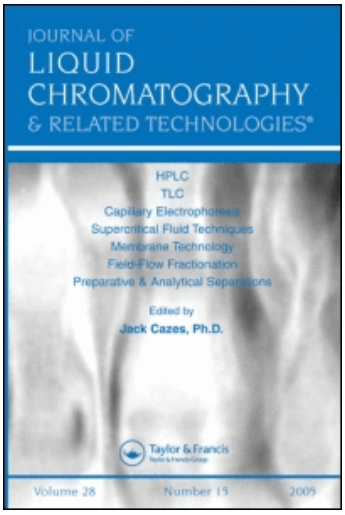

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The Effect of Organic Modifier in the Mobile Phase on the Separation of Bile Acids and its Fluorescent Derivatives in Inclusion Chromatography

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THE EFFECT OF ORGANIC MODIFIER IN THE MOBILE PHASE ON THE SEPARATION OF BILE ACIDS AND ITS FLUORESCENT DERIVATIVES IN INCLUSION CHROMATOGRAPHY

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ABSTRACT

The effect of organic modifier (methanol, acetonitrile, tetrahydrofuran) in the mobile phase on the separation of free bile acids and its fluorescent derivatives in reversed-phase high-performance liquid chromatography using cyclodextrin as a mobile phase additive was examined. Both methanol and acetonitrile were effective on the separation of free bile acids but the former was more effective than the latter on that of 3-(1-anthroyl)bile acids or bile acid 24-pyrenacyl esters. On the contrary tetrahydrofuran prevented the host-guest interaction.

INTRODUCTION

In recent years considerable attention has been focused on the utilization of cyclodextrin (CD) or crown ether as a stationary or mobile phase in chromatography (inclusion

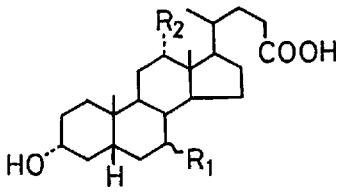
chromatography) owing to their ability to form inclusion complex and give specific retention behavior of various organic compounds [1, 2]. In previous papers we reported the much improved separation of estrogens [3], cardiac steroids [4] and bile acids [5] has been observed by the addition of the suitable CD to the mobile phase in reversed-phase high-performance liquid chromatography (HPLC). Since the formation of CD inclusion complex in the liquid phase proceeds more easily in an aqueous solution, the use of an aqueous-organic solvent as a mobile phase is necessary for this chromatography. Methanol was usually recommended as an organic modifier in the mobile phase, because of the solubility of CD and small preventive effect on the formation of host-guest inclusion complex [6]. Acetonitrile or tetrahydrofuran was also used as an organic modifier [7, 8], but the details of which have not been clarified.

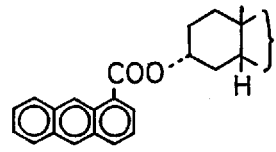
In this paper we examined the effect of organic modifier (methanol, acetonitrile, tetrahydrofuran) in the mobile phase on the separation of free bile acids and its fluorescent derivatives (Fig. 1) in reversed-phase HPLC using CD as a mobile phase additive.

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; 10.5





3-(1-anthroyl)bile acid

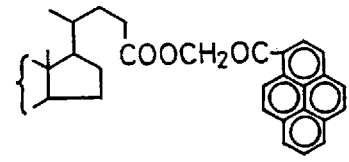
cholic acid (CA) :R_1=$\overset{H}{\underset{OH,}{}}$ R_2=OH

chenodeoxycholic acid (CDCA) :R_1=$\overset{H}{\underset{OH,}{}}$ R_2=H

deoxycholic acid (DCA) :R_1=H, R_2=OH

ursodeoxycholic acid (UDCA) :R_1=$\overset{OH,}{\underset{H}{}}$ R_2=H

lithocholic acid (LCA) :R_1=R_2=H



bile acid 24-pyrenacyl ester

FIGURE 1. Structures of free bile acids and its fluorescent derivatives.

methyl residues/mol) was prepared and donated by Kao Co. (Tokyo). Bile acids were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo). 1-Anthroyl cyanide and 1-bromoacetylpyrene were purchased from Wako Pure Chem. Ind., Ltd. (Osaka, Japan). Solvents were purified by distillation prior to use.

