Journal of Chromatography, 323 (1985) 163–170
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17,336

APPLICATIONS OF THERMOSPRAY IONISATION LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY TO COMPOUNDS OF PHARMACEUTICAL INTEREST

D. A. CATLOW
Imperial Chemical Industries PLC, Pharmaceutical Department, Pharmaceuticals Division, Hurdsfield Industrial Estate, Macclesfield, Cheshire SK10 2NA (U.K.)

SUMMARY

The use of a thermospray ionisation liquid chromatography–mass spectrometry interface designed for use on a magnetic sector instrument is described. Two examples of its on-line application to pharmaceutical compounds are described, and some of the problems encountered with the technique are discussed. Some possible solutions to these problems are considered.

INTRODUCTION

In the eleven years since reports of on-line liquid chromatography–mass spectrometry (LC–MS) first appeared[1], many different approaches to this inherently-difficult interfacing problem have been tried[2–4], each showing early promise but few really maturing into a routine analytical tool. The latest in this long line of pretenders to the LC–MS throne is thermospray ionisation (TSP), introduced by Blakley and Vestal in 1980[5], subsequently improved upon by them[6] and now available commercially from several of the major manufacturers. One such commercial device has been designed to overcome the problems attendant upon operation with a magnetic sector instrument, and was used for the work described in this paper.

The examples discussed represented "real" problems, and utilised the thermospray interface as part of an on-line LC–MS system. During the course of these preliminary investigations into the thermospray LC–MS behaviour of pharmaceutical compounds several problems were encountered which required solving before TSP could be used as a routine analytical tool. These problems and some possible solutions to them are discussed below.

EXPERIMENTAL

The TSP interface was manufactured by Kratos Analytical Instruments, Manchester, U.K. and was connected to a Kratos MS 25 mass spectrometer. The interface has been described elsewhere[7]. Thermocouples positioned at the tip of the capillary and in the vapour stream after the sampling orifice to the ion optics provided read-

0021-9673/85/$03.30

outs of the vaporiser temperature and jet temperature respectively. The vaporiser temperature was optimised for each compound but was typically 250°C.

The mass spectrometer was scanned from 600 to 30 daltons with a cycle time of 5 sec, under the control of a Kratos DS 55 data system. An accelerating voltage of 2 kV was used.

High-performance liquid chromatography (HPLC) utilised a Kratos pump for the on-line work, with a Gilson 302 pump and "Holochrome" variable-wavelength UV detector for off-line work.

RESULTS AND DISCUSSION

During the development of any drug it is frequently necessary to identify the impurities in that drug formed during the synthesis, or the decomposition products formed as a result of, *e.g.* stability testing. Until recently, gas chromatography (GC)–MS was the method of choice for this work, but the increasing complexity of modern pharmaceutical compounds has led to a widespread use of HPLC as the chromatographic technique of choice. This technique is capable of detecting very low levels of impurities, helping to maintain the extremely high purity of many pharmaceutical compounds. It therefore becomes necessary to follow the synthetic route back one or more stages to find related impurities at a level high enough for combined LC–MS. The first example fell into this category and concerned the drug Nolvadex.

A crude sample of the drug was examined by LC and LC–MS using a reversed-phase column with acetonitrile–tetrahydrofuran (THF)–0.1 *M* ammonium acetate solution (300:75:125) as the eluent phase at a flow-rate of 1 ml min^{-1}. (As discussed later, the use of this phase caused problems which were attributed to the

