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Examples of preparative chiral chromatography on an amylose-based chiral stationary phase in support of pharmaceutical research

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ABSTRACT

The analytical and preparative separation of two compounds of pharmaceutical interest was achieved on an amylose-based chiral stationary phase. Analytical HPLC method development was performed to screen various chiral stationary phases and mobile phases. The effect of loading on the preparative separation was also investigated. The preparative methods developed allowed for the isolation of gram quantities of individual enantiomers with an enantiomeric purity of >98%.

INTRODUCTION

The study of the biological effects of individual enantiomers is an important step in the drug development process [1]. At Searle these studies are performed during the early development phases of the compound. For these studies to be performed, small quantities (100 mg–1 g) of the individual enantiomers must be generated. There are two approaches for obtaining enantiomerically pure chemicals. These are (1) asymmetric synthesis of the desired isomer and (2) resolution of a racemic mixture into individual isomers. While asymmetric synthesis is useful when large quantities of enantiomers are required, the time required to develop the synthesis can make this approach impractical when only small quantities 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, indirect chromatographic resolution and direct chromatographic resolution. The time required to develop a recrystallization method 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 [2]. 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 some limitations to this approach. First, high enantiomeric purity of the derivatizing agent is essential. Also, the 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 chromatography and the reaction to remove the derivatives after chromatography. The preparative chromatography group at Searle has found the use of preparative chiral chromatography a more efficient approach for the generation of small quantities of individual enantio-

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mers [3–6]. The use of preparative chiral chromatography to generate individual enantiomers of drug candidates is used by numerous companies in the pharmaceutical industry [7–9]. In addition, an excellent review of preparative chiral chromatography, with many references to pharmaceutical separations, has recently been published [10].

This paper reports on the preparative chromatographic separation of the enantiomers of two compounds of pharmaceutical interest, SC-51146 ethyl ester and SC-53864. Extensive analytical HPLC method development was performed to determine the stationary phase and mobile phase which gave the best enantiomeric separation. Loading studies were then performed to determined the optimum conditions for the purification.

EXPERIMENTAL

Equipment

The analytical chromatograph consisted of a Spectra-Physics (San Jose, CA, USA) SP9700 pump or a Waters Associates Model 590 solvent-delivery system and a U6K injector or Waters Intelligent Sample Processor (Milford, MA, USA), a Kratos Model 783 variable-wavelength detector (Ramsey, NJ, USA), a Linear Model 585 recorder (Hackensack, NJ, USA), and Digital Equipment Corporation VAX 11/785 computer with Searle chromatography data system.

The preparative liquid chromatograph consisted of a Beckman Model 450 data system/controller, two Model 101 pumps with preparative heads, a Model 165 variable-wavelength detector with a 5-mm semi-preparative flowcell (Berkeley, CA, USA), or a Gilson Model 116 variable-wavelength detector (Middleton, WI, USA) and a Kipp and Zonen Model BD41 two-channel recorder (Delft, Netherlands). A Rheodyne Model 7125 syringe-loading sample injector (Cotati, CA, USA) equipped with a 10-ml loop (Valco, Houston, TX, USA) or a Gilson Model 401 dilutor in combination with a Rheodyne electrically actuated Model 7010 injector or a Gilson Model 231 autosampler equipped with a 10-ml loop were used for sample injection. The column effluent was fractionated using a Gilson Model FC220 or Model 202 fraction collector.

Materials

The chiral stationary phases were obtained from Daicel (Tokyo, Japan) through Regis Chemical (Morton Grove, IL, USA) as prepacked 10-μm analytical (250 mm × 4.6 mm I.D.) and preparative columns (500 mm × 10 mm I.D.). All samples for purification were synthesized in the laboratories of Searle (Skokie, IL, USA). The solvents were reagent grade or better and obtained from a variety of sources.

