Journal of Chromatography, 562 (1991) 13–29
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5561

Particle beam liquid chromatography–mass spectrometry on a double-focusing sector instrument

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ABSTRACT

A commercially available particle beam interface developed by the Vestec Corporation has been coupled to a double-focusing high-resolution mass spectrometer (VG ZAB 2F) via a momentum separator. An ultraviolet detector was in-line with the interface and allowed monitoring of the eluting materials. With this instrumentation, complete electron-impact (EI) mass spectra on synthetic mixtures of steroids, nucleosides, and several derivatives of amino acids were recorded. Amongst these the EI mass spectra of 2,4-dinitrophenyl, 9-fluorenylmethoxycarbonyl and phenylthiohydantoin derivatives were obtained. The standard LC conditions used here (250 mm × 4.6 mm I.D., reversed-phase C_{18} column, 1.0 or 1.5 ml/min flow-rate, isocratic or gradient programming) allowed separation of the mixtures. The resulting mass spectra were compared with those obtained using a direct insertion probe. The agreement was excellent in most cases. Sensitivity measurements were performed on cholesterol and caffeine. A complete low-resolution mass spectrum can be obtained on 100 ng of cholesterol. Single-ion monitoring of the $M^{+\cdot}$ of 200 pg of caffeine gave a 4:1 signal-to-noise ratio at *m/z* 194. Complete high-resolution mass spectra were obtained on 5 μg of cholesterol (loop injection) and on every peak of a five-steroid mixture of 5 μg each. The accuracy of the mass measurements were better than 5 m.m.u. for most cases. Three to four scans could be obtained for each liquid chromatographic peak. Mass-analyzed ion kinetic energy spectra were recorded on the $M^{+\cdot}$ of 2.5 μg of cholesterol at *m/z* 386. Similarly the B/E linked scan of the same ion was recorded on 2.5 μg and 500 ng.

INTRODUCTION

The combination of liquid chromatography (LC) with mass spectrometry (MS) has been extensively investigated in the last eighteen years. Some of the first attempts of obtaining complete low-resolution electron-impact (EI) mass spectra from LC effluents used a motor-driven movable insertion probe [1]. The analytes and solvents were placed on a gold gauze probe tip or absorbing material, the solvents were removed under vacuum, and the probe was introduced into the ion source. Some of the ideas of this interface were eventually incorporated into the moving belt interface. One of the many reviews on this subject has been published recently [2]. It has been clear from the start that availability of libraries of EI mass spectra and the characteristic fragmentation of organic molecules in the EI mode made the moving belt interface a very desirable addition to many MS laboratories. Direct liquid introduction (DLI) [3] was another method of coupling the LC

0378-4347/91/$03.50

instrument to a mass spectrometer. Here the LC effluent is split in a ratio of 100:1 and the 1% is introduced into the ion source. The resulting mass spectra have a chemical ionization (CI) type of appearance. The advent of microbore columns has allowed to introduce all of the effluents into the ion source. [4].

Another approach to interface the liquid chromatograph to the ion source of a mass spectrometer has been described more recently [5]. A monodisperse aerosol generation interface for combining LC with MS (MAGIC-LC–MS) has been constructed and good quality EI mass spectra could be obtained on LC peaks. An improved version of this interface has been described recently [6]. Several commercial interfaces of this type, called particle beam (PB) interface, have become available in the last year, mainly on quadrupole instruments. Although it was clearly shown that this type of interface can operate on a sector type mass spectrometer [5], little information is available on the potential of coupling the PB interface to a double-focusing high-resolution mass spectrometer.

A "Universal Interface" for coupling liquid chromatographs to analytical instruments has been introduced at the 1989 Pittsburgh Conference by the Vestec Corporation. This interface encompasses a vaporizer with a replaceable tip, a membrane diffusion cell, and a momentum separator. The effluents from the LC column are vaporized and most of the solvents removed in the membrane diffusion cell. The eluents are carried to a dual-stage momentum separator. Here most of the helium is removed and the analytes can be directly ionized by various ionization techniques. A detailed description of this interface and its operation has been reported [7].

The present article describes the connection of this interface to a VG ZAB 2F double-focusing high-resolution mass spectrometer and its operation in various modes.

EXPERIMENTAL

The Gilson high-performance liquid chromatographic (HPLC) system (Gilson Medical Electronics, Middleton, WI, U.S.A.) used here consists of a 302 and a 305 programmable piston pump, an 805 manometric module, an 811B dynamic mixer, a Model 231 injector, and the Model 116 UV dual-wavelength detector. The HPLC column was a C_{18}, 10 μm particle size (Spheri-10 RP-18), 250 mm × 4.6 mm I.D. reversed-phase column from Brownlee Labs. (Santa Clara, CA, U.S.A.). A 20- or 100-μl loop was used in the injector. The UV detector was in-line with the Universal Interface (Vestec, Houston, TX, U.S.A.). Thus the total-ion current trace obtained from the MS data system could be direclty compared with the UV chromatogram obtained from the UV detector. The Universal Interface is connected to the momentum separator via a Teflon tubing (*ca.* 1 m length, 6 mm O.D. and 4 mm I.D.). The first stage of the momentum separator was pumped with a 25 m^3/h and the second stage with an 11.5 m^3/h Edwards direct drive mechanical pump (Edwards High Vacuum, Crawley, U.K.). This

resulted in an ion source pressure of $8 \cdot 10^{-6}$ Torr. The output of the momentum separator is connected to a standard VG ion source of the VG ZAB 2F (VG Analytical, Manchester, U.K.) via a quartz tube (4 mm O.D., 2 mm I.D.). The temperature of the momentum separator was 135°C and the ion source temperature was maintained at 220°C. In a typical experiment the parameters of the Universal Interface were set as follows: input helium pressure, 1 bar; tip temperature, 160°C; separator temperature 45°C; spray temperature, 70°C. The ion source temperature was 220°C, the electron energy was 70 eV, the accelerating voltage was 7.0 kV, and the trap current was 100 μA.

All spectra [low-resolution, high-resolution, mass-analyzed ion kinetic energy (MIKES) and B/E-linked scans] were recorded with the UPACS II data system described previously [8]. This system has been expanded and transferred to a Harris H800 dual-CPU computer (Harris, Fort Lauderdale, FL, U.S.A.).

