Retention Mechanisms in Chiral Chromatography: LC-MS Analysis using Macrocyclic Glycopeptide Chiral Stationary Phases

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Predicting Chiral Selectivity

- Retention mechanisms in chiral separations are highly complex and analyte
specific, therefore many columns and conditions must be screened to ensure that
optimum conditions are found.

- The approach of using ‘experience’ is not as effective as it is with typical reversed-
phase method development.

- Why Not?
**β-Blocker Separation on Chirobiotic T**

Column Name: CHIROBIOTIC T

- Length: 25 cm
- Diameter: 0.46 cm
- Particle Size: 5 μm
- Mobile Phase: methanol; 15mM ammonium formate
- Flow Rate: 1 ml/min
- Temperature: 25 °C
- Detector: UV (220 nm)
- Injection Volume: 3 μL

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>tR</th>
<th>k’</th>
<th>Selectivity</th>
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<tbody>
<tr>
<td>1</td>
<td>clenbuterol</td>
<td>8.5</td>
<td>2.4</td>
<td>1.20</td>
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<tr>
<td>2</td>
<td>clenbuterol</td>
<td>9.8</td>
<td>2.9</td>
<td>1.11</td>
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<td>3</td>
<td>metoprolol</td>
<td>10.6</td>
<td>3.2</td>
<td>1.11</td>
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<td>metoprolol</td>
<td>11.5</td>
<td>3.6</td>
<td>1.19</td>
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<td>5</td>
<td>sotalol</td>
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<td>8</td>
<td>atenolol</td>
<td>19.3</td>
<td>6.7</td>
<td>-</td>
</tr>
</tbody>
</table>

Retention Time (min)
Ephedrine/Pseudoephedrine

Chiral AGP Column
10 mM ammonium phosphate/
1 mM octanoic acid

Chirobiotic T
Methanol:Acetic Acid:TEA
Modern Chiral Stationary Phases

Polymeric

Synthetic
- Methacrylate
- Polycyclic amine-2

Natural
- Cellulose
- Amylose
- Proteins

Small molecule ligands
- Copper complex-2
- π-complex
- Crown ether
- Cyclodextrin-12
- Macrocyclic glycopeptides-6

Supelco Phases
CHIROBIOTIC Chiral Stationary Phases

- **Macrocyclic glycopeptides** provide a multi-modal chiral surface capable of a wide variety of different interactions.

- To date, there are 6 types of CSPs commercially available.

- Subtle differences between them help to reveal the dominant mechanisms that lead to enantiomeric recognition.

- Among these mechanisms, **ionic interactions** dominate for ionizable molecules.

- **Macrocyclic glycopeptides CSPs provide a valuable source of separations for polar molecules.**
NOTE: V2, T2 differ from V, T in the chemistry used to bond the glycopeptide to the silica. The V2 and T2 often give higher selectivity for many applications and both have higher capacity for prep.

<table>
<thead>
<tr>
<th>CHIROBIOTIC V, V2</th>
<th>Vancomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIROBIOTIC T, T2</td>
<td>Teicoplanin</td>
</tr>
<tr>
<td>CHIROBIOTIC R</td>
<td>Ristocetin</td>
</tr>
<tr>
<td>CHIROBIOTIC TAG</td>
<td>Teicoplanin Aglycone</td>
</tr>
</tbody>
</table>

- **Broad based** chiral stationary phases for basic, acidic and neutral molecules
- **Chemically** bonded to pure silica (>4 linkages), so very robust
- **Stable** to high flow rates and pressures
Macrocyclic glycopeptides (CHIROBIOTICs)

A. Profile view of the aglycone basket of space-filling molecular models

B. Stick figures

VANCOMYCIN  TEICOPLANIN  RISTOCETIN A
Mobile Phase Types for CHIROBIOTIC CSPs

• **Polar Ionic Mode** – a *non-aqueous* mobile phase. Unique to CHIROBIOTICS: fast, perfect for prep, MS detection
  • for *ionizable* molecules – any acid or base

• **Reversed Phase** – MS compatible, ideal for manufacturing QC, bioanalysis
  • for *all types* of molecules

• **Normal Phase** –
  • about 15% of all applications
Using LC-MS as a Tool to Study Retention and Selectivity in Chiral Separation

To more quickly investigate the many variables that contribute to retention and selectivity in chiral separations, the use of a composite set of probes would be beneficial.

