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Analytical supercritical fluid chromatography using fully automated column and modifier selection valves for the rapid development of chiral separations

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Abstract

Automated analytical supercritical fluid chromatography is used to separate enantiomers of pharmaceutical compounds in the drug discovery laboratory. Modification of a commercial instrument to incorporate a six-way column selection valve, multiple chiral columns based on derivatized cellulose or amylose, and a four-way modifier selection valve provides a powerful combination for the rapid development of chiral separations. A wide set of columns and conditions can be tested sequentially, including unattended operation overnight. This paper shows that similar racemic compounds, even those from the same molecular class, are separated using different column and modifier combinations. Therefore, the use of program-controlled column and modifier selectors has great advantages. Using the fully automated system, the optimal chiral separation of several compounds can be obtained unattended within 24 h.

Keywords: Enantiomer separation; Automation; Pharmaceutical analysis; Supercritical fluid chromatography

1. Introduction

Chiral separations have an important impact in drug discovery. The US Food and Drug Administration (FDA) requires investigators to evaluate the safety and effectiveness of therapeutic drugs if they contain asymmetric centers. Such drugs will exist as racemic mixtures of two enantiomers, of which one may have quite different pharmacological and/or toxicological effects than the other. A significant number of prescribed drugs are racemates, and in most cases their efficacy would be improved by removing the unwanted enantiomer [1–3]. At the early stage of drug discovery only a small amount (50 mg–10 g) of each enantiomer is needed for testing, and pure enantiomers can often be obtained faster by chiral separation than by conventional chiral synthesis. Furthermore, with the recent introduction of simulated moving bed (SMB) separations, the use of chromatography for commercial scale separation of kilogram amounts of enantiomers is increasing [2,4–6].

Supercritical fluid chromatography (SFC) has proven to be a very useful and efficient tool for the separation of chiral compounds [3]. The technique has shown distinct advantages over traditional high-performance liquid chromatography (HPLC) both on the analytical scale for purity assessments, as well as

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on a larger preparative scale for separation of racemic mixtures and isolation of large quantities of the individual enantiomers. Due to a much higher theoretical plate count, SFC often shows better resolution and shorter run times than HPLC under comparable conditions [3,7–9]. Furthermore, the substitution of hexane in HPLC by CO_2 in SFC greatly reduces organic solvent consumption – both techniques use small percentages (up to 25%) of polar organic modifiers. This solvent reduction is particularly important on a preparative scale where grams of a compound can be separated by SFC and collected in less than 100 ml of solvent, rather than in multi-liter quantities of HPLC mobile phase. It is estimated, based on the cost of solvent and solvent disposal, CO_2 cost, energy cost, cycle time and operator cost (everything except the initial capital investment in the instrumentation), that the average cost of a preparative chiral separation using SFC is half the cost of running a comparable HPLC purification [10].

A large number of chiral stationary phases are commercially available, often based on modified cellulose and amylose derivatives. The same column packings can be used with either HPLC or SFC. However, unlike some chromatographic methods (e.g., reversed-phase HPLC), the prediction of which column and modifier combination will provide the best separation is almost impossible [1,2]. Optimal conditions vary greatly and are compound-specific. It is not uncommon for a slight change in just one functional moiety on a molecule – such as changing a methyl to an ethyl group – to require different columns and/or modifiers to achieve the desired resolution of the respective enantiomers.

Automated column and/or solvent selection using HPLC has been reported several times in the past [11,12]. To our knowledge, this is the first demonstration of these techniques for the improved efficiency of SFC methods development of chiral separations. At the time we began, commercially available analytical SFC instruments did not offer a column selection valve. After installing a six-port column selection valve as a prototype on our SFC, Berger Instrument now offers it as a standard option on their systems. Automated valve switching allows a scientist to set up large experimental design sequences to run unattended. This greatly reduces the time spent by the scientist in developing methods compared to a manual, serial injection process.

This paper will demonstrate the wide variety of chromatographic conditions required to support the diverse nature and high number of samples in a drug research laboratory. Furthermore, we will discuss how the use of automated column and modifier selection increases both laboratory productivity and the quality of chiral separations.