RESULTS AND DISCUSSION

Low-resolution mass spectrometry

The LC–MS system described here has been assembled from commercial parts. The Vestec Separator™ interface was purchased as part of the LC–MS interface for the Vestec 201 thermospray (TSP) and EI package. The adaptation to the VG ZAB 2F high-resolution mass spectrometer was particularly easy since the flange of the momentum separator from Vestec fits a standard 20-mm flange. It was connected to the port normally used for the gas chromatographic–mass spectrometric (GC–MS) interface. The mass spectrometer is isolated from the LC system via a stainless-steel Whitey ball valve. Thus the mass spectrometer and the LC system can be operated independently from each other.

The total system was tested first using repetitive injections of 1 μg of cholesterol in 20 μl of methanol. The spraying solvent was water containing 0.1% trifluoroacetic acid (TFA)–acetonitrile (1:1) at a flow-rate of 1 ml/min. Various parameters of the interface were adjusted to give a maximum response of the molecular ion of cholesterol at *m/z* 386. Full scans on 100 ng and 1 μg were obtained and are shown in Fig. 1. As can be seen the bottom trace (1 μg) is identical to a spectrum obtained using a direct insertion probe. The top trace corresponding to 100 ng contains some ions due to background. Thus the ions at *m/z* 57 and 69 are at least partly due to solvent and the ion at 446 is due to background from the momentum separator. Note that three or four scans could be obtained for each injection of 100 ng of cholesterol. Thus the spectrum shown (top trace in Fig. 1) represents only a fraction of the material present in the source at a given moment. Single-ion monitoring (SIM) of the $M^{+\cdot}$ ion at *m/z* 386 showed that 10 ng of cholesterol gave a response of 4 × over background. Similar experiments were performed on five injections of caffeine. SIM of the $M^{+\cdot}$ of caffeine at *m/z* 194 showed a detection limit of 200 pg for each injection of the caffeine solution. This indicates that the sensitivity of the PB technique, as is the

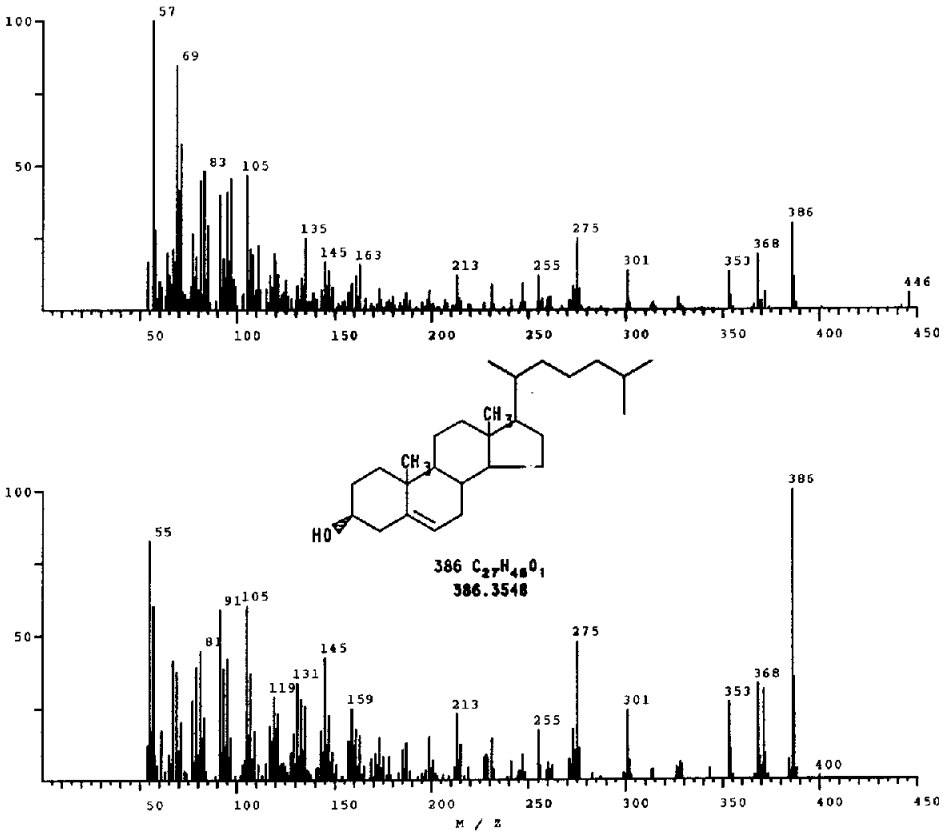

Fig. 1. Comparison of EI mass spectra of cholesterol obtained by loop injections on PB interface. Top, 100 ng; bottom, 1 μg.

case of many other MS techniques, will be a function of the structure and stability of the analytes under investigation.

A synthetic mixture of five steroids which contained 100 ng/μl each of medrol, medrol acetate, 17-hydroxyprogesterone, provera, and progesterone (the steroids are listed by increasing retention times) were separated on the LC column. The chromatography was run in the isocratic mode: acetonitrile–water containing 0.1% TFA (70:30). The flow-rate was 1.0 ml/min and the wavelength used to monitor the components was 260 nm. The resulting total-ion chromatogram (sum of the raw ion intensities for each scan) is displayed in Fig. 2. As can be seen the LC peaks are not of the same abundance even though the same amount (1 μg) of each steroid was injected on the column. This difference can be explained by considering the mass spectra of peak 2 and peak 5. These are shown in Figs. 3 and 4, respectively. In the mass spectrum corresponding to the second LC peak, there

