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Preparative chromatographic resolution of enantiomers using polar organic solvents with polysaccharide chiral stationary phases

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Abstract

The preparative chromatographic resolution of racemic mixtures is rapidly becoming a standard approach for the generation of enantiomers in pharmaceutical research and development. This paper will discuss the optical resolution of numerous pharmaceutical intermediates and final products using polar organic solvents with polysaccharide chiral stationary phases. The advantages of this approach compared to more traditional mobile phases for preparative separations will be presented. In addition the ability to reverse elution order using polar organic solvents will be presented.

Keywords: Enantiomer separation; Preparative chromatography; Pharmaceutical analysis

1. Introduction

The necessity to generate individual enantiomers for testing has become a growing priority in pharmaceutical research and development. This necessity is directly related to the increased knowledge of the effect differing enantiomers have in biological systems. There are two approaches to obtaining enantiomerically pure chemicals. These are: (1) asymmetric synthesis of the desired enantiomer and (2) resolution of a racemic mixture into individual enantiomers. While asymmetric synthesis is useful when larger quantities of enantiomers are required, the time required to develop the synthesis can make this approach impractical when small quantities (<1 kg) are needed. An additional disadvantage of asymmetric synthesis is the generation of only one of the enantiomers. Resolution of a racemic mixture has the advantage of producing both enantiomers, essential for testing. Resolution methods include recrystallization, enzymatic resolution, indirect chromatographic resolution and direct chromatographic resolution. The time required to develop a recrystallization method or an enzymatic resolution can also make this an inefficient approach for the generation of small quantities of enantiomers. Indirect chromatographic resolution, involving derivatization of the enantiomers to form a pair of diastereomers, followed by separation on an achiral stationary phase is useful for the generation of individual enantiomers [1]. While the separation of diastereomers is usually easier than the separation of enantiomers, and has the advantage of being easier to scale up, there are limitations to this approach. First, high enantiomeric purity of the derivatizing agent is essential. Also, removal of the derivative after chromatographic separation should not cause racemization of the desired enantiomer. Finally the use of indirect chromatographic separation adds two steps to the synthesis, the reaction to form the derivatives prior to

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chromatography and the reaction to remove the derivatives after chromatography. The Separations Group at Searle has found the use of preparative chromatography using chiral stationary phases (CSPs) to be the most efficient approach for generation of small (<1 kg) quantities of enantiomers [2–6]. In past years the preparative resolution of racemic mixtures has rapidly become a standard approach for the generation of enantiomers in pharmaceutical research and development [7–12].

Over the past 10 years the number of CSPs available for chiral separations has grown rapidly. These advances have made it possible to develop an analytical enantioseparation for nearly any racemic mixture. This is not always the case for the development of high productivity preparative enantioseparations. The traditional mobile phases for many CSPs is an alcohol–hydrocarbon combination. Unfortunately many pharmaceutical compounds have poor solubility in these mobile phase combinations. In addition to maximum separation, high solubility is required for a high productivity preparative separation [13]. Initial work has been reported on the use of polar organic solvents (methanol, ethanol, acetonitrile) with polysaccharide based CSPs [14,15]. Racemates have increased solubility in these solvents compared to alcohol–heptane mixtures. In addition, racemates often exhibit short retention ($k'<3$) with these solvents. Short retention is another requirement for high productivity separations. [16,17] The initial work has shown higher productivities for preparative separations developed using this approach compared to traditional alcohol–hydrocarbon mobile phases.

This paper discusses the optical resolution of numerous pharmaceutical intermediates and final products using polar organic solvents with polysaccharide CSPs. The advantages of this approach compared to more traditional mobile phases for preparative separations will be presented. Finally the ability to reverse elution order using polar organic solvents will be presented.

2. Experimental

2.1. Equipment

The analytical chromatograph consisted of a Hewlett-Packard 1050 pump (San Fernando, CA, USA), a Waters Intelligent Sample Processor (Milford, MA, USA), a Kratos 757 variable-wavelength detector (Ramsey, NJ, USA), a Kipp and Zonen BD41 two-channel recorder (Delft, The Netherlands) and Digital Equipment Corp. VAX 11/785 computer with Searle chromatography data system.

Three preparative chromatographs were used for this work. The first consisted of two Rainin (Woburn, MA, USA) SD-1 pumps, a Model UV-M detector and a Kipp and Zonen BD41 two-channel recorder. A Rheodyne (Cotati, CA, USA) Model 7125 syringe loading sample injector equipped with a 10-ml loop (Valco, Houston, TX, USA) or a Gilson Model 401 Dilutor (Middleton, WI, USA) in combination with a Rheodyne electrically actuated Model 7010 injector was used for sample injection. The column effluent was fractionated using a Gilson Model 202 fraction collector. The second preparative chromatograph consisted of two Rainin SD-1 pumps, a Model UV-M detector and a Kipp and Zonen BD41 two-channel recorder. A separate Rainin SD-1 pump was used for sample injection. The third chromatograph consisted of a Separations Technology (Wakefield, RI, USA) ST1000XP preparative chromatography system. For some purifications the CSP was packed into a 8 cm I.D. Prochrom (Indianapolis, IN, USA) dynamic axial compression column. The final bed length varied depending on the amount of CSP packed into the column. See Results and discussion for additional details.

For the experiments utilizing a chiral detector, a Jasco (Easton, MD, USA) Model CD-995 Circular Dichroism Chiral Detector was installed into the fluid stream after the UV detector.

2.2. Materials

The CSPs were obtained from Chiral Technologies (Exton, PA, USA) as prepacked 10-μm analytical (250 mm×4.6 mm) and preparative columns (250 mm×20 mm) or as 20-μm prepacked (500 mm×50 cm or 500 cm×100 cm) columns or as bulk packing (20 μm). All chemicals for purification were synthesized in the laboratories of Searle (Skokie, IL and St. Louis, MO, USA). The solvents and reagents

were reagent grade or better and obtained from a variety of sources.

3. Results and discussion

The compounds discussed in this paper are proprietary intermediates or final compounds being tested in the research and development laboratories of Searle. While no information regarding the structure of the compounds being separated is presented, the approach for separation discussed in this paper is generic and could be used for the separation of any low-molecular-mass (<500) racemates.

3.1. Compound 1

A 90-g amount of Compound 1 was submitted for separation. This compound was closely related to a compound previously separated in our laboratories [15], thus no analytical method development was performed. The analytical separation of this compound is shown in Fig. 1. This method produced short retention as well as good separation ($k'=1.11$ and 1.98, $\alpha=1.78$). This analytical method was scaled up to preparative loadings. The desired enantiomer eluted second using these conditions. Because of this and the need to maximize yield, the amount of racemate applied to the column was only increased until touching bands were observed. The preparative separation of 5.8 g is shown in Fig. 2. Baseline separation of the enantiomers was achieved in 20 min. To maximize the throughput, the technique of overlapping injections was used. In overlapping injections (also called boxcar injections) the second injection is made prior to all the chemical eluting from the first injection. Overlapping injections allowed an injection to be made every 10 min instead of every 20 min. Automation was used to make injections and perform automated peak collection. A 90-g amount of racemate was processed in 15 injections. A purity of >99% enantiomeric excess (ee) and a yield of 98% was obtained for both enantiomers.

