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Analysis of Bile Acids by Mass Fragmentography —Application of 1:1 Mixture Technique by Use of Stable Isotope labeled Compounds—

Mass fragmentography developed by Sweeley, *et al.*[1] is not only very sensitive but also highly specific for qualitative and quantitative analyses of organic compounds because the informations concerning to characteristic fragment ions and their inherent intensities in combination with gas chromatographic retention time are obtained by this technique. Therefore, this has been used as one of the most excellent techniques for microdetermination of drugs and endogenous substances in biological fluids. Consequently, a great number of valuable applications have been reported as reviewed by Gorden and Frigerio.[2]

In clinical field, however, it is feared that this analysis may be affected by unexpected impurities in biological fluids because of the existance of different kinds of them due to the nature of disease and individual difference of patients. If a single ion monitoring technique is applied to the above sample, therefore, it will be difficult to assure that no contaminants contribute to a peak on mass fragmentogram.

In order to solve this serious problem, a few techniques utilizing the relative intensities as a profitable information for qualification have been developed.[3-5] Owing to the limitation of mass range in multiple ion detection with a mass spectrometer of sector type, however, the above techniques often require a tedious operation which compells to repeat monitoring over all characteristic fragment ions for identification.

In this connection, the present communication provides a convenient technique which enables to free from the limitation and also the mesurements of the ratio of peak heights or areas for qualification.

The principle of the presented technique bases upon the monitoring over a pair of adjointing characteristic fragment ions and these ions are obtained from the derivatives prepared with a mixture of protium and deuterium labeled reagents in the ratio of 1:1. Hence, it seems easy to detect the aimed compounds only by observing the peaks which keep the definite ratio on a mass fragmentogram, and moreover, it is expected to obtain an useful information that no contaminants are contained in these peaks.

Thus, the authors applied this technique to authentic cholic acid followed by the analysis of bile acids in rat serum. Cholic acid was esterified by using the equal molar of methanol and perdeuteromethanol saturated with dry hydrogen chloride at room temperature. After 1 hr, the excess reagents were evaporated to almost dryness and the residue was trimethylsilylated with trimethylsilyl imidazole.

The mass spectrum of these derivatives of cholic acid is shown in figure 1. It is observed that the characteristic fragment ions keeping its methyl ester group are accompanied with the new fragment ions (m/e=371, 461, and 626) which shifted to 3 mass units from the m/e=368, 458 and 623 corresponding to native ones. Furthermore, figure 1 suggests the occurence of little isotope effect in the esterification process because the ratio of the intensities of these doublet peaks agreed closely with that of the mixture of methanol and perdeuteromethanol.

In the case where the present technique is applied to analysis of bile acids in rat serum, the paired peaks due to dihydroxycholanoic acids are easily distinguished from many other

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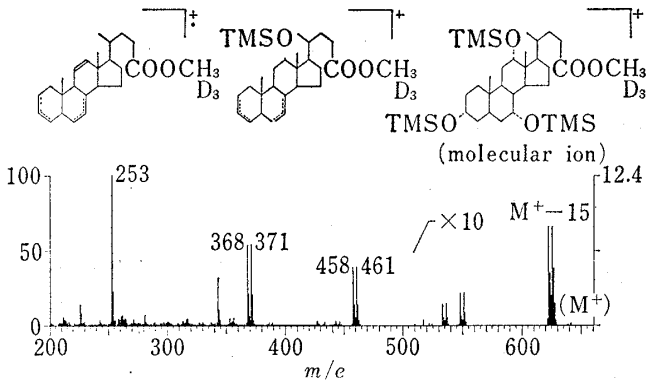

Fig. 1. Mass Spcetrum of Methyl/Trideuteromethyl Ester-O-Trimethylsilyl Ether Derivatives of Cholic Acid

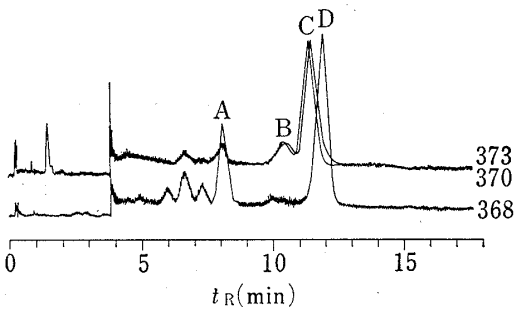

Fig. 2. Mass Fragmentogram of Bile Acids (A: Cholic, B: Deoxycholic and C: Chenodeoxycholic Acids) and Cholesterol (D) in Rat Serum monitored over Characteristic Fragment Ions

an LKB-9000 GC-MS System equipped with MID was employed, column: 3% Poly-I 2 m on Chromosorb W-HP, column temp.: 260°, ionization current: 60 μA ionization volt.: 20 eV, accelerating volt: 3.5 kV, ion source temp.: 290°, carrier gas (Helium): 30 ml/min

peaks as shown in figure 2. And also these doublet fragment ions having the same structure but the different mass number should have given a free interval of the *m/e* values if the paired peaks had not shown a definite ratio. Thus, it is expected to obtain more satisfactory results by using more suitable reagents.

Moreover, this technique will be applied conveniently to analysis of endogenous substances which are difficult to use a conventional 1: 1 mixture technique,[6] and also enable to carry out simultaneously qualitative and quantitative analyses in picogram level of endogenous substances with high reliability.

The authors hope the present technique to be used for the investigation of biological substances in the field of clinical pharmacology and biochemistry.

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Fluorometric Assay of Bisulfite

One of the most commonly used methods for the determination of sulfite or bisulfite is the West-Gaeke method[1] and modified colorimetric methods[2] using acid-bleached pararo-

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