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INTERFACE FOR ON-LINE LIQUID CHROMATOGRAPHY–MASS SPECTROSCOPY ANALYSIS

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SUMMARY

An interface is described that permits the on-line operation of a liquid chromatograph and quadrupole mass spectrometer combination. The interface consists of two differentially pumped chambers through which a wire train system continually takes a sample of the column eluent, evaporates the solvent, and subsequently vaporizes the residual solute in the ionizing chamber of the mass spectrometer. The feasibility of the system is confirmed and its use as an alternative method for probe injection is demonstrated. Examples of its use with a liquid chromatograph in the separation of a fermentation extract are given and the results are compared with those obtained using the instrument as a probe sampling system to provide spectra from fractions collected from the simple chromatographic separation of the same mixture. The sensitivity of the present system is *ca.* 10^{-6}–10^{-5} g/ml of solute, which is only just adequate for liquid chromatography separations if the full separation potential of the column is to be realized. The quadrupole mass spectrometer used in this work was aged and had poor resolution. It is estimated that a modern quadrupole with improved resolution fitted with suitable interfaces would provide sensitivities of 10^{-7}–10^{-6} g/ml, which would cope with the majority of separation problems where 1 to 2 mg of a complex sample were available.

INTRODUCTION

As a result of the rapid developments that have taken place in the field of liquid chromatography (LC), the improved separations have provided complex chromatograms of multicomponent mixtures where, in many cases, the identities of the individual solutes are unknown. In fact, the progress of LC is following that of gas chromatography (GC), where having separated the individual components of the mixture the question remained as to their identity. In GC this was solved by coupling the gas chromatograph to the mass spectrometer or the infrared spectrometer. It follows that the need to couple a liquid chromatograph with a suitable spectroscopic system is now becoming necessary and although this has already been achieved with the UV spectrometer, the spectra have limited value in determining molecular structure. The ideal combination which should provide both structural information and high sensitivity is the liquid chromatograph–mass spectrometer (LC–MS).

To date mass spectra of column eluents have been obtained by trapping the solute peaks, concentrating the solutions and placing the solutes in the mass spectrometer by a probe sampling technique. This procedure is time consuming particularly if a large number of fractions are collected as the probe sampling technique is a 10- to 20-min operation for each fraction. A more efficient approach would seem to be to link the liquid chromatograph directly to the mass spectrometer.

There are two basic procedures by which the mass spectrometer could be linked to the liquid chromatograph. The eluent from the liquid chromatograph or a portion thereof could be fed directly into the mass spectrometer and employing the chemical ionization procedure, utilizing the solvent employed in the LC development as the chemical ionization agent, chemical ionization spectra would be obtained.

This method can probably provide the highest sensitivity but imposes restrictions on the solvent that can be used for development in the LC system. Further, as gradient elution is often essential to provide the necessary range of separation, the composition of the column eluent and thus the chemical ionization agent will continually change during the separation and confuse the spectra obtained. Such a system would also inhibit the use of the more conventional electron impact ionization, where, in general, more useful information is provided for structural determination.

The second approach to coupling a liquid chromatograph to a mass spectrometer is the use of a wire transport system similar to that used in the wire transport detector. In principle, the eluent passes over a moving wire which would carry a sample of the eluent into an evaporation chamber where the solvent would be removed leaving the solute deposited on the wire. The wire would then pass through a suitable interface directly into the ionization chamber of the mass spectrometer where it would be heated and the vapor ionized to provide the necessary spectra. Such a system can provide electron impact spectra or chemical ionization spectra as required but would be completely independent of the solvent used for development in the liquid chromatograph and thus maintain the versatility and capabilities of the separation technique. It is, however, unlikely to have the equivalent sensitivity to the previous procedure utilizing eluent sampling directly to the mass spectrometer.

The system based on the wire transport mechanism has already been reported[1] and in its original form was just adequate for use with LC separations, as it only had a sensitivity of 2×10^{-5} g/ml of column eluent. This sensitivity was equivalent to that of the refractometer detector. It did, however, demonstrate the practicability of operating a wire transport system through the ion source of a quadrupole mass spectrometer while maintaining sufficiently low pressures for effective operation.

This paper will describe modifications that have been carried out to improve the sensitivity and give examples of its application in providing spectra of chromatographic eluents.

THE LC–MS SYSTEM

The basic LC–MS instrument has been previously discussed[1] but a brief description of the apparatus will be given together with modifications that have been carried out.

