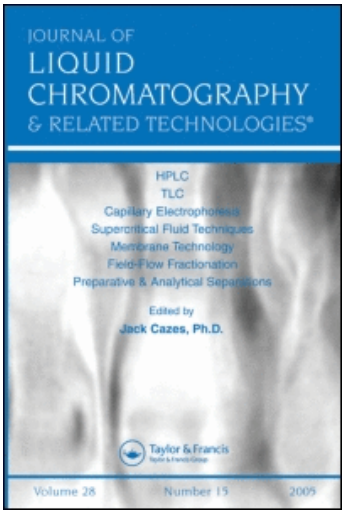


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Effects of Alcohol Modifiers on the Separation of 1-Alkoxycarbonyl-Alkyl-Pyrrolidine-3-Carboxylic Acid Alkyl Ester Enantiomers on Polysaccharide-Based Stationary Phases

S. Lin[a]; C. Engelsma[a]
[a] Chemical Development Department Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Michigan, USA

To cite this Article Lin, S. and Engelsma, C.(1997) 'Effects of Alcohol Modifiers on the Separation of 1-Alkoxycarbonyl-Alkyl-Pyrrolidine-3-Carboxylic Acid Alkyl Ester Enantiomers on Polysaccharide-Based Stationary Phases', Journal of Liquid Chromatography & Related Technologies, 20: 14, 2181 — 2192
To link to this Article: DOI: 10.1080/10826079708006555
URL: http://dx.doi.org/10.1080/10826079708006555

J. LIQ. CHROM. & REL. TECHNOL., 20(14), 2181–2192 (1997)

EFFECTS OF ALCOHOL MODIFIERS ON THE SEPARATION OF 1-ALKOXYCARBONYL-ALKYL-PYRROLIDINE-3-CARBOXYLIC ACID ALKYL ESTER ENANTIOMERS ON POLYSACCHARIDE-BASED STATIONARY PHASES

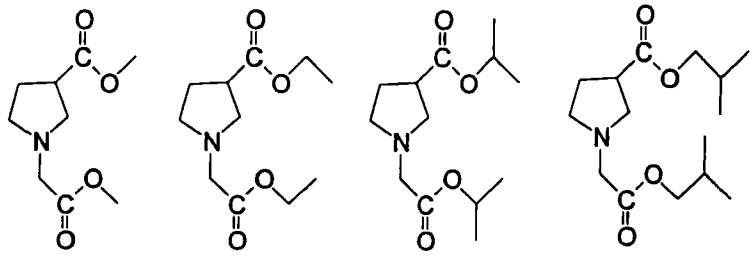
Sechoing Lin,* Cara Engelsma

Chemical Development Department
Parke-Davis Pharmaceutical Research Division
Warner-Lambert Company
188 Howard Avenue
Holland, Michigan 49424, USA

ABSTRACT

1-Alkoxycarbonylalkyl-pyrrolidine-3-carboxylic acid alkyl esters (**1**, **2**, **3** and **4**, see Figure 1 for structures) are the synthetic intermediates used for the large-scale synthesis of PD 151832. PD 151832 is a highly potent m1 subtype selective muscarinic agonist expected to be useful for patients with Alzheimer's disease. The mobile phase consisting of hexane/2-propanol/diethylamine has been previously shown to resolve the enantiomers of compounds **1**, **2** and **4** on a Chiralpak AS column and compound **3** on a Chiralpak AD column.[1]

In the current study, the nature of the alcohol modifier in mobile phase was varied and the resulting change in stereoselectivity was found to depend on compound and column type. Superior separations can often be achieved by using an



1 2 3 4

Figure 1. Structures of 1-Alkoxycarbonylalkyl-pyrrolidine-3-carboxylic Acid Alkyl Esters (**1**, **2**, **3** and **4**).

alcohol modifier other than the commonly used 2-propanol or ethanol on the same column. The use of different alcohol modifiers in mobile phase to enhance the enantiomeric resolution can provide useful and less expensive alternatives to the approach of using multiple columns in chiral methodology development.

