Chiral Purification using Macrocyclic Glycopeptide CSPs with Simulated Moving Bed Technology

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Chirality has long been regarded as one of the critical issues in the discovery and drug design processes of the pharmaceutical and biotechnologies industries. During the early development stages, the pharmacokinetic aspects of the chiral drugs need to be addressed because each enantiomer can behave differently in terms of absorption, distribution, metabolism, and excretion in clinical studies. Therefore, any chiral molecules under development, small amounts of pure enantiomers are needed for such studies.

LC chiral stationary phases (CSPs), made by bonding macrocyclic glycopeptides, have demonstrated wide chiral selectivity and excellent robustness since their introduction in 1995.
The advances of simulated moving bed (SMB) technologies have been successfully applied for many large-scale binary separations of sugars, hydrocarbons and other small molecules. Thus, a small scale, bench-top SMB would expand the use of this powerful separation technique in obtaining gram quantities of enantiomers in a very short period of time.

- This presentation will explore the methodology of utilizing macrocyclic glycopeptide CSPs in a bench-top SMB prototype system to purify chiral pharmaceutical drugs. Various mobile phase systems will be employed. The advantages of this approach over traditional batch preparative chromatography will be discussed in terms of overall throughput, purity of enantiomers, and the reduction of solvent waste. The ease of method development and ruggedness of this system plus the chiral stationary phases will also be demonstrated.
Success Factors for Chiral Prep Applications

- Selectivity
- Solubility
- Sample Recovery
- Solvent Composition/Consumption
- Throughput/Productivity
- Robustness
True Moving Bed (TMB) Chromatography

The switch time in Zones 2 and 3 must be greater than the residence time of B and less than the residence time of A.
SMB Introduction

• 1961 Broughton and Gerhold (UOP), US patent
  - Petrochemical industry
  - Sugars, amino acids purification
• 1989-present, large scale chiral purifications
  - Novasep and Knauer etc.
• Continuous countercurrent chromatography
• 4/8/12/16 HPLC columns-in 4 or 3 zones
• Each column has 2 Inlets (Feed/Eluent), 2 outlets (Extract/Raffinate) and a connection valve between columns
• Continuous valves/ports switching at a fixed time interval in the direction of the eluent flow
• Binary separations
Batch vs. SMB

- Batch process requires >10,000 plates to achieve baseline separation
- SMB uses <500 plates and works with very little separation.

Optimized Batch Chromatography

Separation zone = 10 % ?

Optimized SMB

Separation zone = 50-70 %

Graphic courtesy of Novasep Inc.
Classical 2-2-2-2 SMB Configuration

- **Feed** (A + B)
- **Raffinate** (B + D)
- **Desorbent (Eluent)** (D)

**Zone 1**
- Desorption of A, Cleaning of adsorbent

**Zone 2**
- Enrichment of A
- Direction of fluid flow and port switching

**Zone 3**
- Enrichment of B

**Zone 4**
- Cleaning of desorbent

**Extract** (A + D)

A is slower moving component
B is faster moving component
Modified 3-2-3 Configuration for Chiral Application

**Raffinate (Peak 1)**
(B + D)

**Feed**
(A + B)

**Zone 1**
Desorption of A, Cleaning of adsorbent

**Zone 2**
Enrichment of A

**Zone 3**
Enrichment of B

**Extract (Peak 2)**
(A + D)

**Desorbent (Eluent)**
(D)
Advantages of 3 Zones for Small-Scale SMB

- No need to recycle desorbent
- Flow of Zone 3 into Zone 1 prevented
- Maximizes options for configuration in Zones 1-3
- Simplifies flow rate optimization
Semba Octave Chromatography System

- A versatile **bench-top** multi-column chromatography system
- Capable of performing SMB and other continuous automated separation protocols
- Suitable for milligram to gram scale purification
- Chiral or protein purification
Key Features

