



SEC Analysis of Insulin and HMW Aggregates

Highlighted FACTS:

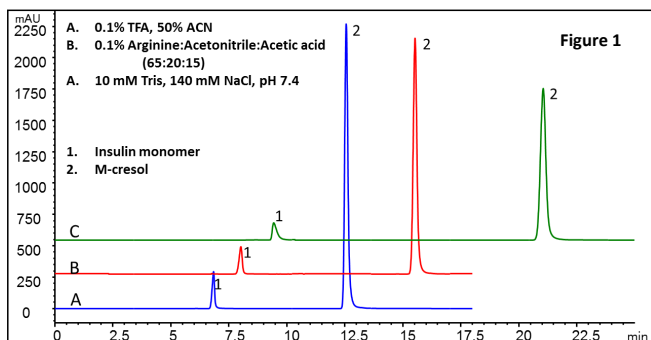
- Zenix®-C SEC-80 (3 mm, 80Å) is suitable for pharmaceutical insulin analysis with different mobile phase systems (salt, USP method and organic volatile mobile phases).
- High resolution between insulin monomers and aggregates can be achieved with a 4.6 x 300 mm column.
- Formulation additives such as 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:

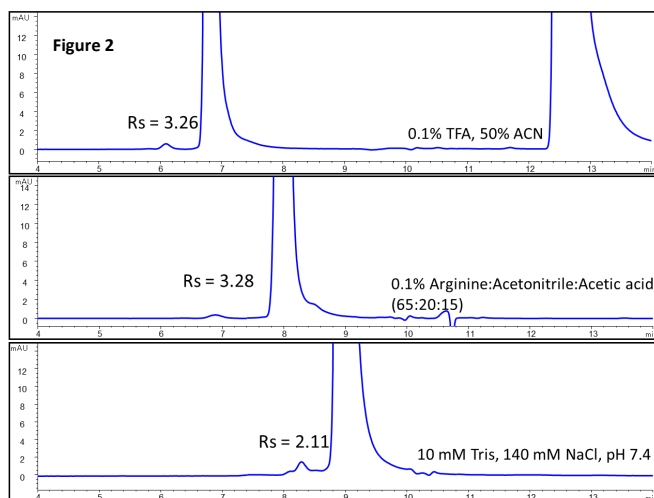
- **Columns:** Zenix®-C SEC-80 (3 mm, 80 Å)
Zenix®-C SEC-150 (3 mm, 150 Å)
- **Column size:** 4.6 x 300 mm, 7.8 x 300 mm

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

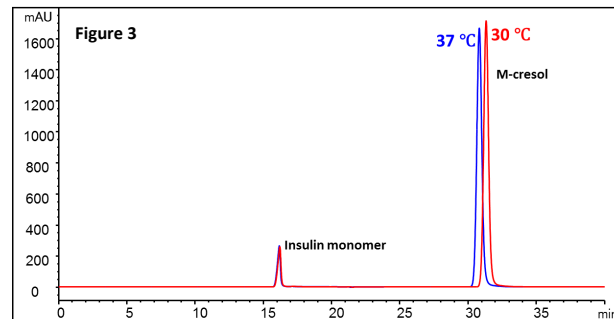


Zoom In-Insulin Lispro (monomer and aggregate)