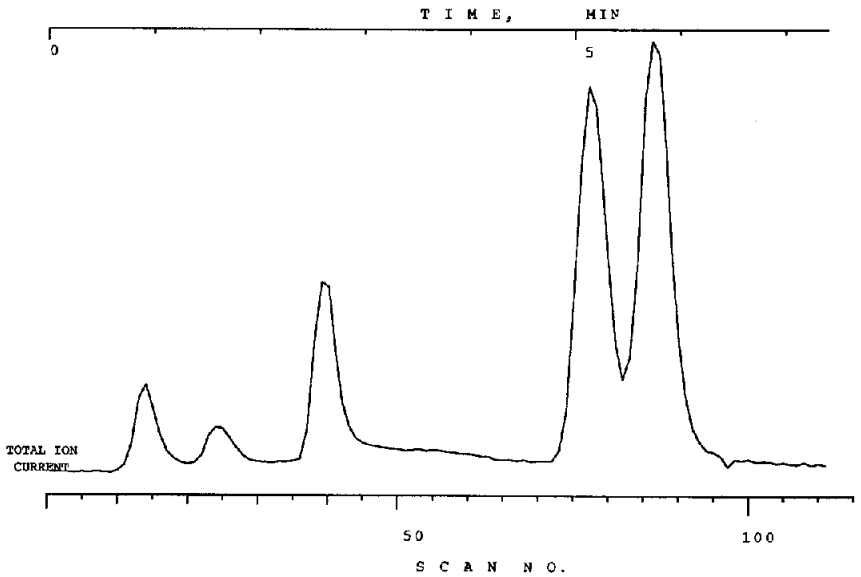

Fig. 2. Total-ion chromatogram of a five-steroid mixture (1 μg each injected on-column).

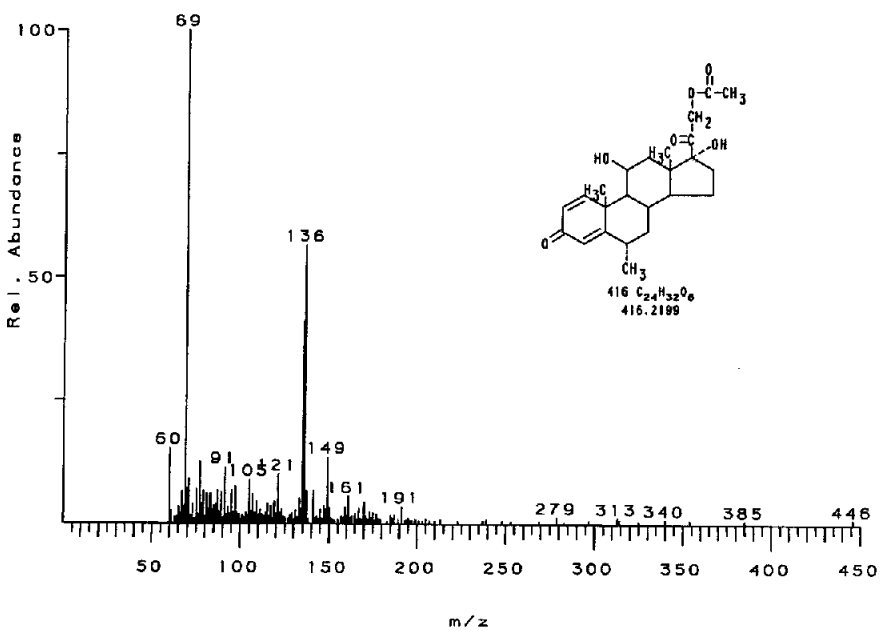

Fig. 3. EI mass spectrum (PB) of component 2 (medrol acetate) of the steroid mixture.

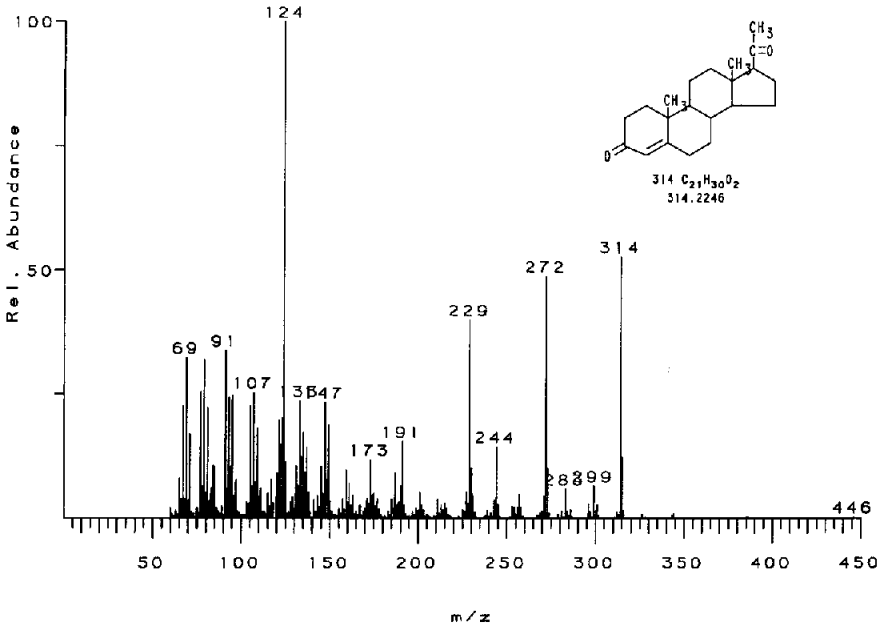

Fig. 4. EI mass spectrum (PB) of component 5 (progesterone) of the steroid mixture.

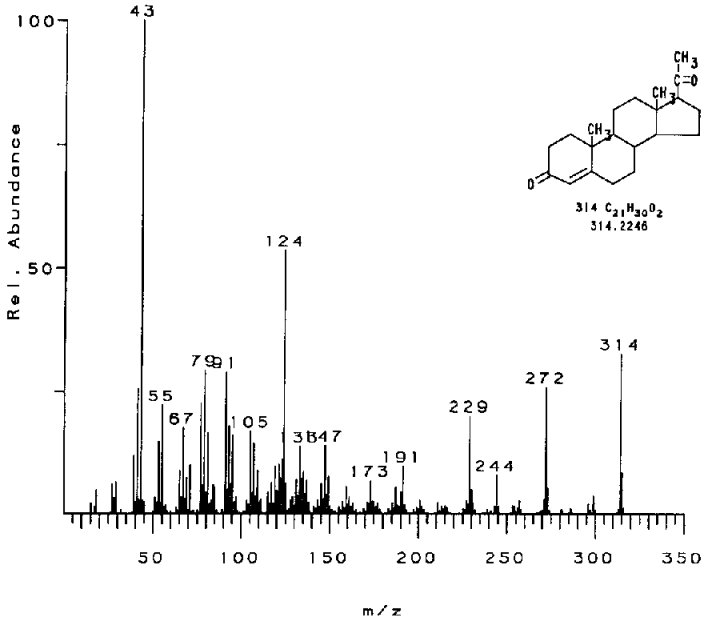

Fig. 5. EI mass spectrum (probe) of progesterone.

is no molecular ion present at *m/z* 416 and there are very few intense fragment ions. The EI mass spectrum is dominated by the ions at *m/z* 135 and 136. The ions at *m/z* 69 and 446 are due to background. Note that the ions due to the steroid (medrol acetate) are much weaker than the ion at *m/z* 69, which is probably due to the presence of TFA in the solvents. On he other hand the mass spectrum corresponding to the fifth LC peak (progesterone) has a strong molecular ion and many abundant fragment ions. The ions at *m/z* 69 and 446 are much less intense in comparison to the most abundant fragment ion of progesterone at *m/z* 124. Note that this mass spectrum is very similar to that obtained by direct insertion probe, which is shown in Fig. 5.