RESULTS AND DISCUSSION

Analytical separation of SC-51146 ethyl ester

To support biological testing, 1 g of each of the enantiomers of SC-51146 (Fig. 1), with an enantiomeric purity of >99.5% was required. Prior to preparative work, the analytical chiral separation of SC-51146 and its immediate precursor, SC-51146 ethyl ester was investigated. Both compounds were investigated at this stage for two reasons. First, the solubility in the mobile phase was a concern. SC-51146 ethyl ester had greater solubility in low-polarity organic solvents than SC-51146. Second, it is usually more efficient to perform the preparative separation on the compound which has the best enantiomeric resolution. The results of the analytical HPLC method development are summarized in Table I. Both cellulose-based (Chiralcel) and amylose-based (Chiralpak) chiral stationary phases (CSPs) were investigated. Initial work was performed on the cellulose based CSP (Chiralcel OD, OC, OJ and OF). None of these stationary phases gave adequate separation and resolution of either SC-51146 or SC-51146 ethyl ester. The next chiral stationary phase investigated was Chiralpak AD. Chiralpak AD is a phenyl carbamate derivative of amylose which is coated on silica gel. The Chiralpak AD column is designed for the separation of compounds with an aromatic group or a bulky substituent at the chiral center. Resolution of both compounds was achieved using an isopropanol–hexane–formic acid mobile phase. Additional method development was performed on the AD column to

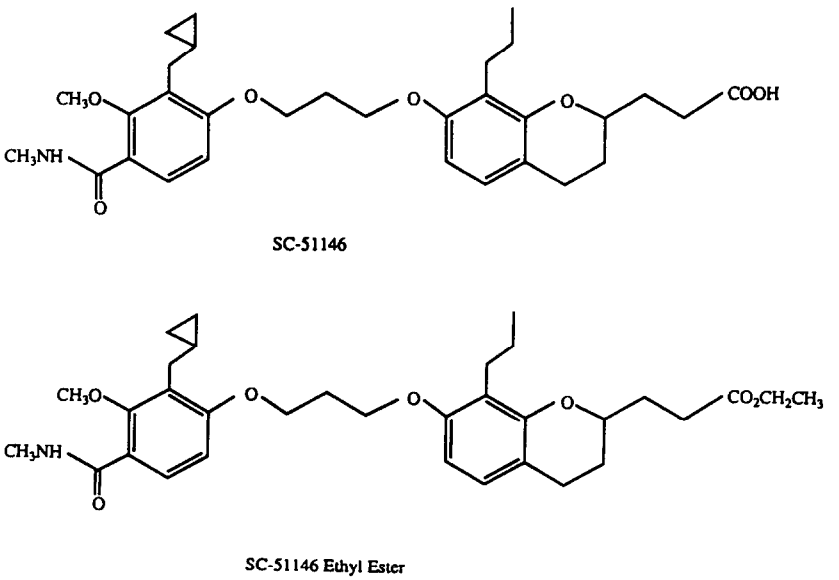


Fig. 1. Structures of SC-51146 and SC-51146 ethyl ester.

determine the effect of a different alcohol and a neutral mobile phase. When formic acid was removed from the mobile phase good separation was seen for both SC-51146 and SC-51146 ethyl ester but poor peak shape was observed for SC-51146. Approximately the same separation was obtained for SC-51146 ethyl ester when ethanol was substituted for isopropanol. Based on these results, it was decided to use a Chiralpak AD column with an isopropanol–hexane mobile phase for the enantiomeric separation of SC-51146 ethyl ester. While the enantiomeric

TABLE I

CHROMATOGRAPHIC RESULTS

HPLC conditions: flow-rate, 1.0 ml/min; detection 235 nm.

