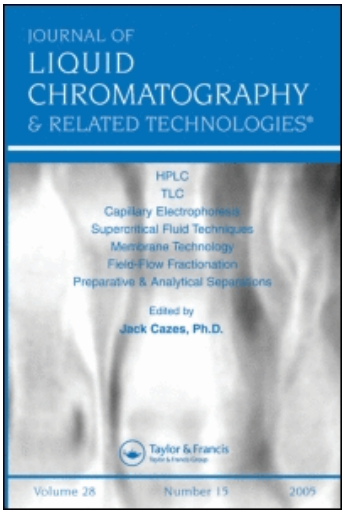

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Development of Preparative Chiral Separations Using an Intelligent Chiral Resolution System

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ABSTRACT

Chiral preparative chromatography is a technology increasingly used in the pharmaceutical industry to deliver enantiomerically pure drug candidates. A strategy for rapid screening of conditions on polysaccharide chiral stationary phases (CSPs) has been developed with an emphasis on preparative applications. Sample solubility is a major limiting factor in preparative chromatography. Most reported analytical chiral separations on polysaccharide CSPs are with eluent systems consisting of mainly hexane and small amounts of alcohol as a modifier. The intelligent chiral resolution system (ICRS) was developed to emphasize polar solvents as the first choice for eluent systems. Examples of this approach, and details on the screening procedure are presented. Using the system, chiral preparative procedures can be developed for most compounds in approximately 3 hours.

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Key Words: Enantiomer separation; Preparative chromatography; Chiral screening; Chiral chromatography; ICRS.

INTRODUCTION

Development of single enantiomer drug candidates has become standard practice in the pharmaceutical industry. In most cases, both isomers of a drug will need to be tested for activity to determine the most applicable candidate. There may also be therapeutic advantages to marketing single isomer drugs, not to mention the potential to extend patent life in some cases. Two approaches toward development of enantiomerically pure drugs are routinely used in the pharmaceutical industry. The racemic approach involves separation techniques including chromatography, as well as, classical resolution procedures to generate isomers, while the chiral approach relies on stereoselective synthetic solutions.[1] The racemic approach utilizing chromatography offers several advantages over traditional recrystallization or stereoselective synthetic procedures. Since racemate is produced, both isomers may be collected and are available for testing. Traditional isolation of the individual isomers by recrystallization as diastereomeric salts can be time consuming. The time required for development of recrystallization or asymmetric synthesis can be excessive, rendering these techniques impractical for generation of small quantities of enantiomers for testing.[2] Time is critical, particularly in the discovery phase, where lead candidates for different targets are identified. Chromatographic method development is generally much faster than traditional procedures involving recrystallization or synthesis development. Availability of techniques, such as simulated moving bed (SMB) chromatography and commercially available quantities of chiral stationary phases (CSP) make chromatography a viable option for all phases of development as well as manufacturing.

Preparative chromatography development must be approached differently than traditional analytical separation techniques. The majority of reported chiral separations in literature are performed under conditions of low alcohol concentration in hexane. With the sensitivity of current HPLC detectors, these separations are completely adequate for most analytical applications where quantitation and limit of detection parameters are a consideration. Unfortunately, most pharmaceutical compounds of interest are not very soluble in these alkane heavy eluent systems. Solubility in the eluent system is desirable for successful scale-up of a preparative chromatography separation. Otherwise, large injection volumes are necessary for reasonable productivity. High mass overloading of a preparative column is desirable rather than volume overloading from a large injection. In batch chromatography, variation in sample diluent may be used to improve solubility as long as the stronger

solvent does not cause precipitation of solute on the column in the weaker eluent, or disrupt the equilibrium state of the column. This cannot be done in continuous techniques such as SMB, since the feed solvent and sample diluent will eventually become the column eluent in the equilibrium state.[3] Thus, method development for preparative chromatography must be done with different criteria than analytical methodology.

