Journal of Chromatography, 346 (1985) 161–166
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 17 933

DIRECT COUPLING OF MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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(Received May 28th, 1985)

SUMMARY

An interface for direct coupling of micro high-performance liquid chromatography with fast atom bombardment mass spectrometry has been developed. The interface was made of fused-silica capillary tubing, the end of which was attached to a stainles-steel frit. The mobile phase contained glycerol, which functioned as the matrix, and the solvent was immediately vaporized on the surface of the frit. The argon beam stroke the surface of the frit and its position was adjusted so that the solute could be effectively ionized. The performance of the interface was examined using bile acids as the test solutes.

INTRODUCTION

Various methods for coupling liquid chromatography (LC) and mass spectrometry (MS) have been investigated, involving direct introduction accompanied by chemical ionization[1], a thermospray method[2,3], a moving-belt method[4], etc. However, these ionizaton techniques are unsuitable for high-molecular-weight compounds, which have been successfully analysed by fast atom bombardment MS (FABMS) and secondary-ion MS(SIMS). A few reports have dealt with the direct combination of LC with FABMS or SIMS[5,6], but techniques have been restricted to the moving-belt method owing to obstruction by the mobile phase solvent. Micro high-performance LC (micro HPLC) can overcome this problem. Micro HPLC combines well with MS because of the low flow-rates of the mobile phase[7,8].

This paper describes the direct coupling of micro HPLC with FABMS using a new interface.

0021-9673/85/$03.30

EXPERIMENTAL

Mass spectrometer

A JMS-DX 300 double-focusing mass spectrometer (JEOL, Tokyo, Japan) was employed without any modification. The argon beam energy was 6 kV and the ion source temperature was kept at 100°C. The interface was introduced from the side of the direct introduction system of the mass spectrometer.

LC–MS interface

The structure of the interface for micro HPLC–FABMS is illustrated in Fig. 1. The interface is made of fused-silica capillary tubing, 40 μm I.D. and 0.19 mm O.D. (Scientific Glass Engineering, Melbourne, Australia), which was glued into the stainless-steel tubing, 0.19 mm I.D. and 0.41 mm O.D. (Hakkoshoji, Tokyo, Japan), with epoxy-resin adhesive. The latter tubing was glued in glass tubing, 0.5 mm I.D. and 3 mm O.D., with epoxyresin adhesive for insulation. The stainless-steel frit (2 μm porosity) was attached to the outlet of the interface tubing, and only the rim of the frit was glued to the cross-section of the glass tubing. The small-diameter (1/16 in.) frit was cut from a thin plate (0.33 mm thick) in the laboratory.

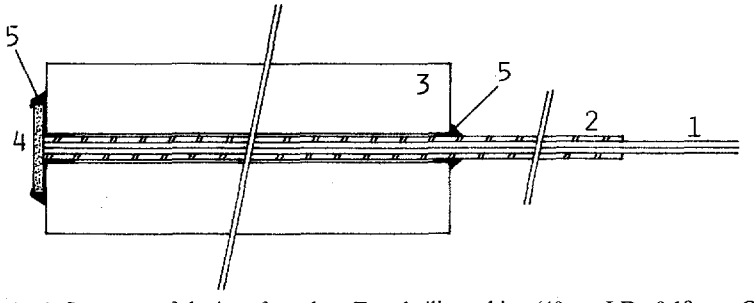

Fig. 1. Structure of the interface. 1 = Fused-silica tubing (40 μm I.D., 0.19 mm O.D.); 2 = stainless-steel tubing (0.19 mm I.D., 0.41 mm O.D.); 3 = glass tubing (0.5 mm I.D., 3 mm O.D.); 4 = stainless-steel frit; 5 = epoxy-resin adhesive.

The effluent from the column passes through the 40 μm I.D. fused-silica capillary and reaches the porous frit. The mobile phase solvent is immediately vaporized on the surface of the frit, and the solute and the matrix (glycerol) are left on the surface and are subjected to bombardment by the argon beam. The solute can be effectively dispersed in the matrix by premixing glycerol with the mobile phase.

