HPLC Enantiomeric Separations of Pharmaceuticals Using Polar Organic Mobile Phases

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Agenda

• Background
• Benefits
• Mechanisms
• Separation Comparisons
• LC-MS Applications
• Optimization
• Screen Results
• Summary
• Conclusions
Background

Polar Organic Mode (POM):
- Astec CYCLOBOND™ (1989) (e.g. 95/5/0.3/0.2, CH₃CN/MeOH/HOAc/TEA)
  - Acetonitrile is a dominant solvent
  - Acid/base additives are to suppress ionization
  - Samples have at least 2 H-bonds capability
- Astec CHIROBIOTIC® (neutral molecules)
- Astec P-CAP, P-CAP-DP
- Cyclofructans
- Polysaccharides (e.g. ASTEC Cellulose DMP)

Polar Ionic Mode (PIM):
- Astec CHIROBIOTIC (2003) (e.g. 100/0.1/0.1, MeOH/HOAc/TEA)
  - Methanol is a dominant solvent
  - CSPs have ionic character
  - Acid/base additives promote ionic interactions for ionizable samples
  - ASTEC CHIROBIOTIC V2
Benefits of Polar Organic Mode (POM)

Selectivity
• Conformational changes of CSPs
• Different interaction mechanisms

Sensitivity
• Less baseline noise in UV detection
• LC-MS compatible for biological samples

Solubility
• Easy sample prep
• Easy scale-up
Mechanism 1: Astec CYCLOBOND CSPs

Reversed phase mode: the most hydrophobic portion of the molecule will form an inclusion complex with the cyclodextrin cavity.

Polar organic mode: CH$_3$CN occupies the cavity, so the chiral molecule lies across the surface and interacts with the upper rim of the cyclodextrin ring.
Mechanism 2: Astec CHIROBIOTIC CSPs

- Macrocyclic glycopeptides provide a multi-modal chiral surface capable of a wide variety of different interactions
- Subtle differences between them provide different, dominant retention mechanisms that lead to enantiomeric recognition
- Among these mechanisms, ionic interactions dominate for ionizable molecules
- A family of 6 columns
- Macrocyclic glycopeptide CSPs provide unique separations for polar, ionic molecules

Vancomycin (CHIROBIOTIC V2/V)
Mechanism 3: Cellulose DMPC Derivative

Cellulose, a linear polymer of D-glucose linked by $\beta(1\rightarrow4)$-glycosidic bonds with several hundreds to over ten thousand units.

DMPC, 3,5-Dimethylphenyl carbamate derivatized cellulosic phase coated onto silica.

hydrogen bonding & dipole stacking

$\pi-\pi$

steric interactions
From NP to POM: Cellulose DMP (Warfarin)

15 cm x 4.6 mm; Flow Rate: 0.5 mL/min; UV: 278 nm

50/50/0.1, EtOH/Hexane/TFA
Selectivity: 2.44
Resolution: 1.61

100/0.1, MeOH/TFA
Selectivity: 1.71
Resolution: 2.34

100/0.1, MeOH/DEA (or IPAmine)
Selectivity: 1.00
Resolution: N/A

100/0.1w%, MeOH/NH$_4$ formate
Selectivity: 1.13
Resolution: 1.40

100/0.2/0.1, MeOH/HOAc/TEA
Selectivity: 1.31
Resolution: 3.71
Separation Comparison: Warfarin

CYCLOBOND I 2000, 25 cm x 4.6 mm
100/0.3/0.2, CH$_3$CN/HOAc/TEA
Flow Rate: 1.0 mL/min, UV: 278 nm

CHIROBIOTIC V, 25 cm x 4.6 mm
30/70, CH$_3$CN/5 mM NH$_4$OAc, pH 4.1
Flow Rate: 1.0 mL/min, UV: 278 nm

Cellulose DMP, 15 cm x 4.6 mm
100/0.2/0.1, MeOH/HOAc/TEA
Flow Rate: 0.5 mL/min, UV: 278 nm

S-form more potent, anticoagulant
Separation Comparison: Mianserin

Cellulose DMP, 15 cm x 4.6 mm
10/90/0.1, IPA/Heptane/DEA
Flow Rate: 0.5 mL/min, UV: 230 nm

Spiked R(-) S(+)

Cellulose DMP, 15 cm x 4.6 mm
100/0.1w%, MeOH/NH₄ formate
Flow Rate: 0.5 mL/min, UV: 230 nm

R(-) S(+) S(+)

CHIROBIOTIC V2, 25 cm x 4.6 mm
100/0.1w%, MeOH/NH₄ formate
Flow Rate: 0.8 mL/min, UV: 230 nm