Selection of the appropriate CSP and solvent combination is critical for a successful preparative separation. The polysaccharide-based CSPs have become phases of choice for many large scale separations in the pharmaceutical industry.[4,5]

A description of the separation mechanism and a number of applications for the polysaccharide CSP have been reported.[6] Other separations of pharmaceutical compounds using polar organic solvents such as ethanol, methanol, or acetonitrile, rather than alkane heavy solvent systems, have also been published.[7] Advantages of these solvent systems include higher sample solubility and, thus, higher mass loading for preparative applications. Various procedures for screening columns and solvent systems in development of chiral separations have been presented.[8] These procedures are based on Plackett-Burnan design studies and indicate that certain solvent/CSP combinations are more selective. Ethanol was reported to be a better solvent on the Chiralpak® AD column, whereas isopropyl alcohol proved superior with the on Chiralcel® OD column for chiral recognition. Data was also presented to indicate the optimal concentration of alcohol in hexane to be approximately 10% by volume.

These studies did not factor in sample solubility as a variable. The intelligent chiral resolution system (ICRS) in this manuscript was developed based on experience in the separation of numerous pharmaceutical intermediates and final drug substances. Experiments are arranged in order of highest probability for successful separation with the highest concentration of polar solvent in the eluent. Examples of separations using polar solvents will be presented, along with a description of the experiments used for selection of the appropriate CSP and solvent combination. Most compounds are separated by the ICRS approach in less than 3 hours.

EXPERIMENTAL

Equipment

The analytical chromatography system used was a Shimadzu Scientific LCMS-QP8000 CL Liquid Chromatograph Mass Spectrometer consisting of LC-10AD vp pumping system, SPD-M10A vp PDA detector, SIL-10AD vp Auto sampler, and six port column switcher containing two FCV-14AH

valves. The mass spectrometer was operated in APCI mode. Preparative chromatography systems were Novasep LC-80 (Novasep Inc., Boothwynn, PA) and Novaprep 800 with Hitachi L7400 uv detector (Hitachi Instruments Inc., Naperville, IL) Prochrom LC80.VE.100 columns, 8 cm i.d., were obtained from Novasep Inc., Boothwynn, PA, and packed to variable length as needed to accommodate approximately 1 kilo of packing material.

Materials

Twenty micron bulk Chiralpak AS, AD, Chiralcel OD, OJ packing material and 4.6 × 150 mm analytical columns packed with 10 micron material were purchased from Chiral Technologies Inc., Exton, PA. Two separate Chiralpak AD columns were used for high alcohol (>60%) and low alcohol (<20%) experiments. Solvent compatibility data for the Chiralpak columns is available on the Chiral Technologies technical data web page.[9]

Trifloroacetic acid (TFA) and dimethylethyl amine (DMEA) were purchased from Aldrich Chemical Company, Milwaukee, WI. Heptane was obtained from Tedia Co., Inc. in Fairfield, OH. Denatured ethanol (EtOH) (contains 5% methanol), methanol (MeOH), isopropyl alcohol (IPA), and acetonitrile (ACN) were purchased from Mays Chemical, Indianapolis, IN.

RESULTS AND DISCUSSION

In the discovery phase of drug development, numerous racemic compounds with different structural characteristics are examined as potential candidates for various therapeutic groups. Due to the number of compounds, rapid development of scaleable preparative chromatographic conditions is necessary. The examples presented in this manuscript are all pharmaceutical intermediates. Structures are provided when possible. The ICRS approach for method development has been successfully applied to over 500 different compounds. The screening system is generic in nature and, therefore, can be applied to widely varying compound classes.

