



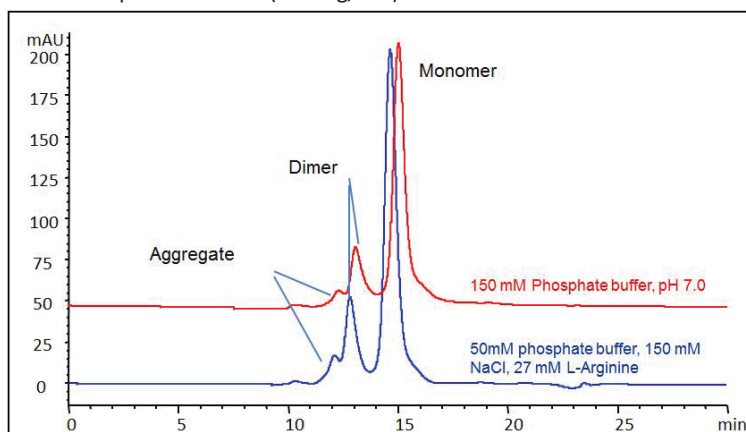
### Highlighted FACTS:

- Fusion proteins are structural hybrids that contain two different proteins, which retain the functional properties from the original proteins.
- Zenix and Zenix-C SEC columns are suitable for aggregation/monomer separation of fusion proteins including Fc fusion and HSA fusion proteins.

- Proteomix SAX and SCX provide high resolution separation of fusion proteins, especially different glycosylation forms, charge variants and fusion proteins from their formulation components.

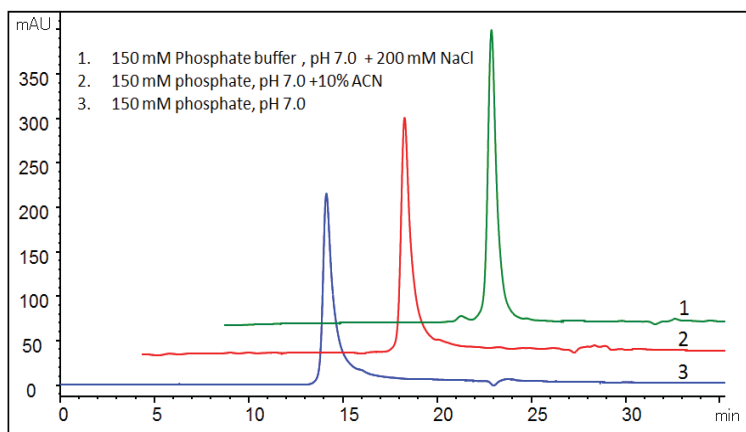
### Fc Fusion Protein 1 - Mobile Phase Effect

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 ).



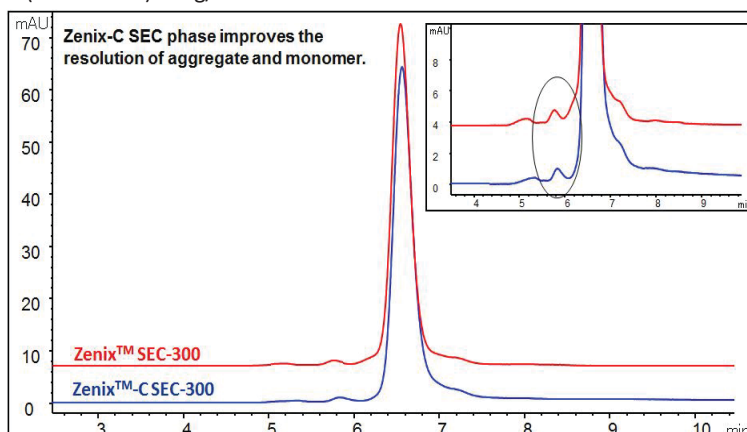
### Fusion Protein 3 - Mobile Phase Effect

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.



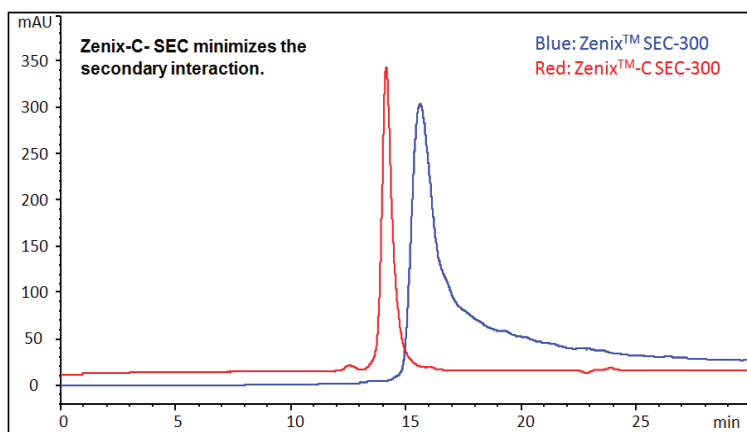
### Fc Fusion Protein 2: rhTNFR-Fc - SEC Phase Comparison

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 - 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.

