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LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY INTERFACING ON A MAGNETIC SECTOR MASS SPECTROMETER

TECHNIQUES AND APPLICATIONS

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SUMMARY

The adaptation of thermospray ionization as a basis for liquid chromatography–mass spectrometry interfacing on a magnetic sector mass spectrometer is described. Typical data are presented together with a discussion of the effect of operating conditions on the quality of these data.

INTRODUCTION

The thermospray process[1] offers a means of vapourizing high flow-rates of solvents and of ionizing samples while these are in solution. It is therefore an important practical method for interfacing a liquid chromatograph and a mass spectrometer. In particular, thermospray ionization can provide molecular ions from polar, higher mass compounds which are not amenable to analysis using more conventional ionization techniques[2]. There is, therefore, a considerable incentive for the use of the thermospray technique with modern magnetic sector mass spectrometers which provide high sensitivity at high mass.

An overall schematic of a thermospray system is shown in Fig. 1. Liquid flow

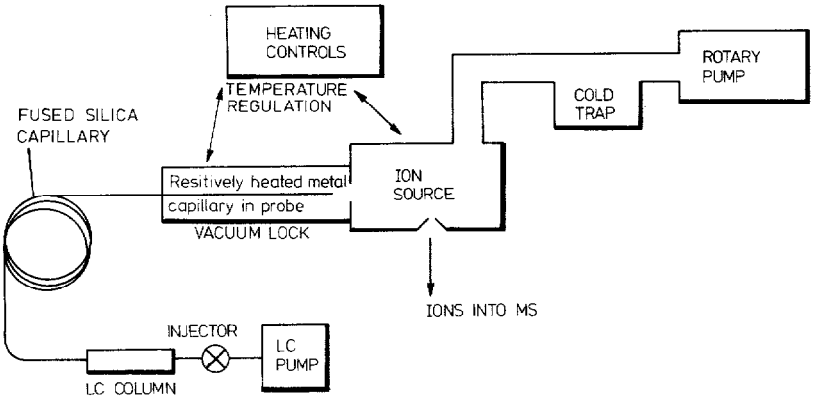

Fig. 1. Schematic diagram of thermospray system.

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from the liquid chromatograph is rapidly vapourized by spraying from a narrow bore, resistively heated, stainless steel capillary. Under optimum conditions, a high proportion of the liquid flow is converted into vapour as it passes through the heated capillary. This vapour then acts as nebulizer gas to convert the remainder of the liquid stream, which still contains the sample in solution, into a directed jet of small droplets which emerges from the end of the capillary. The size of these droplets is further reduced by evaporation during their passage into the ion source itself.

In the presence of an added electrolyte, such as ammonium acetate, although the solution is overall electrically neutral, the statistical distribution of charge ensures that each droplet carries a slight excess positive or negative charge. The field due to this excess charge is then sufficient to cause the evaporation of sample ions from very small droplets[3]. Thus sample ions may be formed from labile molecules by a mild, in-solution, process in the absence of an energetic, external ionization source.

The other major feature of any thermospray system is a rough pumping line attached directly to the ion source to remove vapourized solvent. In this way the load on the source-housing pumping system is greatly reduced and comprises only a much smaller flow of vapour that leaks out from the ion source itself. Thus, the addition of this pumping line permits the direct sampling of aqueous flows in excess of 1 $cm^3\ min^{-1}$. In effect the whole flow from a standard 4.6 mm I.D. liquid chromatograph column may be introduced into the mass spectrometer.

If thermospray ions are to be analysed using a magnetic sector instrument then, since these ions are formed during the spraying process, it is necessary to maintain the capillary from which the droplets originate at the same high potential as the ion source itself. The resistively heated capillary is therefore mounted in a probe which can be introduced through the standard vacuum lock so that the capillary enters and achieves the same potential as the source block. The other end of the capillary is connected to the column by a length of narrow bore fused-silica capillary to provide a maximum leakage current of *ca.* 100 μA through 0.1 *M* aqueous ammonium acetate with a source potential of 4 kV.

The provision of a cold trap in the line used to pump the ion source ensures that condensible vapours do not reach the rotary pump and that the pressure in this line is reduced to a value that is too low to sustain any measurable electrical leakage.

