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Liquid Chromatographic Resolution of Vigabatrin and Its Analogue γ-Amino Acids on Chiral Stationary Phases Based on (3,3'-Diphenyl-1,1'-binaphthyl)-20-crown-6†

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Two chiral stationary phases (CSPs) based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 bonded covalently to silica gel were applied for the first time to the resolution of racemic vigabatrin and its analogue γ-amino acids and the resolution results were compared to those on the commercially available Crownpak CR(+) based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 coated dynamically onto octadecylsilica gel. While vigabatrin was not resolved at all on Crownpak CR(+), it was resolved quite well on the two CSPs. Among four vigabatrin analogue γ-amino acids, only two were resolved on Crownpak CR(+), but three were resolved on the CSP (CSP **1**) containing residual silanol groups and all of four were resolved on the CSP (CSP **2**) containing residual silanol group-protecting *n*-octyl groups. The improved lipophilicity in CSP **2** was proposed to be responsible for its superiority to CSP **1** for the resolution of vigabatrin and analogue γ-amino acids. In addition, the composition of aqueous mobile phase was found to affect the chiral recognition behaviors for the resolution of vigabatrin and analogue γ-amino acids on CSP **2**.

Key Words : Chiral stationary phase, Chiral crown ether, Enantiomeric resolution, Liquid chromatography, Vigabatrin, γ-Amino acids

Introduction

Liquid chromatographic chiral stationary phases (CSPs) based on chiral crown ethers have been known to be very useful for the resolution of racemic compounds containing a primary amino group.[1] Especially, a CSP based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 covalently bonded to silica gel (CSP **1**, Fig. 1) was very successful in the resolution of racemic α-amino acids,[2] β-amino acids,[3] cyclic and non-cyclic amines,[4] amino alcohols,[4] various fluoroquinolone antibacterials,[5] tocainide (antiarrhythmic agent) and its analogues,[6] and aryl-α-amino ketones.[7] Another CSP prepared by treating CSP **1** with n-octyltriethoxysilane (CSP **2**, Fig. 1) was also very successful in the resolution of α-amino acids,[8] β-amino acids,[3] cyclic and non-cyclic amines,[8] amino alcohols,[8] various fluoroquinolone antibacterials,[9] tocainide (antiarrhythmic agent) and its analogues,[10] and aryl-α-amino ketones,[11] the chiral recognition efficiency of CSP **2** being even greater than that of CSP **1**. However, CSP **1** and CSP **2** have not been utilized in the resolution of vigabatrin and its analogue γ-amino acids.

Vigabatrin (**3**, 4-amino-5-hexenoic acid, Fig. 2), a structural analogue of the inhibitory neurotransmitter γ-amino acid (4-aminobutyric acid, GABA, Fig. 2), is an anticonvulsant drug used for the treatment of epilepsy.[12] Vigabatrin is a chiral compound. Even though only the (*S*)-(+)-enantiomer has been reported to posses pharmacological activity,[13] vigabatrin is supplied as a racemic mixture. In addition, the two enantiomers of vigabatrin have been reported to show different pharmacokinetics and pharmacological activity after epileptic patients was treated with racemic vigabatrin.[14]

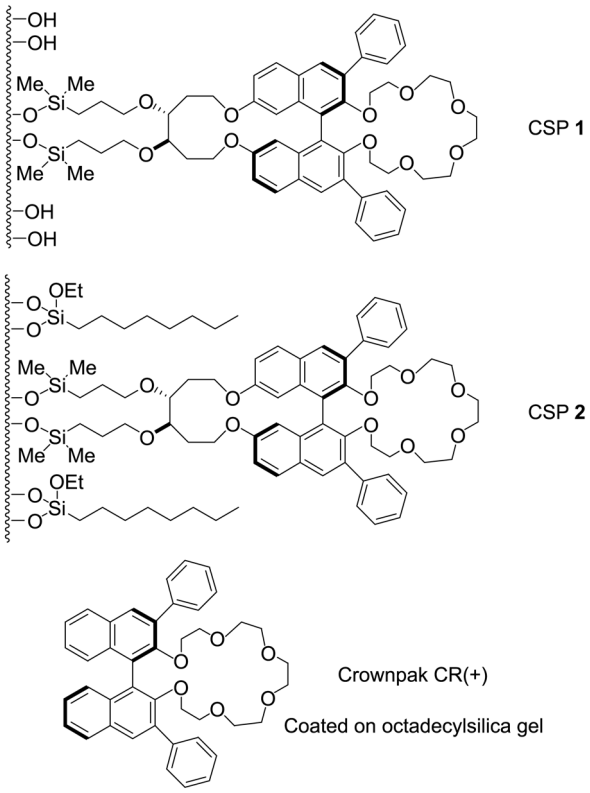

Figure 1. Structures of CSP**1**, CSP **2** and Crowmpak CR(+).