Micro HPLC apparatus

A micro HPLC system was assembled from Micro Feeder MF-2 (Azumaden-kikogyo, Tokyo, Japan) equipped with a gas-tight syringe MS-GAN 025 (Terumo, Tokyo, Japan) as a pump, an ML-422 micro valve injector (0.02 μl; Jasco, Tokyo, Japan), a fused-silica column (50 × 0.26 mm I.D.) packed with ODS-Hypersil-5 (5 μm; Shandon, Runcorn, U.K.) and a UVIDEC-100 II UV spectrophotometer (Jasco) equiped with a small-volume (0.05 μl) flow cell, as shown in Fig. 2. Line filters (2 μm) are placed between the LC pump and the injector and between the column and

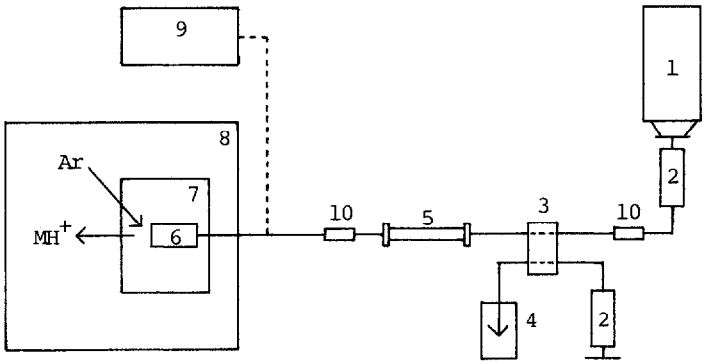

Fig. 2. Diagram of the apparatus. 1 = Micro Feeder; 2 = gas-tight syringe; 3 = sample injector; 4 = waste reservoir; 5 = separation column; 6 = interface; 7 = ion source; 8 = mass spectrometer; 9 = UV spectrophotometer; 10 = line filters.

the interface, in order to filter the mobile phase and the column effluent. The line filters are composed of unions in which a laboratory-cut stainless-steel thin frit is placed. A 1/16 in. union was used for the former line filter and a 1/32 in. union for the latter. The separation column was prepared as described previously[9].

Reagents

HPLC-grade distilled water, acetonitrile, glycerol and ammonium bicarbonate were supplied by Wako (Osaka, Japan). The mobile phase and the sample solution were filtered with a membrane filter (0.45 μm) before use. Bile acids were supplied from Sigma (St. Louis, MO, U.S.A.) or P-L Biochemicals (Milwaukee, WI, U.S.A.).

RESULTS AND DISCUSSION

In FABMS the solute must be dispersed in a matrix such as glycerol, so that it can be effectively ionized. The solute is manually mixed with the matrix in a batch process, which is unsuitable for HPLC–FABMS coupling. Premixing of glycerol with the mobile phase permits on-line dispersion of the solute in the matrix and allows the solute to be loaded with the sample injector. The concentration of glycerol in the mobile phase affects the sensitivity and the retention of the solute.

The mobile phase was a mixture of glycerol and ethanol. The ion current of glycerol was increased as the proportion of glycerol increased up to 15%, which indicates that the sensitivity increases as the proportion of glycerol increases. The ion current of glycerol in the mobile phase containing 15% glycerol was 1.2–1.3 times greater than in the mobile phase containing 10% glycerol. However, glycerol increased the viscosity of the mobile phase, and thus a proportion of 10% was selected for this work.

The flow-rate of the mobile phase should be carefully chosen because it strongly affects the pressure in the mass spectrometer. Ideally the vaporization rate of the glycerol should be the same as the rate of supply. When the flow-rate was 0.52 μl/min, the pressure arround the inlet of the diffusion pump was quite stable ($7 \cdot 10^{-6}$–$8 \cdot 10^{-6}$

Torr). Higher flow-rates of the mobile phase led to infavourable observations, such as unstable vaporization or electric discharge in the ion gun. These problems will be overcome by improving the vacuum system.

Fig. 3 demonstrates the repeated ion monitoring of 20 ng of sodium taurocholate (mol. wt. 537) without the separation column. The peak width obtained with FABMS detection was around twice that obtained with UV detection, and tailing was observed in the former. These deteriorations may be due to band spreading of the solute on the frit and the slow ionization speed. The reproducibility of the peak height was satisfactory. The signal at *m/z* 369 corresponds to the protonated tetramer of glycerol and shows the stability of ionization. It should be noted that the ion current of glycerol is decreased when the solute is ionized.