• Eight column positions, accommodates a variety of column sizes
• Proprietary valve block design minimizes dead volume
• Four available inlet and four available outlet channels per column, plus shut-off between columns
• Up to 270 psi operating pressure
• Four independently-controlled pumps
• Non-metallic flow path, compatible with chemical and biological samples and solvents
• Easy-to-use software interface for valve and pump control
Key Measurements to Determine SMB Conditions

• Column properties
  - Single column volume \( V \)
  - Extra-column dead volume \( V^D \)
  - Retention time of inert tracer at flow rate \( Q = t_0 \)

• Sample properties
  - Analyte resolution, solubility, viscosity
  - Retention time of A at flow rate \( Q = t^{R_A} \)
  - Retention time of B at flow rate \( Q = t^{R_B} \)
  - Loading studies (expecting nonlinear adsorption isotherms)
Key Parameters for SMB Conditions

• Switch time: $t^*$
• External flow rates: $Q_{\text{Eluent}} + Q_{\text{Feed}} = Q_{\text{Extract}} + Q_{\text{Raffinate}}$
• 3-zone internal flow rates – simple to calculate
  - $Q_1 = Q_{\text{Eluent}}$
  - $Q_2 = Q_1 - Q_{\text{Extract}}$
  - $Q_3 = Q_2 + Q_{\text{Feed}}$
Henry Constants Determination

\[ H_i = \left( \frac{t_{R_i} - t_o}{t_o} \right) \times \frac{\varepsilon}{(1 - \varepsilon)} \]

(Selectivity = \( H_2/H_1 \))

HPLC RUN

\( t_{R_i} \) = retention time of component i (small pulse)

\( t_o \) = retention time of inert tracer

\( \varepsilon \) = overall void fraction of column = \( t_o \times Q/V \),

where \( V \) = column volume, \( Q \) = flow rate
Equilibrium Theory: under ideal conditions separation depends on generalized flow rate ratios in the 4 zones

\[ q_{i} = H_{i} c_{i} \rightarrow t_{Ri} = t_{M} \left[ \frac{1 + (1 - \varepsilon_{\text{total}})}{\varepsilon_{\text{total}}} H_{i} \right] \quad \text{[Nicoud, 1992]} \]

- \( t_{A1} \leq t_{\text{switch}} \)
- \( t_{B2} \leq t_{\text{switch}} \leq t_{A2} \)
- \( t_{B3} \leq t_{\text{switch}} \leq t_{A3} \)
- \( t_{\text{switch}} \leq t_{B4} \)

Equilibrium Theory:

\[
m_{j} = \frac{Q_{j}^{\text{SMB}} t_{\text{switch}} - V \varepsilon_{\text{total}}}{V (1 - \varepsilon_{\text{total}})}
\]

- \( H_{A} < m_{1} < \infty \)
- \( H_{B} < m_{2} < H_{A} \)
- \( H_{B} < m_{3} < H_{A} \)
- \( m_{4} < H_{B} \)

\( H_{i} \): Henry coefficient – linear adsorption coefficient

\( m_{j} \): flow rate ratio in zone \( j \)

\( Q_{j}^{\text{SMB}} \): volume flow rate in SMB zone \( j \)

Migliorini, Morbidelli et al. (2)
\[ m_3 > m_2 \]
\[ H_B < m_2 < H_A \]
\[ H_B < m_3 < H_A \]

\[ m_j = \frac{Q_j^{SMB} t_{\text{switch}} - V \varepsilon_{\text{total}}}{V(1 - \varepsilon_{\text{total}})} \]

W = optimal operating point = maximal productivity
Practical Effects of Parameters ($m_2/m_3$) Adjustment

**Eluent flow/Switch time**
- Increase
- Decrease

**Extract flow rate**
- Increase
- Decrease

**Feed flow rate**
(Affecting $m_3$ only)
- Increase
- Decrease

Mathematical equation:

\[ m_j = \frac{Q_j^{SMB} t_{\text{switch}} - V \varepsilon_{\text{total}}}{V (1 - \varepsilon_{\text{total}})} \]
Sample Overload: Langmuirian or anti-Langmuirian?