Many chiral separations derived from traditional eluent combinations of alcohol and hexane are not scaleable. Multiple factors must be considered when choosing appropriate preparative conditions. These include the quality of the separation, compound compatibility with the CSP and eluent, removal of solvent from fractions, and availability of the CSP. Sample solubility in the eluent is often overlooked as a variable when chiral separations are developed. Utilizing polar solvents as eluent systems for chiral separations, rather than traditional alcohol/hexane, is advantageous for preparative separations in that most pharmaceutical

compounds are more soluble in these systems. In order to test this concept, a series of 84 different compounds were separated with traditional or polar organic solvents. These data indicate that a slightly higher number of separations were possible with the polar organic solvents, but both techniques are viable for chiral separations. However, when separations were scaled up and the amounts of material purified by the two techniques were compared, it is obvious that the polar organic solvent systems are more applicable for preparative scale applications. These data are summarized in Fig. 1. Separations for two of the compounds are illustrated in Fig. 2, utilizing ACN as eluent on a Chiralpak AD. These chromatograms were generated on a 4.6 × 150 mm Chiralpak AD column with a flow rate of 1.0 mL/min. The 5-member ring ketone compound was evaluated as candidate for SMB. The estimated productivity on a 8 column (5 cm ID × 9 cm) with 800 g CSP was 0.42-kg racemate/day/kg CSP.[10]

Based on these data, a systematic approach to chiral method development for preparative applications was developed utilizing polar organic solvent combinations, initially, followed by traditional alkane based eluents containing lower concentration of IPA or EtOH. Heptane was used as the preferred alkane substituent. The sequence of experiments and eluent systems for ICRS is presented in Table 1. Analytical chromatograms are generated on 4.6 × 150 mm 10 micron columns with a flow rate of 1.0 mL/min and detection by UV diode array and mass spectrometer with APCI probe. The mass spectrometer confirms identification of the desired compounds when other substituents are present in the samples.

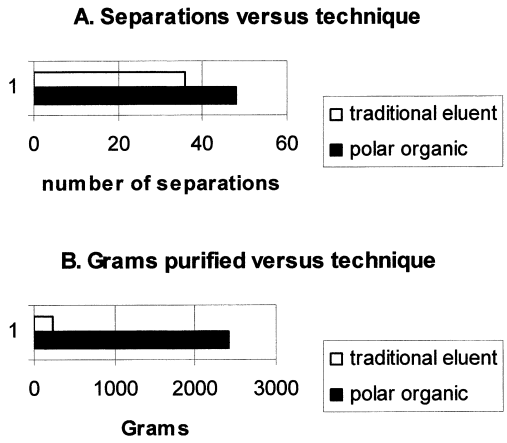

Figure 1. Comparison of polar organic mode separations vs. traditional eluent. (A) Number of separations vs. technique; (B) grams purified vs. technique.

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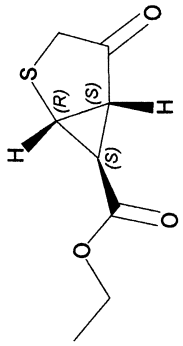

