*Journal of Chromatography*, 358 (1986) 201–207
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 484

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

## II. APPLICATION TO GRADIENT ELUTION OF BILE ACIDS*

YUKIO ITO*, TOYOHIDE TAKEUCHI and DAIDO ISHII
*Department of Applied Chemistry, Faculty of Engineering, Nagoya University, Chikusa-ku, Nagoya-shi 464 (Japan)*
MASASHI GOTO
*Research Centre for Resource and Energy Conservation, Nagoya University, Chikusa-ku, Nagoya-shi 464 (Japan)*
and
TOKUO MIZUNO
*Analytical Instruments, Technical and Engineering Division, Jeol Ltd., 1418 Nakagami, Akishima-shi 196 (Japan)*
(Received December 31st, 1985)

SUMMARY

A new system without a moving belt has been developed for direct coupling of micro high-performance liquid chromatography with fast atom (xenon or argon) bombardment mass spectrometry. The structure of the interface was basically the same as used previously, but the mass spectrometer was modified by adding both a liquid nitrogen trap between the ion source housing and the diffusion pump, and a position adjuster for the interface. Stable ionization of the solute in a glycerol matrix is achieved at flow-rates below 2 μl/min. The system was applied to the analysis of bile acids by gradient elution chromatography. Steady baselines were observed in the mass chromatograms.

## INTRODUCTION

Direct coupling of liquid chromatography (LC) and mass spectrometry (MS) is expected to become a very powerful analytical tool. Interfaces for LC–MS have been developed by many researchers. The three popular interfaces are direct liquid introduction[1,2], thermospray[3,4] and moving belt[5,6] methods. Fast atom bombardment MS (FAB-MS)[7,8] and secondary ion MS (SIMS)[9] are especially suitable for the analysis of high-molecular-weight, polar or thermally labile compounds. How-

* For part I, see *J. Chromatogr.*, 346 (1985) 161.

0021-9673/86/$03.50 

ever, the coupling of FAB-MS–LC has been restricted to the moving belt method[10] in which the test solute is ionized in a liquid matrix such as glycerol. We have developed an on-line interface without a moving belt for a micro high-performance LC (micro HPLC)–FAB-MS system[11]. Micro HPLC combines well with MS because of the low flow-rates[12] (1–10 μl/min), which allow the introduction of the total effluent into a mass spectrometer. The application of this unique interface to gradient elution chromatography will be described in this paper.

## EXPERIMENTAL

### *Mass spectrometer and LC–MS interface*

A JMS-DX300 double-focusing mass spectrometer (Jeol, Tokyo, Japan) was employed with the following modifications. A liquid-nitrogen trap was added between the ion source housing and the diffusion pump in order to improve the ability of evacuation. The 8-in. vacuum chamber cover of the ion source housing was modified to allow the introduction of the interface through the centre part of the cover. This modification also allows the movement of the interface (backward and forward) in the ionization chamber to achieve optimum positioning. Xenon or argon atoms with 6 keV of energy were used for the bombardment. The reconstructed ion chromatogram (RIC) range was set at 350–600 *m/z*.

The structure of the interface used for micro HPLC–FAB-MS is the same as shown in Fig. 1 of the preceding paper[11]. The interface was made of fused-silica capillary tubing, 40 μm I.D. and 0.19 mm O.D., and a stainless-steel frit (2 μm porosity) was attached to the cross-section of the capillary tubing. Glycerol was premixed in the mobile phase (*ca.* 10%) to serve as the matrix of FAB ionization. The effluent from the column passes through the capillary tubing and reaches the porous frit. The mobile phase solvent is immediately vapourized on the surface of the frit, while the solute and the matrix (glycerol) are left on the surface and are subjected to bombardment by the argon or xenon beam.