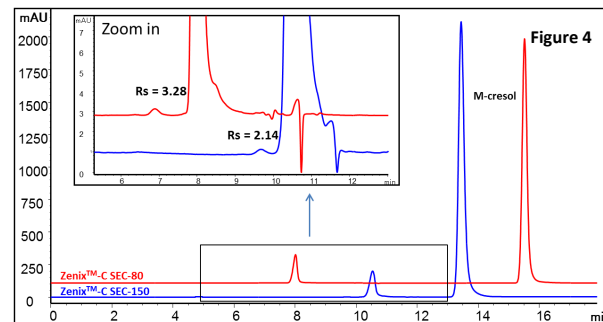
Column Temperature — Insulin Lispro

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



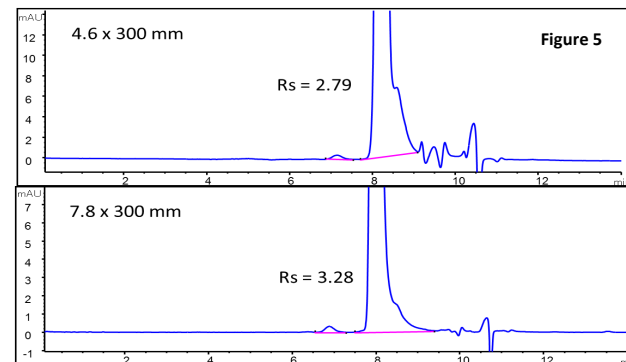
Pore Size Effect — Insulin Lispro

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



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

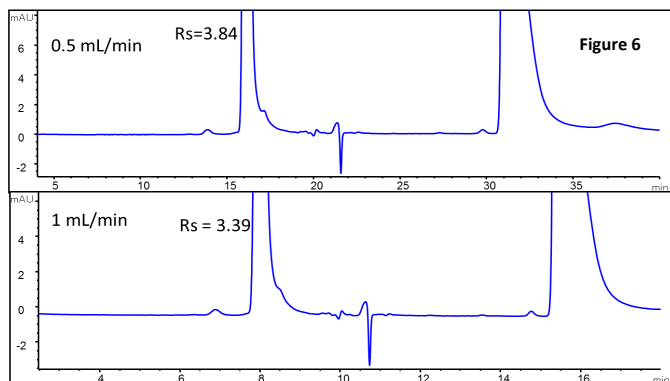




SEC Analysis of Insulin and HMW Aggregates

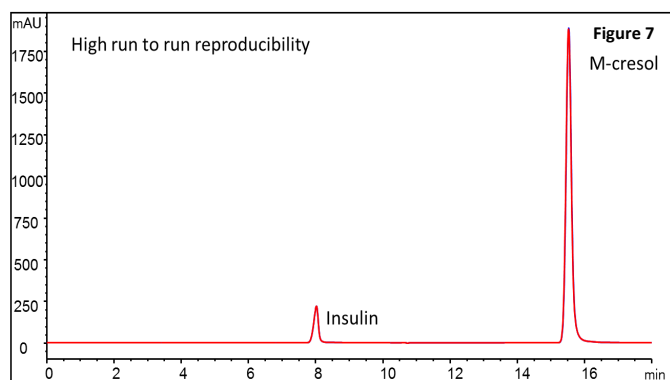
Flow Rate Effect– Insulin Lispro

Column: Zenix®-C SEC-80 (3 μ m, 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 μ L;
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol



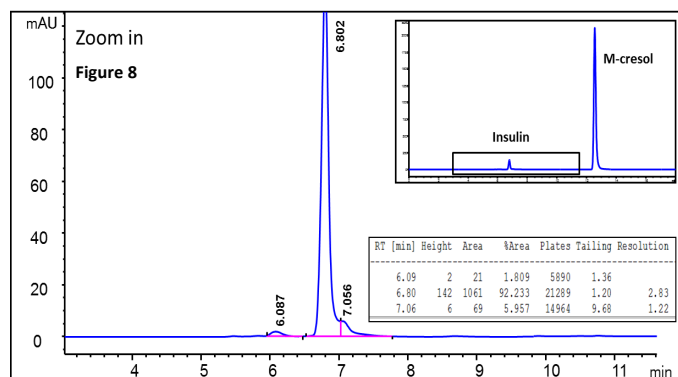
Duplicate Injections of Insulin Lispro

Column: Zenix®-C SEC-80 (3 μ m, 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 μ L;
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol



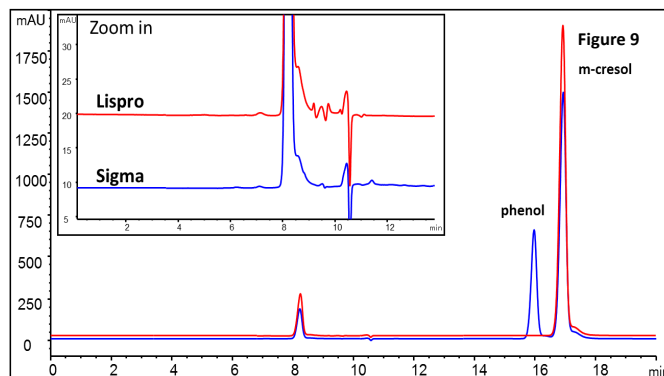
Degraded Insulin on Zenix®-C SEC-80

Column: Zenix®-C SEC-80 (3 μ 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 μ L;
Sample: Degraded insulin 3.75 mg/mL in phosphate buffer containing m-cresol (40 hours at 60 °C)



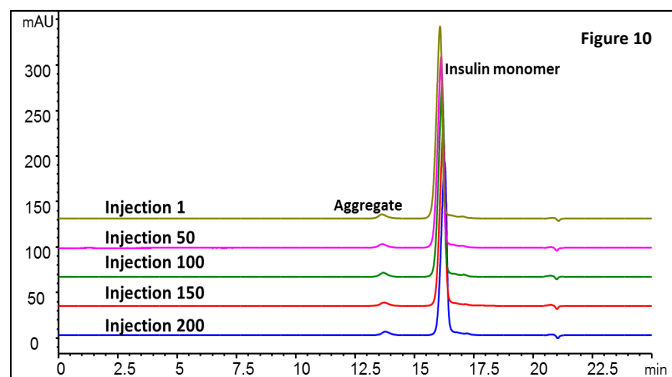
Insulin Analysis on Zenix®-C SEC-80

Column: Zenix®-C SEC-80 (3 μ m, 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 μ L; 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



Life Time Test-Insulin Analysis on Zenix®-C SEC-80

Column: Zenix®-C SEC-80 (3 μ m, 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 μ L;
Sample: Insulin 4 mg/mL in 0.01 N HCl, Pressure: 52 bar



Insulin Analysis Parameters-Life Time Test

injection	RT	Plates	Tailing	Rs	Area % dimer
1	16.08	19767	0.79	4.32	3.37
50	16.13	20116	0.79	4.43	3.21
100	16.17	20327	0.8	4.44	3.18
150	16.22	21287	0.82	4.42	3.34
200	16.28	21629	0.83	4.44	3.15
RSD/%	0.48	3.86	2.25	1.16	3.04