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FRACTIONAL SAMPLING INTERFACE FOR COMBINED LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY WITH ^{252}Cf FISSION FRAGMENT-INDUCED IONIZATION

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SUMMARY

Time-of-flight mass spectrometry (MS) utilizing ^{252}Cf fission fragment-induced ionization and desorption of non-volatile compounds is suitable as a universal detector in high-performance liquid chromatography (HPLC). The LC–MS combination is achieved by nebulizing the unsplit effluent (*ca.* 0.5 cm^3 min^{-1}) into a vacuum chamber (*ca.* 10^{-4} bar) and vacuum-drying the non-volatile compounds on collector foils. The permanent storage of the separated compounds on twelve discrete sample foils decouples the LC and MS operations. Stepping the disk interface periodically, each sample is transported into the ion source for the non-destructive MS analysis. It can be repeated afterwards and samples may be recovered for other analyses.

INTRODUCTION

The capabilities of high-performance liquid chromatography (HPLC) in the quantitative analysis of therapeutic agents[1] (actually all organic compounds with low volatility) are generally recognized. However, the use of mass spectrometry (MS) as a universal detection method[2] should overcome some problems of sensitivity, selectivity and reproducibility. Chemical ionization, field desorption and laser- or keV–MeV particle-induced desorption are soft ionization techniques[3] suitable for organic solids[4].

Common to all LC–MS combinations is the interface problem, *i.e.*, the need to separate the eluent from the dissolved compounds which must be transferred into the ion source. Commercial systems offering chemical ionization and induced desorption (fast atom bombardment) utilize a moving belt interface[5]. This performs continuous sampling of the effluent at atmospheric pressure, assists evaporation of the mobile phase using an infrared heater and overcomes the pressure decrease towards the ion source by three differentially pumped vacuum stages. The semi-permanent storage of the chromatographically separated compound on the belt decouples the LC and MS operations.

In order to use ^{252}Cf fission fragment-induced desorption[6] we have modified

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these interface solutions to obtain a stepping disk interface, which performs fractional sampling in a vacuum drying process. Again the retention time scale is transformed into a spatial distribution. As the ^{252}Cf fission fragment-induced ionization is virtually non-destructive, this sampling procedure results in a permanent storage of the collected compounds. Thus the mass spectrometry can be performed both on-line and off-line with respect to the LC sampling.

EQUIPMENT

The linkage of the LC–MS system is shown in Fig. 1 The dissolved compound is purified (when necessary extracted from the matrix) in a preparative procedure. The modular liquid chromatograph is run in the reversed-phase mode with isocratic elution. The effluent is fed unsplit, in some instances by-passing the UV detector, into the interface, which, together with the time-of-flight mass spectrometer (TOF MS), was designed and assembled at our laboratory.

The LC–MS interface

The polar effluent, typically methanol–water at a flow-rate of 0.5 $cm^3\ min^{-1}$, emerges from the stainless-steel LC capillary (0.2 mm I.D.) directly into the evacuated sampling chamber (Fig. 2). The last section of the capillary (*ca.* 2 cm) is heated to prevent plugging by freezing and to achieve proper nebulization of the effluent. When the heating power is properly adjusted, the expansion process results in a hardly visible mist formation. The resulting aerosol impinges on a 2 μm thick foil, where the non-volatile compounds are collected in a vacuum-drying process. A very thin layer of frozen effluent (water) indicates proper collection by freeze-drying

A PTFE disk (Fig. 3) sandwiched between two flanges serves as a sample carrousel and spans the pressure difference between the sampling chamber (*ca.* 10^{-4} bar) and the ion source (*ca.* 10^{-9} bar). The fractional sampling of the chromatogram in up to twelve fractions, due to twelve discrete sample positions, is sufficient for