Since LC-MS has the ability to separate in the mass/charge dimension, it should be possible to run a composite set of probes to assess the impact of operational parameters on enantiomeric selectivity for many analytes simultaneously.
Focus on CHIROBIOTIC CSPs

Chirobiotic stationary phases operate well in both reversed-phase and polar ionic modes (low buffer concentrations in polar organic solvents). Highly amenable to the use of LC-MS systems.

In this study, the LC-MS approach for a set of basic probes differing in $pK_a$ values, hydrophobicity and molecular weight is first validated, then utilized to probe the impact of buffer (salt) type, buffer concentration and acid/base ratio on retention, and selectivity.

In addition, the approach is utilized to screen several Chirobiotic stationary phases to identify unique selectivity.
Secondary Possible Outcomes

Move from traditional UV screening to LC-MS based screening protocol
  • Increase throughput – multiple samples/run
  • Decrease false-positives
Resolve issues with traditionally-used modifiers
  • TFA
  • TEA
  • Non-volatile salts
Overcome additional obstacles
**Experimental**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument</td>
<td>Waters/Micromass ZQ, Single Quadrupole, Waters Alliance 2690</td>
</tr>
<tr>
<td>Column</td>
<td>Chirobiotic T, 150 cm x 4.6 mm, 5 µm</td>
</tr>
<tr>
<td>Temperature</td>
<td>35°C</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>1 mL/min</td>
</tr>
<tr>
<td>Mobile Phase</td>
<td>0.1%, w/v ammonium acetate in methanol (Polar Ionic Mode)</td>
</tr>
<tr>
<td>Detection</td>
<td>ESI, Positive Ion Mode, scan range m/z 150–500</td>
</tr>
<tr>
<td>Inj. Vol.</td>
<td>5 µL</td>
</tr>
</tbody>
</table>
Composite Test Probe- Basic Analytes

- Synephrine, m/z 168
- Chloramphetamine, m/z 170
- Methylenedioxyamphetamine (MDA) m/z 180
- Normetanephrine, m/z 184 (base peak m/z 166 –H₂O)
- Fenfluramine, m/z 232
- Bupropion, m/z 240
- Midodrine, m/z 255
- Propranolol, m/z 260
- Metoprolol, m/z 268
- Chlorpheniramine, m/z 275
- Pentazocine, m/z 286
- Norfluoxetine, m/z 296
- Fluoxetine, m/z 310
- Verapamil, m/z 455

Metoprolol
pK$_a$ Values for Probe Analytes

Variation in pK$_a$ Values

<table>
<thead>
<tr>
<th>Analyte</th>
<th>pK$_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td>5</td>
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<tr>
<td>Noradrenaline</td>
<td>6</td>
</tr>
<tr>
<td>Pentazocine</td>
<td>7</td>
</tr>
<tr>
<td>norLSDA</td>
<td>8</td>
</tr>
<tr>
<td>Synephrine</td>
<td>9</td>
</tr>
<tr>
<td>norfluoxetine</td>
<td>10</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>11</td>
</tr>
<tr>
<td>Propranolol</td>
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<tr>
<td>chloramphetamine</td>
<td>9</td>
</tr>
<tr>
<td>MDA</td>
<td>10</td>
</tr>
<tr>
<td>Fenfluramine</td>
<td>10</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>11</td>
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</tbody>
</table>

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Log P Values for Probe Analytes

Variation in Log P Values

- Normetanephrine
- Normetanephrine [INN; BAN]
- Synephrine
- Metoprolol [BAN; INN; USAN]
- MDMA
- Buproprion
- dexamfetamine
- Phenylpropanolamine
- (R)-Carpropanolamine
- Flunitrazepam
- Fluoxetine [BAN; INN; USAN]
- Verapamil [BAN; INN; USAN]
- Fenfluramine
- Fluoxetine [BAN; INN; USAN]
- Norfluoxetine
- Fluoxetine [INN; USAN]
- Verapamil [BAN; INN; USAN]
- Pentazocine
Molecular Weights for Probe Analytes

Variation in Molecular Weight

- Synephrine
- chlorphamine
- MDA
- Normetanephrine
- Fenfluramine
- Bupropion
- Metadren (INN; BAN)
- Propranolol
- Metaprolol (INN; USAN)
- Noradrenaline
- norfluoxetine
- Fluoxetine (INN; USAN)
- Verapamil (INN; USAN)
- Pentazocine
- Midodrine (INN; BAN)
- Propranolol (BAN; USAN)
- Bupropion (INN; USAN)
Validation of LC-MS Approach

Chirobiotic T has been shown to be effective for the separation of β-blocker enantiomers (1-2).