INTRODUCTION

The HPLC separation of chiral compounds is increasingly important with a large number of new potential chiral drugs. Useful HPLC separations of racemic mixtures were developed by testing columns with different chiral stationary phases. This way of approaching chiral methods development requires considerable effort and can become extremely expensive.

Polysaccharide-based stationary phases have found many successful applications and are among the most widely used stationary phases for enantiomeric separations with the commonly recommended hexane/2-propanol or hexane/ethanol as the mobile phase.[2,3] The effects of mobile phase modifiers, particularly the alcohol on the stereoselectivity of the polysaccharide-based stationary phases, have been demonstrated.[4-8]

It was found that an alcohol modifier other than 2-propanol or ethanol can be superior. 1-Alkoxycarbonylalkyl-pyrrolidine-3-carboxylic acid alkyl esters are important intermediates towards synthesis of PD 151832, a highly potent

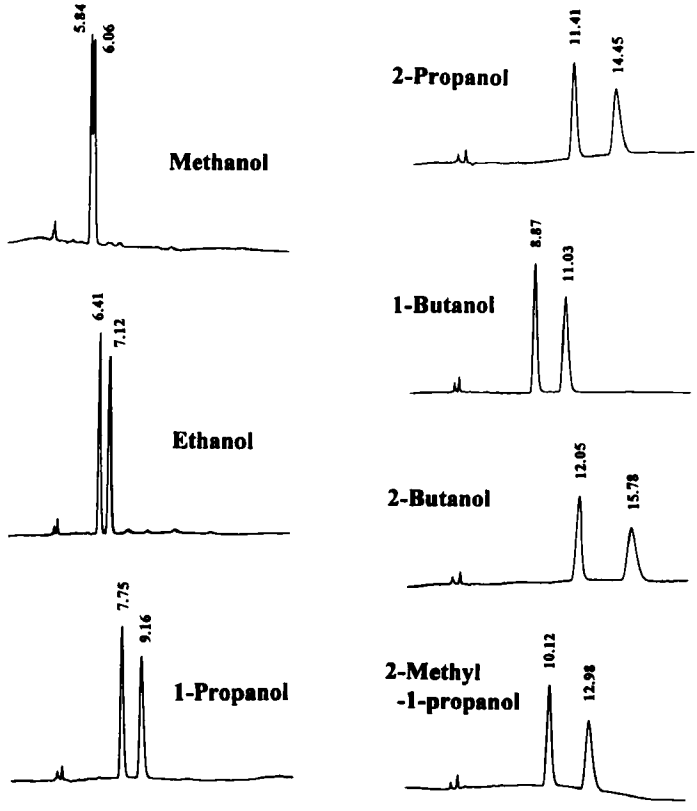


Figure 2. Separation of a Racemic Mixture of Compound **1** using Various Alcohol Modifiers; Column: Chiralpak AS, Mobile Phase: Hexane/Alcohol/DEA (950/50/1), Flow Rate: 1.0 mL/min, Detection: UV @ 230nm, Sample Amount Injected: ~21μg.

m1 subtype selective muscarinic agonist potentially useful for the treatment of neurodegenerative disorders.[9] It was our desire to resolve these early intermediates. In our previous work,[1] two columns were successfully employed to perform the chiral separation for all four compounds using hexane/2-propanol/diethylamine.

It would be advantageous, if the desired chiral separation can be accomplished with one column for all four compounds by simply changing the alcohol modifier.