†This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.

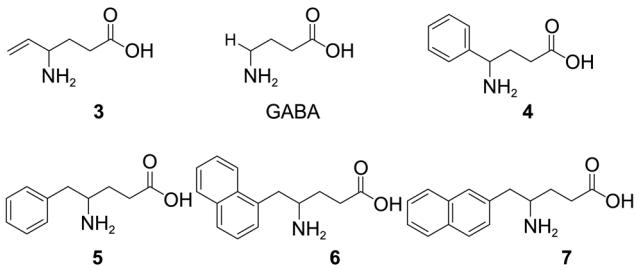

Figure 2. Structures of vigabatrin (**3**), γ-aminobutyric acid (GABA), 4-amino-4-phenylbutanoic acid (**4**), 4-amino-5-phenylpentanoic acid (**5**), 4-amino-5-(1-naphthyl)pentanoic acid (**6**) and 4-amino-5-(2-naphthyl)pentanoic acid (**7**).

In this instance, the determination of the enantiomeric composition of vigabatrin is very important. For the determination of the content of vigabatrin in plasma or serum, gas chromatography-mass spectrometry (GC-MS) or gas-liquid chromatography (GLC) has been utilized.[15] Vigabatrin derivatized with chiral derivatizing agents have been resolved as diastereomers by high performance liquid chromatography (HPLC) or by capillary electrophoresis (CE) method.[14,16] However, the direct resolution of vigabatrin by HPLC without derivatization is rare. Recently, a CSP based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was successfully utilized in the resolution of vigabatrin and its analogue γ-amino acids.[17] In addition, macrocyclic glycopeptide antibiotic CSPs based on teicoplanin aglycone were used for the resolution and quantification of the two enantiomers of vigabatrin and γ-amino acids.[18] As another example for the direct resolution of vigabatrin and its analogue γ-amino acids, in this study, we report that CSP **1** and CSP **2** can be successfully applied to the resolution of vigabatrin and its analogue γ-amino acids.

Experimental

Chromatography was performed with an HPLC system consisting of a Waters model 510 HPLC Pump (Milford, MA, USA), a Rheodyne model 7725i injector (Rohnert Park, CA, USA) with a 20 μL sample loop, Waters 486 tunable absorbance detector (Milford, MA, USA) and Younglin Autochro data module (Software: YoungLin Autochro-WIN 2.0 plus) (Seoul, Korea). Chiral columns packed with CSP **1** (250 × 4.6 mm i.d.) and CSP **2** (150 × 4.6 mm i.d.) were available from prior studies.[2,8] Crownpak CR(+) column (150 × 4.6 mm i.d., Fig. 1) was commercially available from Daicel Chemical Industries (Tokyo, Japan). Vigabatrin (**3**) and its analogue γ-amino acids (**4**-**7**) shown in Fig. 2 were available from prior study.[17] The temperature of the chiral columns was maintained at 20 °C by using a Julabo F30 Ultratemp 2000 cooling circulator (Seelbach, Germany). Injection samples were prepared by dissolving analytes in water (vigabatrin) or in methanol (other γ-amino acids) at a concentration of 1.0 mg/mL and an injection size was typically 3 μL.