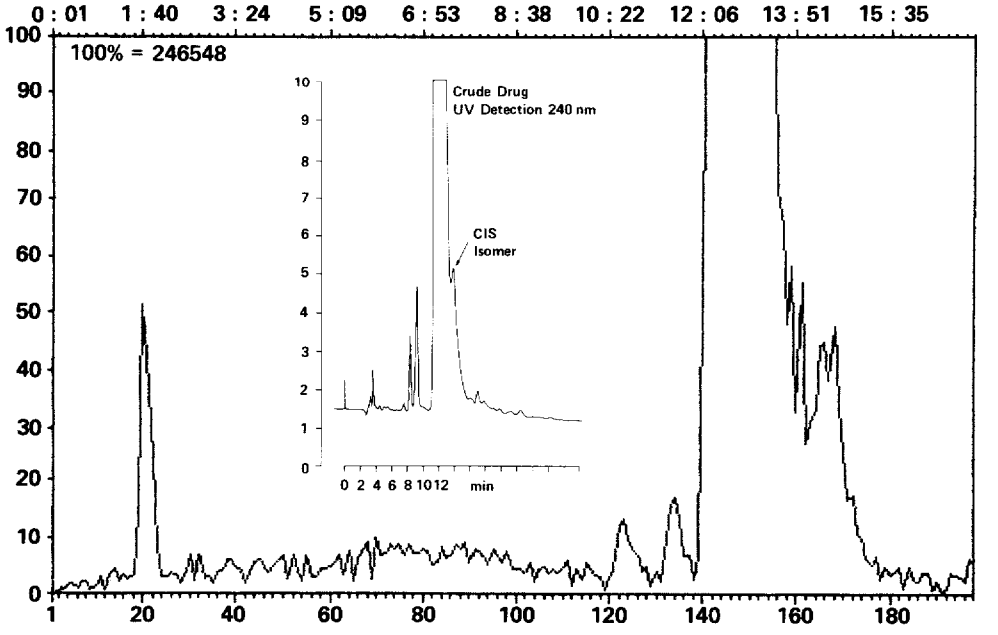

Fig. 1. Crude drug by thermospray LC–MS. Inset: UV chromatogram at 240 nm.

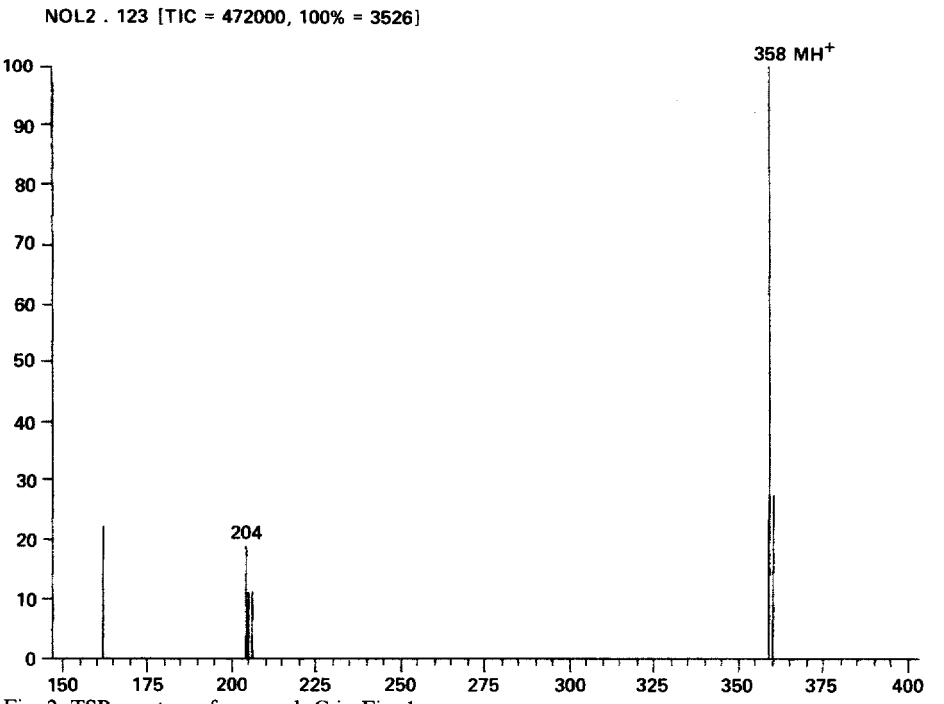

Fig. 2. TSP spectrum from peak C in Fig. 1.

presence of THF.) The UV chromatogram (240 nm) and the reconstructed ion chromatogram (RIC) are shown in Fig. 1. The correspondence between the two chromatograms is immediately apparent, and it was possible to obtain mass spectra from these relatively low level (< 1%) impurities, from which relative molecular mass information could be derived. peaks B and C for example, both appeared to be homologues of the parent compound, probably *cis* and *trans* isomers. Peak D is the *cis* isomer of the main component, confirmed by synthesis and subsequent retention time measurements.

