Liquid Chromatographic Resolution of Amino Acid Esters of Acyclovir Including Racemic Valacyclovir on Crown Ether-Based Chiral Stationary Phases

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ABSTRACT Valacyclovir, a potential prodrug for the treatment of patients with herpes simplex and herpes zoster, and its analogs were resolved on two chiral stationary phases (CSPs) based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 covalently bonded to silica gel. In order to find out an appropriate mobile phase condition, various mobile phases consisting of various organic modifiers in water containing various acidic modifiers were applied to the resolution of valacyclovir and its analogs. When 30% acetonitrile in water containing any of 0.05 M, 0.10 M, or 0.15 M perchloric acid was used as a mobile phase, valacyclovir and its analogs were resolved quite well on the two CSPs with the separation factors (α) in the range of 2.49 ~ 6.35 and resolutions (R_S) in the range of 2.95 ~ 12.21. Between the two CSPs, the CSP containing residual silanol protecting *n*-octyl groups on the silica surface was found to be better than the CSP containing residual silanol groups. *Chirality 00:000–000, 2015.* © 2015 Wiley Periodicals, Inc.

KEY WORDS: chiral stationary phase; enantiomer separation; liquid chromatography; valacyclovir

Acyclovir (Fig. 1) is a highly effective antiviral drug for the treatment of patients with herpes simplex and herpes zoster.[1] However, the bioavailability of the drug at appropriate oral doses is limited to about 20%. Consequently, multiple higher oral doses or intravenous doses is necessary to reach sufficient clinical results.[1] The low oral bioavailability of acyclovir might be due to weak intestinal absorption of the drug. To improve the bioavailability of acyclovir, various amino acid esters of acyclovir were developed as potential prodrugs.[2] After oral administration, amino acid esters of acyclovir have been known to be well absorbed from the intestinal tract and then rapidly and extensively hydrolyzed to acyclovir, increasing plasma levels of acyclovir. In particular, L-valyl ester of acyclovir, valacyclovir (**1**) (Fig. 1), was found to show an oral bioavailability of 3–5 times greater than that of acyclovir itself.[3,4] Interestingly, intestinal absorption of amino acid esters of acyclovir was found to be stereoselective, with a preference for L-isomers.[2,5] In this instance, determination of the enantiomeric composition of amino acid esters of acyclovir is important.

For the determination of enantiomeric composition of chiral drugs, liquid chromatographic enantioselective resolution method with chiral stationary phases (CSPs) has been known to be very effective.[6] While the resolution of amino acid esters of acyclovir on liquid chromatographic CSPs is quite rare, Chiralpak AD column has been used to separate the two enantiomers of racemic valacyclovir[3] and Crownpak CR (+) column has been used to identify L-valacyclovir in the presence of some impurities.[7]

Previously, we developed two CSPs based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 (CSP **I** containing residual silanol groups on the silica surface and CSP **II** containing residual silanol protecting *n*-octyl groups on the silica surface, Fig. 2) covalently bonded to silica gel.[8,9] The two CSPs have been applied to the resolution of racemic compounds containing a primary amino group. For example, α-amino acids,[8,9] β-amino acids,[10] primary amines and amino alcohols,[9,11] fluoroquinolone antibacterials,[12,13] racemic catinone (central nervous system stimulant),[14,15] and tocainide (antiarrhythmic agent)[16,17] were resolved very well on CSP **I** and CSP **II**. In every case, CSP **II** was found to show greater chiral recognition than CSP **I**. Amino acid esters of acyclovir including racemic valacyclovir contain one primary amino group. Consequently, they are expected to be resolved on CSP **I** and CSP **II**. However, CSP **I** and CSP **II** have not been applied to the resolution of racemic valacyclovir and other amino acid esters of acyclovir. In this study, we report the liquid chromatographic resolution of various amino acid esters of acyclovir on CSP **I** and CSP **II**.

EXPERIMENTAL

A high-performance liquid chromatography (HPLC) system consisting of a Waters model 515 HPLC pump (Milford, MA), a Rheodyne model 7725i injector (Rohnert Park, CA) with a 20 μL sample loop, a Waters 2487 dual absorbance detector, and a YoungLin Autochro data module (Software: YoungLin Autochro-WIN 2.0 plus) was used for the liquid chromatography. The chiral column temperature was maintained at 20 °C by using a JEIO TECH VTRC-620 cooling circulator (Daejeon, Korea). Chiral columns packed with CSP **I** and CSP **II** were available from prior studies.[8,9] Injection samples were prepared by dissolving each analyte in methanol at a concentration of 1.0 mg/mL and an injection size was typically 2.0 μL.