Apparatus

HPLC was carried out on a JASCO TRI ROTAR chromatograph equipped with a JASCO UVIDEC-100-II ultraviolet detector (UV) (Japan Spectroscopic Co., Ltd., Tokyo) or Hitachi F-1000 fluorescence detector (FL) (Hitachi Ltd., Tokyo) at a flow rate

of 1 ml/min unless otherwise stated. A YMC-GEL C_8 (5 μm) column (15 cm x 0.4 cm i.d.) (Yamamura Chemical Co., Kyoto, 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 $NaNO_3$ (UV) or MeOH (FL: Ex. 280 nm, Em. 320 nm).

Derivatization Methods

The derivatizations of bile acids with 1-anthroyl cyanide and 1-bromoacetylpyrene were done according to the procedure described by Goto et al. [9] and Kamada et al. [10], respectively.

RESULTS AND DISCUSSION

The Solubility of CDs in the Mobile Phase

The solubility of various CDs (α, β, $\gamma$and Me-β-CDs) in the aqueous mobile phase using methanol, acetonitrile or tetrahydrofuran as an organic modifier was examined . Each CD was dissolved in the examined mobile phase at the concentration of 0.5, 1, 2, 3, 4 or 5 mM and the mixture was kept at room temperature overnight. The maximum concentration of added CD that did not show turbidity was listed in TABLE 1. The solubility of each CD in aqueous-methanol was the largest among these three aqueous-organic solvent systems, except for β-CD in methanol-water (1:1 and 3:2). Among the examined CDs, Me-β-CD was most soluble in all the examined mobile phase. These data are helpful

TABLE 1

The Solubility of Each CD in the Mobile Phase Containing Organic Modifier

a) MeOH-H_2O System

	CD			
	α	β	Me-β	γ
1:1*	5**	3	5	5
3:2	5	2	5	5
2:1	5	2	5	5
3:1	5	1	5	5
4:1	5	0.5	5	3
5:1	3	0.5	5	2
6:1	3	0.5	5	2
7:1	2	0.5	5	1
8:1	2	0.5	5	0.5
9:1	2	0.5	5	0.5

b) CH_3CN-H_2O System

	CD			
	α	β	Me-β	γ
1:1*	5**	5	5	5
3:2	4	1	5	2
2:1	1	0.5	5	0.5
3:1	0.5	-	5	-
4:1	-	-	5	-
5:1	-	-	5	-

c) Tetrahydrofuran-H_2O System

	CD			
	α	β	Me-β	γ
1:1*	5**	5	5	5
3:2	5	4	5	3
2:1	5	2	5	2
3:1	2	0.5	5	0.5
4:1	1	-	5	-
5:1	-	-	2	-

* Organic solvent : H_2O
** mM

for the selection of suitable mobile phase containing CD and prompted us to use Me-β-CD as the host compound in the following experiments.

The Effect of Tetrahydrofuran in the Mobile Phase

Recently Higashidate et al. used the inclusion chromatography for the separation of bile acid dansylhydrazones