2. Experimental

2.1. Materials

Carbon dioxide (SFC grade) was obtained from National Welder (Durham, NC, USA). All chiral compounds were synthesized in-laboratory. Methanol and isopropanol (IPA) were HPLC-grade solvents from EM Science (Gibbstown, NJ, USA). The ethanol used was from McCormick Distilling Co. (Weston, MO, USA). Triethylamine (TEA) and trifluoroacetic acid (TFA) were obtained from J.T. Baker (Phillipsburg, NJ, USA).

2.2. Chiral stationary phases

Columns packed with Chiralpak AD and Chiralpak AS amylose derivatives, Chiralcel OD cellulose carbamate derivative and Chiralcel OJ cellulose ester derivative were purchased from Chiral Technologies (Exton, PA, USA)[1]. Hereafter columns are referred to only with the two-letter designation, e.g., AD or OJ. Column dimensions were 250×4.6 mm I.D. Particle size for all columns was 10 μm.

2.3. Instrumentation

SFC was performed on a Berger (Newark, DE, USA) equipped with a SFC pump, a modifier pump, an automated injector, a column oven and a UV diode array detector. The column selection valve (TCM-2030) for six columns is now available from Berger. The modifier selection valve for four modifiers was obtained from VICI Valco Instruments (Houston, TX, USA). All the free contact closures on the SFC were used for the column selection valve, therefore an additional contact closure was added to

[1]Chiralpak AD, Chiralpak AS, Chiralcel OD and Chiralcel OJ are trademarks of Daicel Chemical Industries, (Chiyoda-ku, Tokyo 100, Japan), which is the parent company of Chiral Technologies.

permit the installation of the modifier selection valve.

2.4. Supercritical fluid chromatography

Samples were dissolved to a concentration of about 1 mg/ml in methanol and 10 μl was injected. Results were all generated at a flow-rate of 2 ml/min, 205 atm pressure, and 40°C (the critical point of CO_2 is 31°C and 73 atm; 1 atm=101 325 Pa). The percentage of modifier was calculated as a volume/volume ratio (v/v). To improve peak shape and separation, 0.1% TEA or 0.1% of TFA (v/v) was added to methanol. At high concentrations of modifier (e.g., 25% IPA), the actual critical temperature of the mixture is probably above 40°C, however chromatography with the mixtures described herein still provides separation of the compounds. The automated analysis was programmed as a sequence of columns, modifiers and chromatographic conditions. All analyses were isocratic, isobaric and isothermal. In the absence of other information, a 25-min runtime was deemed sufficient to elute the compounds. Between each change of column, modifier, or modifier concentration, a 20-min equilibration time was programmed in. The wavelength for absorption measurement for each figure is listed in the figure legend.

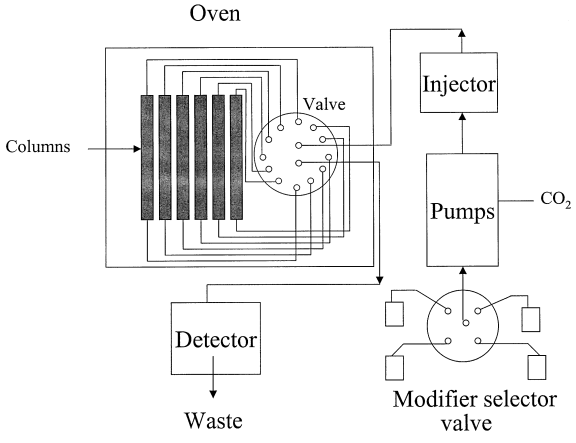

Fig. 1. Diagram of the automated analytical SFC showing column switching and modifier selection valves.

3. Results and discussion

The modification to our commercial instrument is shown in Fig. 1. To maintain temperature, the column-switching valve was installed inside the chromatographic oven. Up to six columns can be accommodated in the oven simultaneously, and can be selected in any order under software control. When not selected, the column is isolated with no fluid flowing through it. The organic modifier is added to the CO_2 fluid prior to the injector valve. To provide for maximum flexibility, we added a second switching valve to allow the selection of one of up to four modifiers. Again, the selection of the composition and the nature of the modifier is under software control.