Similar experiments were performed on a synthetic mixture of 9-fluorenylmethoxycarbonyl (FMOC) derivatives of five common D- and L-amino acids (D-Phe, L-Val, D-Trp, L-Leu, L-Met). These derivatives are widely used in peptide synthesis and as a fluorescence tag which allows LC detection of amino acids in the lower femtomole range [9]. The HPLC conditions used were similar to those described above with the exception that a linear gradient was run at a flow-rate of 1.5 ml/min. The starting solvent mixture was acetonitrile–water containing 0.1% TFA (40:60) and was programmed linearly till a 80:20 ratio of acetonitrile–water was attained in 15 min. The total-ion chromatogram shown in Fig. 6 corresponds to an injection of 500 ng of each component on column. As can be seen, at the end of the run (after scan 225) the column bleed is quite noticeable. A mass spectrum of this bleed is shown in Fig. 7. Two very intense ions are present at *m/z* 75 and 313. Accurate mass measurements (see below) of these two ions indicated that the

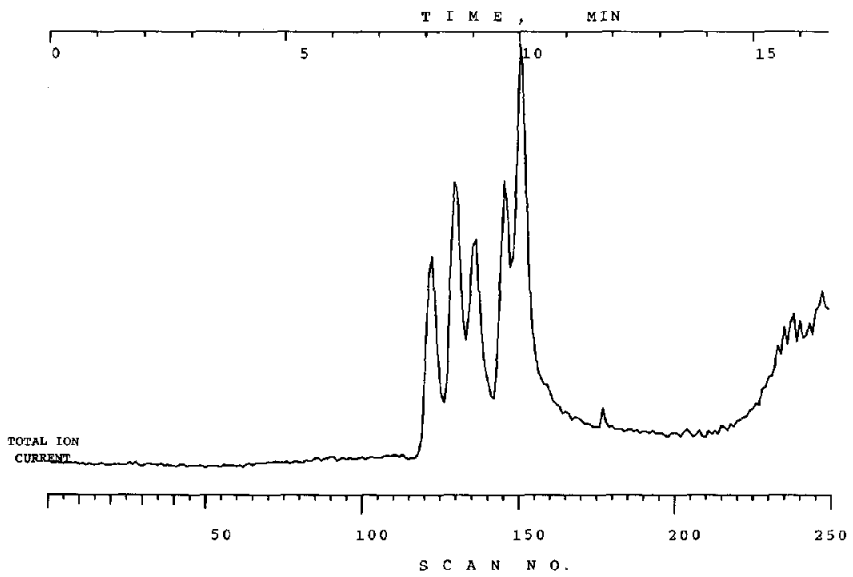

Fig. 6. Total-ion chromatogram of five FMOCs of amino acids (500 ng each injected on-column).

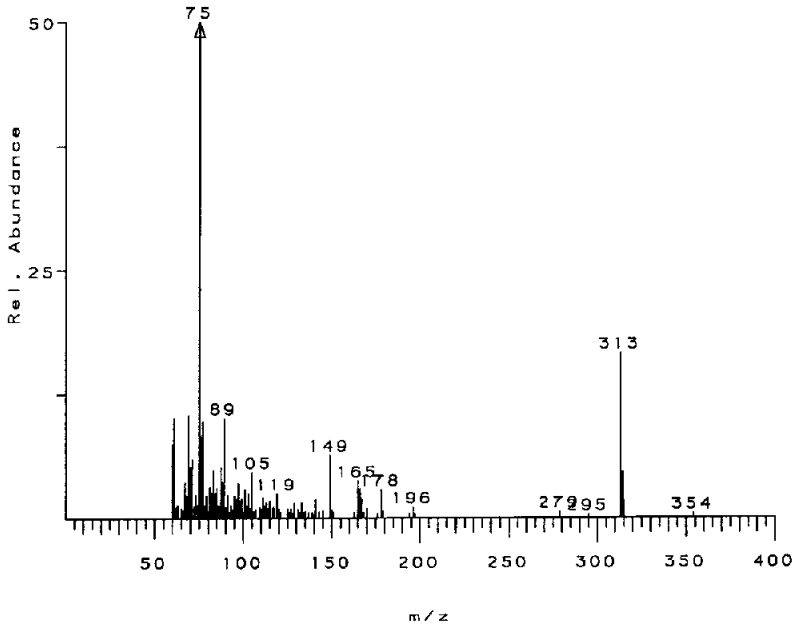

Fig. 7. EI mass spectrum (PB) of column bleed.

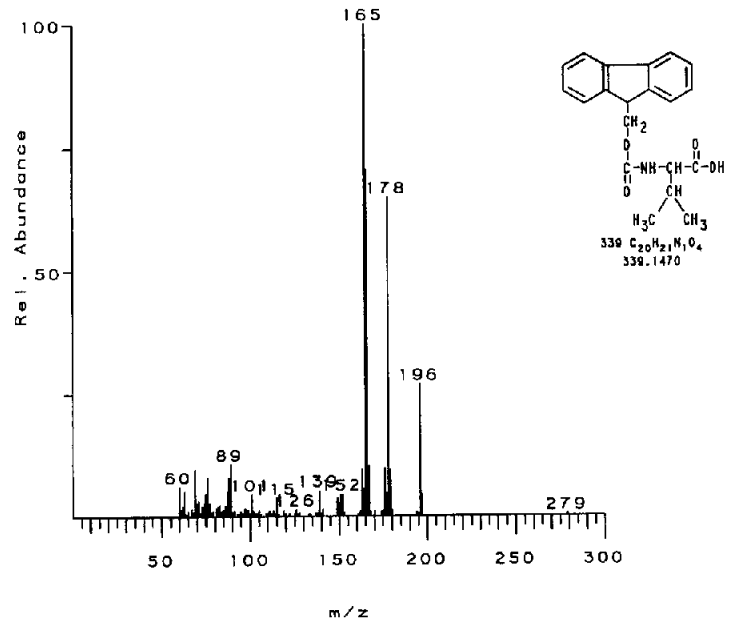

Fig. 8. EI mass spectrum (PB) of FMOC derivative of an amino acid.