Racemic valacyclovir (**1**) and its analogs (**2–5**), shown in Figure 1, were prepared via a procedure (Fig. 3) modified slightly from the procedure reported for the preparation of L-valacyclovir.[18] As an example, the detailed synthetic procedure for the preparation of racemic valacyclovir is described as follows. Racemic *N*-t-Boc-valine (0.25 g, 1.15 mmole), which was prepared by treating racemic valine with di-*tert*-butyl

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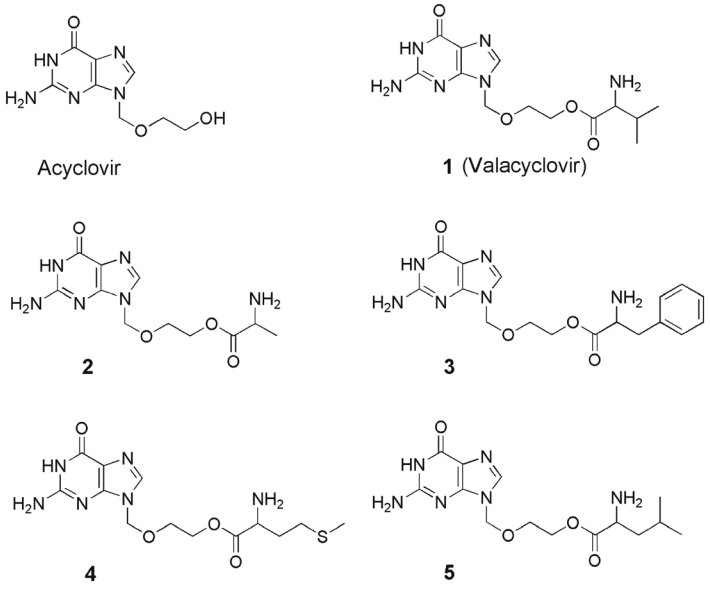

Fig. 1. Structures of acyclovir, valacyclovir (**1**) and its analogs **2–5**.

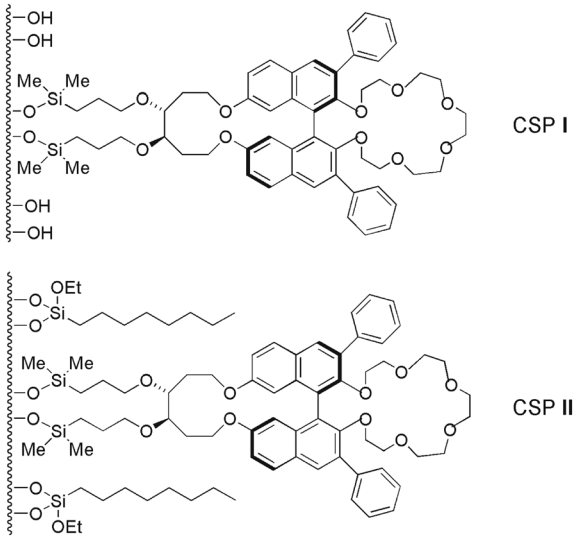

Fig. 2. Structures of CSP **I** and CSP **II**.

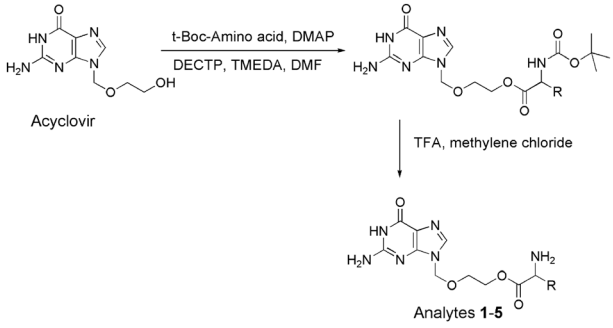

Fig. 3. Scheme for the preparation of valacyclovir (**1**) and its analogs **2–5**.

