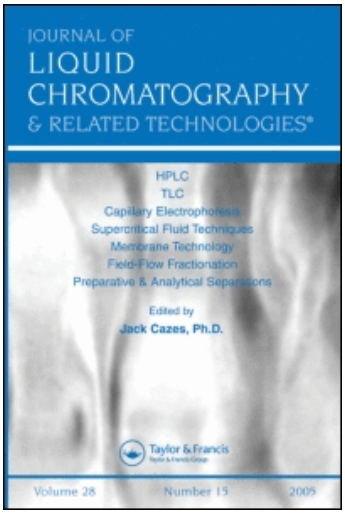


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Influence of the Kind of Alcoholic Modifier on Chiral Separation on a Pirkle Model Chiral Column

Mu-Bin Huang[a]; Guo-Sheng Yang[a]; Qing Dai[b]; Xin-Ling Guo[a]; Huai-Jing Yuan[a]; Ru-Yu Gao[b]; Qin-Sun Wang[b]; Ai-Qin Du[a]; Gao-Lan Li[a]
[a] Department of Chemistry, Shandong University, Jinan, China [b] National Laboratory of Elemento-Organic Chemistry, Nankai University, Tianjin, China

To cite this Article Huang, Mu-Bin , Yang, Guo-Sheng , Dai, Qing , Guo, Xin-Ling , Yuan, Huai-Jing , Gao, Ru-Yu , Wang, Qin-Sun , Du, Ai-Qin and Li, Gao-Lan(1998) 'Influence of the Kind of Alcoholic Modifier on Chiral Separation on a Pirkle Model Chiral Column', Journal of Liquid Chromatography & Related Technologies, 21: 12, 1797 — 1806
To link to this Article: DOI: 10.1080/10826079808005892
URL: http://dx.doi.org/10.1080/10826079808005892

J. LIQ. CHROM. & REL. TECHNOL., 21(12), 1797–1806 (1998)

INFLUENCE OF THE KIND OF ALCOHOLIC MODIFIER ON CHIRAL SEPARATION ON A PIRKLE MODEL CHIRAL COLUMN

Mu-Bin Huang,[1] Guo-Sheng Yang,[1] Qing Dai,[2] Xin-Ling Guo,[1]
Huai-Jing Yuan,[1] Ru-Yu Gao,[2] Qin-Sun Wang,[2] Ai-Qin Du,[1] Gao-Lan Li[1]

[1] Department of Chemistry
Shandong University
Jinan 250100, China

[2] National Laboratory of Elemento-Organic Chemistry
Nankai University
Tianjin 300071, China

ABSTRACT

Separation of the enantiomers of two series of seven organic phosphonates on π acidic N-(3,5-dinitrobenoyl) leucine chiral stationary phase is described. Enantiomer resolution is highly dependent on the mobile phase composition and the structure of the enantiomers. One of seven enantiomers can be separated using hexane-2-propanol mobile phase, other enantiomers can be partly separated only. The six out of seven enantiomers are separated and the retention times of all enantiomers are significantly decreased using ethanol instead of 2-propanol. The separation of all enantiomers is hardly affected by varying column temperature (10-40°C).

INTRODUCTION

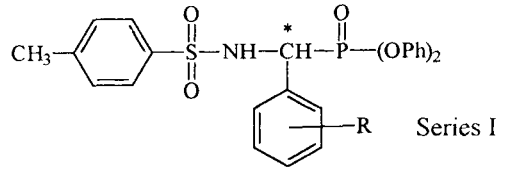
In recent years, the chromatographic separation of enantiomers by high performance liquid chromatography (HPLC) upon chiral stationary phase (CSP_s) has developed to be an important method. The Pirkle's CSP developed by Pirkle and co-workers was one of the common known and the best useful chiral stationary phases.[1-3] A series of N-(3,5-dinitrobenzoyl)-α-amino acid derived CSP_s were a kind of Pirkle's CSP and had been proven to be effective for determination of enantiomer purity, absolute configuration, and preparative-scale separation of a variety of enantiomers.[4-6] The separation of the enantiomers of a variety of phosphonates as the 3,5-dinitrobenzamide derivatives on the different Pirkle's CSP had been reported.[7-10] The mixture of hexane and 2-propanol was frequently used as mobile phase in the above-mentioned separation, although reversed phase solvent (methanol-water) was also reported.[7-9] Pescher et al.[11] reported the enantiomeric resolution of tertiary phosphine oxides on Pirkle's CSP by varying the nature of the mobile phase, which included the mixtures of hexane and ethanol, 2-propanol, n-butanol, tert-butanol, chloroform, tetrahydroforan etc. The influence of varying the nature of the mobile phase on chiral separation was considered. Zief et al.[12] have similar reports also.

