



Sepax Technologies

Size Exclusion Columns for Tough Sample Separation

Mathew George, Helen Gu, Tingzhou Wu and Xueying Huang

***Sepax Technologies, Inc
Newark, Delaware 19711***



Sepax Technologies

www.sepax-tech.com

Background of SEC for Biological Molecules

In General: Size exclusion chromatography is one of the major tools for analysis and quality control of biopharmaceuticals, such as antibodies, PEGylated proteins, oligos and peptides. In ideal situation, the biological molecules will not have any interactions with the packing materials and elute based on their size. However, in real samples, it is much more complicated and often a challenge to analyze the sample without interference.

Case 1: Due to electrostatic or hydrophobic interactions, non-specific interactions often occur and the sample elutes later, showing tailing peaks, and in worst case the sample sticks to the packing material and does not elute.

Case 2: Matrix interference. For example, the protein sample contains Tween 20 (polysorbate). The polysorbate gives a disturbing peak just where the protein elutes.

Solution: Sepax offers a total solution for SEC separation challenges by developing very comprehensive packing materials with **7 pore sizes** from 100 to 2000Å (100, 150, 250, 300, 500, 1000 and 2000Å), **3 surface chemistries** of Nanofilm, SRT&Zenix, SRT-C&Zenix-C and **3 particle sizes** of 3, 5 and 10µm.

Examples of applications: 1) Proteins in tween-80 and 20, 2) 5-min high resolution separation of MAb, and 3) PEGlyated protein separation.



Sepax Technologies

www.sepax-tech.com

Comprehensive SEC Resins for Biological Separations

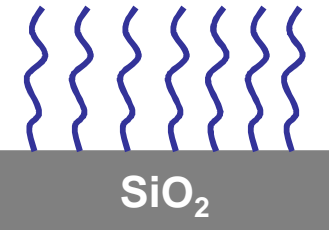
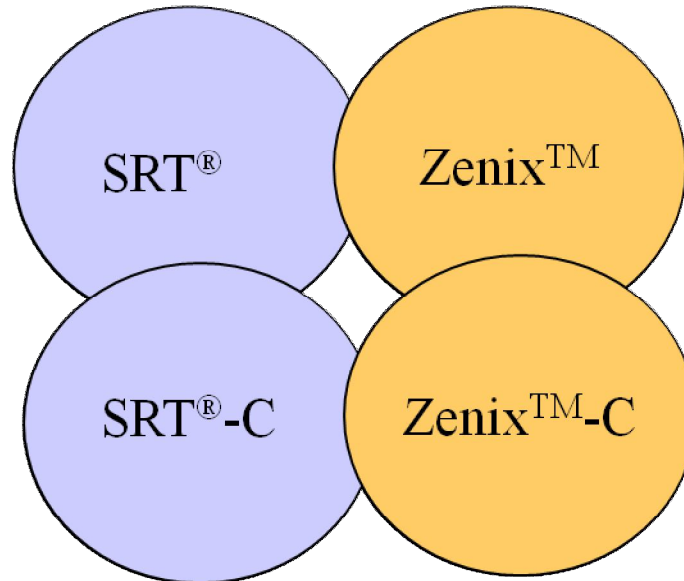
SEC Packings

SRT & SRT-C

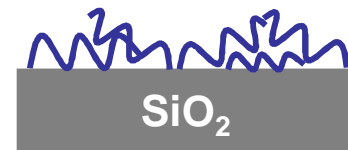
- 5µm Particle
- 100 – 2000Å pore size
- Higher stability
- Great reproducibility

Zenix & Zenix-C

- 3µm Particle
- Sole Vendor
- Highest efficiency
- Highest resolution



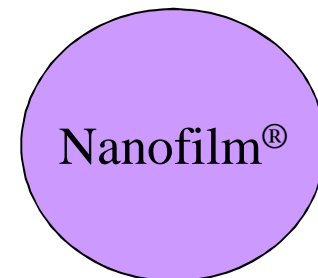
SRT & Zenix Phases
Stand-up Monolayer



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

- 5µm Particle
 - Nanometer thick hydrophilic coating
 - Especially suitable for membrane protein separation
- Christopher B. Roth, Michael A. Hanson and Raymond*

