Journal of Chromatography A, 1218 (2011) 3529–3536

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

A high throughput approach to purifying chiral molecules using 3 μm analytical chiral stationary phases via supercritical fluid chromatography

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ARTICLE INFO

Article history:
Received 20 January 2011
Received in revised form 16 March 2011
Accepted 28 March 2011
Available online 4 April 2011

Keywords:
SFC
Chiral
Purification

ABSTRACT

In this study we describe a fast 2.5 min gradient chiral screening method that utilizes 3 μm particles CSPs. An empirical approach to scale-up from the 2.5 min gradient method to an isocratic preparative method is described. We also evaluate the use of 5 μm preparative columns that are 150 mm in length versus the industry standard of 250 mm. Finally, we evaluate eleven different CSPs against 46 compounds, 23 commercially available and 23 internal compounds from a variety of projects. All 46 compounds were separated using the 2.5 min gradient method. Assuming an R_s of 1.0 or greater, the Chiralpak AD column from Chiral Technologies proved most useful, followed by the Cellulose-1 from Phenomenex. The Cellulose-4, a novel stationary phase from Phenomenex, provided the third most separations of the eleven columns tested. For the 46 compounds tested, the Chiralcel OJ column from Chiral Technologies outperformed Phenomenex's version, the Lux Cellulose-3.

1. Introduction

Given the complex nature of biological targets, medicinal chemists are increasingly synthesizing compounds that have at least one chiral center [1,2]. FDA regulations state that compounds need to be tested in their enantiomerically pure state as the individual enantiomers may have vastly different pharmacological and toxicity profiles [3,4]. While creating an asymmetric synthesis route is desirable, it is often time consuming and expensive. Chiral chromatography is one of the fastest and easiest means to separate chiral compounds. Recently, the use of supercritical fluid chromatography (SFC) has become the method of choice in pharmaceutical discovery research labs for quickly separating and purifying chiral compounds. SFC has many advantages over traditional liquid chromatography, most notably supercritical carbon dioxide (sCO_2) has much lower viscosity and a much higher diffusivity rate than traditionally used solvents [2,5–12]. As a result, high flow rates can be used without losing efficiency resulting in shorter run times and much faster equilibration times while providing excellent efficiency.

The use of small particles (i.e. sub-2 μm) has recently become very popular in the world of HPLC for its ability to speed up analysis and reduce solvent usage without sacrificing resolution [13–16]. It is only natural that this trend would find its way into SFC applications. With SFCs low backpressure due to the low viscosity of carbon dioxide, it is a perfect fit for using small particles to increase resolution and speed. While there have been reports about using small particles for chiral analysis using normal phase approaches [17,18], we have not seen these small particles used in SFC chiral separations.

With the increasing demands of creating chirally pure compounds, there is a need to increase the throughput of the chiral screening process, especially in the research phase of development. In this study we outline a screening process that involves the use of a 3 μm particle size for the chiral stationary phases (CSPs) instead of using the traditional 5 μm particle size. The use of a 3 μm particle size allows for shorter column lengths combined with high flow rates that can significantly reduce analysis times without sacrificing resolution. A 2.5 min (approximately 3.5 min from injection to injection) method with a 1.5 min gradient for each CSP column is described along with the subsequent scale up to purification. Additionally, we show how effective a 150 mm prep column with 5 μm particles can be compared to the traditionally used 250 mm column for smaller quantity samples (<150 mg). Finally, we offer a comparison of how eleven different CSPs performed separating 23 commercial and 23 proprietary racemic compounds.

2. Experimental

2.1. Chemicals

Carbon dioxide was obtained from Praxair (Des Moines, IA). Methanol (MeOH), isopropyl alcohol (IPA) and ethyl alcohol (EtOH) were purchased from EMD chemicals (Gibbstown, NJ,

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doi:10.1016/j.chroma.2011.03.066

USA). Diethyl amine (DEA), benzoin, 1-phenylethane-1,2-diol, 1,1′-bi-2-naphthol, mianserin, thalidomide, indapamide, flurbiprofen, ibuprofen, warfarin, sulindac, clenbuterol HCl, chlorpheniramine maleate, norphenylephrine HCl, mandelamide, 1-1′ binaphthyl 2,2′ diamine, propanol HCl, ketoprofen, and bendroflumethiazide were from Sigma–Aldrich (St. Louis, MO, USA). Chlorthalidone and atenolol were purchased from Spectrum Chemical (Gardena, CA, USA). Flavanone was purchased from Enzo (Plymouth Meeting, PA, USA). 2,2,2 trifluoro 1-9anthrylethanol was purchased from Oakwood Products (West Columbia, SC, USA). *Trans*-Stilbene oxide and homatropine HBr were purchased from TCI (Tokyo, Japan).

2.2. Analytical instrumentation

The analytical instrument was a Berger SFC unit (Thar Technologies, Pittsburgh, PA, USA). The unit consisted of FCM 1200 flow control module, a dual pump control module, a TCM1200 thermal control module (temperature controlled from 7 to 150 °C), a six position column selection valve, and a six position solvent control valve. The SFC was equipped with an Agilent 1100 photodiode array detector with a high-pressure flow cell (Agilent Technologies, Palo Alto, CA, USA). The auto sampler/injector was a CTC HTC PAL Analytics from Leap Technologies (Carrboro, NC, USA). The mass spectrometer was a Waters (Milford, MA, USA) ZQ single quadruple with an electro spray ionization (ESI) source coupled to the SFC. The software used in the analyses was a Berger Massware™ v.4.03 and MassLynx™ v.4.1. In order to minimize the run time, we reduced the dead volume in the analytical system to make sure our chromatography was producing the sharpest peaks possible. The instrument was re-plumbed using 0.05 mm i.d. tubing throughout the instrument with the exception of the tubing in the column switcher which remained at 0.1 mm i.d. Although this added inlet pressure, it reduced the retention times of the peaks by over 0.2 min. The inlet pressure ranged from 240 to 280 bar over the course of the gradient, well below the system limitations of 400 bar. An approximately 1 mg/mL solution was made for all of the compounds analyzed and dissolved in methanol. A few drops of formic acid were added as needed to help with the solubility.