Further evaporation of the droplets following the initial spraying process is accomplished by means of heaters embedded in the source block which transfer heat to the droplets in flight between the capillary and the ion extraction region. This heat imput is controlled by a thermocouple which senses the spray temperature after ion extraction.

The spectrum of riboflavin (Fig. 2) is typical of data obtained by thermospray ionization, *i.e.* in the presence of ammonium acetate and without the use of a filament as an external ionization source. For the analysis of compounds of low proton affinity, or in cases where the necessary electrolyte concentration cannot be maintained in the solvent used for elution, a filament may be used. In this case the filament provides reagent gas ions from the solvent and the sample is then ionized by ion-molecule processes. The spectrum of chloramphenicol (Fig. 3) is typical of data obtained in the filament-on mode.

With the present instrumental configuration, the filament is made from thoriated iridium wire and the electrons are accelerated through a maximum potential of

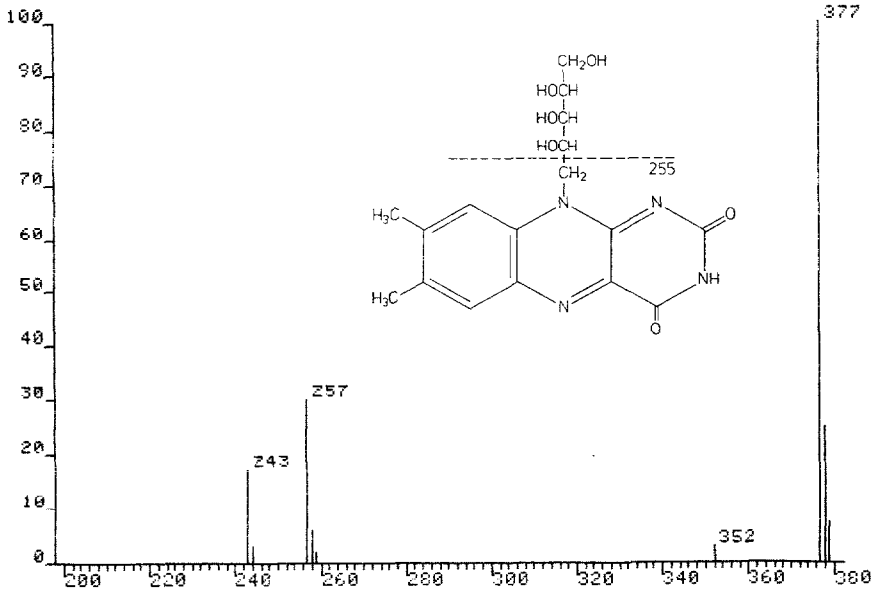


Fig. 2. Thermospray ionization of riboflavin (mol.wt. 376) introduced in 0.1 *M* aqueous ammonium acetate solution.

500 V. The sensitivity in the filament-on mode is somewhat less than that obtained by thermospray ionization unless the flow-rate is lowered. Thus, filament-on operation is more compatible with the direct introduction of the whole flow from a 2 mm

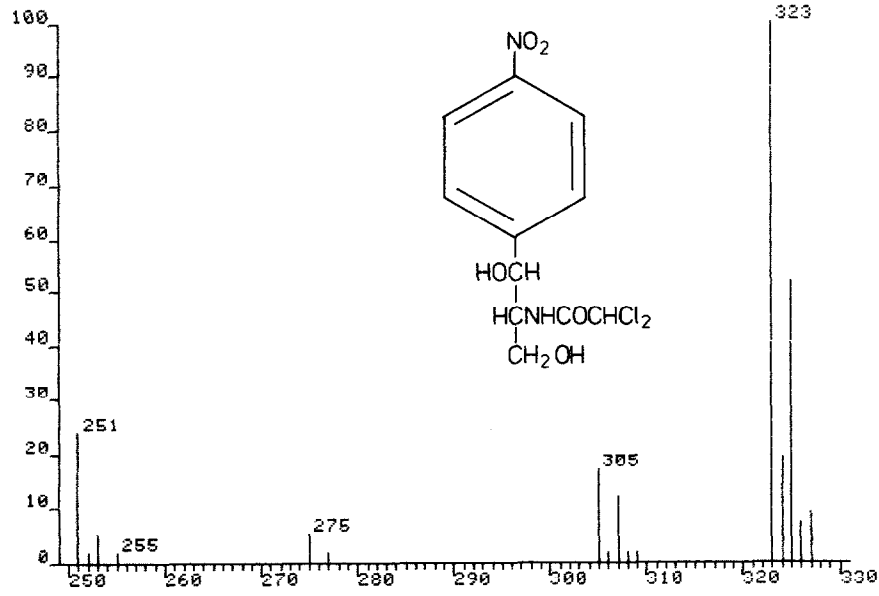


Fig. 3. Filament-on ionization of chloramphenicol (mol.wt. 322) introduced in aqueous methanol.

