Resolution of Tocainide and Its Analogues on Liquid Chromatographic Chiral Stationary Phases Based on (+)-(18-Crown-6)-2,3,11,12-tetracarboxylic Acid

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Two liquid chromatographic chiral stationary phases (CSPs) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid were successfully applied in the resolution of racemic tocainide and its analogues. In the resolution of tocainide, especially, the CSP containing N-CH_3 amide tethering groups was quite effective, showing clear baseline resolution (R_S: 2.66) with reasonable enantioselectivity (α: 1.25). Consequently, the CSP containing N-CH_3 amide tethering groups is expected to be useful to monitor the enantiomeric composition of tocainide in clinical samples. In addition, the chromatographic behaviors for the resolution of tocainide and its analogues on the two CSPs were found controllable by varying the content and the type of organic and acidic modifiers in aqueous mobile phase.

Key Words : Liquid chromatography, Enantioseparation, Chiral stationary phase, Tocainide

Introduction

Tocainide is a well-known antiarrythmic agent.[1] As shown in Figure 1, tocainide has one stereogenic center. Consequently, the two enantiomers consisting of racemic tocainide are expected to show different pharmacological behaviors. Actually, the stereoselective disposition of tocainide in healthy young volunteers, in patients with acute ventricular arrhythmias and in rats and mice was reported.[2] It was also reported that the *R*-(−)-enantiomer was eliminated more rapidly from plasma than the *S*-(+)-enantiomer.[3] In addition, the *R*-(−)-enantiomer was reported to be three times more potent than the *S*-(+)-enantiomer.[4] In this instance, exact determination of the enantiomeric composition of tocainide is important.

Various methods are available for the determination of enantiomeric composition of chiral drugs. However, the liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has been known to be the most accurate and convenient means of determining the enantiomeric composition of chiral drugs.[5] Previously, for example, several CSPs based on alpha 1-acid glycoprotein[6] and modified diallyl-tartardiamide[7] were used in the liquid chromatographic resolution of tocainide. Crown ether-based CSPs, which have been known to be useful for the resolution of racemic compounds containing a primary amino group,[8] are expected to be also useful for the resolution of tocainide because it contains one primary amino group. Actually, optically active (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 dynamically coated on octadecyl silica gel (CROWNPAK CR, Daicel Chemical Industries) was utilized in the resolution of tocainide.[9] But the baseline resolution was not observed (R_S: 0.97). Recently, a very effective CSP (CSP **1**, Figure 1) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was developed in our laboratory and successfully applied in the resolution of α-amino acids,[10] primary amines,[11] primary amino alcohols[12] and fluoroquinolone compounds containing a primary amino group.[13] In addition, CSP **1** was also briefly applied in the resolution of tocainide.[10,14] More recently, a modified CSP (CSP **2**, Figure 1) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was developed in our laboratory by simply replacing the N-H hydrogens of the two tethering groups of CSP **1**.[15] CSP **2** was reported to be superior to CSP **1** in the resolution of racemic primary amines. In the resolution of α-amino acids and primary amino alcohols, CSP **1** and CSP **2** were complementary with each other. However, the chromatographic behaviors for the resolution of tocainide on CSP **1** and CSP **2** have not been systematically studied yet. In this study, we wish to explore the characteristics of CSP **1** and CSP **2** for the resolution of tocainide and its analogues shown in Figure 1.

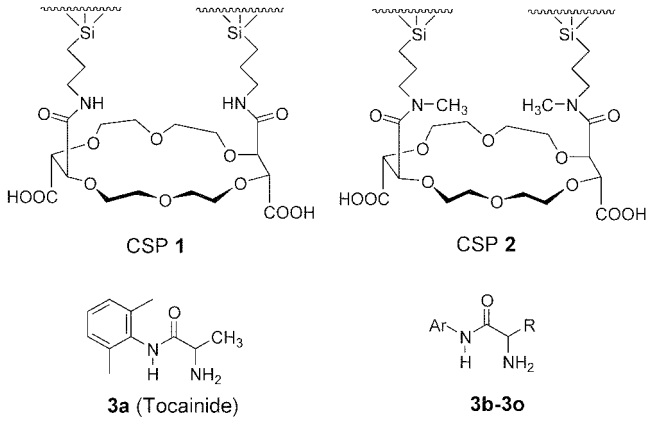

Figure 1. Structures of CSP **1**, CSP **2**, tocainide (**3a**) and tocainide analogues (**3b**-**3o**).