In this study, the β-blocker metoprolol was first injected alone and then in the presence of the 13 additional compounds; significant retention time overlap was observed.

The success of the approach was confirmed and then utilized to investigate the impact of buffer type, concentration and acid/base ratio on retention and selectivity in Polar Ionic Mode.


2. Bell, D., C. Aurand, J. Claus, D. Schollenberger and J. Jones, Chiral LC-MS Analysis of Drug Substances (Beta-Blockers) from Plasma Using Macroyclic Glycopeptide Chiral Stationary Phases, Pittcon 2009, Poster Tuesday PM.
Comparison of Metoprolol Alone and in Probe Mix

Note a slight variation in enantiomer response due to ion-suppression by coeluting peaks; however, retention and selectivity is not compromised.

Chirobiotic T, 0.1% NH₄Ac in Methanol (Polar Ionic Mode), ESI+ (Extracted Ion Current).
Probe Mix using 0.1% Ammonium Acetate in Methanol

Set 1: Extracted Ion Current (XIC)

Fenfluramine
Normetanephrine
MDA
Chloramphedrine
Synephrine
Probe Mix using 0.1% Ammonium Acetate in Methanol

Set 2:

1. Midodrine was not observed under these conditions.
Probe Mix using 0.1% Ammonium Acetate in Methanol

Set 3:

Verapamil
Fluoxetine
Norfluoxetine
Pentazocine
Chlorpheniramine
Chiral Retention Mechanisms: Impact of Buffer Type on Retention and Enantiomeric Selectivity

0.1% wt/v ammonium acetate, ammonium formate and ammonium trifluoroacetate were prepared in methanol.

Retention and selectivity were monitored as a function of the buffer type.

The complex probe mixture was run using multiple injections to confirm system equilibration.
Comparison of Ammonium Formate with Ammonium Acetate – Metoprolol in Probe Mix

Chirobiotic T (Polar Ionic Mode), ESI+ (Extracted Ion Mode).
Slow Equilibration with Ammonium TFA

metoprolol_T_0.1%ATFA in methanol

~100 min
~90 min
~40 min
~30 min
~20 min
Ion-Suppression using Ammonium TFA

Anion has a major effect on response, a slight effect on retention, and no significant effect on selectivity.
Chiral Retention Mechanisms: Impact of Buffer Concentration on Retention and Enantiomeric Selectivity

The complex probe mixture was run using 0.1%, 0.075% and 0.05% ammonium formate (AF) in methanol.

Retention and selectivity were monitored as a function of the buffer concentration.
Impact of Buffer Concentration on Metoprolol Retention and Selectivity

Concentration has an effect on retention, but no major effect on selectivity or response.

0.1% AF

0.075% AF

0.05% AF
Impact of Buffer Concentration on Retention and Selectivity for Complex Probe Mix

Concentration of ammonium formate has an effect on retention, but no major effect on selectivity or response.
Impact of Buffer Component Ratio on Retention and Selectivity

13 mM ammonium hydroxide and 13 mM formic acid were independently prepared in methanol.
The complex sample was run using acid:base ratios of 3:1, 1:1 and 1:3.
Retention and enantiomeric selectivity were monitored.
Runs were repeated to ensure equilibration.
Impact of Buffer Component Ratio on Metoprolol Retention and Selectivity

Ratio creates a significant change in retention plus some change in selectivity.

Ratio is base:acid

1:3
1:1
3:1

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Impact of Stationary Phase Chemistry for Three CSPs on Retention and Selectivity

Chirobiotic V2, TAG and R were run using the 1:1 mobile phase to assess the impact of stationary phase on the set of basic analytes.

Instrument: Waters/Micromass ZQ, Single Quadrupole, Waters Alliance 2690
Column: Chirobiotic V2, TAG and R, 150 x 4.6 mm
Temperature: 35° C
Flow Rate: 1 mL/min
Mobile Phase: Ammonium formate in methanol (13 mM)
Detection: ESI, Positive Ion Mode, scan range m/z 150–500
Inj. Vol.: 5 µL
Chirobiotic V2 Shows Selectivity Towards Fluoxetine and Norfluoxetine

Unique selectivity between V2 phase and certain solutes shows up in complex probe mix.

Verapamil
Fluoxetine
Norfluoxetine
Pentazocine
Chlorpheniramine
Chirobiotic TAG Shows Selectivity Towards the Amphetamines

Unique selectivity between TAG phase and certain solutes shows up in complex probe mix.