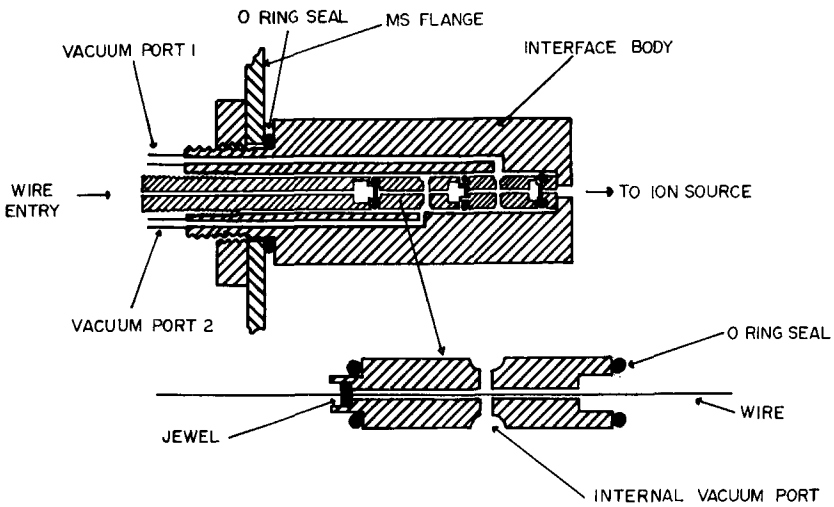

Fig. 1. The LC–MS interface. Reproduced by courtesy of the Ciba Foundation.

Interface

A diagram of the interface is shown in Fig. 1. The main body of the interface is constructed of brass and is fitted to the side flanges of the Finnigan mass spectrometer such that the interfaces are re-entrant to the ion source and terminate a few millimeters from the electron beam. The interface itself consists of two chambers separated and terminated by ruby jewels 1/10 in. in diameter and 0.018 in. thick. The jewels in the left-hand interface have central apertures 0.010 in. in diameter where the sample is introduced into the mass spectrometer. The jewels in the right-hand interface where the wire leaves the mass spectrometer to the winding spool have central apertures 0.007 in. in diameter. In this respect the interfaces differed from the original form[1] and result in lower pressures being obtained in the ion source. The larger-diameter apertures on the feed side of the spectrometer are employed to reduce scuffing of the wire and possibly loss of solute. The first chamber of each interface is connected directly to a 150 l/min rotary pump, which reduces the pressure in the first chamber to about 0.1 mm of mercury. The second chamber of each interface is connected to an oil diffusion pump backed by a 150 l/min rotary pump. The pressure in the second chamber of each interface was reduced by this system to about 5 to 10 μmHg. The entrance and the exit apertures were fitted with a helium purge T junction. Helium passed through the T junction replaced the air entering the mass spectrometer through the interfaces. In this way background spectra from air contaminants were reduced. The T junction also afforded a method of introducing methane or other suitable gases if chemical ionization spectra were required. The wire winding mechanism was that employed in the wire transport detector manufactured by Philips Chromatography and was mounted horizontally in front of the mass spectrometer ion source. Wire from the supply spool passed around an insulated brass pully through the feed interface, to the ion source and finally out through the second interface. From the second interface the wire passed over a second earthed brass pully to the winding capstan of the winding

mechanism. The wire could be passed through the system at rates of 2.5, 5, 7.5, 10 and 12.5 cm/sec. In normal operation the maximum wire speed of 12.5 cm/sec was employed. The sample was vaporized in the source by ohmic heating and this was achieved by passing a current through the wire from the insulated pully to the earth pully from an appropriate d.c. power supply. In practice, it was found that the optimum current required to volatilize the solute on the wire varied with the volatility of the respective solute. However, a compromise current of 175 to 200 mA provided adequate heating for the majority of solutes so far examined. To assemble the apparatus the wire was threaded through the feed interface (left hand), through the ion source of the mass spectrometer, then through the right-hand interface and subsequently the interfaces were bolted onto the mass spectrometer body. A photograph of the prototype instrument is shown in Fig. 2.

