2.1 mm ID Size Exclusion chromatography for Protein Separation

Native volatile buffer
200 mM ammonium acetate, pH 7.0
Vs.
Native salt buffer
150 mM sodium phosphate, pH 7.0

Native volatile buffer can be applied to native protein SEC-LC-MS analysis without denaturing the protein.

Reference: Anal. Chem. 2012, 84, 2843–2849,
Native Intact Mass Determination of Antibodies Conjugated with Monomethyl Auristatin E and F at Interchain Cysteine Residues
BSA SEC Analysis

Column: Zenix-C SEC-300 (3 µm, 300 Å, 2.1 x 300 mm)
Mobile phase: See chromatogram
Flow rate: 0.08 mL/min; Detector: UV 280 nm; Column temperature: 25 °C
Injection volume: 1 µL, Sample: BSA 1 mg/mL

200 mM Ammonium Acetate, pH 7
150 mM Phosphate Buffer, pH 7
Monoclonal antibody analysis

Column: Zenix-C SEC-300 (3 µm, 300 Å, 2.1 x 300 mm)
Mobile phase: See chromatogram
Flow rate: 0.08 mL/min; Detector: UV 280 nm; Column temperature: 25 °C
Injection volume: 1 µL, Sample: mAb 1 mg/mL
Antibody drug conjugate #1-cysteine conjugate with cleavable linker

Column: Zenix-C SEC-300 (3 µm, 300 Å, 2.1 x 300 mm)
Mobile phase: See chromatogram
Flow rate: 0.08 mL/min; Detector: UV 280 nm; Column temperature: 25 °C
Injection volume: 1 µL, Sample: ADC #1 1 mg/mL

![Chromatogram](chart.png)
Antibody drug conjugate #2-cysteine conjugate with non-cleavable linker

Column: Zenix-C SEC-300 (3 µm, 300 Å, 2.1 x 300 mm)
Mobile phase: See chromatogram
Flow rate: 0.08 mL/min; Detector: UV 280 nm; Column temperature: 25 ºC
Injection volume: 1 µL, Sample: ADC #2 1mg/mL

![Chromatogram of antibody drug conjugate #2 with non-cleavable linker](chart.png)

- **200 mM Ammonium Acetate, pH 7**
- **150 mM Phosphate Buffer, pH 7**
Antibody drug conjugate #3 - Lysine conjugate

Column: Zenix-C SEC-300 (3 μm, 300 Å, 2.1 x 300 mm)
Mobile phase: See chromatogram
Flow rate: 0.08 mL/min; Detector: UV 280 nm; Column temperature: 25 ºC
Injection volume: 1 μL, Sample: ADC #3 1mg/mL