In this paper, two series of organic phosphonates (Series I: O,O-diphenyl-(p-methylbenzenesulfonamido)-aryl-methylphosphonates, Series II:O,O-diphenyl-{[(p-methylbenzesulfonamido)-acetyl]amido} aryl-methyl phosphorates) were directly separated on the π acidic N-(3,5-dinitrobenoyl) leucine CSP. The influence of the ethanol, 2-propanol modifier and the different ratio between alcohol and hexane on chiral separation was discussed.

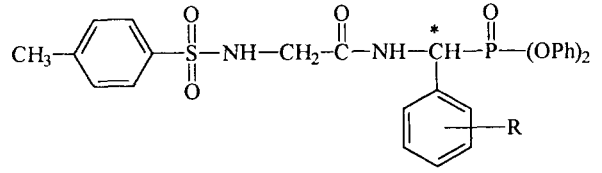
EXPERIMENTAL

Materials

Two series of seven chiral organic phosphonate compounds were synthesized by National Laboratory of Elemento-Organic Chemistry, Nankai University. The separation of these compounds has not been reported. The general structures of the compounds are presented in Figure 1. The structure of the substituents "R" are (1)p-Cl (2)p-Me (3)H (4)p-NO_2 (5)p-OMe in series I and (6) p-Cl (7)p-OMe in series II, respectively. These compounds were dissolved in ethanol, then diluted with eluent. Solutions with approximate concentration of 1 mg/mL were used for injection. All solvents were filtered by a 0.5μ filter and degassed in vacuum before use.



Series I



Series II

Figure 1. The general structures of compounds used in this study. Series I O,O-diphenyl, (p-methyl-benzenesulfonamido)-aryl-methylphosphonates. Series II O,O-diphenyl{[(p-methyl-benzenesulfonamido)-acetyl]- amido} aryl methylphosphonates.

Apparatus

The chromatography was performed with Shimadzu (Japan) modular liquid chromatography equipped with CR-6A integrator, SPD-10A UV-Vis detector, and LC-10AD solvent delivery system.

Chromatographic Conditions

The chiral column[8] was 250 × 4.6 mm and the structure of chiral stationary phase was shown in Figure 2. The mobile phases used were the mixture of 2-propanol-n-hexane and ethanol-n-hexane with different compositions. Flow-rate was maintained at 1 mL/min. Column temperatures were 10, 20, 25, 30, and 40°C. SPD-10A UV-Vis detector was at 230 nm.

RESULTS AND DISCUSSION

The mixture of n-hexane and 2-propanol was frequently used as mobile phase on the Pirkle's CSP for the chiral separation. Therefore, in order to optimize the separation, the effects of different ratio between 2-propanol and n-

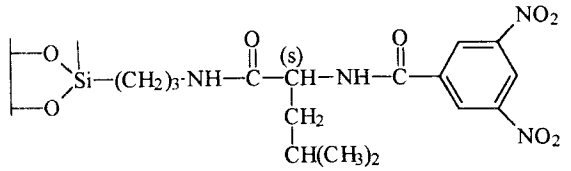


Figure 2. The structure of chiral stationary phase used in this study.

Table 1

k_1', k_2' and α Values for Chiral Separation of Organic Phosphonate Compounds at Different Mobile Phase Compositions of Isopropyl Alcohol and n-Hexane*