3.2. Compound 2

A 550-g amount of compound 2 was submitted for separation. The analytical method developed for this separation utilized a Chiralpak AD CSP with ethanol–heptane (50:50) mobile phase. While this method gave good separation ($\alpha=1.88$) and short retention (k' for second enantiomer=1.60), solubility of the racemate was poor in ethanol–heptane. Analytical high-performance liquid chromatography (HPLC) method development was performed to see if the ethanol percentage in the mobile phase could be increased and still achieve enantioselectivity. The

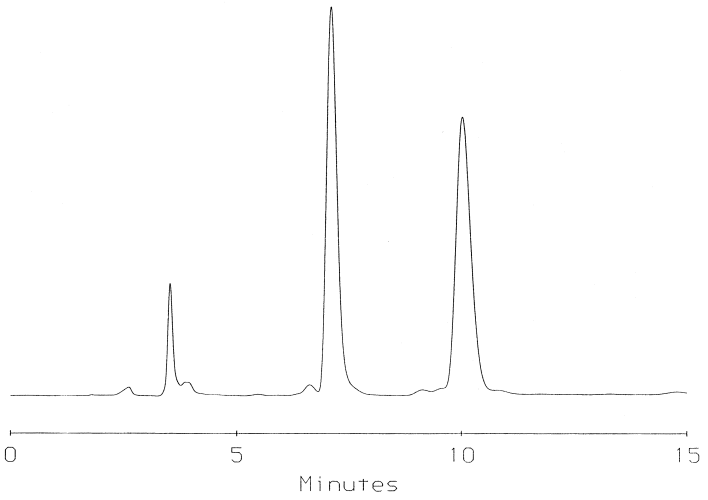

Fig. 1. Analytical HPLC separation of compound 1. Analysis conducted on Chiralpak AS column 10 μm (250 mm×4.6 mm I.D.), detection at 220 nm, 0.1 AUFS. Mobile phase: acetonitrile, flow-rate 1 ml/min.

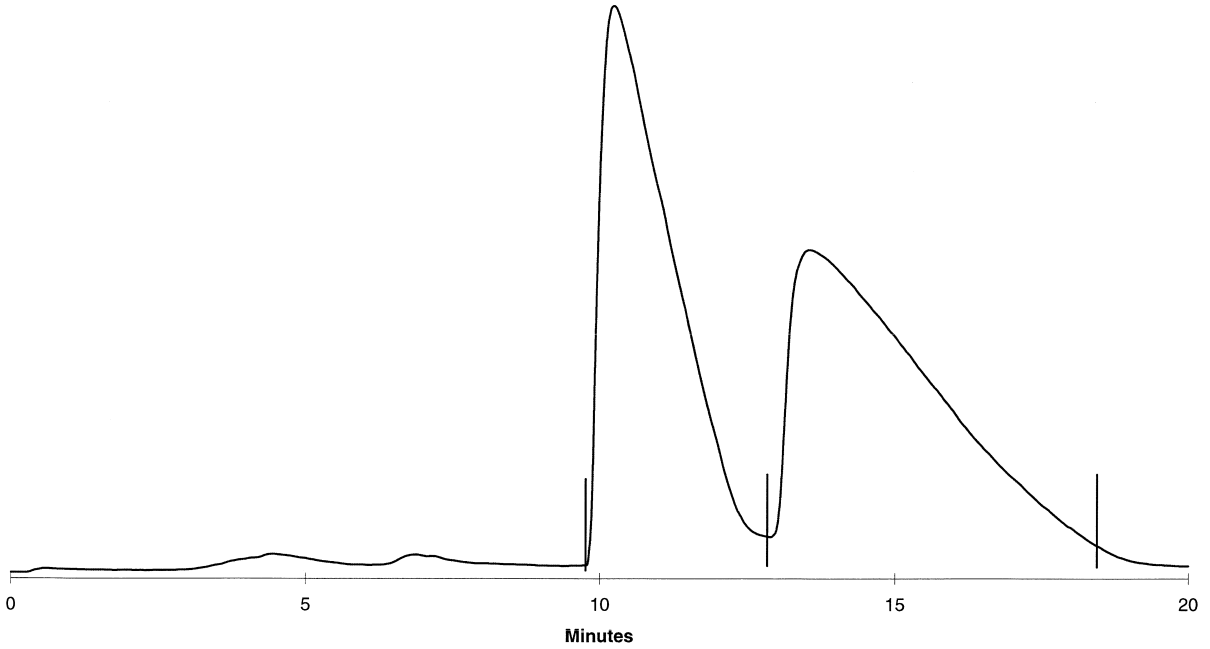

Fig. 2. Preparative HPLC separation of compound 1. The purification was conducted on Chiralpak AS column 20 μm (500 mm×100 mm I.D.), with a mobile phase of acetonitrile. A flow-rate of 500 ml/min, detection at 220 nm and a loading of 5.8 g (25 mg/ml in acetonitrile) were used.

results of these analyses, summarized in Table 1, show that increasing the ethanol content has minimal effect on retention and no effect on separation. The analytical separation using ethanol as a mobile phase is shown in Fig. 3. This separation was scaled to preparative loadings using a Prochrom DAC column containing 1.5 kg of 20 μm Chiralpak AD CSP. The desired enantiomer eluted first under these conditions. This allowed increased amounts of racemate to be injected on the column while still achieving sufficient purity and yield. The preparative separation of 6.2 g of racemate is shown in Fig. 4. Overlap injections were made every 10 min. A 550-g amount was processed in approximately 90 injections. The desired first enantiomer was isolated with purity of >98% ee and in 98% yield. The second enantiomer was isolated with purity of 98% ee and in 92% yield.

3.3. Compound 3

A 9.6-g amount of compound 3 was submitted for separation. The analytical method developed previously for this separation used a mobile phase of

Table 1
Chromatographic results for compound 2

Column	Mobile phase	$k_1'^{a}$	$k_2'^{b}$	α
Chiralpak AD	Ethanol–heptane (50:50, v/v)	0.85	1.60	1.88
Chiralpak AD	Ethanol–heptane (60:40, v/v)	0.68	1.28	1.88
Chiralpak AD	Isopropanol–heptane (70:30, v/v)	0.58	1.10	1.89
Chiralpak AD	Ethanol–heptane (80:20, v/v)	0.56	1.05	1.89
Chiralpak AD	Isopropanol–heptane (90:10, v/v)	0.58	1.12	1.92
Chiralpak AD	Ethanol	0.68	1.33	1.96

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.