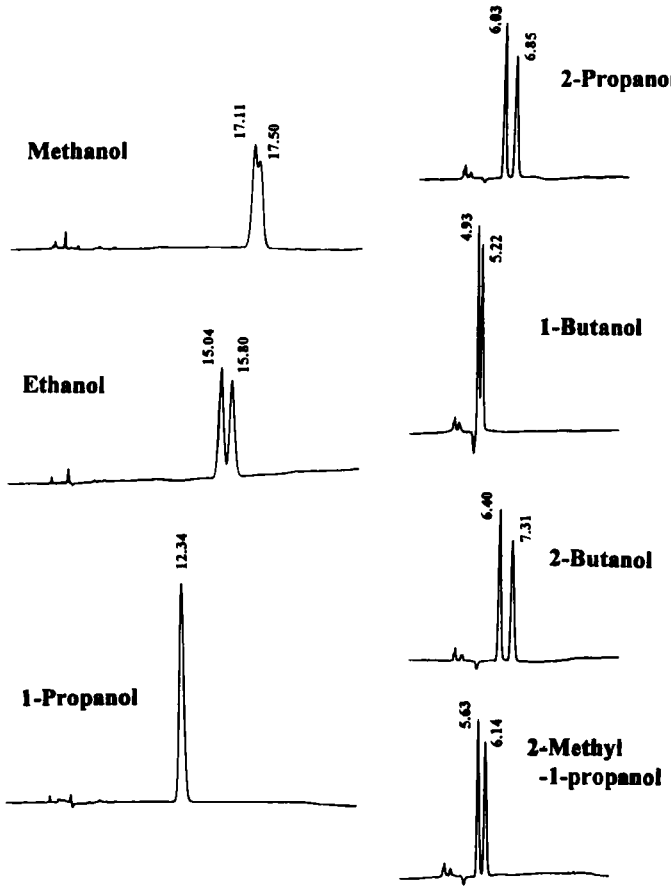


Figure 3. Effect of Alcohol Modifiers on the Separation of a Racemic Mixture of Compound **1** using a Chiralpak AD column; Mobile Phase: Hexane/Alcohol/DEA (980/20/1), Flow Rate: 1.0 mL/min, Detection: UV @ 230 nm, Sample Amount Injected: ~21 μg.

EXPERIMENTAL

Equipment

The liquid chromatographic system consisted of a Hitachi L-6200 intelligent pump, a Micromeritics 728 autosampler, a Valco injector with a 20 μL loop, a Hitachi L-4000 variable wavelength UV detector, a Waters 410

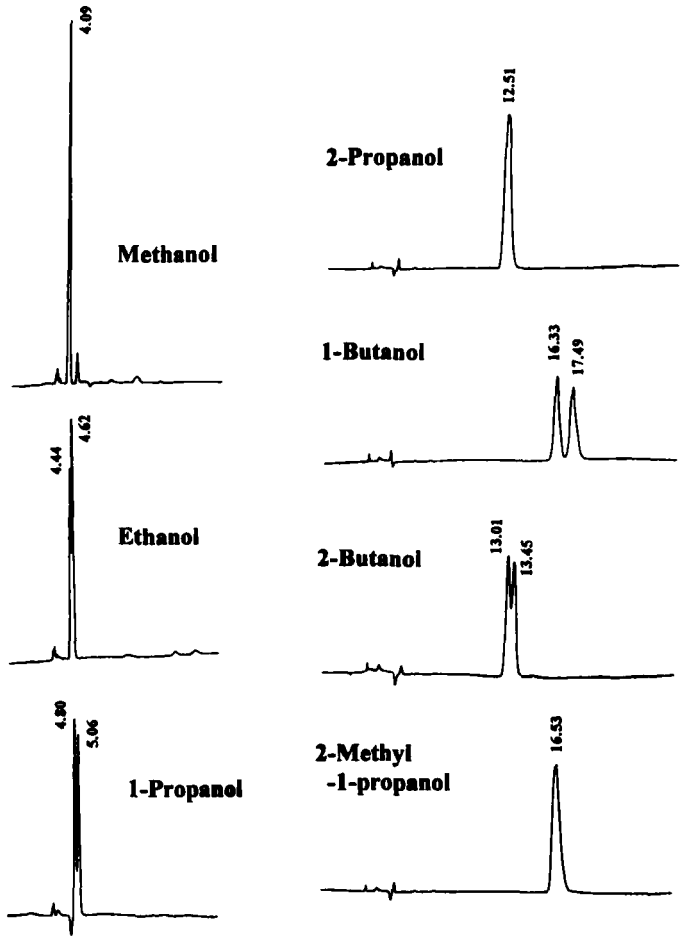


Figure 4. Effect of Alcohol Modifiers on the Separation of a Racemic Mixture of Compound **4** using a Chiralpak AS column; Mobile Phase: Hexane/Alcohol/DEA (980/20/1), Flow Rate: 1.0 mL/min, Detection: UV @ 230 nm, Sample Amount Injected: ~21 µg.