2.3. Preparative instrumentation

The preparative SFC instrument was a Berger Multigram™ II from Thar Technologies (Pittsburgh, PA, USA). The system components included the Electronics Control Module (ECM)-2500, the Separator Control Module (SCM)-2500, SD-1 modifier and CO_2 delivery pumps Berger Enhanced, direct expansion probe chiller, ventilated collection cabinet, Knauer 2501 detector, and Julabo FT 401 Chiller Berger Enhanced. The injector was a Cavaro XL 3000 Syringe Pump from Tecan Systems (San Jose, CA, USA). Software used in the purification was Berger SFC Pronto™ v1.5.305.15.

2.4. Chiral columns

The analytical chiral columns we used in our study included the Chiralpak AD-3, Chiralcel OJ-3, Chiralpak AS-3, Chiralpak IA-3, Chiralpak IB-3, and Chiralpak IC-3 from Chiral Technologies (West Chester, PA, USA). These columns are referred to as AD, OJ, AS, IA, IB, and IC, respectively throughout the paper. We also screened the Lux Cellulose-1, Lux Cellulose-2, Lux Cellulose-3, Lux Cellulose-4, and Lux Amylose-2 from Phenomenex (Torrance, CA, USA). These columns are referred to as Celluose-1, Cellulose-2, Cellulose-3, Cellulose-4, and Amylose-2, respectively throughout the paper. The dimensions of all the analytical columns were 50 mm × 4.6 mm I.D. with 3 μm particle size. We have the analogous 5 μm, 21.2 mm × 250 mm and/or 5 μm, 21.2 mm × 150 mm preparative columns in all of the phases with the exception of the Cellulose-3. A list comparing the various column chemistries is displayed in Table 1.

2.5. Analytical SFC screening method and calculations

The analytical screening method utilized a gradient. Mobile phase A was supercritical carbon dioxide and mobile phase B was one of the following: methanol, ethanol, or isopropanol. One-tenth of a percent of diethylamine or 0.1% triethylamine was added when the analyte was basic. The gradient started at 10% B and ramped to 55% over 1.5 min at a rate of 30% per minute. The gradient was kept at 55% for one minute. Once the method ended, the system automatically returned to its original 10% starting percentage. There is no re-equilibration built into the method. Instead, the authors rely upon the system equilibrating during the injection cycle which can take from 30 to 90 s depending on how quickly the system can stabilize its pressure after switching to a new column. The column volume is roughly 0.5 mL, so even with the 30 s delay time, there are still approximately 5 column volumes going through the column. Due to approximately 1 mL of volume in the tubing from the solvent switching valve to the modifier pump, 90 s of equilibration time was needed to re-equilibrate the analytical columns when switching solvents (i.e. going from MeOH to EtOH). The flow rate was 5.0 mL/min with an outlet pressure of 120 bar. The total runtime was 2.5 min. The column oven temperature was 40 °C and the nozzle temperature was 60 °C. Each injection was 5 μL. Twenty-three commercially available and 23 proprietary compounds were screened in the study on 11 different chiral columns.

Retention times were obtained from the UV trace generated from Masslynx™ v4.1. The UV wavelength ranged from 214 nm to 254 nm depending on the compound's lambda max. The resolution (R_s) was calculated by using the traditional chromatographic equation:

$$R_s = \frac{2(t_2 - t_1)}{(tw_1 + tw_2)}$$

where t_1 and t_2 are the retention times and tw_1 and tw_2 are the peak widths at base height for peaks 1 and 2 [19].

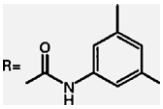
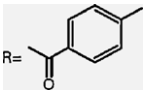
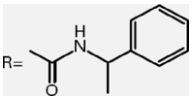
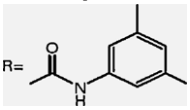
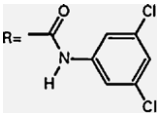
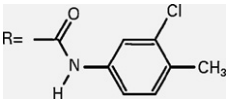
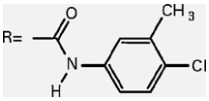
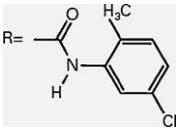
2.6. Preparative analysis conditions for warfarin and mandelamide

All of the preparative methods had mobile phase A as supercritical carbon dioxide and mobile phase B as methanol. The percentage of A versus B varied based on the compound. The flow rate was 70 mL/min for all chromatograms and each injection was approximately 1 mL. The UV wavelength was either 230 nm or 214 nm. The outlet pressure was set to 100 bar and the column oven temperature was 40 °C.

3. Results and discussion

The compounds were selected with the intent to cover a broad range of chemical space that would also resemble typical medicinal chemistry compounds. The commercially available compounds were compiled from referencing previous evaluations of novel CSPs [20–22]. Of the commercial compounds selected, 11 were neutral, 4 were acidic and 9 were basic, see Fig. 1. Of the 23 proprietary compounds, 22 basic and one was neutral. None were acidic. The 23 samples covered 8 eight different projects and were structurally diverse. All of the compounds were subjected to the same 2.5 min method. Carbon dioxide and methanol (with 0.1%DEA for basic compounds) was always the first solvent combination screened. Carbon dioxide and ethanol (with 0.1%DEA for basic compounds) was next, followed by carbon dioxide and isopropanol

Table 1
List of columns tested.