Experimental Section

An HPLC system consisting of a Waters model 515 HPLC

pump (Milford, MA, USA), a Rheodyne model 7725i injector with a 20 μL sample loop (Cotati, CA, USA), a YoungLin M720 Absorbance detector (variable wavelength) (Seoul, Korea) and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus) (Seoul, Korea) was used in obtaining the chromatograms for the resolution of tocainide and its analogues on CSP **1** and CSP **2**. The temperature of the chiral column was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator (Julabo Labortechnik GMBH, Seelbach, Germany). Chiral columns (250 mm × 4.6 mm I.D.) packed with CSP **1** and CSP **2** were available from previous studies.[10,15] Each of racemic and optically active tocainide and its derivatives prepared in this laboratory was dissolved in water (usually 2.5 mg/mL) and then used for the resolution on CSP **1** and CSP **2**. The usual injection volume was 0.1 μL. The chromatographic parameters were calculated based on the column void volume measured by injecting 2,6-lutidine, which was incidentally found to elute faster than any solvent used in the mobile phase.[13] All data used for the calculation of chromatographic parameters were collected from three runs for each sample. Between runs, deviation was negligible.

HPLC grade methanol (CH_3OH) and acetonitrile (CH_3CN) were obtained from Fisher Scientific (Springfield, NJ, USA). Water was deionized by using Milli-Q water Purification System from Millipore (Bedford, MA, USA). Sulfuric acid (H_2SO_4) was from Matsunoen Chemicals (Osaka, Japan) and perchloric ($HClO_4$) and acetic acid (CH_3COOH) were from Junsei Chemicals (Tokyo, Japan). Tocainide (**3a**) and its derivatives (**3b**-**3o**) were prepared in both optically active and racemic forms from corresponding α-amino acids as following. All chemicals used in the preparation of tocainide and its derivatives were from Aldrich (Milwaukee, Wisconsin, USA). Optically active or racemic α-amino acids were treated with di-*tert*-butyldicarbonate in the presence of triethylamine to afford *N*-*t*-BOC-α-amino acids. *N*-*t*-BOC-α-Amino acids were treated with aniline, 2,6-dimethylaniline or benzylamine in the presence of EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline) in methylene chloride to afford *N*-*t*-BOC-α-amino amides. Finally, treatment of *N*-*t*-BOC-α-amino amides in methylene chloride with HCl gas evolved from the reaction of H_2SO_4 and NaCl produced racemic and optically active tocainide and its analogues as their HCl salts. The structures of tocainide and its analogues thus prepared were identified with ^{1}H NMR spectra.

Results and Discussion

Resolution of racemic tocainide (**3a**) and its analogues (**3b**-**3o**) on CSP **1** and CSP **2** is summarized in Table 1. All data shown in Table 1 were obtained under an identical chromatographic condition with the mobile phase of 80% methanol in water containing 10 mM sulfuric acid. The elution orders shown in Table 1 were determined by injecting configurationally known samples.

As shown in Table 1, clear baseline separation of the two enantiomers of tocainide (**3a**) was not observed on CSP **1** as denoted by the resolution factor (R_S: 0.92). However, when the 2,6-dimethylphenyl group of tocainide was changed into phenyl (**3b**) or benzyl group (**3c**), the enantioselectivity (α) and the resolution (R_S) were improved quite much. In contrast, the resolution of tocainide (**3a**) is much more effective on CSP **2** than on CSP **1** in terms of the separation (α) and the resolution factor (R_S). Clear baseline resolution (R_S: 2.66) with reasonable enantioselectivity (α: 1.25) was observed on CSP **2**. Consequently, CSP **2** is expected to be successfully utilized in monitoring the enantiomeric composition of tocainide in clinical samples. In the resolution of

Table 1. Resolution of tocainide (**3a**) and its analogues (**3b**-**3o**) on CSP **1** and CSP **2**[a]

