

The Challenge:

Are you working with a sample that shows a tendency to stick to traditional size exclusion resins with delayed elution time, low recovery, varying HMWS, or excessive tailing?

Sepax SEC C-line columns!

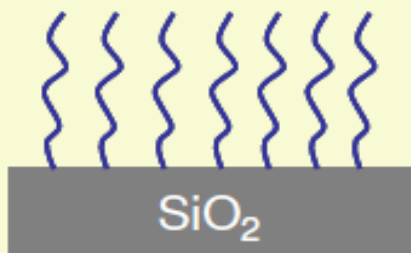
Some comparison examples on the two different surface chemistries on size exclusion separation

- ADC
- Fusion Protein
- mPEG-peptide
- Hydrophobic Peptide
- MAb
- Glycoprotein
- PEGylated peptide
- Membrane Protein

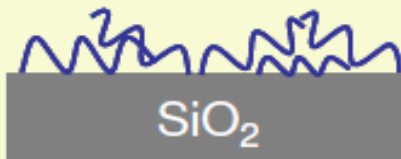


SEC phases

Difference in Phase Structures



SRT and Zenix Phase
Stand-up Monolayer



SRT-C and Zenix-C Phase
Lay-down Monolayer

Tough samples:

ADC, Derivatized MAb,
Pegylated Protein,
Membrane Protein,
“Super sticky protein”,
and etc.

Characteristics	Zenix, SRT	Zenix-C, SRT-C
Particle size	3 μm , 5 μm	3 μm , 5 μm
Pore size ($\text{\AA}$)	Zenix 80, 100, 150, 300, SRT 100, 150, 300, 500, 1000 & 2000	Zenix-C 80, 100, 150, 300 SRT-C 100, 150, 300, 500, 1000 & 2000
Surface structure	Chemically bonded stand-up monolayer	Chemically bonded lay-down monolayer
Applications	Versatile for mAbs, proteins, peptides, oligonucleotides, virus, VLPs, and water- soluble polymers	Ideal for hydrophobic samples such as insulin or ADC, hydrophobic mAb, pegylated peptides and proteins

SRT SEC-10 (10 μm) is the latest addition for semi-prep and prep scale separation.
SRT SEC-1000 and 2000 are suitable for very large biomolecule separation.

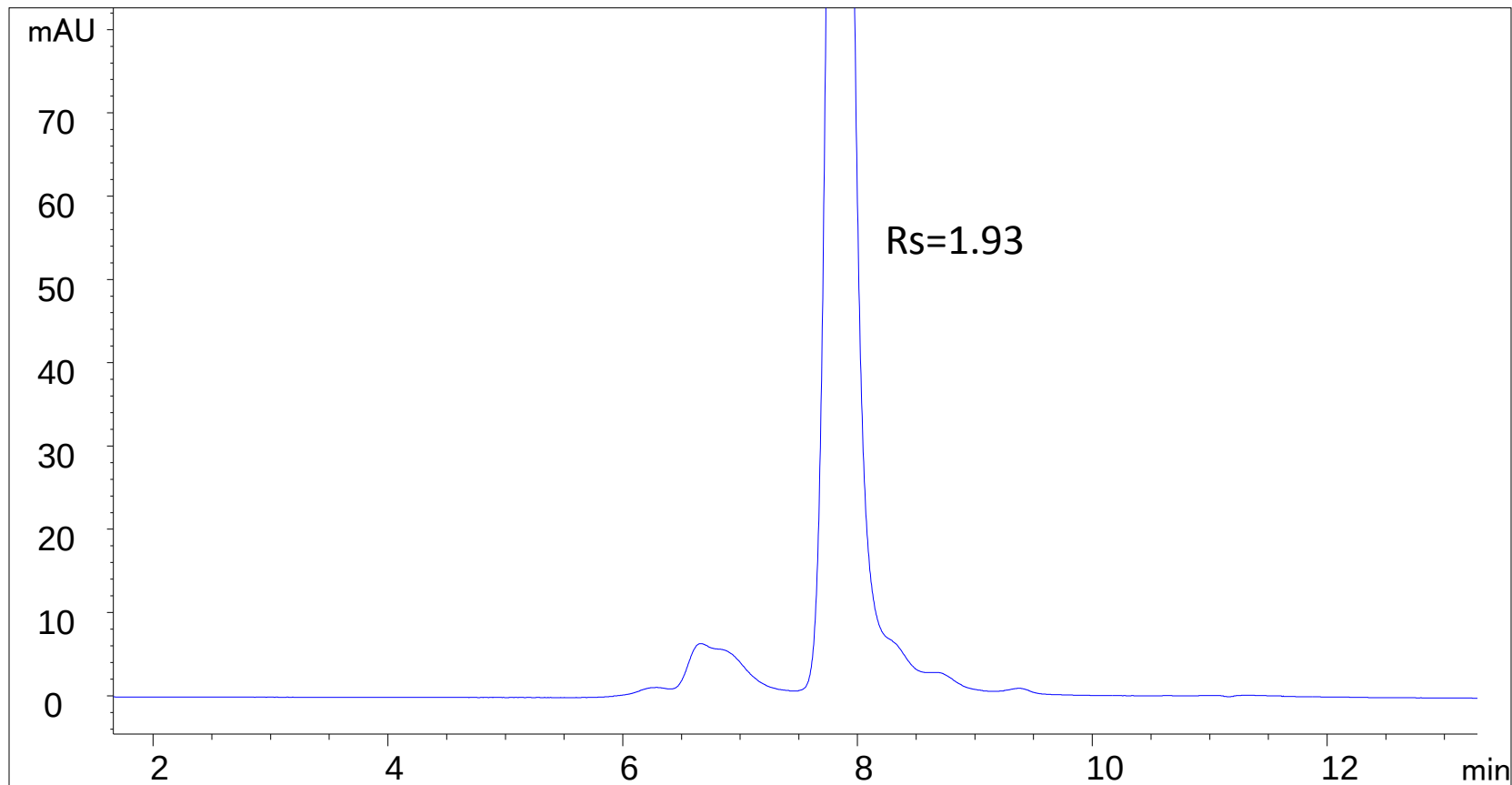
Herceptin Analysis on Zenix™ SEC-300 (7830)

Column: Zenix™ SEC-300, (3 μm , 300 Å, 7.8 x 300 mm)

Mobile phase: 150 mM phosphate buffer

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

Injection volume: 10 μL ; Samples: Herceptin 2.34 mg/mL



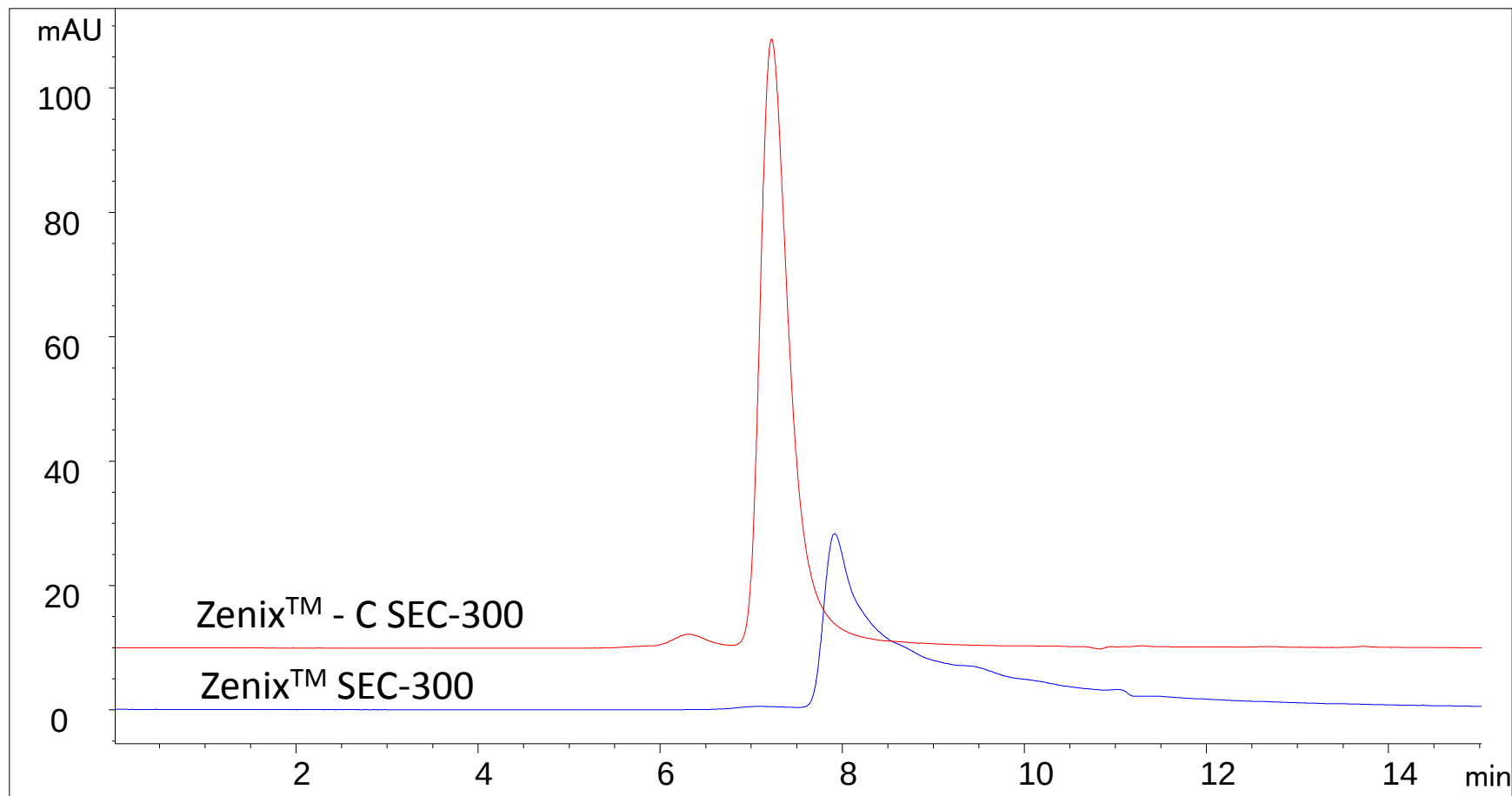
Herceptin Lysine ADC Analysis on SEC-300 (7830)