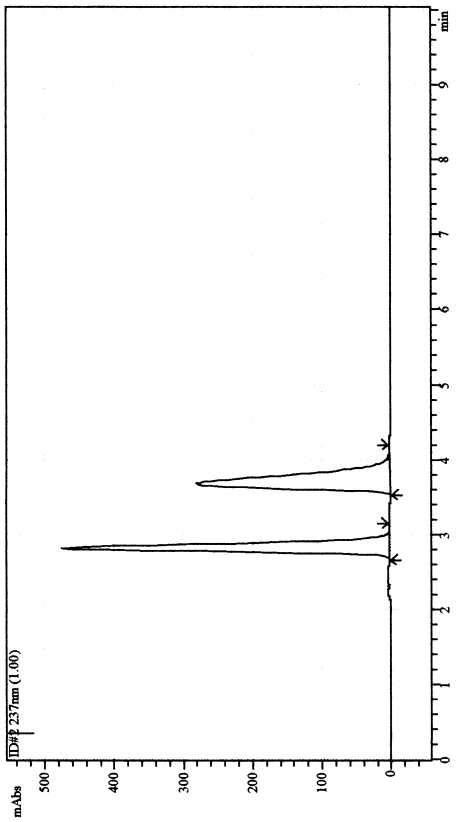

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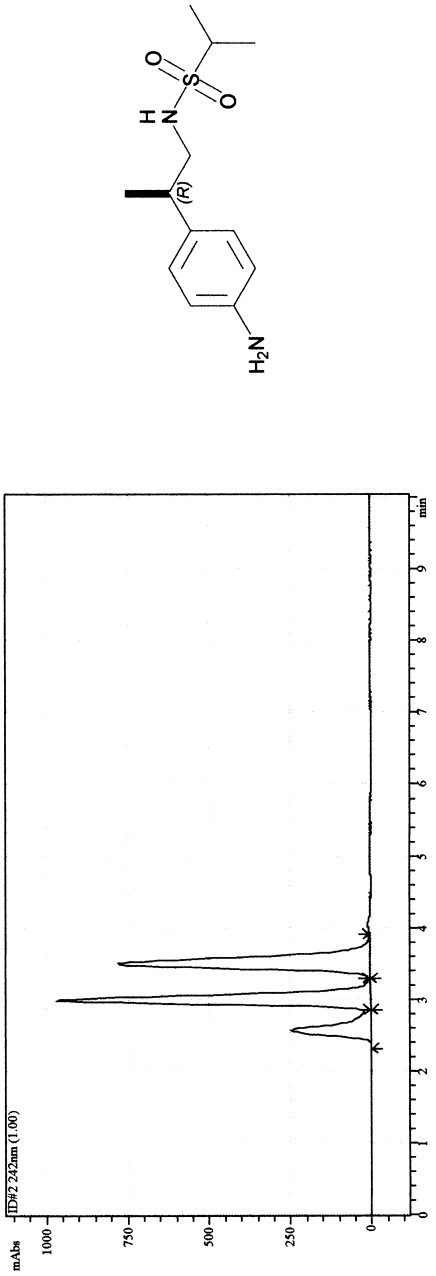

Figure 2. Separation of pharmaceutical intermediates with acetonitrile on Chiralpak AD.

Table 1. Intelligent chiral resolution system experimental design.

Experiment	Column	Eluent[a]
1	Chiralpak AD	100% EtOH
2	Chiralpak AD	100% MeOH
3	Chiralpak AD	100% Acetonitrile
4	Chiralcel OD	10% IPA/heptane
5	Chiralpak AD	10% IPA/heptane
6	Chiralcel OJ	20% IPA/heptane
7	Chiralpak AS	20% IPA/heptane
8	Chiralcel OD	10% EtOH/heptane
9	Chiralpak AD	10% EtOH/heptane
10	Chiralcel OJ	20% EtOH/heptane
11	Chiralpak AS	20% EtOH/heptane

[a]Eluent may contain 0.1% trifluoroacetic acid for acidic compounds or 0.1% dimethylethyl amine for basic compounds.

Intelligent chiral resolution system screening is designed to match the CSP and eluent system most likely to yield a successful preparative system. In many cases, additional experiments are necessary to yield the final optimal conditions. Examination of the structure and some knowledge about chemical stability and solubility are essential before beginning method development. It does little good to develop a method for a compound using heptane if the compound is only partially soluble in that solvent (<50 mg/mL). Compounds that contain basic functionality will require the addition of DMEA, whereas compounds containing acidic moieties, such as carboxylic acid or phenol may require TFA in the eluent system. We have found 0.1% acid or base to be sufficient for most cases. The systematic approach starting with polar solvents often reveals separations not found by random "shot gun" screens. The three polar solvent experiments (1–3) on Chiralpak AD are run in 10 min cycles with 5 min equilibration between runs. Other remaining low alcohol experiments (4–11) are run in 15 min cycles with 5 min equilibration. The total time for all experiments is approximately 3 hours. Running all experiments has separated or indicated starting conditions for 95% of all compounds submitted for purification. Polar organic solvent combinations are also useful on Chiralcel OJ and Chiralpak AS CSPs. The OJ and AS are evaluated with polar solvents when the conditions in Table 1 do not yield a separation. Baseline separations are often obtained rapidly after few experimental observations. Many times, separations are achieved with more than one experiment. Figure 3 illustrates separation of a compound utilizing both the traditional IPA/heptane eluent and EtOH as eluent on Chiralpak AD column. The 30%

