Universal Chiral Screening System for Optimized Method Development

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Abstract

Due to the highly complex and interdependent retention mechanisms that manifest themselves into retention and enantioselectivity of chiral separations, predicting what stationary phase/mobile phase combination will provide separation for a given chiral analyte is difficult and usually impossible. In a relatively short period of time, column screening at the inception of method development provides a complete picture of possible chromatographic systems that offer selectivity of desired chiral analytes. The data acquired from screening can form the basis of many development efforts leading to analytical methods and also provide a fall back point, should unforeseen events arise.

In this report, a semiautomated HPLC chiral screening protocol that includes a combination of 12 different stationary phases and 6 different elution systems is described. The protocol includes normal-phase, polar organic, polar ionic and reversed-phase modes of elution. Stationary phases range from cellulosic/amylosic chemistries to the more polar macrocyclic glycopeptide phases, each exhibiting unique separation qualities for a wide range of analyte structures. Examples of the approach toward the development of analytical methods will be highlighted.
Objectives

The aim of this study is to screen an established set of 32 diverse chiral compounds in a newly developed reversed-phase and normal-phase chiral screening protocol to:

1. Develop a more comprehensive chiral screening protocol for customers.
2. Compare the results of the new chiral screen to those of the old screen.
3. Gain a better understanding of the highly complex chiral retention mechanisms.
Experimental

The set of racemic probes shown below were chosen to represent acidic, basic and neutral molecules of varying pKa value, molecular weight and hydrophobicity.

This set of probes was first run using a traditional chiral screening protocol that encompassed reversed-phase, polar ionic (PI) and polar organic (PO) modes using 6 different stationary phase chemistries.

The same set of probes were then run using a newly developed protocol that, in addition to the 3 modes above, also utilizes normal phase mode. In addition, the column selection was increased to 12 different chemistries. Each of the protocols are described below in detail.
Instrumentation

Waters 2690 HPLC Systems, equipped with either a 996 (NP/POM modes) or a 2996 (RP/PIM modes) PDA detector and a 6 position column changer.

- column: 25 cm x 4.6 mm I.D., 5 µm particles
- flow rate: 1 mL/min.
- temp.: ambient (NP/POM), 35 °C (RP/PIM)
- samples: 1 mg/mL in methanol (RP/PIM), 75:25 heptane:IPA (NP/POM)

Compounds Screened

- Bupropion
- Midodrine
- Normetanephrine
- DL-3,4-dihydrophenylalanine
- Pentazocine
- Verapamil
- Norfluoxetine
- Chlorpheniramine
- Synevirine
- Metoprolol
- Propranolol
- 6-Fluoro-DL-tryptophan
- DL-p-Chloramphetamine
- MDA
- Fluoxetine
- Fenfluramine
- Baclofen
- Arginine
- Mandelic Acid
- Flurbiprofen
- Fenoprofen
- Ketorolac
- Thiocic Acid
- N-Acetylhomocysteinethiolactone
- 5-Methyl-5-phenylhydantoin
- Furoin
- Ethylmandelate
- Omeprazole
- Clopidogrel
- Benzoin
- Triadimenol
- Propiconazole
Log D (7.4) Distribution
Old Screen Protocol

- 12 different chromatographic systems
- 3 mobile phase set of conditions run using 6 different stationary phase chemistries as a front line screening.

**Columns:**
- CHIROBIOTIC™
  - V2
  - T
  - TAG
- CYCLOBOND™
  - β-CD
  - DMP
  - HP-RSP

**Mobile Phases:**
- Polar-Ionic Mode (PIM)
  - 100:0.1:0.1, methanol:acetic acid:triethylamine
- Reversed-Phase (RP)
  - 70:30, 20 mM ammonium acetate (pH 4.0):acetonitrile
- Polar-Organic Mode (POM)
  - 95:5:0.3:0.2, acetonitrile:methanol: acetic acid:triethylamine.
New Screen Protocol

- 33 different chromatographic systems
- 2 instruments running simultaneously
  - Normal Phase (NP) System
  - Reversed-Phase (RP) System

Normal Phase Screen

- 18 different column mobile phase combinations
- All mobile phases run on all columns at ambient temperature

Columns:
- Kromasil® CelluCoat
- Kromasil AmyCoat
- Kromasil TBB
- Kromasil DMB
- (R,R) P-CAP-DP
- (R,R) P-CAP

Mobile Phases:
- Normal Phase 1 (NP1)
  - 80:20, heptane: isopropanol (with 0.1% TEA and 0.1% TFA)
- Normal Phase 2 (NP2)
  - 50:25:25, heptane: isopropanol (with 0.1% TEA and 0.1% TFA):MTBE
- Polar Organic Mode (POM)
  - 95:5, acetonitrile:isopropanol (with 0.1% TEA and 0.1% TFA)
New Screen Protocol (contd.)