No	Series	R	5% i-PrOH			10% i-PrOH			15% i-PrOH		
			k_1'	k_2'	α	k_1'	k_2'	α	k_1'	k_2'	α
1	I	p-CL	9.994	11.013	1.102	4.685	5.141	1.097	3.157	3.447	1.092
2	I	p-Me	13.158	14.370	1.092	5.852	6.379	1.090	3.840	4.164	1.085
3	I	H	12.065	12.065	1.000	6.344	6.344	1.000	3.810	3.810	1.000
4	I	p-NO_2	19.655	22.107	1.125	7.912	8.928	1.128	5.350	5.993	1.120
5	I	p-OMe	21.469	26.629	1.240	8.099	9.639	1.190	5.426	6.419	1.183
			20% i-PrOH			30% i-PrOH			40% i-PrOH		
6	II	p-Cl	6.844	8.590	1.255	4.282	5.275	1.232	2.928	3.590	1.226
7	II	p-OMe	13.623	17.471	1.282	6.968	8.721	1.252	4.593	5.671	1.235

* Column temperature: 20°C, flow rate: 1.0 mL/min.

hexane in mobile phase on the chiral separation were investigated. The separation data, capacity factor, k', and separation factor, α, were indicated in Table I. The results showed that the k' values were decreased and α values were not notably changed with increasing the concentration of 2-propanol in mobile phase for all compounds. In addition, the magnitude of k' and α values was related to the nature of the substituent "R". Among compounds 1-5, the k' and α values of compound 5 (R=P-OMe) were the largest and the baseline separation was achieved when concentration of 2-propanol was 5 % (see Figure 3 A). The separation of compound 3 (R=H) did not occur and the other compounds were all only partly separated (see Figure 3 B) in a range of 5 %-40 % 2-propanol in mobile phase. The data in Table 1 showed that the k' and α values of compound 6 and 7 (Series II) were larger than that of compounds 1-5 (Series I). Perhaps this was due to the differences in their molecular structures.

The only difference in the structures between Series I and Series II compounds was that the $-CH_2-CO-NH-$ group was included in the compounds of Series II. The k' and α values of compound 7 (R=P-OMe) were also larger than that of compound 6(R=P-Cl). The experimental results indicated that the compound 5 with R=p-OMe was the best easily separated and the retention time was 90 min, but no separation or only partly separation was obtained for other compounds using 2-propanol as modifier.

In the present experiment, it was found that the retention times of all compounds were longer when the 2-propanol was used as modifier in mobile phase. For example, the retention time of compound 1-5 was 40-90 min with 5 % 2-propanol concentration and that of compounds 6 and 7 was 30-50 min with 20 % 2-propanol concentration, but the separation of enantiomers were poor, except for the compound 5.

Recently it was found that the change of the modifier from 2-propanol to ethanol and methanol leads to an improvement of the chiral separation on chiralpak AD and chiral OD column.[13-14] In Ref. 11, the mixture of hexane-ethanol-chloroform was used as the optimized ternary mobile phase for the enantiomeric resolution of tertiary phosphine oxides on Pirkle's CSP. Therefore, the use of the more polar ethanol, instead of 2-propanol as modifier in mobile phase, was performed and the separation data were indicated in Table 2.

The results indicated that the k' and α values were decreased with increasing the concentration of ethanol in mobile phase for all compounds. For a constant analysis time, the greater the steric hindrance, the higher must be the alcohol concentration.[11] (Comparison of the data in Table 1 and Table 2). On the other hand, when the concentrations of 2-propanol and ethanol in mobile phase were all 5 %, the k' values were obviously decreased for all compounds and the α values of compound 1-3 were obviously increased and those of compound 4-5 were slightly decreased under the ethanol modifier. Similarly when the concentrations of 2-propanol and ethanol in mobile phase all were 20 %, k' values were significantly decreased and α values were slightly decreased under ethanol modifier for compound 6 and 7.

The baseline separation of compound 1,2 3 and 5 was achieved when the concentration of ethanol in mobile phase was 5% (see Figure 3C), but the baseline separation of compounds 6 and 7 was obtained when 10 % ethanol concentration was used in mobile phase (see Figure 3D). However, the chiral separation of compound 4 ($R=P-NO_2$) was not essentially improved whether 2-propanol or ethanol was used as a modifier. The reason for this probably is related to strong electro-attracting nature of the substituent-nitro group ($-NO_2$).