Compound	Mobile phase	Column	k_1' [a]	k_2' [b]	α	R_s
SC-51146	Isopropanol–hexane–formic acid (10:90:0.1, v/v/v)	OD	5.31	5.82	1.10	0.90
SC-51146 ethyl ester	Isopropanol–hexane–formic acid (10:90:0.1, v/v/v)	OD	3.96	4.35	1.10	1.01
SC-51146	Isopropanol–hexane–formic acid (20:80:0.1, v/v/v)	OC	6.26	6.26	1.00	0.00
SC-51146 ethyl ester	Isopropanol–hexane–formic acid (20:80:0.1, v/v/v)	OC	6.12	6.12	1.00	0.00
SC-51146	Isopropanol–hexane–formic acid (10:90:0.1, v/v/v)	OJ	10.42	12.74	1.22	0.91
SC-51146 ethyl ester	Isopropanol–hexane–formic acid (10:90:0.1, v/v/v)	OJ	12.10	12.10	1.00	0.00
SC-51146 ethyl ester	Isopropanol–hexane (30:70, v/v)	OF	8.40	8.40	1.00	0.00
SC-51146	Isopropanol–hexane–formic acid (10:90:0.1, v/v/v)	AD	4.88	6.14	1.26	2.93
SC-51146 ethyl ester	Isopropanol–hexane–formic acid (10:90:0.1, v/v/v)	AD	2.91	3.60	1.24	2.42
SC-51146	Isopropanol–hexane (10:90, v/v)	AD	11.52	12.94	1.12	0.61
SC-51146 ethyl ester	Isopropanol–hexane (10:90, v/v)	AD	3.10	3.82	1.23	2.37
SC-51146 ethyl ester	Ethanol–hexane (10:90, v/v)	AD	2.60	3.26	1.25	2.79
SC-51146 ethyl ester	Isopropanol–hexane (5:95, v/v)	AD	9.32	11.52	1.24	2.81

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

separation was approximately the same for SC-51146 and SC-51146 ethyl ester, the preparative separation was performed at the ethyl ester stage for two reasons. The first was the increased solubility of the ethyl ester compared to the free acid. The second reason was, at the time of this work the Chiralpak AD column had only been recently introduced. Since little information was available on the stability of the column to acid, we were leery of exposing the column to acid for long periods of time. Since that time the manufacturer has come out with operating conditions that recommend trifluoroacetic acid or acetic acid (up to 0.5%) as acidic modifiers [11]. The analytical separation of SC-51146 ethyl ester is shown in Fig. 2. Table II summarizes the capacity factor (k'), separation factor (α), and resolution (R_s) for the enantiomeric separation of SC-51146 ethyl ester.

Preparative separation of SC-51146 ethyl ester

The analytical HPLC method shown in Fig. 2 was scaled up to preparative loadings. In order to maximize the throughput of the preparative method, experiments were performed to determine the effect of increasing sample load on the separation. Sample sizes of between 10 and 218 mg were investigated. These studies showed that no pure second eluting enantiomer could be isolated at injection sizes greater than 20 mg. In addition, a maximum throughput for the first

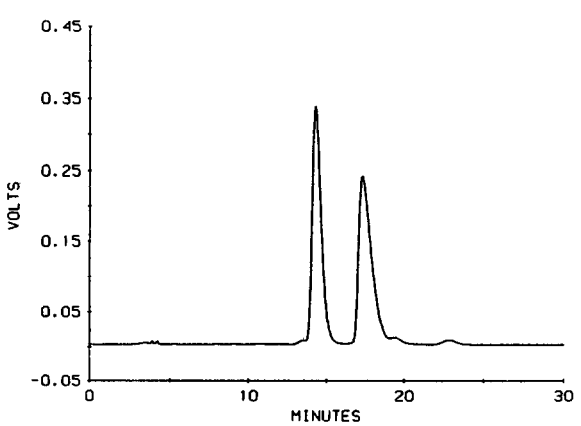


Fig. 2. Analytical HPLC separation of SC-51146 ethyl ester. Analysis conducted on Chiralpak AD column (250 mm × 4.6 mm I.D.), detection at 235 nm, 0.1 AUFS. Mobile phase: isopropanol–hexane (10:90 v/v), flow-rate 1 ml/min.