Column: Zenix™ SEC-300, Zenix™ - C SEC-300 (3 μm, 300 Å, 7.8 x 300 mm)

Mobile phase: 150 mM phosphate buffer

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

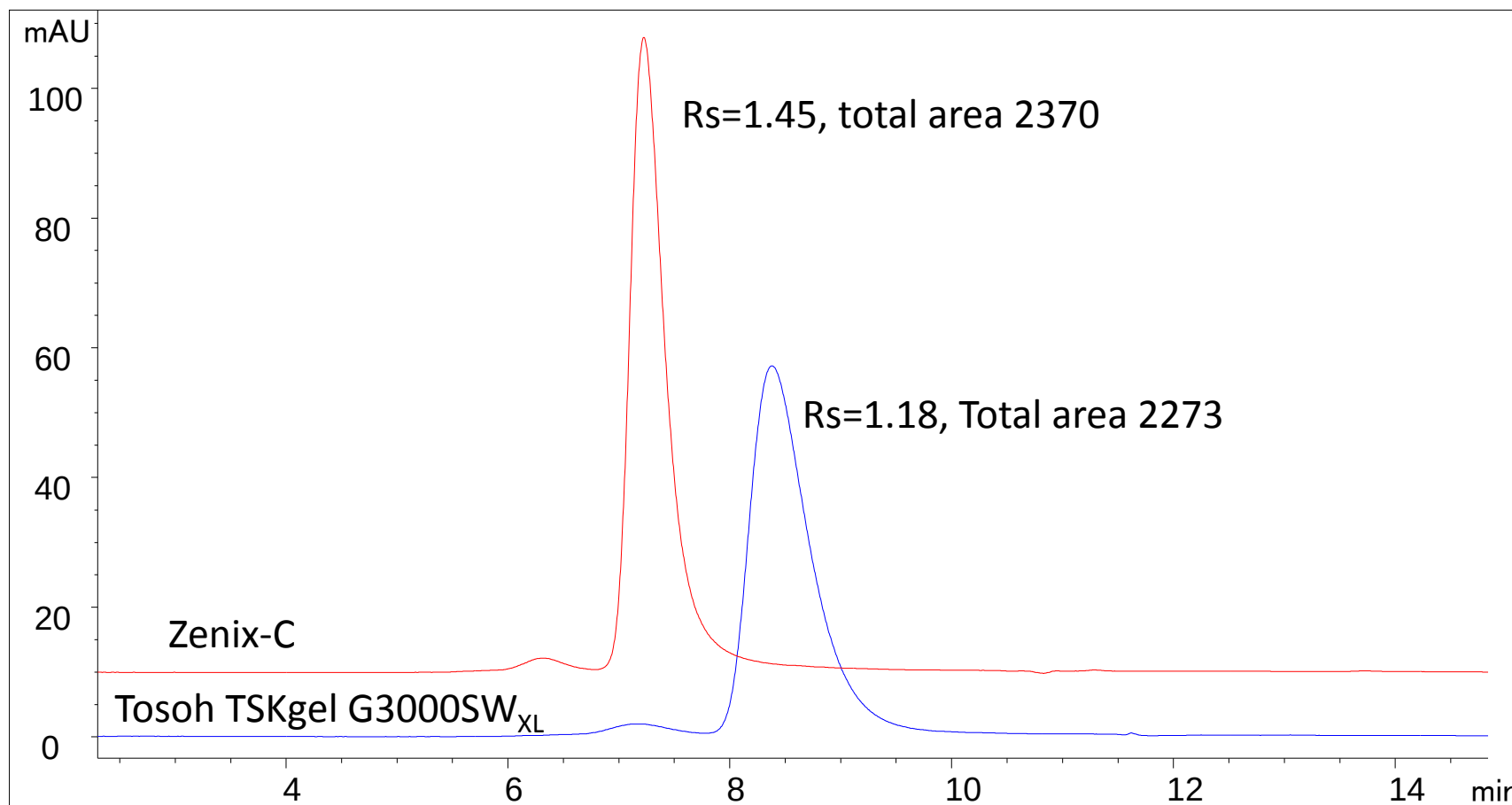
Injection volume: 10 μL; Samples: Herceptin-lysine conjugate 2.05 mg/mL



Herceptin-Lysine Drug Conjugate Analysis

Zenix-C SEC-300 vs. Tosoh TSKgel G3000SW_{XL}

Column: Zenix® - C SEC-300 (3 μm , 300 $\text{\AA}$, 7.8 x 300 mm) , Tosoh TSKgel G3000SW_{XL} (5 μm , 250 $\text{\AA}$, 7.8 x 300 mm)
Mobile phase: 150 mM phosphate buffer, pH 7.0; Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 $^{\circ}\text{C}$
Injection volume: 10 μL ; Samples: Herceptin-lysine drug conjugate 2.05 mg/mL



Disclaimer: TSKgel and Tosoh Bioscience are registered trademarks of Tosoh Corporation; Comparative separations may not be representative of all applications.



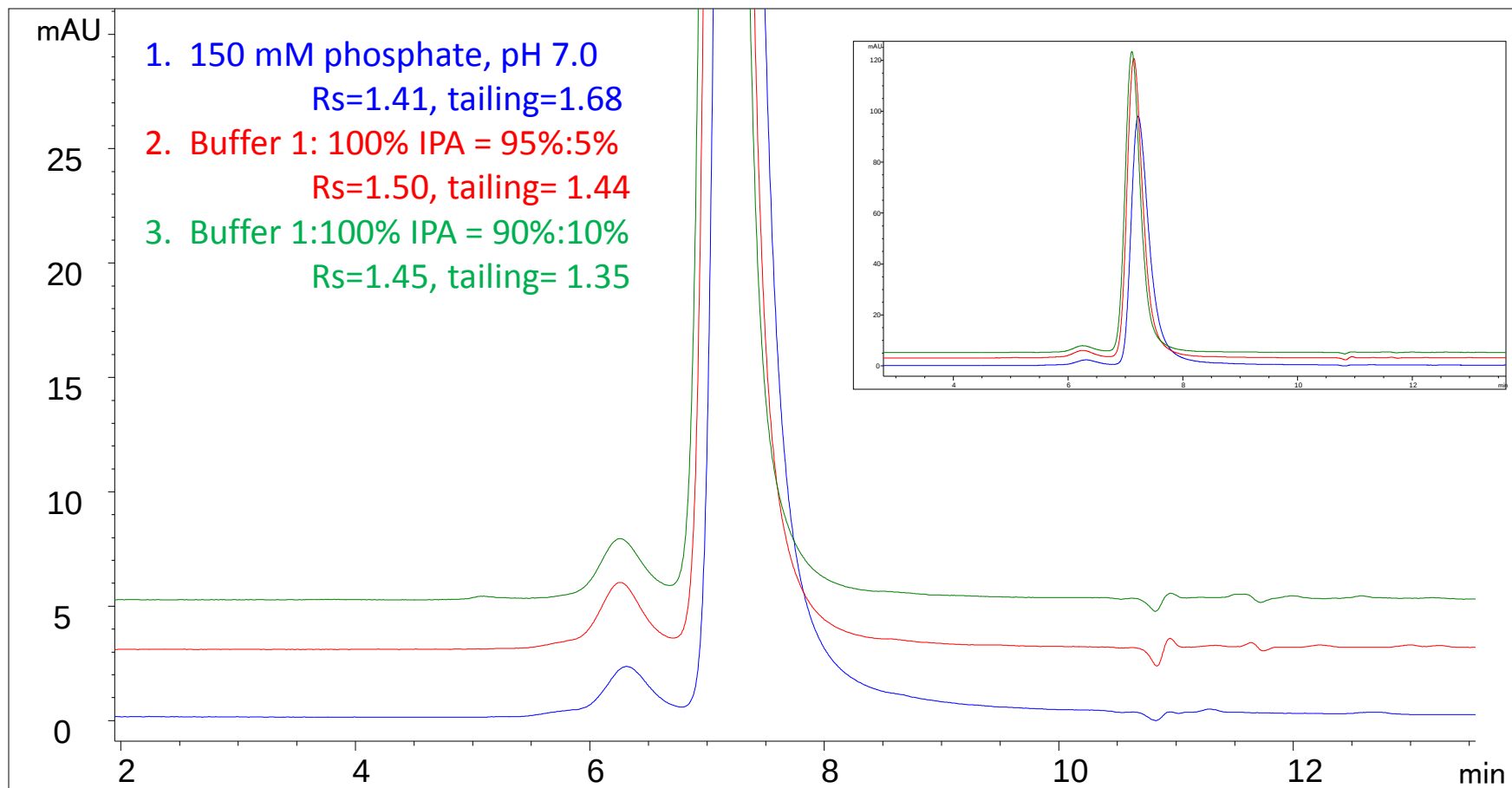
Herceptin Lysine ADC Analysis on SEC-300 (7830)