### *Micro HPLC apparatus*

Fig. 1 shows the HPLC system used. It was assembled using a Micro Feeder MF-2 (Azumadenkikogyo, Tokyo, Japan) pump equipped with a gas-tight syringe

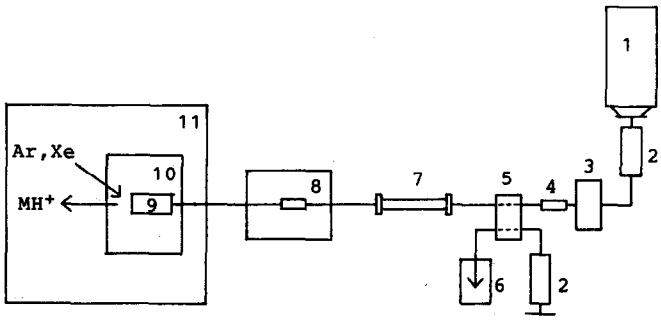


Fig. 1. Diagram of the apparatus. 1 = Micro feeder; 2 = gas-tight syringe; 3 = gradient maker; 4 = line filter; 5 = sample injector; 6 = waste reservoir; 7 = separation column; 8 = UV spectrophotometer; 9 = interface; 10 = ion source housing; 11 = mass spectrometer.

MS-GAN 050 (0.5 ml; Terumo, Tokyo, Japan), a laboratory-built gradient maker, a micro valve injector (0.02 μl; Jasco, Tokyo, Japan), a home-made separation column and a UVIDEC-100 II UV spectrophotometer (Jasco) equipped with a small volume (0.05 μl) flow cell. The separation column was made from fused-silica tubing (100 × 0.26 mm I.D.) and packed with Hypersil ODS (5 μm; Shandon, Runcorn, U.K.) as described elsewhere[13]. The gradient maker constists of a mixing vessel and a magnetic stirrer Model SS-5 (Toyokagaku-sangyo, Tokyo, Japan). The mixing vessel is a modified gas-tight syringe with a volume of 109 μl, as described in a previous paper[14]. The resulting gradient profile is exponential and depends on the volume of the mixing vessel and the mobile phase flow-rate. Glycerol–acetonitrile–10 m*M* ammonium bicarbonate (9:18:73) was employed as the initial solution, and glycerol–acetonitrile–10 m*M* ammonium bicarbonate (9:50:41) was supplied from the pump. The flow-rate for gradient elution was 1.04 μl/min.

*Reagents*

Cholic acid (C), deoxycholic acid (DC) and lithocholic acid (LC) were purchased from Sigma (St. Louis, MO, U.S.A.). Glycocholic acid (GC), sodium salts of glycodeoxycholic acid (GDC), glycolithocholic acid (GLC), taurocholic acid (TC), taurodeoxycholic acid (TDC) and taurolithocholic acid (TLC) were purchased from P-L Biochemicals (Milwaukee, WI, U.S.A.). Other reagents were obtained from Wakko (Osaka, Japan), unless otherwise stated.

## RESULTS AND DISCUSSION

The system equipped with a liquid-nitrogen trap provides stable ionization at flow-rates up to 2 μl/min when using an aqueous mobile phase. Without the trap,

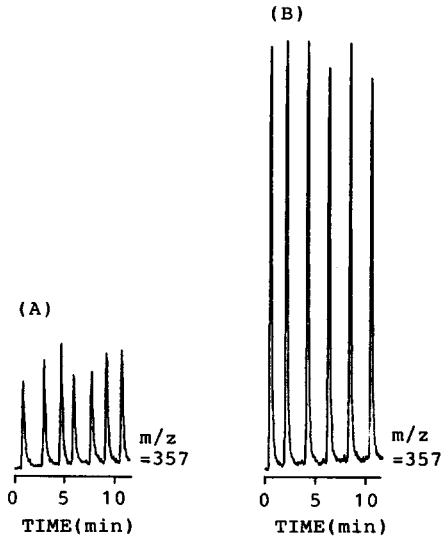


Fig. 2. Effect of bombarding atoms on ionization of a bile acid. (A) argon bombardment; (B) xenon bombardment. Mobile phase, glycerol–acetonitrile–water (10:27:63); flow-rate, 2.08 μl/min; sample, 20 ng of deoxycholic acid.

the upper limit of flow-rates is 0.5 μl/min. Fig. 2 shows the effect of bombarding atoms on ionization of a bile acid. The interface was directly connected to the sample injector and 20 ng of deoxycholic acid (mol.wt. 392) were injected as the sample $(M - 2H_2O + H)^+$ (*m/z* 357) was monitored as the base peak ion. Glycerol–acetonitrile–water (10:27:63) was used as the mobile phase with a flow-rate of 2.08 μl/min. The ionization chamber temperature was kept at 190°C. The sensitivity and reproducibility of the results obtained using xenon bombardment show appreciable improvement over those obtained with argon bombardment. This is due to the more efficient sputtering achieved with xenon bombardment.