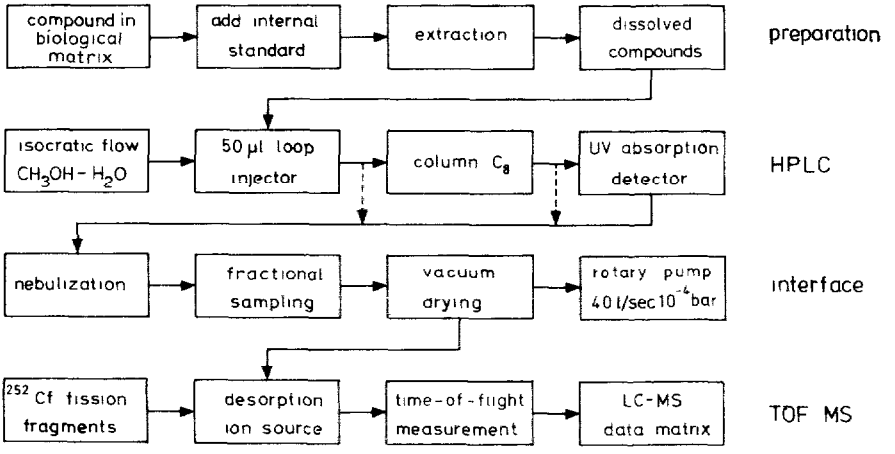

Fig 1. Flow chart of the combined LC–MS system

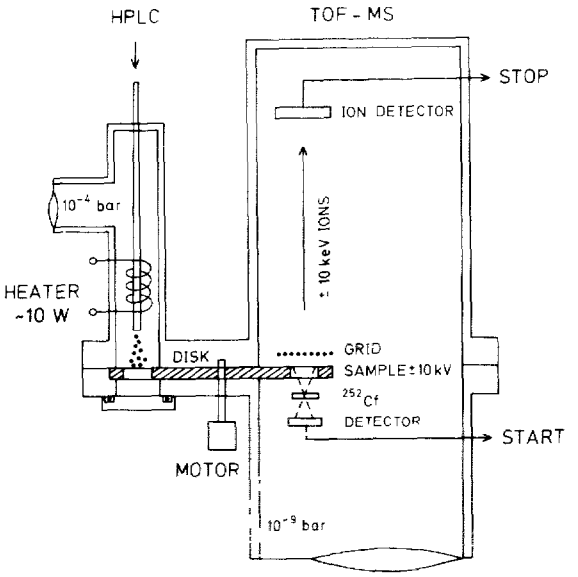

Fig 2. Time-of-flight mass spectrometer with disk interface for HPLC effluent (cut vertically along B–C in Fig 3)

most LC–MS applications. In many instances (routine analyses) it is even possible to cut a single fraction rather than monitoring the complete chromatogram.

Fractionation can be periodic (typically 1 min^{-1}) or adjusted to the chromatographic separation. The first sample foil may be analysed in the ion source (cut C in Fig 3) while the fifth foil is collected in the sampling chamber (cut B in Fig. 3) and so on. In this instance the sampling time per fraction limitis the data acquisition time. To avoid this limitation, the mass spectrometric analysis can be performed after the sampling procedure (off-line).

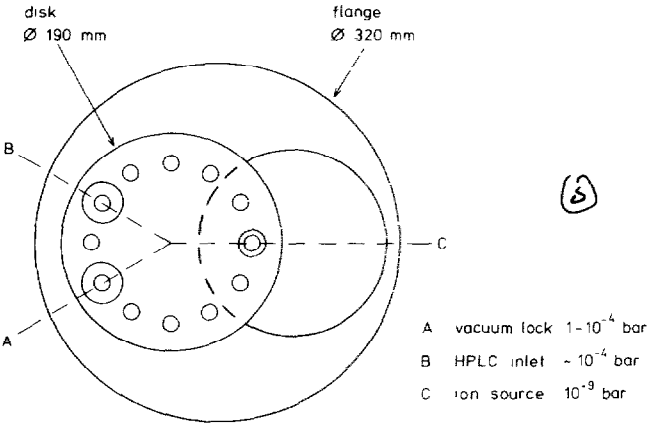

Fig 3 Top view of the sample changing disk indicating the typical pressures at the three main sections.

The time-of-flight mass spectrometer

Each sample is transported by the PTFE insulating disk into the ion source, where it is set at the acceleration potential (±10 kV) and irradiated with fission fragments (Fig. 2). The sudden (< 1 nsec) perturbation in the sample caused by each penetrating fission fragment produces quasimolecular and fragment ions of the compound. As all ions are accelerated to the same kinetic energy-to-charge ratio, E/z, their mass-to-charge ratio m/z can be determined from a time-of-flight t measurement ($m/z = 2E/zv^2 = \alpha t^2$, with a calibration constant α, velocity v)

The acquisition of each time-of-flight spectrum in a multi-channel-analyser lasts 1–10 min (depending on the sample coverage of 0.1–1 μg cm^{-2})[7,8], but it results in a complete mass spectrum (*e.g.*, m/z 1–1000) without scanning. Further, it can be repeated to obtain results on positive and negative ions from the same sample (or to obtain better statistics), as the analysis is non-destructive.