dicarbonate in the presence of triethylamine in a mixed solvent of dioxane and water, was dissolved in dimethyl formamide (DMF, 20 mL) in a 250-mL round-bottom flask. To the solution were added acyclovir (0.15 g, 0.67 mmole), 4-(dimethylamino)pyridine (DMAP, 0.01 g, 0.082 mmole), and *N*,*N*,*N*',*N*'-tetramethylethylenediamine (TMEDA, 0.70 mL, 4.67 mmole). The reaction mixture was stirred at 5 °C for 20 min and then *O*,*O*'-diethyl chlorothiophosphate (DECTP, 0.30 mL, 1.91 mmole) was added drop by drop. The reaction mixture was stirred at 5 °C for 2 h and then at room temperature for 12 h. Water (100 mL) was added to the reaction mixture. The reaction mixture was heated to 90 °C and then cooled to 5 °C slowly with stirring. The precipitates were collected and washed with water to afford *N*-*t*-Boc-valacyclovir (0.19 g, 66.9% yield). ^{1}H NMR (CD_3OD, ppm) δ 7.85 (s, 1H), 5.48 (s, 1H), 4.25-4.19 (m, 2H), 3.99-3.97 (d, 1H), 3.81-3.77 (m, 2H), 2.02-1.98 (m, 1H), 1.43 (s, 9H), 0.91-0.86 (m, 3H). *N*-*t*-Boc-valacyclovir (0.05 g, 0.12 mmole) was dissolved in methylene chloride (2 mL) in a 30-mL round-bottom flask. To the solution was added trifluoroacetic acid (0.5 mL) and the whole mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated by using a rotary evaporator to afford to racemic valacyclovir. ^{1}H NMR (CD_3OD, ppm) δ 8.09 (s, 1H), 5.56 (s, 2H), 4.41-4.45 (m, 3H), 4.28-4.34 (m, 2H), 2.12-2.18 (m, 1H), 0.99 (d, 6H).

RESULTS AND DISCUSSION

Racemic valacyclovir (**1**) and its analogs (**2–5**) were resolved on CSP **I** and CSP **II**. As an effort to find out the most widely applicable mobile phase condition, the five analytes were resolved with variation of the type and content of organic modifier in water containing perchloric acid (0.10 M) and the resolution results are summarized in Table 1. In every case, the L-enantiomer was found to be eluted faster than the D-enantiomer. As an organic modifier, methanol (MeOH), ethanol (EtOH), or acetonitrile (ACN) was tested. Among the three organic modifiers, acetonitrile was found to show the best resolution results. As shown in Table 1, 30% acetonitrile in water containing 0.10 M perchloric acid was found to be better than 30% ethanol or 30% ethanol in water containing 0.10 M perchloric acid as a mobile phase in terms of both the separation factors (α) and the resolutions (R_S) for the resolution of analytes **1–5** on CSP **I** and CSP **II**. When the content of acetonitrile in water was changed from 15% to 30% and then to 50%, the retention factors (k_1) for the first eluted enantiomers were found to decrease continuously in every case on both CSPs. As an example, the chromatograms for the resolution of

TABLE 1. Resolution of racemic valacyclovir (1) and its analogs (2–5) on CSP I and CSP II with variation of the content of methanol (MeOH), ethanol (EtOH), or acetonitrile (ACN) in water as a mobile phase containing 0.10 M perchloric acid ($HClO_4$)[a]

CSP	Organic modifier in water	**1**			**2**			**3**			**4**			**5**		
		k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
CSP **I**	MeOH (30%)	1.30	2.43	2.50	3.06	2.85	3.96	6.38	2.85	3.62	5.97	5.04	4.29	4.10	4.90	4.71
CSP **I**	EtOH (30%)	0.68	2.45	2.04	1.57	2.86	3.50	2.88	2.85	3.39	3.13	5.04	5.13	2.16	4.79	4.53
CSP **I**	ACN (30%)	0.87	2.88	3.22	2.08	2.97	4.82	3.94	3.46	5.04	3.83	5.27	5.43	3.13	5.47	6.13
CSP **I**	ACN (15%)	1.43	2.61	3.71	3.22	2.85	4.69	7.43	3.10	5.06	6.77	5.08	6.75	5.03	5.11	6.50
CSP **I**	ACN (50%)	0.35	3.16	2.65	1.14	3.15	4.33	1.15	3.69	4.84	1.61	5.36	6.36	1.20	5.64	6.23
CSP **II**	MeOH (30%)	0.60	2.34	2.65	1.13	2.97	4.59	3.02	2.99	3.54	2.14	5.52	4.28	1.77	5.11	4.26
CSP **II**	EtOH (30%)	0.22	2.63	2.52	0.41	3.30	4.25	1.03	2.94	4.20	0.89	5.89	7.17	0.73	5.30	6.06
CSP **II**	ACN (30%)	0.45	2.77	4.43	0.87	3.18	8.00	1.86	3.62	6.51	1.67	6.01	8.90	1.41	6.10	8.56
CSP **II**	ACN (15%)	0.81	2.62	5.41	1.47	3.11	8.32	4.09	3.35	6.10	3.17	5.83	9.24	2.80	5.80	8.51
CSP **II**	ACN (50%)	0.12	3.80	2.63	0.36	3.84	6.40	0.42	4.47	7.00	0.53	7.06	11.42	0.38	7.64	9.55