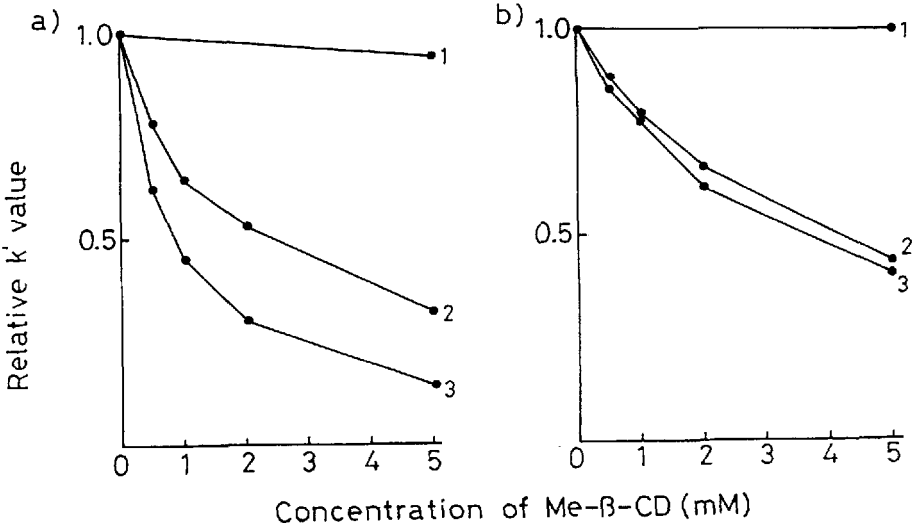


FIGURE 2. Effect of organic modifier on the retention of UDCA fluorescent derivatives.
a) 3-(1-Anthroyl)UDCA
1. Tetrahydrofuran-0.1% KH_2PO_4(pH 4.0) (20:21), 2. CH_3CN-0.1% KH_2PO_4(pH 4.0) (12:5), 3. MeOH-0.1% KH_2PO_4(pH 4.0) (9:2) containing Me-β-CD as indicated.
Detection, FL (Ex. 370 nm, Em. 470 nm).
b) UDCA 24-pyrenacyl ester
1. Tetrahydrofuran-H_2O (10:13), 2. CH_3CN-H_2O (5:3), 3. MeOH-H_2O (4:1) containing Me-β-CD as indicated.
Detection, FL (Ex. 370 nm, Em. 440 nm).

and reported that the addition of tetrahydrofuran in the mobile phase invalidated the effect of CD [8], but the details of which have not been clarified. The data prompted us to examine the phenomenon more precisely. The effect of Me-β-CD contents in the mobile phase using three different organic modifiers on the relative capacity factor (Rk') of 3-(1-anthroyl)UDCA (Fig. 2a)

and UDCA 24-pyrenacyl ester (Fig. 2b) was investigated. The Rk' values decreased significantly with increasing concentration of Me-β-CD in the mobile phase using methanol or acetonitrile as an organic modifier, but the Rk' values were almost unchanged by the addition of the CD in the mobile phase containing tetrahydrofuran. Although any plausible explanation has not been done, the effect of CD in the inclusion chromatography has been disappeared in the presence of tetrahydrofuran in the mobile phase. According to these data the following experiments have been done by using methanol or acetonitrile as an organic modifier.

The Effect of Organic Modifier in the Mobile Phase on the Separation of Free Bile Acids

The effect of methanol or acetonitrile as an organic modifier in the mobile phase on the separation of free bile acids was examined by using Me-β-CD as a host compound (TABLE 2). The Rk' values of CDCA and UDCA using acetonitrile as an organic modifier in the mobile phase were more influenced than those obtained with methanol. But the Rk' values of other bile acids were not so different from each other in the both mobile phases. The separation of five free bile acids using methanol or acetonitrile as an organic modifier was shown in Fig. 3a and 3b, respectively. Both chromatograms showed satisfactory separations but acetonitrile gave the better one. The relationship between the retention behaviors and structures of bile acids in this

TABLE 2

The Effect of Organic Modifier in the Mobile Phase on the k' Value of Free Bile Acids

Bile Acid	k' Value			
	Concentration of Me-β-CD			
	0 mM		2 mM	
CA	10.35[a]	6.54[b]	8.88[a] (0.86)[c]	5.32[b] (0.81)
CDCA	6.75	7.43	4.20 (0.62)	3.53 (0.47)
DCA	7.19	8.49	6.35 (0.88)	7.40 (0.87)
UDCA	7.55	9.13	1.92 (0.25)	1.33 (0.15)
LCA	10.31	9.76	4.15 (0.40)	4.01 (0.41)

a) MeOH-0.5% KH_2PO_4(pH 4.0) system. b) CH_3CN-0.5% KH_2PO_4(pH 4.0) system. c) Rk' value. The k' value obtained without CD was taken as 1.0. Detection, UV (222 nm).

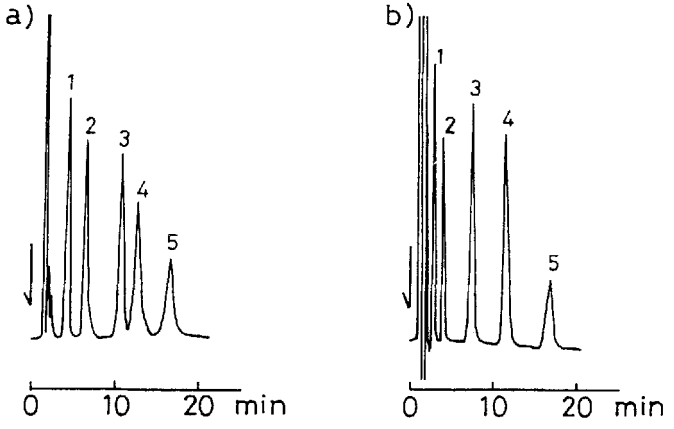


FIGURE 3. Separation of free bile acids.
1: UDCA 2: CA 3: CDCA 4: DCA 5: LCA
Conditions: mobile phase, a) MeOH-0.5% KH_2PO_4(pH 4.0) (3:1) containing 0.5 mM Me-β-CD. b) CH_3CN-0.5% KH_2PO_4(pH 4.0) (4:5) containing 1 mM Me-β-CD; detection, UV (222 nm).

