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THE IMPROVED METHOD OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF INDIVIDUAL BILE ACIDS: FREE AND GLYCINE-CONJUGATED BILE ACIDS

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The improved method of high performance liquid chromatographic separation of bile acids, free and conjugated with glycine, is described. The analysis of free and glycine-conjugated bile acids is based on the esterification of the carboxylic group of bile acids with 4-bromomethyl-7-methoxycoumarin (BMC). These derivatives of bile acids can be separated by high performance liquid chromatography and detected on high sensitivity with a fluorescence detector.

In 1976, we already described the high performance liquid chromatographic analysis of individual bile acids, which are free, glycine- and taurine-conjugated[1]. Since BMC has been reported as an excellent reagent for derivatizing fatty acids[2], the derivatization of bile acids with BMC was attempted to improve chromatographic analysis of individual bile acids.

As we found that the derivatives of bile acids with BMC possess characteristic and strong fluorescence, the application of this character for the analysis of these bile acids was done.

Materials and Methods

The bile acids were obtained from Sigma, St. Louis. Mo., Calbiochem, San Diego, Calif. and BMC was obtained from Regis Chemical Co.. A Jasco Trirotor high pressure liquid chromatograph was used throughout this work. The instrument was fitted with a Jasco FP-550 spectrofluorometer, a gradient elution accessory and Sic Intelligent Integrator 5000A. SC-02 Column, which was commercially available from Jasco Co. for reversed-phase liquid chromatography, was used for the analysis of derivatives of bile acids. On the analysis of free and glycine-conjugated bile acids, the condition was as follows: column, SC-02 (25 cm x 0.46 cm i.d.); mobile phase, A-70% methanol in H_2O, B-85% methanol in H_2O; gradient slope, 1.5%/min; flow rate, 1 ml/min; detector, fluorescence λ_{ex}= 360 nm, λ_{em}= 410 nm.

Procedure

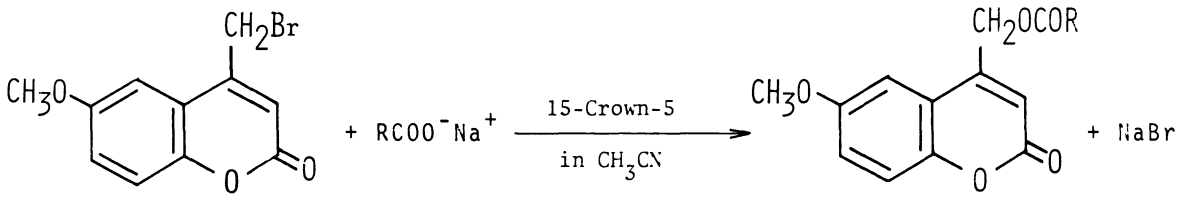
The sodium salts of bile acids were treated with BMC and 15-crown-5 in acetonitrile for 1 h at room temperature as illustrated in Scheme. Jugding from each peak area on the chromatogram, this reaction proceeds quantitatively. Especially, the structure of the coumarin derivative of cholic acid was secured by the spectroscopic analysis.

Results and Discussion

The separation of these coumarin esters of individual bile acids including unconjugated (free) and glycine-conjugated bile acids was investigated by using high performance liquid chromatography. The separation is very clear and simple as shown in Fig. 1. Each peak is equivalent with 50 ng of bile acids. The estimated detection limit was about 10 ng for any individual bile acid.

From these results, we could achieve the improvement of the detectability of bile acids by

the derivatization with BMC and the simplification of procedure, compared with our previous studies[1,3]. Accordingly, this method described in this paper is applicable for the estimation of individual free and glycine-conjugated bile acids in serum.



Scheme

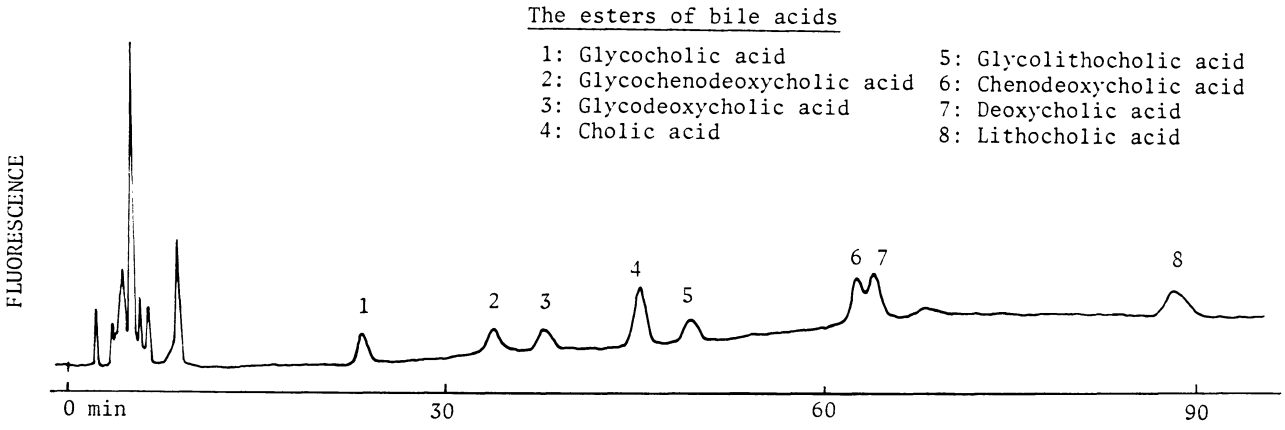


Fig. 1. Liquid chromatographic analysis of free and glycine-conjugated bile acids.

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