Results and Discussion

Previously, vigabatrin was attempted to be resolved without derivatization by HPLC using the Crownpak CR(+) based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 dynamically coated onto octadecyl silica gel, but it was not successful.[19] The chiral selector of Crownpak CR(+), (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6, is identical to that of CSP **1** and CSP **2**. In this instance, we tried to compare the chiral recognition abilities of Crownpak CR(+), CSP **1** and CSP **2** for the resolution of vigabatrin (**3**) and its analogue γ-amino acids (**4**-**7**). The chromatographic results for the resolution of vigabatrin (**3**) and its analogue γ-amino acids (**4**-**7**) on Crownpak CR(+), CSP **1** and CSP **2** are summarized in Table 1. As shown in Table 1, vigabatrin was not resolved at all on Crownpak CR(+), but analytes **4** and **5** were resolved. Analytes **6** and **7** were found to be not eluted from the chiral column probably because of the long retention times. The chromatographic resolution on Crownpak CR(+) was performed with the use of 15% methanol in water containing sulfuric acid (10 mM) as a mobile phase. Because of the dynamically coated nature of the Crownpak CR(+), the use of a mobile phase containing more than 15% methanol results in leaching of the chiral crown selector from the chiral column.[20] Consequently, we did not try to use other mobile phase condition.

Vigabatrin was resolved best with the separation factor (α) of 1.45 and the resolution (R_S) of 1.86 on CSP **1** when 80% acetonitrile in water containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) was used as a mobile phase as shown in Table 1. The identical mobile phase was applied to

Table 1. Resolution of vigabatrin (**3**) and its analogue γ-amino acids **4**-**7** on Crownpak CR(+), CSP **1** and CSP **2**[a]

Analytes	Crownpak CR(+)			CSP **1**			CSP **2**		
	k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
3	1.26	1.00		1.43	1.45	1.86	2.11	1.73	2.19
4	8.70	1.45	2.17	1.01	2.11	3.08	1.66	2.56	4.74
5	8.52	1.11	0.94	0.68	1.17	1.25	0.68	1.29	3.83
6				0.68	1.00	–	1.94	1.10	0.51
7				0.70	1.08	0.59	1.91	1.59	2.13

[a]Mobile phase: 15% Methanol in water containing sulfuric acid (10 mM) on Crownpak CR(+), 80% acetonitrile in water containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) on CSP 1 and 80% methanol in water containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) on CSP 2. Flow rate: 0.5 mL/min. Detection: 214 nm UV for analyte **3** and 254 nm UV for analytes **4**-**7**. Temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution factor.

the resolution of other analytes on CSP **1**. Analytes **4** and **5** were also resolved well on CSP **1**, but analyte **6** was not resolved at all and analyte **7** was resolved only slightly with the resolution (R_S) of 0.59. In contrast, vigabatrin was resolved with the separation factor (α) of 1.73 and the resolution (R_S) of 2.19 on CSP **2** when 80% methanol in water containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) was used as a mobile phase as shown in Table 1. Under the identical mobile phase condition, other analytes (**4**-**7**) were resolved on CSP **2**. In every case, CSP **2** was found to be better than CSP **1** in the resolution of vigabatrin and its analogue γ-amino acids. Previously, it was concluded from the chromatographic results for the resolution of various racemic primary amino compounds on CSP **1** and CSP **2** that the protection of the residual silanol groups of CSP **1** with lipophilic *n*-octyl groups can considerably improve its chiral recognition ability.[3,8-11] Removal of the non-enantioselective interaction between the residual silanol groups and racemic analytes by the protection of the residual silanol groups of CSP **1** with n-octyl groups might be responsible for the higher chiral recognition ability of CSP **2** compared to that of CSP **1**.[3] However, this effect should be limited under highly polar aqueous mobile phase condition. Instead, the additional *n*-octyl protecting groups make CSP **2** more lipophilic than CSP **1** and the improved lipophilicity of CSP **2** might be responsible for the higher chiral recognition ability of CSP **2** compared to that of CSP **1** even though the reason is not clear yet.[3]

