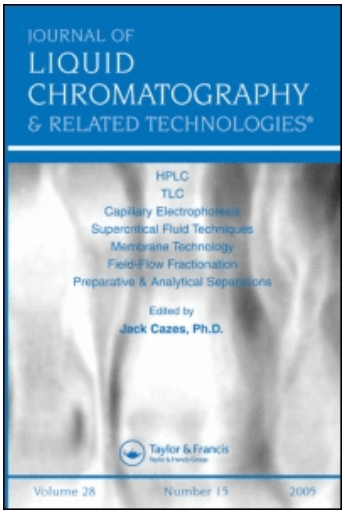


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Chromatographic Behavior of Bile Acids Using Cyclodextrin in Mobile Phase of High Performance Liquid Chromatography

Kazutake Shimada[a]; Yoshihiro Komine[a]; Tomoyuki Oe[a]
[a] Faculty of Pharmaceutical Sciences, Kanazawa University, Kanazawa, Japan

To cite this Article Shimada, Kazutake , Komine, Yoshihiro and Oe, Tomoyuki(1989) 'Chromatographic Behavior of Bile Acids Using Cyclodextrin in Mobile Phase of High Performance Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 12: 4, 491 — 500
To link to this Article: DOI: 10.1080/01483918908051752
URL: http://dx.doi.org/10.1080/01483918908051752

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(4), 491-500 (1989)

CHROMATOGRAPHIC BEHAVIOR OF BILE ACIDS USING CYCLODEXTRIN IN MOBILE PHASE OF HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

KAZUTAKE SHIMADA*, YOSHIHIRO KOMINE AND TOMOYUKI OE

Faculty of Pharmaceutical Sciences
Kanazawa University
13-1 Takara-machi
Kanazawa 920, Japan

ABSTRACT

The chromatographic behavior of bile acids was examined by the addition of cyclodextrin to the mobile phase of reversed-phase high performance liquid chromatography. The separation of bile acids was much improved by the addition of β-cyclodextrin. The capacity factors of bile acids having a 12 α-hydroxyl group were not so influenced but those of other bile acids were decreased sharply by the additives.

The response of fluorescence labeled derivative of bile acid to a fluorescence detector was raised with an addition of cyclodextrin to the mobile phase.

INTRODUCTION

In the previous paper we reported the use of cyclodextrin (CD) in the mobile phase, which is of great advantage to the separation of isomeric estrogens, cardenolides and their fluorescence detection in reversed-phase high performance liquid

chromatography (HPLC) [1, 2]. In recent years considerable attention has been directed to the biodynamics of bile acids in patients with hepatobiliary diseases, and the development of reliable methods is urgently required for the analysis of profiles of bile acids in biological materials (Figure 1). HPLC was widely used for the separation and determination of bile acids without prior hydrolysis and/or solvolysis [3, 4]. On the other hand Snopek et al. used CDs as leading electrolyte additives in isotachophoresis for the separation of bile acids [5]. These data prompted us to examine chromatographic behavior of bile acids using CD in the mobile phase of reversed-phase HPLC. The effect of CD on the fluorescence detector response has also been investigated.

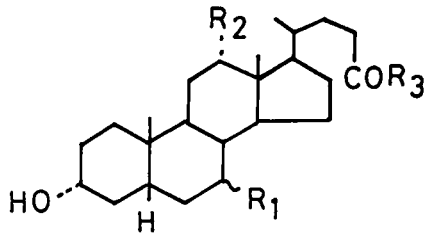
MATERIALS AND METHODS

Materials

CDs were kindly supplied by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan). Heptakis-(2,6-di-O-methyl)-β-CD (Me-β-CD) was prepared and donated by Kao Co. (Tokyo). Bile acids and 4-bromomethyl-7-methoxycoumarin were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). 1-Anthroyl cyanide was purchased from Wako Pure Chem. Ind., Ltd. (Tokyo). Solvents were purified by distillation prior to use.