EXPERIMENTAL

The LC–MS analysis as displayed in Fig. 1 is utilized routinely to investigate the pharmacokinetics of the anti-neoplastic agent Etoposide (VP16-213) using the homologous compound Teniposide (VM26) as an internal standard[9]. For example, a 1 cm^3 serum sample is spiked with 20 μg of VM 26 and a chloroform extraction is performed. The effluent (methanol–acetonitrile–water, 2:1:1; 500 μl min^{-1}) is sampled on up to twelve foils, typically 0.5–1 min per fraction. The resulting data of matrix retention time *versus* ion mass (Fig 4) shows the quasimolecular ions $[M + H]^+$ and $[M + Na]^+$ at m/z 589 and 611 for VP16-213 (mol. wt. 588) and at m/z 657 and

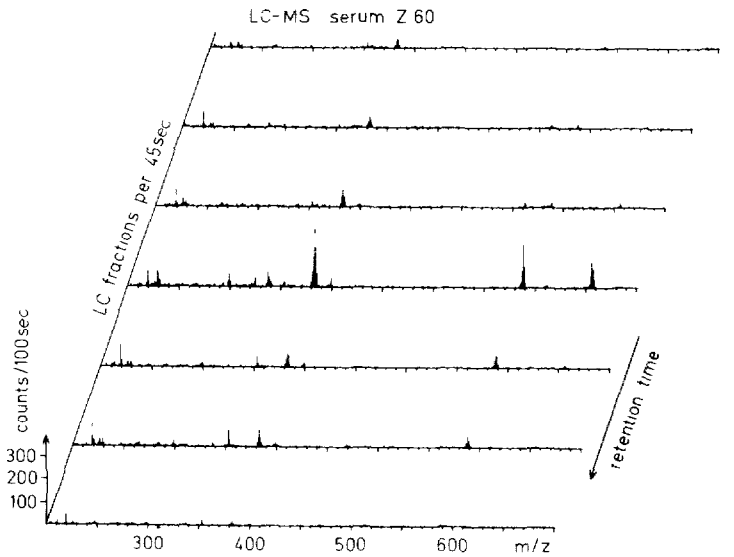

Fig 4 An LC MS data matrix given by seven spectra of seven consecutive fractions The sample was taken from a patient 2 h after a 250-mg infusion of VP16-213 (mol wt 588) and spiked with 20 μg cm^3 of VM26 (mol wt 656)

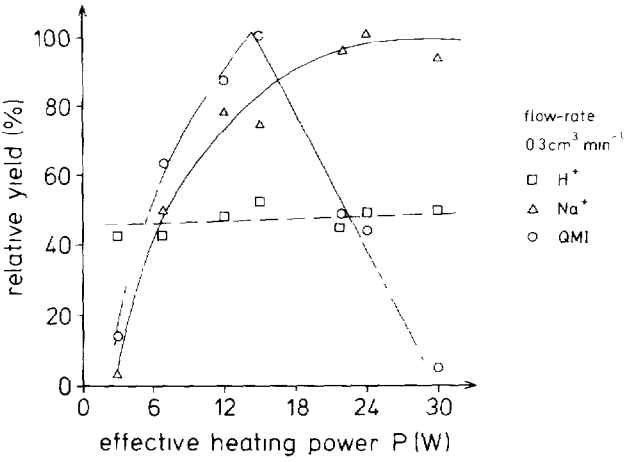

Fig. 5 Relative yields of quasimolecular ions (QMI) from Verapamil (*m/z* 453–456), Na^+ and H^+ at a flow-rate of 0 3 cm^3 min^{-1} (methanol–water) as a function of the effective heating power deposited at the end of the LC capillary

679 for VM 26 (mol. wt. 656). The retention time for both compounds differs only *ca.* 0.5 min. The concentration of VP16-213 is calculated from the quasimolecular ion intensity using a calibration graph[9].

The performance of the disk interface, *i.e.*, the collection efficiency of the non-volatile compounds and their mass spectrometric detection, depends on the following parameters: the flow-rate and composition of the polar eluent (*i.e.*, proportion of water), the heat deposited at the end of the LC capillary and the capillary nozzle–collector foil spacing. A systematic investigation of these parameters was performed by eluting the antiarrhythmic drug Verapamil (mol. wt 454) with a polar eluent (methanol–water, 3·1).

A typical result is shown in Fig. 5. obtained by using a constant flow-rate and varying the heating power, *P*. The unaffected intensity of H^+ ions demonstrates stable conditions of the MS operation. Hence the fluctuations of the yields of the other ions resemble the variations in collection efficiency. The different behaviour between the quasimolecular ions (*m/z* 453–456) and Na^+ ions indicates differences in volatility and probability that the sample sticks on the collector surface.

The complex situation for the collection of Verapamil molecules is displayed in Fig. 6 as a function of flow-rate, *F*, and effective heating power, *P*. Optimal collection efficiency is achieved at about $P/F = 35$ W min cm^{-3}, resulting in perfect nebulization of the effluent. The effluent splashes at lower and vaporizes at higher heating power.

