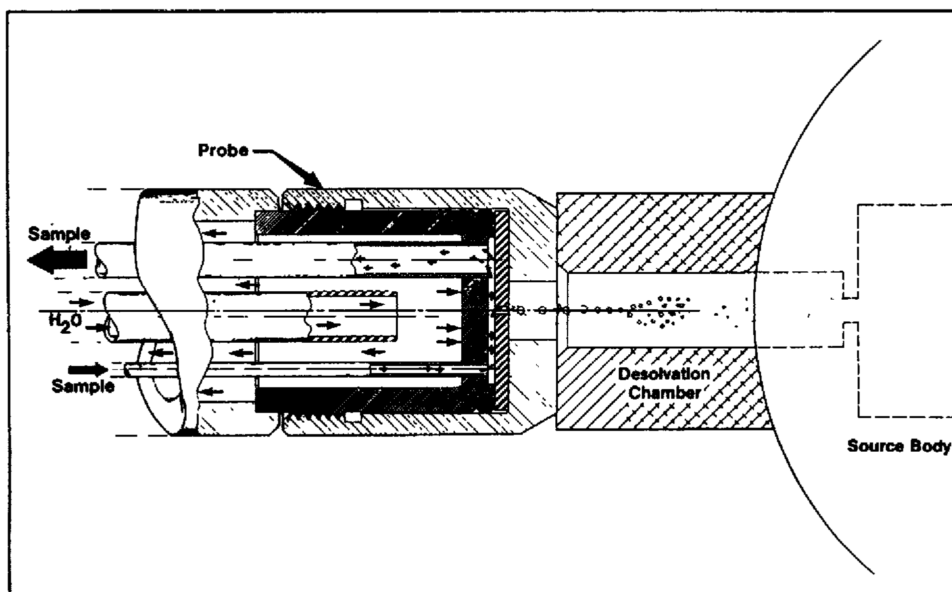

Fig. 1. Schematic diagram of a direct liquid introduction interface (Hewlett-Packard).

with a cryopump, and this increases the sensitivity, in terms of amount of sample injected, 20–100-fold.

Owing to the relatively large liquid flow entering the mass spectrometer, the pressure in the ion source region is relatively high (ca. 100 Pa), so only (positive or negative) chemical ionization can be used. The eluent from the HPLC column acts as a reagent gas, and this means a further restriction. If gradient methods are used in HPLC, the proton affinity of the reagent gas (mixture) may change during the chromatographic process and this may affect the mass spectra. However, this rarely presents a practical problem. On the positive side, a small amount of high-proton-affinity additive may either be added to the HPLC eluent or introduced through a gas line directly into the ion source. This will form the dominant reagent ions, so there are some possibilities of selecting CI and HPLC conditions separately. With negative ionization the reagent gas usually acts only as a moderator gas, so the composition of the eluent is not so critical as with positive ionization. It is also possible to change the character of negative CI with additives, e.g., by the addition of a small amount of halogen compounds, acting by ion attachment [10].

A very important feature of DLI is that thermally very labile molecules can be studied, and some examples will be discussed below. The reason for this is that the sample may be ionized without the need for contact with hot surfaces. The diaphragm is kept at about room temperature (by water cooling). The droplets are formed without heating and evaporation of the solvent from the surface keeps their temperature down; it has been calculated [11] that a droplet of acetonitrile at room temperature will freeze in an adiabatic process after evaporation of only 23% of the liquid! In fact, heating the desolvation chamber is necessary only to stop freezing, and not to heat the liquid. The only part of the instrument which is kept at a high temperature is the ion source (usually at 100–300°C), but the sample molecules normally should not come into contact with the walls of the source, only with the reagent gas (in a low vacuum). This provides much less efficient heat transfer, and only for a short time, probably a few milliseconds.