Fig. 3 shows a typical gradient separation of a standard mixture of nine bile acids. The bile acids were dissolved in ethanol, and a 0.02-μl sample containing 40 ng of each bile acid was injected with a micro valve injector. The drift of the baseline obtained with UV detection at 205 nm reflects the gradient profile of acetonitrile in the mobile phase. The nine bile acids are separated well by the gradient elution. Nine peaks are seen in the RIC of FAB-MS detection, while only seven peaks are observed with UV detection due to small absorptivity of free bile acids at this wavelength. The signal at *m/z* 461 in the mass chromatograms corresponds to the protonated pentamer of glycerol and shows the stability of ionization. The base peak signals of the tested bile acids were monitored. The peak width obtained with FAB-MS detection is 1.5 times wider than that obtained with UV detection. This is due to both band spreading in the UV cell and on the frit of the interface, and slow ionization speed. The temperature of the ion source affects the ionization stability significantly. As the concentration of water in the mobile phase decreases, the ion source temperature has to be decreased to keep the temperature of the interface constant. Otherwise, the ion intensity of glycerol is changed. This is probably due to a decrease in the amount of glycerol on the frit at temperatures higher than the optimal value. When the temperature is lower than the optimal value, unstable vapourization of the mobile phase

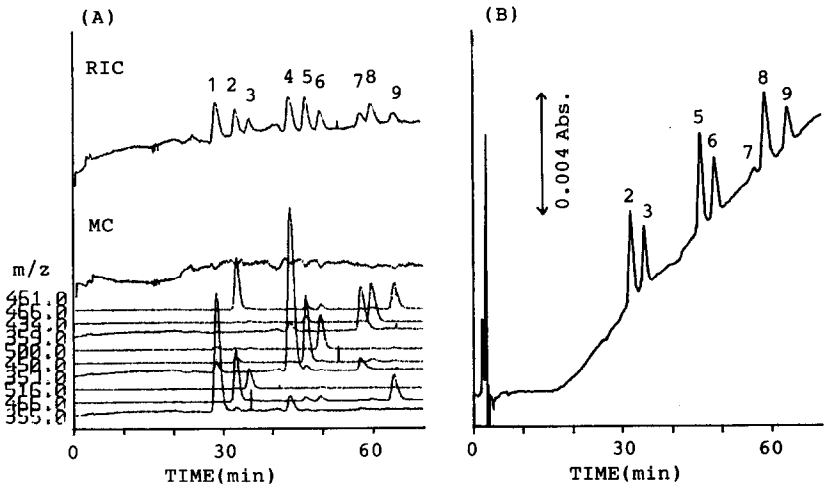


Fig. 3. Separation of standard bile acids with gradient elution. (A) FAB-MS (RIC and MC) detection; (B) UV detection. Column, Hypersil ODS (5 μm), 100 × 0.26 mm I.D.; mobile phase, see text; flow-rate, 1.04 μl/min; RIC, *m/z* 350–600; wavelength of UV detection, 205 nm; sample, 40 ng each. 1 = C, 2 = GC, 3 = TC, 4 = DC, 5 = GDC, 6 = TDC, 7 = LC, 8 = GLC, 9 = TLC.

occurs. In the gradient elution, the initial temperature was set at 160°C and the final temperature was changed to 150°C, manually according to the concentration of acetonitrile in the mobile phase.

This system allows the subtraction of the background interferences present in the sample matrix, leading to high sensitivity. Fig. 4 shows the mass spectra corresponding to the peak (A) and the valley (B) of the RIC for taurodeoxycholic acid (mol.wt. 499) shown in Fig. 3. The bottom trace (C) in Fig. 4 is the subtracted spectrum A − B, in which the background due to the matrix is clearly removed. The peaks at $m/z$ = 464, 500, 517 and 538 correspond to $(M - 2H_2O + H)^+$, $(M + H)^+$, $(M + NH_4)^+$ and $(M + K)^+$, respectively. The spectra obtained by this method coincide with those obtained by a conventional off-line batch method.