first ion at m/z 75 is due to $(CH_3)_2Si{=}OH^+$ and the second ion at m/z 313 corresponds to the elemental composition of $C_{19}H_{41}OSi^+$. The EI mass spectra of the five amino acid derivatives looked very similar. They are dominated by four intense fragment ions (m/z 165, 166, 178, and 196) which originate from the fluorenylmethoxy part of these derivatives. A typical EI mass spectrum of the FMOC derivatives obtained in this run is shown in Fig. 8.

A derivative that was widely used in peptide and protein sequencing [10] is the 2,4-dinitrophenyl (2,4-DNP) derivative. A mixture of four 2,4-DNP derivatives of D,L-β-alanine, D,L-norvaline, D,L-norleucine, and D,L-methionine was analyzed. The LC conditions were similar to the above used for the FMOC derivatives. The eluting peaks were monitored at 254 nm, and 20 μl of the mixture containing 1 μg of each component were injected on-column. Three out of the four components gave good total-ion currents and EI mass spectra. However, none showed a molecular ion. Thus a fraction of the mixture was derivatized with an excess of ethereal diazomethane and injected direclty onto the LC column. The total-ion chromatogram is shown in Fig. 9. The 2,4-DNP-methyl esters (1 μg each) of the amino acids listed above eluted in the following order: D,L-β-Ala, D,L-Met, D,L-NorVal and D,L-NorLeu. All four derivatives gave excellent EI mass spectra which showed molecular ions. However, the column bleed was quite intense for the fourth compound (D,L-NorLeu) as is shown in Fig. 10. Thus the ions at m/z 75 and 313 are due to column bleed and have been explained in the previous paragraph. Note that at the end of the chromatogram the total-ion current has been decreased by a factor of ten starting from scan 230 to normalize

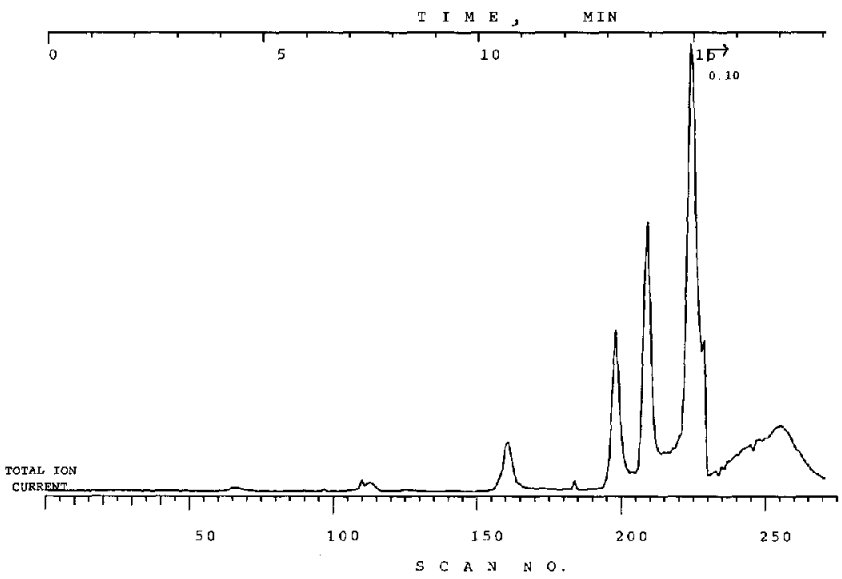

Fig. 9. Total-ion chromatogram of 2,4-DNP-methyl ester derivatives of four amino acids.

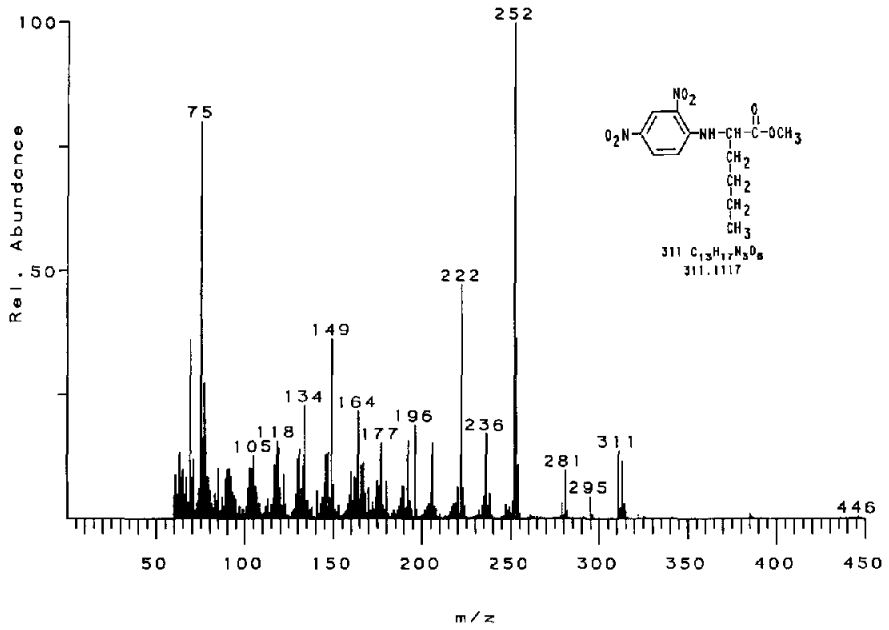

Fig. 10. EI mass spectrum (PB) of 2,4-DNP-methyl ester derivative of D,L-NorLeu.