In order to see the effect of mobile phase composition on the chiral recognition ability of CSP **2** for the resolution of vigabatrin and its analogue γ-amino acids, we selected three analytes (**3**, **4** and **7**) and resolved them on CSP **2** with the variation of the composition of aqueous mobile phase. The chromatographic results for the resolution of selected three analytes (**3**, **4** and **7**) on CSP **2** with the variation of the composition of aqueous mobile phase are summarized in Table 2. As shown in Table 2, as the content of methanol in water containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) was increased from 30% to 50% and then to 80%, the retention times denoted by the retention factors (k_1) decrease continuously (see entry a in Table 2). Especially, the retention factor for analyte **7**, which is probably most lipophilic, was found to decrease dramatically. The representative chromatograms for the resolution of analyte **4** on CSP **2** with the variation of the methanol content in aqueous mobile phase containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) shown in Fig. 3 clearly demonstrate the retention behaviors. As the content of methanol in aqueous mobile phase is increased, the mobile phase polarity decrease and consequently, the lipophilic interaction between the analyte and the quite lipophilic CSP is expected to decrease. In this instance, the retention of the analyte decreases. The separation factors (α) and resolutions (R_S) for the resolution of analyte **3** and **4** were found to increase as the content of methanol in aqueous mobile phase is increased, but those for the resolution of analyte **7** were found not to change notably. The use of acetonitrile instead of methanol in aqueous mobile phase containing sulfuric acid (10 mM) and ammonium acetate (1.0 mM) was found to be also useful for the resolution of selected three analytes (**3**, **4** and **7**) on CSP **2** (see entry a in Table 2).

Acidic modifier added to the mobile phase is used to protonate the primary amino group of analytes. The resulting primary ammonium ion ($R\text{-}NH_3^+$) of analyte has been

Table 2. Resolution of selected analytes **3**, **4** and **7** on CSP **2** with the variation of the composition of aqueous mobile phase[a]

	Mobile phase	**3**			**4**			**7**		
		k_1	α	Rs	k_1	α	Rs	k_1	α	Rs
a	30% CH_3OH + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	3.37	1.23	1.08	8.68	1.90	2.62	62.85	1.57	2.19
	50% CH_3OH + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	3.30	1.27	1.40	5.00	1.98	3.20	13.77	1.59	2.83
	80% CH_3OH + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	2.11	1.73	2.19	1.66	2.56	4.74	1.91	1.59	2.13
	50% CH_3CN + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	3.06	1.27	2.02	1.84	2.20	5.12	2.44	1.33	2.60
	80% CH_3CN + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	3.01	1.45	1.47	1.36	3.20	6.98	1.01	1.26	1.36
b	80% CH_3OH + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	2.11	1.73	2.19	1.66	2.56	4.74	1.91	1.59	2.13
	80% CH_3OH + 10 mM $HClO_4$ + 1.0 mM $CH_3CO_2NH_4$	2.46	1.62	1.99	1.85	2.50	4.76	2.14	1.56	2.36
	80% CH_3OH + 10 mM TFA + 1.0 mM $CH_3CO_2NH_4$	2.29	1.00		2.55	2.49	4.81	1.98	1.56	2.87
	80% CH_3OH + 10 mM AcOH + 1.0 mM $CH_3CO_2NH_4$	2.24	1.32	1.31	1.72	1.97	2.90	1.54	1.34	1.75
c	80% CH_3OH + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	2.11	1.73	2.19	1.66	2.56	4.74	1.91	1.59	2.13
	80% CH_3OH + 5 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	2.74	1.31	1.48	1.55	2.65	4.97	1.80	1.61	3.00
	80% CH_3OH + 2 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	2.71	1.31	1.47	1.78	2.46	2.06	2.01	1.42	2.21
	80% CH_3OH + 1 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	2.79	1.31	1.21	1.88	2.19	2.02	2.01	1.43	2.21
d	50% CH_3CN + 10 mM H_2SO_4 + 1.0 mM $CH_3CO_2NH_4$	3.06	1.27	2.09	1.84	2.20	5.21	2.44	1.31	2.60
	50% CH_3CN + 10 mM H_2SO_4 + 0.5 mM $CH_3CO_2NH_4$	4.24	1.24	2.02	4.64	2.22	6.43	6.11	1.29	5.97
	50% CH_3CN + 10 mM H_2SO_4 + 0.1 mM $CH_3CO_2NH_4$	7.21	1.26	1.86	8.33	2.13	3.43	10.70	1.27	3.05
	50% CH_3CN + 10 mM H_2SO_4 + 0.0 mM $CH_3CO_2NH_4$	7.93	1.26	1.82	10.45	2.48	3.42	13.02	1.33	2.68

[a]Flow rate: 0.5 mL/min. Temperature: 20 °C. Detection: 210 or 254 nm UV. k_1: Retention factor of the first eluted enantiomer.