	Analytes		CSP **1**			CSP **2**		
	R	Ar	k_1	α	R_S	k_1	α	R_S
3a	Methyl	2,6-Dimethylphenyl	1.05 (S)	1.17	0.92	4.93 (S)	1.25	2.66
3b		Phenyl	1.82 (S)	1.73	2.52	8.39 (S)	1.80	6.33
3c		Benzyl	1.38 (S)	1.44	2.10	6.23 (S)	1.81	6.45
3d	Isopropyl	2,6-Dimethylphenyl	0.10	1.00		0.38 (S)	1.66	2.56
3e		Phenyl	0.34 (S)	2.10	2.56	1.81 (S)	2.37	8.90
3f		Benzyl	0.30 (S)	1.42	1.00	1.43 (S)	2.00	6.81
3g	Isobutyl	2,6-Dimethylphenyl	0.25 (S)	1.17	0.25	1.17 (S)	1.24	1.71
3h		Phenyl	1.35 (S)	5.00	4.00	7.64 (S)	4.12	13.98
3i		Benzyl	1.07 (S)	2.39	5.50	5.16 (S)	2.75	11.05
3j	Benzyl	2,6-Dimethylphenyl	0.58 (S)	1.00		2.28 (S)	1.08	0.69
3k		Phenyl	2.29 (S)	3.72	5.33	10.22 (S)	3.28	11.25
3l		Benzyl	2.08 (S)	2.19	3.29	8.70 (S)	2.65	11.14
3m	Phenyl	2,6-Dimethylphenyl	1.49 (S)	2.05	3.52	9.27 (S)	1.86	5.32
3n		Phenyl	1.55 (S)	3.50	5.50	10.85 (S)	2.47	9.57
3o		Benzyl	1.60 (S)	2.58	4.89	11.11 (S)	2.20	9.50

[a]Mobile phase: 80% methanol in water + sulfuric acid (10 mM). Flow rate: 0.5 mL/min. Detection: 210 nm UV. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α: Separation factor. R_S: Resolution factor.

tocainide analogues **3b** and **3c**, CSP **2** was also more effective than CSP **1** in terms of both the separation (α) and the resolution factors (R_S).

When the methyl group at the chiral center of tocainide is changed into a larger group, the resolution was generally improved on both CSP **1** and CSP **2** except for the resolution of the analogues containing a 2,6-dimethylphenyl group as an Ar group. As shown in Table 1, analytes containing a 2,6-dimethylphenyl group as an Ar group (**3d**, **3g** and **3j**), which were originated from valine, leucine and phenylalanine, were not resolved at all or resolved with only marginal separation factors. However, an analyte (**3m**) originated from phenylglycine was resolved quite well on both CSP **1** and CSP **2** even though it contains a 2,6-dimethylphenyl group. When the Ar group of analytes was phenyl or benzyl group, their resolutions were quite excellent on both CSP **1** and CSP **2**.

Between CSP **1** and CSP **2**, in the resolution of analytes including tocainide containing a 2,6-dimethylphenyl group, CSP **2** is better than CSP **1** in terms of both the enantioselectivity (α) and the resolution (R_S) except for the resolution of analogue **3m** as shown in Table 1. In the resolution of other tocainide analogues containing a phenyl or a benzyl group as an Ar group, CSP **2** is much better than CSP **1** in terms of the resolution (R_S). However, the enantioselectivities denoted by the separation factors (α) for the resolution of tocainide analogues containing a phenyl or a benzyl group as an Ar group are greater on CSP **1** than on CSP **2** in some cases or worse on CSP **1** than on CSP **2** in other cases, depending on the structure of analytes as shown in Table 1.