TABLE II

VALUES FOR ANALYTICAL SEPARATION OF ENANTIOMERS OF SC-51146 ETHYL ESTER AND SC-53864

See Figs. 3 and 6 for HPLC conditions.

Compound	k_1' [a]	k_2' [b]	α	R_s
SC-51146 ethyl ester[c]	3.74	4.75	1.27	2.51
SC-53864[d]	2.70	3.38	1.25	2.91

[a] Capacity factor for first eluting enantiomer.
[b] Capacity factor for second eluting enantiomer.
[c] Chiralpak AD, isopropanol–hexane (10:90, v/v), 235 nm.
[d] Chiralpak AD, isopropanol–hexane (3:97, v/v), 215 nm.

eluting enantiomer was reached at an injection size of 20 mg.

Factors which influenced our decision on the loading to use for the purification included: cost of the chemical, chemical composition and solubility in the mobile phase. SC-51146 is a difficult molecule to synthesize, requiring numerous chemical steps. We therefore had to maximize the isolated yields of each of the enantiomers. Additional impurities were detected in the racemic SC-51146 ethyl ester. These impurities needed to be removed during the enantiomer separation. Finally, SC-51146 ethyl ester has a solubility of *ca.* 10 mg/ml in the mobile phase. Based on the need to maximize the isolated yields, the separation between the enantiomers and between the enantiomers and impurities in the sample and the low solubility, an injection size of 10 mg was chosen for processing of the remainder of the chemical.

With an injection size of 10 mg, numerous injections would be required to generate one gram of each of the enantiomers. To reduce the manhours required for this purification, automation of pump control, sample injection and fraction collection was used. A chromatogram for an automated injection is shown in Fig. 3. Eluent fractionation was based on time. A total of six fractions were made. The first two fractions removed the impurity eluting just before the first enantiomer. The third cut was pure first enantiomer, a fourth cut was made across the valley of the two peaks. The final two fractions

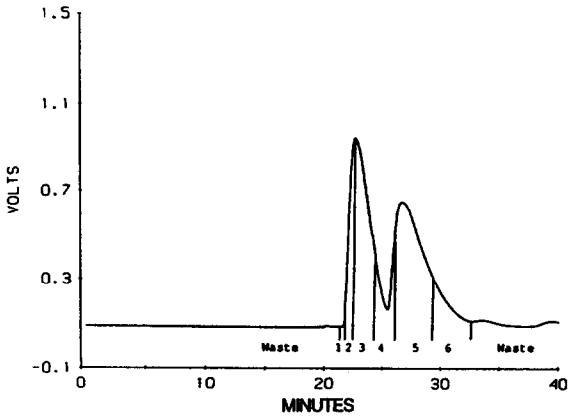


Fig. 3. Chromatogram of the preparative resolution of the enantiomers of SC-51146 ethyl ester. The purification was conducted on a Chiralpak AD column, 10 μm, (500 mm × 10 mm I.D.), with a mobile phase of isopropanol–hexane (8:92, v/v). A flow-rate of 8 ml/min, detection at 235 nm, and a loading of 10 mg/injection were used. Fractions: 1 = impurity–1st enantiomer (40:60); 2 = impurity–1st enantiomer (2:98); 3 = 100% 1st enantiomer; 4 = 1st enantiomer–2nd enantiomer (43:57); 5 = 1st enantiomer–2nd enantiomer (0.5:99.5); 6 = 100% 2nd enantiomer.