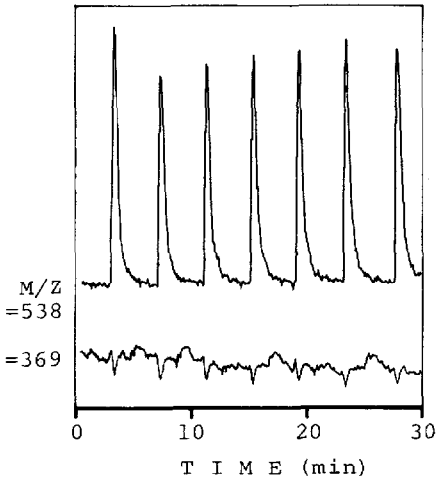

Fig. 3. Ion monitoring of bile acid and glycerol. Upper trace, *m/z* 538; lower trace, *m/z* 369. Mobile phase, glycerol–acetonitrile–water (10:27:63); flow-rate, 0.52 μl/min; sample, 20 ng of sodium taurocholate.

Fig. 4 demonstrates the separation of sodium taurocholate and sodium taurodeoxycholate 50 ng of each injected). The reconstructed ion chromatogram (RIC) and mass chromatograms are shown in the figure. The signals at *m/z* 516 and *m/z* 500 correspond to protonated taurocholic acid and protonated taurodeoxycholic acid, respectively. The RIC range was set at 450–560 in order to avoid interference from signals due to the glycerol background. Fig. 4 also shows a chromatogram obtained with UV detection. Band broadening or tailing observed with FABMS detection will be improved by adjusting the structure of the interface, the amount of glycerol used and the energy of bombarding particles.

This system allows the subtraction of the background interference due to the matrix from the solute signal, leading to high sensitivity. Figs. 5 and 6 show mass spectra of sodium taurocholate and sodium taurodeoxycholate, respectively. These spectra give structural information and are nearly the same as those obtained with the conventional off-line batch method.

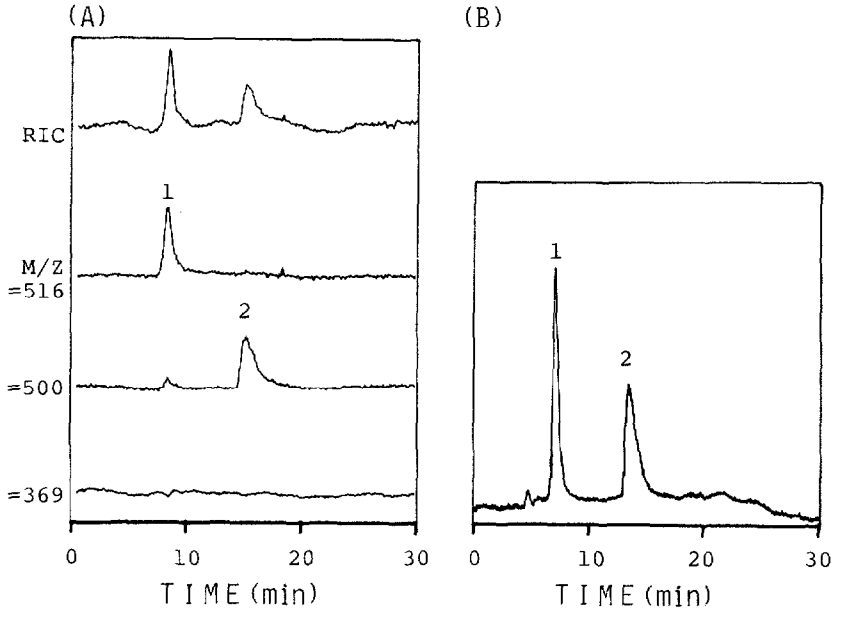

Fig. 4. Separation of sodium taurocholate and sodium taurodeoxycholate. (A) FABMS detection; (B) UV detection. Column, ODS-Hypersil-5 (50 × 0.26 mm I.D.); mobile phase, glycerol–acetonitrile–1 m*M* ammonium bicarbonate (10:22.5:67.5); flow-rate, 0.52 μl/min; RIC, *m/z* 450–560; wavelength of UV detection, 200 nm. Solutes: 1 = sodium taurocholate; 2 = sodium taurodeoxycholate.