Reversed Phase Screen
- 15 different column mobile phase combinations
- Run at 35 °C

Columns:
- CHIROBIOTIC
  - V2
  - T
  - TAG
- CYCLOBOND
  - β-CD
  - DMP
  - HP-RSP

Mobile Phases:
- Reversed-Phase1 (RP1)
  - 70:30, 20 mM ammonium acetate (pH 4.0):acetonitrile
- Reversed-Phase2 (RP2)
  - 50:50, 20 mM ammonium acetate (pH 4.0):methanol
- Polar-Ionic Mode (PIM)
  - 100:0.1:0.1, methanol:acetic acid:triethylamine
Results: Statistics of Old Screen

• 72% Success Rate*
• Success Rates of Each Column:
  – CHIROBIOTIC TAG = 28%
  – CHIROBIOTIC V2 = 27%
  – CHIROBIOTIC T = 22%
  – CYCLOBOND I 2000 = 8% (mostly in POM)
  – CYCLOBOND I 2000 HP-RSP = 20% (mostly in RP)
  – CYCLOBOND I 2000 DMP = 8% (mostly in POM)
• Mobile Phase Success Rate
  – RP = 16%
  – PIM = 34%
  – POM = 9%

* Success is interpreted as any sign of enantiomeric selectivity; not necessarily baseline separation.
Statistics of New Screen

• 94% Success Rate
• 8 compounds that showed no enantiomeric selectivity on the old screen showed selectivity on the new screen
• Success Rates of Each Column:
  – Kromasil CelluCoat = 28%
  – Kromasil AmyCoat = 33%
  – Kromasil TBB = 12%
  – Kromasil DMB = 11%
  – (R,R) P-CAP-DP = 12%
  – (R,R) P-CAP = 6%
  – CHIROBIOTIC TAG = 25%
  – CHIROBIOTIC V2 = 23%
  – CHIROBIOTIC T = 21%
  – CYCLOBOND I 2000 = 2%
  – CYCLOBOND I 2000 HP-RSP = 20%
  – CYCLOBOND I 2000 DMP = 9%
Statistics of New Screen (contd.)

Mobile Phase Success Rate

- NP1 = 28%
- NP2 = 16%
- POM = 7%
- RP1 = 10%
- RP2 = 18%
- PIM = 17%

- Of all hits, % observed in one of NP modes (NP1, NP2, or POM) = 54%.
- Of all hits, % observed in one of RP modes (RP1, RP2, or PIM) = 46%.
- Of all hits observed in RP2 and/or RP1, % of hits seen in RP2 but not RP1 = 28%.
- Of all hits observed in RP2 and/or RP1, % of hits seen in RP1 but not RP2 = 0%.
- When hits are seen in both RP2 and RP1, both resolution and selectivity in RP2 is equal to or better than that seen in RP1.
- Is RP1 really necessary for screening purposes? Could we take out RP1 and screen the Cyclobonds in POM on RP system?
Selected Observations

• Here are some of the benefits of the new protocol over the old.
• In general, more systems are identified that could lead to successful methods using the updated protocol.
  – More options to develop methods amenable to the desired outcome (i.e., preparative, LC-MS compatible).
  – More options to separate enantiomeric pairs from other impurities, diastereomers, etc.
Bupropion

Old Screen: 3 hits

<table>
<thead>
<tr>
<th>Chromatogram</th>
<th>Column</th>
<th>Mode</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chirobiotic TAG chromatogram" /></td>
<td>Chirobiotic TAG</td>
<td>PIM</td>
<td>Separation</td>
</tr>
<tr>
<td><img src="image2.png" alt="Chirobiotic V2 chromatogram" /></td>
<td>Chirobiotic V2</td>
<td>PIM</td>
<td>Separation</td>
</tr>
<tr>
<td><img src="image3.png" alt="Cyclobond 2000 HP-RSP chromatogram" /></td>
<td>Cyclobond 2000 HP-RSP</td>
<td>RP</td>
<td>Separation</td>
</tr>
</tbody>
</table>
**Bupropion** (contd.)

**New Screen: 7 hits**

<table>
<thead>
<tr>
<th>Chromatogram</th>
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<th>Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Chromatogram" /></td>
<td>Cellucoat</td>
<td>Separation</td>
<td>NP1</td>
</tr>
<tr>
<td><img src="image2.png" alt="Chromatogram" /></td>
<td>Cellucoat</td>
<td>Separation</td>
<td>NP2</td>
</tr>
<tr>
<td><img src="image3.png" alt="Chromatogram" /></td>
<td>AmyCoat</td>
<td>Separation</td>
<td>POM</td>
</tr>
<tr>
<td><img src="image4.png" alt="Chromatogram" /></td>
<td>R,R P-CAP-DP</td>
<td>Separation</td>
<td>NP1</td>
</tr>
<tr>
<td><img src="image5.png" alt="Chromatogram" /></td>
<td>R,R P-CAP-DP</td>
<td>Separation</td>
<td>NP2</td>
</tr>
<tr>
<td><img src="image6.png" alt="Chromatogram" /></td>
<td>CHIROBIOTIC TAG</td>
<td>Separation</td>
<td>PiM</td>
</tr>
<tr>
<td><img src="image7.png" alt="Chromatogram" /></td>
<td>CHIROBIOTIC T</td>
<td>Separation</td>
<td>RP2</td>
</tr>
</tbody>
</table>
Bupropion (contd.)