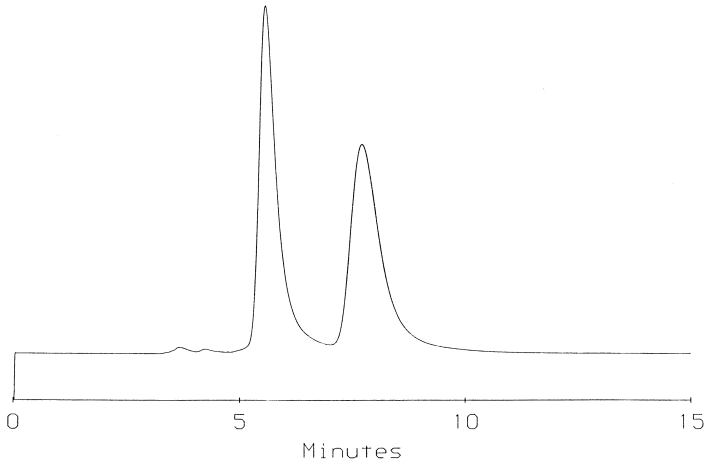

Fig. 3. Analytical HPLC separation of compound 2. Analysis conducted on Chiralpak AD column 10 μm (250 mm×4.6 mm I.D.), detection at 254 nm, 0.1 AUFS. Mobile phase: ethanol, flow-rate 1 ml/min.

isopropanol–heptane. The compound had poor solubility in this mobile phase. An alternate system needed to be developed for preparative separation. A sample of this racemate was sent to Chiral Technologies to be investigated in their screening service. From their work a mobile phase of methanol with Chiralpak AS CSP was recommended. The analytical separation using these conditions is shown in Fig. 5. The k' values for the first and second enantiomer were 1.10 and 1.69, respectively. The α value was 1.53. This method was scaled up to preparative loadings. Since the quantity to be resolved was small, a 2 cm I.D. pre-packed column was used. The separation of 150 mg of racemate is shown in Fig. 6.

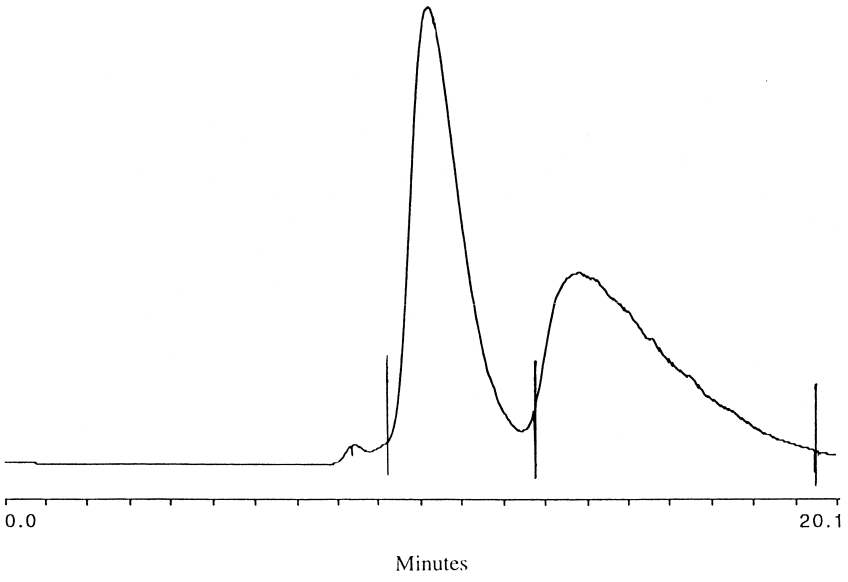

Fig. 4. Preparative HPLC separation of compound 2. The purification was conducted on Chiralpak AD column 20 μm (470 mm×80 mm I.D.), with a mobile phase of ethanol. A flow-rate of 300 ml/min, detection at 254 nm and a loading of 6.2 g (50 mg/ml in ethanol) were used.

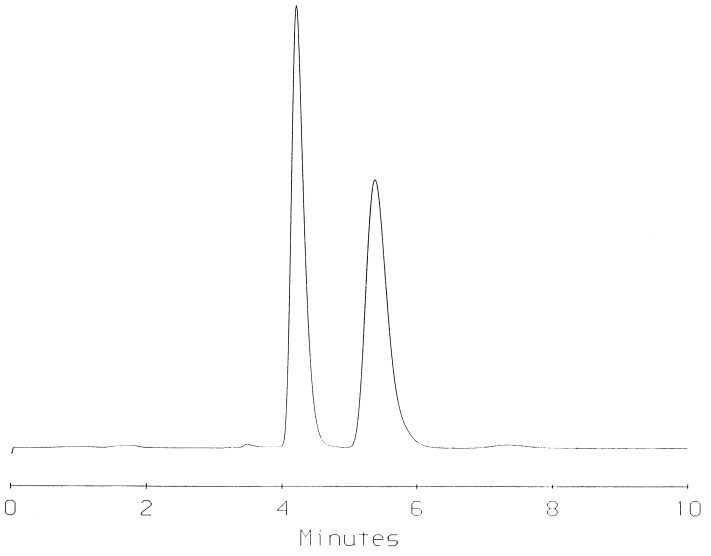

Fig. 5. Analytical HPLC separation of compound 3. Analysis conducted on Chiralpak AS column 10 μm (250 mm×4.6 mm I.D.), detection at 254 nm, 0.1 AUFS. Mobile phase: methanol, flow-rate 1 ml/min.

Both enantiomers were required to be isolated pure. A 9.6-g amount of racemate was resolved in 64 injections. Both enantiomers were isolated with >99.5% ee and near quantitative yield. HPLC analysis of the racemate shows the presence of other impurities. Because of these impurities injections could be made only every 8.8 min. If these impurities were not present the injection time could be reduced to 5 min. To maximize productivity these impurities should be removed prior to the enantioseparation. Since for this separation the amount of material to be resolved was small, an achiral purification was not performed prior to the chiral separation.

3.4. Compound 4

The optical resolution of 193 g of compound 4 was required for Discovery testing. Initial CSP and mobile phase screening showed that the Chiralpak AS CSP and an ethanol–heptane mobile phase gave the best enantioseparation. Solubility studies showed the compound to be extremely insoluble. Maximum solubility of 5 mg/ml was observed in ethanol. Prior to preparative separation additional analytical HPLC analyses were performed to determine the effect of increasing levels of ethanol on the separation. This work is summarized in Table 2. These data showed that as the ethanol percentage increases, retention decreases with only a minimal decrease in separation. Mobile phase combinations of ethanol and methanol were also explored. Separation decreases with increases in methanol. For the preparative scale-up a mobile phase of methanol–ethanol (10:90, v/v) was used. The analytical HPLC separation using these conditions is shown in Fig. 7. Preparative loadings were increased until touching bands were obtained. A maximum load of 15 g of racemate was possible before overlap of peaks occurred. This corresponds to an injection volume of 3000 ml. As the preparative separation in Fig. 8 shows this large injection volume had minimal effect on the separation. The 193 g was processed in 13 injections. Overlapping injections were made every 29 min. Both enantiomers were isolated with purities greater than 99% ee and in near quantitative yield.

