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Chiral separation of diltiazem, trimetoquinol and related compounds by micellar electrokinetic chromatography with bile salts

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ABSTRACT

The separation of optically isomeric diltiazem hydrochloride, trimetoquinol hydrochloride and related compounds by micellar electrokinetic chromatography was investigated employing four bile salts as chiral surfactants. The chiral separation of diltiazem hydrochloride and trimetoquinol hydrochloride was successfully achieved by use of sodium taurodeoxycholate under neutral conditions, although enantiomers of carboline derivatives A and B and 2,2'-dihydroxy-1,1'-dinaphthyl were resolved with all the bile salts under conditions from neutral to alkaline. The chiral separation of diltiazem-related compounds was affected by the structure of the samples in addition to the effects of bile salt structures and pH of the buffer solutions. Application to the optical purity testing of trimetoquinol hydrochloride by the area percentage method is described. A possible chiral separation mechanism is briefly mentioned.

INTRODUCTION

Micellar electrokinetic chromatography (micellar EKC or MEKC) has been developed for the separation of non-ionic compounds by the use of capillary zone electrophoresis (CZE). This method is based on micellar solubilization and electrophoretic migration of the micelle[1-3], that is, the solutes are separated by the differential distribution between the micelle and the surrounding aqueous phase and the differential migration of the two phases. Hence this method can be classified as an individual kind of chromatographic technique from the viewpoint of the separation principle, although it is performed with the same apparatus as in CZE.

Micellar EKC has many advantages for the separation of drugs in addition to high theoretical plate numbers. The selectivity has been much improved, even for the

separation of water-soluble ionic compounds, e.g., water-soluble vitamins[4] and β-lactam antibiotics[5], in comparison with CZE, although some of these solutes can be separated by the usual CZE. Moreover, corticosteroids and benzothiazepin analogues, which are insoluble in water or lipophilic and are not separated by CZE, have been successfully separated by this method[6]. The determination of drugs in preparations by the internal standard method has also been studied with the same reproducibility as in HPLC[6-10]. The determination of antibiotics in human plasma by micellar EKC was successful using a direct sample injection method[11-13]. The plasma proteins, which can interfere with the drug analysis, were solubilized by the micelle employed and hence eluted later than the drugs. That is, pretreatment of the plasma sample such as deproteinization or extraction was not necessary in micellar EKC. Separations of closely related isotopic compounds have also been successfully achieved by micellar EKC[14,15].

Recently, chiral separation by EKC has been investigated, using various techniques. In micellar EKC, chiral separation was achieved by employing a chiral surfactant such as bile salts[16,17] or by using a mixed micelle of sodium dodecyl sulphate (SDS) and some chiral additives such as N,N-didecyl-L-alanine in the presence of copper(II)[18] or digitonin[19]. Chiral separation of dansylated DL-amino acids was achieved by EKC with cyclodextrin derivatives, which have ionic groups in the molecules, based on inclusion formation[3].

In a previous paper, we described the chiral separation of carboline derivatives A and B and 2,2'-dihydroxy-1,1'-dinaphthyl by micellar EKC with bile salts, discussing the effects of pH and bile salts species on the chiral recognition[17]. These solutes were successfully separated with all the bile salts employed at pH 7.0 and 9.0. Chiral separation of dansylated DL-amino acids has been also successfully achieved by micellar EKC using bile salts[16]. In that work, the separation was successful with sodium taurodeoxycholate (STDC) at pH 3.0 or 7.0, and the best chiral separation of these solutes was accomplished with acidic (pH 3.0) buffer solutions. A tentative separation mechanism was also described.

In this paper, we describe the chiral separation of some drugs, especially diltiazem hydrochloride, trimetoquinol hydrochloride and related compounds, by micellar EKC with bile salts, which are readily available chiral surfactants. Enantiomers of diltiazem hydrochloride and trimetoquinol hydrochloride were successfully separated with STDC under neutral conditions, based on the differential solubilization of each isomer to the bile salt micelle. The effects of bile salt species, the pH of buffer solutions and the structure of samples are also described. This method was successfully applied to the purity testing of trimetoquinol hydrochloride.