Another point to be emphasized is that the sample does not need to be vaporized, in contrast to most other LC–MS interfaces. After formation of the droplets the (volatile) HPLC eluent evaporates from the droplets; this process may leave the sample molecules in the gas phase, perhaps with some solvent molecules attached. It is not true, as stated in some LC–MS reviews [12], that only relatively volatile compounds can be studied. As an example, vitamin B_{12} has been studied using a DLI interface with negative ionization [13]. The spectrum shows the

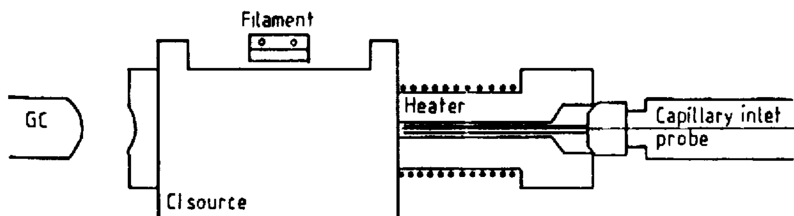

Fig. 2. Schematic diagram of a capillary inlet interface probe with external heater assembly [15].

$(M-H)^-$ ion as the base peak. Vitamin B_{12} is a complex, non-volatile, thermally labile compound, and the results compare favourably with those obtained by other soft ionization methods [14].

There is a recent interest in modified DLI interfaces. One is capillary connection of the recently developed open-tubular HPLC system to a mass spectrometer (Fig. 2). In this instance the liquid flow-rate is so low (ca. 0.5 μl/min) that it is possible to obtain EI spectra [15,16]. However, this method is too new to evaluate its potential yet.

Magic interface

The monodisperse aerosol-generating interface for chromatography (Magic) [6,7] has recently been commercially developed in a version known as the particle beam (BP) interface [6]. This device (Fig. 3) differs from the "conventional" DLI discussed above mainly in the desolvation process. The liquid jet is broken up into small droplets due not to inherent surface instability in the jet but to an orthogonal gas flow (the monodisperse aerosol generator, see Fig. 4) at atmospheric pressure. A dispersion gas is used to prevent clustering of the droplets. Desolvation also occurs at atmospheric pressure, where heat transfer is more efficient. An aerosol beam separator connects the desolvation chamber to the

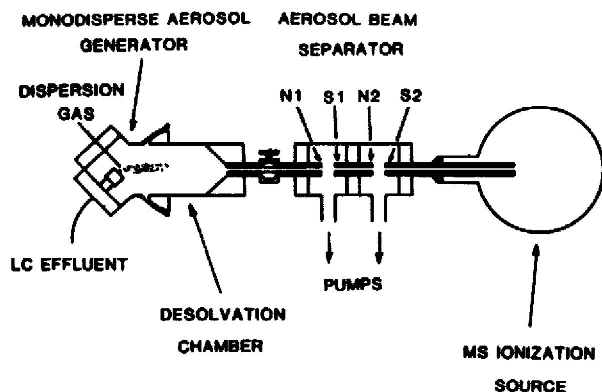

Fig. 3. Schematic diagram of Magic interface [6]. N1, N2 = nozzles, S1, S2 = skimmers.

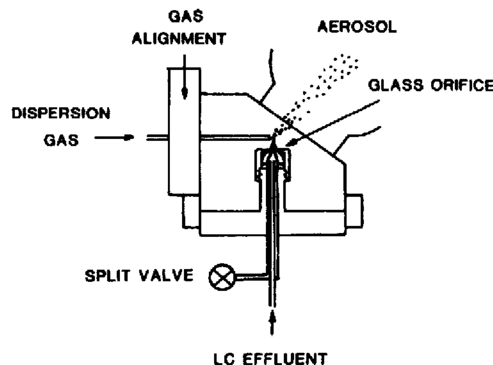

Fig. 4. Monodisperse aerosol generator [6].

mass spectrometer ion source. Liquid flow-rates of 0.1–0.5 ml/min are optimal, which means that no or only small splitting is necessary, improving the sensitivity. Probably the most important advantage of this interface is that complete separation of the sample from the eluent gives a free choice between the EI and classical CI modes. Its sensitivity, however, appears to be low for non-volatile compounds [17].