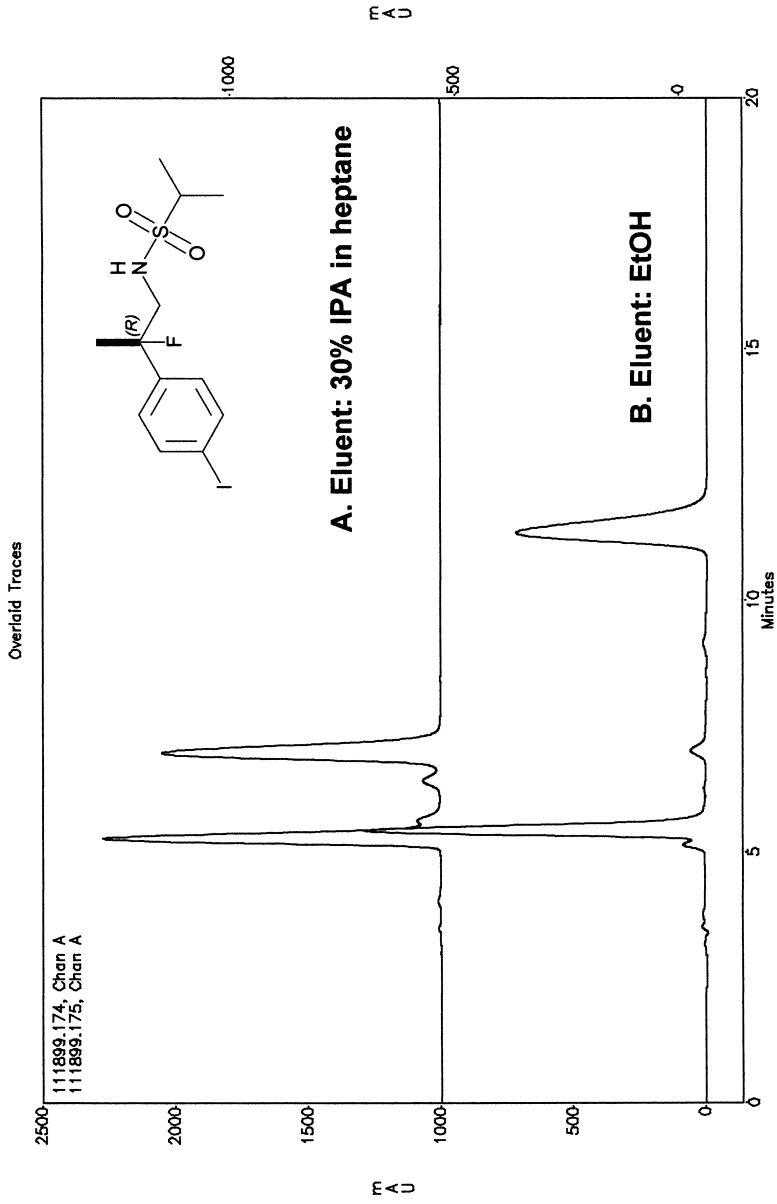

Figure 3. Comparison of separation with traditional chiral eluent (A) to polar organic (B) on Chiralpak AD.

IPA/heptane eluent was developed after observations from experiment 5 in Table 1. The separation using the heptane system did not scale up efficiently, due to lower resolution on the preparative column and low solubility in the IPA/heptane solvent system. The EtOH eluent, however, provided increased resolution and improved solubility, allowing 4.0 g loading on the 8×32 cm preparative column. Separation was complete in approximately 15 min.