When faced with a separation request, we will typically set up a series of injections on four of the columns we have found to be most successful for our applications, using one of four common modifiers. This provides 16 different column/modifier combinations, even more variations if multiple concentrations of each modifier are programmed. The sample is then sequentially injected with each of the defined conditions/columns; and can operate without further attention for many hours. At the end of the experiment, the analyst has two-dimensional array of chromatograms, representing different columns and modifier conditions on the two axes; and can assess which column and modifier are most appropriate to achieve separation. If necessary, further experiments can be designed to more precisely define the optimum conditions for separation.

This array of conditions is particularly useful in a laboratory where both analytical-scale and preparative-scale analyses are being conducted, because the separation requirements of the two experiments are quite different. In an analytical scale separation, one is interested primarily in speed; resolution between the isomers need only be sufficient to accurately determine enantiomeric purity. For a preparative separation, where the column will likely be heavily overloaded, a large separation between isomers is critical, even if that separation requires more time.

Since the installation of the column and modifier selection valves, we have analyzed all of our separation requests, representing a wide variety of chemi-

cal classes using the automated system. We have been able to identify conditions that provide a baseline separation for all of them. The examples shown below (see structures of the compounds in Fig. 2) were chosen to give clear examples of how each variable – column, modifier solvent and modifier concentration – can have a significant affect on enantiomeric resolution.

Compound **1** is a racemic indanylidine under investigation for pain relief. It was injected in one experiment using four different columns (AD, OD, AS and OJ) and three different modifiers (MeOH, EtOH and MeOH containing 0.1% TFA). The experiment was automated and injected overnight, so no analyst involvement was required after the initial set-up. The results are shown in Fig. 3. For this compound, the AS and OJ columns gave little or no separation, AD gave baseline separation, and OD using ethanol as modifier gave by far the best resolution. While enantiomeric purity on an analytical scale could be assessed using any one of several column/modifier combinations (e.g., AD, 10% methanol; or OD, 10% methanol containing 0.1% TFA), for preparative-scale separation, the large separation provided by the OD column and 10% ethanol is preferred.

In another experiment, five different compounds were injected in a single experiment using four different columns (AD, OD, AS and OJ) and four different modifiers (MeOH, EtOH, MeOH containing 0.1% TEA and IPA). A total of 80 injections was made over a span of 36 h. The following three examples from this experiment show the results obtained for compounds **2**, **3** and **4**.

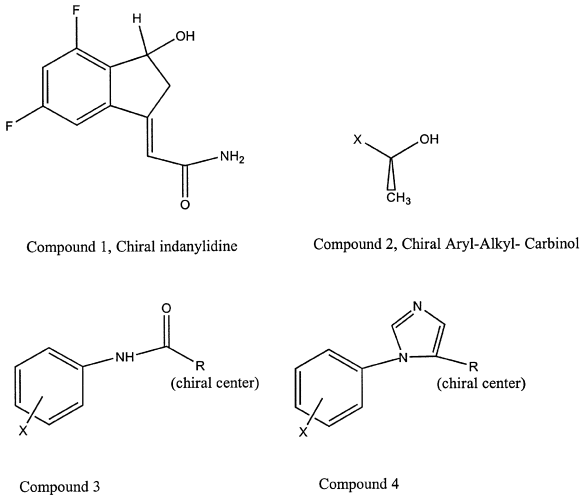

Fig. 2. Partial structures of the chiral compounds **1**–**4** separated in this work.

For compound **2**, an aryl-alkyl carbinol, the automated optimization showed that the OJ column, using 25% IPA as modifier, provided the best resolution (Fig. 4d). The AD column with 25% IPA provided some separation (Fig. 4a), whereas the other 14 injections showed no separation at all. A subsequent optimization of modifier concentration was conducted to evaluate the effect of varying the percentage of modifier being used (Fig. 4e and f). Reducing the amount of IPA resulted in longer retention times, but resolution was improved slightly. Ultimately the preparative separation of 300 mg of compound was achieved using the OJ column and 15% IPA.

For compound **3**, an aromatic amide, the best resolution was obtained on an AS column using 20% IPA. Separation was also obtained on the AD and OJ columns, but with less resolution than on the AS column. The OD column gave no separation at all. A subset of the chromatograms obtained, namely those obtained with 20% IPA as modifier, is shown in Fig. 5; but other modifiers were also investigated on the same set of columns during the course of the overnight optimization.