Apparatus

HPLC was carried out on a Shimadzu LC-6A chromatograph equipped with a Shimadzu SPD-6A ultraviolet detector (UV; 210 nm)(Shimadzu Co., Ltd., Kyoto, Japan) or Hitachi F-1000 fluorescence detector (FL)(Hitachi Ltd., Tokyo) at a flow rate of



I :R_1=$\overset{H}{\underset{OH}{\lessdot}}$, R_2=OH

II :R_1=$\overset{H}{\underset{OH}{\lessdot}}$, R_2=H

III:R_1=H, R_2=OH

IV :R_1=$\overset{OH}{\underset{H}{\lessdot}}$, R_2=H

V :R_1=R_2=H

a :R_3=OH

b :R_3=$NHCH_2COOH$

c :R_3=$NHCH_2CH_2SO_3H$

FIGURE 1. Structures of Bile Acids.

1 ml/min. A Develosil ODS-5 (5 μm) column (15 cm x 0.4 cm i.d.) (Nomura Chemical Co., Seto, Japan) was used at ambient temperature. The pH of the mobile phase was adjusted with H_3PO_4. The dead volume was determined by the use of $NaNO_3$.

Derivatization Methods

The derivatizations of bile acids with 4-bromomethyl-7-methoxy coumarin and 1-anthroyl cyanide were done according to the procedures described by Okuyama et al. [6] and Goto et al. [7], respectively.

Detector Response of Fluorescence Labeled Derivatives

4-Bromomethyl-7-methoxy coumarin (FL; Ex. 360 nm, Em. 410 nm) and 1-anthroyl cyanide (Ex. 370 nm, Em. 470 nm) derivatives of ursodeoxycholic acid (IVa) were subjected to HPLC using

acetonitrile/H_2O (2:1) and acetonitrile/0.5% KH_2PO_4 (pH 4.0)(3:1) containing 5 mM Me-β-CD as a mobile phase, respectively. FL response was examined by measuring the peak area ratio relative to the value obtained with the above solvent system without Me-β-CD.

RESULTS AND DISCUSSION

Effect of CD in the Mobile Phase on the Retention

It has previously been disclosed that β- and γ-CDs were the most effective modifiers for the retentions of estrogens and digitoxin, respectively [1, 2]. The effects of α-, β- and γ-CD contents in the mobile phase on capacity factor (k') of chenodeoxycholic acid (IIa) were examined as before (Figure 2). Among the CDs examined, β-CD was most effective in decreasing the k' value and the same behavior was observed on other bile acids. On the basis of these data, further study was carried out with β-CD. The capacity factors of unconjugated bile acids having a 12 α-hydroxyl group (Ia, IIIa) were not so influenced but those of other unconjugated bile acids (IIa, IVa, Va) were decreased

TABLE 1

Effect of β-CD on the Retention of Unconjugated Bile Acids

Bile Acids	k' Value	
cholic acid (Ia)	2.84[1)]	2.19[2)]
chenodeoxycholic acid (IIa)	10.59	3.73
deoxycholic acid (IIIa)	12.32	10.29
ursodeoxycholic acid (IVa)	4.00	0.69
lithocholic acid (Va)	----[3)]	16.89

1) acetonitrile/0.5% KH_2PO_4 (pH 4.0) (4:5). 2) acetonitrile/0.5% KH_2PO_4 (pH 4.0)(4:5) containing 5 mM β-CD. 3) The compound was not eluted within 30 min. t_0=1.09 min.