Differential Refractometer equipped with a column oven, and a Hitachi D-2500 Chromato-integrator. The analytical columns were Chiralcel OD-H, OJ, Chiralpak AS and AD. All of the columns were 250 x 4.6 mm I.D., and 10 microns in particle size except OD-H which was 5 microns. They were purchased from Chiral Technologies, Inc, Exton, PA.

Table 1

Effects of Mobile Phase Alcohol Modifiers on the Enantiomeric Separations of Compounds 1, 2, 3, and 4 on Various Columns with a Flow Rate of 1.0mL/min and a Mobile Phase of Hexane/Alcohol/DEA (950/50/1)

Alcohol Modifier	Compound 1			Compound 2			Compound 3			Compound 4		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Chiralpak AD												
methanol	no separation			----	----	<0.5	no separation			no separation		
ethanol	----	----	<0.5	----	----	<0.5	----	-----	<0.5	0.76	1.11	0.96
1-propanol	no separation			1.02	1.06	0.61	0.55	1.08	0.54	0.61	1.16	1.63
1-butanol	2.05	1.13	1.88	no separation			----	----	<0.5	0.81	1.07	0.64
2-propanol	----	----	<0.5	0.93	1.10	1.19	0.55	1.11	1.09	0.62	1.13	1.07
2-butanol	1.40	1.06	0.73	1.04	1.11	1.26	0.64	1.13	1.14	0.73	1.12	1.22
2-methyl-1-propanol	----	----	<0.5	no separation			----	----	<0.5	0.82	1.06	0.52
Chiralpak AS												
methanol	0.90	1.09	0.80	no separation			no separation			no separation		
ethanol	1.10	1.21	2.29	0.55	1.12	0.80	no separation			no separation		
1-propanol	1.43	1.30	3.47	0.67	1.16	1.31	no separation			0.30	1.14	0.52
1-butanol	1.79	1.39	4.03	0.77	1.22	1.79	no separation			0.31	1.18	0.74
2-propanol	2.80	1.34	4.01	1.28	1.22	2.23	no separation			0.56	1.25	1.65
2-butanol	3.00	1.46	4.79	1.41	1.34	3.28	----	----	<0.5	0.64	1.27	2.00
2-methyl-1-propanol	2.36	1.44	3.06	1.01	1.33	1.87	no separation			0.43	1.22	1.20
Chiralcel OJ												
methanol	no separation			----	----	<0.5	no separation			no separation		
ethanol	no separation			no separation			no separation			no separation		
1-propanol	2.72	1.05	0.61	no separation			no separation			0.39	1.22	1.35
1-butanol	3.15	1.07	0.94	1.43	1.06	0.50	no separation			0.49	1.14	0.85
2-propanol	----	----	<0.5	no separation			no separation			no separation		
2-butanol	2.87	1.06	0.77	----	----	<0.5	no separation			no separation		
2-methyl-1-1.03propanol	2.97	1.06	0.73	no separation			no separation			0.41	1.24	
Chiralcel OD-H												
methanol	no separation			no separation			no separation			no separation		
ethanol	no separation			no separation			no separation			no separation		
1-propanol	----	----	<0.5	no separation			no separation			no separation		
1-butanol	----	----	<0.5	0.78	1.08	0.63	no separation			no separation		
2-propanol	1.51	1.07	0.85	----	----	<0.5	no separation			no separation		
2-butanol	1.86	1.06	0.80	----	----	<0.5	no separation			no separation		
1-methyl-1-propanol	----	----	<0.5	----	----	<0.5	no separation			no separation		

Table 2

Effects of Mobile Phase Alcohol Modifiers on the Enantiomeric Separations of Compounds 1, 2, 3, and 4 on a Chiralpak AD Column with a Flow Rate of 1.0mL/min