In order to see the effect of the content and the type of organic and acidic modifier in aqueous mobile phase on the resolution of tocainide (**3a**) and its analogues (**3b**-**3o**) on CSP **1** and CSP **2**, we selected four analytes including tocainide and resolved them on CSP **1** and CSP **2**. Their resolution behaviors are summarized in Table 2 and Table 3. As shown in Table 2 and Table 3, resolution of tocainide (**3a**) and its selected analogues (**3i**, **3k** and **3m**) on CSP **1** and CSP **2** is very much dependent on the content of organic modifier in aqueous mobile phase (see entry a in Table 2 and Table 3). When the content of organic modifier such as methanol or acetonitrile in aqueous mobile phase increases, the retention of analytes denoted by the retention factors (k_1) also increases on both CSP **1** and CSP **2**. These trends are exactly consistent with those for the resolution of amino acids and primary amines on CSP **1** and CSP **2** reported previously.[10,11,15] As the content of organic modifier increases, the aqueous mobile phase becomes less polar and more hydrophobic. In this instance, the hydrophilic interaction between polar-protonated analytes and the mobile phase decreases and consequently, the retention is expected to increase as the content of organic modifier in aqueous mobile phase increases. The separation (a) and the resolution factors (R_S) increase continuously as the content of organic modifier in aqueous mobile phase increases. As an example, the chromatograms illustrating the chromatographic trends for the resolution of tocainide on CSP **1** and CSP **2** with the variation of the content of methanol in aqueous mobile phase are presented in Figure 2.

Table 2 and Table 3 also show that the chromatographic behaviors for the resolution of tocainide (**3a**) and its selected analogues (**3i**, **3k** and **3m**) on CSP **1** and CSP **2** are dependent on the content and the type of acidic modifier in aqueous mobile phase (see entry b in Table 2 and Table 3). Previously, it was reported that complexation of ammonium ion ($R\text{-}NH_3^+$) inside the cavity of the crown ether ring is essential for the chiral recognition.[10] In this instance, an

Table 2. Resolution of tocainide (**3a**) and three selected its analogues (**3i**, **3k** and **3m**) on CSP **1** with the variation of the content and the type of organic and acidic modifier in aqueous mobile phase[a]

	Mobile phase	3a			3i			3k			3m		
		k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
a	30% CH_3OH + H_2SO_4 (10 mM)	0.39	1.00		0.58 (S)	1.34	1.31	1.52 (S)	1.77	2.83	0.85 (S)	1.59	2.25
	50% CH_3OH + H_2SO_4 (10 mM)	0.59	1.00		0.81 (S)	1.69	3.05	1.86 (S)	2.45	5.17	1.16 (S)	1.70	3.24
	80% CH_3OH + H_2SO_4 (10 mM)	1.05 (S)	1.17	0.92	1.07 (S)	2.39	5.50	2.29 (S)	3.72	5.33	1.49 (S)	2.05	3.52
	50% CH_3CN + H_2SO_4 (10 mM)	0.17	1.00		0.14 (S)	1.83	1.32	0.27 (S)	2.74	3.79	0.22 (S)	1.92	2.18
	80% CH_3CN + H_2SO_4 (10 mM)	0.44	1.00		0.21 (S)	2.28	3.56	0.35 (S)	3.46	7.00	0.35 (S)	2.28	4.73
b	80% CH_3OH + H_2SO_4 (1 mM)	1.35 (S)	1.17	0.83	1.44 (S)	2.26	4.90	3.44 (S)	3.43	6.43	1.61 (S)	2.10	4.16
	80% CH_3OH + H_2SO_4 (5 mM)	1.03 (S)	1.15	0.78	0.93 (S)	2.31	2.95	2.11 (S)	3.32	5.21	1.23 (S)	2.17	4.58
	80% CH_3OH + H_2SO_4 (10 mM)	1.05 (S)	1.17	0.92	1.07 (S)	2.39	5.50	2.29 (S)	3.72	5.33	1.49 (S)	2.05	3.52
	80% CH_3OH + H_2SO_4 (15 mM)	1.01 (S)	1.15	0.90	0.85 (S)	2.31	5.37	1.82 (S)	3.20	5.70	1.16 (S)	2.21	4.91
	80% CH_3OH + $HClO_4$ (1 mM)	0.69 (S)	1.19	0.41	1.83 (S)	2.91	5.68	1.91 (S)	3.18	6.08	0.89 (S)	2.02	2.92
	80% CH_3OH + $HClO_4$ (5 mM)	0.65 (S)	1.14	0.50	1.48 (S)	3.42	7.68	1.33 (S)	3.10	5.12	0.83 (S)	2.19	4.26
	80% CH_3OH + $HClO_4$ (10 mM)	0.71 (S)	1.15	0.73	1.54 (S)	3.42	6.75	1.32 (S)	3.02	4.46	0.92 (S)	2.22	4.76
	80%CH_3OH + CH_3CO_2H (1 mM)	4.42 (S)	1.27	1.54	14.56(S)	2.19	5.00	16.14(S)	3.92	5.15	6.32 (S)	1.95	4.43
	80%CH_3OH + CH_3CO_2H (5 mM)	1.96 (S)	1.27	1.25	9.74 (S)	2.11	5.33	11.81(S)	3.54	6.79	3.04 (S)	2.07	3.73
	80%CH_3OH + CH_3CO_2H (10 mM)	1.65 (S)	1.25	1.01	1.32 (S)	2.16	5.86	7.47(S)	3.54	5.18	2.35 (S)	2.07	3.34

