



## SEC Analysis of Insulin and HMW Aggregates

### Highlighted FACTS:

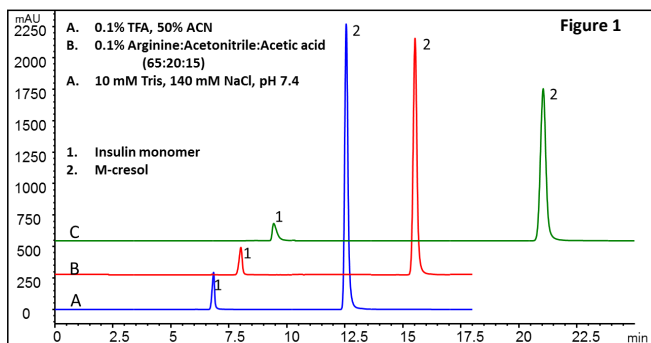
- Zenix-C SEC-80 (3 mm, 80Å) is suitable for pharmaceutical insulin analysis with different mobile phase systems (salt, USP method and organic volatile mobile phases).
- High resolution between insulin monomers and aggregates can be achieved with a 4.6 x 300 mm column.
- Formulation additives such as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

### Order Information

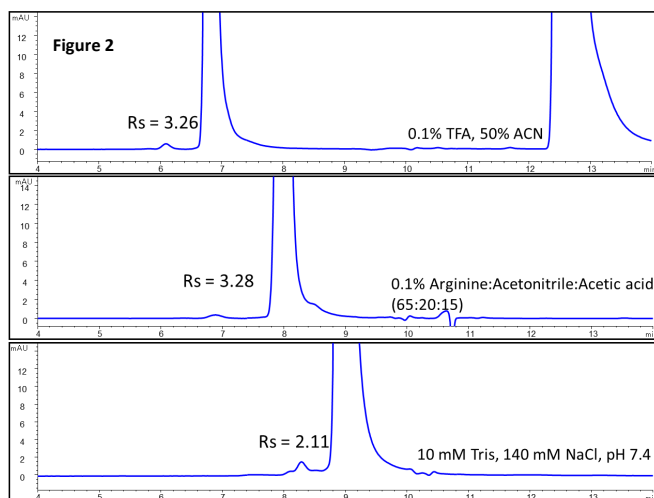
233080-4630	Zenix-C SEC-80, 3 $\mu$ m, 80 Å, 4.6 x 300 mm
233080-7830	Zenix-C SEC-80, 3 $\mu$ m, 80 Å, 7.8 x 300 mm
233150-7830	Zenix-C SEC-150, 3 $\mu$ m, 150 Å, 7.8 x 300 mm

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

Column: Zenix-C SEC-80 (3  $\mu$ m, 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  $\mu$ L; Sample: Insulin Lispro 3.47 mg/mL in phos-