3.5. Compound 5

Compound 5 is an intermediate to a compound being evaluated in Searle Discovery. In support of this project 66 g of compound 5 needed to be separated. Analytical HPLC method development produced two methods which gave separation of the

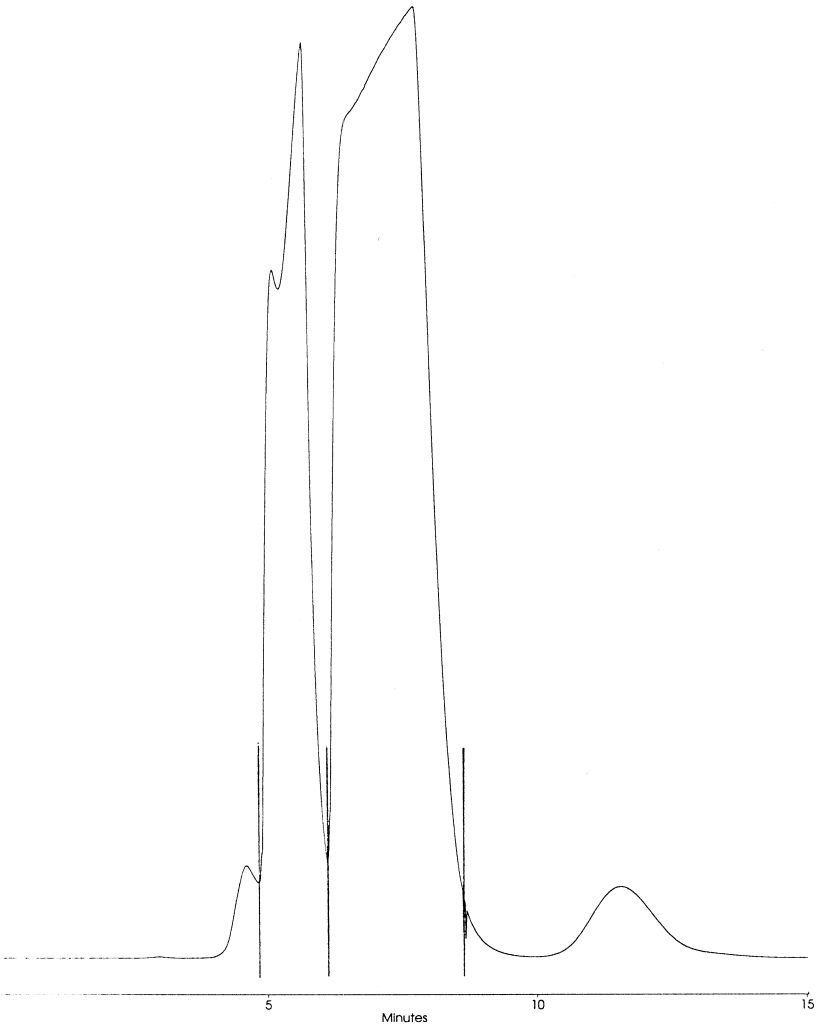

Fig. 6. Preparative HPLC separation of compound 3. The purification was conducted on Chiralpak AS column 10 μm (250 mm×20 mm I.D.), with a mobile phase of methanol. A flow-rate of 13 ml/min, detection at 254 nm and a loading of 150 mg (50 mg/ml in methanol) were used.

two enantiomers. Method 1 used a Chiralpak AD CSP with an ethanol–heptane (40:60, v/v) mobile phase. Method 2 used a Chiralpak AS CSP and a mobile phase of acetonitrile. Both methods had short retention (k' for second enantiomer<3) and good separation ($\alpha>2$). Due to better solubility of the racemate in acetonitrile this method was chosen for preparative scale-up. The analytical HPLC separation of compound 5 is shown in Fig. 9. The peak shape for the second eluting enantiomer is broad, especially for a peak with such short retention ($k'\sim0.4$). While there was some concern that this would make the preparative separation more difficult, it was decided to scale up this method because of the good solubility and the large separation ($\alpha=3.6$). The enantiomer required for Discovery activities was the second eluting enantiomer. It is well known that isolation of the second eluting enantiomer is more difficult due to tailing of the first peak into the second peak. This was especially true with this separation. Utilizing a 50 cm×10 cm I.D. Chiralpak AS column loads of 0.7, 1.4 and 2.1 g of racemate were made. Two

Table 2
Chromatographic results for compound 4 [Chiralpak AS (250 mm×4.6 mm), flow-rate 1 ml/min]

Methanol (v/v)	Ethanol (v/v)	Heptane (v/v)	k_1'[a]	k_2'[b]	α
	50	50	2.54	6.84	2.69
	60	40	1.77	4.77	2.70
	70	30	1.37	3.64	2.66
	80	20	1.10	2.93	2.65
	90	10	1.01	2.62	2.60
	100		1.04	2.54	2.45
10	90		0.87	2.09	2.39
20	80		0.81	1.88	2.33
30	70		0.70	1.60	2.27
40	60		0.70	1.56	2.21
50	50		0.71	1.52	2.14
60	40		0.76	1.55	2.03
70	30		0.82	1.62	1.96
80	20		0.90	1.71	1.90
90	10		0.99	1.86	1.87
100			1.10	2.00	1.81

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.

fractions were collected with the fraction cut occurring at the valley of the two peaks. In all three loads overlap was seen. The yields for the first eluting peak for these three injections were 80%, 76% and 64%, respectively. Based on these results it was decided the separation would be performed in two steps. The first step would remove the bulk of the first eluting enantiomer. The fraction enriched in second enantiomer would be re-purified to isolate the second enantiomer. The preparative HPLC separation of 1.4 g of racemate is shown in Fig. 10. Injections were made every 5 min. This separation was automated to produce pure first enantiomer and an overlap fraction containing an 11:89 mixture of the two enantiomers. This overlap fraction was dried down and re-purified at a load of 1 g per injection. The preparative HPLC separation is shown in Fig. 11. Using this two pronged approach the first eluting enantiomer was isolated with >99% ee and a yield of >98%. The second eluting enantiomer was iso-

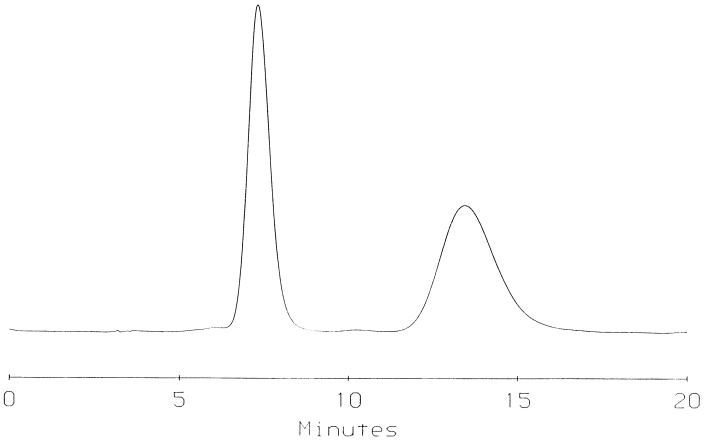

Fig. 7. Analytical HPLC separation of compound 4. Analysis conducted on Chiralpak AS column 10 μm (250 mm×4.6 mm I.D.), detection at 254 nm, 0.1 AUFS. Mobile phase: methanol–ethanol (10:90, v/v), flow-rate 1 ml/min.