The spectrum shown in Fig. 2 from peak C is typical of the type of spectra obtained from these impurities. Fig. 3 shows the spectrum of the major component. In neither case is there much fragmentation; one advantage of a sector instrument in this respect is the possibility of performing collision-induced dissociation (CID) experiments as an aid to structural elucidation, although this course has yet to be investigated.

Peak A in Fig. 1 is interesting in that examination of the spectra indicated the presence of at least three components (see Fig. 4); this is confirmed by a closer examination of the UV chromatogram. The apparent loss in resolution in the RIC trace is thought to be due largely to the slow scan speed of the mass spectrometer. It does however, show the utility of an MS detector for method development, as the presence of multiple components can be demonstrated and the LC eluent composition altered to effect a better separation.

In the second example, what initially appeared to be a relatively simple case of identifying a single related impurity in a steroid sample produced some unexpected

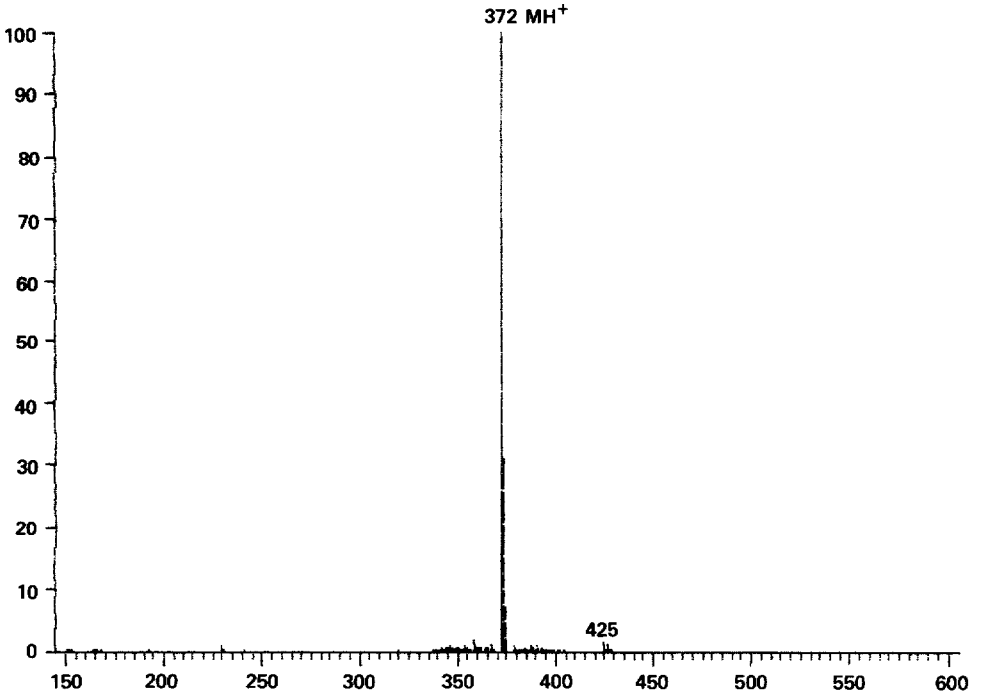

Fig. 3. TSP spectrum of the drug Nolvadex.