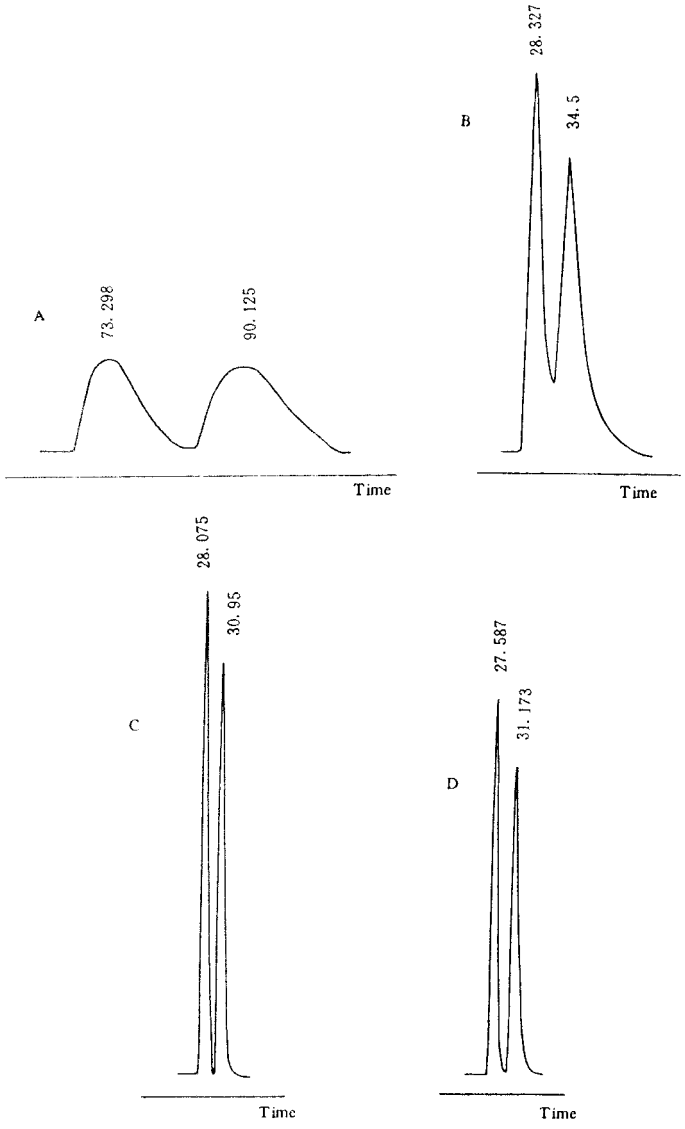


Figure 3. Influence of the alcoholic modifier on the chiral separation. Chromatographic condition: flow-rate, 1 mL/min; column temperature, 20°C; mobile phase:alcohol:n-haxane, A: 5% 2-propanol, B: 20 % 2-propanol, C: 5% ethanol, D: 10% ethanol. A and C: compound 5, B and D: compound 6.

Table 2

k_1', k_2', and α Values for Chiral Separation of Organic Phosphonate Compounds at Different Mobile Phase Compositions of Ethanol and n-Hexane*

No.	Series	R	5% EtOH k_1'	5% EtOH k_2'	5% EtOH α	10% EtOH k_1'	10% EtOH k_2'	10% EtOH α
1	I	p-Cl	8.121	9.191	1.137	3.360	3.658	1.089
2	I	p-Me	7.000	8.478	1.211	3.222	3.506	1.088
3	I	H	8.041	8.987	1.178	3.497	3.820	1.092
4	I	p-NO_2	8.511	9.096	1.069	3.766	4.007	1.064
5	I	p-OMe	7.902	8.813	1.115	3.360	3.703	1.090
			10% EtOH			**20% EtOH**		
6	II	p-Cl	6.986	8.012	1.147	3.182	3.606	1.134
7	II	p-OMe	12.082	13.946	1.155	4.490	5.062	1.128

* Column temperature: 20°C, flow rate: 1.0 mL/min.

The improvement of the chiral separation of compound 3 (R=H) was the largest, its chiral separation only occurred when the ethanol was used as a modifier. These results show that the chiral separation of the most compounds was significantly improved by using ethanol instead of 2-propanol as a modifier, but the degree of improvement of the enantiomer separation was significantly influenced by the nature of substituent "R". The result was similar to the report by Pirkle,[10] in which the degree of enantiomer separation depends predominantly on the c-aryl substituent.

