Fusion Protein Separation

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.

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, pl 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, pl 6.8-7.0.

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

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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 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, pl 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, pl 5.0, HSA conjugated peptide in diabetes treatment).

**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);
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, pl 5.0, 50 mg/mL, dilute to 10 mg/mL).

**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, pl 5.0, HSA conjugated peptide in diabetes treatment), HSA 2 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, pl 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, pl 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, pl 5.0, HSA conjugated peptide in diabetes treatment.

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