Column name	Column chemistry	Structure	Immobilized?
Chiralpak AD	Amylose tris (3,5-dimethylphenylcarbamate)		N
Chiralcel OJ	Cellulose tris (4-methylbenzoate)		N
Chiralpak AS	Amylose tris [(S)-α-methylbenzylcarbamate]		N
Chiralpak IA	Amylose tris (3,5-dimethylphenylcarbamate)	See chiralpak AD	Y
Chiralpak IB	Cellulose tris (3,5-dimethylphenylcarbamate)		Y
Chrialpak IC	Cellulose tris (3,5-dichlorophenylcarbamate)		Y
Cellulose-1	Cellulose tris (3,5-dimethylphenylcarbamate)	See chiralpak IB	N
Cellulose-2	Cellulose tris (3-chloro-4-methyl phenylcarbamate)		N
Cellulose-3	Cellulose tris (4-methylbenzoate)	See chiralcel OJ	N
Cellulose-4	Cellulose (4-chloro-3-methyl phenylcarbamate)		N
Amylose-2	Amylose (5-chloro-2-methyl phenylcarbamate)		N

(with 0.1%TEA for basic compounds). We switch to 0.1%TEA instead of 0.1%DEA with isopropanol because if we do not get good separation with the first two solvent systems we want to try to drastically change the mobile phase in an effort to find a method that will provide us with good results. We did not attempt to study the chromatographic differences between diethylamine and triethylamine. We did not add an additive for acidic compounds as the peak shapes were very good without the presence of an additive. In an effort to increase our purification throughput, if adequate separation ($R_s \geq 1.0$) was found using methanol (or methanol with 0.1% DEA) as the co-solvent, then EtOH and IPA were not analyzed. In our experience, approximately 80% of the time methanol (or MeOH with DEA for basic analytes) is able to obtain a resolution greater than 1.0. Only the best separation for each column was reported if multiple co-solvents were analyzed. Separation was found for all 46 compounds.

Since our ultimate goal in our lab is to purify racemic mixtures, most of the CSPs were selected in this study because we already had a matching preparative column of that phase. Additionally, we have a 99% plus success rate using the polysaccharide columns. These included the Chiralpak AD, Chiralpak AS, Chiralpak IA, Chiralpak IB, Chiralpak IC, Chiralcel OJ, Lux Cellulose-1, Lux Cellulose-2, and Lux Amylose-2. We decided to add two novel stationary phases from Phenomenex: the Lux Cellulose-3 (comparable to the Chiralcel OJ) and the Lux Cellulose-4. Table 1 shows the chemistry differences between the 11 columns. It is important to note that the three immobilized columns are capable of handling any solvent where as the coated columns are limited to a select few solvents. Having more solvent freedom obviously increases a column utility, however we did not screen against any solvent other than the four previously mentioned. The results from all of these columns were included in the study.

The goal of the study was four-fold. The first goal was to demonstrate that a 2.5 min method can be successfully used for screening chiral columns. Secondly, we wanted to demonstrate the ease of scaling up from a 2.5 min method. Thirdly, we want to demonstrate the effectiveness of a 150 mm length prep column as opposed to the more commonly used 250 mm length. Finally, we wanted to evaluate 11 different CSPs on their usefulness for prep chromatography.

3.1. Evaluation of the 2.5 min screening method

Fig. 2 shows the comparison of 5 μm particles with a 4.6 mm × 100 mm column and a 6 min method versus 3 μm particles on a 4.6 mm × 50 mm column and the aforementioned 2.5 min method. The stationary phase in both columns was the Cellulose-1 and the analyte was a 1 mg/mL solution of TSO. The flow rate was kept constant for both runs at 5 mL/min. Despite using a column that was half as long, the 3 μm column allowed for the analysis time be cut by more than half without sacrificing any resolution.

Fig. 3 shows the separation of warfarin on the 11 different CSPs using the 2.5 min method with methanol as the co-solvent. All of the columns demonstrated some separation, with all but three