Fenfluramine
Normetanephrine
MDA
Chloramphetamine
Synephrine
Changing the CSP stationary phase is still the most useful means of altering enantiomeric selectivity.

Each of the Chirobiotic phases showed selectivity toward different analytes (except R, which is generally more applicable to acidic compounds).

Other than very general trends, selectivity remains unpredictable, necessitating a column screening approach to method development.

When CSPs are compatible, LC-MS can make this easier by allowing mixtures of different enantiomers to be screened simultaneously.
Results of Fluoxetine Column Screen

(+/-)-Fluoxetine underwent the primary screening protocol and yielded positive results.

CHIROBIOTIC V2 in both RP and PIM provided excellent selectivity while CYCLOBOND I 2000 DNP showed some selectivity in RP.

<table>
<thead>
<tr>
<th>Spectrum</th>
<th>Column</th>
<th>mode</th>
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<td></td>
<td>CHIROBIOTIC TAG</td>
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<td>PIM</td>
<td>No Separation</td>
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<tr>
<td></td>
<td>CHIROBIOTIC V2</td>
<td>RP</td>
<td>Separation</td>
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<tr>
<td></td>
<td>CHIROBIOTIC V2</td>
<td>PIM</td>
<td>Separation</td>
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<tr>
<td></td>
<td>CHIROBIOTIC T</td>
<td>RP</td>
<td>No separation</td>
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<tr>
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<td>CHIROBIOTIC T</td>
<td>PIM</td>
<td>No Separation</td>
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<tr>
<td></td>
<td>Cyclobond I 2000</td>
<td>RP</td>
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<td>Cyclobond 2000 HP-RSP</td>
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<td></td>
<td>Cyclobond 2000 DNP</td>
<td>POM</td>
<td>Unknown</td>
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</table>

- Screening results are viewed in a tabular form for easy review and comparison.
Fluoxetine

Column: CHIROBIOTIC V2, 100x2.1mm
Mobile Phase: 13 mM NH4Formate in MeOH
Flow Rate: 0.2mL/min
UV: 230nm
P1: 3.36 min (S- form)
P2: 3.83 min (R- form)
Selectivity = 1.27
Extracted Ion Currents of other β-blockers From Rat Plasma

- Clenbuterol
- Pindolol
- Alprenolol
- Salbutamol
Conclusions

The utility of LC-MS to study the impact of variables on retention and selectivity simultaneously for a large sample set in chiral separations has been demonstrated.

Variables such as buffer type, buffer concentration, acid/base ratio and column phase chemistry were investigated in polar ionic mode. Equilibration problems were observed with ammonium TFA in methanol—further work is planned to investigate this phenomenon.

Selectivity was impacted the greatest by changing stationary phase, confirming the need for column screening as a first step in method development.
Once selectivity has been observed on a CSP, the adjustment of buffer component ratios appears to have the greatest impact on enantiomeric resolution

Both the type of buffer salt and the concentration can be used to manipulate peak shape and retention, but have limited impact on selectivity

Batch screening shows excellent potential for speeding up the column selection process.

Further work is planned to rapidly investigate similar variables in reversed-phase and polar organic modes using Cyclodextrin and other CSPs using this batch LC-MS screening technique.
Supelco Chiral Analytical Services
Chiral Analytical Service Offerings

Chiral LC Screening Protocol

For primary screening purposes, the mobile phase compositions are as follows:

- **PI** = 100:0.1:0.1, methanol:acetic acid: triethylamine
- **RP1** = 70:30, 20 mM ammonium acetate (pH 4.0):acetonitrile
- **RP2** = 50:50, 20 mM ammonium acetate (pH 4.0):methanol
- **PO** = 95:5, acetonitrile:isopropanol (with 0.1% TEA and 0.1% TFA)
- **NP1** = 80:20, heptane: isopropanol (with 0.1% TEA and 0.1% TFA)
- **NP2** = 50:25:25, heptane: isopropanol (with 0.1% TEA and 0.1% TFA):MTBE
## Comprehensive Column Screen