The data presented for compound **4** demonstrate the dramatic effect of changing modifier on the separation achieved with a specific column (Fig. 6). The resolution obtained using IPA was by far the best of the four modifiers used. It is interesting to note that compounds **3** and **4** were analogues for the same project (X and R are the same in both molecules); but their best separations were obtained using different columns. The conditions obtained in this optimization were used to monitor enantiomeric purity on an analytical scale during chiral syntheses of both compounds.

The multi-position valving has a number of advantages beyond the rapid optimization of separations for preparative work. Because more complete data is obtained for each compound analyzed, we get a clearer sense of what separations work best for a given project or compound class. The column/modifier matrix (as seen in Fig. 3) is a convenient way to

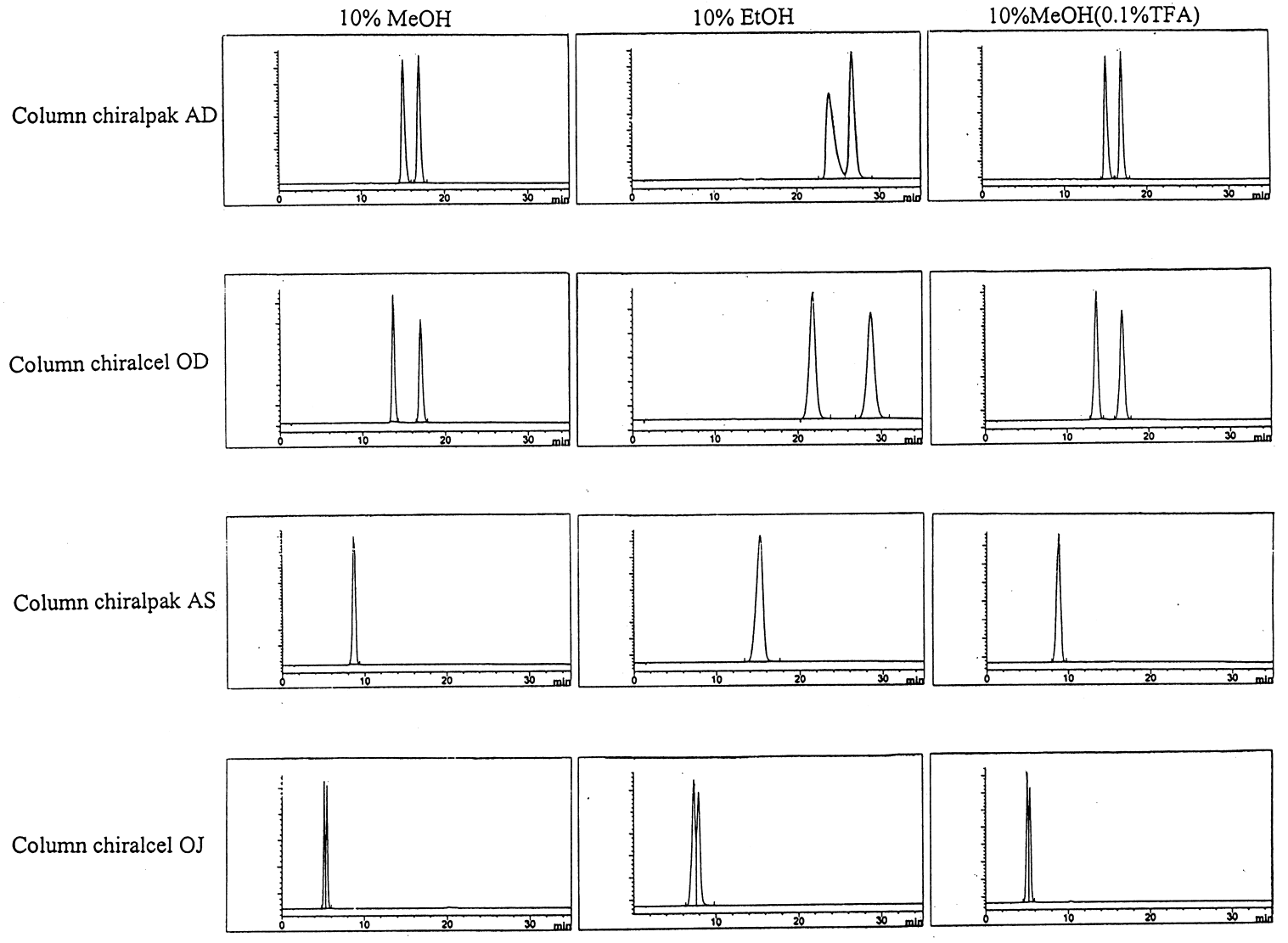

Fig. 3. Chromatograms of **1**, injected on four different columns using 10% MeOH, 10% EtOH and 10% MeOH containing 0.1% TFA. UV absorbance at 280 nm is monitored.