C. Stevens, J. Mol. Biol. (2008) 376, 1305-1319



Nanofilm®



Sepax Technologies

www.sepax-tech.com

Features of SRT, Zenix, SRT-C & Zenix-C phases

Characteristics	SRT	Zenix	SRT-C	Zenix-C
Resolution	High	Highest, Shorter column for faster separation	High	Highest, Short column for faster separation
Efficiency	High	Doubled from 5 μm	High	Doubled from 5 μm
Selectivity	Same for SRT and Zenix		Same for SRT-C and Zenix-C	
Recommended Sample Types	Monoclonal antibodies, proteins, peptides, nucleic acids, oligonucleotides, virus, and water-soluble polymers		"Tough samples" such as <i>hydrophobic proteins</i> like insulin, membrane protein <i>monoclonal antibodies derivatized with polymer branches</i> , e.g. polypeptide, PEG.	



Sepax Technologies

www.sepax-tech.com

Zenix SEC-150: High Resolution and High Loading

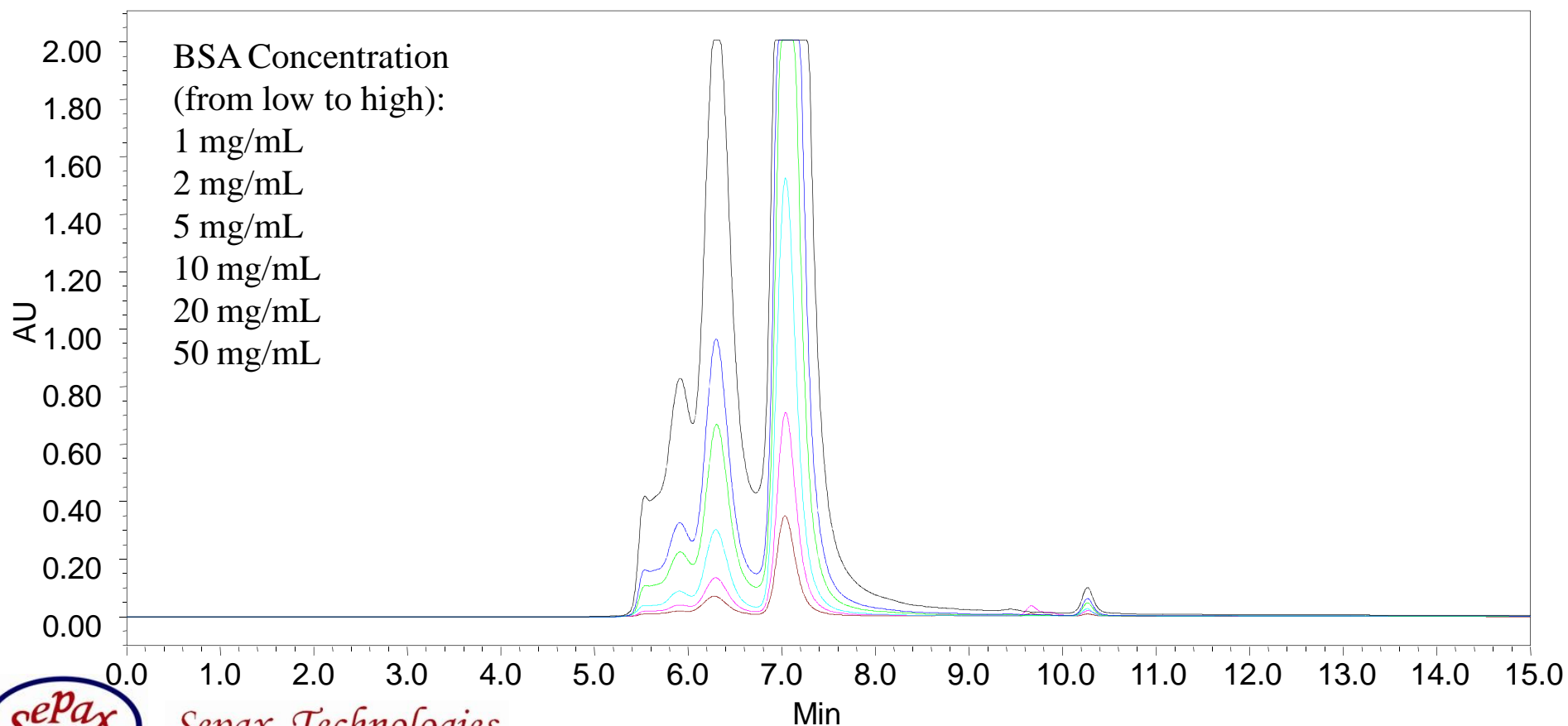
Column: Zenix SEC-150 (3 μ m, 7.8x300 mm)