I.D. column into the mass spectrometer whereas sensitivity using thermospray ionization optimises at a flow-rate of *ca.* 1 cm^3 min^{-1}.

Adjustment of the source block temperature affects both the degree of fragmentation and chromatographic integrity. Fig. 4 shows mass chromatograms extracted from repetitive scan data recorded during the elution of adenosine. With a

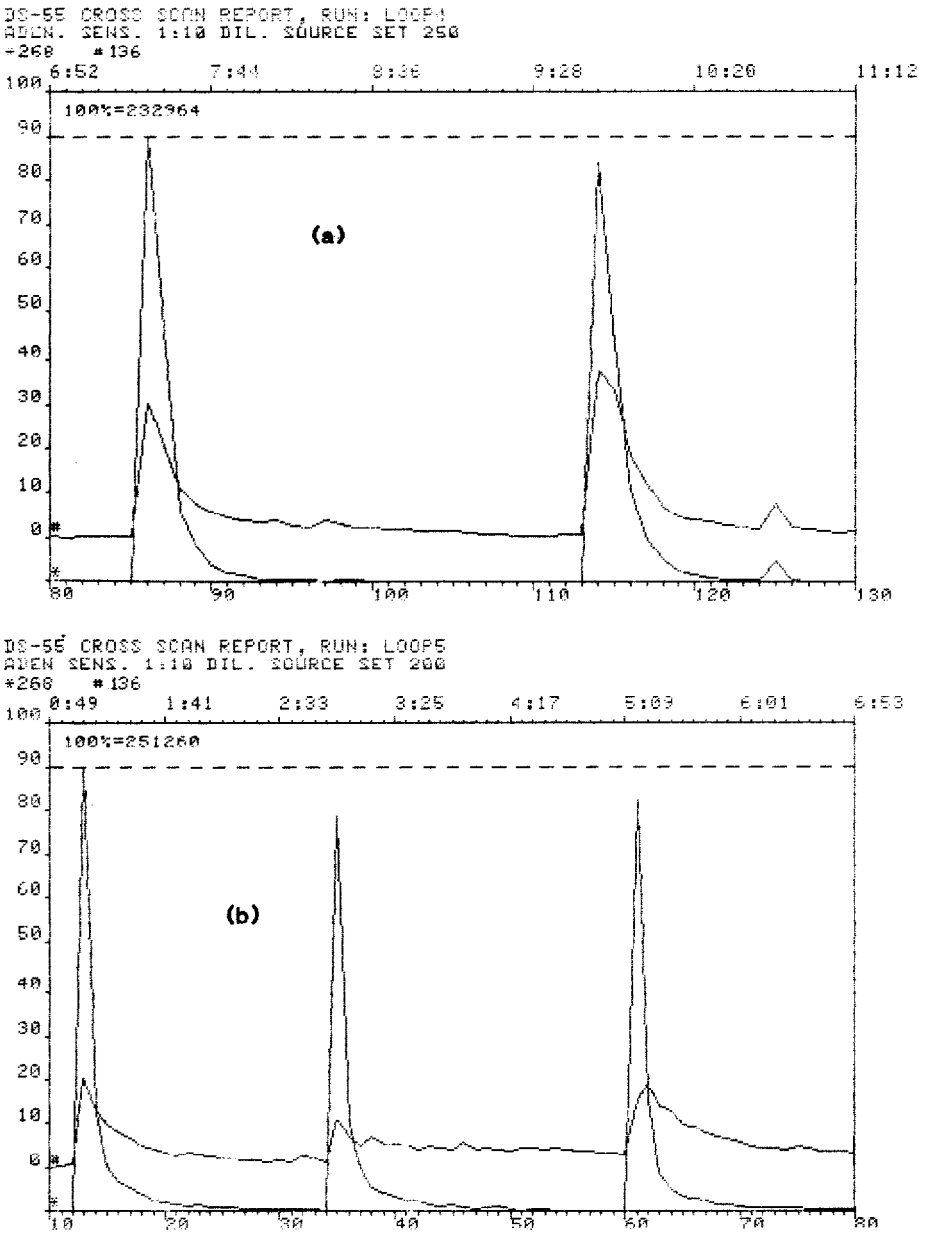


Fig. 4.

