Fusion Protein Separation on SEC and SAX

Highlighted FACTS:

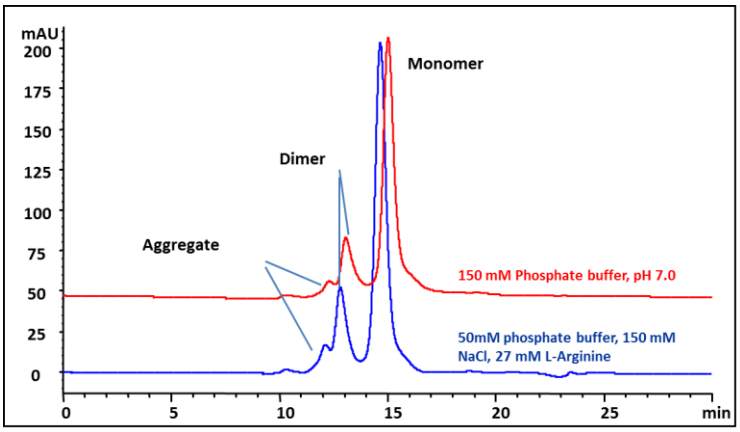
- Fusion proteins are structural hybrids that contain two different proteins, which retain the functional properties from the original proteins.
- Zenix and Zenix-C SEC are suitable for aggregation/monomer separation of fusion proteins including Fc fusion and HSA fusion protein.
- Proteomix SAX and SCX provide high resolution separation of fusion proteins, especially different glycosylation forms, charge variants and fusion proteins from their formulation components.

Order Information

213300-7830	Zenix SEC-300, 3µm, 300 A 7.8 x 300 mm
233300-7830	Zenix-C SEC-300, 3µm, 300 A 7.8 x 300 mm
213300-7805	Zenix SEC-300, 3µm, 300 A 7.8 x 50mm
401NP5-4625	Proteomix SAX-NP5, 5µm, NP 4.6 x 250 mm

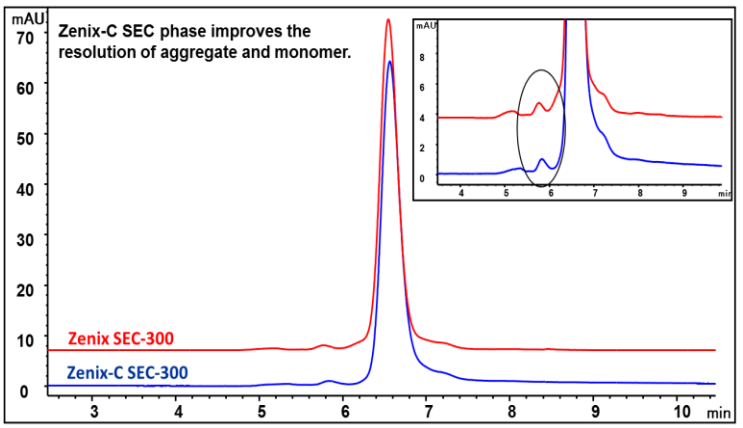
Fc Fusion Protein 1 - Mobile Phase Effect

Column: Zenix SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase 1: 150 mM Phosphate buffer, pH 7.0
Mobile phase 2: 50mM phosphate buffer, 150 mM NaCl, 27 mM L-Arginine, pH 7.0; Flow rate: 0.5 mL/min; Detector: UV 280 nm; Column temperature: Room temperature; Injection volume: 50 µL; Pressure: 50 bar; Sample: CHO cell expressed Fc-fusion protein 160 kD (2.0 mg/mL)



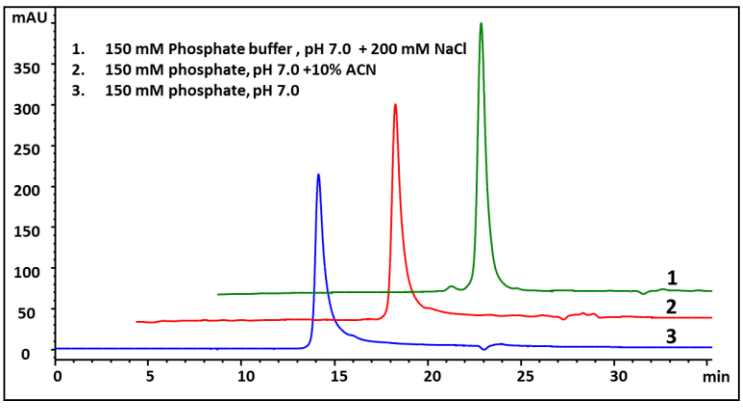
Fc Fusion Protein 2: rhTNFR-Fc - SEC Phase Comparison

Column: Zenix SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Zenix-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25°C; Injection volume: 10 µL; Samples: Recombinant human tumor necrosis factor receptor-Fc (rhTNFR-Fc) 2 mg/mL in water



