Chiral LC/MS/MS Analysis with Polysaccharide-based Stationary Phases for Basic and Neutral Stereoisomeric Pharmaceutical Compounds

Liming Peng, Tivadar Farkas and Swapana Jayapaian
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA (www.phenomenex.com)
The hyphenation of the resolving power of chiral HPLC with the sensitivity of MS detection is highly desired in drug metabolism and pharmacokinetic studies of stereoisomers in the drug discovery process. Derivatives of polysaccharides are the most widely used chiral stationary phases due to their wide chiral recognition ability, high loading capacity, and durability. As normal phase is favorable for the principal mechanism of chiral recognition – hydrogen bonding interaction – the majority of chiral separations with polysaccharide phases are performed in normal phase using hexane and alcohol modifiers as mobile phase components. However, these mobile phases are highly flammable and are not compatible with atmospheric pressure ionization (API) MS ion sources. Developing rapid chiral LC separations compatible with MS ionization interfaces while preserving both chromatographic resolution and sensitivity in MS detection has proven to be a great challenge to analytical scientists.

We present the results of a systematic feasibility study of using polysaccharide-based chiral stationary phases (CSPs) coupled with API-MS/MS detection for the analysis of various pharmaceutical racemates in reversed phase (RP) elution mode.
Experimental Conditions

**HPLC System:** HP 1100 series (www.agilent.com)

**Pump:** G1312A (Binary Pump)

**Autosampler:** G1329A ALS

**MS Detector:** API 3000 LC/MS/MS with ESI (TurbolonSpray®) (www.Appliedbiosystems.com)

- TurbolonSpray - ESI, Positive Ion
- Mode; MRM; heater gas flow 5000 cc/min; heater temperature 400 °C.

**Flow Rate:** 0.2 mL/min

**Injection Volume:** 5 µL

**Concentration:** 0.2 – 1.0 µg/mL of racemates

**Columns:**
- Lux 3 µm Cellulose-1
- 150 x 2.0 mm
- Lux 3 µm Cellulose-2
- 150 x 2.0 mm
- Lux 3 µm Amylose -2
- 150 x 2.0 mm
- Gemini®-NX 3 µm C18
- 50 x 2.0 mm

**Mobile Phases:**
1. Acetonitrile/Methanol : 5 mM \( \text{NH}_4\text{HCO}_3 \)
2. Acetonitrile/Methanol: 5 mM \( \text{NH}_4\text{HCO}_3 + 0.025 \% \text{ DEA}^* \)
3. Acetonitrile/Methanol: 5 mM CH\(_3\)COONH\(_4\)

* DEA - Diethylamine
Figure 2. LC/MS/MS Responses of Representative Racemates

- **Ketamine**
- **Isoxsuprine**
- **Nisoldipine**
- **Trimipramine**
- **Norketamine**
- **Nifenolol**

**LC/MS/MS responses**

- 0.1% Formic acid/Acetonitrile
- 5 mM Ammonium Bicarbonate/Acetonitrile

**Conc.: 200 ng/mL**
**Column: Gemini-NX 3 µm**
**50 x 2.0 mm**
Figure 3. Effect of Modifier and Additive on Enantiorecognition

XIC of +MRM (1 pair): 225.0/165.2 amu from Sample... Max. 8.5e3 cps.

2 4 6 8 10 12 14
Time, min

0.0 1.0e5 2.0e5 3.0e5 4.0e5 5.0e5
Intensity, cps

Nifenolol 225.0/165.2
Lux Cellulose-2
60:40/MeOH: 5 mM NH₄HCO₃

XIC of +MRM (1 pair): 295.4/100.0 amu from Sample... Max. 1.8e5 cps.

2 4 6 8 10 12 14 16 18 20 22 24
Time, min

0.0 5.0e4 1.0e5 1.5e5 1.8e5
Intensity, cps

Trimipramine 295.4/100.0
Lux Cellulose-2
50:50/CH₃CN: 5 mM NH₄HCO₃

XIC of +MRM (1 pair): 225.0/165.2 amu from Sample... Max. 1.6e4 cps.

2 4 6 8 10 12 14
Time, min

0.0 5000.0 1.0e4 1.5e4
Intensity, cps

Nifenolol 225.0/165.2
Lux Cellulose-2
60:40/MeOH: 5 mM NH₄HCO₃ +0.025 % DEA

XIC of +MRM (1 pair): 295.4/100.0 amu from Sample... Max. 1.5e5 cps.