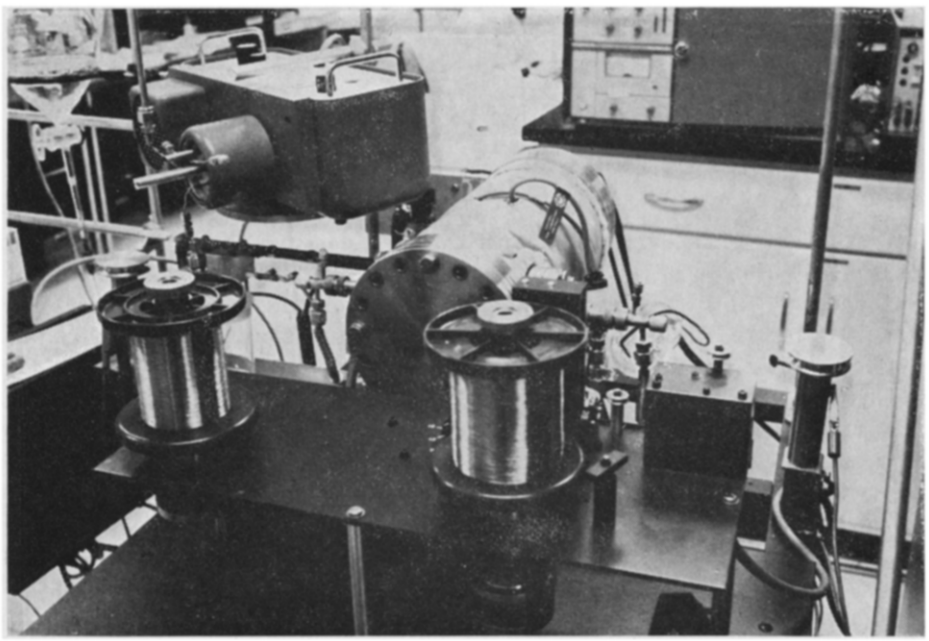

Fig. 2. Prototype instrument. Reproduced by courtesy of the Ciba Foundation.

To eliminate the need for disassembling the mass spectrometer at the end of each spool of wire, the spools were interchanged on the winding mechanism and the empty spool rewound for subsequent use. It was found that little or no contamination from previous runs was experienced possibly due to the fact that the column eluent washed the wire clean of any previous solute residues at the same time as depositing the fresh sample. As opposed to the original version[1], the mass spectrometer used in this work was fitted with a magnetically constrained source. The use of the magnetically constrained source in conjunction with the modified jewels constitutes the main changes in the instrument from the original prototype.

Operation

The mass spectrometer was operated under normal conditions except that the filament current employed was about 3 mA. The instrument was used with the standard data handling system supplied for the Finnigan mass spectrometer and a typical set of operating conditions is given in Table I. The chromatographic column was situated such that the terminal 1/16- to 1/4-in. Swagelok column coupling rested about 1/2 mm above the moving wire. Under these conditions the wire was always immersed in the column eluent despite eluent leaving the column in drops. The column employed was 50 cm long 4.6 mm I.D. and packed with 10 μ Partisil silica gel, a slurry packing procedure being used. The column was connected to a Milton Roy minipump, which in turn was fed by the apparatus used for incremental gradient elution which has been previously described[2]. A stop-flow method of injection was used and samples of approximately 2 to 6 mg of sample were used for each chromatogram depending on the complexity of the mixture. Subsequent to a separation the column was regenerated in the manner described by Scott and Kucera[3].

TABLE I

MASS SPECTROMETER OPERATING CONDITIONS

Filament current	3 mA
Electron energy	60 V
Source pressure	3×10^{-6} mm Hg
Mass range	60–199; 200–399; 400–650
Integration time	3; 5; 8
Sample/AMU	1; 1; 1
Threshold	1
Attenuation	5
Mass range setting	high
Mass run time	400 min
Delay between scans	3 sec

In the determination of sensitivity 0.01% solutions of diazepam in a 10% tetrahydrofuran–chloroform mixture were dropped onto the wire in a continuous stream and the total ion current was monitored. The sensitivity was taken as that concentration of diazepam that would provide a constant ion current signal equivalent to twice the noise level.

The sensitivity measured in this way for diazepam was 4×10^{-6} g/ml. This showed an increase in sensitivity over the original prototype (2.5×10^{-5} g/ml). The wire carried 10 μl/min of eluent into the mass spectrometer at the maximum wire speed. Thus, assuming a complete spectrum was obtained in one sec, this would correspond to a total mass of *ca.* 7×10^{-10} g of solute per spectrum. The modified jewel system resulted in the pressure in the ion source being about 3×10^{-6} mm of mercury which was reduced to an apparent pressure of 1×10^{-6} mm of mercury when helium was introduced into the T junctions. The use of helium reduced the noise level by a factor of 3, which together with the effect of the magnetically constrained source accounted for the overall six-fold increase in sensitivity over that of the original prototype.