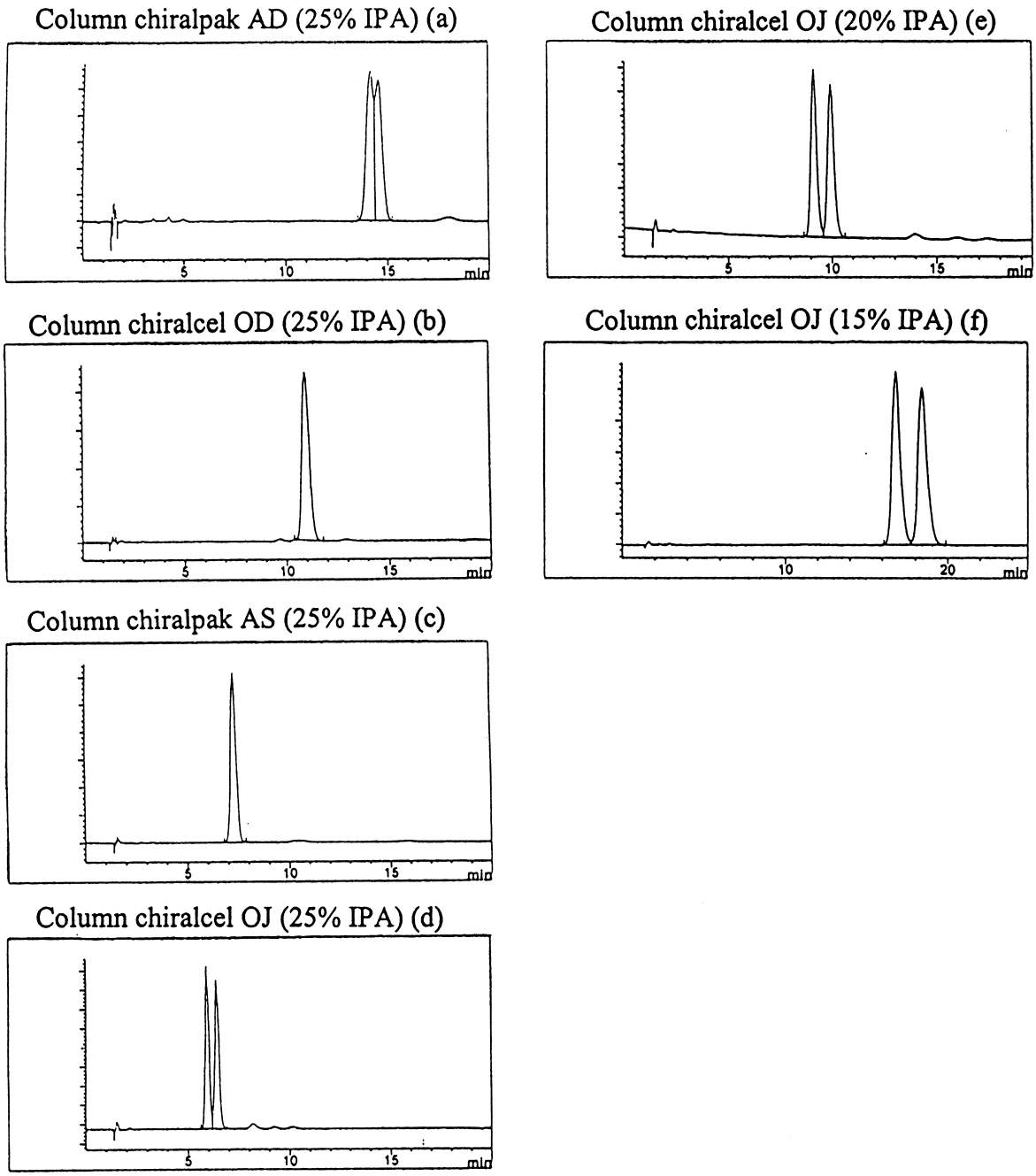

Fig. 4. Compound **2**, injected on four different columns (as labeled) using isopropanol (IPA). UV absorbance at 254 nm is monitored.