TABLE 3

The Effect of Organic Modifier in the Mobile Phase on k' Value of Bile Acid Fluorescent Derivatives

a) 3-(1-Anthroyl)derivative

Bile Acid Derivative	k' Value: Concentration of Me-β-CD 0 mM		2 mM	
CA	5.62*	6.59**	4.35*(0.77)***	5.45**(0.83)
CDCA	12.43	12.14	6.76 (0.54)	8.10 (0.67)
DCA	12.42	11.30	7.78 (0.63)	8.49 (0.75)
UDCA	12.73	14.04	3.77 (0.30)	7.40 (0.53)
LCA	13.47	14.98	3.52 (0.26)	9.58 (0.64)

* MeOH-0.1% KH_2PO_4(pH 4.0) system. ** CH_3CN-0.1% KH_2PO_4(pH 4.0) system. *** Rk' value calculated as described in TABLE 2. Detection, FL (Ex. 370 nm, Em. 470 nm).

b) Bile Acid 24-Pyrenacyl Esters

Bile Acid Derivative	k' Value: Concentration of Me-β-CD 0 mM		2 mM	
CA	8.76*	9.15**	8.33*(0.95)***	8.66**(0.95)
CDCA	8.36	7.26	6.03 (0.72)	6.53 (0.90)
DCA	9.02	7.85	7.57 (0.84)	7.57 (0.96)
UDCA	6.90	12.54	4.21 (0.61)	8.26 (0.66)
LCA	9.54	9.84	6.54 (0.69)	6.94 (0.71)

* MeOH-H_2O system. ** CH_3CN-H_2O system. *** Rk' value. Detection, FL (Ex. 370 nm, Em. 440 nm).

chromatography using acetonitrile as an organic modifier has been discussed previously [5].

The Effect of Organic Modifier on the Separation of Bile Acid Fluorescent Derivatives

The effect of organic modifier in the mobile phase on the separation of 3-(1-anthroyl)bile acids and bile acid 24-pyrenacyl

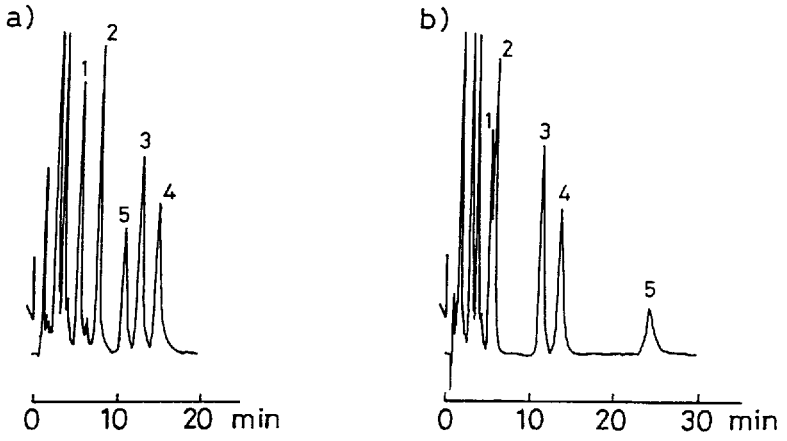


FIGURE 4. Separation of 3-(1-anthroyl)bile acids.
1: UDCA 2: CA 3: CDCA 4: DCA 5: LCA
Conditions: mobile phase, a) MeOH-0.25% KH_2PO_4(pH 4.0) (7:2) containing 3.5 mM Me-β-CD. b) CH_3CN-0.25% KH_2PO_4(pH 4.0) (7:3) containing 5 mM Me-β-CD; Flow rate, 1.5 ml/min; detection, FL (Ex. 370 nm, Em. 470 nm).