[a]Flow rate: 0.5 ml/min. Detection: 254 nm UV. Column temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution.

valacyclovir (**1**) on CSP **I** and for the resolution of analyte **5** on CSP **II** with the variation of the content of acetonitrile in water containing 0.10 M perchloric acid are presented in Figure 4. In general, the retention behaviors for the resolution of racemic primary amino compounds on crown ether-based CSPs have been explained by the balance between the hydrophilic interaction of the analytes with the mobile phase and the lipophilic interaction of the analytes with the CSP.[19,20] In reverse phase chromatography, the lipophilic interaction of analytes with the CSP might be an important factor for the retention of analytes, especially when the CSP is lipophilic. Both CSP **I** and CSP **II** might be lipophilic because of the 3,3'-diphenyl-1,1'-binaphthyl group and CSP **II** is expected to be even more lipophilic because of the additional *n*-octyl groups.[15] As the content of acetonitrile in water is increased, the polarity of mobile phase should decrease. In this instance, the lipophilic interaction between the analytes and the CSP is expected to decrease and, consequently, the retention of analytes decreases as the content of acetonitrile in water is increased. The decreasing trends of the retention factors are expected to be more significant with the more lipophilic CSP. Actually, the decreasing trends of the retention factors are more significant with CSP **II** than with CSP **I,** as expected. For example, the retention factors for the resolution of valacyclovir (**1**) on CSP **I** and CSP **II** were decreased by 76% and 85%, respectively, when the acetonitrile content was changed from 15% to 50%, indicating that the decreasing trends of the retention factors are more significant on the latter CSP. In general, the retention times on CSP **II** are expected to be greater than those on CSP **I** because of the higher lipophilicity of CSP **II**. However, the retention factors (k_1) on CSP **II** were smaller than those on CSP **I,** as shown in Table 1. The dipolar interactions between the primary ammonium ions ($R\text{-}NH_3^+$) of analytes and the residual silanol groups on the silica surface of CSP **I** might be more effective in retaining the analytes than the improved lipophilic interactions between the analytes and CSP **II**. In this instance, the retention factors (k_1) on CSP **II** could be smaller than those on CSP **I**, but the exact reason is not yet clear. When the content of acetonitrile in water was changed from 15% to 30% and then to 50%, the separation factors (α) were found to increase in every case for the resolution of analytes **1–5** on CSP **I** and CSP **II**. As the content of acetonitrile in water is increased, the retention times decrease for both of the two enantiomers, for example, as shown in Figure 4. Retention of two enantiomers on the CSPs is expected to be controlled by the nonenantioselective and enantioselective interactions. The nonenantioselective interactions such as dipolar interactions and lipophilic interactions might be exerted equally on the two enantiomers, but the enantioselective interactions should be exerted more effectively on the more retained enantiomer than the less retained enantiomer. As the content of acetonitrile in water is increased, the nonenantioselective interactions such as lipophilic interactions between the analytes and the CSPs should decrease and the retention times of the two enantiomers decrease, but the decreasing trends are less significant with the more retained enantiomers because of the more significant enantioselective interactions. As an example, the retention factor of the less retained enantiomer for the