record the results of many separations over time. As patterns emerge of which combinations of modifier and column work best for a compound class, those conditions can be tried first in the automated optimization. If an early injection shows an acceptable level of separation, the optimization can be aborted

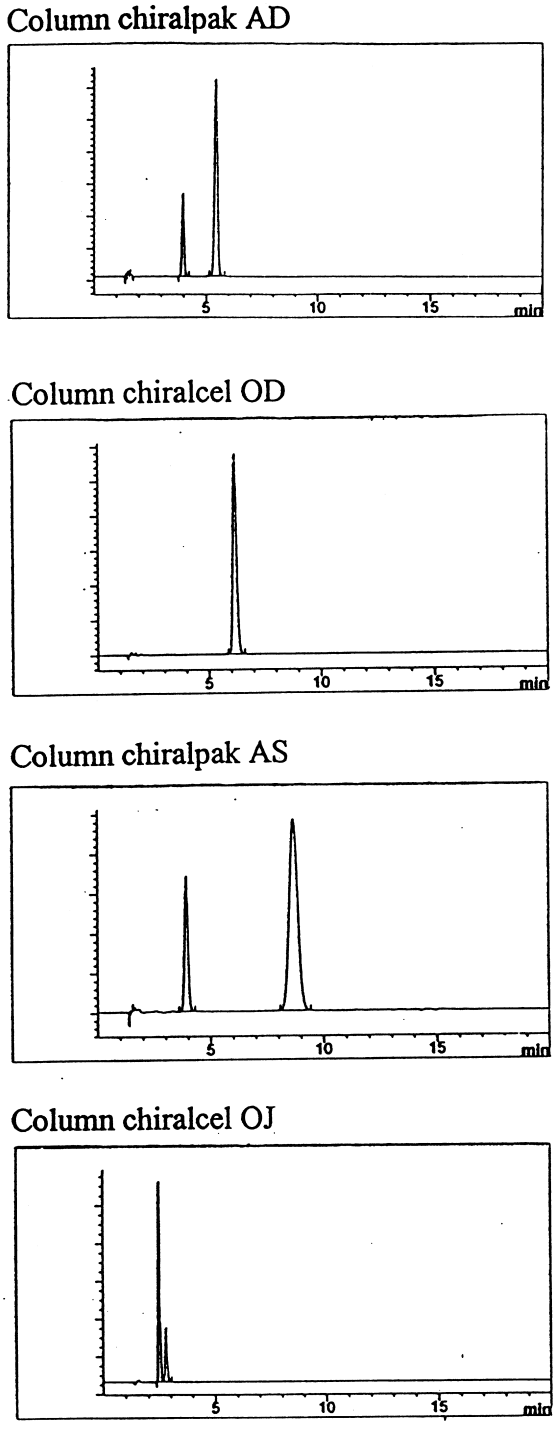

Fig. 5. Compound **3**, injected on four different columns (as labeled) using 20% IPA. UV absorbance at 254 nm is monitored.