6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
Time, min

0.0 5.0e4 1.0e5 1.5e5 1.8e5
Intensity, cps

Trimipramine 295.4/100.0
Lux Cellulose-2
45:55/CH₃CN: 5 mM NH₄HCO₃

XIC of +MRM (1 pair): 225.0/165.2 amu from Sample... Max. 3881.0 cps.

5 10 15 20 25 30
Time, min

0 1000 2000 3000 3881
Intensity, cps

Nifenolol 225.0/165.2
Lux Cellulose-2
45:55/MeOH: 5 mM NH₄HCO₃ + 0.025 % DEA 35 min

XIC of +MRM (1 pair): 295.4/100.0 amu from Sample... Max. 4.8e4 cps.

2 4 6 8 10 12 14 16 18 20 22 24
Time, min

0.0 1.0e4 2.0e4 3.0e4 4.0e4 4.8e4
Intensity, cps

Trimipramine 295.4/100.0
Lux Cellulose-2
50:50/CH₃CN: 5 mM NH₄HCO₃ + 0.025 % DEA

Broader peaks & delayed RT

Decreased signal

Decreased signal
Figure 4. **Effects of Temperature and Additive on Enantiorecognition**

![Graphs showing effects of temperature and additive on enantiorecognition](Image)

- **Adrenaline 180.0/124.1**
  - Lux Cellulose-2
  - 50:50/MeOH: 5 mM NH₄HCO₃
  - @ ambient
  - Max. 4.4e5 cps.
  - Max. 1.9e5 cps.

- **Lorazepam 321.4/275.1**
  - Lux Cellulose-2
  - 80:20/MeOH: 5 mM NH₄HCO₃
  - @ ambient
  - Max. 8.8e4 cps.
  - Max. 2.3e4 cps.
Figure 5a. Effect of Buffer on Chiral Resolution in RP LC/MS/MS on Lux Cellulose-1
Figure 5b. Effect of Buffer on Chiral Resolution in RP LC/MS/MS on Lux Cellulose-1

**Zopiclone 245.1/217.3**
60:40/CH3CN: 5 mM NH4HCO3

**Reboxetine 314.3/176.2**
60:40/CH3CN: 5 mM NH4HCO3

**Nomifensine 239.3/44.1**
60:40/CH3CN: 5 mM NH4HCO3

**Mebeverine 430.4/149.1**
60:40/CH3CN: 5 mM NH4HCO3

**Napropamide 272.4/129.4**
60:40/CH3CN: 5 mM NH4HCO3

**Zopiclone 245.1/217.3**
60:40/CH3CN: 5 mM NH4AC

**Reboxetine 314.3/176.2**
60:40/CH3CN: 5 mM NH4AC

**Nomifensine 239.3/44.1**
60:40/CH3CN: 5 mM NH4AC

**Mebeverine 430.4/149.1**
60:40/CH3CN: 5 mM NH4AC

**Napropamide 272.4/129.4**
60:40/CH3CN: 5 mM NH4AC
Figure 6. Enantioresolution in RP LC/MS/MS on Lux Cellulose-1

- Dimethindene (293.4/248.3 amu) in 90:10 MeOH: 5 mM NH₄HCO₃, Max. 1.5e6 cps.
- Bendrofluazide (420.0/289.1 amu) in 80:20 MeOH: 5 mM NH₄HCO₃, Max. 1.1e5 cps.
- Etozolin (285.2/200.2 amu) in 90:10 MeOH: 5 mM NH₄HCO₃, Max. 9.5e5 cps.
- Homatropine (276.3/124.2 amu) in 80:20 MeOH: 5 mM NH₄HCO₃, Max. 8.3e5 cps.
- Isoamarine (299.4/104.2 amu) in 90:10 MeOH: 5 mM NH₄HCO₃, Max. 1.5e6 cps.
- Nisoxetine (272.3/44.1 amu) in 40:60 CH₃CN: 5 mM NH₄HCO₃, Max. 1.0e6 cps.
- Mianserin (265.3/208.3 amu) in 90:10 MeOH: 5 mM NH₄HCO₃, Max. 1.6e5 cps.
- Halofantrine (500.3/142.3 amu) in 90:10 MeOH: 5 mM NH₄HCO₃, Max. 1.6e4 cps.
- Meclizine (391.2/201.1 amu) in 70:30 CH₃CN: 5 mM NH₄HCO₃, Max. 4.1e6 cps.
Figure 7a. Enantioresolution in RP LC/MS/MS on Lux Cellulose-2

- Tolperisone 246.3/98.2 amu from Sample...
  Max. 2.1e5 cps.