Continuous-flow fast atom bombardment

An important new technique is continuous-flow FAB [18–22], also known as dynamic FAB or on-line FAB. The technical solution is very simple and similar to the first LC–MS interfaces: liquid at a few µl/min enters a mass spectrometer ion source through a narrow capillary (Fig. 5). The main differences are that the eluent contains a few percent of viscous fluid, such as glycerol or thioglycerol, and the liquid does not evaporate in the capillary tube but forms a small droplet on the "target" at the mass spectrometer end of the capillary. The atom beam is directed to this liquid droplet and a conventional FAB process takes place. Some interface designs include an absorbent wick beside the metal target; this creates, instead of a droplet, a thin liquid film running over the target. Continuous-flow FAB, in addition to being a relatively simple LC–MS interface, offers advantages over conventional FAB, even if compound separation is not required: sample preparation may be less critical; the "suppression" effect [23,24] is less critical; the chemical background is decreased and the sensitivity is improved; and continuous sampling of liquids, and therefore reaction monitoring or process control, becomes possible.

Thermospray

Discovery of thermospray ionization was a major step in LC–MS interfacing [25–27]. In the thermospray process a liquid flow of about 1–2 ml/min is rapidly vaporized in a capillary tube. In this way a supersonic jet of vapour with entrained fine droplets is formed, and at least part of the droplets will be electrically charged.

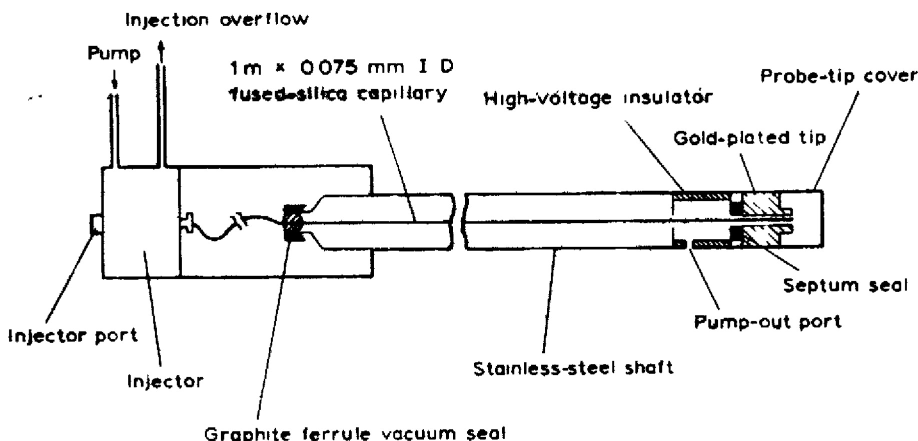

Fig. 5. Schematic diagram of continuous flow FAB probe [20].

These will continue to vaporize while travelling through the heated source. As the size of a droplet is reduced, the electric field at the liquid surface will increase until ions present in the droplet are ejected. Therefore, an external source of ionization is not essential. As the pressure in this region is relatively high, the ions formed in this way will subsequently undergo ion–molecule reactions, in a similar way to conventional chemical ionization. A schematic diagram of a thermospray LC–MS source is shown in Fig. 6.

In thermospray the rapid and controlled heating of the capillary is a critical parameter. Early systems suffered from rapid temperature changes, but this has been overcome by direct electrical heating of the stainless-steel capillary. In this way, temperature programming of the interface for gradient elution also became possible. For the thermospray process to be efficient, the mobile phase should contain a high percentage of water and an electrolyte. To study ionic samples no additional salt may be necessary, but for other compounds a ca. 0.1 *M* solution of ammonium acetate proved to be very advantageous. To extend the applicability of thermospray to eluents with low water contents, post-column addition of water or buffers has been developed [28].

It was found that the sensitivity of the method described above, the so-called filament-off thermospray, depends very much on the sample to be analysed, and many compounds are not even observed. To obtain a more uniform response the filament-on thermospray was developed [29]. In this technique electrons emitted by a conventional filament in the ion source help to ionize the sample. Instead of the filament an electric discharge can also be used, as indicated in Fig. 6: the technique is then also known as "plasma spray" or "plasma discharge ionization". Apart from increased sensitivity for some compounds, it has been found that plasma spray requires less careful control of the vaporizer temperature.

Spectra obtained with the thermospray interface contain only a few fragment ions, indicative of a "soft" chemical ionization. Both positive and negative ionization are possible. Similarly to DLI, the eluent acts as the reagent gas. It is interesting and important that, in spite of the rapid heating in the interface, thermally labile compounds can be studied with good results.