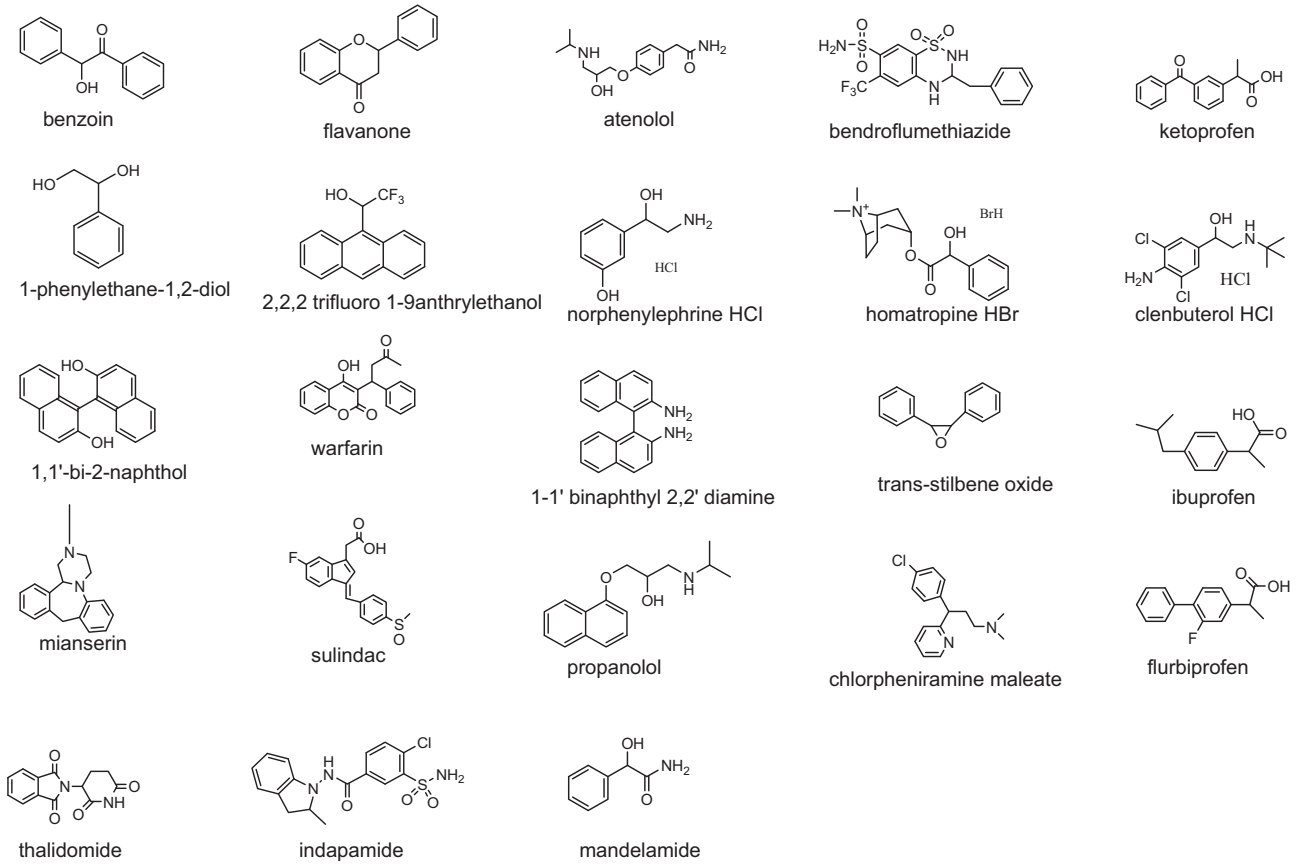


Fig. 1. The 23 commercially available racemic compounds.

of the columns having a resolution greater than 1.3. Additionally, the resolution can easily be increased by running under isocratic conditions as seen in Fig. 4a and b. The example of warfarin clearly demonstrates the utility of short, fast gradients using small particles. Again, separation was found for all 46 compounds analyzed using the 2.5 min method. Although separation was found for all the samples, it is important to note that not all of them can be easily scaled up for preparative separation if obtaining high purities for both enantiomers is desired. Only two compounds had a resolution less than 0.7, ketoprofen (R_s = 0.42) and a proprietary compound (R_s = 0.55). [Data not shown].

3.2. Scaling up to prep scale from the 2.5 min analytical method

After selecting a method (normally the analytical method that provides the largest resolution), a 2.5 min isocratic method is run.

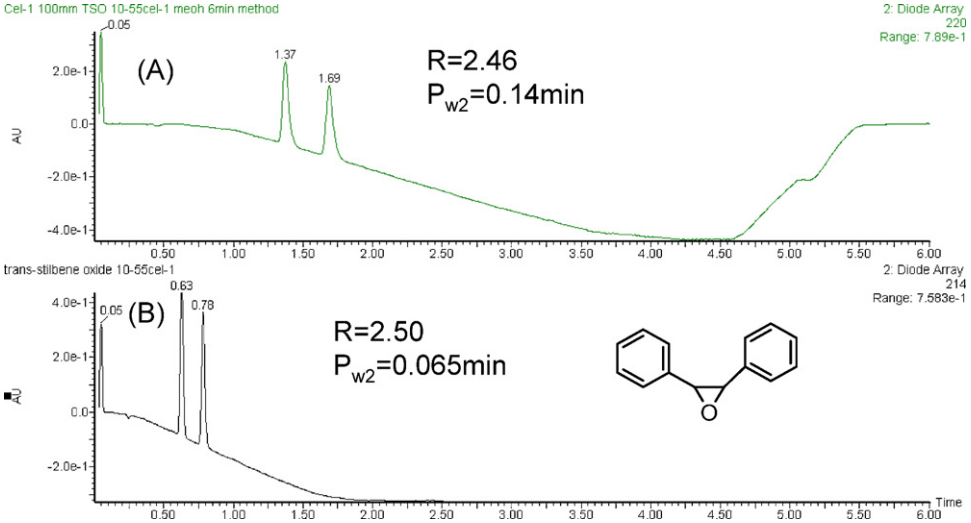


Fig. 2. Comparison of using 4.6 mm × 100 mm, 5 μm (A) versus 4.6 mm × 50 mm, 3 μm (B). The gradient for (A) was hold at 10% for 0.5 min, then 10–55% gradient in 3 min, hold for 1 min, then re-equilibrate at 1.0 min. The gradient for (B) was 10–55% in 1.5 min, hold for 1 min. Both flow rates were 5 mL/min on the Cellulose-1, UV set to 220 nm. Mobile phase B was methanol.