<table>
<thead>
<tr>
<th>Column</th>
<th>Chromatographic Modes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI</td>
</tr>
<tr>
<td>CHIROBIOTIC® TAG</td>
<td>X</td>
</tr>
<tr>
<td>CHIROBIOTIC® V2</td>
<td>X</td>
</tr>
<tr>
<td>CHIROBIOTIC® T</td>
<td>X</td>
</tr>
<tr>
<td>CYCLOBOND®</td>
<td>X</td>
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<tr>
<td>CYCLOBOND®</td>
<td>X</td>
</tr>
<tr>
<td>CYCLOBOND®</td>
<td>X</td>
</tr>
<tr>
<td>Kromasil® CelluCoat</td>
<td>X</td>
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<tr>
<td>Kromasil® AmyCoat</td>
<td>X</td>
</tr>
<tr>
<td>Kromasil® TBB</td>
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</tr>
<tr>
<td>Kromasil® DMB</td>
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<tr>
<td>Astec (R,R) P-CAP™-DP</td>
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<tr>
<td>Astec (R,R) P-CAP™</td>
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</tr>
</tbody>
</table>
Modern Chiral Stationary Phases

Polymeric

Synthetic
- Methacrylate
- Polycyclic amine-2

Natural
- Cellulose
- Amylose
- Proteins

Small molecule ligands
- Copper complex-2
- $\pi$-complex
- Crown ether
- Cyclodextrin-12
- Macrocyclic glycopeptides-6

Supelco Phases
Following this initial run:

- If successful in providing positive separation, the conditions are verified on a separate analytical system and may include minimal optimization. The enantiomers are identified as (+) and (-) using a Chiralyser detection system.

- If the initial run is unsuccessful or marginally successful, manual method development is conducted by our laboratory experts based on their experience with similar compounds and information from the primary screen. Successful separations are verified and enantiomers are identified (+/-)

- Reports are generated and provided to the customer regardless of outcome
HPLC Chiral Screening Report

Analyte Description: (S)-Pentazocine-HCl (CAS: 84024-15-3)
Superco Sample No.: R&D Application Request #1387
Quote No.: 
Report lo: Internal

The sample has been tested through our method development protocol employing 3 Astec CHIROBIOTIC® (V2, T, TAG), 3 Astec CYCLOBOND® (I 2000 (underivatized), I 2000 DMP and I 2000 HP-RSP) and 3 normal phase (Kromasil® C8uCoat, Kromasil® TBB, Kromasil® DMB, Astec (R,R) F-CAP™-RP, and Astec (R,R) F-CAP™) columns with a combination of mobile phases encompassing polar ionic (PI), reversed-phase (RP), normal-phase (NP) and polar organic (PO) chromatographic modes of operation.

For primary screening purposes, the mobile phase compositions are as follows:
- PI = 100:0 1:0.1, methanol:acetic acid : triethylamine
- RP1 = 70:30, 20 mM ammonium acetate (pH 4.0):acetonitrile
- RP2 = 50:50, 20 mM ammonium acetate (pH 4.0):methanol
- PO = 95:5, acetonitrile:isopropanol (with 0.1% TEA and 0.1% TFA)
- NP1 = 80:20, heptane:isopropanol (with 0.1% TEA and 0.1% TFA)
- NP2 = 30:25:25, heptane:isopropanol (with 0.1% TEA and 0.1% TFA):MTBE

Results of Primary Screen:
The following combinations of stationary phase and chromatographic mode provided evidence of enantiomer selectivity:

- AmyCoat: NP1 mode
- CHIROBIOTIC T: PI mode
- CelluCoat: NP1 mode
Example Screening Report

Summary of Primary Screen

CHIROBIOTIC T
PIM Mode
Example Screening Report

Kromasil AmyCoat Normal Phase Mode
The separation observed on the AmyCoat in NP1 was then confirmed on a second system. Please refer to Figure 1 below.

Figure 1. Confirmation of the separation of (±) Pentazocine on Kromasil AmyCoat in NP1

UV

Impurities and solvent peaks
(±) Pentazocine enantiomers

Conditions:

Column: Kromasil AmyCoat, 25 cm x 4.6 mm I.D., 5 µm particles (cat. no. K08570C48)
Mobile Phase: 80:20 heptane:isopropanol (with 0.1% TEA and 0.1% TFA)
Temperature: 25 °C
Flow Rate: 1.0 mL/min
Detection: UV at 220 nm
Injection Volume: 10 µL
Sample: 0.2 mg/mL in 75:25, heptane:ethanol

Peak 1 retention time (Rt): 5.153 min.
Peak 2 retention time (Rt): 5.738 min.

To increase the resolution of the two enantiomers, the amount of isopropanol within the mobile phase was decreased to 10%. This decrease in mobile phase strength gave baseline separation of the pentazocine enantiomers, as seen in Figure 2.
In order to obtain the optical rotation of each enantiomer, a CHIRALYSER-MP polarimeter was utilized in line with the UV detector. The CHIRALYSER demonstrated the elution order to be the (+) enantiomer followed by the (-) enantiomer. Please refer to Figure 2.