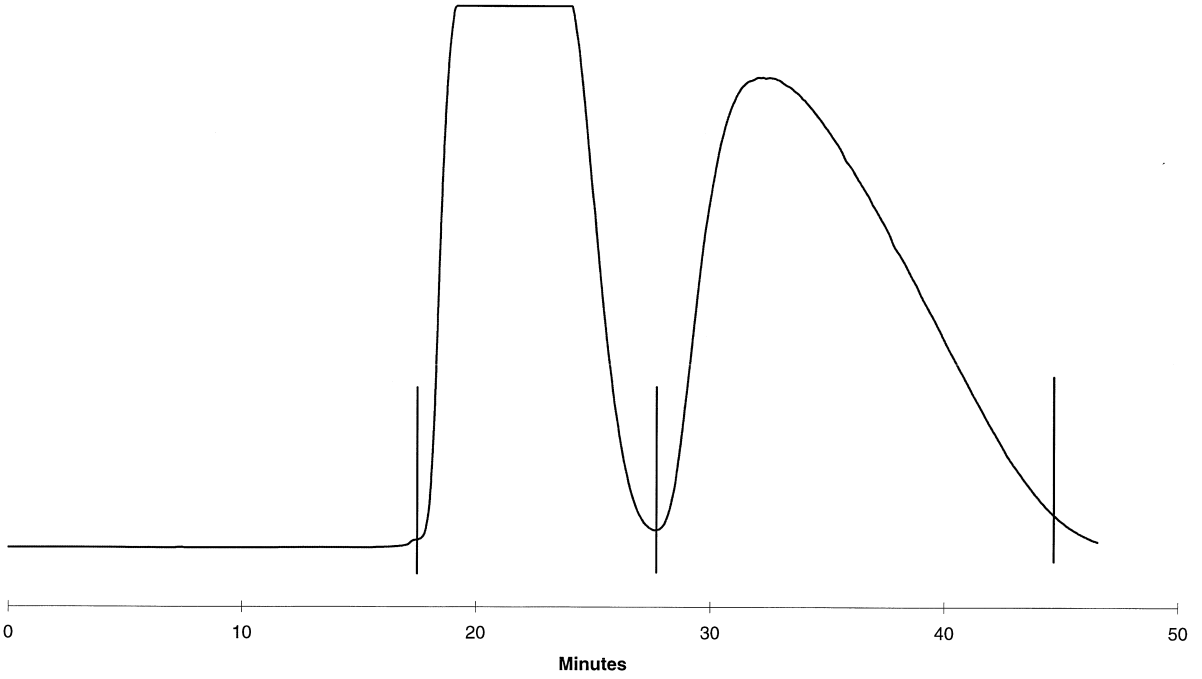

Fig. 8. Preparative HPLC separation of compound 4. The purification was conducted on Chiralpak AS column 20 μm (500 mm×100 mm I.D.), with a mobile phase of methanol–ethanol (10:90, v/v). A flow-rate of 500 ml/min, detection at 254 nm and a loading of 15 g (5 mg/ml in ethanol) were used.

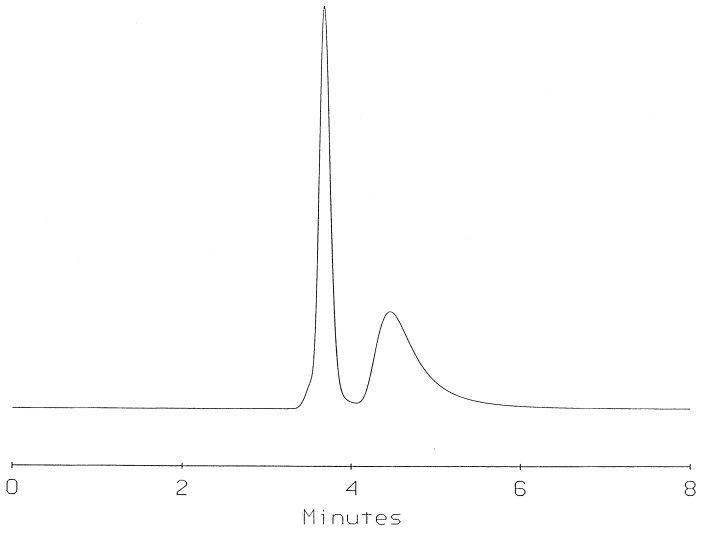

Fig. 9. Analytical HPLC separation of compound 5. Analysis conducted on Chiralpak AS column 10 μm (250 mm×4.6 mm I.D.), detection at 254 nm, 0.1 AUFS. Mobile phase: acetonitrile, flow-rate 1 ml/min.

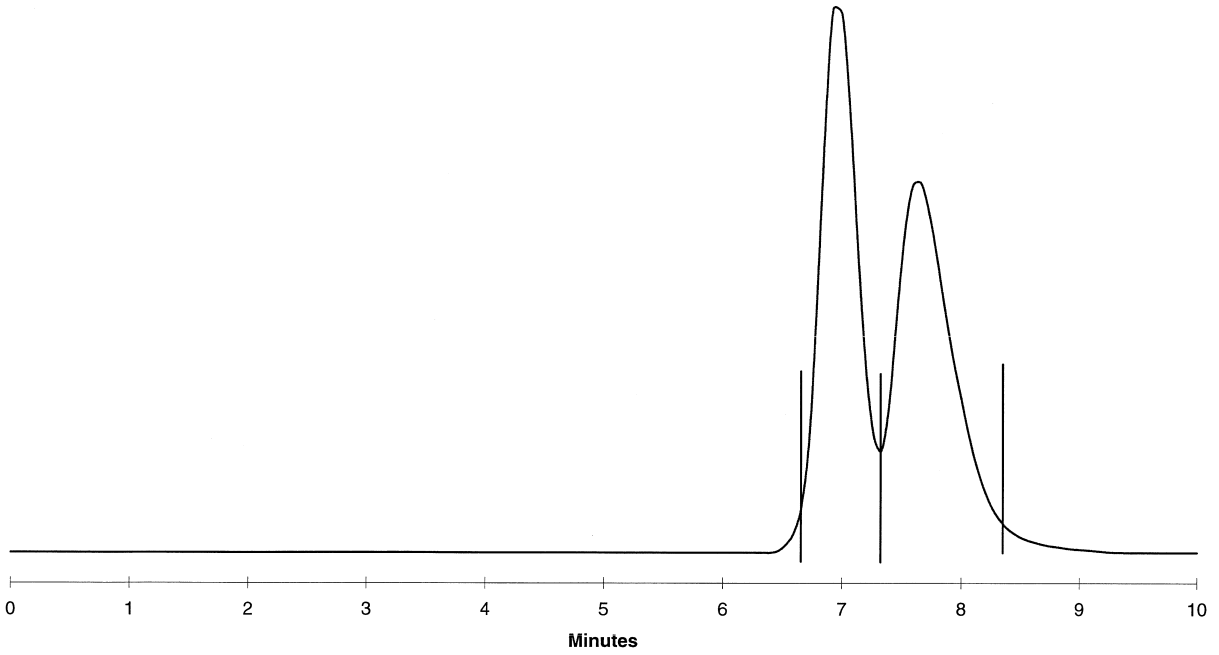

Fig. 10. Preparative HPLC separation of compound 5. The purification was conducted on Chiralpak AS column 20 μm (500 mm×100 mm I.D.), with a mobile phase of acetonitrile. A flow-rate of 500 ml/min, detection at 254 nm and a loading of 1.4 g (20 mg/ml in acetonitrile) were used.

lated with >99.5% ee and a yield of approximately 90%.