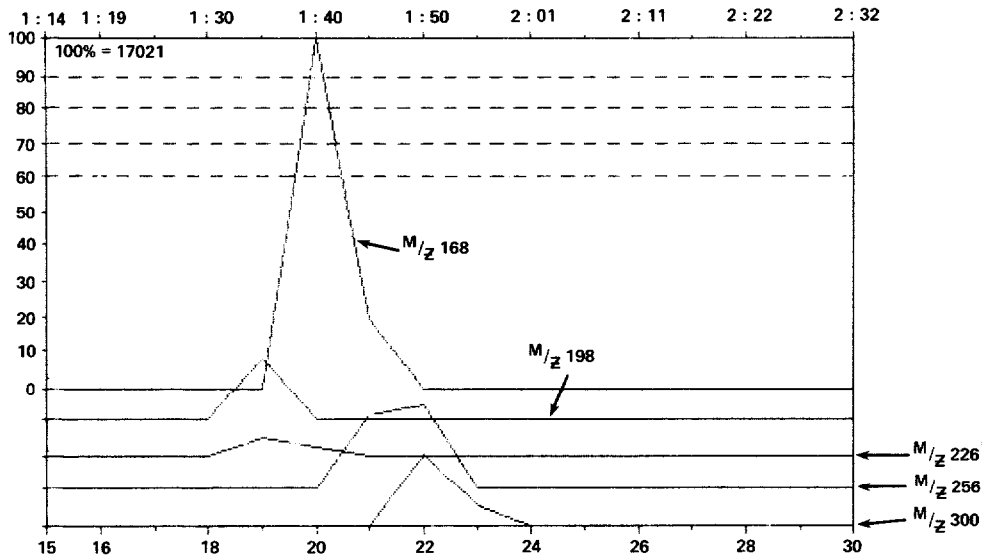

Fig. 4. Mass chromatograms showing the presence of multiple components in peak A from Fig. 1.

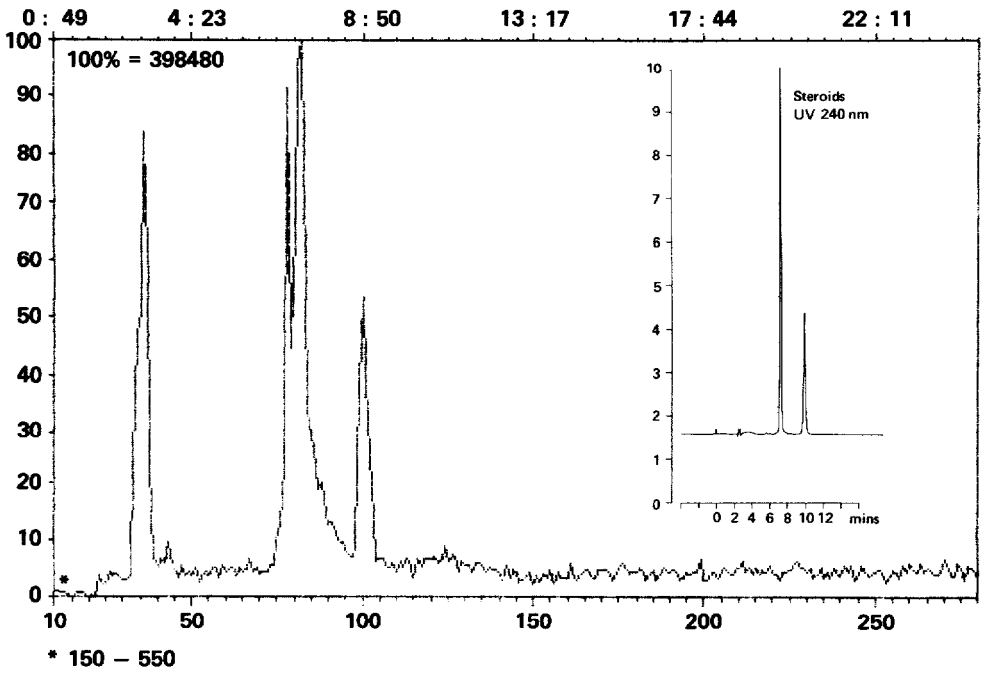

Fig. 5. Steroid mixture by thermospray LC–MS. Inset: UV chromatogram at 240 nm.