The ICRS screen will usually provide information about the best CSP, but further optimization of solvent combinations may be necessary. This is illustrated in Fig. 4. The screen indicated partial separation using IPA/heptane (experiment 5), but with insufficient resolution and poor peak shape. The addition of methanol improved peak shape and resolution. The low UV response for this compound on the analytical syste, (A) necessitated a large injection volume and, therefore, accentuated the solvent injection peak. The preparative chromatogram (B) on the 8×32 cm column, illustrates a 1.0 g loading with separation achieved using a shave/recycle technique. Shave/recycle chromatography involves shaving the front and back of the mix, while the center of the mixture is passed through the column a second time prior to collection of the desired isomers. In this example, both isolated isomers had enantiomeric excess $>97.5\%$.

Figure 5 illustrates the results obtained from a compound containing a carboxylic acid functionality. Partial separation occurred on the Chiralpak AD with EtOH (1), MeOH (2), and 10% EtOH/heptane (9). Partial separation also occurred on the Chiralcel OD with IPA/heptane (3). Chromatogram 5 on Chiralpak AD does not indicate separation, but rather carry over from previous injection. Acetonitrile was not evaluated for separation of these compounds. No chiral recognition occurred on the Chiralpak AS. The optimal conditions were achieved in experiment 10 on the Chiralcel OJ using 20% EtOH/heptane with 0.1% TFA as modifier. These compounds were soluble in the EtOH/heptane eluent system; thus, further optimization with polar solvent, such as MeOH on the Chiralpak AD, was not necessary. These chromatograms also illustrate previous observations involving the best solvent/CSP combination for recognition. Ethanol/heptane combinations are better than IPA/heptane combinations on the Chiralpak AD for chiral recognition, whereas, IPA/heptane combinations are usually better than EtOH/heptane on the Chiralcel OD.

Chromatograms from the ICRS screen of a compound containing a tertiary amino acid constituent are presented in Fig. 6. The Chiralpak AD provided excellent separation of these enantiomers using both pure EtOH (1), 10% EtOH/heptane (8), and 20% IPA/heptane (11). Again, EtOH, rather than IPA, appears to be the optimal solvent for chiral recognition and peak shapes on the Chiralpak AD column. Chiral recognition did not occur on the other CSPs in this study.

The next step in development for a true intelligent system involves incorporation of analysis software that will schedule experiments as needed

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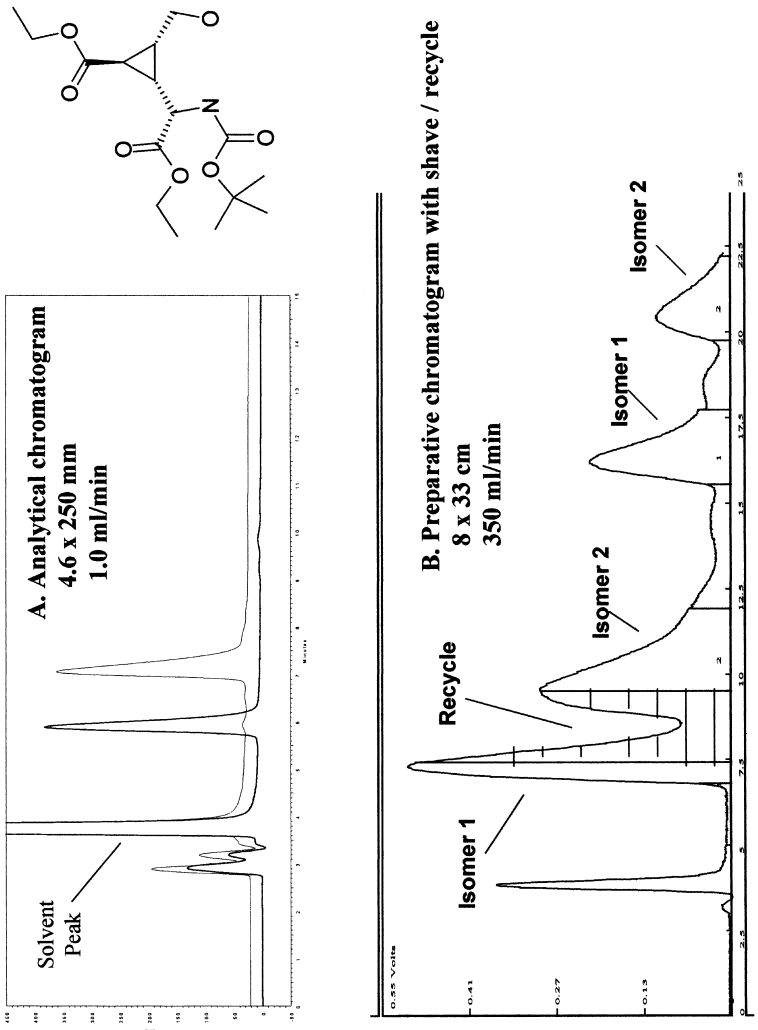