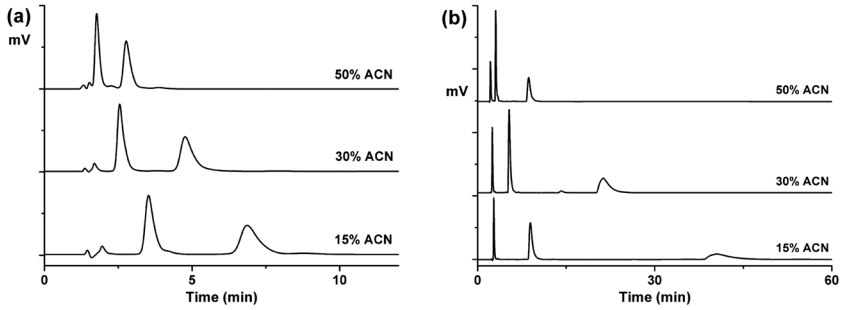

Fig. 4. Comparison of the chromatograms for the resolution of (**a**) valacyclovir (**1**) on CSP **I** and (**b**) analyte **5** on CSP **II** with the use of 15%, 30%, or 50% acetonitrile (ACN) in water containing perchloric acid (0.10 M) as a mobile phase. Flow rate: 0.5 ml/min. Detection: 254 nm UV. Temperature: 20 °C.

resolution of valacyclovir (**1**) on CSP **II** decreases by 85% (retention factor of the less retained enantiomer decreases from 0.81 to 0.12; see Table 1) while the retention factor of the more retained enantiomer decreases by 78% (retention factor of the more retained enantiomer decreases from 2.12 to 0.46; these values are calculated from the data shown in Table 1) when the content of acetonitrile in water is changed from 15% to 50%. Consequently, the separation factor (α) should increase as the content of acetonitrile in water is increased on CSP **I** and CSP **II**. In contrast, the resolutions (R_S) for the resolution of analytes **1–5** on CSP **I** and CSP **II** were found not to show any consistent trend with the variation of the content of acetonitrile in water.

As an acidic modifier in mobile phase, four different acids such as sulfuric acid, trifluoroacetic acid, acetic acid, and perchloric acid were tested. The acidic modifier added to the mobile phase has been proposed to protonate the primary amino group of analytes and then the resulting primary ammonium ion ($R\text{-}NH_3^+$) of analytes can form energetically different two transient diastereomeric complexes inside the cavity of the crown ether ring of the crown ether-based CSPs.[19,20] In this instance, acidic modifier in the mobile phase is essential for the chiral recognition. The chromatographic results for the resolution of five analytes on CSP **I** and CSP **II** with the variation of the type and content of acidic modifier in 30% acetonitrile in water as a mobile phase are summarized in Table 2. When sulfuric acid was used as an acidic modifier, the retention factors (k_1) for the resolution of five analytes on CSP **I** and CSP **II** were quite small. In some cases (analyte **1** on CSP **I**, analytes **1** and **2** on CSP **II**), the retention factors were too small to be useful when sulfuric acid was used as an acidic modifier. In contrast, the retention factors (k_1) for the resolution of five analytes **1–5** on CSP **I** and CSP **II** were quite large when acetic acid was used as an acidic modifier. The large retention factors need long analytical times and a large volume of mobile phase. Considering these results, it is concluded that sulfuric acid or acetic acid is not appropriate as an acidic modifier for the resolution of valacyclovir and its analogs on CSP **I** and CSP **II**.

When trifluoroacetic acid or perchloric acid was used as an acidic modifier in mobile phase, the retention factors were small enough to be useful and the separation factors and resolutions were quite good. Between the two acidic modifiers, perchloric acid was found to be always better than trifluoroacetic acid as an acidic modifier in terms of both the separation factors and resolutions. As shown in Table 2, the separation factors and resolutions for the five analytes are always greater with the use of perchloric acid (0.1 M) than with the use of trifluoroacetic acid (0.1 M) as an acidic modifier on both CSP **I** and CSP **II**. These results indicate that perchloric acid is the best acidic modifier in 30% acetonitrile in water as a mobile phase.