[a]Flow rate: 0.5 mL/min. Detection: 210 nm UV. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. α: Separation factor. R_S: Resolution factor.

Table 3. Resolution of tocainide (**3a**) and three selected its analogues (**3i**, **3k** and **3m**) on CSP **2** with the variation of the content and the type of organic and acidic modifier in aqueous mobile phase[a]

	Mobile phase	3a			3i			3k			3m		
		k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
a	30% CH_3OH + H_2SO_4 (10 mM)	1.38 (S)	1.13	0.96	2.47 (S)	1.82	5.55	5.43 (S)	2.13	5.68	4.06 (S)	1.68	4.50
	50% CH_3OH + H_2SO_4 (10 mM)	2.10 (S)	1.19	1.69	3.34 (S)	2.21	8.21	7.40 (S)	2.70	8.99	5.71 (S)	1.75	5.20
	80% CH_3OH + H_2SO_4 (10 mM)	4.93 (S)	1.25	2.66	5.16 (S)	2.75	11.05	10.22(S)	3.28	11.25	9.27 (S)	1.86	5.32
	50% CH_3CN + H_2SO_4 (10 mM)	0.62 (S)	1.19	1.26	0.77 (S)	2.18	6.72	1.30 (S)	2.70	8.45	1.43 (S)	1.56	4.27
	80% CH_3CN + H_2SO_4 (10 mM)	1.22 (S)	1.17	2.04	0.99 (S)	2.33	10.73	1.48 (S)	2.82	12.40	2.11 (S)	1.53	5.30
b	80% CH_3OH + H_2SO_4 (1 mM)	6.08 (S)	1.32	2.83	9.24 (S)	2.88	10.04	19.66(S)	3.85	11.42	11.94(S)	1.99	4.62
	80% CH_3OH + H_2SO_4 (5 mM)	4.86 (S)	1.25	2.38	5.13 (S)	2.78	10.49	11.04(S)	3.39	12.21	8.87 (S)	1.89	5.51
	80% CH_3OH + H_2SO_4 (10 mM)	4.93 (S)	1.25	2.66	5.16 (S)	2.75	11.05	10.22(S)	3.28	11.25	9.27 (S)	1.86	5.32
	80% CH_3OH + H_2SO_4 (15 mM)	4.00 (S)	1.21	2.34	3.94 (S)	2.78	11.91	7.70 (S)	3.21	11.62	7.75 (S)	1.88	6.35
	80% CH_3OH + $HClO_4$ (1 mM)	0.81 (S)	1.52	1.59	3.83 (S)	2.14	3.70	2.77 (S)	4.43	4.63	1.38 (S)	2.40	4.00
	80% CH_3OH + $HClO_4$ (5 mM)	1.62 (S)	1.36	2.29	5.74 (S)	2.54	7.26	3.90 (S)	3.60	6.32	1.99 (S)	1.99	4.90
	80% CH_3OH + $HClO_4$ (10 mM)	1.93 (S)	1.29	2.07	6.04 (S)	2.59	7.74	3.97 (S)	3.28	7.12	3.41 (S)	1.91	5.84
	80%CH_3OH + CH_3CO_2H (1 mM)	0.16 (S)	2.28	0.78	1.21 (S)	1.93	1.71	1.30 (S)	3.43	2.92	0.25 (S)	4.02	2.31
	80%CH_3OH + CH_3CO_2H (5 mM)	0.16 (S)	2.17	1.04	1.38 (S)	1.96	1.67	1.31 (S)	3.92	3.04	0.32 (S)	3.72	2.68
	80%CH_3OH + CH_3CO_2H (10 mM)	0.22 (S)	1.88	1.11	1.61 (S)	1.71	1.89	1.35 (S)	4.31	3.20	0.41 (S)	3.25	2.72