results. The UV and MS chromatograms from the sample on the same reversed-phase column used above, with acetonitrile–0.1 *M* ammonium acetate (45:35) as eluent at 1 ml min^{-1}, are shown in Fig. 5, and it can be seen from this that there are two extra peaks in the RIC chromatogram. Neither component could be identified from the extremely weak spectra obtained, but they are obviously non-UV absorbing species, possibly hydrocarbons. Some peak broadening is apparent in the RIC chromatogram, and it is thought that the TSP conditions in this experiment deviated considerably from the optimum. A TSP spectrum of the minor steroid component, retention time 10 min, is shown in Fig. 6.

During the course of this work, several problem areas were identified, each of which would affect the routine operation of the TSP interface. These are listed and discussed below.

(i) The addition of ammonium acetate to the eluant phase can considerably alter the chromatography when compared to the same system in the absence of the buffer. In Fig. 7 for example, the effect of the addition of ammonium acetate to the eluant can clearly be seen, and, whilst it is still possible to reproduce chromatograms using the mass spectrometer as a detector, it does become very difficult to correlate this data with the original non-buffered chromatography.

The post column addition of ammonium acetate using a zero dead volume T-piece, or the simple arrangement shown in Fig. 8, should alleviate this problem.

(ii) Trace analysis, such as that in the first example, requires the injection of relatively large amounts of the major component, typically > 50 μg in order to detect impurities at the 0.1–0.2% level (equivalent to 50–100 ng of each impurity). Whilst the amount of material is within the loading capability of the HPLC column, it

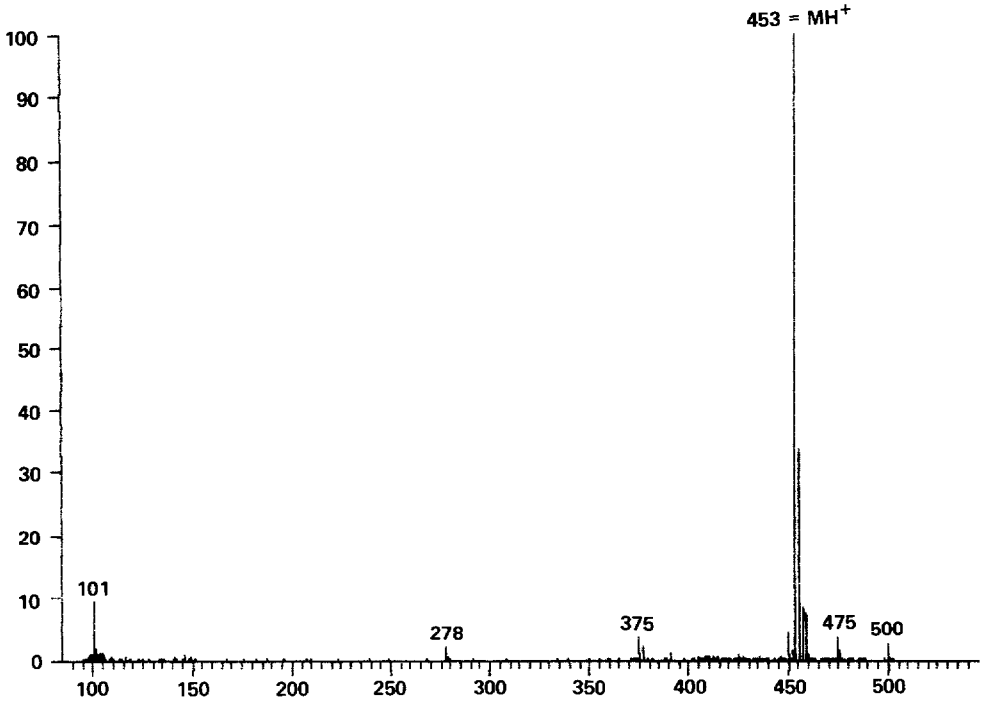

Fig. 6. TSP spectrum of peak of retention time 10 min (UV) in Fig. 5.