EXPERIMENTAL

Apparatus

Separation was performed in a 650 mm × 50 μm I.D. (effective length 500 mm) fused-silica capillary tube (Scientific Glass Engineering, Ringwood, Victoria, Australia). A high voltage (up to +25 kV) was applied with a Model HJLL-25PO d.c. power supply (Matsusada Precision Devices, Kusatsu, Shiga, Japan). Detection was achieved by measuring the on-column UV adsorption at 210 nm with a Uvidec-100-VI (Jasco, Tokyo, Japan) with a time constant of 0.05 s. A Chromatopac C-R5A

(Shimadzu, Kyoto, Japan) was used for data processing. The micellar EKC apparatus used was the same as described previously[4].

Reagents and samples

Sodium cholate (SC), sodium taurocholate (STC), sodium deoxycholate (SDC) and sodium taurodeoxycholate (STDC), purchased from Nacalai Tesque (Kyoto, Japan), were dissolved in 0.02 M phosphate–borate buffer of pH 7.0 or 9.0 at a concentration 0.05 M and these micellar solutions were passed through a membrane filter (0.45 μm) prior to use. Sudan III from Nacalai Tesque was used as a tracer of the micelle[2]. All other reagents and solvents were of analytical-reagent grade from Katayama Kagaku (Osaka, Japan) and used without further purification.

The structures of diltiazem hydrochloride [(SS)-form], its related compounds, trimetoquinol hydrochloride [(S)-form] from Tanabe Seiyaku (Osaka, Japan) and tetrahydropapaveroline from Aldrich (Milwaukee, WI, U.S.A.) are given in Tables I and II. Diltiazem hydrochloride is a calcium antagonist with coronary vasodilatory activity. Trimetoquinol hydrochloride is a bronchodilator. Antipodes of diltiazem hydrochloride, its related compounds (except for D7 and D8) and trimetoquinol hydrochloride were obtained from Tanabe Seiyaku. Enantiomers of 2,2'-dihydroxy-1,1'-dinaphthyl, 2,2,2-trifluoro-1-(9-anthryl)ethanol, purchased from Nacalai Tesque, and carboline derivatives A and B, which are new hepatoprotective agents synthesized at Tanabe Seiyaku, were also studied. These solutes were dissolved in water or methanol at concentrations of 0.2 mg/ml (D2 and D10) and 1 mg/ml (others) to give adequate peak heights. A known amount of racemic trimetoquinol hydrochloride was added to (S)-trimetoquinol hydrochloride in the optical purity testing.
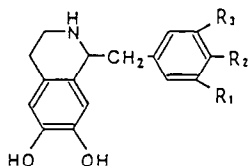
TABLE I

STRUCTURE OF DILTIAZEM HYDROCHLORIDE AND RELATED COMPOUNDS
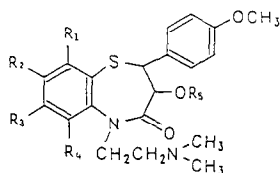
Solute	R_1	R_2	R_3	R_4	R_5
D1 (diltiazem)	H	H	H	H	COCH$_3$
D2	H	H	H	H	H
D3	Cl	H	H	H	COCH$_3$
D4	Cl	H	H	H	H
D5	H	Cl	H	H	COCH$_3$
D6	H	Cl	H	H	H
D7	H	H	Cl	H	COCH$_3$
D8	H	H	Cl	H	H
D9	H	H	H	Cl	COCH$_3$
D10	H	H	H	Cl	H

TABLE II

STRUCTURE OF TRIMETOQUINAL AND TETRAHYDROPAPAVEROLINE

Compound	R_1	R_2	R_3
Trimetoquinol	OCH$_3$	OCH$_3$	OCH$_3$
Tetrahydropapaveroline	OH	OH	H

Procedure

A sample solution was introduced into the positive end of the tube by siphoning (about 10 cm height, 5–10 s) and micellar EKC was performed at ambient temperature (*ca.* 20°C). The injection volume in the system was of the order of 1 nl. The detailed procedure has been described previously[4].