- Isoamrinene 299.4/104.2 amu from Sample...
  Max. 2.8e5 cps.

- Methaqualone 251.3/132.3 amu from Sample...
  Max. 9.1e4 cps.

- Napropamide 272.4/129.4 amu from Sample...
  Max. 4.5e5 cps.

- Thalidomide 259.1/84.2 amu from Sample...
  Max. 1.4e4 cps.

- Omeprazole 328.3/120.2 amu from Sample...
  Max. 3021.8 cps.

- Tetramisole 398.4/124.2 amu from Sample...
  Max. 1.0e6 cps.
Figure 7b. Enantioresolution in RP LC/MS/MS on Lux Cellulose-2

XIC of +MRM (14 pairs): 246.3/98.2 amu from Sample... Max. 5.5e5 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>2.0e5</th>
<th>4.0e5</th>
<th>5.5e5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>3.98</td>
<td>3.63</td>
<td>2.46</td>
<td>1.20</td>
</tr>
</tbody>
</table>

Tolperisone 246.3/98.2 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 299.3/104.3 amu from Sample... Max. 3.4e5 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>3.4e5</th>
<th>5.0e5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>3.12</td>
<td>2.46</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Isoamarine 299.3/104.3 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 311.2/243.3 amu from Sample... Max. 4.1e6 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>2.0e6</th>
<th>4.0e6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>5.42</td>
<td>6.08</td>
<td>5.99</td>
</tr>
</tbody>
</table>

Bifonazole 311.2/243.3 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 285.2/200.2 amu from Sample... Max. 7.5e5 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>5.0e5</th>
<th>7.5e5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>4.15</td>
<td>5.69</td>
<td>5.12</td>
</tr>
</tbody>
</table>

Etozolin 285.2/200.2 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 362.3/91.2 amu from Sample... Max. 5.1e4 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>2.0e4</th>
<th>4.0e4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>4.56</td>
<td>6.71</td>
<td>1.93</td>
</tr>
</tbody>
</table>

Butaclamol 362.3/91.2 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 466.2/184.0 amu from Sample... Max. 7.5e5 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>5.0e5</th>
<th>7.5e5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>4.26</td>
<td>5.19</td>
<td>4.52</td>
</tr>
</tbody>
</table>

Cisapride 466.2/184.0 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (14 pairs): 293.3/154.3 amu from Sample... Max. 3.7e5 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>3.7e5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>5.39</td>
<td>7.20</td>
</tr>
</tbody>
</table>

Ambucetamid 293.3/154.3 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 381.1/125.1 amu from Sample... Max. 7.3e5 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>5.0e5</th>
<th>7.3e5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>7.39</td>
<td>8.67</td>
<td>6.92</td>
</tr>
</tbody>
</table>

Econazole 381.1/125.1 80:20/CH3CN: 5 mM NH4HCO3

XIC of +MRM (13 pairs): 500.3/142.3 amu from Sample... Max. 1.0e4 cps.

<table>
<thead>
<tr>
<th>Time, min</th>
<th>0.0</th>
<th>5000.00</th>
<th>1.00e4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity, cps</td>
<td>11.59</td>
<td>10.95</td>
<td>11.90</td>
</tr>
</tbody>
</table>

Halofantrine 500.3/142.3 80:20/CH3CN: 5 mM NH4HCO3
### Figure 7c. Enantioresolution in RP LC/MS/MS on Lux Cellulose-2