The pH values of the mobile phases consisting of 30% acetonitrile in water containing 0.10 M sulfuric, acetic, trifluoroacetic, or perchloric acid measured at 20 °C were 0.89, 2.98, 1.05, and 0.99, respectively. The retention times of analytes seem to be dependent on the mobile phase pH values. When the pH value of the mobile phase is quite low (0.89 for 0.10 M sulfuric acid), acid anion concentration is expected to be relatively high and, consequently, the ionic strength of the mobile phase increases. In this instance, primary ammonium ions ($R\text{-}NH_3^+$) of analytes are expected to be hydrated significantly by the aqueous mobile phase and then the protonated analytes could be distributed to the mobile phase more significantly than to the stationary phase.[20] Then the retention times of ionic analytes should be relatively short. However, when the pH value of the mobile phase is high (2.98 for 0.10 M acetic acid), the ionic strength of the mobile phase is relatively low and, consequently, the retention times of ionic analytes should be relatively long. The pH values of the mobile phase containing 0.10 M perchloric acid is slightly lower than that of the mobile phase containing 0.10 M trifluoroacetic acid, but the retention times are longer with the former mobile phase than with the latter mobile phase. The lipophilicity of acid anion, ClO_4^-, is known to be greater than that of CF_3COO^-.[21] Consequently, primary ammonium ions ($R\text{-}NH_3^+$) of analytes containing counter acid anion, ClO_4^-, are expected to be retained longer than those containing CF_3COO^-. However, the trends of the separation factors and resolutions with the variation of the type of acidic modifier in aqueous mobile phase are not clear.

When the concentration of perchloric acid was increased from 0.05 M to 0.10 M, the retention factors (k_1) increased

TABLE 2. Resolution of racemic valacyclovir (1) and its analogs (2–5) on CSP I and CSP II with variation of the content of acidic modifier such as sulfuric acid (H_2SO_4), trifluoroacetic acid (TFA), acetic acid (AcOH), or perchloric acid ($HClO_4$) in 30% acetonitrile (ACN) in water as a mobile phase[a]

CSP	Acidic modifier in mobile phase	**1**			**2**			**3**			**4**			**5**		
		k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
CSP **I**	H_2SO_4 (0.10 M)				0.16	2.74	1.17	0.31	3.69	2.77	0.32	5.82	4.14	0.25	6.14	3.95
CSP **I**	TFA (0.10 M)	0.29	2.79	1.95	0.67	2.84	3.26	1.26	3.36	4.36	1.39	5.11	5.41	1.08	5.31	5.68
CSP **I**	AcOH (0.10 M)	10.4	2.05	3.42	16.0	2.49	3.93	29.4	3.05	4.61	24.8	4.76	4.60	25.7	4.78	5.59
CSP **I**	$HClO_4$ (0.10 M)	0.87	2.88	3.22	2.08	2.97	4.82	3.94	3.46	5.04	3.83	5.27	5.43	3.13	5.47	6.13
CSP **I**	$HClO_4$ (0.05 M)	0.61	2.73	2.95	1.44	2.87	4.40	2.43	3.40	5.22	2.82	5.07	6.81	2.12	5.26	6.44
CSP **I**	$HClO_4$ (0.15 M)	0.68	3.02	3.32	1.78	3.02	4.63	2.86	3.58	5.88	3.32	5.31	7.24	2.44	5.56	6.91
CSP **II**	H_2SO_4 (0.10 M)							0.19	3.45	3.31	0.20	4.85	4.56	0.12	5.61	4.60
CSP **II**	TFA (0.10 M)	0.21	2.42	2.56	0.36	2.75	4.38	0.77	3.26	6.34	0.70	5.63	9.79	0.60	5.52	8.29
CSP **II**	AcOH (0.10 M)	6.79	1.64	2.00	10.4	2.27	5.80	13.7	2.83	2.81	14.7	4.38	5.48	11.5	4.56	3.21
CSP **II**	$HClO_4$ (0.10 M)	0.45	2.77	4.43	0.87	3.18	8.00	1.86	3.62	6.51	1.67	6.01	8.90	1.41	6.10	8.56
CSP **II**	$HClO_4$ (0.05 M)	0.35	2.49	3.70	0.64	3.00	6.84	1.21	3.45	6.42	1.16	5.66	10.82	0.96	5.70	9.26
CSP **II**	$HClO_4$ (0.15 M)	0.31	3.04	4.80	0.69	3.33	7.79	1.29	3.79	8.17	1.27	6.22	12.21	1.02	6.35	10.08

[a]Flow rate: 0.5 ml/min. Detection: 254 nm UV. Column temperature: 20 °C. k_1: Retention factor of the first eluted enantiomer. α: Separation factor. R_S: Resolution.