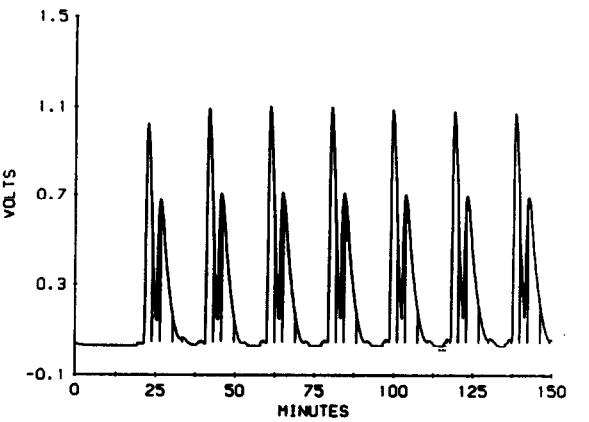
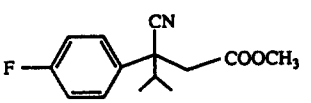


Fig. 4. Chromatogram of the automated preparative resolution of the enantiomers of SC-51146 ethyl ester. Injections were made every 20 min. See text for conditions.



SC-53864

Fig. 5. Structure of SC-53864.

were pure second enantiomer. Using the automated system, a total of 530 injections were made. Injections were made for 20 h per day over the course of 9 days. To maximize the throughput, the technique of overlapping injections was used. In overlapping 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 twenty minutes instead of every forty minutes. A chromatogram for automated injections is shown in Fig. 4. From the 530 injections a total of 1.59 g of first eluting enantiomer and 1.21 g of second eluting enantiomer was isolated. Enantiomeric purity for both enantiomers was greater than 99.5%.

Preparative separation of SC-53846

Multigram quantities of each of the enantiomers of SC-53864 (Fig. 5), with an enantiomeric purity of >98% was requested. SC-53864 was an intermediate for many of the final products synthesized for one of Searle's research projects. The analytical HPLC separation of SC-53864 is shown in Fig. 6. Table II summarizes the capacity factor (k'), separation factor (α), and resolution (R_s) for the enantiomeric separation of SC-53864. Preparative loadings of 10 and 20 mg sample per gram packing were investigated to determine the effect of loading on the separation. These loadings correspond to 250 and 500 mg on a 500 mm × 10 mm I.D. column, respec-

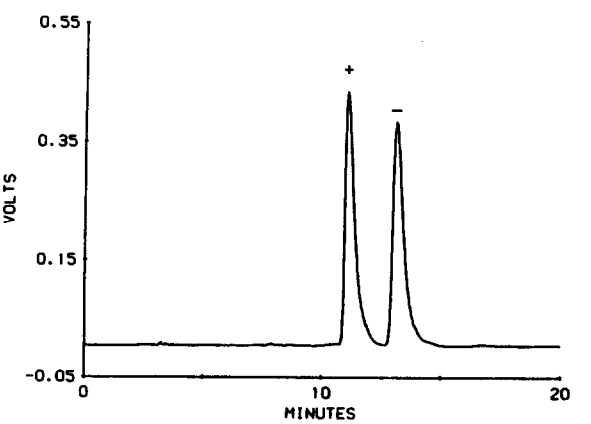


Fig. 6. Analytical HPLC separation of SC-53864. Analysis conducted on Chiralpak AD column (250 mm × 4.6 mm I.D.), detection at 215 nm, 0.1 AUFS. Mobile phase: isopropanol–hexane, (3:97, v/v), flow-rate 1.0 ml/min.

TABLE III

RESULTS OF PREPARATIVE EXPERIMENTS FOR SC-53864

HPLC conditions: Chiralpak AD (500 mm × 10 mm I.D.) containing approximately 25 g of packing; mobile phase, isopropanol–hexane (3:97, v/v); flow-rate, 7 ml/min.