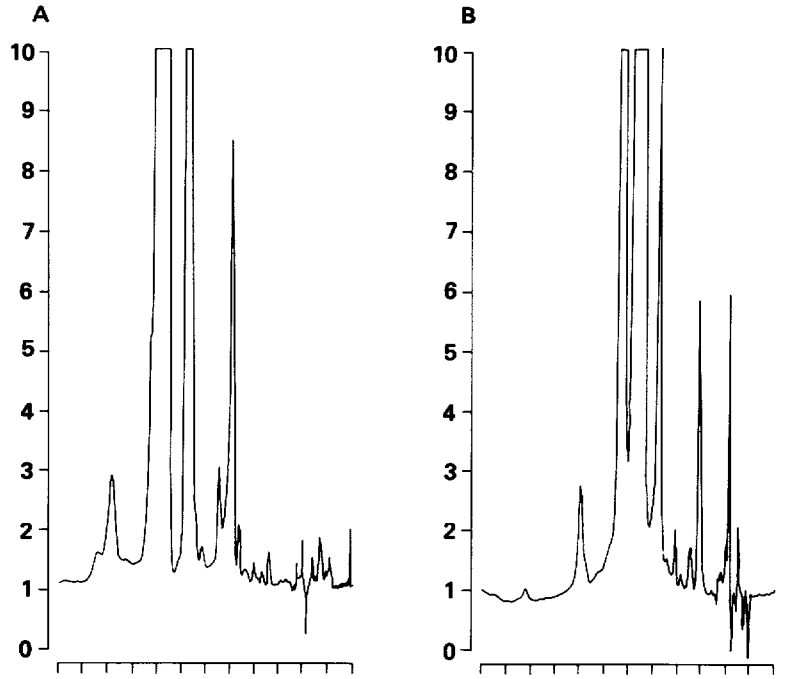

Fig. 7. Effect of adding 0.1 *M* ammonium acetate to the eluent phase on the chromatography of a drug synthesis mixture. Mobile phase: (A) acetonitrile–water (45:55); (B) acetonitrile–0.1 *M* ammonium acetate (45:55).

appears that it can cause problems of precipitation within the TSP capillary, resulting in blockages. The arrangement in Fig. 8 could easily be envisaged to effectively "cut" the major component from the interface, to help prolong the operational life of each capillary, or to provide a quick way of introducing tuning solutions into the flowing eluent using a second injection valve.

(iii) As discussed earlier, the use of an eluent phase containing THF resulted in blockage of the TSP capillary after only 2–3 h of operation. A viscous red liquid could be seen emanating from the end of the capillary. This did not happen when the same column–phase combination, but without THF, was used, and it was tentatively assumed that polymerisation of the THF component was occurring within the capillary. It is possible, although no evidence is to date available, that other