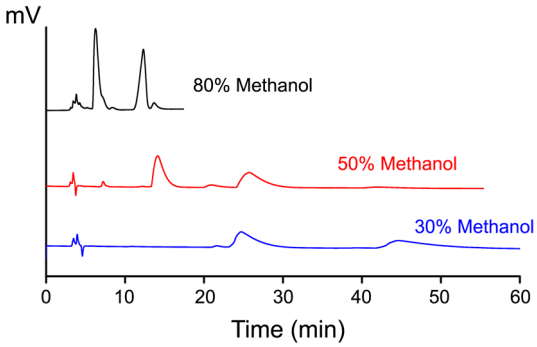

Figure 3. Chromatograms for the resolution of 4-amino-4-phenylbutanoic acid (**4**) on CSP **2** with the variation of the content of methanol in water containing sulfuric acid (10 mM) and ammonium acetate (1 mM). Flow rate: 0.5 ml/min. Temperature: 20 °C. Detection: 254 nm UV.

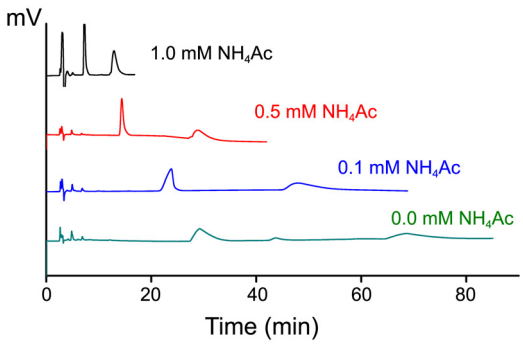

Figure 4. Chromatograms for the resolution of 4-amino-4-phenylbutanoic acid (**4**) on CSP **2** with the variation of the content of ammonium acetate in 50% acetonitrile in water containing sulfuric acid (10 mM). Flow rate: 0.5 ml/min. Temperature: 20 °C. Detection: 254 nm UV.

proposed to be used for the enantioselective complexation inside the cavity of the crown ether ring of the CSP.[1] As an acidic modifier we tested sulfuric acid, perchloric acid, trifluoroacetic acid (TFA) and acetic acid (AcOH). As shown in Table 2 (entry b), sulfuric acid and perchloric acid were found to be quite effective as an acidic modifier in the mobile phase for the resolution of the three selected analytes. Especially, sulfuric acid was found to be most effective for the resolution of vigabatrin (**3**) in terms of the separation factor and resolution. Trifluoroacetic acid was not effective at all for the resolution of vigabatrin (**3**), but quite effective for the resolution of analytes **4** and **7**. Acetic acid was less effective than sulfuric acid or perchloric acid for the resolution of the three selected analytes in terms of the separation factors and resolutions.

Changing the content of sulfuric acid in the mobile phase from 1 mM to 5 mM and then to 10 mM does not affect the chiral recognition behaviors significantly. Vigabatrin (**3**) was resolved best when the content of sulfuric acid in aqueous mobile phase was 10 mM, but analytes **4** and **7** were resolved best when the content of sulfuric acid in aqueous mobile phase was 5 mM (see entry c in Table 2).

Ammonium acetate added to the mobile phase is used to reduce the retention times of analytes. Especially, the retention times of analytes on CSP **1** and CSP **2** have been controlled by adding ammonium acetate to the mobile phase.[8-11] The competition for the complexation inside the cavity of the crown ether ring of the CSP between the ammonium ion (NH_4^+) from ammonium acetate and the primary ammonium ion ($R\text{-}NH_3^+$) of analyte is believed to reduce the retention times of analytes.[8-11] Without ammonium acetate, the retention factors of analytes are quite large as shown in Table 2 (entry d). As the content of ammonium acetate in aqueous mobile phase is increased, the retention factors decrease significantly, but the separation factors do not change significantly. The representative chromatograms showing the retention behaviors for the resolution of analyte **4** on CSP **2** with the variation of the content of ammonium acetate in aqueous mobile phase are shown in Fig. 4. The addition of ammonium acetate to aqueous mobile phase was found to improve the resolution factor for vigabatrin (**3**) continuously. The addition of ammonium acetate (until 0.5 mM) to aqueous mobile phase was also found to improve the resolutions for analytes **4** and **7**. However, further addition of ammonium acetate (1.0 mM) to aqueous mobile phase was found to diminish the resolutions for analytes **4** and **7**. The addition of a certain amount of ammonium acetate to aqueous mobile phase is expected to increase the mass transfer rate of analytes within the chiral column. In this instance, the peak tailing is diminished and the resolution is expected to increase. However, the addition of ammonium acetate further can reduce the retention times of the two enantiomers quite much and then the resolution which is related to the difference between the retention times of two enantiomers can be diminished.