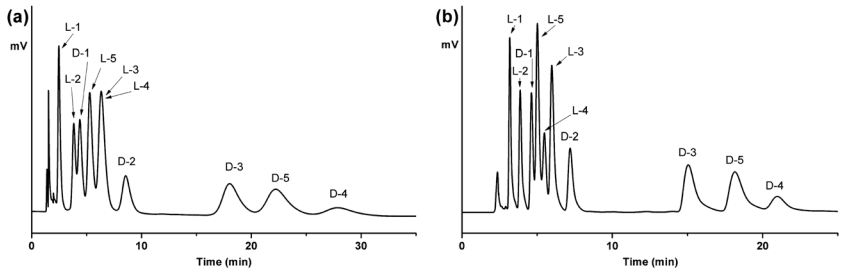

Fig. 5. Chromatograms for the resolution of the mixture of analytes **1–5** on (**a**) CSP **I** and (**b**) CSP **II** with the use of 30% acetonitrile in water containing perchloric acid (0.10 M) as a mobile phase. Flow rate: 0.5 ml/min. Detection: 254 nm UV. Temperature: 20 °C.

in every case. However, when the concentration of perchloric acid was increased further from 0.10 M to 0.15 M, the retention factors (k_1) decreased. In contrast, the separation factors (α) for the resolution of five analytes on CSP **I** and CSP **II** were found to increase steadily as the concentration of perchloric acid in aqueous mobile phase is increased. The resolutions (R_S) were found not to show consistent trends with the variation of perchloric acid concentration in aqueous mobile phase, but analytes **1**, **3**, **4**, and **5** show the highest resolutions on CSP **I** and CSP **II** when the concentration of perchloric acid is 0.15 M. Between the two CSPs, CSP **II** was found to be better than CSP **I** except for the resolution of valacyclovir (**1**) in terms of the separation factors (α) and CSP **II** was found to be always better than CSP **I** in terms of the resolutions (R_S) when 30% acetonitrile in water containing perchloric acid was used as a mobile phase. The protection of the residual silanol groups on the silica surface of CSP **I** with *n*-octyl groups in CSP **II** is expected to remove the nonenantioselective interaction sites and consequently improve the chiral recognition.[9] Overall, the separation factors are always greater than 2.49 and the resolutions are greater than 2.95 on CSP **I** and CSP **II** with the use of 30% acetonitrile in water containing perchloric acid. In this instance, 30% acetonitrile in water containing any of 0.05 M, 0.10 M, or 0.15 M perchloric acid is expected to be useful for the resolution of valacyclovir and its analogs. Previously, valacyclovir was reported to be resolved on a Chiralpak AD column with the separation factor of 2.00 and a resolution of 4.10.[3] In contrast, the separation factors for the resolution of valacyclovir on CSP **I** and CSP **II** were 3.02 and 3.04, respectively, when 30% acetonitrile in water containing 0.15 M perchloric acid was used as a mobile phase. The resolution values for the resolution of valacyclovir on CSP **I** and CSP **II** were 3.32 and 4.83, respectively, under the identical mobile phase condition. From these results, both CSP **I** and CSP **II** are concluded to be much better than a Chiralpak AD column for the resolution valacyclovir in terms of separation factor. CSP **II** is also concluded to be better than Chiralpak AD column for the resolution of valacyclovir in terms of resolution.

In order to elucidate the usefulness of 30% acetonitrile in water containing perchloric acid as a mobile phase for the resolution of valacyclovir and its analogs, as an example, a mixture of five analytes was resolved on CSP **I** and CSP **II** with the use of 30% acetonitrile in water containing 0.10 M perchloric acid as a mobile phase, as shown in Figure 5. For the resolution of a mixture of five analytes on CSP **I**, two peaks corresponding to L-**3** and L-**4** are merged and, consequently, only nine peaks are observed (Fig. 5a). In contrast, all 10 peaks corresponding to each enantiomer of five analytes are clearly observed for the resolution of a mixture of five analytes on CSP **II** (Fig. 5b).

In conclusion, two CSPs (CSP **I** and CSP **II**) based on (3,3'-diphenyl-1,1'-binaphthyl)-20-crown-6 covalently bonded to silica gel were found to be quite successful for the resolution of valacyclovir and its analogs. When 30% acetonitrile in water containing any of 0.05 M, 0.10 M, or 0.15 M perchloric acid was used as a mobile phase, the separation factors (α) were in the range of 2.49 ~ 6.35 and the resolutions (R_S) were in the range of 2.95 ~ 12.21 on the two CSPs. From these results, it is concluded that either of the two CSPs can be successfully utilized for the determination of the enantiomeric composition of valacyclovir and its analogs with the use of 30% acetonitrile in water containing any of 0.05 M, 0.10 M, or 0.15 M perchloric acid as a mobile phase.

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