Alcohol Modifier	Compound 1			Compound 2			Compound 3			Compound 4		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
Hexane/Alcohol/DEA (950/50/1)												
methanol	no separation			----	----	<0.5	no separation			no separation		
ethanol	----	----	<0.5	----	----	<0.5	----	----	<0.5	0.76	1.11	0.96
1-propanol	no separation			1.02	1.06	0.61	0.55	1.08	0.54	0.61	1.16	1.63
1-butanol	2.05	1.13	1.88	no separation			----	----	<0.5	0.81	1.07	0.64
2-propanol	----	----	<0.5	0.93	1.10	1.19	0.55	1.11	1.09	0.62	1.13	1.07
2-butanol	1.40	1.06	0.73	1.04	1.11	1.26	0.64	1.13	1.14	0.73	1.12	1.22
2-methyl-1-propanol	----	----	<0.5	no separation			----	----	<0.5	0.82	1.06	0.52
Hexane/Alcohol/DEA (980/20/1)												
methanol	----	----	<0.5	----	----	<0.5	no separation			no separation		
ethanol	4.46	1.04	0.90	----	----	<0.5	1.17	1.07	1.04	1.39	1.11	1.31
1-propanol	no separation			2.05	1.06	0.98	1.06	1.10	1.18	1.20	1.19	2.41
1-butanol	3.94	1.12	2.30	no separation			1.15	1.10	1.42	1.43	1.09	1.09
2-propanol	no separation			1.67	1.09	1.39	1.00	1.11	1.26	1.13	1.11	1.38
2-butanol	----	----	<0.5	2.26	1.09	1.58	1.41	1.12	1.55	1.62	1.11	1.73
2-methyl-1-propanol	no separation			no separation			1.49	1.06	0.81	1.85	1.06	0.63

Chemicals

Hexane, methanol, 2-propanol, and 2-butanol (HPLC grades) were obtained from EM Science, Gibbstown, NJ. Ethanol (absolute) was purchased from Aaper Alcohol and Chemical Company, Shelbyville, KY. 1-Propanol, 1-butanol, 2-methyl-1-propanol (HPLC grades), and diethylamine (redistilled, 99.5%) were obtained from Aldrich Chemical Co., Inc., Milwaukee, WI. Racemic 1-alkoxycarbonylalkyl-pyrrolidine-3-carboxylic acid alkyl esters were synthesized in the Chemical Development Department, Parke-Davis Pharmaceutical Research Division, Holland, MI.

Chromatographic Conditions

The mobile phase was hexane/alcohol/diethylamine (DEA) in an appropriate volume ratio. The flow rate was either 1.0 or 0.6 mL/min. The

Table 3

Effects of the Flow Rate on the Enantiomeric Separations of Compounds 1, 2, 3, and 4 on a Chiralpak AD Column Using a Mobile Phase of Hexane/Alcohol/DEA (980/20/1)

Alcohol Modifier	Compound 1			Compound 2			Compound 3			Compound 4		
	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s	k_1'	α	R_s
1.0 mL/min												
methanol	----	----	<0.5	----	----	<0.5	no separation			no separation		
ethanol	4.46	1.04	0.90	----	----	<0.5	1.17	1.07	1.04	1.39	1.11	1.31
1-propanol	no separation			2.05	1.06	0.98	1.06	1.10	1.18	1.20	1.19	2.41
1-butanol	3.94	1.12	2.30	no separation			1.15	1.10	1.42	1.43	1.09	1.09
2-propanol	no separation			1.67	1.09	1.39	1.00	1.11	1.26	1.13	1.11	1.38
2-butanol	----	----	<0.5	2.26	1.09	1.58	1.41	1.12	1.55	1.62	1.11	1.73
2-methyl-1-propanol	no separation			no separation			1.49	1.06	0.81	1.85	1.06	0.63
0.6 mL/min												
methanol	----	----	<0.5	----	----	<0.5	no separation			no separation		
ethanol	4.55	1.04	0.96	2.69	1.04	0.63	1.20	1.07	1.00	1.43	1.11	1.64
1-propanol	no separation			2.03	1.06	1.28	1.04	1.10	1.42	1.18	1.19	2.60
1-butanol	3.92	1.12	2.33	no separation			1.13	1.10	1.49	1.41	1.08	1.38
2-propanol	no separation			1.73	1.09	1.48	1.04	1.11	1.50	1.17	1.11	1.52
2-butanol	2.97	1.03	0.56	2.24	1.09	1.70	1.39	1.12	1.85	1.60	1.11	2.00
2-methyl-1-propanol	no separation			no separation			1.48	1.06	0.89	1.84	1.06	1.00