Column: Zenix™ - C SEC-300 (3 μm , 300 Å, 7.8 x 300 mm)

Mobile phase: as indicated

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

Injection volume: 10 μL ; Samples: Herceptin lysine conjugate 2.05 mg/mL



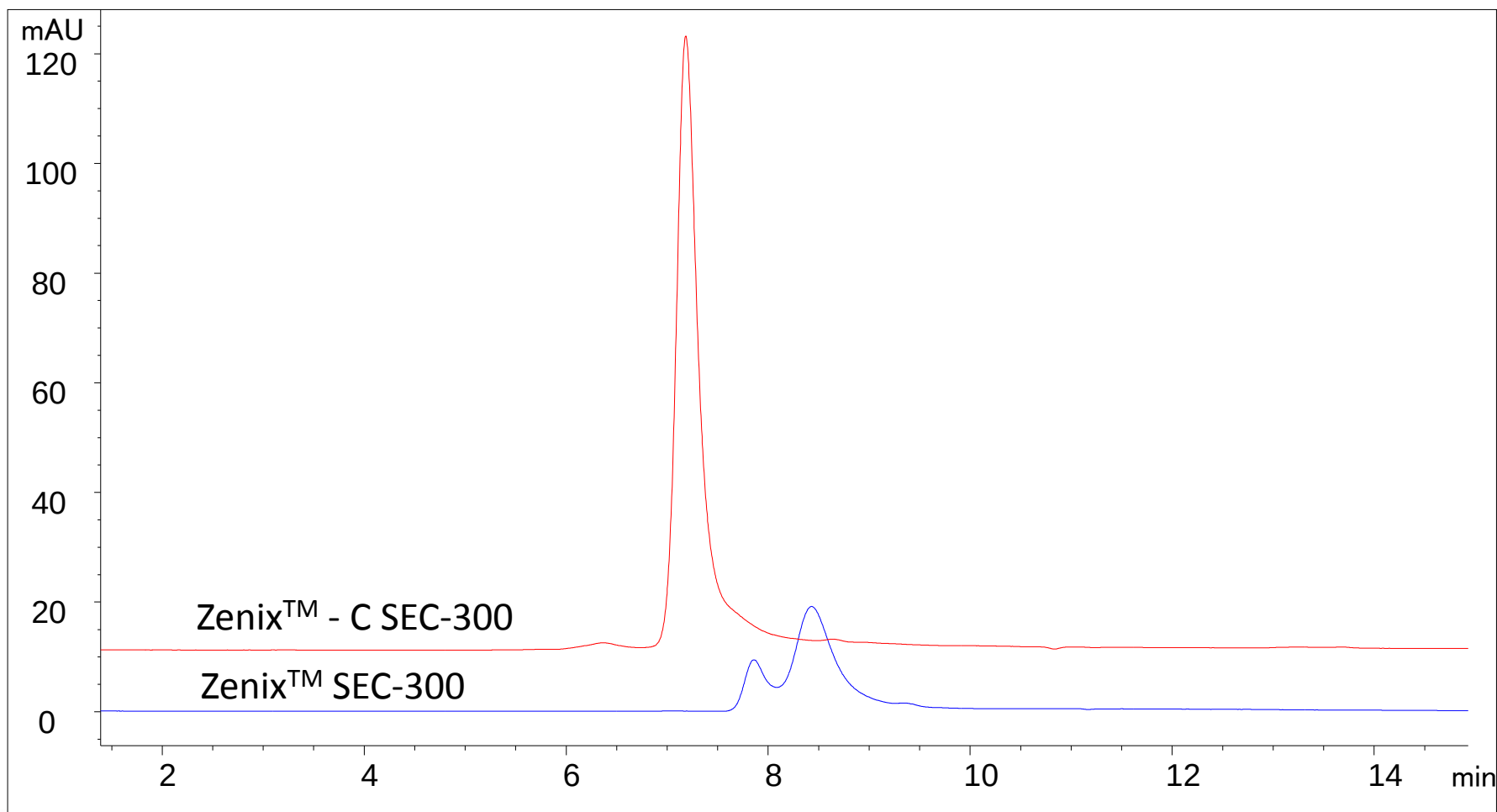
Herceptin Cysteine ADC (cleavable linker) Analysis on SEC-300 (7830)

Column: Zenix™ SEC-300, Zenix™ - C SEC-300 (3 μm, 300 Å, 7.8 x 300 mm)

Mobile phase: 150 mM phosphate buffer

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

Injection volume: 10 μL; Samples: Herceptin Cysteine ADC with cleavable linker 2.5 mg/mL



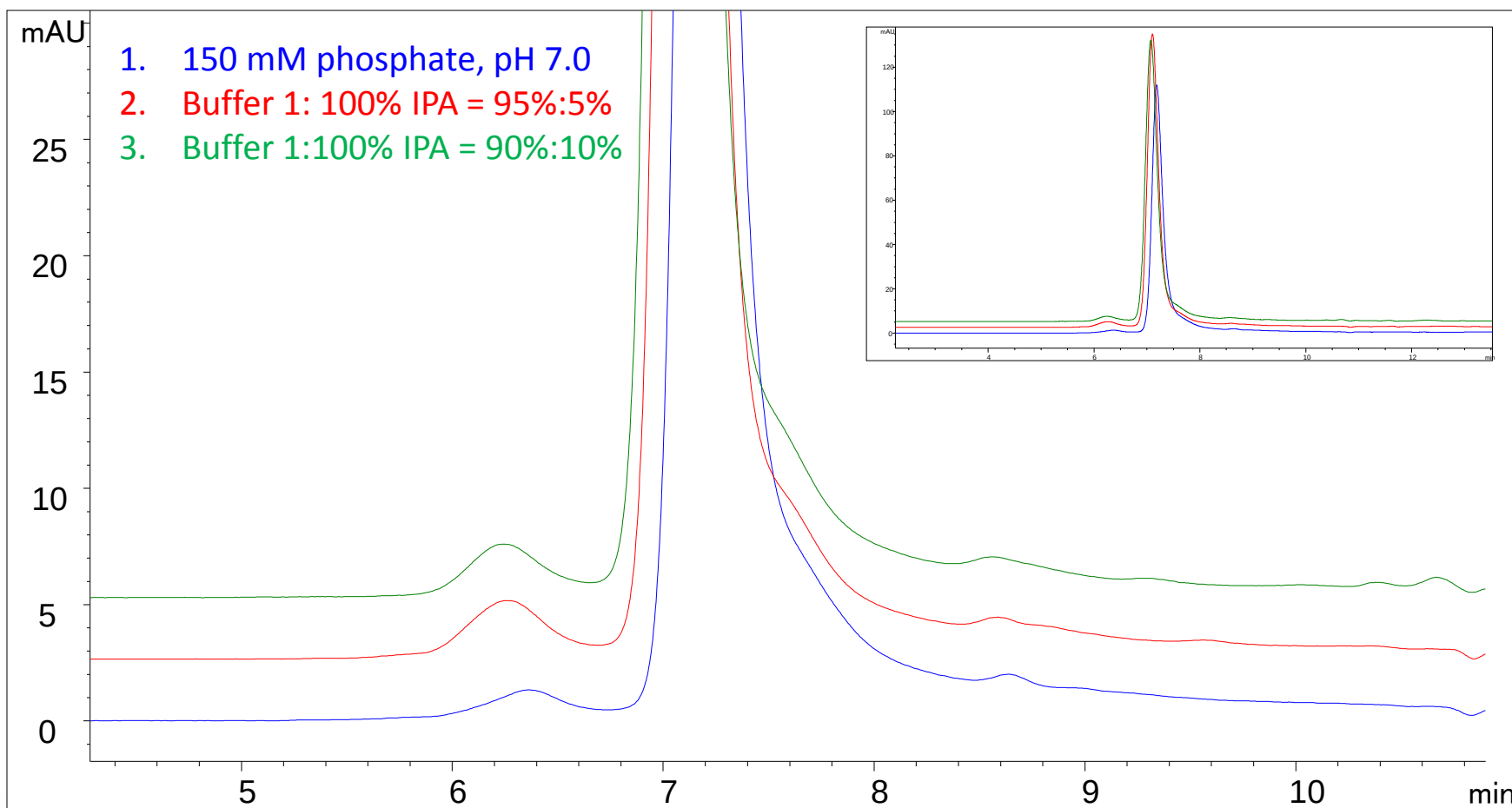
Herceptin Cysteine ADC Analysis on SEC-300 (7830)

Column: Zenix™ - C SEC-300 (3 μm , 300 Å, 7.8 x 300 mm)

Mobile phase: as indicated

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

Injection volume: 10 μL ; Samples: Herceptin Cysteine ADC with cleavable linker 2.5 mg/mL



Antibody drug conjugate Analysis on SEC-300 (7830)