In order to obtain a good peak shape, enantioselectivity and reproducibility of the migration times, it was necessary to wash the capillary tube with an alkaline solution as follows: the capillary was filled with a 0.1 M potassium hydroxide solution with a manually operated syringe and allowed to stand for 30 min, flushed with water and allowed to stand for 5 min, then finally filled with the working solution. The capillary tube was washed out every week.
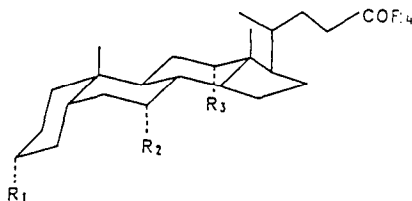
RESULTS AND DISCUSSION

Chiral separation with bile salts

The structure of the bile salts employed in this study is given in Table III. Bile salts form small or primary micelles with up to ten monomers by the hydrophobic

TABLE III

STRUCTURE OF BILE SALTS

Bile salt	Abbreviation	R_1	R_2	R_3	R_4
Sodium cholate	SC	OH	OH	OH	ONa
Sodium taurocholate	STC	OH	OH	OH	NHCH$_2$CH$_2$SO$_3$Na
Sodium deoxycholate	SDC	OH	H	OH	ONa
Sodium taurodeoxycholate	STDC	OH	H	OH	NHCH$_2$CH$_2$SO$_3$Na

interaction between the non-polar faces of the monomers[20]. This may be considered as one of the reasons for the characteristic solubilization capability of the bile salt micelle[6,21] in comparison with long-chain alkyl-type surfactants such as SDS. The enantiomeric separation of diltiazem hydrochloride, trimetoquinol hydrochloride, carboline derivatives A and B, 2,2′-dihydroxy-1,1′-dinaphthyl and 2,2,2-trifluoro-1-(9-anthryl)ethanol was investigated with four kinds of bile salts (0.05 M) under the neutral (pH 7.0) and alkaline (pH 9.0) conditions. Chiral separation was successfully achieved except for 2,2,2-trifluoro-1-(9-anthryl)ethanol and the detailed chiral separation of carboline derivatives A and B and 2,2′-dihydroxy-1,1′-dinaphthyl was reported previously[17]. From the results, it was concluded that the structure of bile salts and the buffer pH significantly affect the recognition of enantiomers.

Chiral separation of diltiazem and related compounds

The chiral separation of diltiazem hydrochloride and related compounds (Table I) was investigated with neutral (pH 7.0) and alkaline (pH 9.0) buffer solutions of the four bile salts. Enantiomers of diltiazem hydrochloride were resolved with only STDC
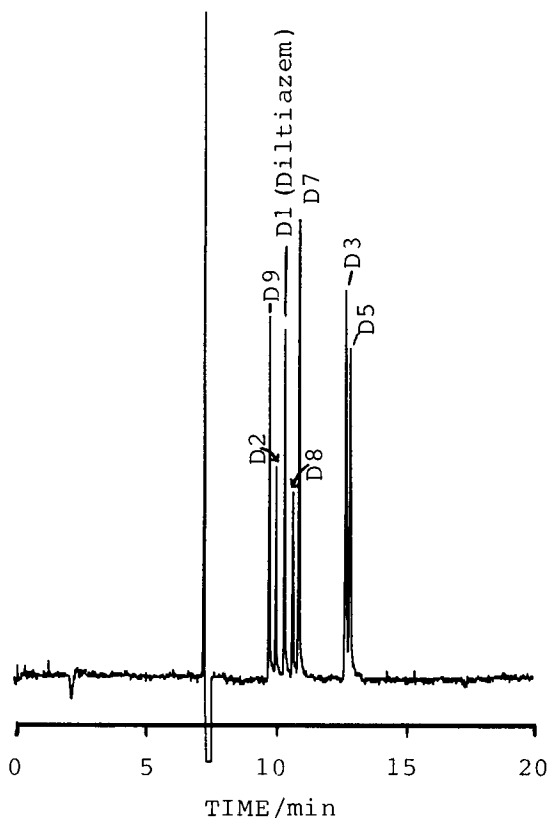

Fig. 1. Micellar EKC separation of seven diltiazem analogues. Samples are indicated with the abbreviations in Table I. Conditions: buffer, 0.05 M STC in 0.02 M phosphate–borate buffer solution of pH 7.0; separation tube, 650 mm × 0.05 mm I.D. (effective length, 500 mm); applied voltage, 20 kV; detection, 210 nm; temperature, ambient.

solutions of pH 7.0. Chiral separation of its related compounds was also successful under the same conditions, except for samples D5 and D6. Under the other conditions, enantiomers of D3 were partially resolved with the SC solution of pH 9.0 and those of D9 and D10 with the STDC solution of pH 9.0.