detection was UV @ 230 nm. The column temperature was maintained at 30°C. The sample was dissolved in mobile phase. The amount of sample injected was 9 to 22 μg unless otherwise stated. The capacity factor of the first eluted peak, k_1', the separation factor, α, and the resolution factor, R_s, were calculated as follows: $k_1' = (t_1 - t_0)/t_0$; $\alpha = (t_2 - t_1)/(t_1 - t_0)$; $R_s = 2(t_2 - t_1)/(w_1 + w_2)$; where t_0 is the time at void volume, t_1 is the retention time of the first eluted peak, t_2 is the retention time of the second eluted peak, w_1 and w_2 are the widths at baseline for the first and second eluted peaks, respectively, and they were obtained by extrapolating the relatively straight sides of the peaks to the baseline.

RESULTS AND DISCUSSION

Table 1 gives k_1', α and R_s for compounds 1, **2**, **3**, and **4** using different alcohols in the mobile phase on Chiralcel OD-H, OJ, Chiralpak AD, and AS

columns, respectively. The Chiralpak AS column gave the best enantiomeric separation for compounds **1** & **2** no matter what alcohol was used. Among the alcohols studied, 2-butanol was more efficient than 2-propanol as the alcohol modifier on this column. The enantiomeric separation of compound **1** using various alcohol modifiers is shown in Figure 2. The Chiralpak AS column afforded better separation for compound **4** using either 2-propanol, 2-butanol or 2-methyl-1-propanol while Chiralpak AD and Chiralcel OJ columns were better when 1-propanol was used. It was very interesting to note from Table 1 that the Chiralpak AD column was the only column which gave reasonable separation for compound **3** and 2-butanol was slightly better than 2-propanol as the alcohol modifier.

Both the flow rate and alcohol amount can be used to enhance enantiomeric separation for all four compounds. The nature of alcohol does not seem to change this aspect. The resolution increased with a reduced amount of alcohol in mobile phase (Table 2) and/or a reduced flow rate (Table 3).

The effects of alcohol modifiers on the separations of compound **1** on a Chiralpak AD column and compound **4** on a Chiralpak AS column using a mobile phase of hexane/ alcohol/DEA (980/20/1) and a flow rate of 1.0 mL/min are illustrated in Figures 3 & 4, respectively. In the case of separating compound **1** on a Chiralpak AD column, 1-butanol clearly was the choice of alcohol modifiers. For separating compound **4** on a Chiralpak AS column, the best alcohol modifier was either 2-butanol or 2-propanol. However, 2-methyl-1-propanol also worked.

Finally, by varying the mobile phase alcohol modifiers, the separations of all four compounds on a Chiralpak AD column could be achieved using 1-butanol for compound **1**, 2-butanol for compounds **2** & **3**, and 1-propanol for compound **4**, respectively. These results are shown in Figure 5.

CONCLUSIONS

For chiral HPLC method development, the choice of the right chiral column often dictates the success of the methodology. The results from this study not only confirm this but also suggest that better separation can be obtained via a change of alcohol modifiers in mobile phase. Although the use of a Chiralpak AS column gave the best separations for compounds **1**, **2** & **4**, the separation of compound **3** required a second chiral column.