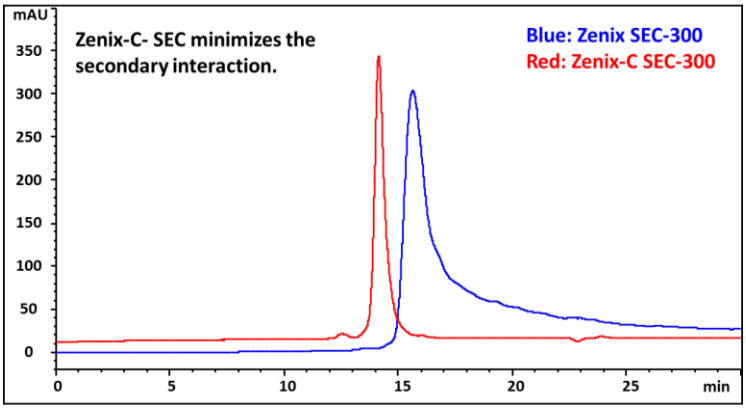
Fusion Protein 3 - Mobile Phase Effect

Column: Zenix-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: as indication; Flow rate: 0.5 mL/min; Detector: UV 214 nm; Column temperature: 25 °C; Injection volume: 10 µL;
Samples: 1 mg/mL fusion protein, MW 170 kD, pI 6.8-7.0 10% retention time offset for presentation purpose



Fusion Protein 3 - SEC Phase comparison

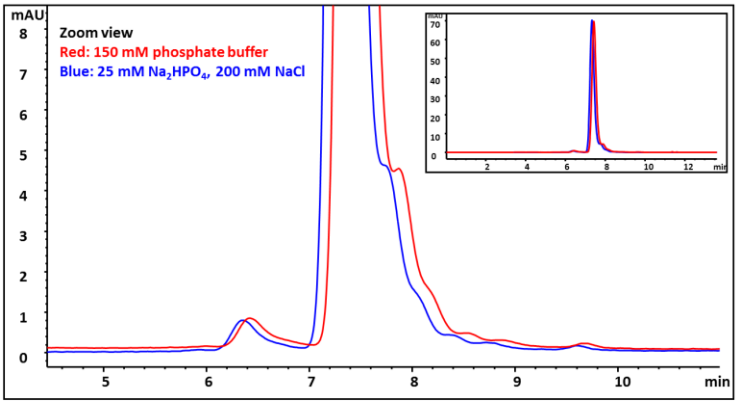
Column: Zenix SEC-300, Zenix-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer (pH 7.0) + 200 mM NaCl;
Flow rate: 0.5 mL/min; Detector: UV 214 nm; Column temperature: 25 °C; Injection volume: 10 µL; Samples: 1 mg/mL fusion protein, MW 170 kD, pI 6.8-7.0



Fusion Protein Separation on SEC and SAX

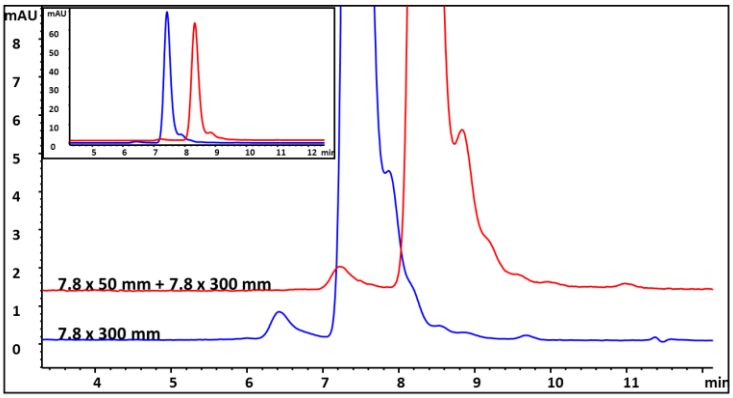
Fusion Protein 4 - Mobile Phase Effect

Column: Zenix SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase 1: 25 mM Na2HPO4, 200 mM NaCl, pH 6.8,
Mobile phase 2: 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25°C; Pressure: 82 bar; Injection volume: 2 µL; Sample: 10 mg/mL Fusion protein (150 kD, pI 5.0, 50 mg/mL, dilute to 10 mg/mL)