Migration of the solutes at pH 7.0 occurs in the order D9, D1 (diltiazem), D7, D3 and D5 in all the bile salt solutions, although D1 migrated faster than D9 at pH 9.0. Each deacetyl form migrated faster than the corresponding acetyl form because of an increase in hydrophilicity, although separation between the acetyl form D5 and its deacetyl form D6 was not achieved with the 0.05 *M* bile salt employed. A typical chromatogram of a mixture of seven diltiazem derivatives with a 0.05 *M* STC solution
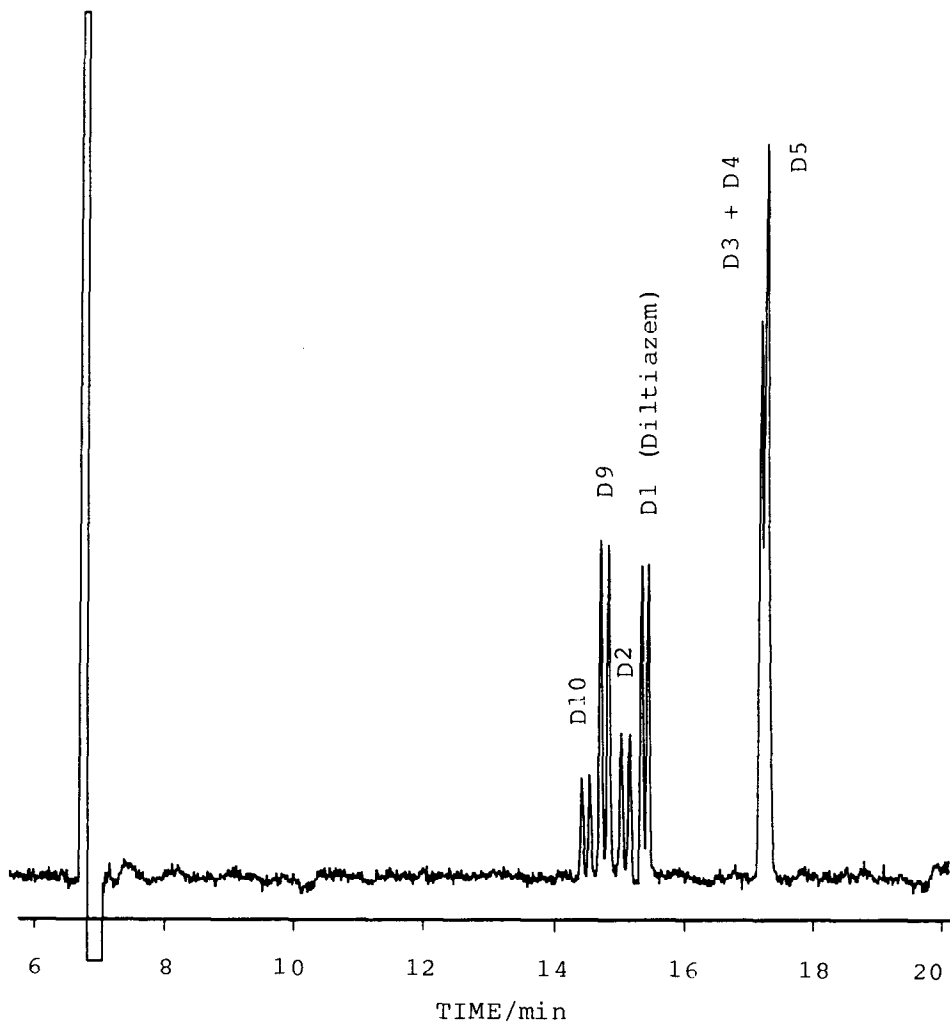

Fig. 2. Chiral separation of diltiazem hydrochloride and related compounds. Samples are indicated with the abbreviations in Table I. Buffer, 0.05 *M* STDC in 0.02 *M* phosphate–borate buffer solution solution of pH 7.0. Other conditions as in Fig. 1.

of pH 7.0 is shown in Fig. 1. Under these conditions, D6 and D10 overlapped with their acetyl forms (D5 and D9) and D4 migrated slightly faster than D3. Chiral separation was not achieved at all.