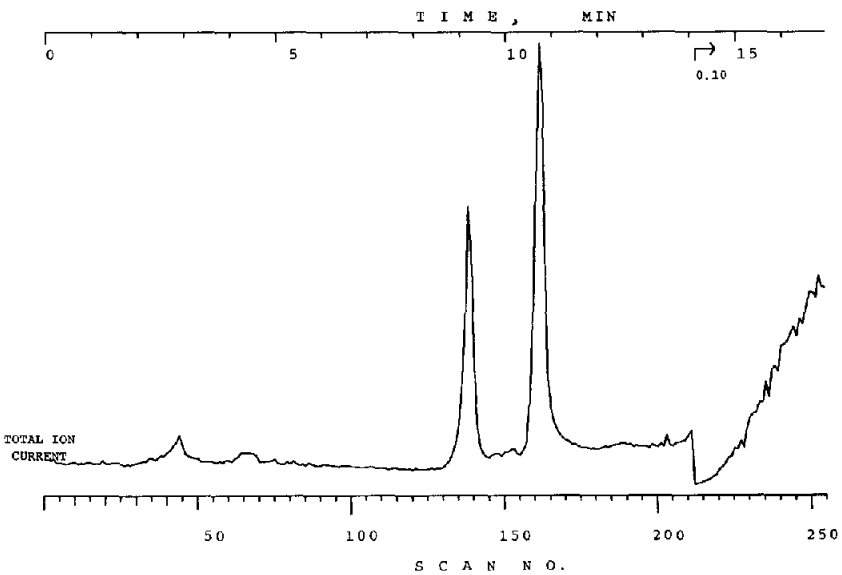

Fig. 11. Total-ion chromatogram of PTH mixture of five amino acids (Asn, Glu, Pro, Trp and Leu), 1 μg each injected.

the LC peaks to the most intense one and not to the bleed. Since UV is transparent to this column bleed it does not appear in the corresponding UV chromatogram. Comparison of the mass spectra obtained from the PB experiment with the published spectra in the literature [11] for the 2,4-DNP-methyl ester derivatives showed in general a very good agreement.

Another derivative that is still widely used in the Edman method of peptide and protein sequencing is the phenylthiohydantoin (PTH) derivative [12]. A complete list of the EI mass spectra of the PTHs of the twenty naturally occurring amino acids has been published [13]. A mixture of five PTHs of Asn, Glu, Leu, Pro, and Trp was injected (1 μg each) on-column. The LC conditions were as follows: 1.5-ml flow, 30:70 acetonitrile–water containing 0.1% TFA, isocratic for 3 min, then gradient till 80:20 acetonitrile–water containing 0.1% TFA in 12 min. The total-ion chromatogram is shown in Fig. 11. Only two LC peaks are apparent. Their corresponding mass spectra are shown in Figs. 12 and 13. They correspond to the EI mass spectra of the PTHs of proline and leucine, respectively, and are nearly identical with the published spectra in the literature [13]. However, the mass spectra of the other three components were very weak and therefore their total-ion current peaks are insignificant. When a fraction of this mixture was treated with diazomethane, the peak corresponding to the PTH-methyl ester of glutamic acid yielded a good total-ion chromatographic peak as well as a good EI mass spectrum. It is not clear at the moment why some of the polar components

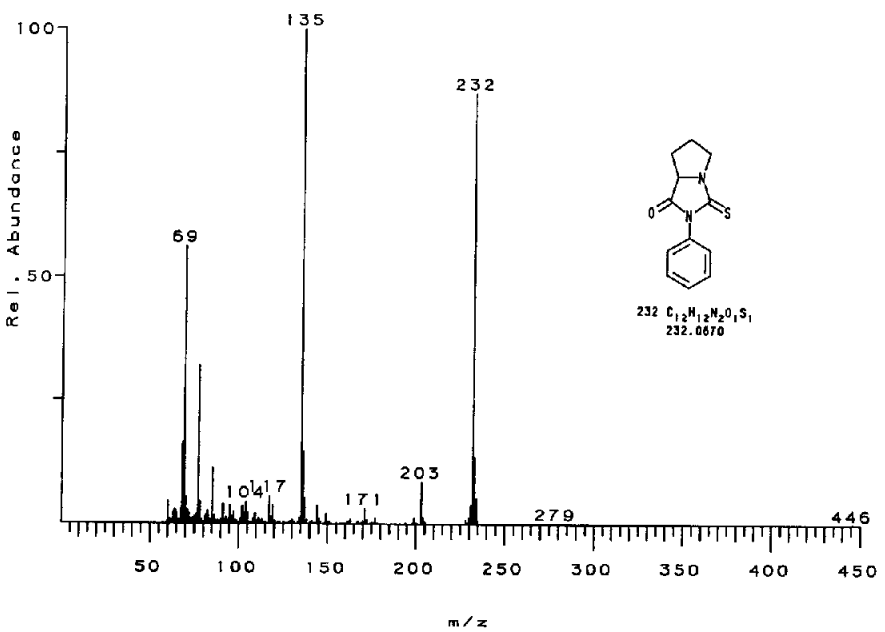

Fig. 12. EI mass spectrum (PB) of first major LC peak (PTH Pro).

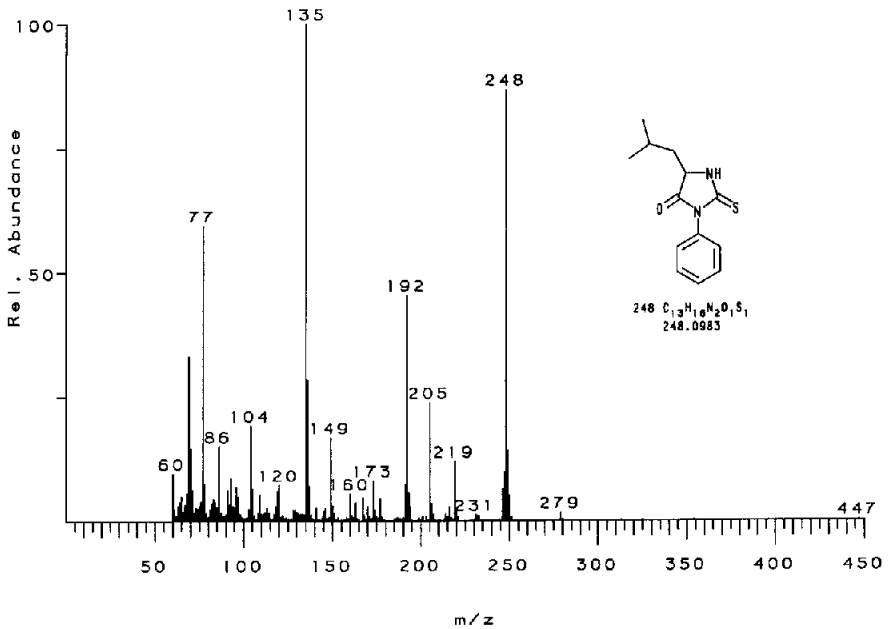

Fig. 13. EI mass spectrum (PB) of second major LC peak (PTH Leu).

