Preparative Chiral Separations and Scale-Up

From Analytical Method Scouting to Preparative Separation
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Polysaccharide Chiral Selectors

Polysaccharides: CHIRALPAK® (Coated Amylose and Immobilized Columns) and CHIRALCEL® (Coated Cellulose)

Normal, Polar Organic, and Reversed Phases, and SFC

✓ First Generation COATED Columns
  AD, AS, AY, AZ, OA, OB, OC, OD, OF, OG, OJ, OK, OX, OZ

✓ Second Generation IMMOBILIZED COLUMNS (Compatible with forbidden normal phase solvents)
  IA, IB, IB-N, IC, ID, IE, IF, IG, IH, IJ, IK
Commercial Applications

- Sertraline (Zoloft) can be resolved under \(n\)-hexane/2-propanol conditions with coated amylose \(tris(3,5\text{-dimethylphenylcarbamate})\).
- Escitalopram (Lexapro) produced with coated amylose \(tris(3,5\text{-dimethylphenylcarbamate})\).
- Levetiracetam (Keppra) produced with coated cellulose \(tris(3,5\text{-dimethylphenylcarbamate})\).
- Radafaxine, which is a potent metabolite of bupropion.
  - \((R)\)-modafinil or Armodafinil was produced for a time with coated amylose \(tris(3,5\text{-dimethylphenylcarbamate})\) with 100% methanol.
    - Required for toxicological and clinical studies. LC (and eventually SMB) conditions were developed on which resulted in productivity of 0.48 kg of racemate kg\(^{-1}\) of CSP and per day.
    - In total, more than 600 kg of racemate were processed via this process.
Start From the Beginning – Method Screening

• Screening provides greatest opportunity to find appropriate separation conditions
  • Changes to the phase system have the greatest effect on selectivity

• Limited mobile phases – solubility is very important
  • High % of hexane generally negatively affects solubility, however it might be required based on selectivity
  • If required, often the addition of DCM can help increase solubility (only for immobilized CSPs)

• Screen on 5 µm (4.6x250mm) columns – 5 µm is smallest particle size for preparative separations
  • Easier transferability – more on this to come!
Start From the Beginning – Method Screening

Conditions
Mobile Phase = 70-30-0.1 = Hex-EtOH-DEA
Flow Rate = 1 ml/min
Sample = 1 mg/ml in EtOH
Start From the Beginning – Method Screening

---ACN=100%  alpha=2.94
---ACN/MeOH=98/2  alpha=2.49
---ACN/MeOH=95/5  alpha=2.02
Start From the Beginning – Method Screening

- CHIRALPAK® IE-5 provided best selectivity

- Need to check solubility and loading to determine productivity
Solubility and Loading

- Solubility of compound in 70-30-0.1 = Hex-EtOH-DEA was 65.20 mg/ml

- Loading is performed using the “touching-band” approach
  - Using a concentrated sample, make increasingly larger injection volumes until the back of Peak 1 touches the front of Peak 2.
  - Can increase the wavelength of detection to load more material on the column – prevents the detector from being swamped.

**Loading Injections**
Blue = 5 µl injection of 65.20 mg/ml
Red = 100 µl injection of 65.20 mg/ml
Green = 125 µl injection of 65.20 mg/ml
Solubility and Loading

- If we don’t look at a higher wavelength, we might falsely believe we’re overloaded

**Loading Injections**
Blue = 5 µl injection of 65.20 mg/ml
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Green = 125 µl injection of 65.20 mg/ml
Solubility and Loading

• If we don’t look at a higher wavelength, we might falsely believe we’re overloaded

Loading Injections
Blue = 125 µl injection at 230 nm
Red = 125 µl injection at 365 nm
Productivity

- With solubility, injection volume, and cycle time, we can calculate productivity

<table>
<thead>
<tr>
<th>CT</th>
<th>21010</th>
</tr>
</thead>
</table>

### Solubility

<table>
<thead>
<tr>
<th>MP Composition</th>
<th>Hex</th>
<th>ETOH</th>
<th>DEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max</td>
<td>70.00</td>
<td>30.00</td>
<td>0.10</td>
</tr>
</tbody>
</table>

- Weight, mg: 32.60
- Volume, ml: 0.50

### Loading

<table>
<thead>
<tr>
<th>CSP</th>
<th>Particle Size, μm</th>
<th>Column Length, mm</th>
<th>Column ID, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE</td>
<td>5</td>
<td>250</td>
<td>4.6</td>
</tr>
</tbody>
</table>

- Cycle Start, min: 6.20
- Cycle End, min: 13.30
- Cycle Time, min: 7.1
- Injection Volume, μl: 125.00
- Sample Concentration, mg/ml: 65.2

- Solubility, mg/ml: 65.20
- Productivity, mg/hr: 66.99

Comments: Solubility could be higher.
Stacked Injections

First injection

Direction of mobile phase

Direction of mobile phase
Stacked Injections

Second injection

Direction of mobile phase

Direction of mobile phase
Stacked Injections

Third injection

Direction of mobile phase

Great – but will it work in the real world?
Stacked Injections

150 ml/min
675 mg / injection
Acetonitrile
Cycle time 4 min
T = 25°C
10.1 g/hr

Column : CHIRALPAK AD
20 µm, 500 x 50 mm
Analytical Productivity Scaled to Preparative Dimensions

- We choose 5 µm 4.6x250 mm length columns for screening and loading because the productivity of a method scales proportionally to the ratio of the column internal diameters (assuming the lengths are the same).