A successful chiral separation of seven enantiomeric diltiazem-related compounds with STDC solutuons of pH 7.0 is shown in Fig. 2. The migration times, capacity factors ($\tilde{k}'$) and separation factors ($\alpha = \tilde{k}'_2/\tilde{k}'_1$) of the solutes shown in Fig. 2 are summarized in Table IV. In micellar EKC, the capacity factor of an electrically neutral solute is given by[2]

$$\tilde{k}' = \frac{t_R - t_0}{t_0(1 - t_R/t_{mc})} \tag{1}$$

where t_0, t_R and t_{mc} are the migration times of an unincorporated solute, the sample and the micelle, respectively. We employed methanol as a tracer of the electroosmotic flow and Sudan III as that of the micelle. Enantiomers of D1, D2, D9 and D10 were successfully resolved and those of D3 and D4, which eluted with the same migration times, were partially resolved with α values around 1.04. Chiral separation of D5 was not successful.

From these results, D5, which has a chlorine atom in the R_2 position (see Table I), has some unfavourable substituents for the chiral recognition in comparison with D1 (diltiazem, no chlorine) or D9, which has a chlorine atom in the R_4 position. The results that D6 (deacetyl form) could not be separated from its acetyl form D5, and the insufficient chiral separation of D3 and D4, each of which has a chlorine atom in the R_1 position, also support the above consideration. That is, it was difficult to recognize enantiomers of diltiazem-related compounds which have some hydrophobic groups in the R_1 or R_2 position with the bile salt micelles. The longer migration times of D3 and D5 compared with those of D1 and D9, which were successfully separated (see Fig. 2), indicate that the region around the R_1, R_2 and p-methoxybenzyl part may be the area of interaction of diltiazem-related compounds with the non-polar part of the bile salt micelle. Solubilization of D3 and D5, in which hydrogen atoms in the R_1 or R_2 position are replaced by chlorine atoms, more than D1 and D9, on the other hand, may reduce the chiral recognition.

TABLE IV

MIGRATION TIMES, CAPACITY FACTORS AND SEPARATION FACTORS OF DILTIAZEM HYDROCHLORIDE AND RELATED COMPOUNDS

0.05 M STDC at pH 7.0; applied voltage, 20 kV; t_0, 6.62 min; t_{mc}, 18.81 min.

Solute	t_{R1} (min)	t_{R2} (min)	$\tilde{k}'_1$	$\tilde{k}'_2$	α
D10	14.06	14.17	4.45	4.62	1.04
D9	14.33	14.44	4.89	5.08	1.04
D2	14.64	14.76	5.46	5.71	1.05
D1 (diltiazem)	14.94	15.05	6.11	6.37	1.04
D4	16.80	16.86	14.39	14.92	1.04
D3	16.80	16.86	14.39	14.92	1.04
D5	16.86	—	14.92	—	—