A recent development is the observation that by using a high repeller voltage

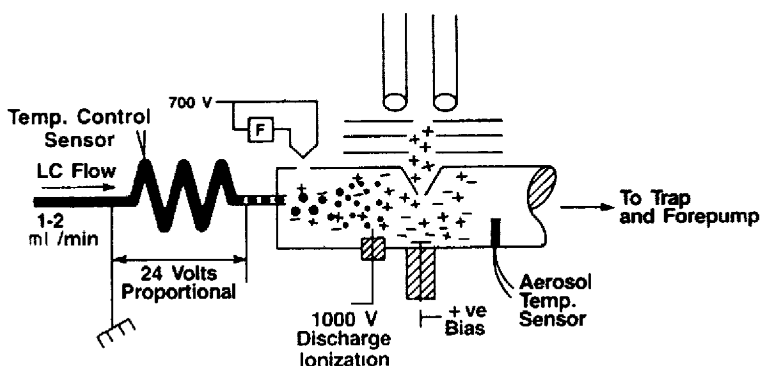

Fig. 6. Schematic diagram of thermospray LC–MS source [31].

(ca. 100 V) the amount of fragment ions can be increased [30–32]. This phenomenon is explained as a collision-induced decomposition occurring in the ion source. This has great importance, as in this way structural information can be obtained on compounds that otherwise do not fragment in thermospray. The process can be compared to an MS–MS experiment carried out, e.g., in a triple quadrupole instrument, provided that at low repeller voltages only one species is formed in the ion source.

Comparison of interfaces

It seems that at present there is no single LC–MS interface suitable for all applications. At present the thermospray technique is the most popular, its main advantages being that it is reliable and does not require special skill to operate. It is ideally suited to conventional packed column reversed phase HPLC, the currently most popular separation technique. Most compounds, including thermally labile compounds, of molecular mass between 200 and 1000 can be easily studied, although this range can be extended. Recent reports of the use of high acceleration voltages suggest that thermospray analyses of peptides and other polar compounds in the mass range 1000–8000 are feasible [33,34]. Also, this interface is commercially available for most mass spectrometers. Its disadvantages are that the sensitivity for some compounds is very low (even in the filament-on mode), so these may not be seen, and that it is suitable only for chemical ionization (so from the few fragment ions only limited structural information can be deduced). This latter problem starts to become less important with the more widespread use of LC–MS–MS methods, and there is still room for improvement in source design [35].

The "conventional" version of DLI is an important, but less popular interface than thermospray. Partly as a consequence and partly as a cause, it is available on fewer instruments. There are more practical problems also, so more experience is necessary to operate it. It is not suitable for high flow-rates, so with conventional packed columns splitting is necessary. However, it is suitable for normal-phase HPLC. Its other characteristics from the user's point of view (suitable only with chemical ionization, etc.) are similar to those of the thermospray technique. As the flow-rates in micro-HPLC are well suited to DLI, but less so to thermospray, the development of micro-HPLC techniques will probably benefit conventional DLI.

Continuous-flow FAB makes it possible to study high-molecular-mass compounds by LC–MS and is becoming frequently used. The thermospray technique, however, is showing improvements in application to heavier compounds. The moving belt provides an alternative for high masses; in fact it is a good all-round method, but, even though it is nearly 15 years old, probably owing to technical difficulties it is not really popular and is losing ground to other interfaces. The Magic interface, on the other hand, will probably compete successfully with thermospray for lower mass compounds of reasonable volatility if it proves to be a reliable, easy-to-use method. The possibility of selecting freely between EI and CI modes is a great advantage.

Finally, specific applications for other interfaces may appear. For example,

some atmospheric ionization [36,37] sources are compatible with high-resolution separation via capillary zone electrophoresis and may well become more popular in the future.

PHARMACEUTICAL APPLICATIONS

LC–MS has a particularly wide range of applications in pharmaceutical research, where the identification of thermally labile and hydrophilic compounds is routinely required [38]. The major areas of application may be summarized as follows:

(i) Detection of known compounds. A mass spectrometer operated in different ionization modes acts as a selective detector, increasing the resolution capabilities of LC; in fact, ion chromatograms are able to separate compounds in mixtures which were unresolved or partially resolved with conventional UV detectors.

(ii) Structure elucidation of unknown compounds in "difficult" mixtures (e.g., biological materials, plant extracts, fermentation broths).