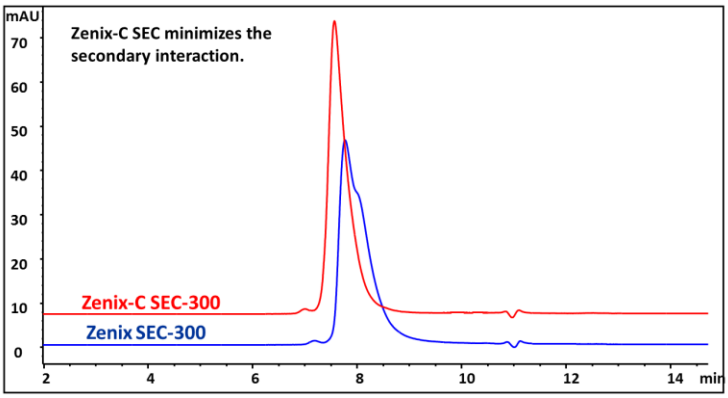
Fusion Protein 4 - Column Length Effect

Column: Zenix SEC-300 (3 µm, 300 Å),
7.8 x 300 mm and 7.8 x 50 mm + 7.8 x 300 mm
Mobile phase: 25 mM Na2HPO4, 200 mM NaCl, pH 6.8;
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25°C; Pressure: 90 bar; Injection volume: 2 µL; Sample: 10 mg/mL Fusion protein (150 kD, pI 5.0, 50 mg/mL, dilute to 10 mg/mL)



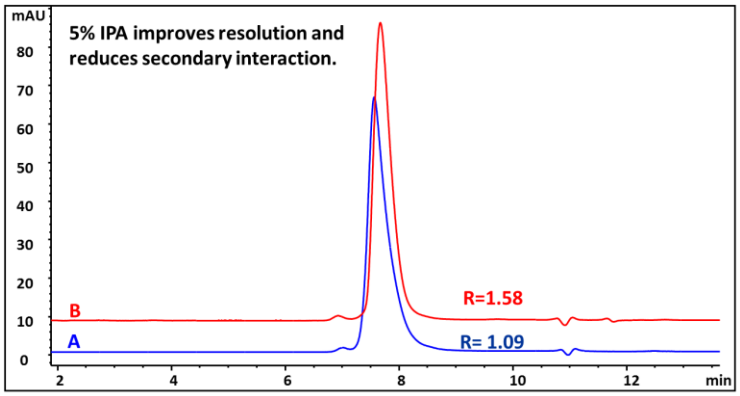
HSA Conjugated Peptide - SEC Phase Comparison

Column: Zenix-C SEC-300, Zenix SEC-300 (3 µm, 300 Å, 7.8 x 300 mm),
Mobile phase: A. 150 mM Phosphate buffer, pH 7.0;
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25°C; Injection volume: 10 µL; Samples: HSA fusion peptide 5 mg/mL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment)



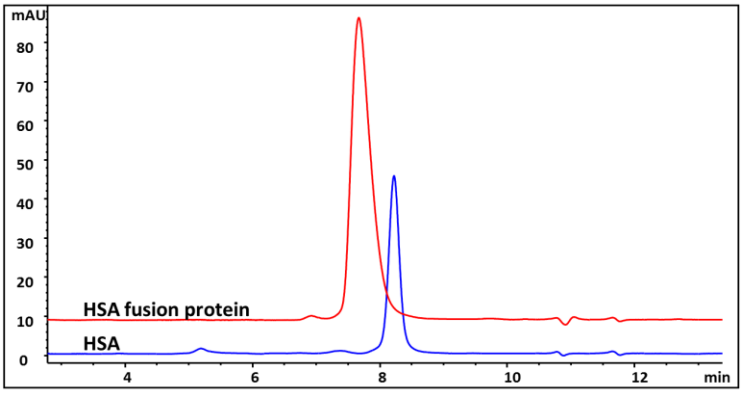
HSA Fusion Protein - Mobile Phase Effect

Column: Zenix-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: A. 150 mM Phosphate buffer, pH 7.0, B: 150 mM Phosphate buffer (pH 7.0) : IPA = 95 : 5 (v/v); Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25°C; Injection volume: 10 µL; Samples: HSA fusion peptide 5 mg/mL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment)



HSA Fusion Protein vs HSA

Column: Zenix-C SEC-300 (3 µm, 300 Å, 7.8 x 300 mm)
Mobile phase: 150 mM Phosphate buffer (pH 7.0) : IPA = 95 : 5 (v/v);
Flow rate: 1.0 mL/min; Detector: UV 280 nm; Column temperature: 25°C;
Injection volume: 10 µL;
Samples: HSA fusion peptide 5 mg/mL (MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment), HSA 2 mg/mL



HSA conjugated peptide Analysis on Proteomix SAX-NP5

Column: Proteomix SAX-NP5 (5 µm, 4.6 x 250 mm)
Mobile phase: Piperazine, Triethanolamine, bis-tris propane and N-methylpiperazine, 20 mM each, A, pH 9.7; B, pH 3.0 (adjust by HCl);
Flow rate: 0.5 mL/min; Detector: UV 280 nm; Column temperature: 30 °C;
Pressure: 139 bar; Injection volume: 10 µL, MW 75 kD, pI 5.0, HSA conjugated peptide in diabetes treatment

Time (min)	Flow (mL/min)	A%	B%
0		35	65
30	0.5	0	100