Chiral separation of trimetoquinol and tetrahydropapaveroline

Enantiomers of trimetoquinol hydrochloride were successfully resolved and those of tetrahydropapaveroline, which is a biosynthetic precursor of morphine and has a similar structure to that of trimetoquinol hydrochloride, were partially resolved with a 0.05 M STDC solution of pH 7.0. A typical chromatogram of these solutes is shown in Fig. 3. Calculated $\bar{k}'$ and α values are summarized in Table V. The capacity factors of these solutes are relatively small compared with those of diltiazem-related compounds and it can be expected that the chiral separation of tetrahydropapaveroline will be improved with increase in the k' value through an increase in surfactant concentration. In micellar EKC, the resolution equation is given by[2]
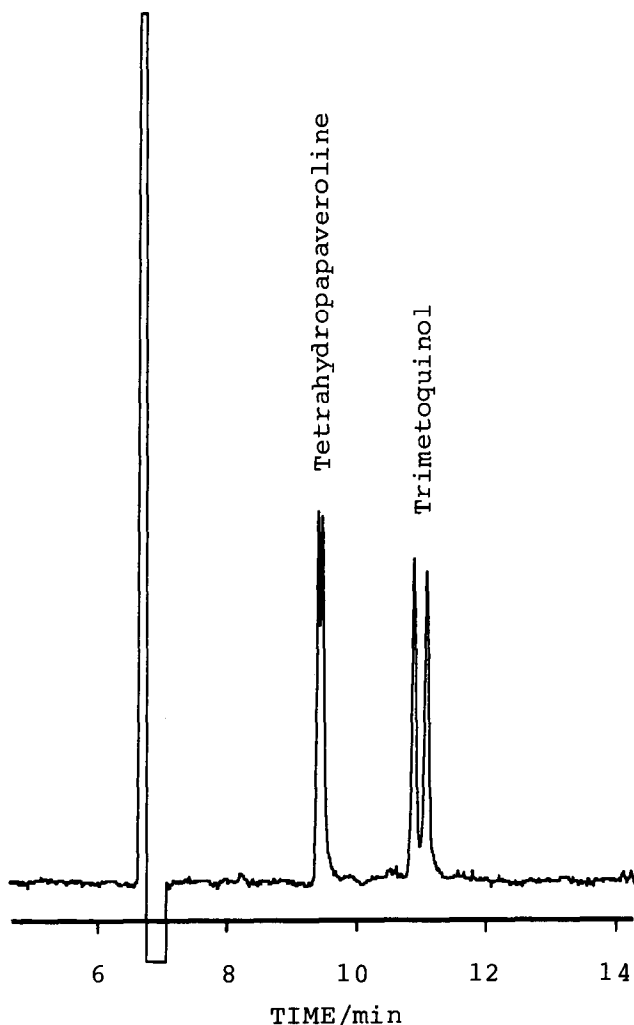

Fig. 3. Chiral separation of trimetoquinol hydrochloride and tetrahydropapaveroline. Conditions as in Fig.

TABLE V

MIGRATION TIMES, CAPACITY FACTORS AND SEPARATION FACTORS OF TRIMETO-QUINOL HYDROCHLORIDE, TETRAHYDROPAPAVEROLINE AND DINAPHTHYL

0.05 M STDC at pH 7.0; applied voltage, 20 kV; t_0, 6.62 min; t_{mc}, 18.81 min.

Solute	t_{R1} (min)	t_{R2} (min)	$\tilde{k}'_1$	$\tilde{k}'_2$	α
Tetrahydropapaveroline	9.36	9.42	0.82	0.85	1.03
Trimetoquinol	10.84	11.04	1.50	1.62	1.07
2,2'-Dihydroxy-1,1'-dinaphthyl	17.72	18.03	28.94	41.56	1.44

$$R_s = \frac{\sqrt{N}}{4}\left(\frac{\alpha - 1}{\alpha}\right)\left(\frac{\tilde{k}'_2}{1 + \tilde{k}'_2}\right)\left[\frac{1 - t_0/t_{mc}}{1 + (t_0/t_{mc})\tilde{k}'_1}\right] \tag{2}$$