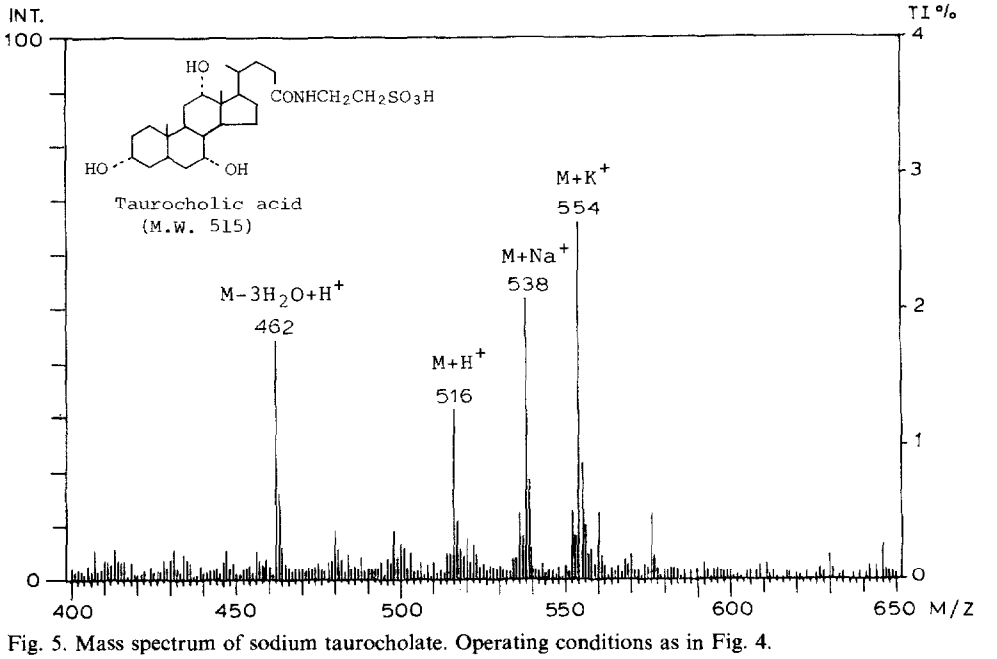

Fig. 5. Mass spectrum of sodium taurocholate. Operating conditions as in Fig. 4.

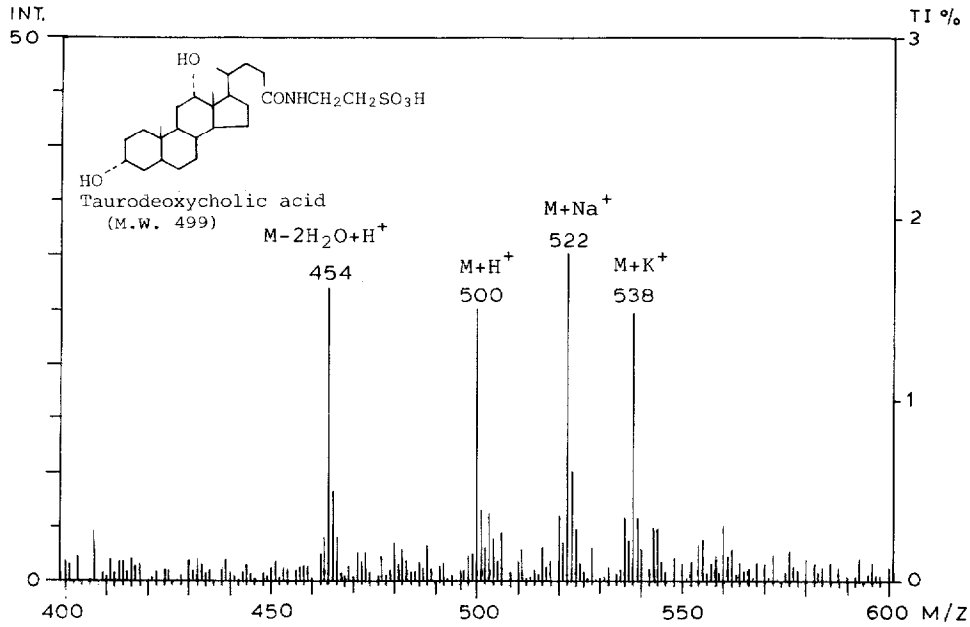

Fig. 6. Mass spectrum of sodium taurodeoxycholate. Operating conditions as in Fig. 4.

CONCLUSION

The developed interface allows the coupling of micro HPLC and FABMS, with the mobile phase premixed with the matrix. The pressure in the ion source could be kept stable. The method will also be suitable as a new sample introduction method for FABMS.

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