[a]Flow rate: 0.5 mL/min. Detection: 210 nm UV. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. In the parenthesis, the absolute configuration of the first eluted enantiomer is presented. a: Separation factor. R_S: Resolution factor.

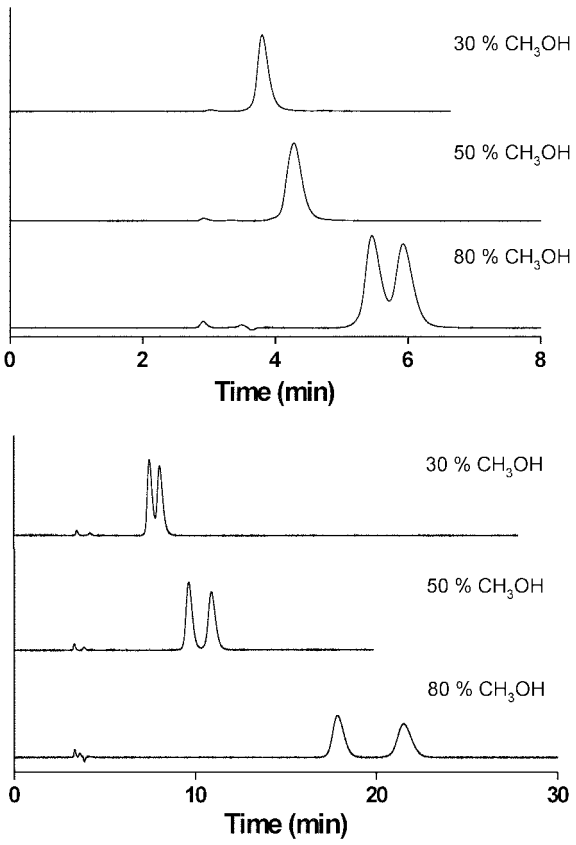

Figure 2. Chromatograms for the resolution of tocainide (**3a**) on (top) CSP **1** and (bottom) CSP **2** with the variation of the content of methanol in aqueous mobile phase. See the footnote to Table 1 for the chromatographic conditions.

acidic modifier added to the aqueous mobile phase is believed to play a role of protonating the primary amino group of analytes and enhancing the diastereomeric complex formation of an analyte inside the cavity of chiral crown ether ring of the CSP.

As shown in Table 2, the retention factors (k_1) generally decrease as the content of acidic modifier in aqueous mobile phase increases. As the content of acidic modifier in aqueous mobile phase increases, the ionic strength of mobile phase increases and consequently, the hydration or the dissolution of polar-protonated analytes by mobile phase is expected to increase. In this instance, polar-protonated analytes are eluted faster and faster as the content of acidic modifier increases. However, the separation (α) and the resolution factors (R_S) do not show significant trends with the variation of the content of acidic modifier in aqueous mobile phase.

As shown in Table 3, the retention factors (k_1) also decrease as the content of sulfuric acid in aqueous mobile phase increases. However, surprisingly, the retention factors (k_1) generally increase as the content of perchloric acid or acetic acid in aqueous mobile phase increases. The acid anions such as ClO_4^- and $CH_3CO_2^-$ are expected to be relatively more lipophilic than SO_4^{2-} based on the previous study concerning the lipophilicity of acid anions.[16] In this instance, as the content of perchloric acid or acetic acid in aqueous mobile phase increases, the lipophilic interaction between the analyte complex ($R\text{-}NH_3^+X^-$ where X^- is acid anion) and the more lipophilic CSP (CSP **2**) is expected to increase and consequently the retention factors (k_1) generally increase.