3.6. Compound 6

The final compound discussed in this paper needed to be separated at 700 g scale to support Discovery activities. The initial HPLC method developed for this compound utilized a Whelk-O CSP with an isopropanol–heptane mobile phase. Under these conditions good separation was obtained ($\alpha=1.8$) but methylene chloride needed to be added to the isopropanol–heptane to achieve solubility. While this approach would work for small scale separations, it was not desirable when 700 g needed to be separated. Compound 6 was analyzed using polar organic solvents with polysaccharide CSPs. No separation was obtained under any of these conditions. At that time it was thought a modifier might be required for the separation. A mobile phase of acetonitrile–0.1% (v/v) trifluoroacetic acid with the Chiralpak AD CSP was explored. This method gave good enantioseparation. Since compound 6 is an ethyl ester there was concern that during work up the compound would be exposed to high acid levels and the ester degrade to the free acid. Milder acids were explored as modifiers for the separation. The results of this work are summarized in Table 3. These analyses showed the type of acid having no effect on the separation. To minimize the chance of compound degradation, acetic acid was chosen as the modifier for the analytical and preparative separation. The analytical HPLC separation using acetic acid as a modifier is shown in Fig. 12. Preparative method development was performed using a 5 cm I.D. column. These experiments showed that the second eluting enantiomer could not be isolated pure even at loadings as low as 300 mg. A two pronged separation approach, like used for compound 5, was chosen. For the initial purification, 1.35 g of racemate was injected. Three fractions were collected; first enantiomer, overlap, and second enantiomer. The preparative separation is shown in Fig. 13. The 700 g of racemate was processed in 543 injections. Injections were made

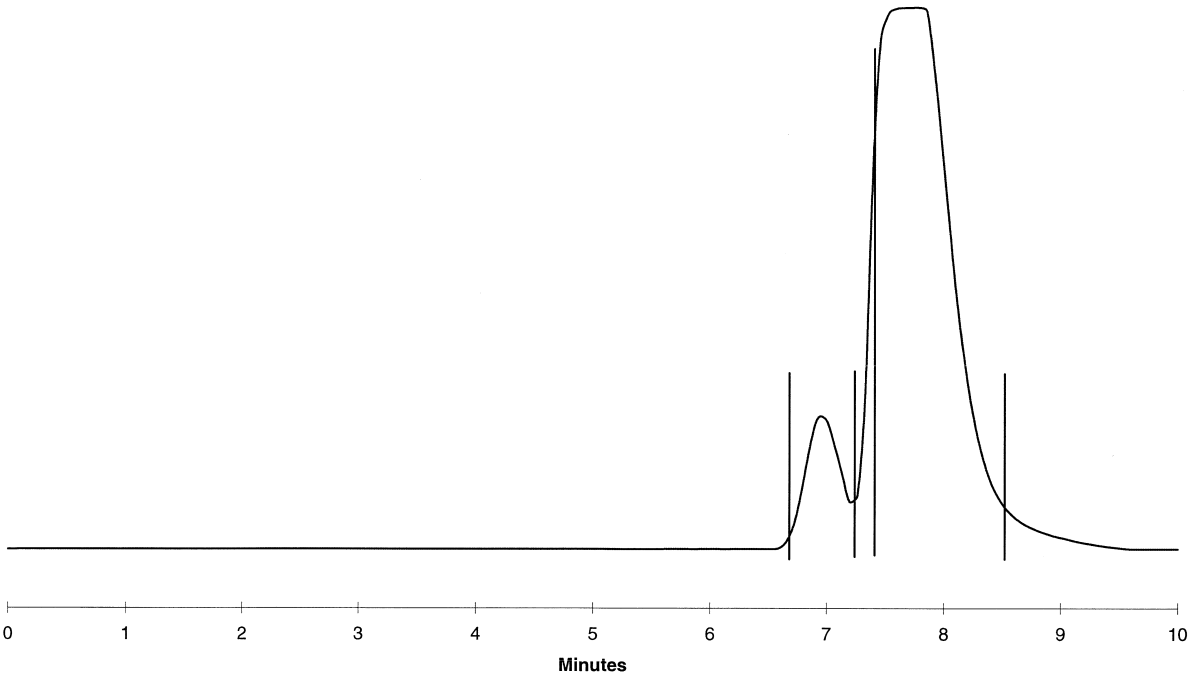

Fig. 11. Preparative HPLC separation of compound 5. The purification was conducted on Chiralpak AS column 20 μm (500 mm×100 mm I.D.), with a mobile phase of acetonitrile. A flow-rate of 500 ml/min, detection at 254 nm and a loading of 1 g (14 mg/ml in acetonitrile) were used.

every 6 min. This separation produced 290 g of pure first enantiomer, 92 g of pure second enantiomer and a 225 g overlap fraction (enantiomer 1–enantiomer 2, 20:80). The overlap fraction was then chromatographed using identical conditions except the injection size was reduced to 1 g. Analysis of the 1-g injection showed that the second enantiomer was isolated at 97% yield with a purity of >95% ee. The first enantiomer was isolated at 87% yield with a purity of >99.5% ee. The separation is shown in Fig. 14. Currently the remainder of the overlap fraction has not yet been processed.

3.7. Reversal of elution order

Earlier in the paper (compound 5) the effect of needing to recover the second eluting enantiomer at high purity and yield was seen; either lower quantities are injected on the column, or an overlap fraction is generated that must be reprocessed For maximum productivity it is desirable to develop a separation with the desired enantiomer eluting first off the column. While reversal of elution order is possible using Pirkle type CSPs that are available in both optical forms, reversal using polysaccharide

Table 3
Chromatographic results for compound 6

Column	Mobile phase	$k_1'^{a}$	$k_2'^{b}$	α
Chiralpak AD	Acetonitrile–0.1% (v/v) trifluoroacetic acid	0.31	0.61	1.95
Chiralpak AD	Acetonitrile–0.1% (v/v) formic acid	0.31	0.61	1.95
Chiralpak AD	Acetonitrile–0.1% (v/v) acetic acid	0.31	0.63	2.01

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.

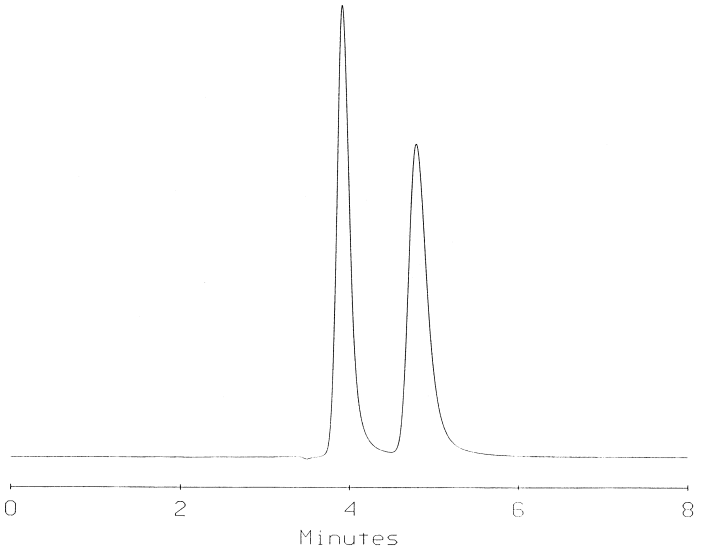

Fig. 12. Analytical HPLC separation of compound 6. Analysis conducted on Chiralpak AD column 10 μm (250 mm×4.6 mm I.D.), detection at 225 nm, 0.1 AUFS. Mobile phase: acetonitrile–0.1% (v/v) acetic acid, flow-rate 1 ml/min.