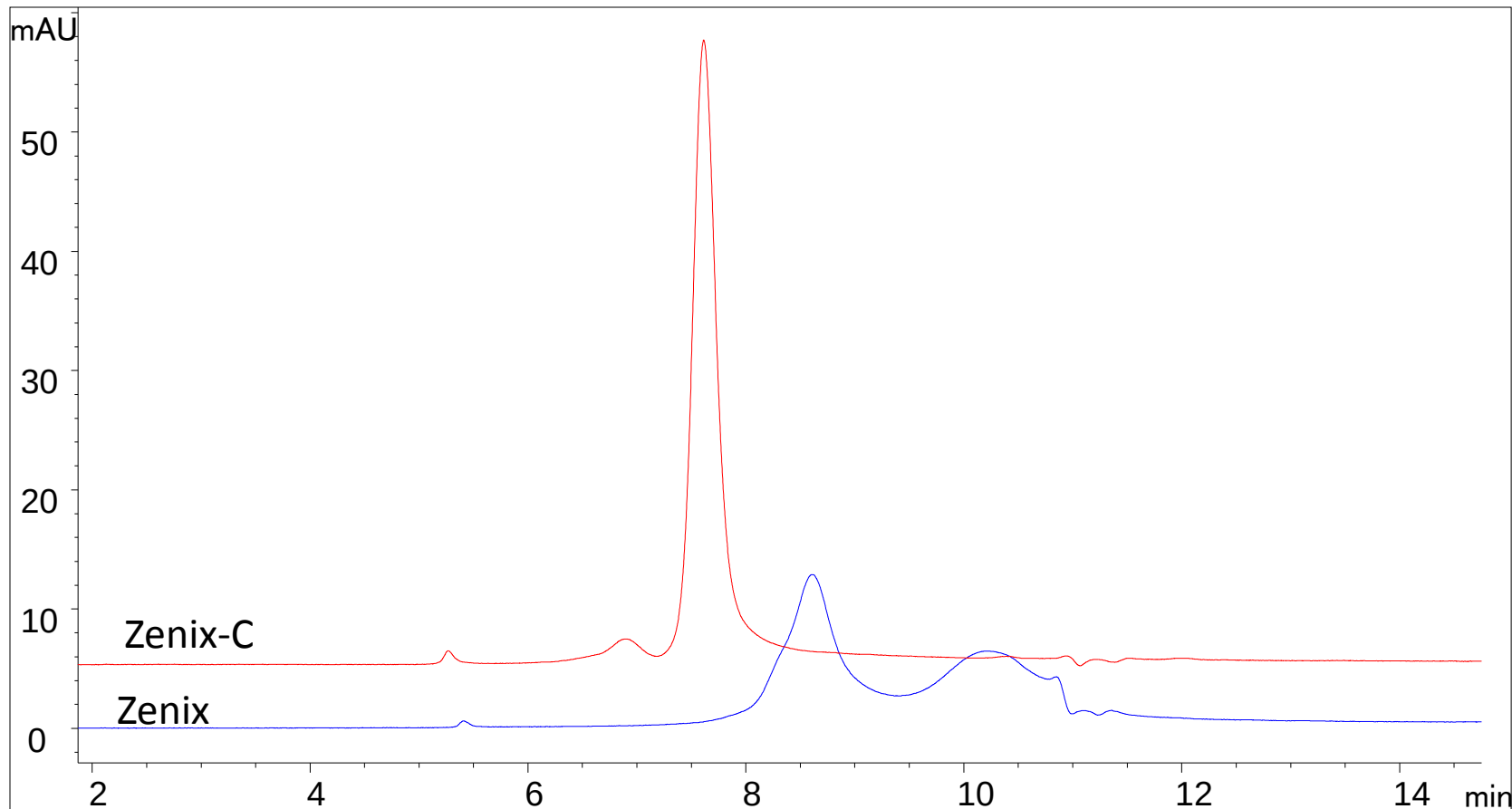
Phase comparison

Column: Zenix™ SEC-300, Zenix™-C SEC-300 (3 μm, 300 Å, 7.8 x 300 mm)

Mobile phase: 150 mM phosphate buffer

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

Injection volume: 20 μL; Samples: 1.68 mg/mL ADC



Zenix-C SEC-300 minimizes the secondary interaction between ADC and resin.



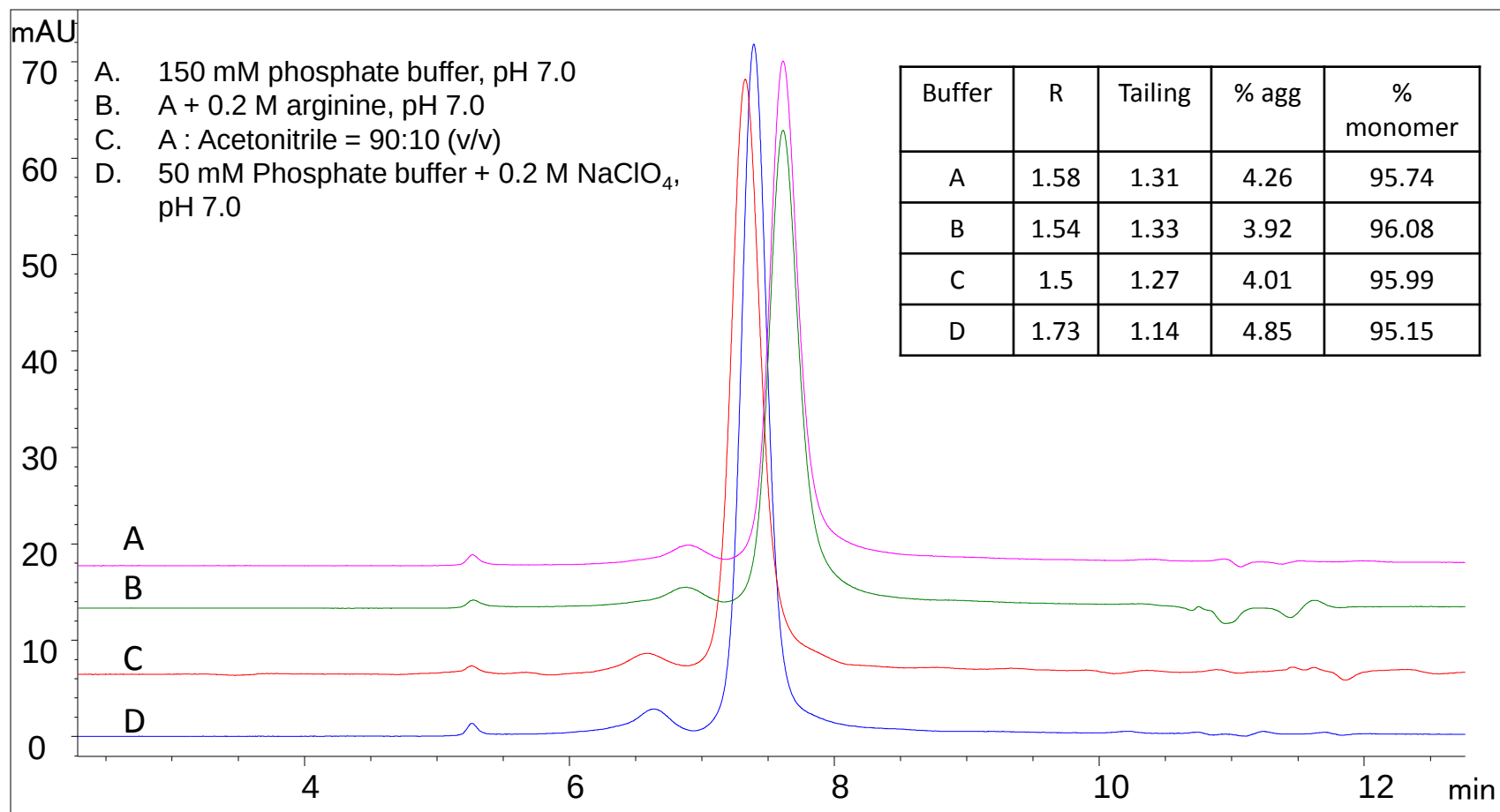
Antibody drug conjugate Analysis on Zenix™-C SEC-300 (7830)

Column: Zenix™-C SEC-300 (3 μ m, 300 Å, 7.8 x 300 mm)

Mobile phase: As indicated

Flow rate: 1 mL/min; Detector: UV 280 nm; Column temperature: 25 °C

Injection volume: 20 μ L; Samples: 1.68 mg/mL ADC



With 10% acetonitrile and 200 mM NaClO₄, total protein recovery, resolution and tailing factor of monomer peak are improved.



