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Analysis of Bile Acids and Cholesterol by Open Tubular Glass Capillary-Selected Ion Monitoring

A method for the baseline separation of the dimethylethylsilyl (DMES) ether derivatives of bile acid ethyl esters and cholesterol was developed by using selected ion monitoring (SIM) with open tublar glass capillary column coated with SE-30. The DEMS ether derivatives of bile acid ethyl esters were eluted in the regular order according to the number of hydroxyl group in the molecule. The detection limit of the DMES ether derivative of lithocholic acid ethyl ester was 5 pg with *S/N* ratio at 5:1.

Keywords——bile acids; profile analysis; dimethylethylsilyl ethers; capillary SIM; WCOT column;

In biomedical field, much attention has been focused on the development of an analytical method for metabolic profile of endogenous substances in biological fluids, because it may provide a valuable information to elucidate their physiological role.

Gas chromatography (GC) equipped with open tubular glass capillary column has already been used as a powerful tool for separation of endogenous substances, especially such as steroids[1] and prostaglandins[2,3] which have very similar structure one another. The combination of high performance capillary GC and extremely sensitive selected ion monitoring (SIM) has been expected to bring great advantages in the analysis of endogenous substances in biological fluids.

GC separation of bile acids has been performed exclusively on packed column with various stationary liquid phases using various derivatives suitable for enhancing the GC resolution.[4–6] However, it may be difficult to apply these conventional methods using a packed column to the analysis of metabolic profile of cholesterol bile acid transformation.

The present communication deals with a complete separation method by GC-SIM with wall-coated open tubular glass capillary column (WCOT) for the compounds related to biotransformation from cholesterol to bile acids.

In this study, bile acids (lithocholic acid: LCA, deoxycholic acid: DCA, chenodeoxycholic acid: CDCA, ursodeoxycholic acid: UDCA and cholic acid: CA) were converted to their

1) A.L. German, C.D. Pfaffenberger, J-P. Thenot, M.G. Horning and E.C. Horning, *Anal. Chem.*, **45**, 930 (1973).
2) J. Maclouf, M. Rigaud, J. Durand and P. Chebroux, *Prostaglandins*, **11**, 999 (1976).
3) F.A. Fitzpatrick, *Anal. Chem.*, **50**, 47 (1978).
4) P. Eneroth and J. Sjövall, in P.P. Nair and D. Krotchervsky (Eds.). "The Bile Acids," vol. I, Plenum Press, New York, 1971. p. 121,
5) K. Imai, Z. Tamura, F. Mashige and T. Osuga, *J. Chromatogr.*, **120**, 181 (1976).
6) H. Miyazaki, M. Ishibashi and K. Yamashita, *Biomed. Mass Spectrom.*, in press.

methyl (Me) ester–trimethylsilyl (TMS) ether, Me ester–dimethylethylsilyl (DMES) ether, ethyl (Et) ester–TMS ether and Et ester–DMES ether[7] derivatives.

It was found that the WCOT provided the excellent resolution for the TMS ethers of cholesterol and bile acid Me (or Et) esters, whereas the separation of these derivatives was very difficult by the use of packed column with non-polar stationary liquid phases such as SE-30 and OV-101. Of these derivatives, moreover, the DMES ether derivatives of bile acid Et esters and cholesterol exhibited the most excellent separation.

The DMES ether derivatives of bile acid Me esters and Et esters were eluted in the regular order according to the number of hydroxyl group in the molecule, whereas in the case of the corresponding TMS ethers the elution order of UDCA and CA was interchanged. Figure 1 shows the selected ion recordings of the TMS ether derivatives of cholesterol and bile acid Me esters and the DMES ether derivatives of cholesterol and bile acid Et esters. Appearance of sharp peak is profitable for the qualification and the quantitation of the trace amount of the compound such as LCA which exhibits hepatoxic action.

The detection limit of the Et ester-DEMS ether derivative of LCA was 5 pg with S/N ratio at 5:1, and the similar results were obtained in the case of cholesterol and other bile acids.

Capillary GC-SIM not only makes it possible to enhance the sensitivity of the microanalysis of bile acids but also is useful for the study of the biotransformation of cholesterol into bile acids.

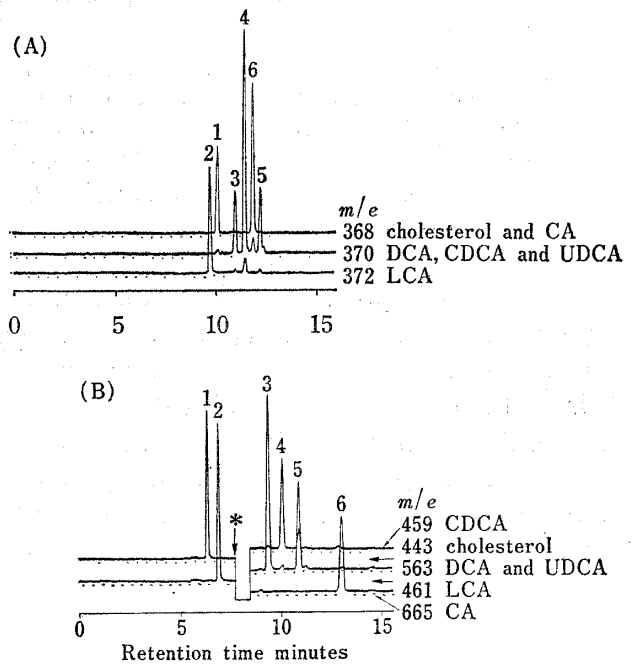

Fig. 1. Selected Ion Recordings of (A) the TMS Ether Derivatives of Bile Acid Me Esters and Cholesterol and (B) the DMES Ether Derivatives of Bile Acid Et Esters and Cholesterol

Bile acids and cholesterol were treated with 5% HCl-methanol (or ethanol) solution and then with trimethylsilyl or dimethylethylsilyl imidazole.[7] A mixture of 10 ng each of these derivatives was injected to a gas chromatograph-mass spectrometer. GC-SIM was carried out by their characteristic ions. 1: cholesterol, 2: LCA, 3: DCA, 4: CDCA, 5: UDCA and 6: CA. An LKB-2091 GC-MS system equipped with the Van den Berg's solventless injector was employed. Column: WCOT, 25 m×0.35 mm I.D. with SE-30 (LKB-Produckter, Stockholm, Sweden), column temperature: (A) 265° and (B) 275°, trap current: 100 μA, ionization voltage: 22.5 eV, ion source temperature: 290°, carrier gas: helium 1.5 ml/min.
*) In selected ion recording of (B), the ions monitored were changed from *m/e* 443 and 461 to *m/e* 459, 563 and 665 at 7.5 min after injection.

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7) H. Miyazaki, M. Ishibashi, M. Itoh and T. Nambara, *Biomed. Mass Spectrom.*, **4**, 23 (1977).