S-active form, antidepressant
Separation Comparison: Tröger’s Base

Cellulose DMP, 15 cm x 4.6 mm
10/90/0.1, IPA/Heptane/DEA
Flow Rate: 0.5 mL/min, UV: 230 nm

Cellulose DMP, 15 cm x 4.6 mm
100/0.1 w%, MeOH/NH₄ formate
Flow Rate: 0.5 mL/min, UV: 230 nm

CHIROBIOTIC V2, 25 cm x 4.6 mm
100/0.1 w%, MeOH/NH₄ formate
Flow Rate: 1.0 mL/min, UV: 230 nm
Polar Organic Mode-Cellulose DMP

100/0.1, MeOH/DEA
Flow Rate: 0.5 mL/min
UV: 230 nm

Diperodon

Homatropine

Indapamide

Mianserin

Tröger’s base
Polar Organic Mode-Cellulose DMP

100/0.1w%, MeOH/NH₄Formate (LC-MS compatible)
Flow Rate: 0.5 mL/min
UV: 230 nm

- **Diperodon**
- **Homatropine**
- **Indapamide**
- **Mianserin**
- **Tröger’s base**
Polar Organic Mode-Cellulose DMP

100% CH₃CN (No additives)
Flow Rate: 0.5 mL/min
UV: 230 nm

Diperodon

Ketoconazole

Mianserin
**Cellulose DMP: NP→POM→NP→POM**

- **Dimension:** 15 cm x 4.6 mm
- **Flow Rate:** 0.5 mL/min
- **Temperature:** 25 °C
- **UV:** 254 nm
- **Samples:** trans-stilbene oxide (NP)/mianserin (POM)

**NP:**
- Pressure: 17 bar
- N eff (P1): 15896
- Selectivity: 1.92
- Resolution: 11.28

**POM:**
- Pressure: 23 bar
- N eff (P1): 13736
- Selectivity: 1.39
- Resolution: 4.19

**NP:**
- Pressure: 17 bar
- N eff (P1): 15583
- Selectivity: 1.87
- Resolution: 10.60

**POM:**
- Pressure: 23 bar
- N eff (P1): 13878
- Selectivity: 1.40
- Resolution: 4.30
Optimization: CHIROBIOTIC (Acid/Base Ratio Effect)

Mianserin

CHIROBIOTIC V2
25 cm x 4.6 mm
1 mL/min
UV: 230nm

100/0.1/0.02, MeOH/HOAc/NH₄OH
16.551

100/0.1/0.05, MeOH/HOAc/NH₄OH
19.579

100/0.1/0.1, MeOH/HOAc/NH₄OH
7.922
Optimization: CHIROBIOTIC (Salt Effect)

Nicardipine

CHIROBIOTIC V2
25 cm x 4.6 mm
1 mL/min
UV: 230 nm

100/0.1w%, MeOH/NH₄ acetate

100/0.1w%, MeOH/NH₄ formate

100/0.05w%, MeOH/NH₄ TFA
Optimization: Polysaccharides (Solvent Effect)

**Ketoconazole**

100/0.1w%, MeOH/NH$_4$formate

Cellulose DMP
15 cm x 4.6 mm
0.5 mL/min
UV: 230 nm

100% CH$_3$CN
### Full Screen Results-1

<table>
<thead>
<tr>
<th>Basic Pharmaceuticals</th>
<th>Cellulose DMP Normal Phase ( k_t )/Selectivity</th>
<th>Cellulose DMP Polar Organic Mode ( k_t )/Selectivity</th>
<th>CHIROBIOTIC V2 Polar Ionic Mode ( k_t )/Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>0.06/1.33</td>
<td>0.18/1.00</td>
<td>3.54/1.00</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>0.86/1.00</td>
<td>0.23/1.00</td>
<td>0.31/1.34</td>
</tr>
<tr>
<td>Citalopram</td>
<td>2.75/1.14</td>
<td>0.26/1.00</td>
<td>2.37/1.12</td>
</tr>
<tr>
<td>Clenbuterol</td>
<td>1.34/1.00</td>
<td>0.03/1.00</td>
<td>1.02/1.22</td>
</tr>
<tr>
<td>Diperodon</td>
<td>No elution</td>
<td>0.73/3.89</td>
<td>0.66/1.00</td>
</tr>
<tr>
<td>Disopyramide</td>
<td>1.65/1.07</td>
<td>0.11/1.02</td>
<td>1.08/1.14</td>
</tr>
<tr>
<td>Esmolol</td>
<td>3.36/1.57</td>
<td>0.09/1.25</td>
<td>1.34/1.12</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>1.09/1.08</td>
<td>0.07/1.02</td>
<td>2.00/1.24</td>
</tr>
<tr>
<td>Homatropine</td>
<td>2.40/1.62</td>
<td>0.08/2.04</td>
<td>0.13/1.00</td>
</tr>
<tr>
<td>Hydroxyzine</td>
<td>1.16/1.23</td>
<td>0.40/1.10</td>
<td>0.71/1.00</td>
</tr>
<tr>
<td>Indapamide</td>
<td>No elution</td>
<td>0.37/2.27</td>
<td>0.26/1.00</td>
</tr>
<tr>
<td>Ketamine</td>
<td>0.80/1.14</td>
<td>0.48/1.00</td>
<td>0.27/1.00</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>No elution</td>
<td>4.31/1.06</td>
<td>0.31/1.00</td>
</tr>
</tbody>
</table>