<table>
<thead>
<tr>
<th>Compound</th>
<th>MRM Transition</th>
<th>Mobile Phase</th>
<th>MRM Transition</th>
<th>Mobile Phase</th>
<th>MRM Transition</th>
<th>Mobile Phase</th>
<th>MRM Transition</th>
<th>Mobile Phase</th>
<th>MRM Transition</th>
<th>Mobile Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reboxetine</td>
<td>314.3/176.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>239.3/44.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>297.2/159.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>313.3/203.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>384.1/337.9</td>
<td>CH3CN: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Nomifensine</td>
<td>239.3/44.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>313.3/203.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>297.2/159.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>314.3/176.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>389.2/315.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Enilconazole</td>
<td>297.2/159.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>314.3/176.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>313.3/203.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>239.3/44.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>302.3/284.0</td>
<td>CH3CN: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Praziquantel</td>
<td>313.3/203.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>297.2/159.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>314.3/176.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>239.3/44.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>389.2/315.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Felodipine</td>
<td>384.1/337.9</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>297.2/159.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>314.3/176.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>239.3/44.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>302.3/284.0</td>
<td>CH3CN: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>389.2/315.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>297.2/159.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>314.3/176.2</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>239.3/44.1</td>
<td>CH3CN: 5 mM NH4HCO3</td>
<td>302.3/284.0</td>
<td>CH3CN: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Lansoprazole</td>
<td>370.4/425.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>346.3/125.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>370.4/252.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>384.1/337.9</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>313.3/203.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Metomizolide</td>
<td>313.3/127.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>346.3/125.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>370.4/252.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>384.1/337.9</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>313.3/127.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Metofoline</td>
<td>346.3/125.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>370.4/252.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>384.1/337.9</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>313.3/127.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>313.3/127.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Metomidate</td>
<td>370.4/252.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>384.1/337.9</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>313.3/127.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>346.3/125.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>370.4/252.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
</tr>
<tr>
<td>Metolazone</td>
<td>384.1/337.9</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>313.3/127.2</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>346.3/125.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>370.4/252.1</td>
<td>MeOH: 5 mM NH4HCO3</td>
<td>384.1/337.9</td>
<td>MeOH: 5 mM NH4HCO3</td>
</tr>
</tbody>
</table>

**Note:** Each compound is analyzed using the specified mobile phase conditions and monitored via MRM transitions at the given amu values. The intensities are measured in counts per second (cps) at various time points (min).
**Figure 8a. Enantioresolution in RP LC/MS/MS on Lux Amylose-2**

- **Nefopam** (254.3/181.4 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 5.9e5 cps)
- **Pheniramine** (241.3/196.3 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 3.2e6 cps)
- **Metomidate** (231.3/127.2 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 6.3e6 cps)
- **Ornidazole** (220.2/128.1 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 2.6e5 cps)
- **Lansoprazole** (370.4/252.1 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 1.1e6 cps)
- **Aminoglutethimid** (233.2/188.3 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 5393.8 cps)
- **Motofoline** (346.3/125.1 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 1.1e5 cps)
- **Enilconazole** (297.2/159.2 amu, 60:40/CH₃CN: 5 mM NH₄HCO₃, Max. 1.2e4 cps)
Figure 8b. Enantioresolution in RP LC/MS/MS on Lux Amylose-2
Figure 9. Enantioseparation of Ketamin and Metabolite on Lux Amylose-2

- XIC of +MRM (3 pairs): 238.1/125.0 amu from Sample 2 (Ketamines-50 % CH3CN) of Ketamines-50 % CH3CN Max. 6.1e4 cps.
- XIC of +MRM (3 pairs): 224.0/125.0 amu from Sample 2 (Ketamines-50%CH3CN) of Ketamines-50%CH3CN Max. 1.6e5 cps.
- XIC of +MRM (3 pairs): 242.1/129.3 amu from Sample 2 (Ketamines-50%CH3CN) of Ketamines-50%CH3CN Max. 1.6e5 cps.
Figure 10. Enantioseparation of Benzodiazepines and β-blockers

Chiral LC/MS/MS experiments

Three different polysaccharide-based chiral...
Chiral LC/MS/MS experiments

Three different polysaccharide-based chiral stationary phases — Lux Cellulose-1 (Cellulose tris[3,5-dimethylphenylcarbamate]), Lux Cellulose-2 (Cellulose tris[3-chloro-4-methylphenylcarbamate]), and Lux Amylose-2 (Amylose tris[5-chloro-2-methylphenylcarbamate]); see Figure 1) were explored in the reversed phase elution mode for the separation of a variety of basic and neutral compounds of pharmaceutical interest, in mobile phases made of 5 mM ammonium bicarbonate or acetate with acetonitrile or methanol, and with MS/MS detection.