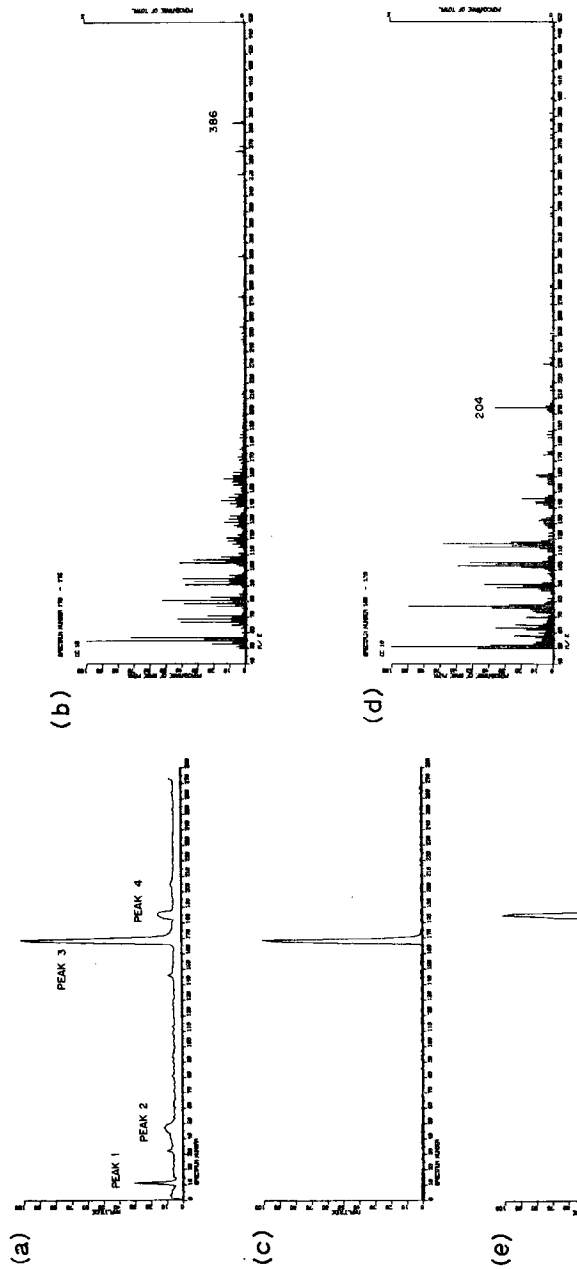

Fig. 3. LC–MS analysis of a synthetic mixture. (a) Reconstructed chromatogram based on total ion current. (b) Mass spectrum of cholesterol. (c) Reconstructed chromatogram based on ion mass 386 (cholesterol). (d) Mass spectrum of phenobarbital. (e) Reconstructed chromatogram based on ion mass 204 (phenobarbital). Reproduced by courtesy of the Ciba Foundation.

Performance of chromatographic mass spectrometer combination

An example of a separation of a synthetic mixture using the LC–MS system is shown in Fig. 3. The mixture contained normal triacontane, cholesteryl laurate, cholesterol and phenobarbital. The total sample was about 2 mg and placed on the column as a 0.6% solution in a mixed solvent heptane–acetone–methanol (1:1:1). Beneath the total ion-current chromatograms in Fig. 3 are shown the reconstructed chromatograms based on peak masses of 386 and 204, significant ions of cholesterol and phenobarbital, respectively. It is seen that good spectra were produced and these are obtained on the