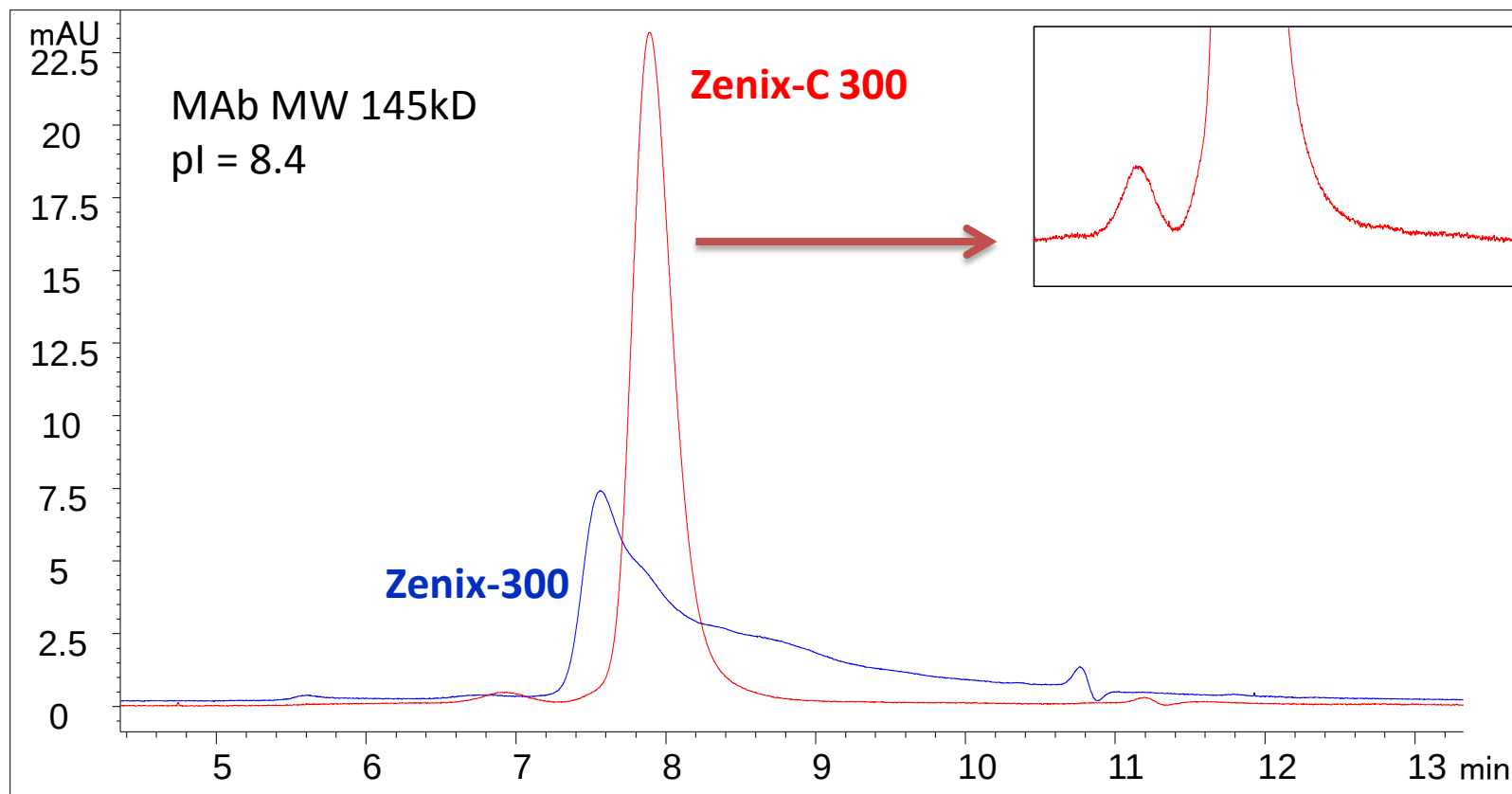
MAb F Analysis on Zenix-300 and Zenix-C 300

Column: Zenix-300, Zenix-C 300 (3 μm , 300 Å, 7.8 x 300 mm)

Mobile phase: 150 mM Sodium Phosphate, pH 7.0, Flow rate: 1.0 mL/min

Detector: UV 280 nm, Column temperature: 25 °C, Pressure: 74 bar

Injection volume: 10 μL , Sample: 1.23 mg/mL MAb F in 10 mM sodium succinate, pH 5.0



C-line SEC outperforms



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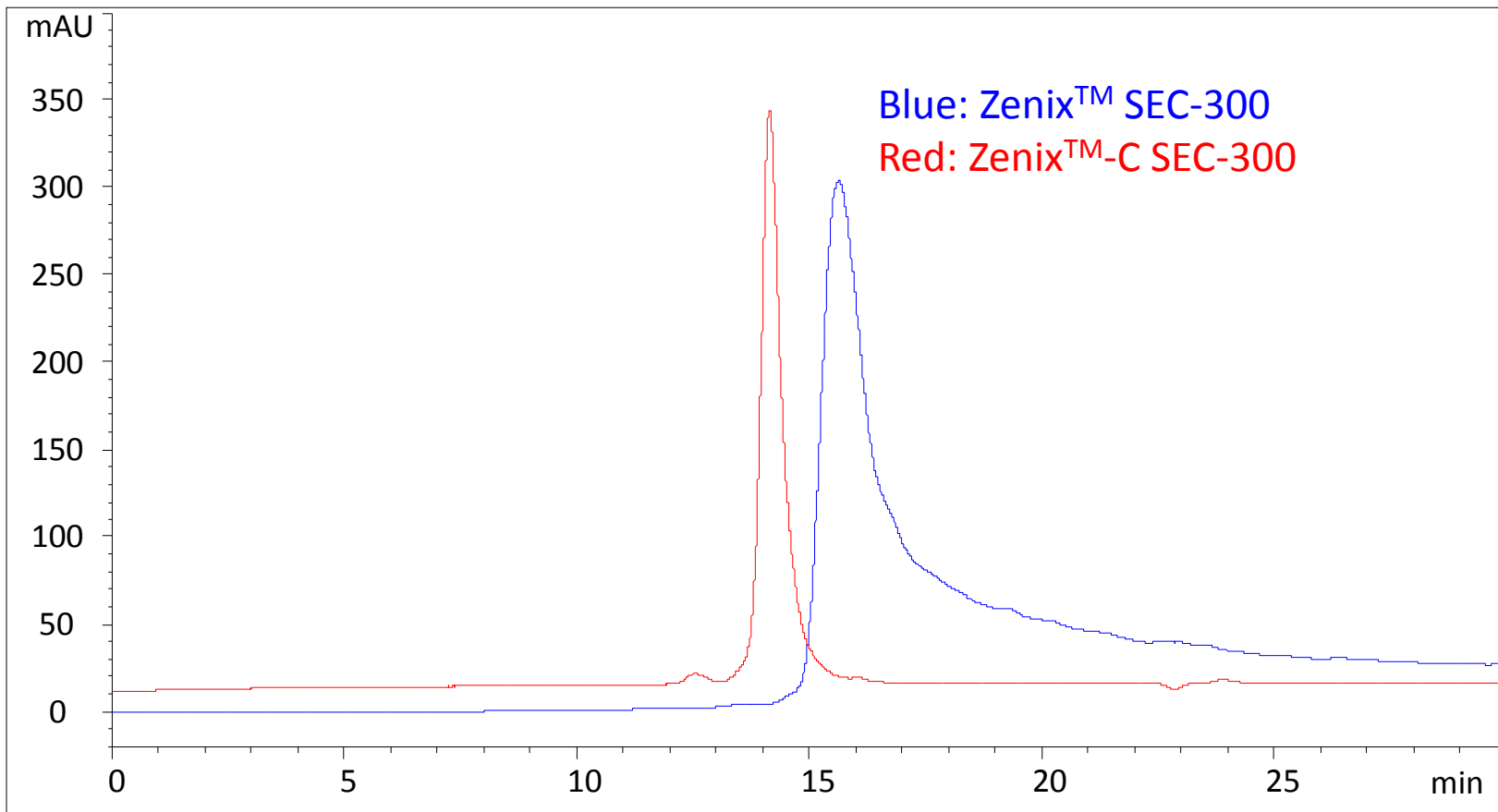
Fusion Protein Analysis on SEC-300 (7830)

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



C-line SEC minimizes the secondary interaction.

Fusion Protein Analysis on Zenix™-C SEC-300 (7830)