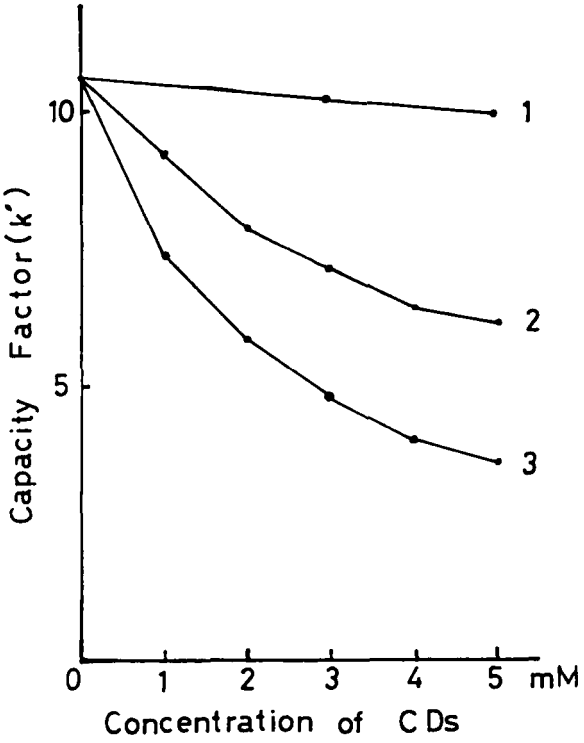


FIGURE 2. Effect of CD on the Retention of Chenodeoxycholic Acid.
1: α-CD 2: γ-CD 3: β-CD
Conditions: mobile phase, acetonitrile/0.5% KH_2PO_4 (4:5) containing each CD as indicated; detection, UV (210 nm).

sharply by the addition of β-CD (Table 1). These phenomena were also observed on glycine or taurine conjugates of bile acids (Figure 3, 4). It is of interest that the formation of the inclusion complex from the solute and β-CD exhibits such a characteristic pattern. It would be helpful to identify the peak on the chromatogram obtained with biological samples.

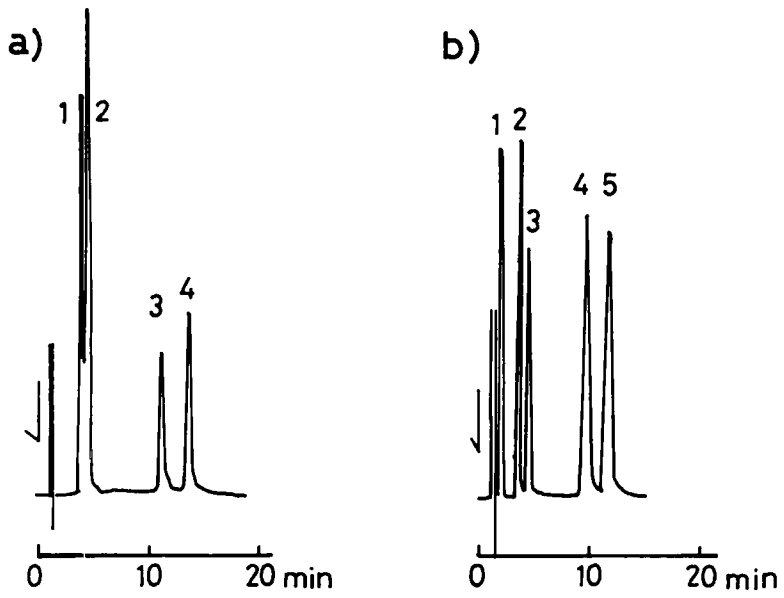


FIGURE 3. Separation of Glycine-Conjugated Bile Acids.
a) 1: Ib, 2: IVb, 3: IIb, 4: IIIb
b) 1: IVb, 2: Ib, 3: IIb, 4: Vb, 5: IIIb
Conditions: mobile phase, a) acetonitrile/0.5% KH_2PO_4 (pH 4.0)(1:2); Glycolithocholic acid (Vb) was not eluted within 30 min under these conditions. b) acetonitrile/0.5% KH_2PO_4 (pH 4.0)(1:2) containing 3 mM β-CD; detection, UV (210 nm; 0.02 AUFS).

Effect of CD in the Mobile Phase on the Separation of Bile Acids

The elution order of cholic acid (Ia) and IVa was reversed and the resolution of IIa and deoxycholic acid (IIIa) (Rs 2.13) was much improved (Rs 7.76) by the addition of β-CD to the mobile phase. It is not easy to separate lithocholic acid (Va) from other unconjugated bile acids under isocratic conditions in a short time [4], because of the higher lipophilicity of Va compared with other unconjugated bile acids. But the addition of β-CD to the mobile phase overcomes this problem as shown in Table