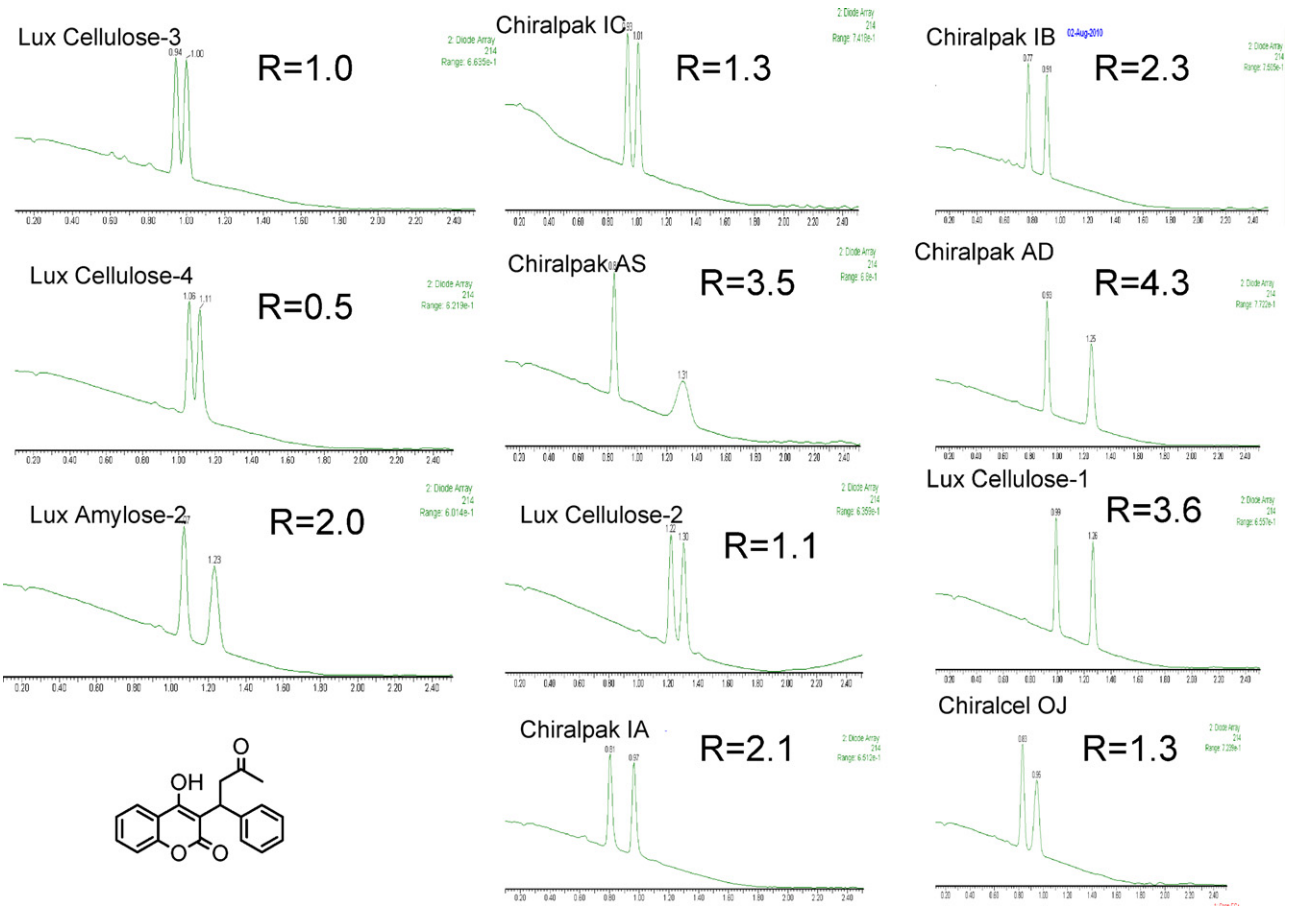


Fig. 3. Chromatograms of warfarin on 11 different CSPs using the 2.5 min method with methanol as a co-solvent.

The percentage of co-solvent is selected based on the chart in Fig. 5. The graph relates the gradient retention times to an isocratic retention time at various percentages of mobile phase B. The data points on the graph represent data collected from standard compounds as well as proprietary compounds on a variety of columns and using a variety of mobile phases. Only percentage increments of 10 percents are displayed to simplify the graph. The graph is independent of which co-solvent is being used. In our laboratory, we have found empirically that an isocratic retention time of about 1.1 min is desired to maximize the separation on the prep scale without making the prep runtimes too long. We recognize there is some variability in the data points, so the isocratic scale up percentage my not be perfectly optimized for each sample. Nevertheless, the figure offers a good starting point to find a suitable isocratic method. In our experience, the isocratic values obtained from the chart have been adequate for preparative separations. Fig. 4a–c shows the 2.5 min method separation, subsequent isocratic run and the purification run of bendroflumethiazide. Using the gradient run of bendroflumethiazine as an example, we can demonstrate the utility of the scale-up chart. First, we use the 1.05 and 1.13 gradient retention times and locate their position on the *x*-axis. Next we extrapolate up the graph until we intersect with a line that is within the 1.1 min range of the *y*-axis. In the case of bendroflumethiazide, the intersection point with 1.1 min on the *y*-axis is in between the 20% and 30% lines. For 1.05, the intersection point is approximately 22%. For 1.13, the intersection point is approximately 26%. Finally, we select a value in between the two percentages. For bendroflumethiazide 25% was chosen and the two peaks eluted at 1.01 and 1.23 min. Notice that the resolution for bendroflumethiazide improves from 1.28 to 1.69 when going from a gradient method to an isocratic method. With an injection of 30.0 mg of bendroflumethiazide, near baseline separation is achieved on the purification run using 25% methanol as seen in Fig. 4c.