(iii) Quantitative analysis. Owing to its selectivity and sensitivity, LC–MS reduces and sometimes eliminates interference problems; hence it is the most reliable technique for determining drugs and/or their metabolites in biological materials in pharmacokinetic, medical, toxicological or forensic studies. It can also be very useful in determining the content and nature of degradation products during accelerated stability studies on new drugs or during complicated extraction and purification processes.

(iv) A special application is the use of LC–MS as a means of introducing thermally very labile compounds into the mass spectrometer.

In our laboratories, the DLI interface has proved extremely useful for the determination of the molecular masses and structures of antibiotics of mass less than 1000 a.m.u. Negative ion (electron-capture) spectra of rifamycins, thermorubin, purpuromycin, kirromycins and polyenes have been obtained. The interface has been regularly used for the structure analysis of chemically modified antibiotics and in helping in the elucidation of novel structures in natural product mixtures with antibacterial activity.

Rifamycins have been studied by various MS techniques such as EI [39–43], field desorption [43,44], FAB and DLI [45]. The main problem in the study of rifamycins especially with EI mode is that these compounds readily decompose thermally prior to ionization. Depending on the design of the source of the mass spectrometer and the operating conditions, often no molecular ions are observed and sometimes structurally significant ions are also missing. Spectra obtained using a DLI interface and negative ionization show high sensitivity, abundant molecular ions and structurally significant fragments (Fig. 7). Many of the most abundant and structurally most significant fragments are shown to be formed by thermal decomposition. These are, perhaps not unexpectedly, difficult to reproduce quantitatively, being critically dependent on source temperature, contaminants in the ion source and the diaphragm and the quality of the liquid spray.

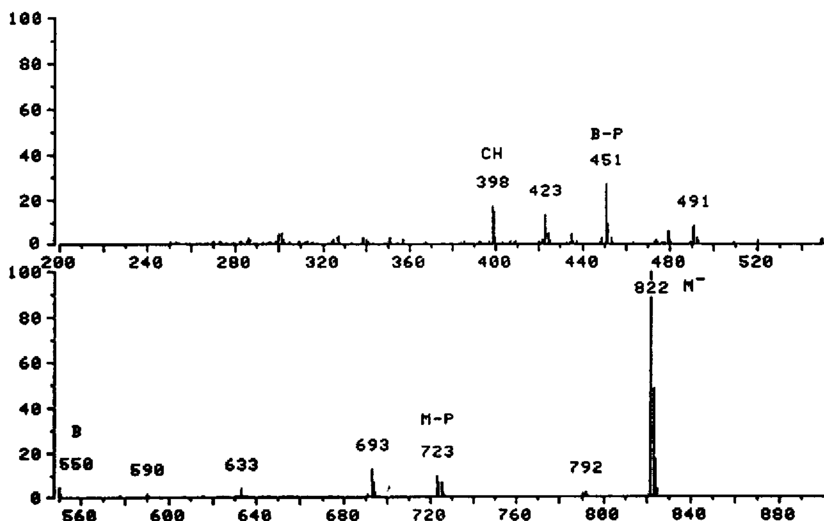

Fig. 7. Negative ion DLI LC-MS of rifampicin [45].

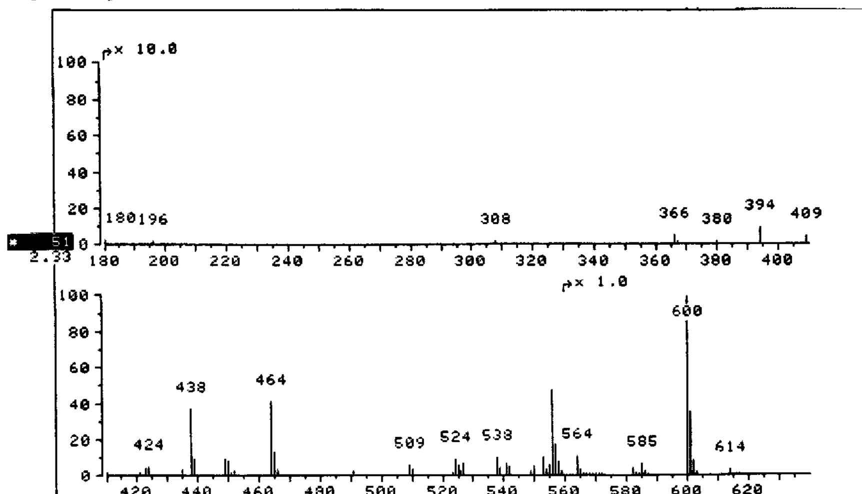

Fig. 8. Negative ion DLI LC-MS of thermorubin.