of these mixtures do not yield good EI mass spectra. A number of parameters of the Universal Interface and of the momentum separator (temperature and helium flow) were studied. However, none of these attempts improved the performance

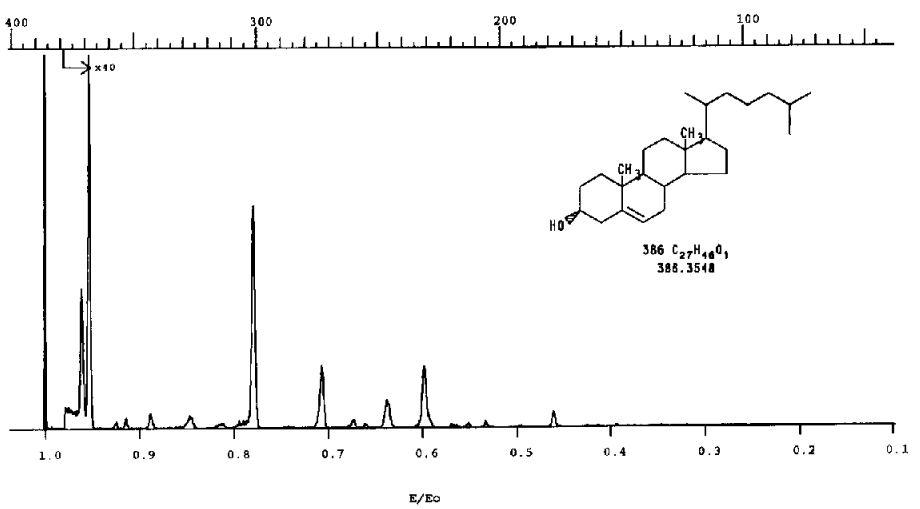

Fig. 14. MIKE scan of $M^{+\cdot}$ of cholesterol at m/z 386 (2.5 μg loop injection).

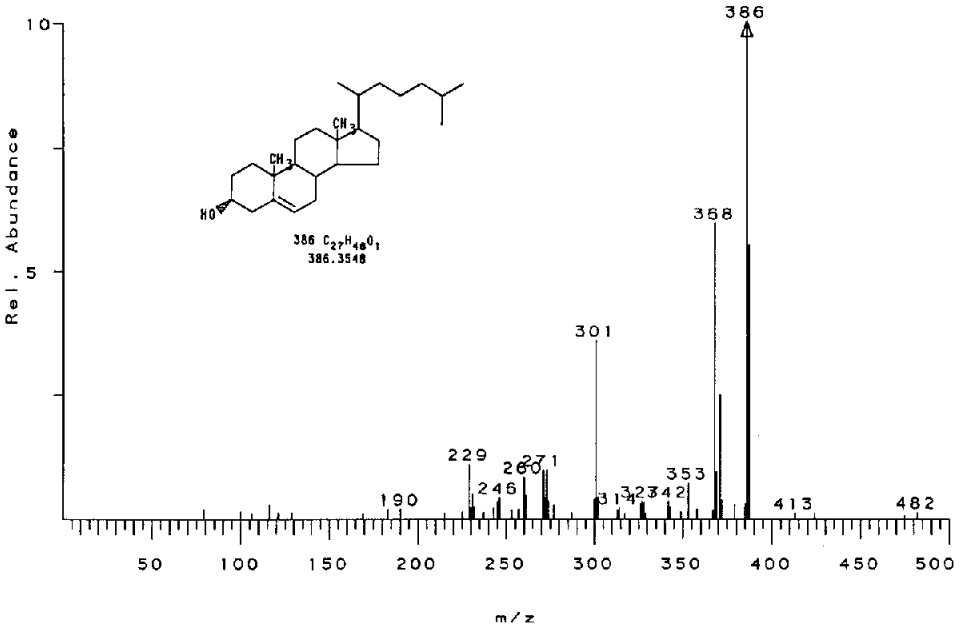

Fig. 15. B/E-linked scan of $M^{+\cdot}$ of cholesterol at m/z 386 (500 ng loop injection).

of this instrumentation. Other experiments are in progress to elucidate this discrimination. It was also observed that dansyl derivatives injected as cyclohexylammonium salts did not yield any EI mass spectra by the particle beam method described here and nucleosides such as cytidine and guanosine also failed to give any reasonable mass spectra. This is not surprising since these compounds also fail to yield mass spectra in the EI ionization mode when run with the direct insertion probe. However, it appears that polar compounds present a special problem for the PB interface and other techniques such as TSP and continuous-flow fast atom bombardment (FAB) interfaces will have to be used for polar compounds.

High-resolution mass spectrometry

The mass spectrometer used in this study is a double-focusing instrument with an ultimate resolution of 100 000. Most of the high-resolution work done on this instrument is at a resolution of 10 000. To obtain accurate mass measurements on compounds introduced into the ion source via the PB interface, a resolution of 3300 was arbitrarily chosen. In the first experiment 5 μg of cholesterol in 100 μl of methanol were injected via loop injections. Three to four scans could be obtained for each injection. The instrument was repetitively scanned from m/z 50 to 710 at a scanning rate of 20 s/dec. Perfluorokerosene (PFK) was constantly admitted