In summary, in this study, we for the first time demonstrated that CSPs based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 bonded covalently to silica gel are quite useful for the resolution of vigabatrin and its analogue γ-amino acids. Between the two CSPs, CSP **2** containing residual silanol group-protecting *n*-octyl groups is better than CSP **1** containing residual silanol groups in the resolution of vigabatrin and its analogue γ-amino acids. We also demonstrated that the chromatographic behaviors for the resolution of vigabatrin and its analogue γ-amino acids on CSP **2** can be controlled by changing the composition of aqueous mobile phase.

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References

1. (a) Hyun, M. H. *J. Sep.Sci.* **2003**, *26*, 242. (b) Hyun, M. H. *Bull. Kor. Chem. Soc.* **2005**, *26*, 1153. (c) Hyun, M. H. *J. Sep.Sci.* **2006**, *29*, 750. (d) Choi, H. J.; Hyun, M. H. *J. Liq. Chromatogr. Rel. Technol.* **2007**, *30*, 853.
2. Hyun, M. H.; Han, S. C.; Lipshutz, B. H.; Shin, Y.-J.; Welch, C. J.

J. Chromatogr. A **2001**, *910*, 359.
3. Choi, H. J.; Ha, H. J.; Han, S. C.; Hyun, M. H. *Anal. Chim. Acta* **2008**, *619*, 122.
4. Hyun, M. H.; Han, S. C.; Lipshutz, B. H.; Shin, Y.-J.; Welch, C. J. *J. Chromatogr. A* **2002**, *959*, 75.
5. Hyun, M. H.; Han, S. C. *J. Biochem. Biophys. Methods* **2002**, *54*, 235.
6. Hyun, M. H.; Min, H. J.; Cho, Y. J. *J. Chromatogr. A* **2003**, *996*, 233.
7. Hyun, M. H.; Tan, G.; Cho, Y. J. *Biomed. Chromatogr.* **2005**, *19*, 208.
8. Hyun, M. H.; Han, S. C.; Choi, H. J.; Kang, B. S.; Ha, H. J. *J. Chromatogr. A* **2007**, *1138*, 169.
9. Choi, H. J.; Cho, H. S.; Han, S. C.; Hyun, M. H. *J. Sep. Sci.* **2009**, *32*, 536.
10. Choi, H. J.; Jin, J. S.; Hyun, M. H. *Chirality* **2009**, *21*, 11.
11. Choi, H. J.; Jin, J. S.; Hyun, M. H. *J. Chromatogr. B* **2008**, *875*, 102.
12. Walker, M. C.; Patsalos, P. N. *Pharmacol. Ther.* **1995**, *67*, 351.
13. Meldrum, B. S.; Murugaiah, K. *Eur. J. Pharmacol.* 1983, *89*, 149.
14. Vermeij, T. A. C.; Edelbroek, P. M.; *J. Chromatogr. B* 1998, *716*, 233.
15. (a) Haegele, K. D.; Schoun, J.; Alken, R. G.; Huebert, N. D. *J. Chromatogr.* **1983**, *274*, 103. (b) Schramm, T. M.; McKinnon, G. E.; Eadie, M. J. *J. Chromatogr. Biomed. Appl.* **1993**, *616*, 39.
16. Zhao, S.; Zhang, R.; Wang, H.; Tang, L.; Pan, Y. *J. Chromatogr. B* **2006**, *833*, 186.
17. Lee, S. J; Cho, H. S.; Choi, H. J.; Hyun, M. H. *J. Chromatogr. A* **2008**, *1188*, 318.
18. (a) Al-Majed, A. A. *J. Pharm. Biomed. Anal.* **2009**, *50*, 96. (b) Pataj, Z.; Ilisz, I.; Aranyi, A.; Forro, E.; Fulop, F.; Armstrong, D. W.; Peter, A. *Chromatographia* **2010**, *71*, S13.
19. Walbroehl, Y.; Wagner, J. *J. Chromatogr. A* **1994**, *685*, 321.
20. Instruction Manual for Crownpak CR(+), Daicel Chemical Industries.