Liquid Chromatographic Enantiomer Separation of Non-steroidal Anti-inflammatory Drugs on Immobilized Polysaccharide Derived Chiral Stationary Phase under Reversed and Normal Phase Mode

Hu Huang, Jing Yu Jin, Joon Hee Hong, Jong Seong Kang,†,* and Wonjae Lee*

*College of Pharmacy, Chosun University, Gwangju 501-759, Korea. *E-mail: wlee@chosun.ac.kr*
†College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea
Received July 27, 2009, Accepted September 30, 2009

Key Words: Enantiomer separation, Chiral stationary phase. Non-steroidal anti-inflammatory drugs

The liquid chromatographic enantiomer separation has become one of the most essential research areas for development of chiral drugs for over two decades.[1] Particularly, the first generation coating type chiral stationary phases (CSPs) derived from polysaccharides have been the most widely utilized for enantiomer resolution of a wide range of chiral compounds.[2] Recently, the second generation covalently immobilized type CSPs based on polysaccharides have been developed and applied.[3-11] The new type CSPs overcome the limitation of the coated type CSPs related to solvent versatility and their applications, since the chiral selectors of polysaccharide derivatives of these CSPs have been immobilized to a silica matrix through covalent bonding.[9-11] The covalently immobilized type CSPs for enantiomer separation had been only employed under normal phase and non-aqueous polar organic solvent mode.[12] However, very recently only a few results employed under reversed phase conditions were reported.[13-16] Compared to normal phase liquid chromatographic enantiomer separation, reversed phase enantiomer separation using aqueous mobile phases is particularly convenient for biological samples of serum or plasma as well as aqueous synthetic analytes.[15] In this study, we present enantiomer resolution of several NSAIDs (non-steroidal anti-inflammatory drugs) on the covalently immobilized type CSP, Chiralpak IA under reversed phase as well as normal phase conditions. Since the same acid additives are used under reversed phase as well as normal phase conditions, this is the first comparative report on both reversed and normal phase liquid chromatographic resolution of NSAIDs regarding acid additive effect using Chiralpak IA.

Table 1 shows the effect of reversed mobile phase on the enantiomer separation of two analytes (ibuprofen and naproxen) on Chiralpak IA. Tables 2 and 3 show the comparative results for the separation of the enantiomers of several NSAIDs on Chiralpak IA under reversed and normal phase conditions, respectively. The chromatographic parameters of resolution factors and capacity factors were considerably influenced by the nature of mobile phase, while selectivity factors in the mobile phase depending upon acid additive were much less influenced (entries 1- 8 in Table 1). As shown in Table 1, 60% MeOH/water (V/V) with methanesulfonic acid among several reversed mobile phases afforded the greatest enantioresolution. The lowest or no enantioselectivity of two analytes was observed in reversed mobile phase conditions using acetonitrile or tetrahydrofuran instead of methanol (entries 9 and 10 in Table 1).

As shown in Tables 1-3, several acid additives were used in both reversed and normal phase. The capacity factors of the analytes generally increase, as the acid additive's acidity as well as concentration in the mobile phase increases. These

Table 1. Effect of reversed mobile phase on the enantiomer separation of ibuprofen and naproxen on Chiralpak IA.

	Analyte	Ibuprofen				Naproxen			
	Mobile phase	α^a	$k_1{}^b$	Rs[c]	Conf.[d]	α^a	$k_1{}^b$	Rs[c]	Conf.[d]
1	60% MeOH/water (V/V) with 20 mM methanesulfonic acid	1.13	7.94	1.81	S	1.24	6.70	3.15	S
2	60% MeOH/water (V/V) with 10 mM methanesulfonic acid	1.13	7.74	1.78	S	1.24	6.64	3.10	S
3	60% MeOH/water (V/V) with 5 mM methanesulfonic acid	1.13	7.67	1.74	S	1.24	6.52	3.01	S
4	60% MeOH/water (V/V) with 10 mM ethanesulfonic acid	1.12	7.26	1.70	S	1.22	6.18	2.89	S
5	60% MeOH/water (V/V) with 10 mM trifluoroacetic acid	1.12	6.63	1.08	S	1.21	5.43	1.89	S
6	60% MeOH/water (V/V) with 5 mM trifluoroacetic acid	1.12	6.36	1.02	S	1.21	5.43	1.77	S
7	60% MeOH/water (V/V) with 10 mM trichloroacetic acid	1.12	6.98	1.03	S	1.20	5.33	1.85	S
8	60% MeOH/water (V/V) with 10 mM acetic acid	1.12	5.98	0.97	S	1.20	5.13	1.64	S
9	40% acetonitrile/water (V/V) with 10 mM methanesulfonic acid	1.06	4.61	1.43	S	1.13	2.96	2.64	S
10	40% tetrahydrofuran/water (V/V) with 10 mM methanesulfonic acid	1.00	4.32	-	-	1.00	3.21	-	-