The solvents used in LC, however, do not affect significantly sensitivity, thermal decomposition or mass spectrometric fragmentation, making it easy to change the chromatographic conditions.

Thermorubin is a thermally labile antibiotic that can be used as a model compound to study the complication introduced in the mass spectra by thermal decomposition. In the EI mode, the molecular ion is weak and easily confused with other peaks. In particular an ion 14 a.m.u. higher in mass has been reported [46,47]. However the DLI negative ion spectrum shows an abundant molecular ion at m/z 600 (Fig. 8), with a weaker ion at m/z 614, and structurally significant fragmentation which will be discussed elsewhere [48].

DLI LC–MS has been applied to study three polyene antibiotics, filipin, amphotericin B and nystatin, and the results were compared with FAB data on the

same samples. The analysis of a 40% pure preparation of filipin complex gave novel information on the structure of the minor component filipin II [49]. The results of this study suggested a complementarity of DLI and FAB, and that combination of data from both techniques could provide a powerful method for the identification of novel polyene structures in submilligram quantities of partially purified isolates with antifungal activity.

REFERENCES

1 J.C. Holmes and F.A. Morell, Appl. Spectrosc., 11 (1957) 86.
2 V.L. Tal'rose, C.V. Karpov, I.G. Gordetskii and V.E. Skurat, J. Phys. Chem., 42 (1968) 1658.
3 C.R. Blakely and M.L. Vestal, Anal. Chem., 55 (1983) 750.
4 H.E. Barth, W.E. Barber, C.H. Lochmuller, R.E. Majors and F.E. Regnier, Anal. Chem., 60 (1988) 387R; and references cited therein.
5 R.P.W. Scott, C.G. Scott, M. Munroe and J. Hess, Jr., J. Chromatogr., 99 (1974) 395.
6 R.C. Willoughby and R.F. Browner, Anal. Chem., 56 (1984) 2626.
7 P.C. Winkler, D.D. Perkins, W.K. Williams and R.F. Browner, Anal. Chem., 60 (1980) 489.
8 W.M.A. Nissen, Chromatographia, 21 (1986) 277.
9 W.M.A. Nissen, Chromatographia, 21 (1986) 342.
10 R.B. Geerdink, F.A. Maris, G.J. de Jong, R.W. Frei and U.A.Th. Brinkman, J. Chromatogr., 394 (1987) 51.
11 P.J. Arpino, P. Krien, S. Vajta and G. Devant, J. Chromatogr., 203 (1981) 117.
12 M.L. Vestal, Science, 226 (1984) 275.
13 M. Dedieu, C. Juin, P.J. Arpino and G. Guiochon, Anal. Chem., 54 (1982) 2375.
14 H.M. Schiebel and H.R. Schulten, Mass Spectrom. Rev., 5 (1986) 249.
15 W.M.A. Niessen and H. Poppe, J. Chromatogr., 385 (1987) 1.
16 W.M.A. Niessen and H. Poppe, J. Chromatogr., 394 (1987) 21.
17 R.F. Browner, P.C. Winkler, D.D. Perkins and L.E. Abbey, Microchem. J., 34 (1986) 15.
18 Y. Ito, T. Takeuchi, D. Ishii and M. Goto, J. Chromatogr., 346 (1985) 161.
19 R.M. Caprioli, W.T. Moore and T. Fan, Rapid Commun. Mass Spectrom., 1 (1987) 15.
20 A.E. Ashcroft, J.R. Chapman and J.S. Cottrell, J. Chromatogr., 394 (1987) 15.
21 D.E. Games, S. Pleasance, E.D. Ramsey and M.A. McDowall, Biomed. Environ. Mass Spectrom., 15 (1988) 179.
22 P. Boulenguer, Y. Leroy, J.M. Alonso, J. Montreuil, G. Ricart, C. Colbert, D. Duquet, C. Dewaele and B. Fournet, Anal. Biochem., 168 (1988) 164.
23 D.H. Williams, C. Bradley, G. Bojesen, S. Santikarn and L.C.E. Taylor, J. Am. Chem. Soc., 103 (1981) 5700.
24 S. Naylor, A.F. Findeis, B.W. Gibson and D.H. Williams, J. Am. Chem. Soc., 108 (1986) 6359.
25 C.R. Blakey, J.J. Carmody and M.L. Vestal, Anal. Chem., 52 (1980) 1636.
26 C.R. Blakey, J.J. Carmody and M.L. Vestal, Clin. Chem., 26 (1980) 1467.
27 C.R. Blakey, J.J. Carmody and M.L. Vestal, J. Am. Chem. Soc., 102 (1980) 5931.
28 R.D. Voyksner, J.T. Bursey and E.D. Pelizzari, Anal. Chem., 56 (1984) 1507.
29 D.A. Garteiz and M.L. Vestal, LC Mag., 3 (1985) 334.
30 D. Zakett and G.J. Kallos, P.J. Savichas, poster presented at the 34th Annual Conference on Mass Spectrometry and Allied Topics, Cincinnati, 1986.
31 W.H. McFadden, D.A. Garteiz and E.G. Siegmund, J. Chromatogr., 394 (1987) 101.
32 C. Lindberg and J. Paulson, J. Chromatogr., 394 (1987) 117.
33 P.J. Rudewicz, S. Lewis, R.W.K. Chapmer and J.K. Wellby, in P. Longevialle, Proceedings of the 11th International Mass Spectrometry Conference, Bordeaux, Aug. 29–Sept. 2, 1988, Heyden & Son, 1989, in press.
34 D.S. Jones, T. Krolik and R.M. Bateman, in P. Longevialle, Proceedings of the 11th International Mass Spectrometry Conference, Bordeaux, Aug. 29–Sept. 2, 1988, Heyden & Son, 1989, in press.
35 L.M. Vestal and M.L. Vestal, in P. Longevialle, Proceedings of the 11th International Mass Spectrometry Conference, Bordeaux, Aug. 29–Sept. 2, 1988, Heyden & Son, 1989, in press.