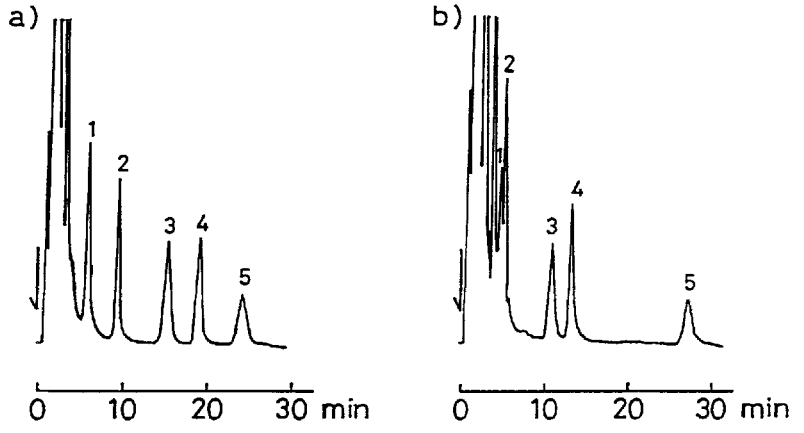


FIGURE 5. Separation of bile acid 24-pyrenacyl esters.
1: UDCA 2: CA 3: CDCA 4: DCA 5: LCA
Conditions: mobile phase, a) MeOH-H_2O (21:5) containing 1 mM Me-β-CD.
b) CH_3CN-H_2O (11:5) containing 2 mM Me-β-CD; Flow rate, 1.5 ml/min; detection, FL (Ex. 370 nm, Em. 440 nm).

esters was examined as described above and the results were shown in TABLE 3. Almost all the Rk' values, especially those of 3-(1-anthroyl)-UDCA and -LCA, obtained with methanol as an organic modifier decreased more significantly than those obtained with acetonitrile. The mixture of five bile acids was derivatized with 1-anthroyl cyanide or 1-bromoacetylpyrene and applied to the HPLC. Both mixtures of the derivatives showed satisfactory separation in the HPLC using methanol as an organic modifier. The peaks corresponding to the excess or decomposed reagents have been eluted near the solvent front and much improved separation from UDCA derivative has been obtained by using this organic modifier (Fig. 4, 5). The elution order of 3-(1-anthroyl)LCA has been changed by the used organic modifier (Fig. 4a, b). The application of the method to the determination of bile acids in biological sample is now under investigation in our laboratories.

Conclusion

Although there was couple of exceptions, the solubility of various CDs in aqueous-methanol has been the largest among the examined twenty two aqueous-organic solvent systems. Me-β-CD has been shown to be the best CD in its solubility in aqueous-organic solvent system. Both methanol and acetonitrile were effective on the separation of free bile acids but the former was more effective than the latter on that of bile acid fluorescent derivatives. The separation of the peaks corresponding to the

derivatization reagents or related compounds from those of the bile acids was clearly done by this organic modifier. On the contrary tetrahydrofuran in the mobile phase prevented the host-guest interaction and showed no effect on the presence of CD.

REFERENCES

1) Ward, T.J. and Armstrong, D.W., Improved cyclodextrin chiral phases: A comparison and review, J. Liquid Chromatogr., 9, 407 (1986) and references cited therein.

2) Hinze, W.L., Application of cyclodextrins in chromatographic separations and purification methods, Sepn. Purifn. Methods, 10, 159 (1981) and references cited therein.

3) 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).

4) Shimada, K., Kurata, Y. and Oe, T., Utility of cyclodextrin in mobile phase for high-performance liquid chromatographic separation of bufadienolides, J. Liquid Chromatogr., 13, 493 (1990).

5) Shimada, K., Komine, Y. and Oe, T., Chromatographic behavior of bile acids using cyclodextrin in mobile phase of high performance liquid chromatography, J. Liquid Chromatogr., 12, 491 (1989).

6) Fujimura, K., Ueda, T., Kitagawa, M., Takayanagi, H. and Ando, T., Reversed-phase retention behavior of aromatic compounds involving β-cyclodextrin inclusion complex formation in the mobile phase, Anal. Chem., 58, 2668 (1986).

7) Fell, A.F., Noctor, T.A.G., Mama, J.E. and Clark, B.J., Computer-aided optimisation of drug enantiomer separation in chiral high-performance liquid chromatography, J. Chromatogr., 434, 377 (1988).

8) Higashidate, S., Hibi, K., Senda, M., Kanda, S. and Imai, K., Sensitive assay system for bile acids and steroids having hydroxyl groups utilizing high-performance liquid chromatography with peroxyoxalate chemiluminescence detection, J. Chromatogr., 515, 577 (1990).

9) 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).

10) Kamada, S., Maeda, M. and Tsuji, A., Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, J. Chromatogr., 272, 29 (1983).