**CHIROBIOTIC V2**

- Polar Ionic Mode
  - 100/0.1w%, MeOH/NH₄ formate
- 10/90/0.1, IPA/Heptane/DEA

**Basic Pharmaceuticals**

- 100/0.1w%, MeOH/NH₄ formate
- 10/90/0.1, IPA/Heptane/DEA
### Full Screen Results-2

<table>
<thead>
<tr>
<th>Basic Pharmaceuticals</th>
<th>Cellulose DMP Normal Phase $k_r$/Selectivity</th>
<th>Cellulose DMP Polar Organic Mode $k_r$/Selectivity</th>
<th>CHIROBIOTIC V2 Polar Ionic Mode $k_r$/Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mefloguine</td>
<td>1.59/1.19</td>
<td>0.07/1.00</td>
<td>2.86/1.36</td>
</tr>
<tr>
<td>Methocarbamol</td>
<td>No elution</td>
<td>0.30/1/35</td>
<td>1.08/1.00</td>
</tr>
<tr>
<td>Methoxyphamine</td>
<td>0.86/1.21</td>
<td>0.07/1.00</td>
<td>1.52/1.16</td>
</tr>
<tr>
<td>Metoprolol</td>
<td>1.25/2.66</td>
<td>0.08/1.38</td>
<td>1.22/1.12</td>
</tr>
<tr>
<td>Mianserin</td>
<td>0.79/1.23</td>
<td>0.96/1.26</td>
<td>0.65/1.98</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>No elution</td>
<td>1.91/1.13</td>
<td>No Elution</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>No elution</td>
<td>1.62/1.07</td>
<td>1.02/1.00</td>
</tr>
<tr>
<td>Promethazine</td>
<td>0.58/1.05</td>
<td>0.47/1.00</td>
<td>1.76/1.68</td>
</tr>
<tr>
<td>Propranolol</td>
<td>2.36/2.22</td>
<td>0.16/1.24</td>
<td>1.60/1.16</td>
</tr>
<tr>
<td>Ritalin</td>
<td>0.66/1.09</td>
<td>0.16/1.00</td>
<td>1.32/1.45</td>
</tr>
<tr>
<td>Thalidomide</td>
<td>No elution</td>
<td>1.20/1.00</td>
<td>0.47/2.97</td>
</tr>
<tr>
<td>Tolperisone</td>
<td>0.41/1.00</td>
<td>0.27/1.00</td>
<td>1.14/1.24</td>
</tr>
<tr>
<td>Troger's base</td>
<td>0.78/1.22</td>
<td>1.33/1.28</td>
<td>0.18/1.00</td>
</tr>
</tbody>
</table>
Summary

Macrocyclic glycopeptides and polysaccharide CSPs can be complementary to one another using polar organic mobile phases

Suggested Sample Screen: 100/0.1w%, MeOH/NH₄ formate

- Astec CHIROBIOTIC V2 and T (TAG)
- Astec Cellulose DMP and “AD”-type phases
- Other CSPs
  - Different derivatives of polysaccharides
  - Immobilized polysaccharides
  - Astec P-CAP (adds 50-70% CH₃CN)
  - Cyclofructans (adds 30-50% CH₃CN)
  - Cinchona alkaloid ion exchange CSP (adds 30-50% CH₃CN)
  - Others
Conclusions

• Polar organic mobile phases provide additional opportunities for chiral selectivity should other types of mobile phases fail

• PIM/POM provide easy sample preparation for polar/ionizable compounds

• No memory effect (quick equilibration)

• LC-MS compatible mobile phases

• Easy scale-up for prep purification

• Straight-forward optimization steps