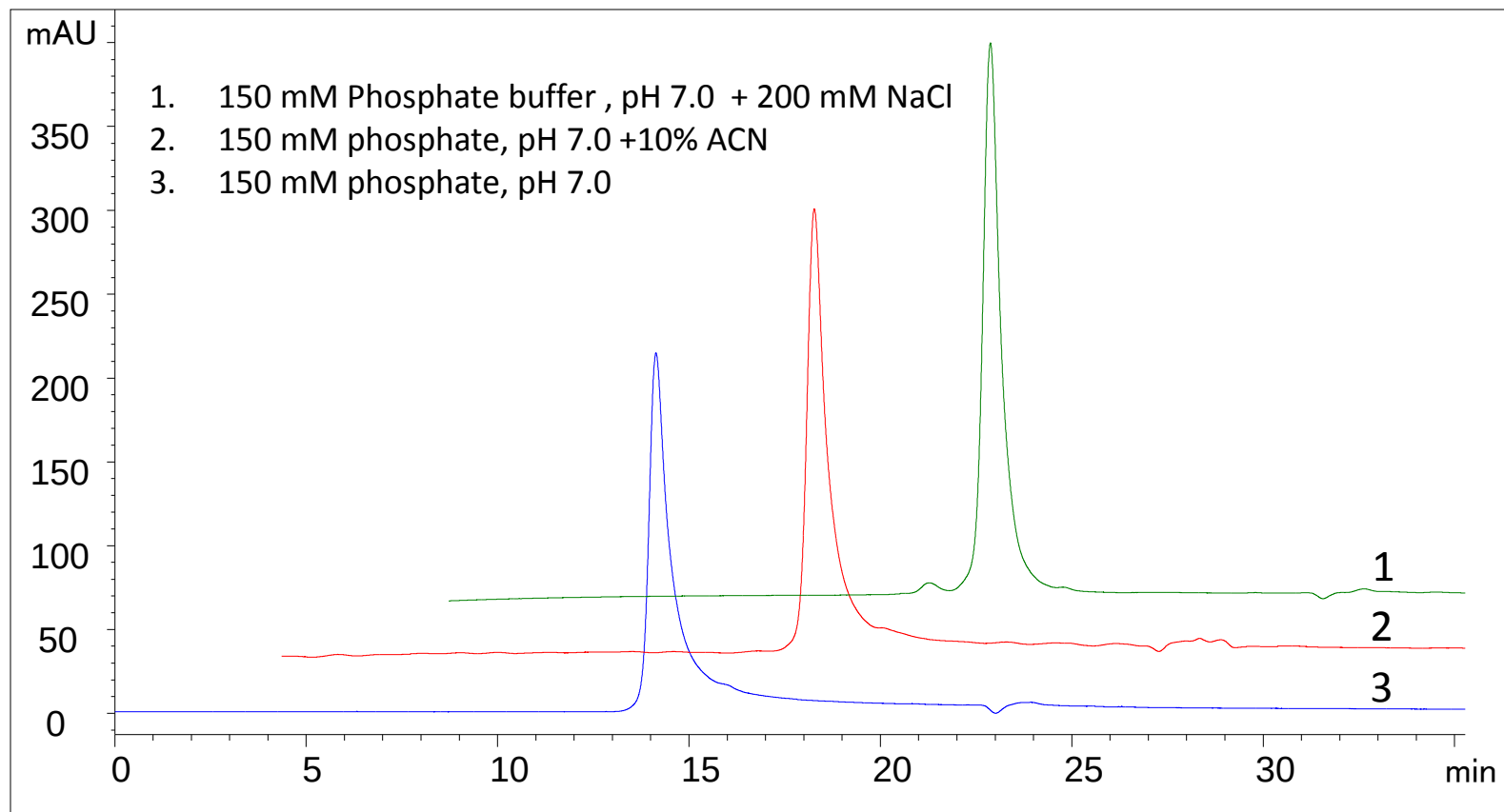
Mobile phase effect

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

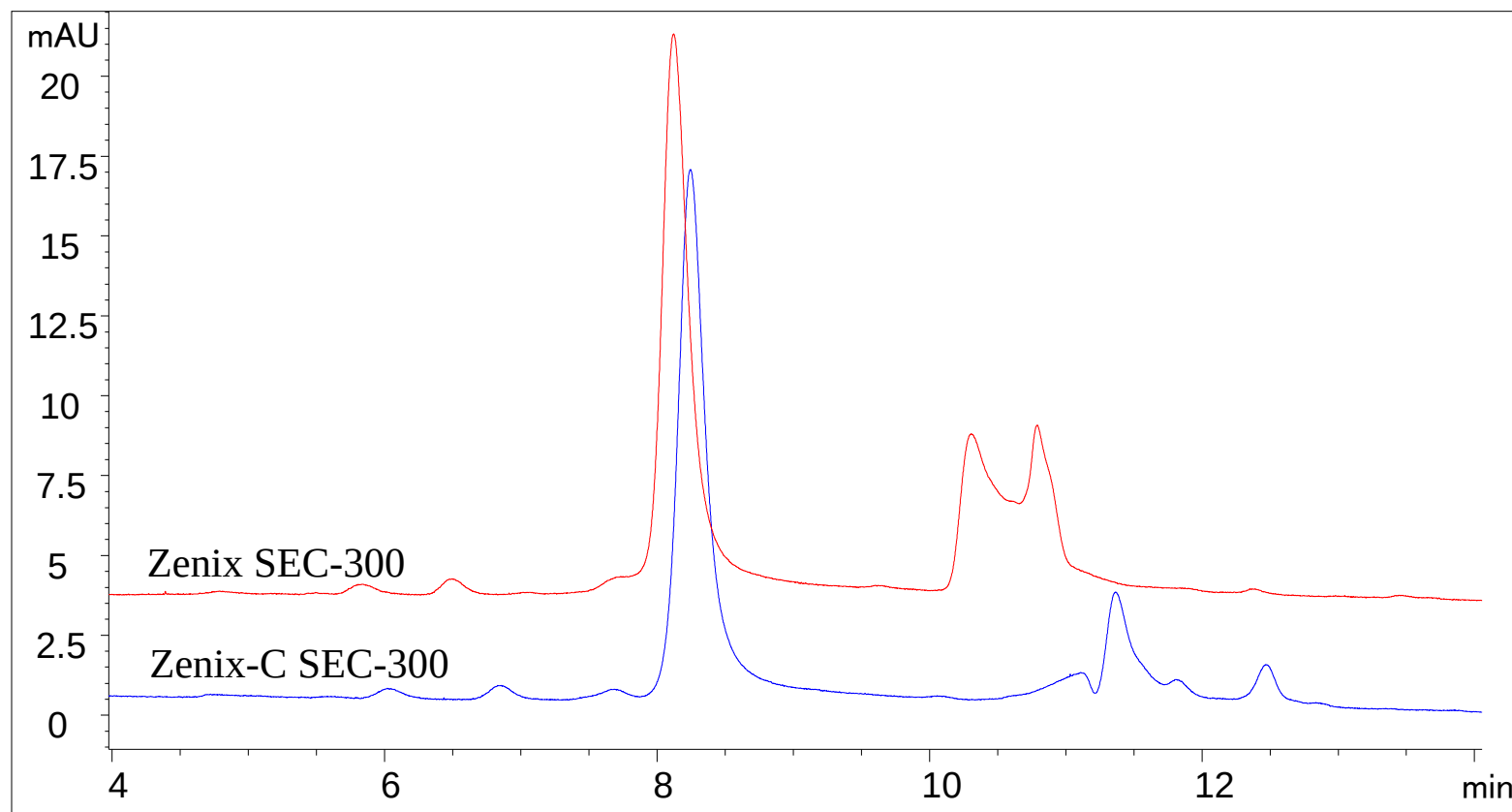


10% retention time offset for presentation purpose



β 2 Glycoprotein I analysis on Zenix-300 and Zenix-C 300

Column: 7.8x300 mm, Mobile phase: 150 mM Sodium Phosphate Buffer pH 7.0, Flow rate: 1.0 mL/min, Sample: β 2 Glycoprotein I (0.5 mg/mL in 1M glycine, 750 mM NaCl, pH 7.4), Injection volume: 20 μ L, Detection: UV 280 nm



Zenix-C gives a better separation



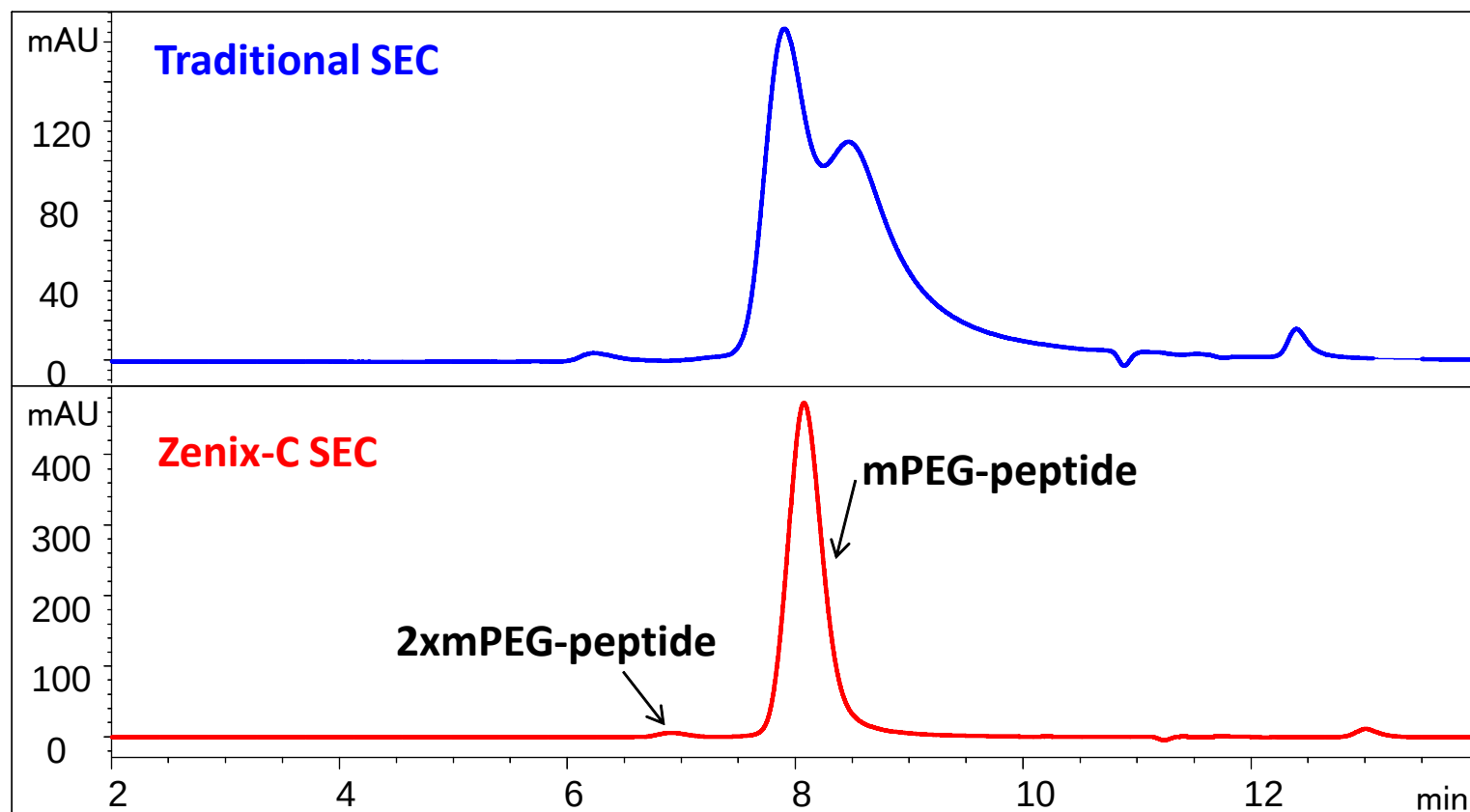
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mPEG-peptide (20 kD PEG + 4 kD peptide)

Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is at 1.0 mL/min with UV214 nm detection. mPEG-peptide concentration is 6 mg/mL, injection volume is 10 μ L.



