Insulin SEC analysis

• Insulin Samples:
  – Lispro, identical in structure to Insulin Human, except that it has lysine and proline at positions 28 and 29, respectively, of chain B, whereas this sequence is reversed in Insulin Human.
  – Sigma human recombinant insulin, P/N 910776
  – Custom insulin sample for column life time study
  – Degraded insulin sample (40 hours at 60 °C)
Insulin-Lispro (Humalog)-mobile phase effect on Zenix™-C SEC-80 (7830)

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

Organic mobile phase minimizes the interaction between sample and column resin.
Zoom in-Insulin Lispro (monomer and aggregate)

Rs = 3.26
0.1% TFA, 50% ACN

Rs = 3.28
0.1% Arginine:Acetonitrile:Acetic acid (65:20:15)

Rs = 2.11
10 mM Tris, 140 mM NaCl, pH 7.4
Column temperature- 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 mL/min, Pressure: 38 bar
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
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

Zenix™-C SEC-80 provides a better resolution for insulin aggregate and monomer separation.
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

Rs = 2.79

Rs = 3.28
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

Slower flow rate gives slightly better resolution at the expense of analysis speed.
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 ºC, Injection volume: 10 µL
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol

High run to run reproducibility
Degraded insulin on Zenix\textsuperscript{TM}-C SEC-80

Column: Zenix\textsuperscript{TM}-C SEC-80 (3 \(\mu\)m, 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 \(\mu\)L,
Sample: Degraded insulin 3.75 mg/mL in phosphate buffer containing m-cresol (40 hours at 60 °C)
Insulin Analysis on Zenix™-C SEC-80 (4630)

Column: Zenix™-C SEC-80 (3 µm, 80 Å, 4.6 x 300 mm)
Mobile phase: 0.1% Arginine:Acetonitrile:Acetic acid (65:20:15), Flow rate: **0.35 mL/min**, Detector: UV 276 nm, Column temperature: 30 °C, Injection volume: 5 µL, Pressure: 90 bar

Sample: **Insulin Lispro** 3.47 mg/mL in phosphate buffer containing m-cresol, **sigma insulin** 3.8 mg/mL in 0.01 N HCl containing phenol and m-cresol

![Graph showing the separation of Insulin Lispro and sigma insulin](image.png)
Life time test

Insulin Analysis on Zenix™-C SEC-80 (7830)

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

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<th>Injection</th>
<th>RT</th>
<th>Plates</th>
<th>Tailing</th>
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