In addition, the use of acetic acid as an acidic modifier afforded opposite trends for the retention of tocainide (**3a**) and its selected analogues (**3i**, **3k** and **3m**) on CSP **1** and CSP **2**. The use of acetic acid as an acidic modifier increases the retention quite much on CSP **1** while it decreases the retention quite much on CSP **2** for the resolution of tocainide

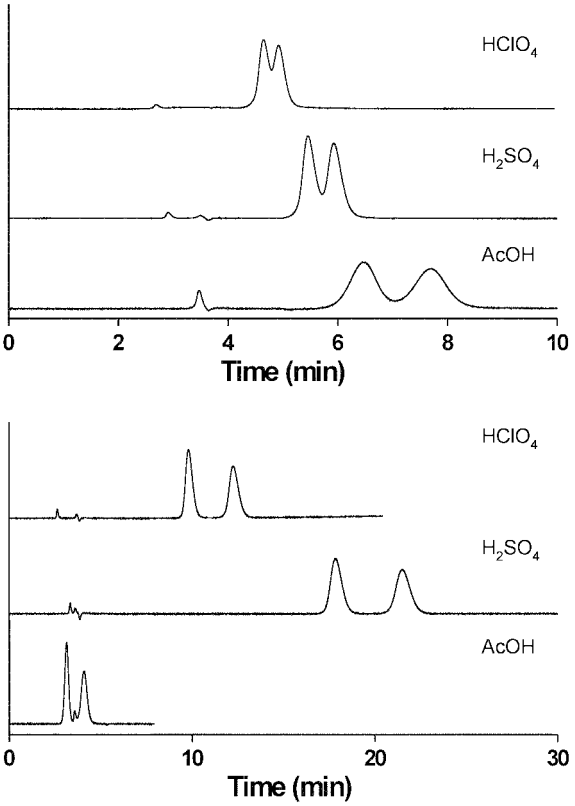

Figure 3. Chromatograms for the resolution of tocainide (**3a**) on (top) CSP **1** and (bottom) CSP **2** with the variation of the type of an acidic modifier (10 mM) in 80% methanol in water as a mobile phase. See the footnote to Table 1 for the chromatographic conditions.

(**3a**) and its selected three analogues (**3i**, **3k** and **3m**) as shown in Table 2 and Table 3. The chromatograms shown in Figure 3 for the resolution of tocainide (**3a**) on CSP **1** and CSP **2** with the variation of the type of acidic modifier in aqueous mobile phase clearly demonstrate these somewhat surprising resolution results. From these results, the retention mechanism on CSP **1** is inferred to be somewhat different from that on CSP **2**. However, the rationale for the different retention behaviors on CSP **1** and CSP **2** with the use of acetic acid as an acidic modifier is not explored yet.

In summary, in this report, we demonstrated that CSP **1** and CSP **2** prepared from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid are quite successful in the resolution of racemic tocainide and its analogues. Especially in the resolution of tocainide, clear baseline separation of the two enantiomers was observed on CSP **2**. Consequently, CSP **2** is expected to be useful to monitor the enantiomeric composition of tocainide in clinical samples. The resolution of tocainide and its analogues on CSP **1** and CSP **2** was also demonstrated to be dependent on the content and the type of organic and acidic modifier in aqueous mobile phase. In general, increasing the content of organic modifier in aqueous mobile phase improves all of three chromatographic parameters such as the retention (k_1), the separation (α) and the resolution factors (R_S) on CSP **1** and CSP **2**. Increasing the content of acidic modifier in aqueous mobile phase diminishes the retention factors (k_1) even though the separation (α) and the resolution factors (R_S) do not show significant trends with the variation of the content of an acidic modifier on CSP **1**. On the contrary, the trends of retention factors (k_1) with the variation of the content of acidic modifier in aqueous mobile phase were not consistent on CSP **2** with the variation of the type of acidic modifier. However, the chromatographic behaviors for the resolution of tocainide and its analogues on CSP **1** and CSP **2** can be controllable by varying the content and the type of organic and acidic modifiers in aqueous mobile phase.

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