Figure 4. Analytical (A) and preparative (B) chromatograms for pharmaceutical intermediate on Chiralpak AD. Eluent: 10% MeOH, 10% IPA, 80% heptane.

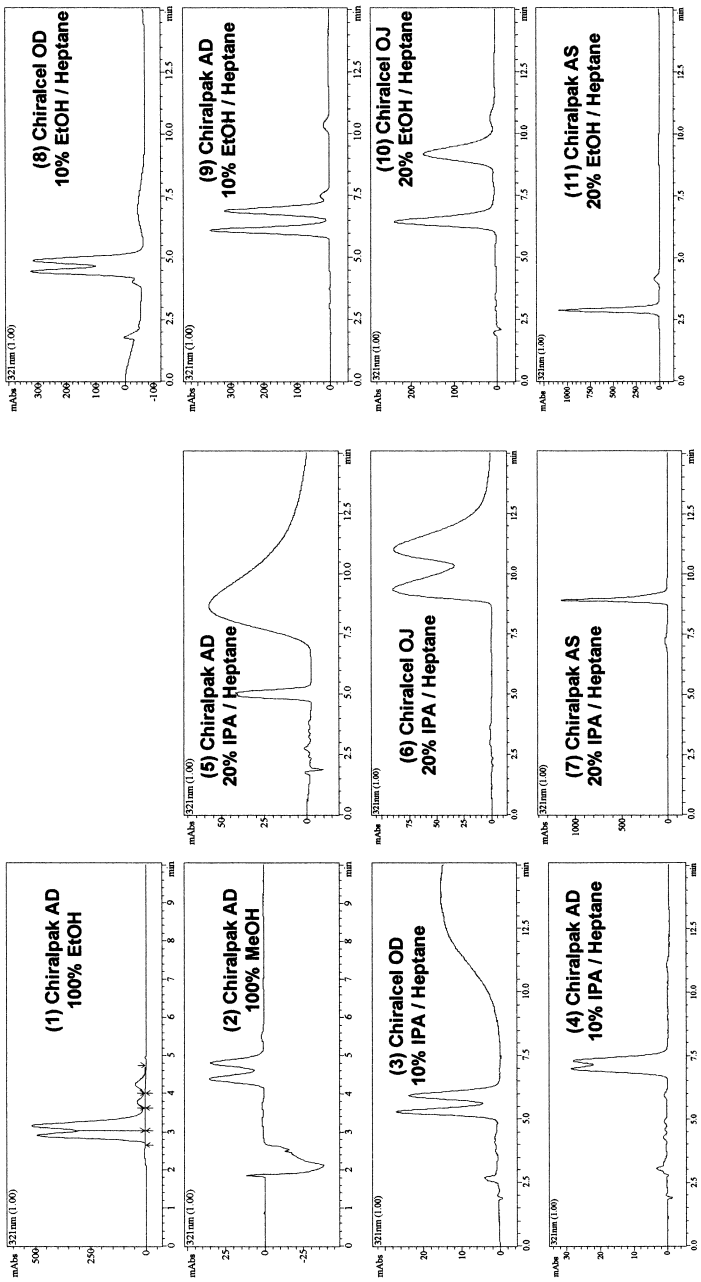

Figure 5. Intelligent chiral resolution system screen of a compound containing a carboxylic acid moiety. Eleven experiments on 4.6 × 150 mm columns; flow rate, 1.0 mL/min; all eluents contain 0.1% TFA.

