SEC Analysis of Insulin and HMW Aggregates

Highlighted FACTS:

- Zenix®-C SEC-80 (3 mm, 80Å) is suitable for pharmaceutical insulin analysis with different mobile phase systems (salt, USP method and organic volatile mobile phases).
- High resolution between insulin monomers and aggregates can be achieved with a 4.6 x 300 mm column.
- Formulation additives such as phenol and cresol can be well separated during a single SEC analysis.
- Zenix®-C SEC-80 exhibits high run to run reproducibility and long column life time with USP standard method.

Technical Specifications:

- **Columns**: Zenix®-C SEC-80 (3 mm, 80 Å)
  - Zenix®-C SEC-150 (3 mm, 150 Å)
- **Column size**: 4.6 x 300 mm, 7.8 x 300 mm

Insulin-Lispro (Humalog) — Mobile Phase Effect on Zenix®-C SEC-80

Column: Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm)
Mobile phase: as indicated; Flow rate: 1 mL/min;
Detector: UV 276 nm; Column temperature: 30 °C;
Injection volume: 10 µL; Sample: Insulin Lispro 3.47 mg/mL in phosphate buffer containing m-cresol

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Pore Size Effect — Insulin Lispro

Column: Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm),
Pressure: 103 bar
Zenix®-C SEC-150 (3 µm, 150 Å, 7.8 x 300 mm),
Pressure: 103 bar
Mobile phase: 0.1% Arginine:Acetonitrile:Acetic acid (65:20:15);
Flow rate: 1 mL/min; Detector: UV 276 nm;
Column temperature: 37 °C and 30 °C; Injection volume: 10 µL;
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol

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Column Size Effect — Insulin Lispro

Column: Zenix®-C SEC-80 (3 µm, 80 Å, 4.6 x 300 mm)
Flow rate: 0.35 mL/min; Injection volume: 5 µL;
Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm)
Flow rate: 1 mL/min; Injection volume: 10 µL;
Mobile phase: 0.1% Arginine:Acetonitrile:Acetic acid (65:20:15);
Detector: UV 276 nm; Column temperature: 30 °C;
Sample: Insulin Lispro 3.47 mg/mL in phosphate buffer containing m-cresol

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Technical Specifications:

- **Columns**: Zenix®-C SEC-80 (3 mm, 80 Å)
  - Zenix®-C SEC-150 (3 mm, 150 Å)
- **Column size**: 4.6 x 300 mm, 7.8 x 300 mm

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**Flow Rate Effect—Insulin Lispro**
Column: Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm)
Mobile phase: 0.1% Arginine:Acetonitrile:Acetic acid (65:20:15); Flow rate: 0.5 and 1 mL/min; Detector: UV 276 nm;
Column temperature: 30 °C, Injection volume: 10 µL;
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol.

**Duplicate Injections of Insulin Lispro**
Column: Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm)
Mobile phase: 0.1% Arginine:Acetonitrile:Acetic acid (65:20:15); Flow rate: 1 mL/min; Pressure: 103 bar; Detector: UV 276 nm;
Column temperature: 30 °C; Injection volume: 10 µL;
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol.

**Degraded Insulin on Zenix®-C SEC-80**
Column: Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm)
Mobile phase: 0.1% TFA, 50% acetonitrile;
Flow rate: 1 mL/min; Pressure: 75 bar; Detector: UV 276 nm;
Column temperature: 30 °C; Injection volume: 10 µL;
Sample: Degraded insulin 3.75 mg/mL in phosphate buffer containing m-cresol.

**Life Time Test—Insulin Analysis on Zenix®-C SEC-80**
Column: Zenix®-C SEC-80 (3 µm, 80 Å, 7.8 x 300 mm)
Mobile phase: 0.1% Arginine:ACN:HAc = 65:20:15; (v/v); Flow rate: 0.5 mL/min; Detector: UV 276 nm;
Column temperature: 40 °C; Injection volume: 25 µL;
Sample: Insulin 4 mg/mL in 0.01 N HCl, Pressure: 52 bar.

**Insulin Analysis Parameters—Life Time Test**

<table>
<thead>
<tr>
<th>Injection</th>
<th>RT</th>
<th>Plates</th>
<th>Tailing</th>
<th>Rs</th>
<th>Area % dimer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.08</td>
<td>19767</td>
<td>0.79</td>
<td>4.32</td>
<td>3.37</td>
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<tr>
<td>50</td>
<td>16.13</td>
<td>20116</td>
<td>0.79</td>
<td>4.43</td>
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<tr>
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<td>20327</td>
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<td>4.44</td>
<td>3.18</td>
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<tr>
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<td>0.82</td>
<td>4.42</td>
<td>3.34</td>
</tr>
<tr>
<td>200</td>
<td>16.28</td>
<td>21629</td>
<td>0.83</td>
<td>4.44</td>
<td>3.15</td>
</tr>
<tr>
<td>RSD/%</td>
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<td>3.86</td>
<td>2.25</td>
<td>1.16</td>
<td>3.04</td>
</tr>
</tbody>
</table>

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