- Scaling Factor = \((\text{Column i.d. #1})^2 / (\text{Column i.d. #2})^2\)

- For a 1 cm prep column, scaling factor is 4.7
  - \((1 \text{ cm})^2 / (0.46 \text{ cm})^2\)

- A 2 cm = 21.2, a 3 cm = 42.5, a 5 cm = 118.1, and an 11 cm = 571.8
Analytical Productivity Scaled to Preparative Dimensions

• For the given method example, the productivity on a 4.6x250 mm analytical column was 66.99 mg/hr, or 621 days for 1 kg

• For a 1 cm prep column, one could achieve 314.8 mg/hr, or 132 days for 1 kg
• For a 2 cm prep column, one could achieve 1.42 g/hr, or 29 days for 1 kg
• For a 3 cm prep column, one could achieve 2.85 g/hr, or 14.6 days for 1 kg
• For a 5 cm prep column, one could achieve 7.91 g/hr, or 5.3 days for 1 kg
• For a 11 cm prep column, one could achieve 38.3 g/hr, or 1.1 days for 1 kg
System Requirements

• Flow rates and injection volumes scale by the same scaling factors – need a system that can achieve the equivalent flow rate and injection volume of the desired column i.d.

• 11 cm column would produce/require 570 ml/min of mobile phase – typically requires large infrastructure to remove solvent from resulting fractions

• 5 cm column would be 118 ml/min, which might be manageable on 20 L rotavaps

• Smaller columns likely manageable on 20 L rotavaps, or something smaller
System Requirements

- A system that can achieve ~120 ml/min can will cover 1 cm prep columns up to 5 cm prep columns.

- Should be able to perform stacked injections to maximize cycle times

- Should be able to inject ~15 ml of sample feed
Start From the Beginning – Method Screening

Conditions
Mobile Phase = 70-30-0.1 = Hex-IPA-DEA
Flow Rate = 1 ml/min
Sample = 1 mg/ml in EtOH
Start From the Beginning – Method Screening

Conditions
Mobile Phase = 60-35-5-0.1 = Hex-IPA-MeOH-DEA
Flow Rate = 1 ml/min
Sample = 1 mg/ml in EtOH
Analytical Scaling to Preparative Separations

CHIRALPAK® IB N-5
MP: 60-35-5-0.1 = Hex-IPA-MeOH-DEA
Flow rate: 1 ml/min
Sample: 30.95 mg/ml in MP
Injection volume:
- 2 µl (0.06 mg) – Blue
- 30 µl (0.93 mg) – Red
- 75 µl (2.32 mg) – Green
- 100 µl (3.1 mg) – Pink
- 125 µl (3.9 mg) – Yellow
Detection: UV 230 nm
Column Dimensions: 250 mm L x 4.6 mm i.d.

Detection: UV 230 nm
Column Dimensions: 250 mm L x 4.6 mm i.d.
Analytical Scaling to Preparative Separations

CHIRALPAK® IB N-5
MP: 60-35-5-0.1 = Hex-IPA-MeOH-DEA
Flow rate: 1 ml/min
Sample: 30.95 mg/ml in MP
Injection volume: 125 µl (3.9 mg)
Detection: UV 230 nm (Red)
        UV 330 nm (Blue)
Column Dimensions: 250 mm L x 4.6 mm i.d.
### Analytical Scaling to Preparative Separations

<table>
<thead>
<tr>
<th>Solubility</th>
<th>Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP Composition</td>
<td>CSP</td>
</tr>
<tr>
<td>Hex</td>
<td>I-PrOH</td>
</tr>
<tr>
<td>60.00</td>
<td>35.00</td>
</tr>
</tbody>
</table>

- **Weight, mg**: 30.95
- **Volume, ml**: 1.00
- **Cycle Start, min**: 4.6
- **Cycle End, min**: 7.60
- **Cycle Time, min**: 3
- **Injection Volume, μl**: 125.00
- **Sample Concentration, mg/ml**: 30.95

**Solubility, mg/ml**: 30.95

**Productivity, mg/hr**: 77.38

- 77.38 mg/hr on a 4.6 mm i.d. analytical column
- 363.7 mg/hr on a 1 cm i.d. semi-prep column
- 1.64 g/hr on a 2.1 cm i.d. prep column
- 3.28 g/hr on a 3 cm i.d. prep column
- 9.2 g/hr on a 5 cm i.d. prep column

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Not All Preparative Loadings Are the Same!
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Questions?