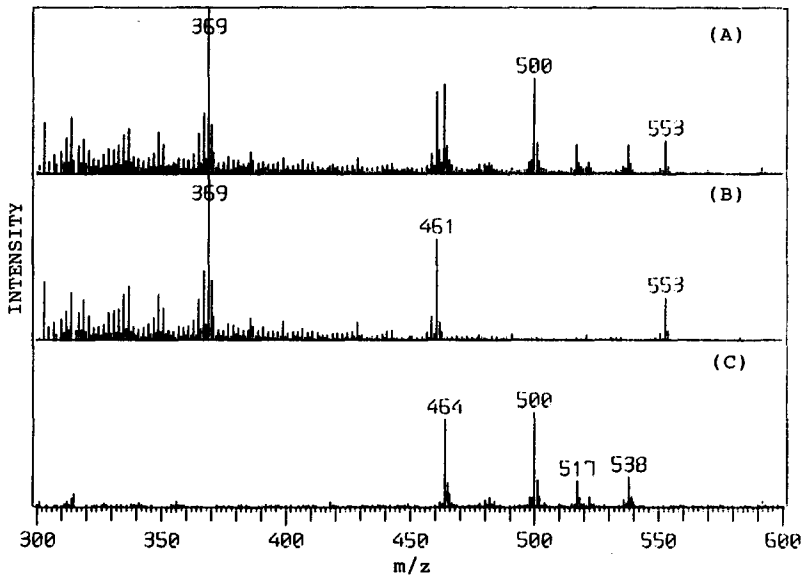


Fig. 4. Mass spectra of taurodeoxycholic acid. (A) Spectrum at the peak in the chromatogram; (B) spectrum at the valley in the chromatogram; (C) subtracted spectrum A − B. Operating conditions as in Fig. 3.

This system was applied to the analysis of bile acids in dehydrated gall bladder as shown in Fig. 5. A 0.5-g sample of dehydrated gall bladder was extracted with 10 ml of ethanol under ultrasonic vibration and filtered through a membrane filter (0.45 μm). A portion (0.02 μl) of the filtrate was injected. The used column cannot separate all the bile acids, but it is possible to observe from the mass spectrum information on whether the peak contains more than one component or not. Fig. 6 shows the mass spectrum of the fifth peak in chromatogram (A) in Fig. 5. It is clear that this peak is composed of two bile acids. By comparing the mass spectrum of each standard bile acid, these components are identified as deoxycholic acid (DC) and glycochenodeoxycholic acid (GCDC). The peaks at $m/z$ = 450, 432 and 414 correspond to $(M + H)^+$, $(M - H_2O + H)^+$ and $(M - 2H_2O + H)^+$ of the former, and the peaks of $m/z$ 485, 375 and 357 correspond to $(M + \text{glycerol} + H)^+$, $(M - H_2O + H)^+$ and $(M - 2H_2O + H)^+$ of the latter, respectively.

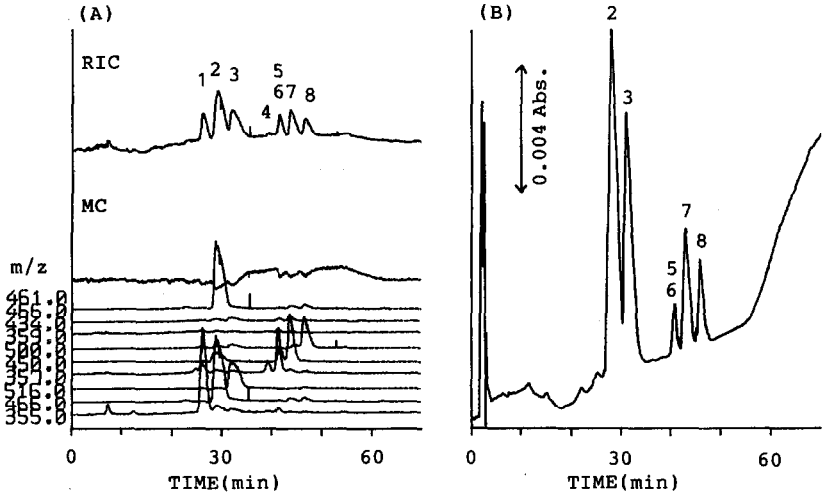


Fig. 5. Separation of bile acids in dehydrated gall bladder. (A) FAB-MS (RIC and MC) detection; (B) UV detection. Operating conditions as in Fig. 3; sample, see text. 1 = C, 2 = GC, 3 = TC, 4 = CDC, 5 = DC, 6 = GCDC, 7 = GDC, 8 = TDC.