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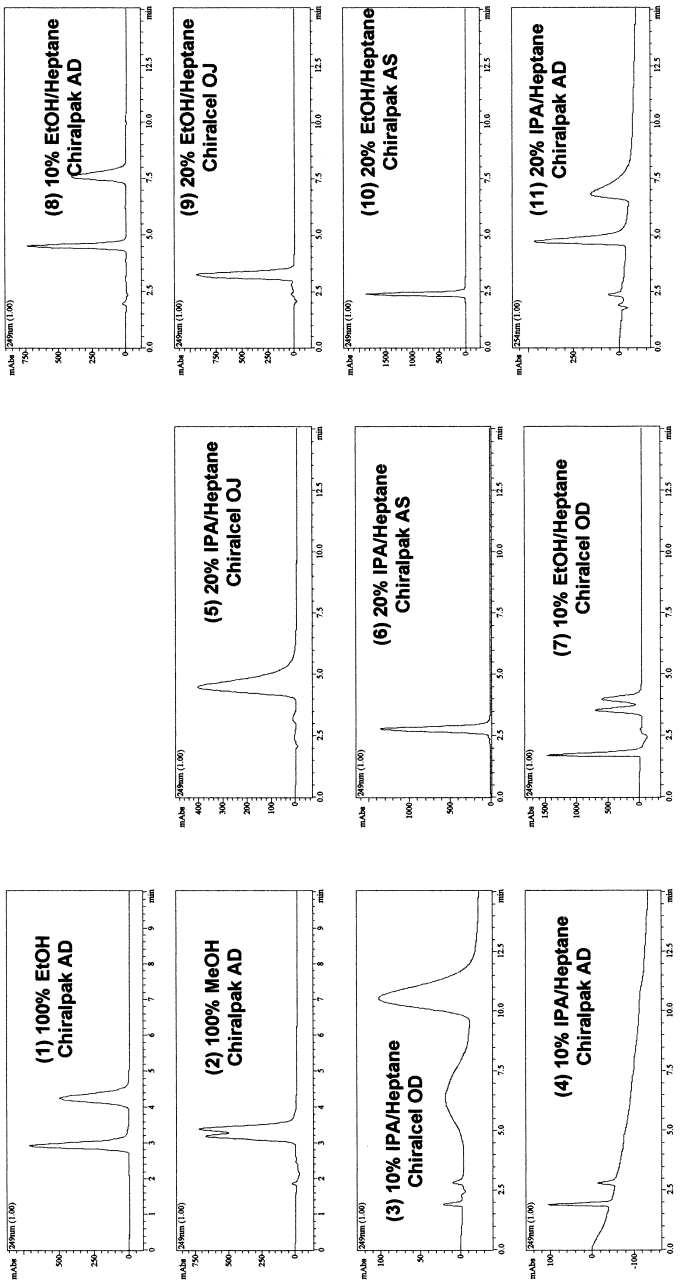

Figure 6. Intelligent chiral resolution system screen of a basic tertiary amino compound. Eleven experiments on 4.6×150 mm columns; flow rate, 1.0 mL/min; all eluents contain 0.1% DMEA.

to achieve optimal enantioseparations. Software for instrument control and analysis of chromatographic results is currently being developed to allow unattended operation of the ICRS development process.

CONCLUSION

Efficient preparative chiral chromatography is a multi-step process involving the development of the enantioseparation followed by preparative scale-up of the separation. Intelligent chiral resolution system assists in the method development segment, identifying viable enantioseparation conditions that are amenable for preparative applications. Future enhancements to ICRS, such as software driven decision trees will decrease the time required to develop separations and, ultimately, decrease turn around time for obtaining enantiomerically pure material.

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