where N is the theoretical plate number of the solute. The last term on the right-hand side is additional to that in the usual HPLC and hence R_s depends considerably on this
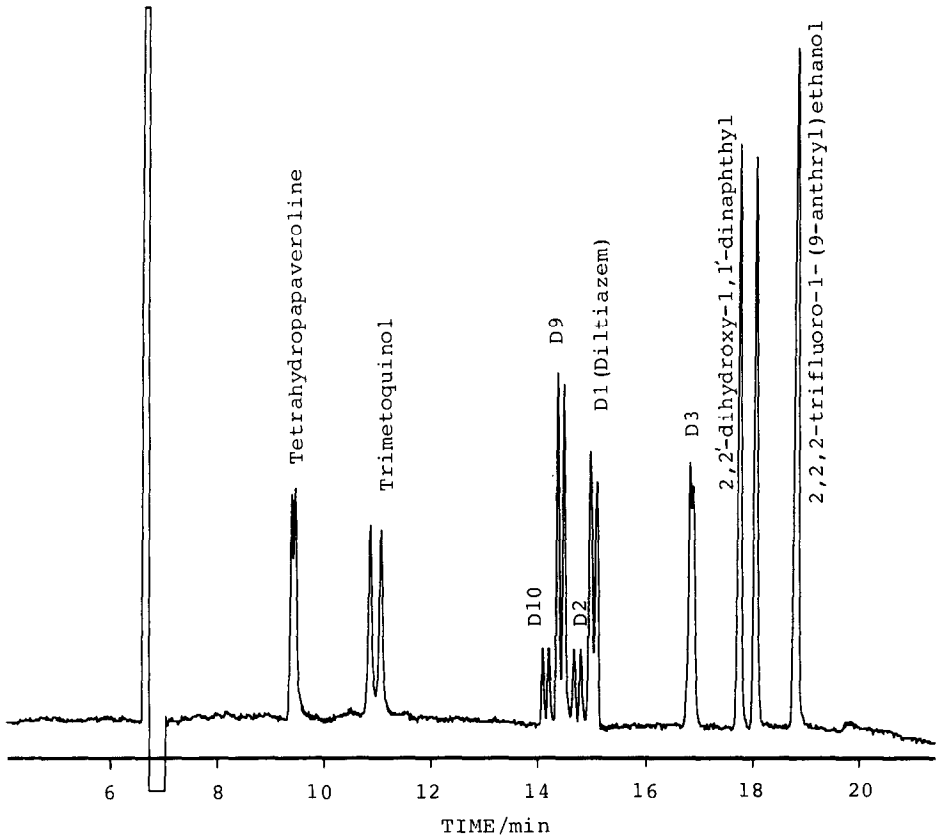

Fig. 4. Chiral separation of trimetoquinol hydrochloride, tetrahydropapaveroline, five diltiazem-related compounds, 2,2'-dihydroxy-1,1'-dinaphthyl and 2,2,2-trifluoro-1-(9-anthryl)ethanol. Conditions as in Fig.

term. With bile salts, the maximum resolution should be obtained when the $\tilde{k}'$ values are between 1 and 2 from the value $t_0/t_{mc} = 0.35$ at a constant N value[2]. The capacity factor of trimetoquinol hydrochloride fitted the above range.

However, to separate several solutes in one run, a 0.05 M concentration was sufficient. A typical chiral separation of nine enantiomeric compounds is shown in Fig. 4, in which t_0 (electroosmotic flow) and t_{mc} were ca. 6.6 and 18.8 min, respectively. The $\tilde{k}'$ and α values of 2,2'-dihydroxy-1,1'-dinaphthyl are given in Table V. Enantiomers of 2,2,2-trifluoro-1-(9-anthryl)ethanol migrated with the same velocity as Sudan III, indicating that it was totally solubilized into the micelle, and were not separated.

Application to the optical purity testing of trimetoquinol hydrochloride was also investigated. Typical chromatograms are shown in Fig. 5. Down to 1% of the minor enantiomer [(R)-form] of the major enantiomer of trimetoquinol hydrochloride [(S)-form] could be detected directly by micellar EKC at a signal-to-noise ratio of 3. The optical purity of five actual batches determined by this method were all more than 99% (not detected).