TABLE I

HIGH-RESOLUTION MASS MEASUREMENTS OF MAJOR IONS OF CHOLESTEROL (5-μg LOOP INJECTION)

m/z	Theory	For	Found	Error (m.m.u.)
213	213.1643	$C_{16}H_{21}$	213.1640	0.28
			213.1611	3.26
			213.1637	0.67
			213.1656	−1.28
255	255.2113	$C_{19}H_{27}$	255.2084	2.90
			255.2109	0.35
			255.2124	−1.09
			255.2095	1.75
275	275.2739	$C_{20}H_{35}$	275.2766	−2.72
			275.2746	−0.70
			275.2730	0.84
			275.2725	1.42
301	301.2895	$C_{22}H_{37}$	301.2930	−3.53
			301.2904	−0.91
			301.2920	−2.45
			301.2888	0.76
353	353.3208	$C_{26}H_{41}$	353.3230	−2.16
			353.4303	0.53
			353.3191	1.71
			353.3207	0.14
368	368.3443	$C_{27}H_{44}$	368.3482	−3.94
			368.3498	−5.53
			368.3468	−2.43
			368.3449	−0.59
386 ($M^{+\cdot}$)	386.3549	$C_{27}H_{46}O$	386.3603	−5.45
			386.3592	−4.33
			386.3543	0.58
			386.3547	0.18

into the ion source from the gas inlet system. Data were acquired at 20 kHz and processed using the UPACS II system. The accurate masses of the major ions of four scans are shown in Table I. As can be seen most of the masses are within the error limits of 5 m.m.u. and can be used to determine elemental compositions. Even weaker ions are determined with similar accuracy, with the exception of $^{12}CH/^{13}C$ overlapping peaks which is a common problem for most sector instruments and is not due to the PB introduction system.

In the next experiment the steroid mixture of five components discussed above was separated on the LC column and three or four scans were recorded on each of the five LC peaks. Here again 5 μg of each component were injected on-column and the eluting material was introduced into the ion source. The results for the

TABLE II

HIGH-RESOLUTION MASS MEASUREMENTS OF MAJOR IONS OF A FIVE-COMPONENT STEROID MIXTURE (5 μg EACH ON-COLUMN)

m/z	Theory	For	Found	Error (m.m.u.)
Peak 2: medrolacetate ($C_{24}H_{32}O_6$; 416.2199)				
135	135.0810	$C_9H_{11}O$	135.0812	−0.18
			135.0812	−0.18
			135.0810	0.0
136	136.0888	$C_9H_{12}O$	136.0874	1.40
			136.0875	1.35
			136.0871	1.72
416	416.2199	$C_{24}H_{32}O_6$	416.2205	−0.65
			416.2184	1.49
Peak 5: progesterone ($C_{21}H_{30}O_2$; 314.2246)				
124	124.0888	$C_8H_{12}O$	124.0882	0.58
			124.0877	1.15
			124.0884	0.42
			124.0868	2.05
229	229.1804	$C_{13}H_{25}O_3$	229.1765	3.82
			229.1828	−2.40
			229.1766	3.77
			229.1752	5.13
272	272.2140	$C_{19}H_{28}O$	272.2142	−0.23
			272.2151	−1.13
			272.2142	−0.14
			272.2167	2.81
314	314.2246	$C_{21}H_{30}O_2$	314.2236	1.02
			314.2216	2.99
			314.2245	0.12

most relevant ions of the most intense LC peak 5 and the weakest peak 2 (see Fig. 2) are reported in Table II. From this table it is apparent that the $M^{+\cdot}$ at *m/z* 416 was present in two scans even though it is very weak and that the mass measurements were satisfactory for all the ions. The results for the other steroid (progesterone), which corresponds to peak 5 of the LC trace, also show quite good mass measurements. The largest deviation in this table is 5.13 m.m.u. and several of the measurements are better than 1.0 m.m.u. The same accuracy and precision is obtained on samples introduced via direct insertion probe.

When smaller amounts of the five-steroid mixture were injected (1 μg each on-column), only one scan could be obtained on the two first LC peaks and only the most abundant ions were mass measured. However, for the other three LC peaks, two or three scans could be obtained and most of the abundant ions were mass-measured.

Metastable ion scans

The VG ZAB 2F is a double-focusing instrument of reversed geometry which means that the ion source is followed by the magnetic sector, which is followed by the electric sector and terminated by the detector. A collision cell is located in the second field-free region between the magnetic and electric sectors. Using this instrument, MIKES and collision-induced dissociation (CID) spectra can be obtained. The computerization of such an instrument has been described some time ago [14]. The programs have been integrated into our new UPACS II data system running on a Harris H800 computer. In addition various methods of linked scanning can also be acquired. Thus it was interesting to obtain such data on well known compounds using the particle beam interface and compare these results with data previously published from this laboratory. The chosen test compound was cholesterol; 2.5 μg and 500 ng loop injections were tried. The spray solvents were acetonitrile–water containing 0.1% TFA (1:1). The MIKES scan of the molecular ion of cholesterol at m/z 386 is shown in Fig. 14. This corresponds to 2.5 μg of cholesterol injected. This can be compared with the results obtained previously [14]. As can be seen both scans are nearly identical. When the same experiment was performed on 500-ng injections only the most abundant metastable peaks could be detected.

A daughter ion scan using the B/E linked scanning method of the same molecular ion at m/z 386 of cholesterol was obtained. The scan in Fig. 15 was recorded on 500 ng (loop injection). This scan is very similar to the one produced when cholesterol is introduced via a direct insertion probe. It shows several of the daughter ions of cholesterol.

CONCLUSION

This study has demonstrated that a commercially available PB interface can be simply connected to a double-focusing high-resolution mass spectrometer. This combination is quite satisfactory for acquiring EI mass spectra from an LC system. The results indicated that these EI spectra are nearly identical with those obtained via direct insertion probe. In addition high-resolution mass measurements on mixtures of steroids separated by LC gave good mass accuracy and precision. The geometry of the mass spectrometer allows the acquisition of MIKES, CID and B/E-linked scans on compounds introduced through the PB inlet system. Regular reversed-phase LC columns were used at flows of 1.0 and 1.5 ml with aqueous solvents either in the isocratic or the gradient modes. The method works well with compounds amenable to EI ionization and will be complementary to CI, TSP, and FAB ionization.

ACKNOWLEDGEMENTS

Technical assistance during the installation of the PB interface and the HPLC

system was provided by Mr. G. E. Bronson and Mr. J. F. Locker. Modifications and improvements of the UPACS II MS data system were performed by Mrs. T. L. Moses and Mr. B. J. D. Vansina. The help of these individuals is greatly appreciated.

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