Effect of mobile phase additives

NH₄HCO₃ and CH₃COONH₄ additives: The responses of representative racemates in 5 mM NH₄HCO₃/CH₃CN mobile phase were compared to the responses in 0.1 % HCOOH/CH₃CN (analysis on C18 stationary phase; Figure 2). The results show that MS/MS responses are comparable or higher in 5 mM NH₄HCO₃/CH₃CN compared to analyte responses in 0.1 % HCOOH/CH₃CN. This proves that ammonium bicarbonate based mobile phases are favorable for MS/MS detection.

The enantioseparation on Lux CSPs was evaluated in both 5 mM CH₃COONH₄/CH₃CN and 5 mM NH₄HCO₃/CH₃CN (Figure 5). In general, NH₄HCO₃ provided similar or occasionally superior resolution to CH₃COONH₄ as mobile phase additive.

Diethylamine (DEA) additive: DEA is a commonly used additive in chiral HPLC with polysaccharide derivatives; unfortunately, it severely suppresses analyte responses in ESI⁺ MS/MS even at low concentration levels as 0.025 % (Figures 3-4). Addition of DEA into mobile phase could improve enantioresolution for very basic compounds (e.g. β-blockers and tricyclic antidepressants); however DEA does not affect the enantioresolution of benzodiazepines, imidazoles or neutral stereoisomers (Figure 6-9). For all these compounds, baseline separation can be achieved without DEA.

Effect of temperature on chiral resolution

Lowering column temperature had little effect on resolution with Lux Cellulose-2 (cellulose tris(3-chloro-4-methylphenylcarbamate); peaks got broader as retention increased (Figure 3 and 4).

In general, decreasing temperature produces slower mass transfer kinetics, resulting in increased retention and decreased column efficiency. Our results (data not presented) show that the effect of column temperature on chiral resolution varies from case to case; it is unpredictable and not significant on any of the CSPs in the temperature range (5 °C - 35 °C) studied.

Effect of organic modifier on chiral resolution

Acetonitrile or methanol organic modifier was used in chiral RP HPLC separations. Increasing the eluting strength of the mobile phase will decrease retention and resolution as shown for nifenolol in Figure 3 and Tolperison in Figure 7. However, once enantiomers eluted later than 10 minutes with only partial resolution, baseline separation can be rarely achieved by decreasing % organic modifier in the mobile phase.

In our study, acetonitrile provides more successful chiral resolution than methanol on Lux CSP in RP mode.

Chiral LC/MS/MS applications

Figures 5-10 demonstrate more than 50 chiral separations on Lux CSPs. Most compounds evaluated here eluted in less than 10 min with baseline resolution in mobile phases of various eluting strength. The results show that Lux 3 µm Cellulose-1 was most successful in separating benzodiazepines and β-blockers, Lux 3 µm Cellulose-2 in separating imidazoles, and Lux 3 µm Amylose-2 in separating antihistamines and imidazoles.
Conclusions

- More than 50 chiral LC/MS/MS analyses are demonstrated on the polysaccharide-based CSPs stationary phases Lux 3 µm Cellulose-1, Lux 3 µm Cellulose-2, and Lux 3 µm Amylose-2 in the reversed phase elution mode.

- Ammonium bicarbonate is the preferred buffer salt with ESI$^+$ MS/MS detection for most basic pharmaceutical stereoisomers. Ammonium acetate is a viable alternative to ammonium bicarbonate but it is less successful in providing baseline resolution.

- Diethylamine (as additional additive) can improve the chiral resolution of strong basic compounds but it has a negative effect on analyte response in ESI$^+$ MS/MS even at low concentration levels (e.g. 0.025 %).

- Decreasing column temperature may not improve chiral resolution on partially separated strong basic stereoisomers because of peak broadening with delayed retention.

- Increasing the organic modifier (CH$_3$CN or MeOH) in the RP mobile phase has the expected effect: it decreases retention and enantioselectivity; adjusting % organic modifier is essential to optimizing chiral resolution.

**Trademarks**
Lux and Gemini-NX are registered trademarks of Phenomenex, Inc in the United States, European Union, and other jurisdictions. TurbolonSpray is a registered trademark of Applied Biosystems. Gemini-NX is patented by Phenomenex U.S. Patent No. 7,563,367

© 2011 Phenomenex, Inc. All rights reserved.