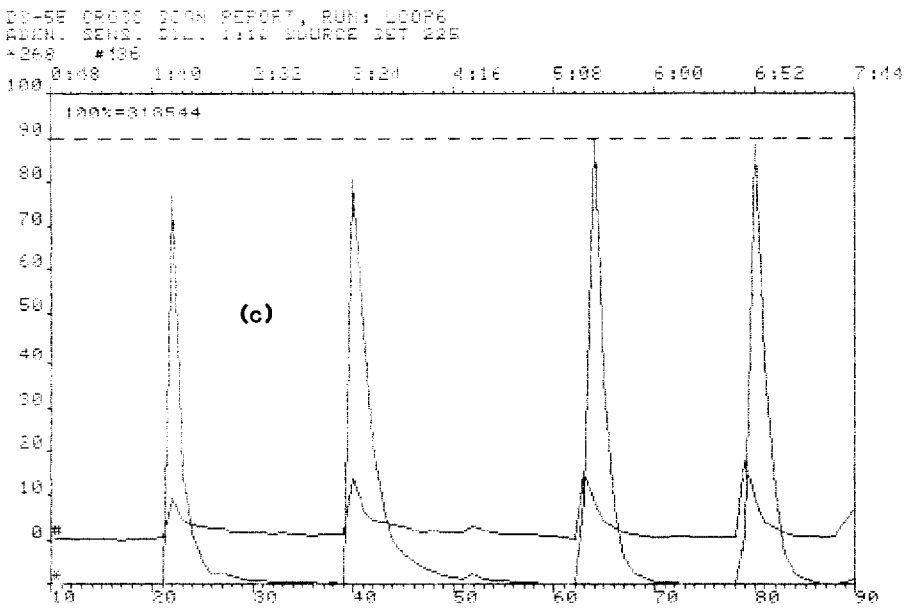


Fig. 4. Mass chromatograms from successive injections of adenosine in 0.1 *M* aqueous ammonium acetate solution. Thermospray ionization. Spray temperatures: (a) 250°C; (b) 200°C; (c) 225°C.

spray temperature following ion extraction of 250°C (Fig. 4a) the mass chromatograms show reasonable time profiles but the fragment ion at *m/z* 136 is relatively intense, being 30–40% compared with the quasimolecular ion (*m/z* 268). With a temperature of 200°C (Fig. 4b) the fragment ion intensity is relatively less but the profiles show an appreciable tail and the background begins to build up following a number of injections. A temperature of 225°C (Fig. 4c) provides an excellent compromise, and under these conditions a spectrum of adenosine such as that shown in Fig. 5 can be obtained.

The intensity of the quasimolecular ion for adenosine and other compounds shows a strong dependence on capillary temperature under thermospray (filament-off) conditions. For example, if the capillary is overheated and the spray dries out, the ion intensity decreases markedly. A similar result ensues if the droplets are too large because insufficient heat is applied to the capillary. The optimum capillary temperature is most obviously a function of solvent composition. It is interesting to note that the *m/z* 136 fragment ion in the spectrum of adenosine does not show the same dependence on capillary temperature, indicating that it is probably formed by some mechanism other than thermospray ionization.

Under optimum source and capillary temperature conditions it is possible to record useful thermospray ionization spectra for a large number of highly labile samples (Figs. 6–8). The spectrum of the less basic cephalexin shows an $(M+NH_4)^+$ ion as well as an $(M+H)^+$ ion (Fig. 6).