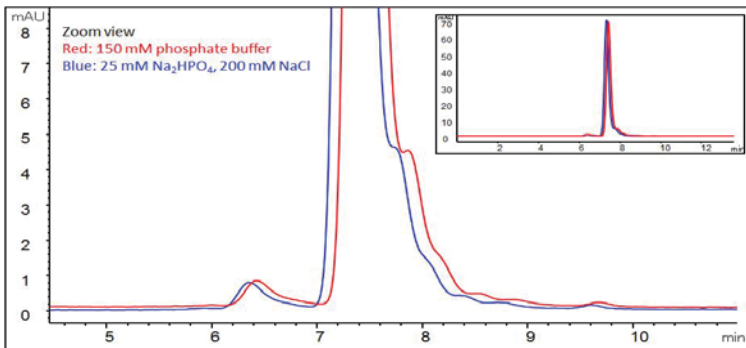




## Fusion Protein Separation

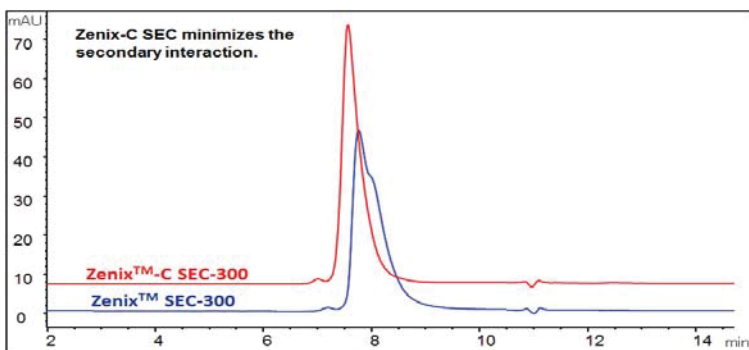
### Fusion Protein 4 - Mobile Phase Effect

Column: Zenix™ SEC-300 (3 μm, 300 Å, 7.8 x 300 mm);  
 Mobile phase 1: 25 mM Na<sub>2</sub>HPO<sub>4</sub>, 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).



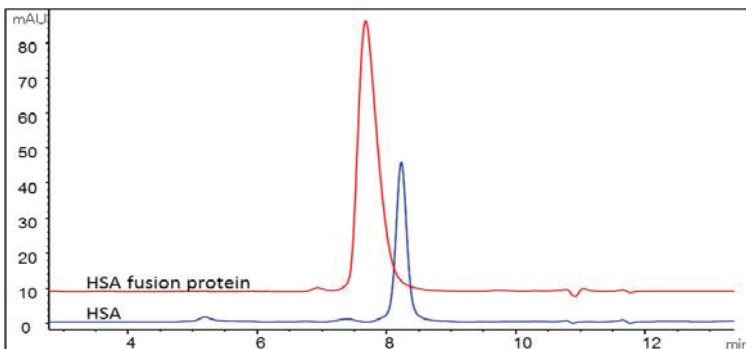
### HSA Conjugated Peptide - SEC Phase Comparison

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).



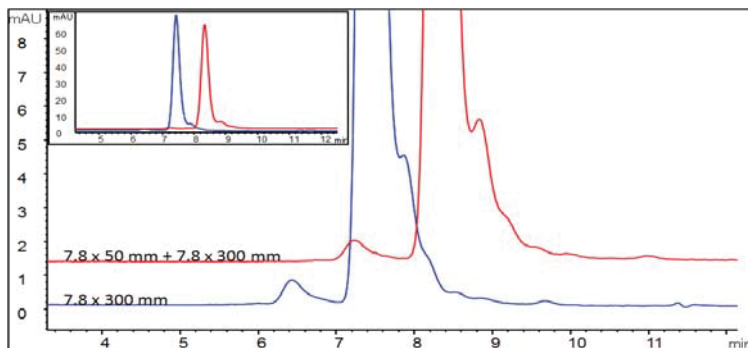
### HSA Fusion Protein vs HSA

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.



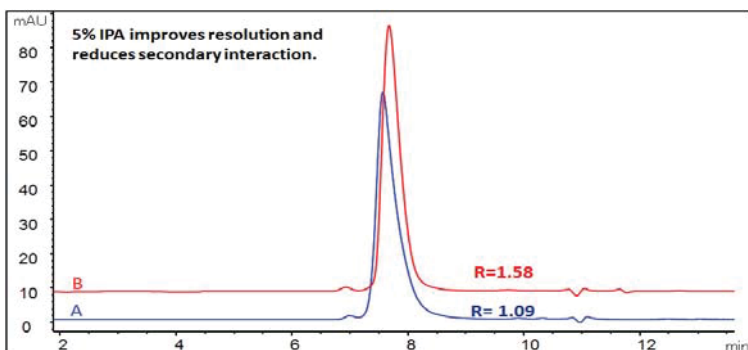
### Fusion Protein 4 - Column Length Effect

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 Na<sub>2</sub>HPO<sub>4</sub>, 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).



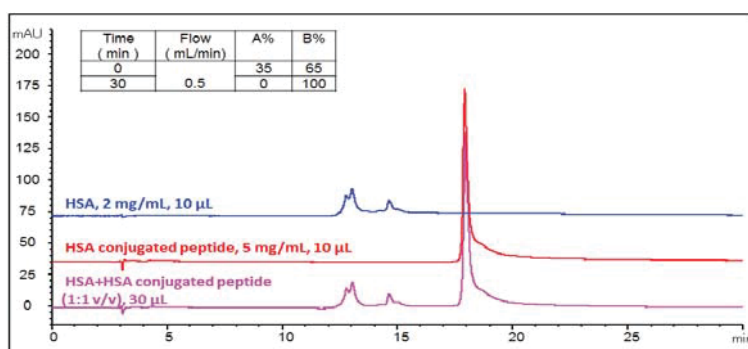
### HSA Fusion Protein - Mobile Phase Effect

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 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.



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