In conclusion, chiral separation by micellar EKC with anionic bile salts is successful when the solutes have a relatively rigid conformation, probably owing to the rigid structure of the bile salt molecule. Enantiomeric compounds, which are positively charged or basic, will be more effectively resolved because of increasing ionic interaction between the solute and the anionic bile salt micelle. However, it will be difficult to predict whether enantiomers can be successfully separated or not because a small difference in the solute structure significantly affects the chiral recognition. In addition to bile salts, other chiral surfactants will be used in micellar EKC similarly to
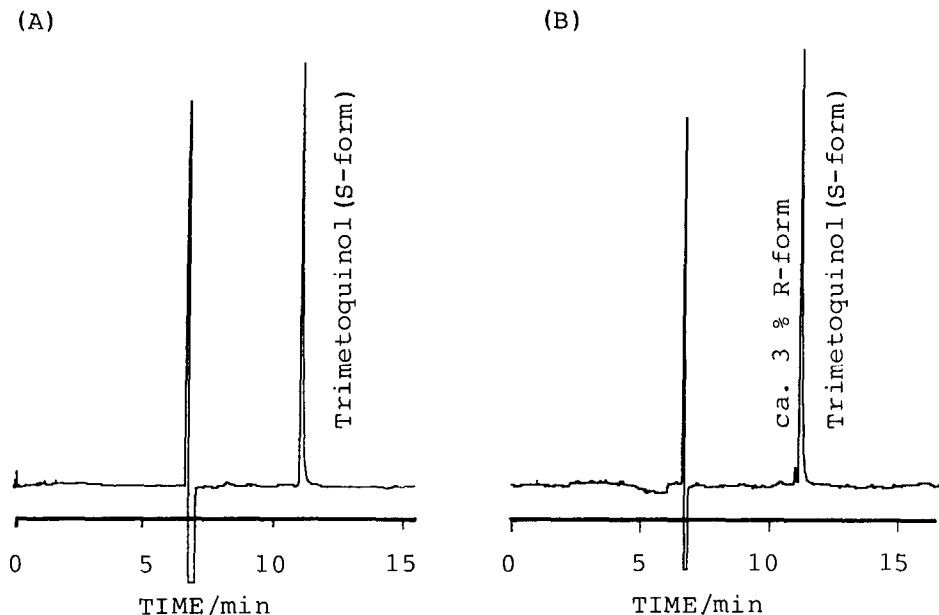

Fig. 5. Optical purity testing of trimetoquinol hydrochloride. (A) Authentic trimetoquinol hydrochloride [(S)-form] and (B) ca. 3% of (R)-form added to A. Conditions as in Fig. 3.

the use of chiral mobile phases in HPLC. Micellar EKC will be widely applied in the future to the direct chiral separation of drugs by use of new chiral surfactants or SDS micelles, mixed with a chiral additive.

REFERENCES

1 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya and T. Ando, *Anal. Chem.*, 56 (1984) 111.
2 S. Terabe, K. Otsuka and T. Ando, *Anal. Chem.*, 57 (1985) 834.
3 S. Terabe, *Trends Anal. Chem.*, 8 (1989) 129.
4 H. Nishi, N. Tsumagari, T. Kakimoto and S. Terabe, *J. Chromatogr.*, 465 (1989) 331.
5 H. Nishi, N. Tsumagari, T. Kakimoto and S. Terabe, *J. Chromatogr.*, 477 (1989) 259.
6 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 513 (1990) 279.
7 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Pharm. Sci.*, 79 (1990) in press.
8 K. Otsuka, S. Terabe and T. Ando, *J. Chromatogr.*, 396 (1987) 350.
9 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 2773.
10 S. Fujiwara, S. Iwase and S. Honda, *J. Chromatogr.*, 447 (1988) 133.
11 T. Nakagawa, Y. Oda, A. Shibukawa and H. Tanake, *Chem. Pharm. Bull.*, 36 (1988) 1622.
12 T. Nakagawa, Y. Oda, A. Shibukawa, H. Fukuda and H. Tanaka, *Chem. Pharm. Bull.*, 37 (1989) 707.
13 H. Nishi, T. Fukuyama and M. Matsuo, *J. Chromatogr.*, 515 (1990) 245.
14 M. M. Bushey and J. W. Jorgenson, *Anal. Chem.*, 61 (1989) 491.
15 M. M. Bushey and J. W. Jorgenson, *J. Microcolumn Sep.*, 1 (1989) 125.
16 S. Terabe, M. Shibata and Y. Miyashita, *J. Chromatogr.*, 480 (1989) 403.
17 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Microcolumn Sep.*, 1 (1989) 234.
18 A. S. Cohen, A. Paulus and B. L. Karger, *Chromatographia*, 24 (1987) 15.
19 K. Otsuka and S. Terabe, *J. Chromatogr.*, 515 (1990) 221.
20 D. Attwood and A. T. Florence, *Surfactant Systems. Their Chemistry, Pharmacy and Biology*, Chapman and Hall, London, 1983, pp. 185–196.
21 H. Nishi, T. Fukuyama, M. Matsuo and S. Terabe, *J. Chromatogr.*, 498 (1990) 313.