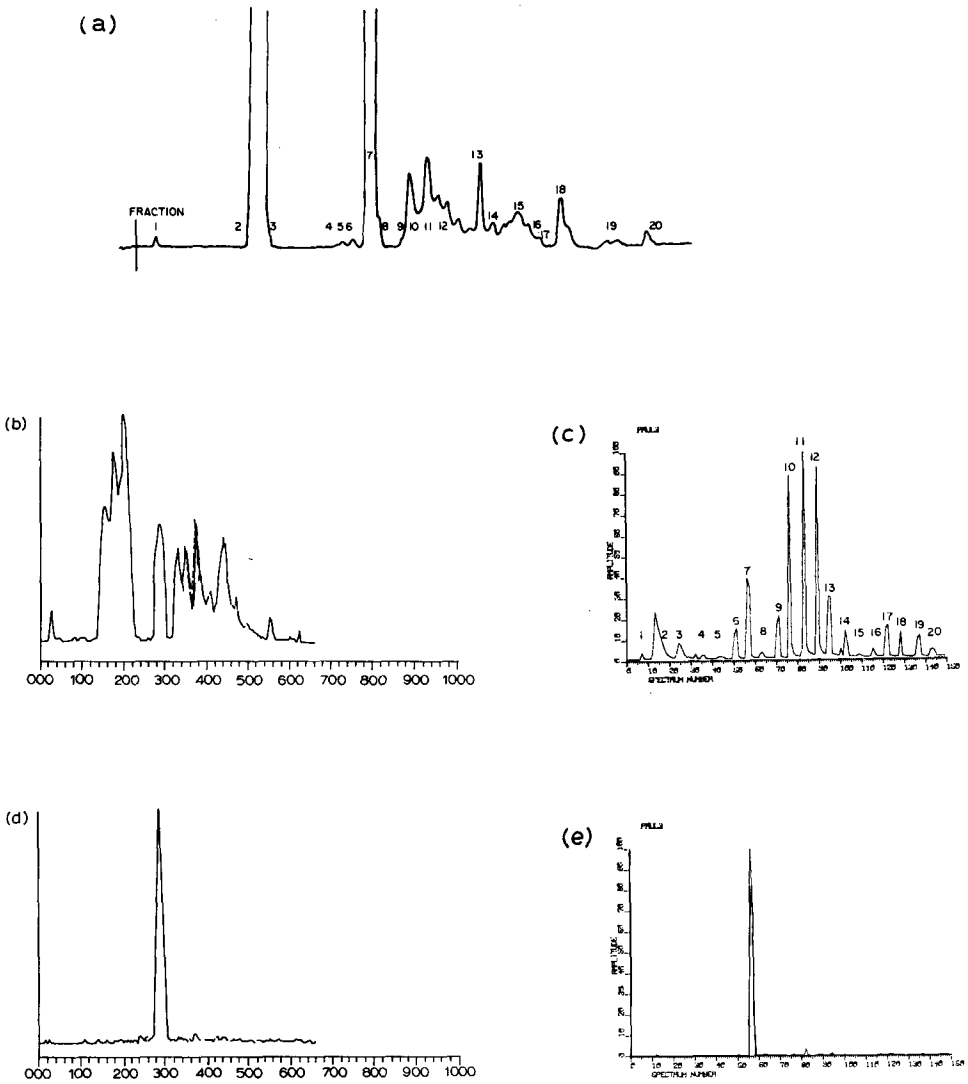

Fig. 4. (a) Chromatogram of a fermentation extract by incremental gradient elution. (b) Total ion-current chromatogram by LC–MS of fermentation extract. (c) Total ion-current trace of fractions sampled directly onto wire. (d) Ion-current trace for mass 327 from LC–MS chromatogram. (e) Ion-current trace for mass 327 from fractions sampled directly onto wire.

original prototype instrument having a sensitivity of 2×10^{-5} g/ml. In Fig. 4 the chromatogram obtained for a fermentation extract in methanol–chloroform is shown and this sample contains solutes that cover a wide range of polarity. The mixture was first chromatographed using the incremental gradient elution technique[2] and the optimum conditions previously described[4] used for the development of the chromatogram. A charge of 2 mg was injected into the column and the detector used was the wire transport detector. The chromatogram produced is shown at the top of Fig. 4. During the chromatographic development fractions were collected for each peak and numbered as shown in the diagram. These were concentrated in a current of nitrogen to about 0.1 ml and each fraction examined using the LC–MS system as a probe injection device. A drop of each sample was spotted onto the wire for a few seconds and the total ion-current traces of these probe samples are shown on the right-hand side of Fig. 4. All the fractions were placed on the moving wire over a period of $8\frac{1}{2}$ min. Thus the spectrum for an individual probe sample was obtained in approximately 25 sec. The sample was then chromatographed on the LC–MS system using a 6-mg charge and the same operating conditions. The chromatogram obtained from the total ion-current monitor is shown as a hard copy on the left-hand side of Fig. 4. It is seen that due to the nature of the hard copy presentation of the chromatogram it is somewhat compressed in size relative to the original using the wire transport detector. However, it is seen that the same pattern of peaks is obtained although owing to the larger charge size employed, the resolution is not as good.