Mobile phase: Flow rate = 0.5 mL/min; Detector UV 254 nm: [a]Selectivity factor. [b]Capacity factor for the first eluted enantiomer. [c]Resolution factor. [d]Indicates the absolute configuration of the second retained enantiomer.

results indicate that the suppression of the ionization of analyte's carboxylic acid group is responsible for the increased retention behavior.[18] Also the greater resolution factors with similar selectivity factors were observed, as the stronger acid additive (for example, methanesulfonic acid: pKa = −1.89, ethanesulfonic acid: pKa = −1.61) was used. Consequently, the strongest methanesulfonic acid among several acid additives gave the greatest resolution and retention, while the weakest acetic acid gave the lowest. Especially, compared to trifluoroacetic acid commonly used as an acid additive under normal phase conditions in Table 3,[2,7,18] the slightly greater enantioresolution in the mobile phase using methanesulfonic acid was obtained. Therefore, it is expected that methanesulfonic acid stronger than trifluoroacetic acid as an acid additive may be usefully applied for enantiomer resolution of chiral acids in normal phase as well as reversed phase conditions.

It is observed that all the elution orders of three examined analytes under both reversed and normal phase conditions were not identical. The S-enantiomers for all examined analytes are eluted later under both reversed and normal phase conditions, except for the R-enantiomer of ketoprofen under reversed phase conditions. This indicates that the chiral recognition interaction in reversed and normal phase condition might be different. Also, the degree of enantioselectivity observed in reversed and normal phase does not parallel the analyte. As an example, no resolution and the lowest enantioselectivity of ibuprofen and naproxen were observed in normal phase condition, respectively, while much greater enantioselectivities of these analytes in reversed phase condition were showed. Therefore, it is expected that the CSP may be complementary employed in dual phase mode for enantiomer resolution of NSAIDs.

The developed analytical resolution method was applied for determination of the enantiomeric purity of a currently marketed S-naproxen drug on Chiralpak IA. It was dissolved in methanol and the analytical sample obtained after filtration was directly injected. The enantiomeric impurity of 0.8% for the marketed S-naproxen drug was determined in the mobile phase of 60% MeOH/water (V/V) with 10 mM methanesulfonic acid. The chromatograms of enantiomer separation of

Table 2. Enantiomer separation of NSAIDs on Chiralpak IA under reversed phase conditions.

Mobile phase	60% MeOH/water (V/V) with 10 mM methanesulfonic acid				60% MeOH/water (V/V) with 10 mM ethanesulfonic acid				60% MeOH/water (V/V) with 10 mM trifluoroacetic acid				60% MeOH/water (V/V) with 10 mM trichloroacetic acid				60% MeOH/water (V/V) with 10 mM acetic acid			
Analyte	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]
Fenoprofen	1.05	8.44	0.79		1.04	7.72	0.62		1.04	6.92	0.33		1.04	6.85	0.32		1.04	6.90	0.22	
Flurbiprofen	1.38	11.66	4.74		1.37	10.89	4.41		1.37	10.47	4.35		1.37	10.42	4.20		1.35	9.69	3.89	
Ibuprofen	1.13	7.74	1.78	S	1.12	7.26	1.70	S	1.12	6.63	1.08	S	1.12	6.98	1.03	S	1.12	5.98	0.97	S
Indoprofen	1.18	16.53	1.21		1.17	14.99	1.16		1.17	14.19	0.92		1.17	13.26	0.91		1.17	13.00	0.85	
Ketoprofen	1.03	5.30	0.38	R	1.03	5.09	0.29	R	1.03	4.66	0.17	R	1.03	4.85	0.14	R	1.00	4.55	-	
Naproxen	1.24	6.64	3.10	S	1.22	6.18	2.89	S	1.21	5.43	1.89	S	1.20	5.33	1.85	S	1.20	5.13	1.64	S

Chromatographic conditions: Flow rate = 0.5 mL/min; Detection UV 254 nm; [a]Selectivity factor. [b]Capacity factor of the first eluted enantiomer. [c]Resolution factor. [d]The absolute configuration of the second eluted enantiomer.