Pegylated Exenatide on Zenix-300 and Zenix-C 300

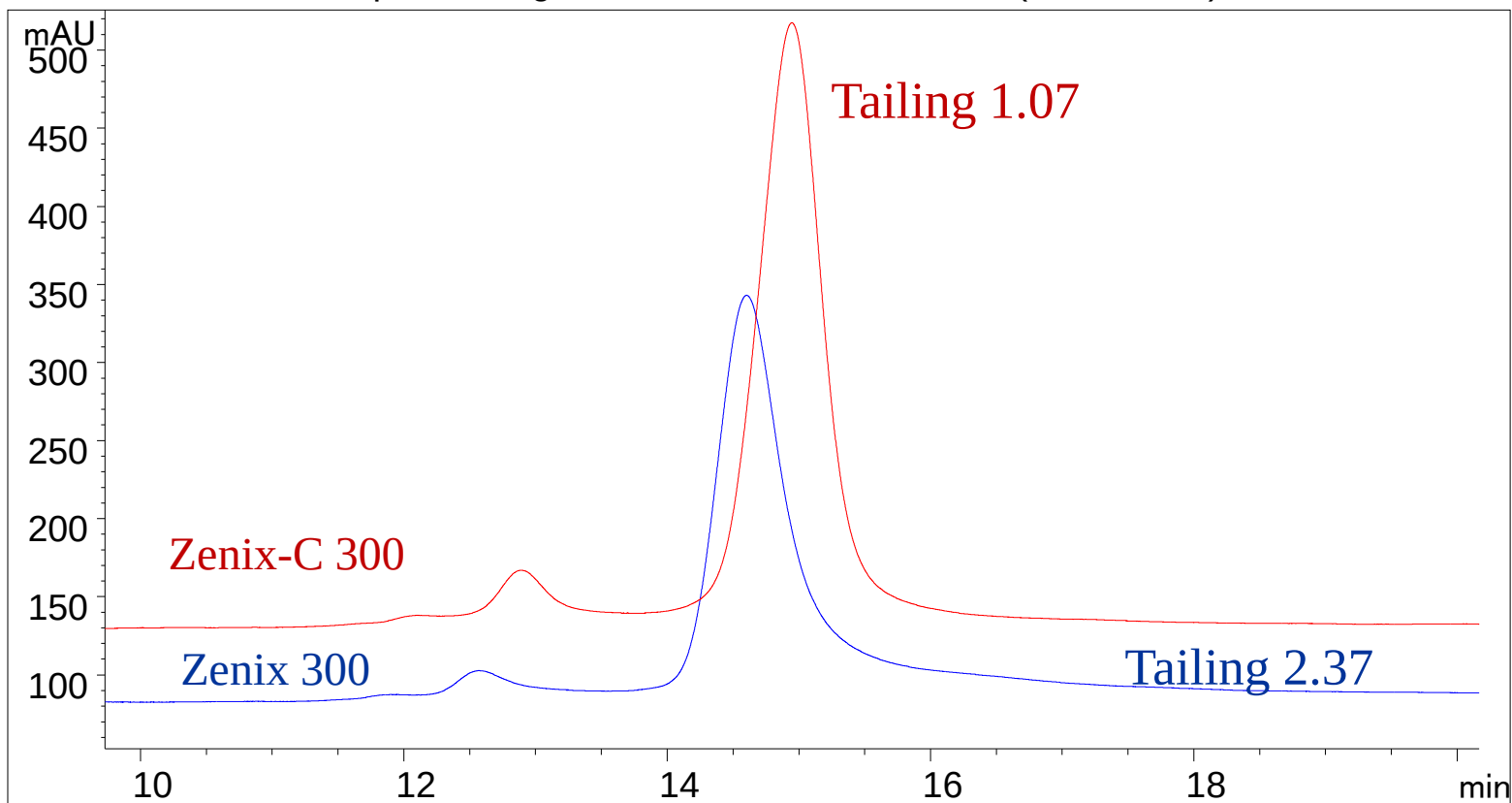
Column: Zenix-300 and Zenix-C 300 (3 μ m, 300 Å, 7.8 x 300 mm)

Mobile phase: 50 mM CH₃COONH₄ : ACN = 90 : 10 (v/v),

Flow rate: 0.5 mL/min

Detector: UV 214 nm, Column temperature: 25 °C, Injection volume: 15 μ L

Pressure: 42 bar, Sample: 3.3 mg/mL PEG-Exenatide in water (PEG 23 kD)



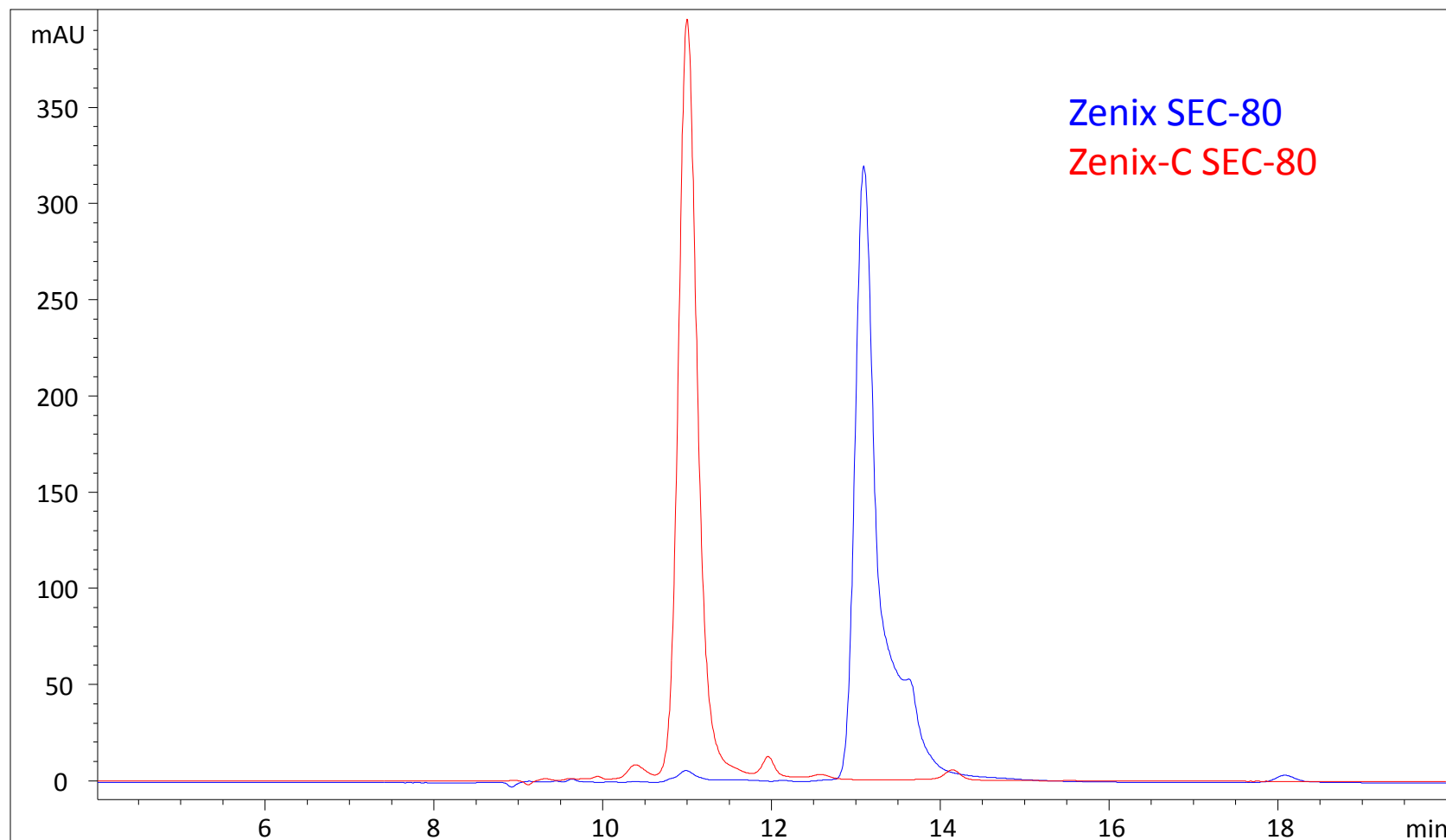
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Angiotensin I Acetate separation on Zenix-80 and Zenix-C 80