A spectrum was obtained from the probe injection sample number 7 and is shown as the lower spectrum in Fig. 5. It is seen that a significant ion mass is 327; thus a reconstructed chromatogram from the ion current of mass 327 was obtained for the probe samples. This is seen as the lower chromatogram on the right-hand side of Fig. 4. Peak 7 is clearly selected and using the same mass 327 a reconstructed chromatogram was obtained from the original ion-current chromatogram from the LC–MS run. This is shown at the bottom of the right-hand side of Fig. 4 and again it is seen that the peak is clearly and unambiguously selected from the mixture. A spectrum was then taken from the respective peak in the LC–MS chromatogram and this is shown at the top of Fig. 5 and can be compared with the spectrum from fraction 7. It is seen that basically the two spectra are identical although the spectrum from fraction 7 obviously contains traces of a contaminating material.

The quadrupole mass spectrometer that was utilized for this work was an old version and had very poor resolving power. As a result of this low resolution the sensitivity of the instrument could not be increased to its normal level. In an attempt to determine the sensitivity of the system, when a modern instrument with specified resolution was used, the sensitivity of the older instrument was increased at the expense of resolution until a single peak became a doublet (due to the increased widths) when processed by the computer. The sensitivity with the apparatus operating under these conditions was estimated at about 5×10^{-7} g/ml. Under the same conditions a sample of vitamin A mother liquor was chromatographed again using incremental gradient elution development. The total ion-current chromatogram obtained for the sample is shown in Fig. 6. The charge placed on the column was *ca.* 1 mg and it is seen that an excellent separation and recording is obtained. In all cases, however, the spectra were not acceptable as each single ion mass was represented as a doublet. In Fig. 7 the separation of a mixture of three fully protected impure di- and tripeptides

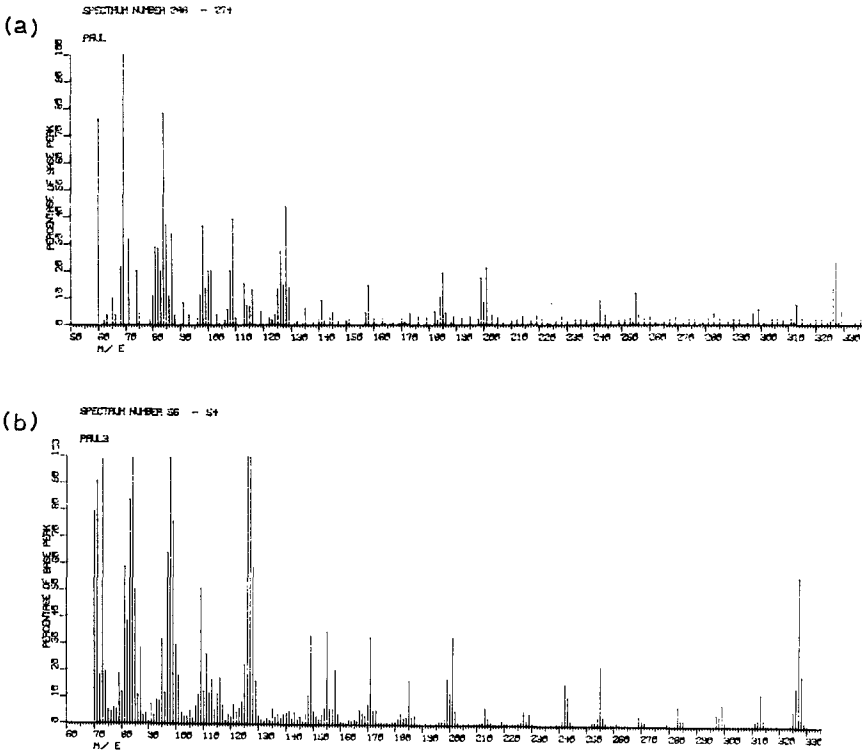

Fig. 5. (a) Spectrum 285–262 from LC–MS chromatogram. (b) Spectrum from fraction 7.

is shown using the same chromatographic conditions and mass spectrometer adjustments. Again approximately 1 mg of total mixture was placed on the column and excellent separations of the three peptides obtained together with many of the impurities. The last three peaks in the chromatogram are the fully protected tri- and two dipeptides, respectively. Assuming that the sensitivity of the modern quadrupole instrument is