Fig. 9 compares the UV detector and mass spectrometer total ion current traces from the separation of a number of basic drugs on a reversed-phase ODS column. An important practical point is that thermospray ionization is still a suitable ioni-

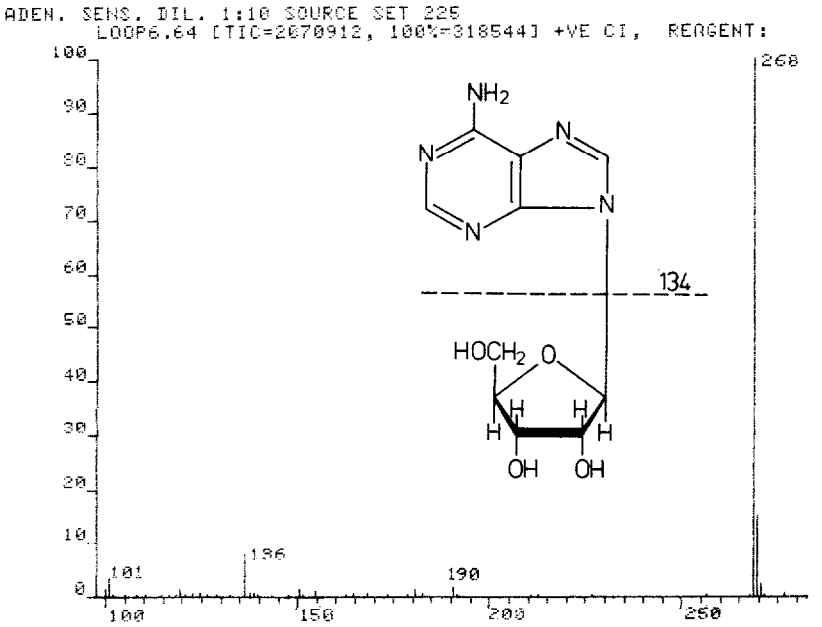


Fig. 5. Thermospray ionization of adenosine (mol.wt. 267) introduced in 0.1 *M* aqueous ammonium acetate solution. Spray temperature 225°C.

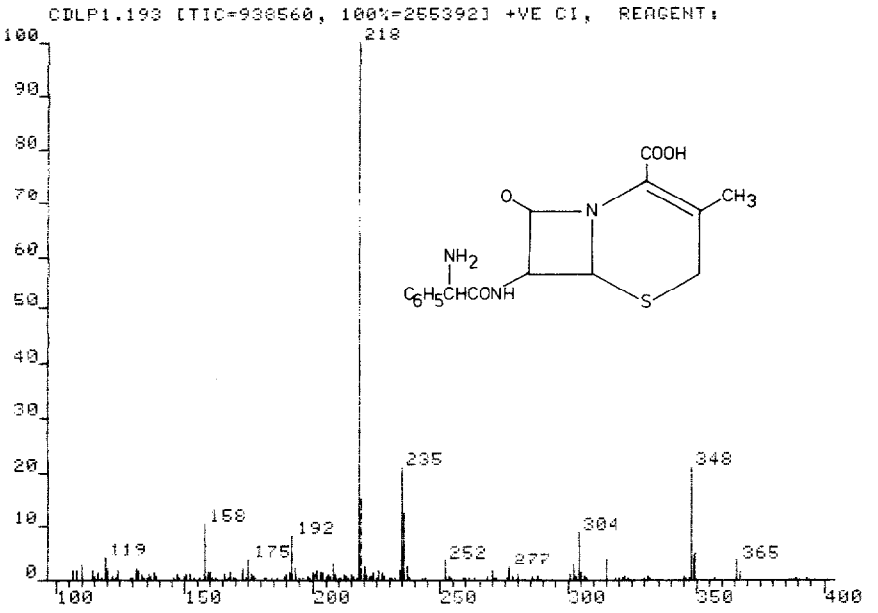


Fig. 6. Thermospray ionization of cephalexin (mol.wt. 347) introduced in 0.1 *M* aqueous ammonium acetate solution.