3.3. Purification of chiral compounds on a 150 mm column

The use of 250 mm columns has long been the industry standard for purifying racemic compounds. Although the diameter of the column varies, the length can be 250 mm or longer. Although a longer column offers more stationary phase to improve the separation and offers higher loading, it is often times not necessary and may be more expensive. Many chiral samples, especially in a discovery chemistry lab, have less than 100 milligrams of racemate. Loading comes less of an issue when the sample size is on the tens of milligrams scale. Because the longer column takes more time to elute the analytes, time and solvent are wasted. An example of using the 150 mm length column is shown in Fig. 4c. Fig. 6 shows a comparison of a 150 mm column and a 250 mm column on the separation of mandelamide. The analytical gradient method gave a resolution of 1.04. Fig. 6b shows that the use of a Chiral Technologies IC 21.2 mm × 150 mm column can offer baseline separation in 35% less time for a 33 mg injection of mandelamide than the same sample and conditions for a Chiral Technologies IC 21.2 mm × 250 mm column, Fig. 6a. However, a high flow rate could also be used on a shorter column without the fear of generating excessive backpressure so even faster separations might be expected. Unfortunately, our MGII instrument has a maximum flow rate of 70 mL/min so we could not explore higher flow rates further.

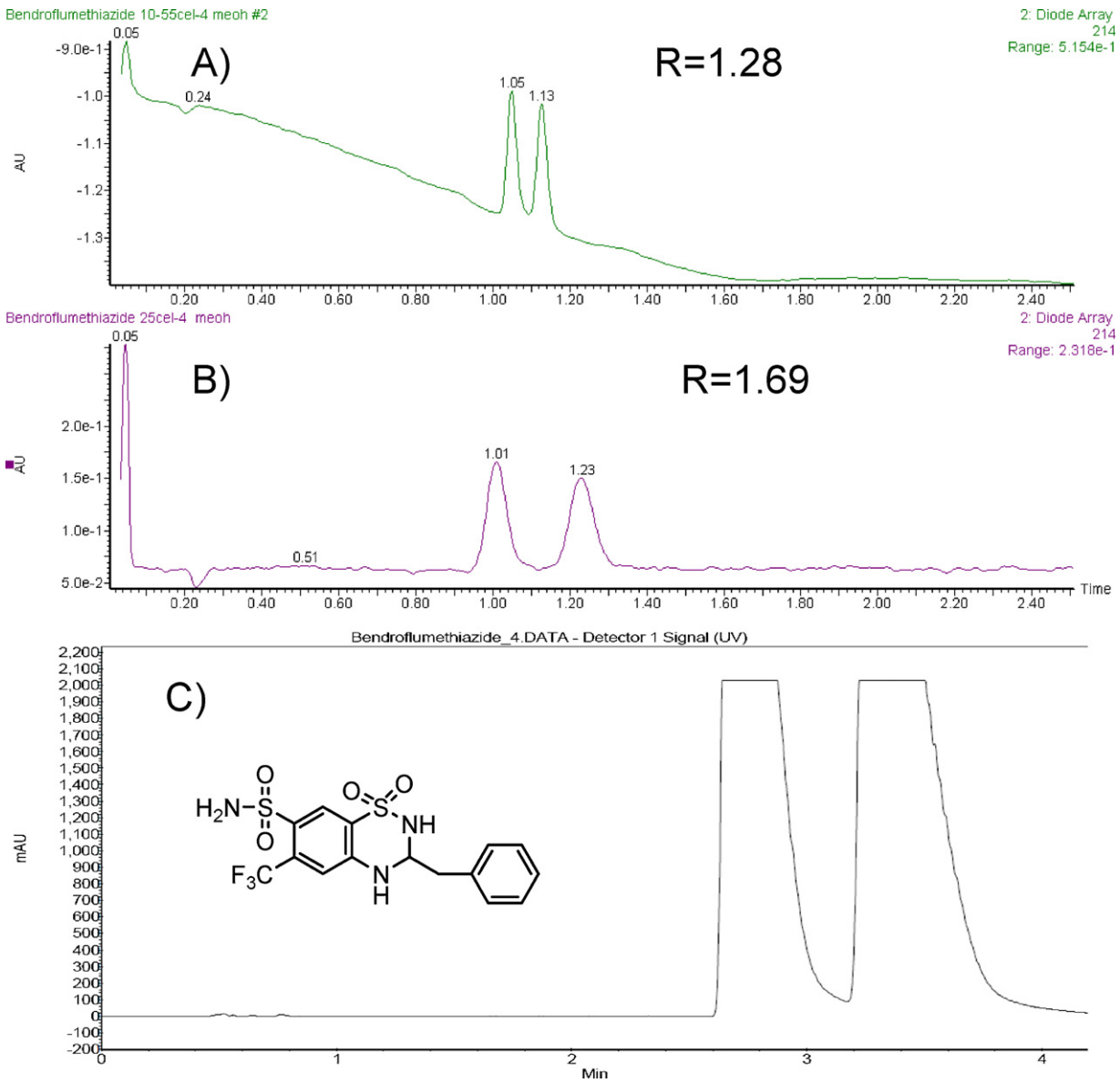


Fig. 4. (A) Separation of bendroflumethiazine using a 2.5 min gradient method. (B) Isocratic separation of bendroflumethiazine at 25% MeOH. (C) Prep run of 30.0 mg of bendroflumethiazine on 21.2 mm × 150 mm, 5 μm Phenomenex Cellulose-4, UV was 214 nm, 25%MeOH at 70 mL/min.