Column: Zenix SEC-80 7.8x300 mm, Flow rate: 1mL/min, Detection: UV 214 and 280 nm,
Mobile phase: 150 mM Sodium Phosphate Buffer pH 7.0, Injection Volume: 5 μ L

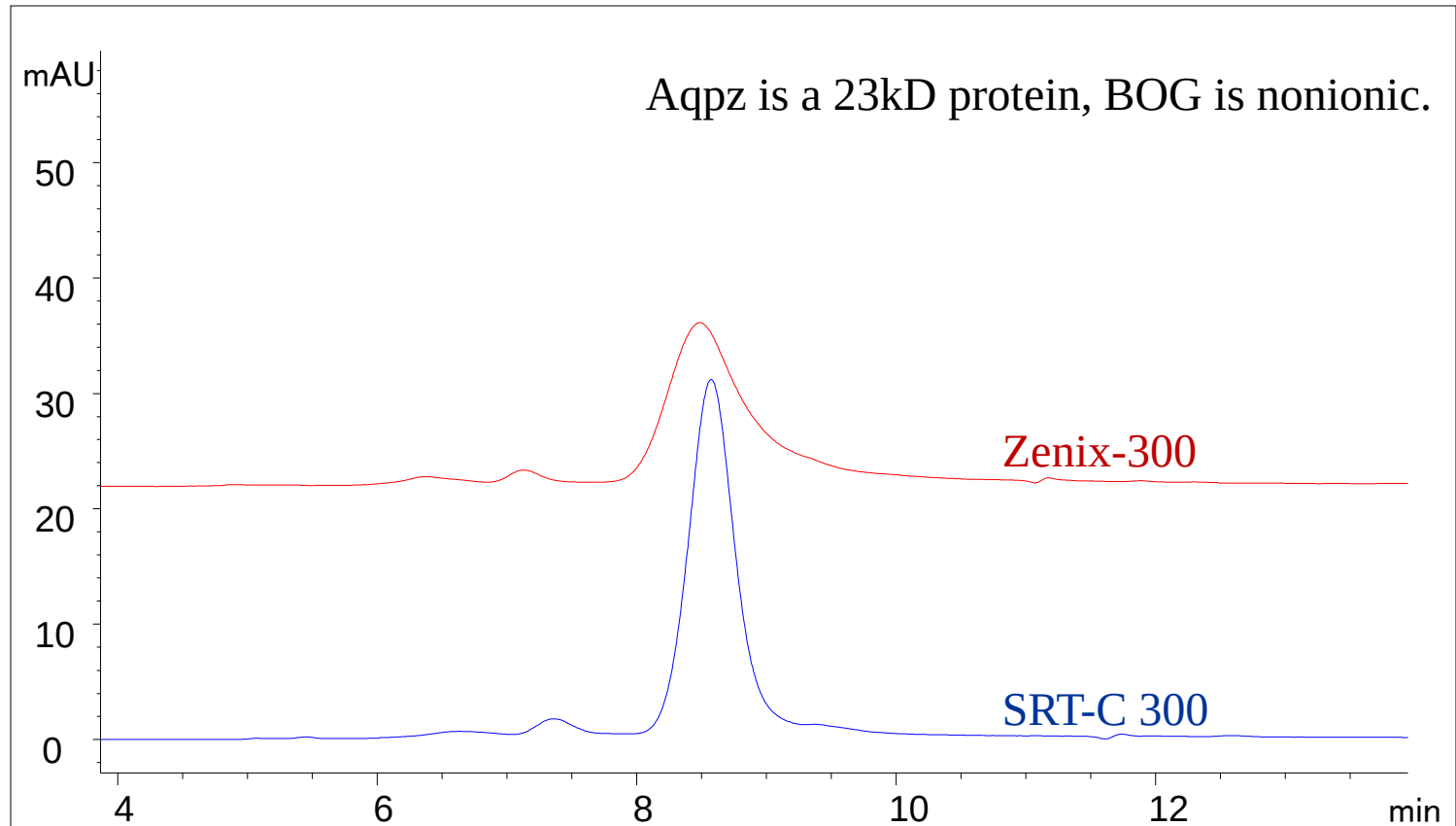


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Membrane protein Aqpz separation on Zenix-300 and Zenix-C 300

Column: SRT-C 300, 7.8x300, Mobile phase: 20 mM TrisHCl, pH 7.0, 190 mM NaCl, 10 mM KCl, **40 mM Octyl glucoside**, Detection: 280nm, Flow: 1 mL/min, Injection: 2 mL of 6 mg/mL



SRT-C 300 gives the best separation with less secondary interaction, baseline separation with the high molecular weight proteins.

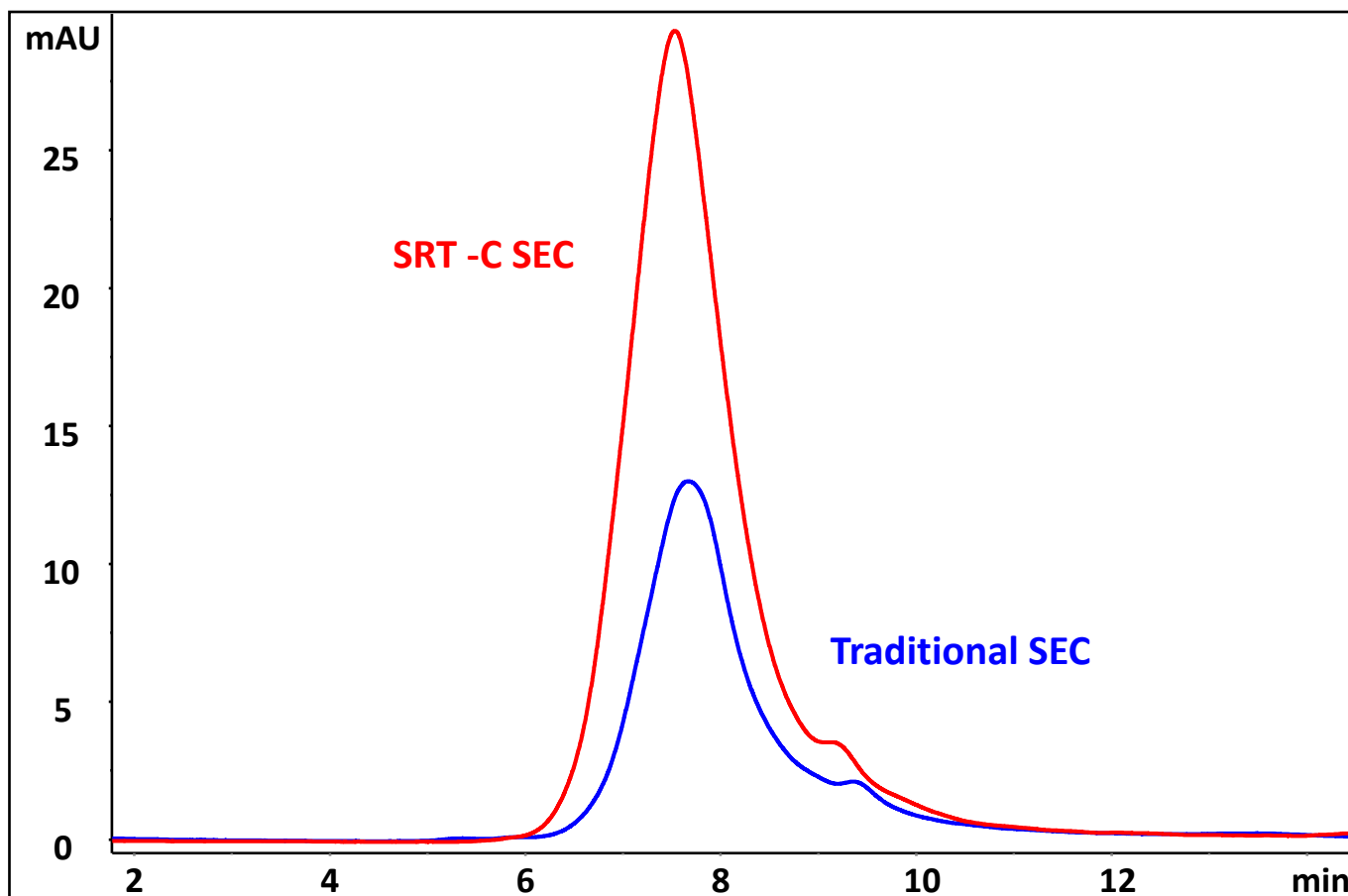


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Bacterial K Channel (16 kD homotetramer) in 0.261% DDM

Column dimension: 4.6 x 300 mm, Mobile phase: 20 mM Tris pH 7.5, 20 mM NaCl, 0.261% DDM,
Detection: 280 nm, Flow rate: 0.35 mL/min, AKTA FPLC system

SRT®-C SEC has the best recovery for Bacterial K Channel in the presence of detergents.



Acknowledgement: Sung Lee at Scripps.



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