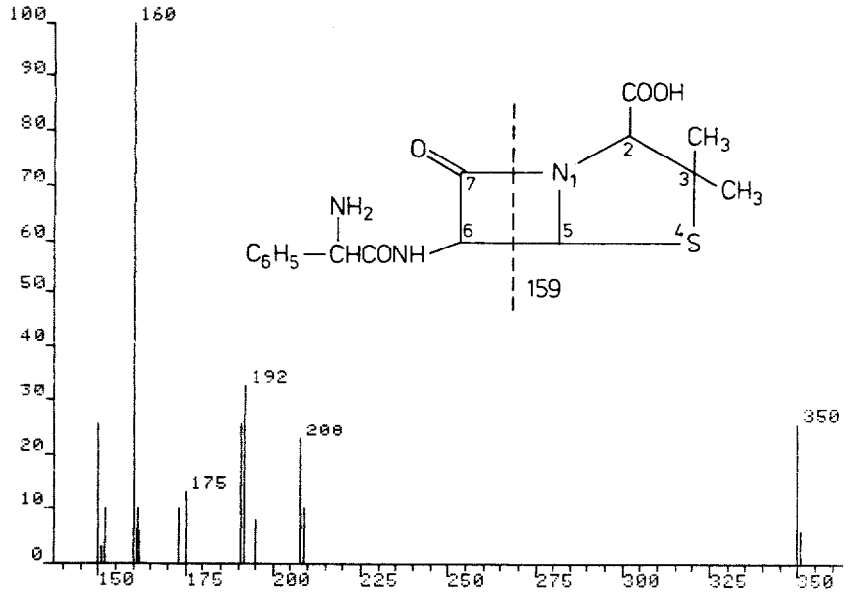


Fig. 7. Thermospray ionization of ampicillin (mol.wt. 349) introduced in 0.1 *M* aqueous ammonium acetate solution.

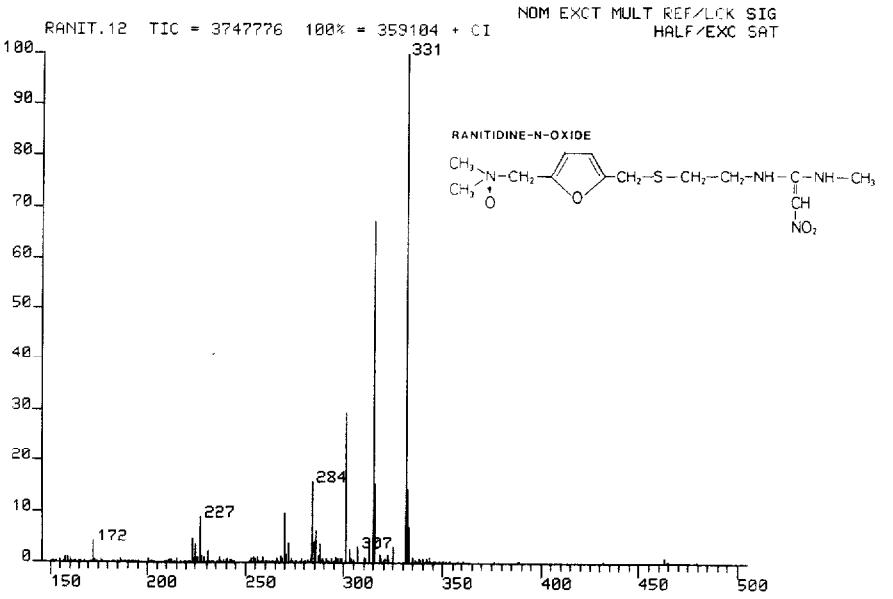
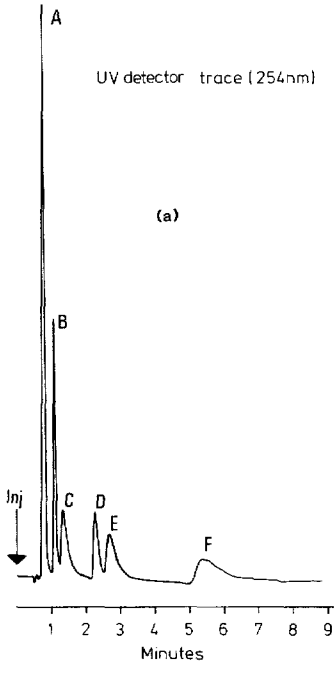


Fig. 8. Thermospray ionization of ranitidine-N-oxide (mol.wt. 330) introduced in a mixture of aqueous ammonium acetate (80%) and acetonitrile (20%).