3.4. Evaluation of the 3 μm CSPs from Chiral Technologies and Phenomenex

The results from the 46 chiral separations are summarized in Fig. 7. The graph is broken into three different qualifiers: number of separations with a resolution greater than 0.3, number of separations with a resolution greater than 1.0 and number of times the CSP offered the largest separation for a given compound (i.e. the largest resolution under gradient conditions). While a resolution less than 0.3 shows some separation of the two enantiomers, it is generally difficult to scale up to prep scale. As a general rule, any separation with a resolution greater than 1.0 scales well. When scaling up, maximizing the separation is desired. Included in Fig. 7 is the number of compounds each column offered the largest resolution. Often times, the method selected for scale up had the largest analytical resolution so the number provides an idea of how often a given prep column would be used. It is important to note again that methanol was screened first and if an adequate separation was found ($R_S \geq 1.0$), ethanol and isopropanol were not explored. Obviously, there is a chance that a compound may separate on a given column if a co-solvent other than methanol were selected. We often omitted running the other two commonly used co-solvents in an effort to closely emulate our screening process.

In terms of providing separations with a resolution greater than 0.3, the Chiralpak AD clearly outperformed the other CSPs by separating 85% of the 46 compounds. The Cellulose-1 was a distant second, separating 63% of the 46 compounds. The newest CSP from Phenomenex, the Cellulose-4, separated the third most at 54%. It was followed closely by the Chiralpak IA (50%) and Cellulose-2 (48%). The Amylose-2 provided the fewest number of separations for the 46 compounds at 26%. In terms of providing separations with a resolution greater than 1.0, the AD offered the most (21) followed by the Cellulose-1 (18) and then the OJ (15). The Amylose-2 separated only 4 of the 46 compounds with a resolution greater than 1.0, the fewest of the eleven CSPs studied. In terms of providing the largest resolution, the AD provided the best result by providing the largest resolution on 13 of the 46 compounds. The Cellulose-1 was second followed by the IC. Interestingly, all of the phases provided the largest resolution for at least one compound. Based on the results from this study, the order of usefulness for scaling

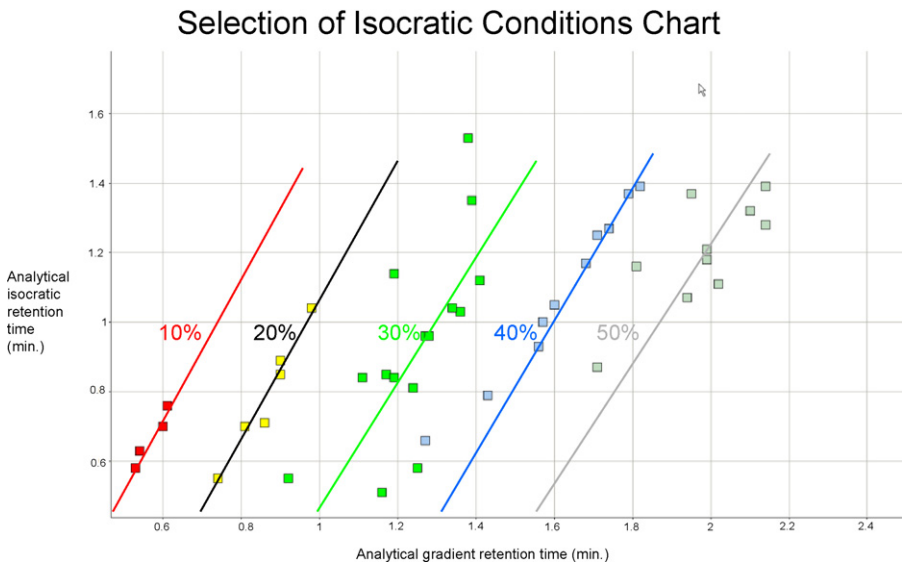


Fig. 5. Chart used to select the correct isocratic conditions. The lines represent the percent co-solvent needed to elute a compound given its initial gradient retention time.