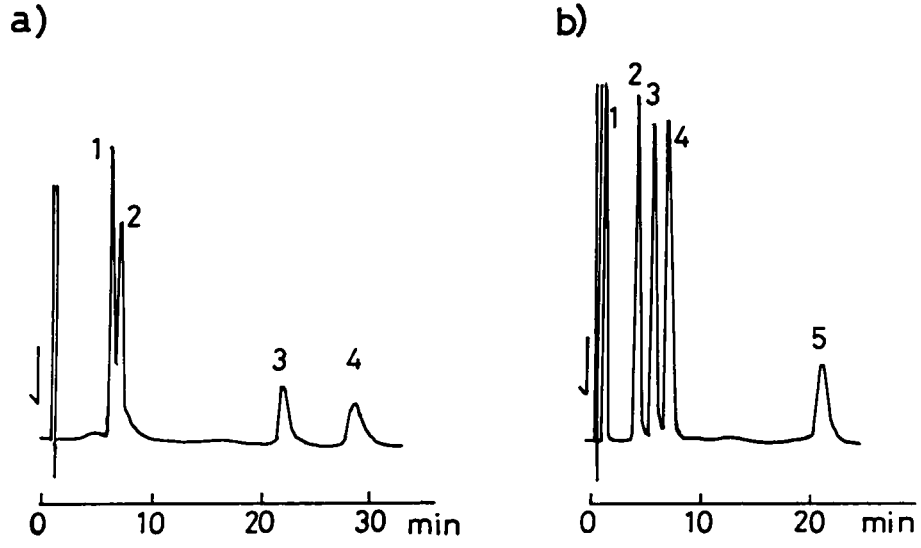


FIGURE 4. Separation of Taurine-Conjugated Bile Acids.
a) 1: IVc, 2: Ic, 3: IIc, 4: IIIc
b) 1: IVc, 2: IIc, 3: Ic, 4: Vc, 5: IIIc
Conditions: mobile phase, a) acetonitrile/0.5% KH_2PO_4 (pH 4.0)(2:5). Taurolithocholic acid (Vc) was not eluted within 30 min under these conditions. b) acetonitrile/0.5% KH_2PO_4 (pH 4.0)(2:5) containing 5 mM β-CD; detection, UV (210 nm; 0.02 AUFS).

1. Each lithocholic acid conjugate (Vb, c) was also eluted with other bile acid conjugates under isocratic conditions using β-CD, respectively (Figure 3b, 4b).

The separation of glycocholic acid (Ib) and glycoursodeoxycholic acid (IVb) has not been satisfactory without β-CD (Figure 3a). The k' value of IVb was more influenced than that of Ib, which has a 12 α-hydroxyl group, by the addition of β-CD and the complete separation of these bile acids has been done. The phenomenon was also compatible with that obtained with glycochenodeoxycholic acid (IIb) and glycodeoxycholic acid

(IIIb)(Figure 3b). The complete separation of taurine-conjugated bile acids (I-Vc) was also accomplished by the addition of β-CD as shown in Figure 4.

Effect of CD on the FL Response

Bile acids are usually monitored by UV detector at 190-210 nm. However, common bile acids have no remarkable UV absorption. Therefore, numerous attempts have been made to improve the sensitivity in detection [3]. Among these, pre-column labeling through the 24-carboxyl or 3 α-hydroxyl group is rather popular. Okuyama et al. developed a HPLC method with FL using 4-bromomethyl-7-methoxy coumarin as a pre-column labeling reagent (VI)[6]. On the contrary Goto et al. developed the reagent possessing both carbonyl nitrile as a reacting group and anthracene as a fluorophore (VII)[7]. The effect of CD in the mobile phase on the FL response was examined using these derivatives of IVa (Figure 5). Me-β-CD instead of β-CD was used as an additive, because of sparing solubility of β-CD in the mobile phase containing more than 50% of acetonitrile as an organic modifier. The fluorescence intensities of both derivatives were approximately one point two times enhanced by the addition of 5 mM Me-β-CD to the mobile phase. These data are compatible with those obtained with estrogens [1].