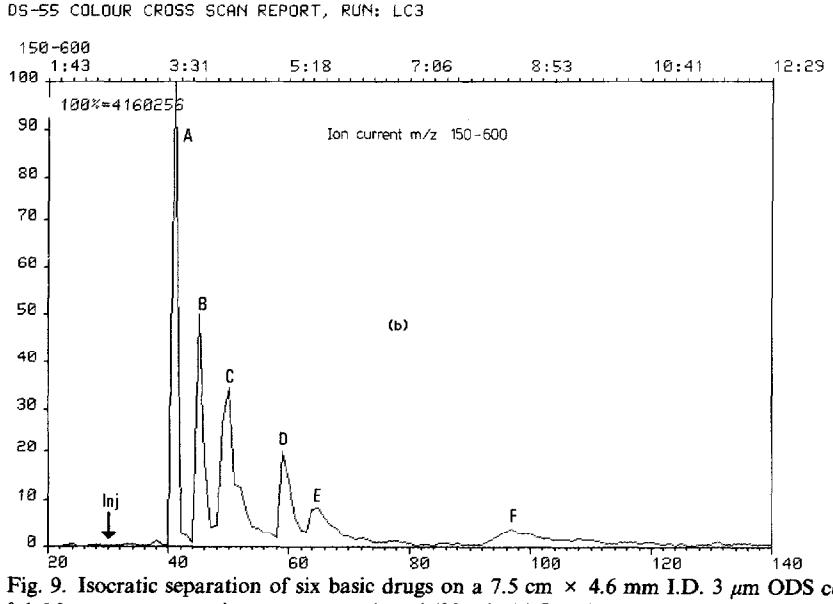


Fig. 9. Isocratic separation of six basic drugs on a 7.5 cm × 4.6 mm I.D. 3 μm ODS column. Solvent, 0.1 *M* aqueous ammonium acetate–methanol (20:80). (a) UV detector trace. (b) MS detector trace: thermospray ionization.

zation technique even when a high percentage of the mobile phase is organic, as in this case. Thus, amitriptyline, which is the last component eluted under these conditions, still provides a very intense spectrum (Fig. 10).

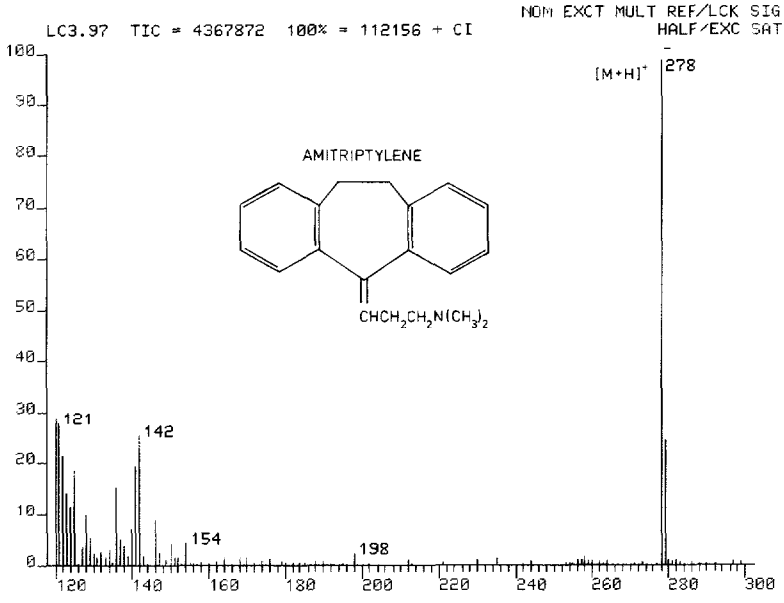


Fig. 10. Thermospray ionization spectrum of amitriptyline (mol.wt. 277) (peak F from the trace shown in Fig. 9b).

The thermospray process has proved to be an excellent basis for the interfacing of liquid chromatography and mass spectrometry. Whilst it is not a suitable method for the analysis of very non-polar compounds such as hydrocarbons, it has been applied successfully to the analysis of a number of other compound types in addition to those described here, *e.g.* drugs and their metabolites, saccharides, prostaglandins and organometallics. Higher molecular weight, underivatized peptides have also been investigated and shown to produce fragment ions suitable for sequencing studies as well as molecular weight information[4].

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