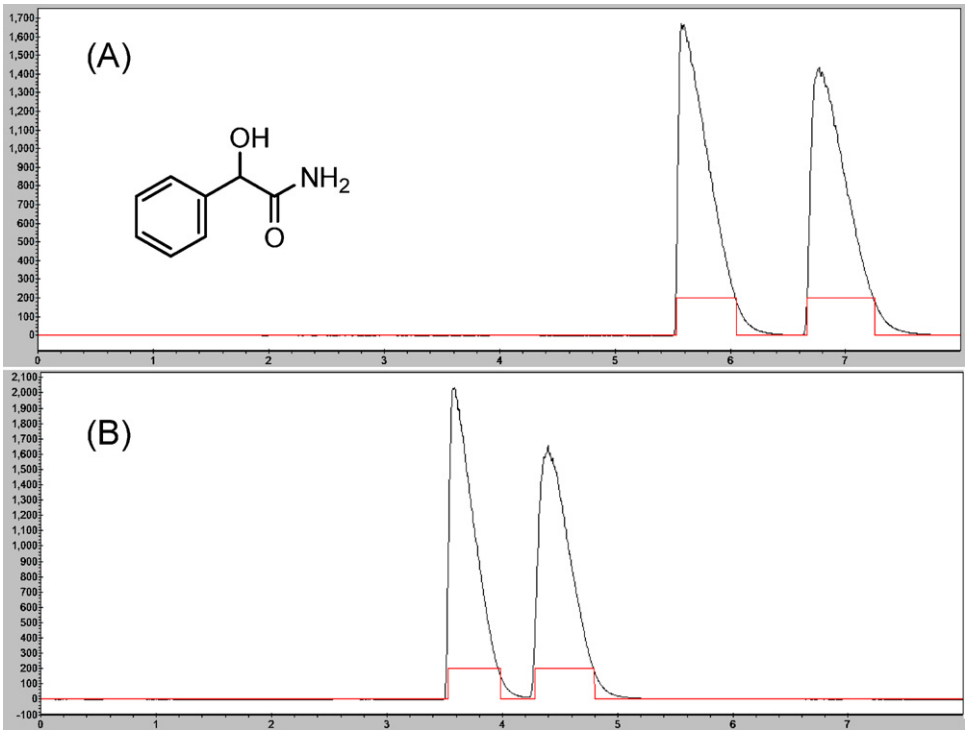


Fig. 6. Purification of approximately 33 mg of mandelamide on Chiralpak IC 21.2 mm × 250 mm, 5 μm column (A) and on a Chiralpak IC 21.2 mm × 150 mm, 5 μm column (B). The UV in both chromatograms is 214 nm and it was run using 10% methanol, 90% CO_2, 70 mL/min.

up to prep is as follows: AD > Cellulose-1 > OJ > IA > IC > Cellulose-4 > AS > Cellulose-3 > Cellulose-2 > IB > Amylose-2.

There were three "pairs" of columns in this study: the AD and its immobilized version the IA, the Cellulose-1 and its immobilized version the IB, and the OJ and the Phenomenex Cellulose-3. The AD outperformed the immobilized IA for separating these 46 compounds under the previously described conditions, although there were some exceptions. The IA offered the largest resolution for three compounds and tied for the largest on another (with the AD). Additionally, thalidomide and 2,2,2 trifluoro 1-9anthrylethanol separated on the IA that did not separate on the AD. The Cellulose-1 also outperformed its immobilized counterpart, the IB, for the 46 compounds under described conditions; but again, there were some exceptions. The IB offered the largest resolution once and separated two compounds that did not separate on the Cellulose-1, norphenylephrine HCl and one proprietary compound. It is important to note that the huge advantage of using the immobilized stationary phases is that they allow for any solvent combinations. It is entirely possible that the IA and IB would perform much better in relation to the coated columns if the full gamut of solvents that are not compatible with the coated phases (dichloromethane, ethyl acetate, methyl *tert*-butyl ether, etc.) were tested. The Cellulose-3 and the OJ column were much more similar than the immobilized and covalently bonded CSPs, although the OJ definitely performed better for these 46 compounds. The OJ separated 19 compounds with 15 separations having a resolution

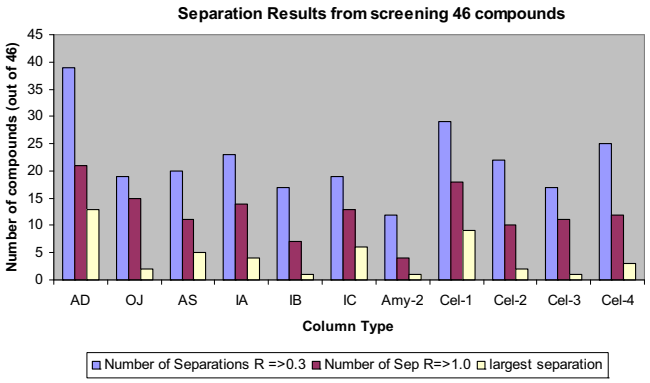


Fig. 7. Screening results from the 23 commercial and 23 proprietary compounds using the 2.5 min method.

greater than 1.0. The Cellulose-3 separated 17 compounds with a mere 11 having a separation greater than 1.0. The OJ also provided the largest resolution for 2 compounds compared to 1 compound for the Cellulose-3.

4. Conclusions

The use of short columns packed with 3 μm CSP particles at high flow rates can dramatically reduce the runtimes for chiral screening while maintaining efficiency. A 1.5 min gradient in a 2.5 method was shown to be very effective for separating 46 different compounds. To date, we have developed methods and purified over 150 chiral compounds since switching to the 2.5 min gradient method. The fast method allows for drastically expedited method development. Often times, chiral columns were screened as part of an overnight run. Now, a six column screening can occur within 20 min. The shortened screening time allows for a user to generate methods very quickly and eliminates the need for overnight runs. Furthermore, it saves time and solvent as once an adequate separation is found no more methods need to be screened.

As expected, the AD proved to be the CSP of choice. The most surprising result was the performance of the Cellulose-4 CSP and it will be incorporated into our first-pass 6 column screen. It was also surprising to see the OJ column was markedly better than the Cellulose-3. However, Chiral Technologies columns analogous to the Cellulose-1 and Cellulose-2, i.e. the Chiralcel OD and the Chiralcel OZ, were not evaluated in this study and therefore no statements concerning comparative success rates can be made. Based on our results and experience, the six columns we have in our primary screen are the following: AD, Cellulose-1, OJ, IC, AS and the Cellulose-4. While 3 μm particle provided good resolution, the use of even smaller particles can have an even larger impact on the speed and resolution of SFC chiral and achiral separations. Furthermore, having an instrument that is built to reduce the system volumes will only improve the separations.

The scale-up from these short methods to prep scale is very fast and easy. The use of 150 mm preparative columns can reduce the purification time while still providing quality separations. Further studies need to be done using even shorter chiral columns combined with smaller particles to further explore the potential of SFC purifications. The use of these shorter columns will hopefully lead to even less solvent usage making SFC an even more effective green technology.

Acknowledgements

The authors would like to thank Martin Struble and Don Schmidt for their guidance during this study.

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