- 6 new hits seen in the new screen, not seen in old screen.
  - 5 new hits from NP screen
  - 4 hits in NP
    - Cellucoat in NP1 and NP2
    - (R,R) P-CAP-DP in NP1 and NP2
  - 1 in POM
    - AmyCoat in POM
  - 1 new hit from RP Screen
    - T in RP2

- Therefore, addition of new columns, NP mode, and RP2 beneficial to screen.
Propiconazole

Old Screen: 3 hits

<table>
<thead>
<tr>
<th>Chromatogram</th>
<th>Column</th>
<th>Mode</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Chromatogram" /></td>
<td>Chirobiotic TAG</td>
<td>RP</td>
<td>Separation</td>
</tr>
<tr>
<td><img src="image" alt="Chromatogram" /></td>
<td>Chirobiotic T</td>
<td>RP</td>
<td>Separation</td>
</tr>
<tr>
<td><img src="image" alt="Chromatogram" /></td>
<td>Cyclobond 2000 HP-RSP</td>
<td>RP</td>
<td>Separation</td>
</tr>
</tbody>
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## New Screen: 9 hits

<table>
<thead>
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<th>Elution</th>
<th>Mode</th>
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</thead>
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<tr>
<td></td>
<td>Cellucoat</td>
<td>Separation</td>
<td>NP1</td>
</tr>
<tr>
<td></td>
<td>Cellucoat</td>
<td>Separation</td>
<td>NP2</td>
</tr>
<tr>
<td></td>
<td>Amycoat</td>
<td>Separation</td>
<td>NP1</td>
</tr>
<tr>
<td></td>
<td>CHIROBIOTIC TAG</td>
<td>Separation</td>
<td>RP1</td>
</tr>
<tr>
<td></td>
<td>CHIROBIOTIC TAG</td>
<td>Separation</td>
<td>RP2</td>
</tr>
<tr>
<td></td>
<td>CHIROBIOTIC T</td>
<td>Separation</td>
<td>RP1</td>
</tr>
</tbody>
</table>
Propiconazole (contd.)

New Screen: 9 hits (contd.)

- 7 new hits not seen in the old screen
  - 3 hits on the NP system
  - 4 new hits on RP2 systems

- Therefore, addition of new columns, NP mode, and RP2 mode beneficial to screen.
Pentazocine

Results of Primary Screen:

The following systems provided evidence of enantiomeric selectivity:

• AmyCoat: NP1 mode
• CHIROBIOTIC T: PI mode
• CelluCoat: NP1 mode
Confirmation of the Separation of Pentazocine

- **Column:** Kromasil AmyCoat, 25 cm x 4.6 mm I.D., 5 µm particles
- **Mobile phase:** 90:10, heptane: isopropanol (with 0.1% TEA and 0.1% TFA)
- **Flow rate:** 1.0 mL/min.
- **Temp.:** 25 °C
- **Det.:** UV at 220 nm
- **Injection:** 10 µL
- **Sample:** 0.2 mg/mL in 75:25, heptane:ethanol
Conclusions

• The new chiral screening protocol, using 33 combinations of columns and mobile phases, shows a much greater success rate than the old screening protocol, using 12 combinations of mobile phases and columns (94% vs. 72%).
• The newly added NP mobile phases and columns account for 54% of the hits observed.
• Room for improvement still:
  – The addition of RP2 within the screening system was very beneficial to the screen.
    – Of the hits seen in RP (excluding PIM), there were 28% more hits seen in RP2 than RP1.
    – There were no hits seen in RP1 that were not seen in RP2.
    – In all cases observed, both resolution and selectivity in RP2 is equal to or better than that seen in RP1.
  – Success rate on the Chirobiotics in the old screen is a little higher than that in new screen, especially in PIM. Maybe because temperature ambient in old screen and 35 °C in new screen. Decreasing the temperature may improve the success rate of the new screen.
  – In the old screen, enantiomeric selectivity on both the CYCLOBOND I 2000 and the DMP was observed mostly in POM mode. Running POM mode on the CYCLOBONDS in the new screening protocol would improve the success rate on the CYCLOBONDS.