Fig. 7.27: Qualitative trends: peak shapes from loading studies and corresponding separation regions in the $M_g$, $M_p$ plane. Left: Langmuirian behavior; middle: Anti-Langmuirian behavior; right: Langmuirian for the second eluted enantiomer; Anti-Langmuirian for the first eluted enantiomer.
Experimental 1. HPLC Results

column: Astec CHIROBIOTIC™ V2, 25 cm x 4.6 mm, 5 μm
mobile phase: 100% MeOH
flow rate: 1.0 mL/min.
sample: 5-methyl 5-phenylhydantoin (2 mg/mL)

Racemic (green)
Extract (blue): >95% pure
Raffinate (red): >99% pure
Experimental 1. SMB Results

- Compound: 5-methyl 5-phenylhydantoin (Selectivity = 1.39)
- Eluent: 100% CH₃OH
- Column (x8): Astec CHIROBIOTIC V2, 5 cm x 10 mm, 15 µm particles
- Henry constant A: 0.80
- Henry constant B: 1.11
- Recovery: 90%
- Results:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Feed Flow</th>
<th>Productivity</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/mL</td>
<td>0.15 mL/min.</td>
<td>20 mg/hr. each enantiomer</td>
<td>Raffinate: 99.8% Extract: 95.7%</td>
</tr>
<tr>
<td>20 mg/mL</td>
<td>0.12 mL/min.</td>
<td>35 mg/hr. each enantiomer</td>
<td>Raffinate: 99.5% Extract: 93.5%</td>
</tr>
<tr>
<td>60 mg/mL</td>
<td>0.15 mL/min.</td>
<td>60 mg/hr. Extract only</td>
<td>Raffinate: 65% Extract: 93.0%</td>
</tr>
</tbody>
</table>

- Throughput of batch chromatography (stacked injections) is 1.5 mg/g CSP/hr.
Typical Concentration Profile from Collections

- **Raffinate**
- **Extract**

Area counts vs Cycles

Stop feeding sample
Experimental 2. HPLC Results

- Sample: Tolperisone (2 mg/mL)
- Mobile Phase: 100/0.1/0.1, MeOH/HOAc/TEA
- Column: Astec CHIROBIOTIC V2, 3 cm x 4.6 mm, 5 μm
- Flow Rate: 0.5 mL/min.

**Racemic (green)**

**Extract (blue):** 94.5% pure

**Raffinate (red):** 75.2% pure
Experimental 2. SMB Results

- Compound: Tolperisone (Selectivity = 1.36)
- Eluent: 100/0.1/0.1 (v/v/v), MeOH/HOAc/TEA
- Column (x8): Astec CHIROBIOTIC V2, 5 cm x 10 mm, 15 μm
- Henry constant A: 3.66
- Henry constant B: 4.96
- Recovery: 80%

- Results:

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Feed Flow</th>
<th>Throughput</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 mg/mL</td>
<td>0.2 mL/min.</td>
<td>0.7 mg/g CSP/hr.</td>
<td>Raffinate: 99.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Extract: 98.0%</td>
</tr>
<tr>
<td>10 mg/mL</td>
<td>0.2 mL/min.</td>
<td>3 mg/g CSP/hr.</td>
<td>Raffinate: 75.2%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Extract: 94.5%</td>
</tr>
</tbody>
</table>

- Throughput of batch chromatography (stacked injections) is 2 mg/g CSP/hr.
Conclusions

• Bench-top SMB is a viable option for small scale (grams quantity) chiral purification with quick turnaround time
• Mobile phase design on Astec CHIROBIOTIC CSPs is suitable for SMB application
  - Both polar organic and polar ionic mode provide good separation with high efficiency and low pressure drop
  - Consisting of 100% methanol, these two mobile phase types do not have sample solubility issues
  - Astec CHIROBIOTIC columns are very rugged and reproducible
• Compared to batch chromatography, SMB provides greener process
  - Less solvent consumption
  - Less sample recovery time
  - Higher throughput
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References