36 B.A. Thomson and L. Danylewych-May, paper presented at the 32st Annual Conference on Mass Spectrometry and Allied Topics, Boston, 1983.

37 T.R. Covey, E.D Lee, A.P. Bruins and J.D. Henion, Anal. Chem., 58 (1986) 1451A.

38 K.H. Schellenberg, M. Linder, A. Groeppelin and F. Erni, J. Chromatogr., 394 (1987) 239.

39 L.F Zerilli and G.G. Gallo, EUR Report 4675, f.i.e., Publications Officielles des Communautés Européennes, Luxembourg, 1972, p. 217.

40 L.F. Zerilli, M. Landi, N. Rimorini and G.G. Gallo, Ann. Chim., 64 (1974) 199.

41 C.-H. Wang, K.-X. Cao, Y.-X Wang and J.-M. Cui, Kangshengsu, 8 (1983) 224.

42 A. Vigevani, M. Bellasio, B. Gioia, L. Marsili, S. Vioglio and G. Franceschi, J. Antibiot., 38 (1985) 1799.

43 A. Vigevani, E. Arlandini, M. Ballabio, G. Franceschi, B. Gioia and S. Vioglio, Farmaco, 40 (1985) 131.

44 L.F. Zerilli, M. Landi, G.G. Gallo, K.H. Maurer and U. Rapp, Biomed. Mass Spectrom., 2 (1975) 307.

45 K. Vekey, D. Edwards and L.F. Zerilli, J. Chromatogr., in press.

46 F. Johnson, B. Chandra, C.R. Iden, P. Naiksatam, R. Kahen, Y. Okaya and S. Lin, J. Am. Chem. Soc., 102 (1980) 5580.

47 M. Turconi, A. De Paoli, P. Ferrari, M. Landi, G. Gallo and B. Cavalleri, Tetrahedron, 42 (1986) 727.

48 D. Edwards and K. Vekey, in preparation.

49 D. Edwards, J. Antibiot., 42 (1989) in press.