The group separation of unconjugated, glycine- and taurine-conjugated bile acids has already been done [3, 4], the use of CD in the mobile phase affords an advantage to the separation of bile acids and the fluorescence detection of their derivatives in the reversed-phase HPLC. Further application of this method to

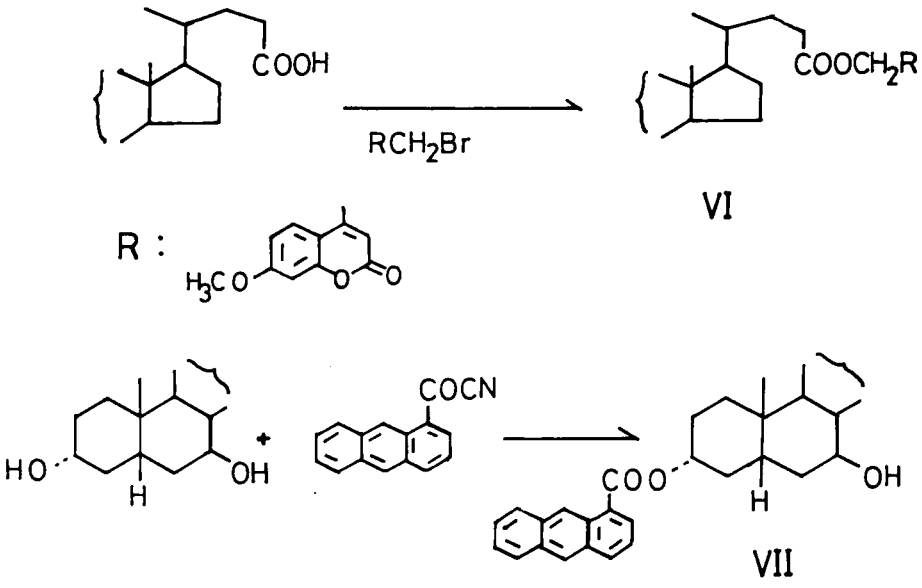


FIGURE 5. Structures of Fluorescence Labeled Bile Acids.

the analysis of bile acids, especially pre-column labeled derivatives, are being conducted in these laboratories, and the details will be reported elsewhere.

ACKNOWLEDGEMENTS

The authors are indebted to professor T. Nambara and Dr. J. Goto (Tohoku University, Sendai, Japan) for his encouragements and suggestions, respectively. Thanks are also due to Nihon Shokuhin Kako Co., Ltd. and T. Nemoto (Kao Co.) for supplying CDs. This work was supported in part by a grant from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1) Shimada, K., Masue, T., Toyoda, K., Takani, M. and Nambara, T., The utility of cyclodextrin in mobile phase for high-performance liquid chromatographic separation of isomeric estrogens, J. Liquid Chromatogr., 11, 1475 (1988).

2) Shimada, K., Oe, T., Kanno, C. and Nambara, T., Utility of cyclodextrin in mobile phase for high performance liquid chromatographic separation of cardenolides, Anal. Sci., 4, 377 (1988).

3) Nambara, T. and Goto, J., The bile acids, Vol. 4, Setchell, K. D. R., Plenum, New York, 1988, pp 43-64 and references cited therein.

4) Nambara, T., Goto, J., Hasegawa, M. and Kato, H., Biological/biomedical applications of liquid chromatography II, Hawk, G.L., Marcel Dekker, New York, 1979, pp 359-374.

5) Snopek, J., Smolková-Keulemansová, E., Jelínek, I., Dohnal, J., Klinot, J. and Klinotová, E., Use of cyclodextrins in isotachophoresis VI. Cyclodextrins as leading electrolyte additives for the separation of bile acids, J. Chromatogr., 450, 373 (1988).

6) Okuyama, S., The improved method of high performance liquid chromatographic separation of individual bile acids: free and glycine-conjugated bile acids, Chem. Lett., 1979, 461.

7) Goto, J., Goto, N., Shamsa, F., Saito, M., Komatsu, S., Suzaki, K. and Nambara, T., New sensitive derivatization of hydroxysteroids for high-performance liquid chromatography with fluorescence detection, Anal. Chim. Acta, 147, 397 (1983).