Table 3. Enantiomer separation of NSAIDs on Chiralpak IA under normal phase conditions.

Mobile phase	10% 2-propanol/hexane (V/V) with 10 mM methanesulfonic acid				10% 2-propanol/hexane (V/V) with 10 mM ethanesulfonic acid				10% 2-propanol/hexane (V/V) with 10 mM trifluoroacetic acid				10% 2-propanol/hexane (V/V) with 10 mM trichloroacetic acid				10% 2-propanol/hexane (V/V) with 10 mM acetic acid			
Analyte	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]	α^a	$k_1{}^b$	Rs^c	Conf.[d]
Fenoprofen	1.25	1.07	3.75		1.25	1.00	3.70		1.25	0.97	3.68		1.23	0.94	2.93		1.22	0.91	2.87	
Flurbiprofen	1.42	0.97	5.80		1.41	0.94	5.77		1.41	0.90	5.44		1.40	0.89	5.32		1.38	0.90	5.16	
Ibuprofen	1.00	0.66	-		1.00	0.65	-		1.00	0.64	-		1.00	0.61	-		1.00	0.63	-	
Indoprofen	1.30	23.08	6.48		1.28	21.07	6.28		1.27	22.35	5.61		1.27	22.17	5.10		1.29	21.29	5.53	
Ketoprofen	1.14	2.75	2.82	S	1.14	2.74	2.82	S	1.13	2.65	2.47	S	1.12	2.39	2.14	S	1.10	2.47	1.92	S
Naproxen	1.09	2.13	1.83	S	1.09	2.10	1.80	S	1.08	2.02	1.62	S	1.07	1.86	1.36	S	1.07	1.86	1.28	S

Chromatographic conditions: Flow rate = 1 mL/min; Detection UV 254 nm; [a]Selectivity factor. [b]Capacity factor of the first eluted enantiomer. [c]Resolution factor. [d]The absolute configuration of the second eluted enantiomer.

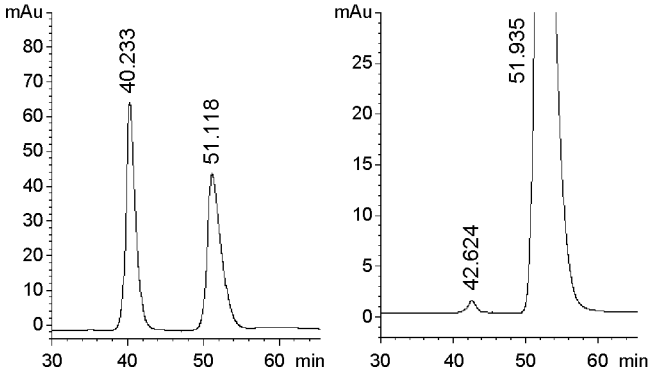

Figure 1. Chromatograms of enantiomer separation of racemic naproxen (the left) and determination of the enantiomeric purity of a currently marketed S-naproxen drug (R : S = 0.8 : 99.2) (the right) under reversed phase condition on Chiralpak IA. Mobile phase: 60% MeOH/water (V/V) with 10 mM methanesulfonic acid. Flow rate: 0.5 mL/min. Detection UV 254 nm. Injection amount: 5 μg.

racemic naproxen and determination of the enantiomeric purity of the currently marketed S-naproxen drug on Chiralpak IA are presented in Figure 1.