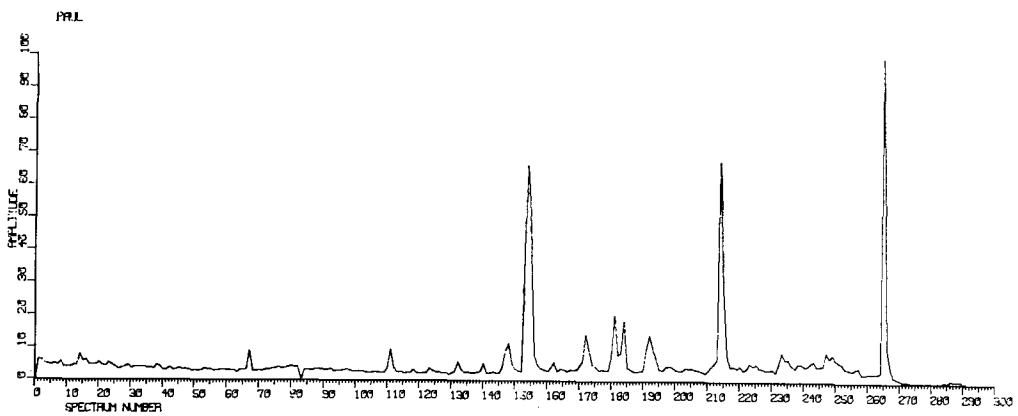

Fig. 6. Total ion-current chromatogram of vitamin A acetate mother liquor.

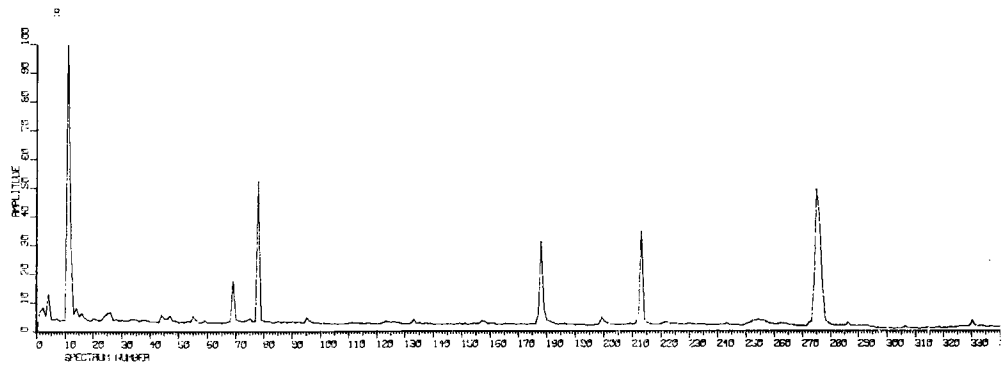

Fig. 7. Total ion-current chromatogram of a mixture of impure protected di- and tripeptides.

simulated by the conditions used above, the overall sensitivity when using such an instrument with the LC–MS system should be more than adequate for the majority of separation problems.

CONCLUSION

It has been shown that two-stage differentially pumped interfaces will permit a wire to pass through the ion source of a quadrupole mass spectrometer while maintaining source pressure at about 3×10^{-6} mmHg. The results also show that vaporization of solute coated on the wire can be achieved by the direct ohmic heating of the wire. The use of the apparatus to provide an alternative probe sampling system together with on-line LC–MS monitoring of column eluents has been demonstrated. The sensitivity of the present system which utilizes a quadrupole mass spectrometer with resolving power significantly below specifications is *ca.* 6×10^{-6} g/ml. The system provides electron impact spectra that as far as has been investigated are the same as those obtained from normal probe injection. The apparatus in its present form is practical for providing LC–MS monitoring but requires significant charges to be placed on the column where trace materials are being identified and this may cause a reduction in column performance by way of lower resolution. The total mass of material required to provide spectra from the present instrument assuming a 1-sec sampling time is 500 pg. Experiments carried out to assess the potential of the system when used with a mass spectrometer providing the normally specified resolution indicated that the sensitivity of the system would be increased to about 5×10^{-7} g/ml, which would be satisfactory for the majority of LC separations. Further sensitivity enhancement might be obtained if required by modification of the interfaces to reduce dead volumes and also by using an alternative means of vaporizing the sample in the ion source. This could be achieved by using heater coils or infrared radiation focused on the wire in the ion source or alternatively by the use of a laser.

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