Figure 2. Optimization and Elution Order Determination of (+) Pentazocine on Kromasil AmyCoat in NP1
Example Screening Report

Conditions:
Column: Kromasil Amoco, 25 cm x 4.6 mm I.D., 5 μm particles (cat. no. K03973048)
Mobile Phase: 50:10, heptane: isopropanol (with 0.1% TEA and 0.1% TFA)
Temperature: 25 °C
Flow Rate: 1.0 mL/min
Detection: UV at 220 nm
Injection Volume: 10 μL
Sample: 0.2 mg/mL in 75:25, heptane:ethanol
Peak 1 retention time (Rt): 13.300 min., Polarity: (+)
Peak 2 retention time (Rt): 17.984 min., Polarity: (-)

Recommendation for Optimization/Conclusions:

1. A primary screen of (±) pentazocine demonstrated selectivity of the enantiomers on the Kromasil Amoco in normal-phase mode. This chiral separation was then confirmed on a second system using the established conditions.

2. Decreasing the isopropanol content of the mobile phase produced a dramatic increase in resolution of the enantiomer peaks. By using the mobile phase 50:10, heptane: isopropanol (with 0.1% TEA and 0.1% TFA), baseline resolution of the enantiomers was achieved in a run lasting less than 20 minutes.

3. Optical rotation determination of the enantiomers by the CHIRALYSER-MP polarimeter showed the elution order to be the (+) enantiomer followed by the (-) enantiomer.

Analyst: Jennifer E. Claus
Date: March 31, 2010

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Chiral Analytical Service Offerings

• **GC chiral column screening:**
  – GC column screening involves manual exploration of 3-4 GC column chemistries by our laboratory experts
  – Samples that require derivatization are verified by GC-MS

• **HPLC chiral method optimization services**
  – Certain customers may be interested in obtaining enantiomerically pure materials
  – Following successful chiral screening or successful reproduction of methods provided by customer, a method optimization and loading study is conducted by our laboratory experts to establish the most efficient means of chromatographically purifying the materials
  – The output of a loading study is approximately 100 mg of purified material and the methodology utilized to obtain such material
Method Development/Optimization
Instrumentation
Small scale enantiomeric purification
- When more than 100 mg of enantiomerically pure material is required, larger scale purifications are necessary
- On the order of 1-2 g of material can be purified based on the method optimization/loading study results
- The output from this study is the enantiomerically pure material to the specifications of the customer (typically >98%) and verification of ee% purity by analytical method established in screening study

Larger scale purification:
- Currently beyond our capabilities, however, we are aiming to work with larger productions sites within Sigma-Aldrich with the goal of establishing a viable means of enabling large scale separations
Purification Instrumentation
Purification Instrumentation
Pricing Structure

Comprehensive method development screen for LC $ 1,100

Comprehensive method development screen for GC $ 950
*50% upcharge for additional chiral centers

Method development for LC and GC  by quotation

Method development, optimization and purification –100mg
$3,000.00

Purification – by quotation
Current Capabilities - Personnel

Personnel:

- Dave Bell – Administrative duties
  - Planning, quotations, monitoring of execution, nondisclosure agreements when necessary
- Jennifer Claus and Jay Jones – execution of laboratory protocols, method development, purification and recovery
- Carmen Santasania/Hugh Cramer/Kathy Stenerson – assistance to chiral services when necessary – primarily execution of chiral screening protocols and purification
- JT Lee – many years of experience in chiral chromatography
  - PhD in Chemistry with Dan Armstrong
- Tracy Ascah – Marketing – much experience in operation of contract laboratory and market awareness
- Denise Wallworth – Vast experience in chiral separations MARKET and customer awareness
Current Capabilities - Instrumentation

Instrumentation:
- LC chiral screening:
  - 2 HPLC systems have been set up for automated execution of the primary screening protocol – currently capable of running 4 screens/day with this setup. Other instruments available in case of overflow, albeit these would require manual switching/sequence building
  - One HPLC system with Chiralyser optical rotation detector used for verification, method tweaking and enantiomer identification
- GC Screening:
  - 2 GC systems including a GC/MS are available – others also available within the building, if necessary
- LC Method development and method optimization:
  - 7 HPLC systems available for method development including UV, fluorescence, RI and ELSD detection
  - 3 LC-MS units are also available
- LC Preparative:
  - 2 automated HPLC preparative systems.
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