CSPs is more of a hit or miss proposition. Preliminary work in our laboratories has shown that reversal in elution order is often seen when switching from a more traditional mobile phase of alcohol–hydrocarbon to a polar organic mobile phase. While this effect is not seen with all compounds, for the

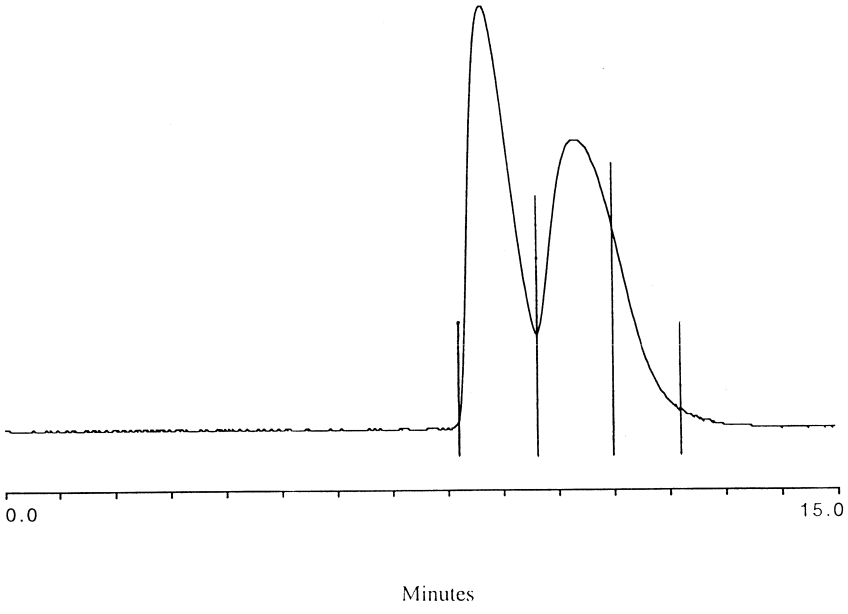

Fig. 13. Preparative HPLC separation of compound 6. The purification was conducted on Chiralpak AD column 20 μm (500 mm×50 mm I.D.), with a mobile phase of acetonitrile–0.1% acetic acid. A flow-rate of 100 ml/min, detection at 300 nm and a loading of 1.35 g (30 mg/ml in acetonitrile) were used.

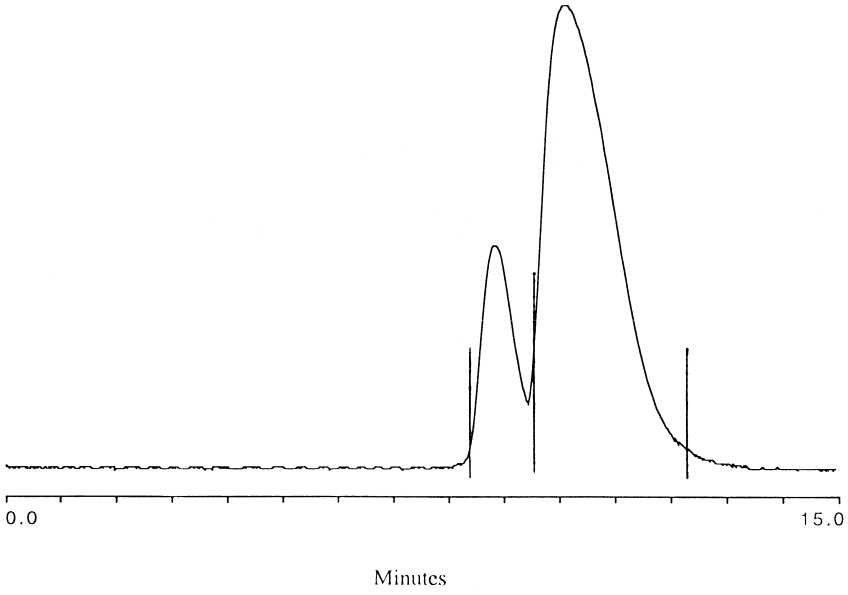

Fig. 14. Preparative HPLC separation of compound 6. The purification was conducted on Chiralpak AD column 20 μm (500 mm×50 mm I.D.), with a mobile phase of acetonitrile–0.1% acetic acid. A flow-rate of 100 ml/min, detection at 300 nm and a loading of 1 g (30 mg/ml in acetonitrile) were used.

compounds we have explored approximately 40% show reversal. Two examples of this effect are shown. The first is compound 7. Fig. 15 shows the separation of the racemate using a Chiralpak AS CSP with an acetonitrile mobile phase. Using these conditions the desired (−) enantiomer eluting second. When the same compound is analyzed using a Chiralpak AD CSP and an ethanol–heptane mobile

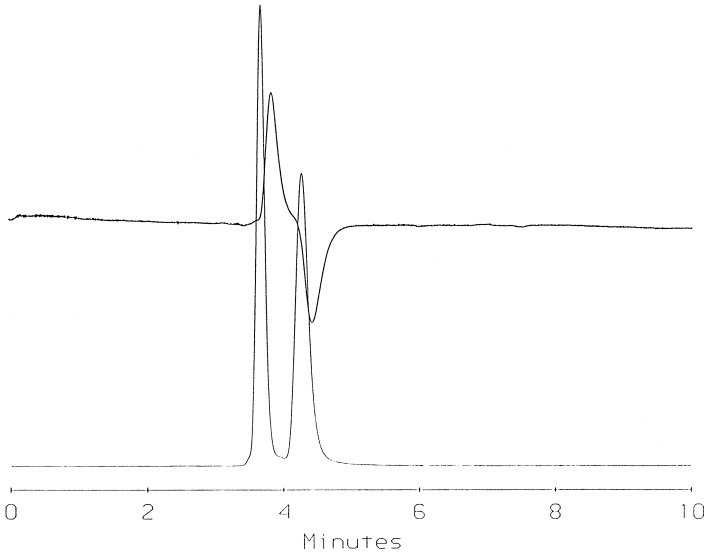

Fig. 15. Analytical HPLC separation of compound 7. Analysis conducted on Chiralpak AS column 10 μm (250 mm×4.6 mm I.D.), detection at 225 nm, 0.1 AUFS. Mobile phase: acetonitrile, flow-rate 1 ml/min.