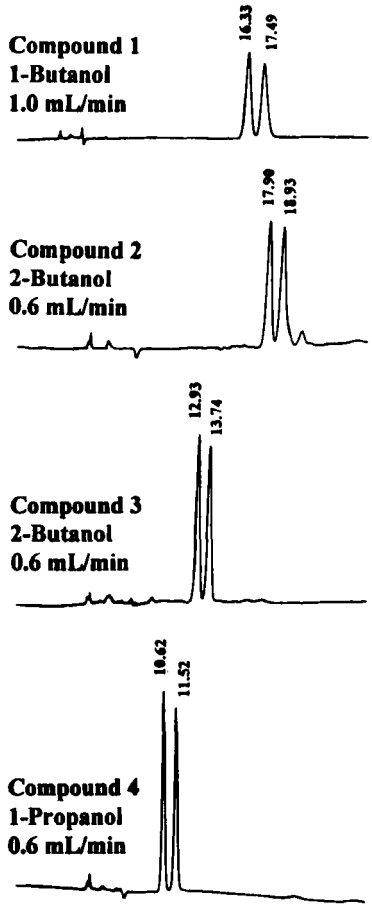


Figure 5. Separations of Racemic Mixtures of Compounds **1**, **2**, **3** & **4** on a Chiralpak AD column by varying the alcohol modifier.
Mobile Phase: Hexane/Alcohol/DEA (980/20/1), Detection: UV @ 230 nm, Sample Amount Injected: ~21 μg.

The change of alcohol modifiers in mobile phase allowed us to separate all four compounds on a Chiralpak AD column. These methods have been routinely employed for screening large-scale resolution conditions for all four compounds in our laboratory.

ACKNOWLEDGMENTS

We gratefully thank Drs. Donald E. Butler and William T. Suggs for their helpful comments, and Ms. Sandy Dokter for the typing of this manuscript.

REFERENCES

1. S. Lin, C. E. Engelsma, N. J. Maddox, B. K. Huckabee, D. M. Sobieray, "Chiral HPLC Separations of 1-Azabicyclo[2.2.1]heptan-3-one and 1-Alkoxycarbonylalkyl-pyrrolidine-3-carboxylic Acid Alkyl Ester Enantiomers on Polysaccharide-Based Stationary Phases," J. Liq. Chrom. & Rel. Technol., 20 (1997), in press.

2. T. Shibata, I. Okamoto, K. Ishii, "Chromatographic Optical Resolution on Polysaccharides and their Derivatives," J. Liquid Chromatogr., **9**, 313-340 (1986).

3. K. Oguni, H. Oda, A. Ichida, "Development of Chiral Stationary Phases Consisting of Polysaccharide Derivatives," J. Chromatogr., A, **694**, 91-100 (1995).

4. I. W. Wainer, M. C. Alembik, E. Smith, "Resolution of Enantiomeric Amides on a Cellulose Tribenzoate Chiral Stationary Phase: Mobile Phase Modifier Effects on Retention and Stereoselectivity," J. Chromatogr., **388**, 65-74 (1987).

5. M. H. Gaffney, R. M. Stiffin, I. W. Wainer, "The Effect of Alcoholic Mobile Phase Modifiers on Retention and Stereoselectivity on a Commercially Available Cellulose-Based HPLC Chiral Stationary Phase: an Unexpected Reversal in Enantiomeric Elution Order," Chromatographia, **27**, 15-18 (1989).

6. K. M. Kirkland, "Optimization of Chiral Selectivity on Cellulose-Based High Performance Liquid Chromatographic Columns using Aprotic Mobile-Phase Modifiers," J. Chromatogr., A, **718**, 9-26 (1995).

7. A. Kunath, F. Theil, K. Jahnisch, "Influence of the Kind of the Alcoholic Modifiers on Chiral Separation on a Chiralpak AD Column," J. Chromatogr., A, **728**, 249-257 (1996).

8. Y. Tang, "Significance of Mobile Phase Composition in Enantioseparation of Chiral Drugs by HPLC on a Cellulose-Based Chiral Stationary Phase," Chirality, **8**, 136-142 (1996).

9. J. Jaen, S. Barrett, M. Brann, M. Callahan, R. Davis, P. Doyle, D. Eubanks, D. Lauffer, L. Lauffer, W. Lipinski, D. Moreland, C. Nelson, C. Raby, R. Schwarz, C. Spencer, H. Tecle, "In Vitro and In Vivo Evaluation of the Subtype-Selective Muscarinic Agonist PD 151832," Life Sciences, **56**, 845-852 (1995).

Received January 17, 1997
Accepted March 5, 1997
Manuscript 4340