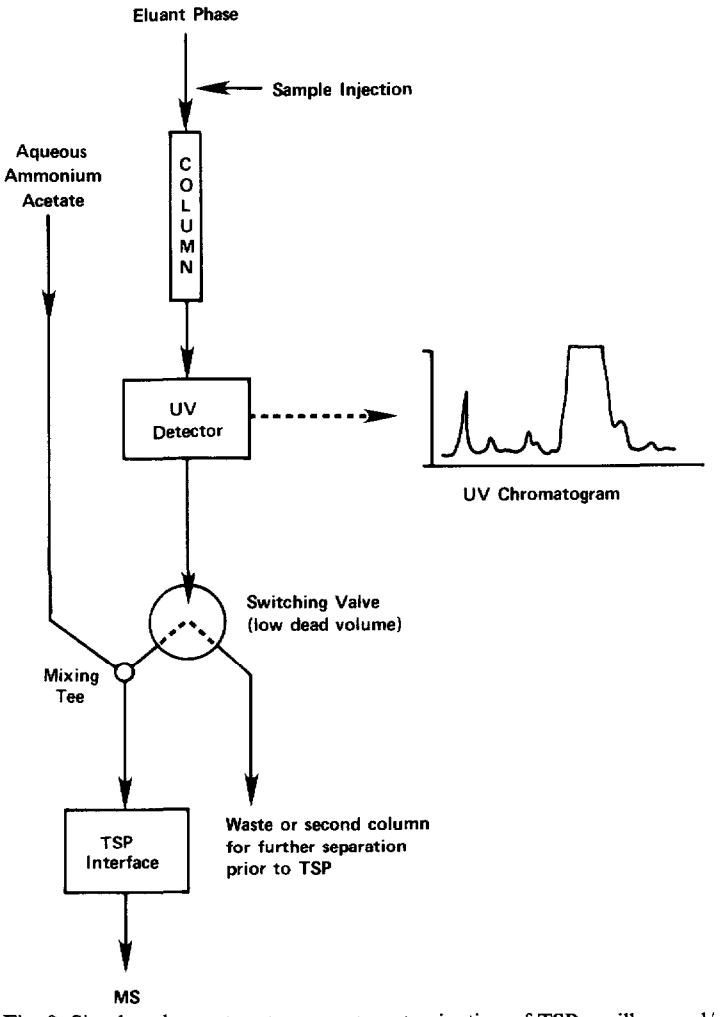

Fig. 8. Simple valve system to prevent contamination of TSP capillary and/or retain original chromatography.

similar solvent additives, such as dioxan, may behave similarly. Some restrictions on the choice of phase would seem to be inevitable.

Blockages also occurred when a system containing 13% (v/v) of an 8% solution of orthophosphoric acid in deionised water was used, although here the blockage occurred in minutes rather than hours and was assumed to be due to the formation of involatile polyphosphoric acid.

The use of 2 mm bore columns, with much lower flow-rates, together with post-column addition of buffer solution, using the arrangement in Fig. 8, may help to overcome this problem to some extent by reducing the level of these components in the eluant stream. However, the attendant dilution of components may make trace analysis impossible.

(iv) It was found necessary to optimise the vaporiser temperature for each compound examined. Recent work[8] indicates that the sensitivity of the TSP process falls with increasing organic content of the eluant phase, at constant vaporiser temperature, suggesting that tuning solutions should be made in the eluent phase, not just ammonium acetate, for optimum results.

CONCLUSIONS

The use of a thermospray LC–MS interface on a magnetic sector instrument operating at 2 kV appears to present no additional problems to those found with other commercial TSP devices. Chromatographic integrity can be maintained, although some peak broadening was observed, the source of which has not yet been identified. Routine operation with on-line LC–MS presents several problems, the solutions to which require a careful appraisal of the entire chromatographic process employed, but with careful operation it should be possible to obtain useful results on a wide variety of pharmaceutical products.

ACKNOWLEDGEMENTS

The author would like to thank Dr. J. Chapman and Dr. J. Pratt, of Kratos Analytical Instruments Ltd., for their expert technical assistance and helpful discussions, and also Imperial Chemical Industries PLC, Pharmaceutical Division, for permission to publish this work.

REFERENCES

1 M. A. Baldwin and F. W. McLafferty, *Org. Mass Spectrom.*, 7 (1973) 1111.
2 N. J. Alcock, C. Eckers, D. E. Games, M. P. L. Games, M. S. Lant, M. A. McDowall, M. Rossiter, R. W. Smith, S. A. Westwood and H.-Y. Wong, *J. Chromatogr.*, 251 (1983) 165–174.
3 N. M. M. Nibbering, *J. Chromatogr.*, 251 (1983) 93–104.
4 P. J. Arpino and G. Guiochon, *Anal. Chem.*, 51 (1979) 682A.
5 C. R. Blakley, J. J. Carmody and M. L. Vestal, *J. Amer. Chem. Soc.*, 102 (1980) 5931–5933.
6 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750–754.
7 J. R. Chapman, S. Fayyid and S. Evans, *32 Ann. Conf. on MS and Allied Topics, San Antonio, TX, 1983*, p. 106.