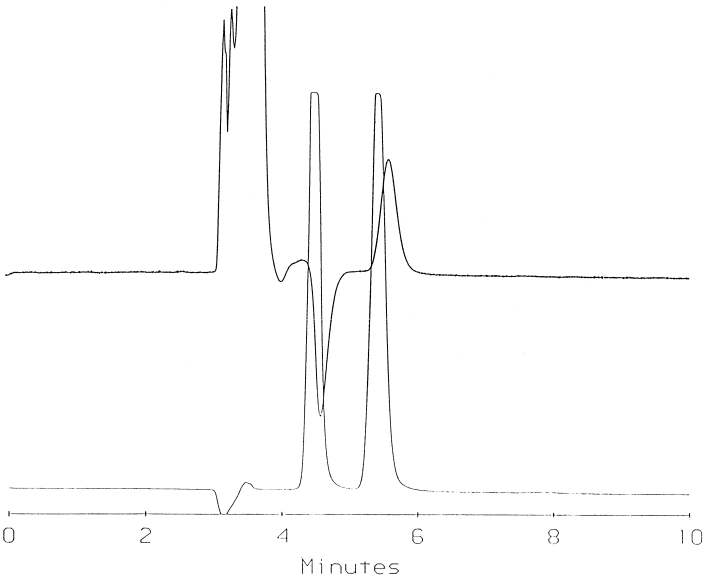

Fig. 16. Analytical HPLC separation of compound 7. Analysis conducted on Chiralpak AD column 10 μm (250 mm×4.6 mm I.D.), detection at 225 nm, 0.1 AUFS. Mobile phase: ethanol–heptane (40:60, v/v), flow-rate 1 ml/min.

phase (Fig. 16), the desired (−) enantiomer elutes first. The second example is compound 1. Fig. 17 shows the separation using Chiralpak AS CSP and acetonitrile mobile phase. The desired (+) enantiomer elutes second. Analysis using a Chiralpak AD CSP and a ethanol–heptane mobile phase (Fig. 18) shows the (+) enantiomer eluting first. Exploring this phenomenon prior to preparative separations may result in higher productivities for a separation. The first purification could be performed at high

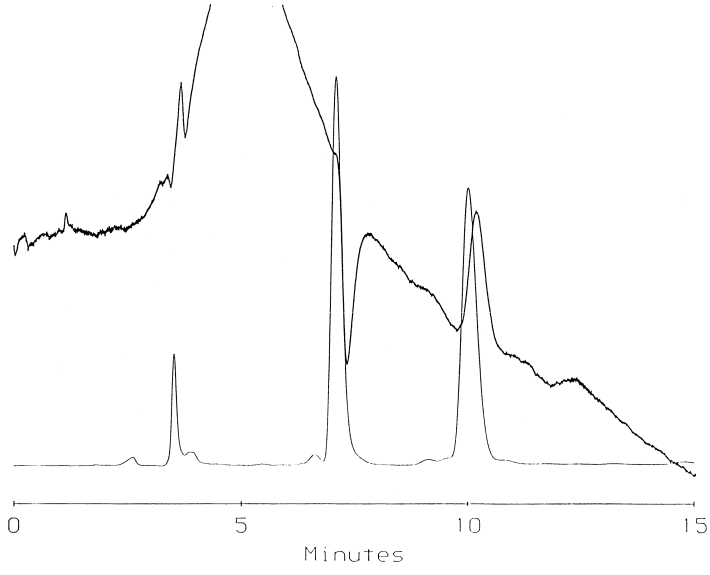

Fig. 17. Analytical HPLC separation of compound 1. Analysis conducted on Chiralpak AS column 10 μm (250 mm×4.6 mm I.D.), detection at 225 nm, 0.1 AUFS. Mobile phase: acetonitrile, flow-rate 1 ml/min.

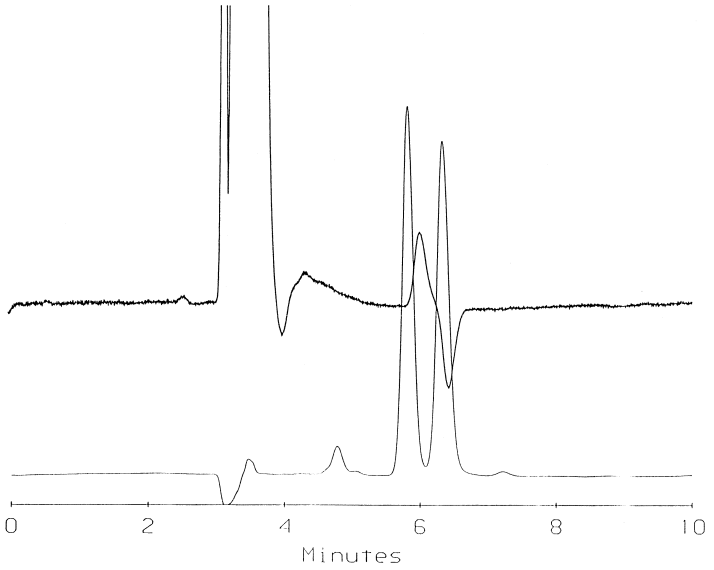

Fig. 18. Analytical HPLC separation of compound 1. Analysis conducted on Chiralpak AD column 10 μm (250 mm×4.6 mm I.D.), detection at 225 nm, 0.1 AUFS. Mobile phase: ethanol–heptane (40:60, v/v), flow-rate 1 ml/min.

loadings, generating the first enantiomer clean. The fraction enriched with the other enantiomer could be processed using the conditions that result in reversal of elution. Isolation of the first eluting peak in this method would allow isolation of both enantiomers at high purity and yield. This approach may not be feasible if the compound exhibits poor solubility in one of the mobile phases. In addition, this approach requires an optical rotation detector during HPLC method development, or alternatively standards of one or both of the enantiomers.

4. Conclusions

The use of polar organic solvents with polysaccharide based CSPs is useful for the preparative resolution of enantiomers. Use of these solvents can alleviate many of the solubility problems encountered with the use of alcohol–hydrocarbon mobile phases. These solvents allow preparative methods with higher productivities to be developed. In addition, the use of single solvents allows easier recycle of solvents, reducing the cost for the preparative separations. Reversal of elution order is possible when switching between polar organic solvents and more traditional alcohol–heptane combinations.

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References

[1] D.R. Brocks, F.M. Pasutto, F. Jamali, J. Chromatogr. 581 (1992) 83.
[2] L. Miller, H. Bush, J. Chromatogr. 484 (1989) 337.
[3] L. Miller, C. Weyker, J. Chromatogr. 511 (1990) 97.
[4] L. Miller, R. Bergeron, J. Chromatogr. 648 (1994) 381.
[5] L. Miller, C. Weyker, J. Chromatogr. A 653 (1994) 219.
[6] L. Miller, D. Honda, R. Fronek, K. Howe, J. Chromatogr. A 658 (1994) 429.
[7] S.B. Thomas, B.W. Surber, J. Chromatogr. 586 (1991) 265.
[8] C.J. Shaw, P.J. Sanfilippo, J.J. McNally, S.A. Park, J.B. Press, J. Chromatogr. 631 (1993) 173.
[9] A. Katti, P. Erlandsson, R. Dappen, J. Chromatogr. 590 (1992) 127.
[10] E.R. Francotte, Switz. Chimia 51 (1997) 717.

[11] E.R. Francotte, in: S. Ahuga (Ed.), Chiral Separations, American Chemical Society, Washington, DC, 1996, p. 271.
[12] E. Kuesters, Switz. Chim. Oggi 14 (1996) 39.
[13] P.D. McDonald, B.A. Bidlingmeyer, in: B.A. Bidlingmeyer (Ed.), Preparative Liquid Chromatography, Journal of Chromatography Library, Vol. 38, Elsevier, Amsterdam, 1987, p. 266.
[14] F. Geiser, presented at Chiral USA, San Francisco, CA, May 1998.
[15] L. Miller, C. Orihuela, R. Fronek, D. Honda, O. Dapremont, J. Chromatogr. A 849 (1999) 309.
[16] S. Golshan-Shirazi, G. Guiochon, Ind. Chromatogr. News 2 (1990) 11–12.
[17] A.M. Katti, presented at the 1995 International Symposium on Preparative Chromatography, Washington, DC, 11 June 1995.