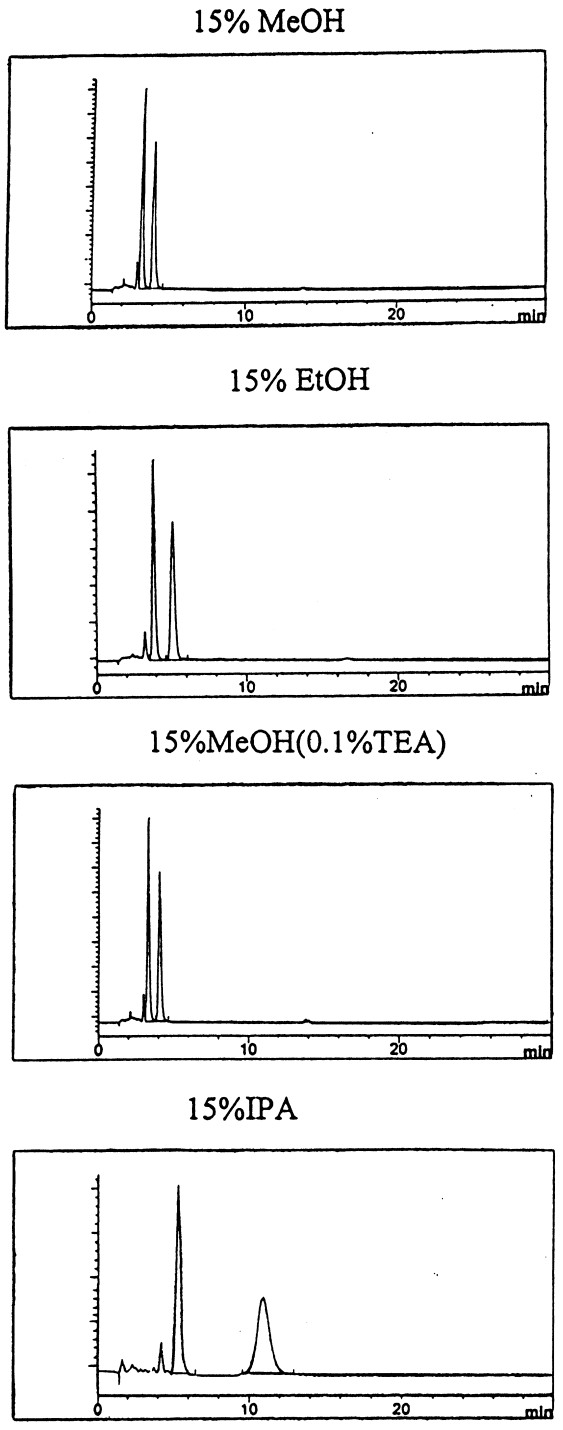

Fig. 6. Compound **4**, injected on the OJ column with various modifiers as labeled. UV absorbance at 254 nm is monitored.

before running through all columns and conditions; and preparative chromatography can begin immediately.

As a demonstration of the variability of columns/conditions required, in a recent analysis, seven different racemic compounds were injected individually onto four columns and separated with four different modifiers on each column. Of the seven, two compounds were resolved best on the AD column, two were resolved best on the AS column, two were resolved best on the OJ column, and one compound on was resolved best on the OD column. For two of the seven compounds, separation was obtained only on one of the four columns. In terms of modifiers, the best results for four of the seven compounds were obtained using methanol, with three compounds resolving best when using IPA. It is clear that no common theme among separations emerges, and random selection of columns and modifiers is a cumbersome way to decide upon the optimum set of separation conditions. The most efficient approach is a systematic combination of columns and modifiers, made possible by the automated valving system described herein.

Finally, by having all the columns and modifiers available all the time, one can conveniently select desired conditions immediately, using only the software. Columns do not have to be changed, thereby decreasing the wear and tear on column fittings caused by repeated tightening and loosening. Modifier reservoirs do not have to be changed. The result is a faster turn-around time and a more efficient use of equipment.

4. Conclusions

SFC using multi-position column and modifier selection allows us to select the best conditions for a chiral separation in a timely manner. Several compounds can be injected on up to six different columns using up to four different modifiers, in a completely unattended mode. If adequate separation is not achieved upon completion of the first automated experiment, only a few subsequent injections are needed to optimize the separation. Using an experimental design concept with three critical parameters – column, modifier, modifier concentration – we are able to achieve near optimum conditions for most compounds overnight. This feature is particularly useful when the method is translated to preparative scale, since greater resolution is required in preparative work. Because structurally similar sets of enantiomers often require different column/modifier combinations, the automated system saves time and ensures the best possible separation.

The system permits a relatively complete characterization of the separation under a variety of conditions. One is not tempted to spend a great effort in optimizing the conditions of a partial separation, when a completely different column or modifier might resolve the enantiomers easily. Further use of the system will allow us to perform a statistical analysis on a larger data set helping to establish the most successful chromatographic parameters for the compounds under investigation in our laboratories, perhaps allowing us to set up more efficient run sequences.

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