In summary, the covalently immobilized Chiralpak IA is proving to be capable of enantiomer separation of NSAIDs under not only normal phase but also reversed phase conditions. The same acid additives for both reversed and normal phase liquid chromatographic resolution were used and the strongest methanesulfonic acid among several acid additives used in this study gave the greatest resolution and retention. Therefore, the greatest enantioseparation was obtained under reversed phase condition of 60% aqueous methanol containing methanesulfonic acid and normal phase condition of 10% 2-propanol in hexane containing methanesulfonic acid. Two analytes (ibuprofen and naproxen) showing no and the lowest enantioseparation in normal phase condition showed much greater enantioseparation in reversed phase conditions. The other analytes showed greater resolution in normal phase conditions than that in reversed phase conditions. Therefore, the CSP might be complementarily applied under reversed or normal phase mode for enantiomer resolution of chiral drugs including NSAIDs. Especially, the reversed phase analytical method using the covalently immobilized type CSP is expected to be a desirable technique for determination of the enantiomeric purity of NSAIDs in intrinsic aqueous biological samples.

Experimental Section

Chromatography was performed at room temperature using an HPLC Breeze system consisting of a Waters model 1525 binary pump, a Rheodyne model 7125 injector with a 20 μL loop, a dual absorbance detector (Waters 2487 detector). Chiralpak IA (250 mm L × 4.6 mm i.d.) was purchased from Daicel Chemical Company (Tokyo, Japan). HPLC-grade methanol, acetonitrile, tetrahydrofuran, hexane and 2-propanol were obtained from J. T. Baker (Phillipsburg, NJ). Methanesulfonic acid, ethanesulfonic acid, trifluoroacetic acid, trichloroacetic acid, acetic acid and all analytes were obtained from Aldrich (Milwaukee, WI) or Sigma (St. Louis, Missouri). A currently marketed S-naproxen drug was obtained from Chosun University Hospital.

Acknowledgments. This study was supported by research funds from Chosun University, 2008.

References

1. *Chiral Separation Techniques: A Practical Approach*, Second revised ed.; Subramanian, G., Ed.; VCH: Weinheim, 2001.
2. Yashima, E. *J. Chromatogr. A* **2001**, *906*, 105.
3. Zhang, T.; Kientzy, C.; Franco, P.; Ohnishi, A.; Kagamihara, Y.; Kurosawa, H. *J. Chromatogr. A* **2005**, *1075*, 65.
4. Li, Y. H.; Jin, J. Y.; Lee, W. *Bull. Kor. Chem. Soc.* **2005**, *26*, 1132.
5. Zhang, T.; Nguyen, D.; Franco, P.; Murakami, T.; Ohnishi, A.; Kurosawa, H. *Anal. Chim. Acta* **2006**, *557*, 221.
6. Ali, I.; Aboul-Enein, H. Y. *J. Sep. Sci.* **2006**, *29*, 762.
7. Jin, J. Y.; Lee, W.; Park, J. H.; Ryoo, J. J. *J. Liq. Chrom. & Rel. Tech.* **2006**, *29*, 1793.
8. Jin, J. Y.; Lee, W.; Park, J. H.; Ryoo, J. J. *J. Liq. Chrom. & Rel. Tech.* **2007**, *30*, 1.
9. Ghanem, A. *J. Sep. Sci.* **2007**, *30*, 1019.
10. Zhang, T.; Nguyen, D.; Franco, P. *J. Chromatogr. A* **2008**, *1191*, 214.
11. Franco, P.; Zhang, T. *J. Chromatogr. B* **2008**, *875*, 48.
12. Thunberg, L.; Hashemi, J.; Andersson, S. *J. Chromatogr. B* **2008**, *875*, 72.
13. Cirilli, R.; Ferretti, R.; Gallinella, B.; Santis, D. E.; Zanitti, L.; Torre, L. F. *J. Chromatogr. A* **2008**, *1177*, 105.
14. Cirilli, R.; Ferretti, R.; Santis, D. E.; Gallinella, B.; Zanitti, L.; Torre, L. F. *J. Chromatogr. A* **2008**, *1190*, 95.
15. Cirilli, R.; Ferretti, R.; Gallinella, B.; Bilia, R. A.; Vincieri, F. F.; Torre, L. F. *J. Sep. Sci.* **2008**, *31*, 2206.
16. Ferretti, R.; Gallinella, B.; Torre, L. F.; Zanitti, L.; Turchetto, L.; Mosca, A.; Cirilli, R. *J. Chromatogr. A* **2009**, *1216*, 5385.
17. Tachibana, K.; Ohnishi, A. *J. Chromatogr. A* **2001**, *906*, 127.
18. Okamoto, Y.; Kawashima, M.; Hatada, K. *Chem. Lett.* **1984**, 739.