The α values of enantiomers 6 and 7 with the 10 % ethanol modifier were more less than that of 20 % 2-propanol modifier, but the k' values were similar to each other. It was noted that the chiral separation could be achieved with 10% ethanol, but can not be achieved with 20 % 2-propanol modifier. Perhaps this is due to significant increase of theory plate number under ethanol modifier. Table 3 illustrates the influence of the kind of alcoholic modifier in mobile phase on theory plate number. From the data in Table 3, it was found that the $N_2^{1/2}/4$ values with 10 % ethanol were larger by 2.6 times than that of 20 % 2-propanol (compound 6 and 7). Resolution (R_s) was directly proportional to $N_2^{1/2}/4$, $(\alpha-1)/\alpha$ and $k'/1+k'$ values, and k' value was similar with two different alcoholic modifiers. By calculation, the increase of N value

Table 3

Influence of the Kind of Alcoholic Modifier in Mobile Phase on Theory Plate Number (N_2/m)*

Compound	2-Proponal:Hexane			Ethanol:Hexane		
	5:95	10:90	20:80	5:95	10:90	20:80
5	1293	2116		15323	13450	
6			1490		11420	10103
7			1345		9358	8002

* N_2 = theory plate number for second-eluting peak, the condition is same as in Table 1.

was good enough to eliminate the decrease of the α value. Thereby, chiral separation was obtained with 10 % ethanol modifier. The chiral separation of compound 5 with 5 % ethanol modifier was similar to that for enantiomers 6 and 7, and its $N_2^{1/2}/4$ value with 5 % ethanol was 3.4 times that of with 5% 2-propanol.

The effects of column temperature on the chiral separation were also investigated in the range of 10-40°C. The result of separation was similar to Ref. 8 and hardly affected by varying column temperature.

CONCLUSION

The experimental results indicated that the direct chiral separation of this class organic phosphorates could be achieved on the Pirkle's chiral stationary phase derived from N-3,5-dinitro benzoyl leucine acid. Enantiomeric separation was highly dependent on the mobile phase composition and the structure of the enantiomers. The separations for six out of seven enantiomers were significantly improved and analytic times were significantly decreased by using ethanol instead of 2-propanol as modifier. The separation of enantiomer with the substituent R=H was most obviously improved (compound 3) and the separation of enantiomer with the substituent R=-NO_2 was hardly changed by using ethanol instead of 2-propanol (compound 4). The result of separation was hardly affected by varying column temperatures (10-40°C).

ACKNOWLEDGMENTS

We would like to thank Prof. Chang-Tai Yan for correcting the manuscript. This work is supported by National Natural Science Foundation of China.

REFERENCES

1. C. J.Welch, J. Chromatogr. A, **666**, 3 (1994).

2. E. Francotte, J. Chromatogr., **576**, 1 (1992).

3. W. H.Pirkle, T. C.Pochapsky, Chem. Rev., **89**, 347 (1989).

4. W. H.Pirkle, J. M. Finn, J. L. Schreiner, B. C. Hamper, J. Am. Chem. Soc., **103**, 3964 (1981).

5. W. H.Pirkle, J. M. Finn, J. Org. Chem., **47**, 4037 (1982).

6. W. H.Pirkle, C. J. Welch, J. Org. Chem., **49**, 138 (1984).

7. W. H.Pirkle, J. A.Burke, J. Chromatogr., **598**, 159 (1992).

8. R. Y. Gao, G. S. Yang, H. X. Shen, R. Y. Chem, Q. Dai, Q. S. Wang, J. Liq. Chromatogr. & Rel. Technol., **19(2)**, 171 (1996).

9. W. H. Pirkle, M. H. Hyun, J. Chromatogr., **322**, 309 (1985).

10. W. H.Pirkle, L. J. Brice, S. Caccamese, G. Principato, S. Failla, J. Chromatogr. A., **721**, 241 (1996).

11. P. Pescher, M. Caude , R. Rosset, A. Tambute, J. Chromatogr., **371**, 159 (1986).

12. M. Zief, L. J. Crane, J. Horvath, J. Liq. Chromatogr., **7(4)**, 709 (1984).

13. A. Kunath, F. Theil, J. Wagner, J. Chromatogr. A., **684**, 162 (1994).

14. A. Kunath, F. Theil, K. Jähnisch, J. Chromatogr. A., **728**, 249 (1996).

Received August 15, 1997
Accepted October 17, 1997
Manuscript 4604