Compound	Loading (mg/g)	First eluting enantiomer		Second eluting enantiomer	
		Percent isolated	mg/h	Percent isolated	mg/h
SC-53864	10	60	225	21	78
	20	30	228	7	55

tively. The results of these studies are summarized in Table III. These studies show that as loading increases, the amount of first eluting and second eluting enantiomer isolated decreases. A maximum throughput for the first and second eluting enantiomer is achieved at a loading of 10 mg/g. Based on the results of the loading study, at a loading of 10 mg/g insufficient quantities of second eluting enantiomer would be isolated. Repurification of the overlap fractions would be required to isolate additional second enantiomer. The purification scheme used for the preparative separation of SC-53864 is shown in Fig. 7. The processing of 8.2 g of racemic material was accomplished in 33 injections of 250 mg each. As with SC-51146 ethyl ester, automation and overlapping injections was used to maximize the throughput. From the first purification, 3.14 g of first enantiomer (enantiomeric purity of 98.5%), 1.17 g of second enantiomer (enantiomeric purity of 98.2%) and 0.47 g of second enantiomer (enantiomeric purity of 100%) were generated. An overlap fraction (3.09 g) with an enantiomeric ratio of 30/70 was also generated. This overlap fraction was repurified in 22 injections of 140 mg each. The injection size was reduced to

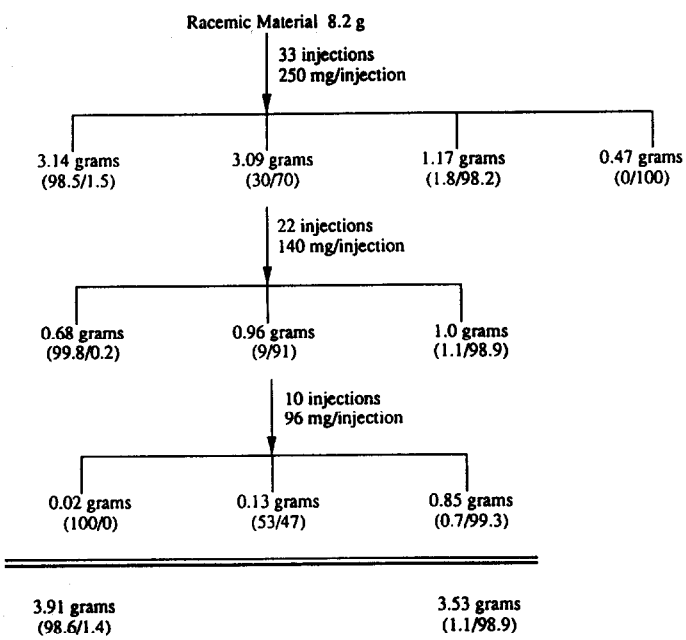


Fig. 7. Purification scheme for the preparative resolution of the enantiomers of SC-53864. See text.

increase the isolated yields of the second enantiomer. This series of injections generated additional first and second enantiomer. A 960-mg overlap fraction with an enantiomeric ratio of 9:91 was also generated. This overlap fraction was purified in 10 injections of 96 mg each, generating first enantiomer and second enantiomer. From the series of purifications, a total of 3.84 g of first eluting enantiomer (purity of 98.6%) and 3.49 g of second enantiomer (purity of 98.9%) were generated. The time required for this purification was four days. The purification scheme developed has more recently been used for the purification of 10 g of additional racemic material, generating *ca.* 4 g of each of the enantiomers.

CONCLUSIONS

Preparative chromatography is a viable alternative to asymmetric synthesis when only small quantities of individual enantiomers are required. The loading used for the purifications depends on the amount of chemical which must be generated, the purity required, the chemical composition of the sample and the cost of the chemical being purified. Extensive analytical method development, exploring various chiral stationary phases and mobile phases should be conducted prior to any preparative chiral separation. Additional compounds in the chemical synthesis, besides the final product, should be investigated to maximize the throughput for the chiral separation. Automation could be utilized to reduce the manual labor required for preparative chiral separations. Preparative chromatography on chiral stationary phases was used for the generation of multigram quantities of pure enantiomers of two compounds in support of pharmaceutical research.

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