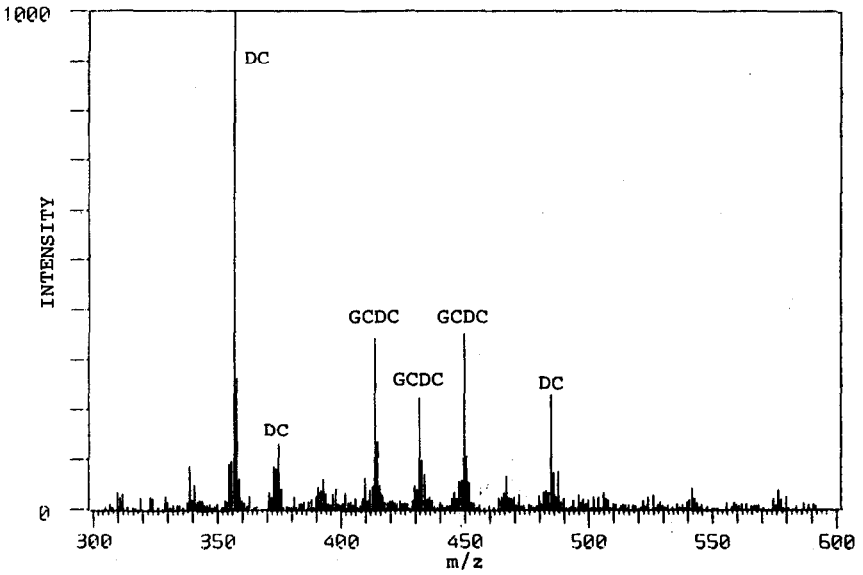


Fig. 6. Mass spectrum of the mixture of deoxycholic acid (DC) and glycochenodeoxycholic acid (GCDC). Operating conditions as in Fig. 3.

## CONCLUSION

The developed system allows the subtraction of interferences due to the matrix (glycerol), providing high sensitivity. This system allows stable ionization even for gradient elution with an aqueous mobile phase. The mass spectra give valuable

qualitative information on the chromatographic peaks. Studies are being conducted to minimize peak broadening. The system will be applied to micro flow injection analysis.

## REFERENCES

1 J. A. Apffel, U. A. T. Brinkman and R. W. Frei, *Anal. Chem.*, 55 (1983) 2280.
2 E. D. Lee and J. D. Henion, *J. Chromatogr. Sci.*, 23 (1985) 253.
3 C. R. Blakley and M. L. Vestal, *Anal. Chem.*, 55 (1983) 750.
4 R. D. Voyksner and C. A. Haney, *Anal. Chem.*, 57 (1985) 991.
5 R. P. W. Scott, C. G. Scott, M. Munroe and J. Hess, Jr., *J. Chromatogr.*, 99 (1974) 395.
6 M. J. Hayes, E. P. Lankmayer, P. Vouros, B. L. Karger and J. M. McGuire, *Anal. Chem.*, 55 (1983) 1745.
7 M. Barber, R. S. Bordoli, R. D. Sedgwich and A. N. Tyler, *J. Chem. Soc. Chem. Commun.*, (1981) 325.
8 K. L. Rinehart, Jr., *Science*, 218 (1982) 254.
9 K. Harada, M. Suzuki and H. Kambara, *Org. Mass Spectrom.*, 17 (1982) 386.
10 J. G. Stroh, J. C. Cook, R. M. Milberg, L. Brayton, T. Kihara, Z. Huang, K. L. Rinehart, Jr. and I. A. S. Lewis, *Anal. Chem.*, 57 (1985) 985.
11 Y. Ito, T. Takeuchi, D. Ishii and M. Goto, *J. Chromatogr.*, 346 (1985) 161.
12 T. Takeuchi, Y. Hirata and Y. Okumura, *Anal. Chem.*, 50 (1978) 657.
13 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 213 (1981) 25.
14 T. Takeuchi and D. Ishii, *J. Chromatogr.*, 253 (1982) 41.