At high power ($P/F \approx 60$ W min cm^{-3}), the resulting vapour jet not only prevents the collection of Verapamil molecules but even removes a pre-collected sample. This washing effect is proved by the following experiment. At 0.3 cm^3 min^{-1} and 12 W, a perfect sample (*ca.* 10 μg cm^{-2}) of Verapamil and its homologous compound D600 (mol. wt. 484) is collected during 1 min. The resulting mass spectrum (Fig. 7a) yields the quasimolecular ions and typical fragments of *m/z* 303 and 333. Now the effective heating power is increased to 30 W, resulting in a vapour jet of the

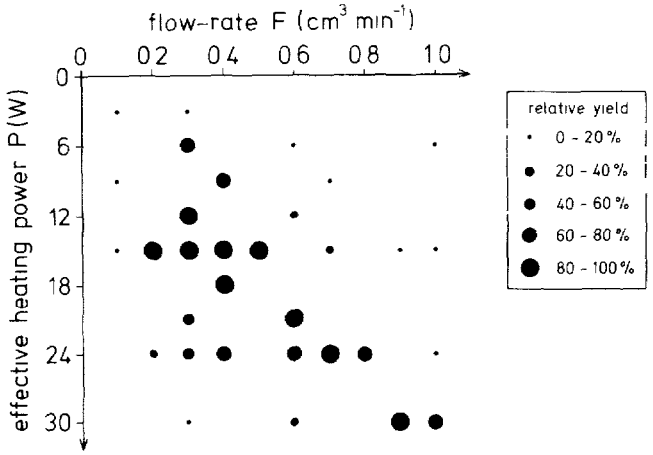

Fig 6 Relative yields of quasimolecular ions from Verapamil as a function of flow-rate, F, and effective heating power, P Optimal efficiency is achieved at $P.F \approx 35$ W min cm^{-3}.

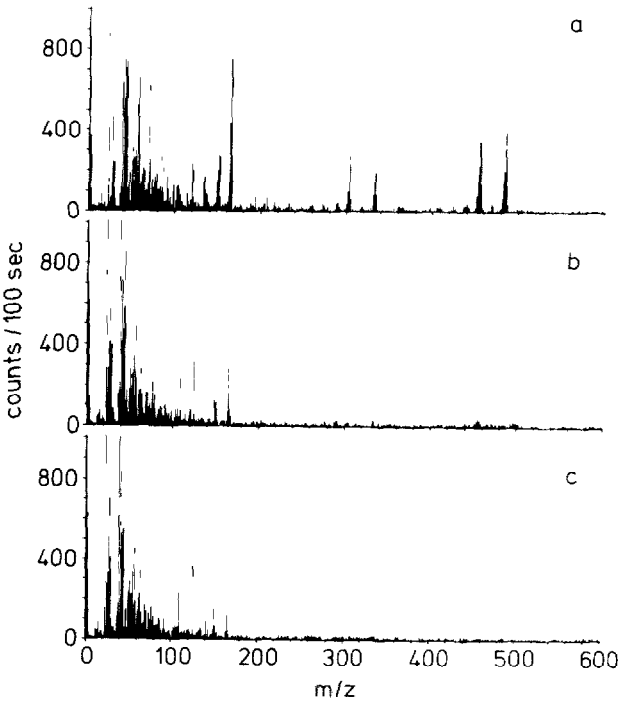

Fig 7 Mass spectra of Verapamil (fragment m/z 303 and QMI m/z 453-456) and its homologous compound D600 (fragment m/z 333 and QMI m/z 483-486) at three stages: (a) after collection under proper nebulization conditions, (b) after cleaning for 10 sec with vapour jet, and (c) after additional cleaning for 20 sec.

pure eluent. The sample is turned back into the sampling chamber, first for 10 sec, analysed (Fig. 7b) and then for a further 20 sec, and analysed again (Fig. 7c). Obviously the pre-collected sample is washed off by the vapour jet within 10–30 sec.

Under optimal nebulization conditions the divergence of the aerosol jet is so small that the diameter of the collected sample can be adjusted in the range 3–10 mm by varying the capillary nozzle–collector foil distance from 2 to 8 cm.

DISCUSSION

The LC–MS interface described here assists nebulization of the unsplit effluent by heating the end of the LC capillary, similarly to the LC–MS thermospray[10], but using approximately ten times less heating power, and collects the aerosol under medium-high vacuum conditions. This deposition and collection process involving vacuum drying is unique by different to all other storage methods, as no infrared heater is involved, which could cause the sample to volatilize or even to pyrolyse.

As the fission fragment-induced ionization is virtually non-destructive (fast ion bombardment to some extent also), our LC–MS system and an HPLC–secondary-ion MS combination[11] operate with permanent storage of the collected chromatographic distribution. As the latter LC–MS technique uses an endless belt (ribbon), which can be prepared for the next sampling cycle by a silver deposition procedure[11], this system allows continuous and unlimited monitoring. The limitation in our method due to the number of collector foils (twelve at present) could be overcome by cleaning the foils after the analysis with a hot vapour jet (as described above). However, any cleaning procedure restricts the re-analysis capability of the LC–MS system.

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