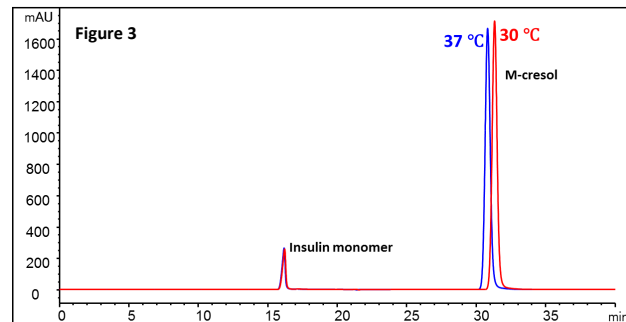


### Zoom In-Insulin Lispro (monomer and aggregate)



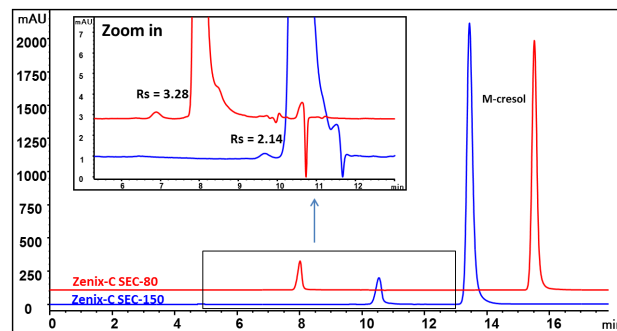
### Column Temperature — Insulin Lispro

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



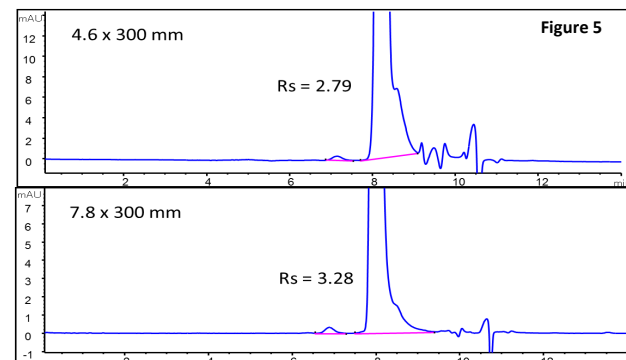
### Pore Size Effect — Insulin Lispro

Column: Zenix-C SEC-80 (3  $\mu$ m, 80 Å, 7.8 x 300 mm),  
Pressure: 103 bar  
Zenix-C SEC-150 (3  $\mu$ m, 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  $\mu$ L;  
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol



### Column Size Effect — Insulin Lispro

Column: Zenix-C SEC-80 (3  $\mu$ m, 80 Å, 4.6 x 300 mm)  
Flow rate: 0.35 mL/min; Injection volume: 5  $\mu$ L;  
Zenix-C SEC-80 (3  $\mu$ m, 80 Å, 7.8 x 300 mm)  
Flow rate: 1 mL/min; Injection volume: 10  $\mu$ L;  
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

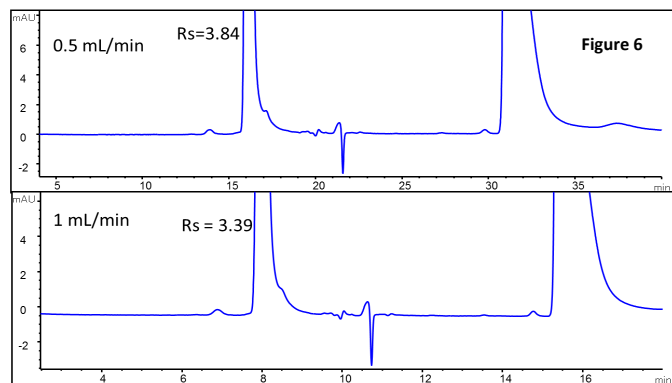




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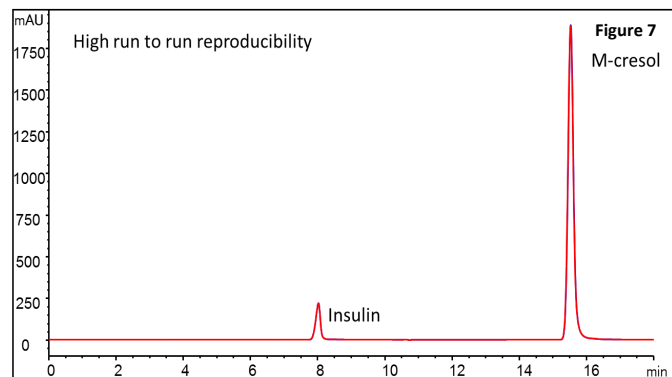
### Flow Rate Effect– Insulin Lispro

Column: Zenix-C SEC-80 (3  $\mu\text{m}$ , 80  $\text{\AA}$ , 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  $^{\circ}\text{C}$ ; Injection volume: 10  $\mu\text{L}$ ;  
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol



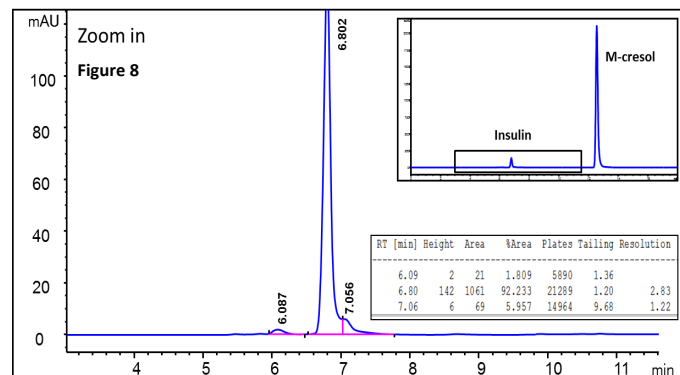
### Duplicate Injections of Insulin Lispro

Column: Zenix-C SEC-80 (3  $\mu\text{m}$ , 80  $\text{\AA}$ , 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  $^{\circ}\text{C}$ ; Injection volume: 10  $\mu\text{L}$ ;  
Sample: Insulin Lispro 3.47 mg/mL in phosphate containing m-cresol



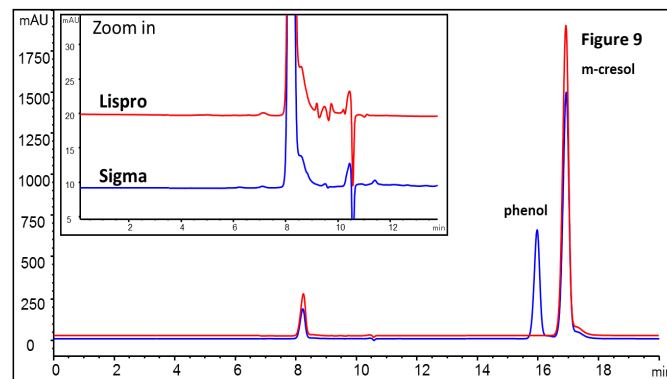
### Degraded Insulin on Zenix-C SEC-80

Column: Zenix-C SEC-80 (3  $\mu\text{m}$ , 80  $\text{\AA}$ , 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  $^{\circ}\text{C}$ ; Injection volume: 10  $\mu\text{L}$ ;  
Sample: Degraded insulin 3.75 mg/mL in phosphate buffer containing m-cresol (40 hours at 60  $^{\circ}\text{C}$ )



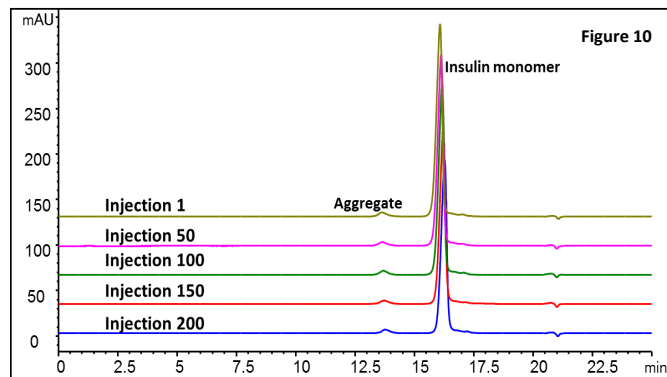
### Insulin Analysis on Zenix-C SEC-80

Column: Zenix-C SEC-80 (3  $\mu\text{m}$ , 80  $\text{\AA}$ , 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  $^{\circ}\text{C}$ ; Injection volume: 5  $\mu\text{L}$ ; 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  $\mu\text{m}$ , 80  $\text{\AA}$ , 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  $^{\circ}\text{C}$ ; Injection volume: 25  $\mu\text{L}$ ;  
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