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

Sample: BSA

Injection volume: 10 μ L

Detection: UV214 nm



Sepax Technologies

www.sepax-tech.com

Zenix SEC-300: High Resolution and High Loading

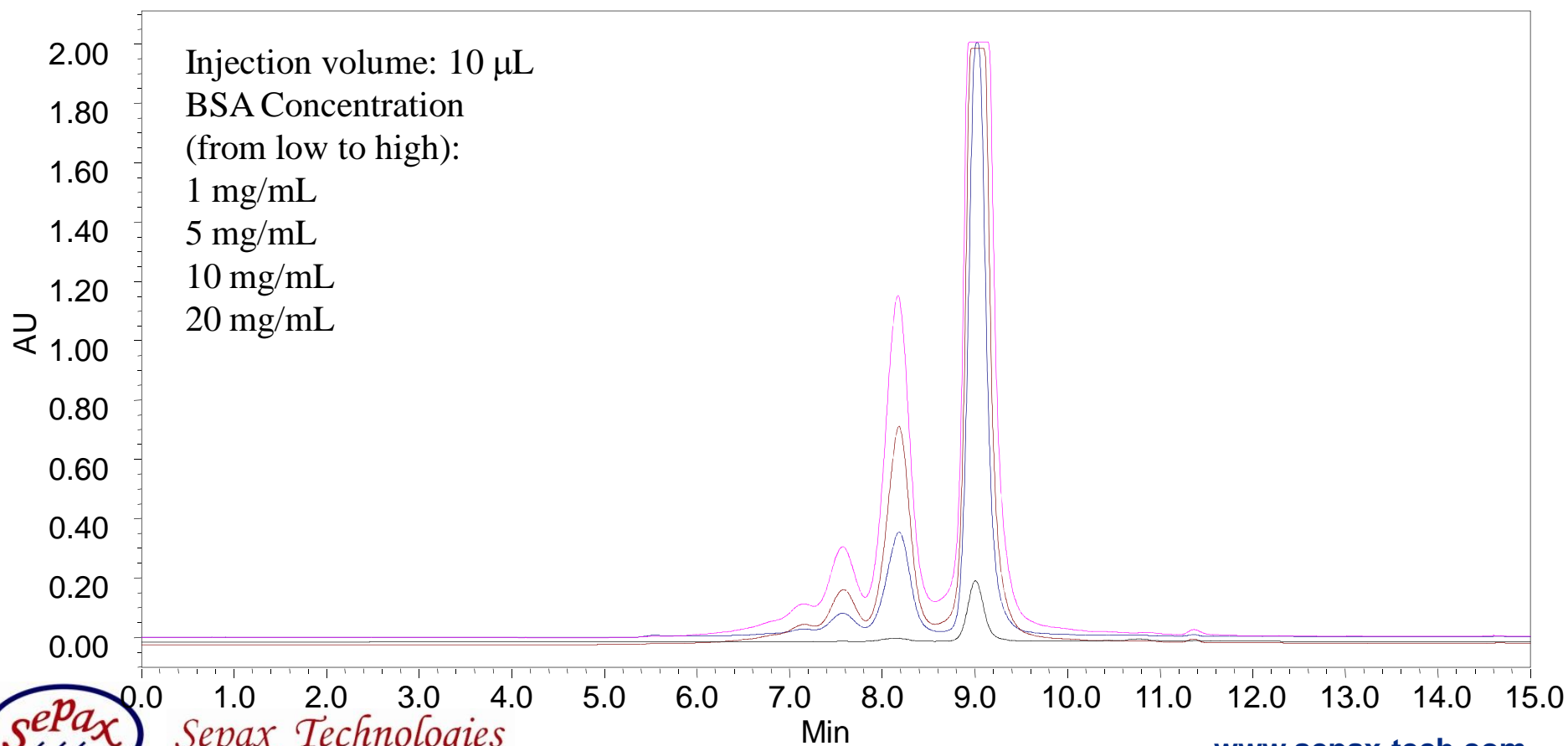
Column: Zenix SEC-300 (3 μm , 7.8x300 mm)

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

Sample: BSA

Injection volume: 10 μL

Detection: UV214 nm



Sepax Technologies

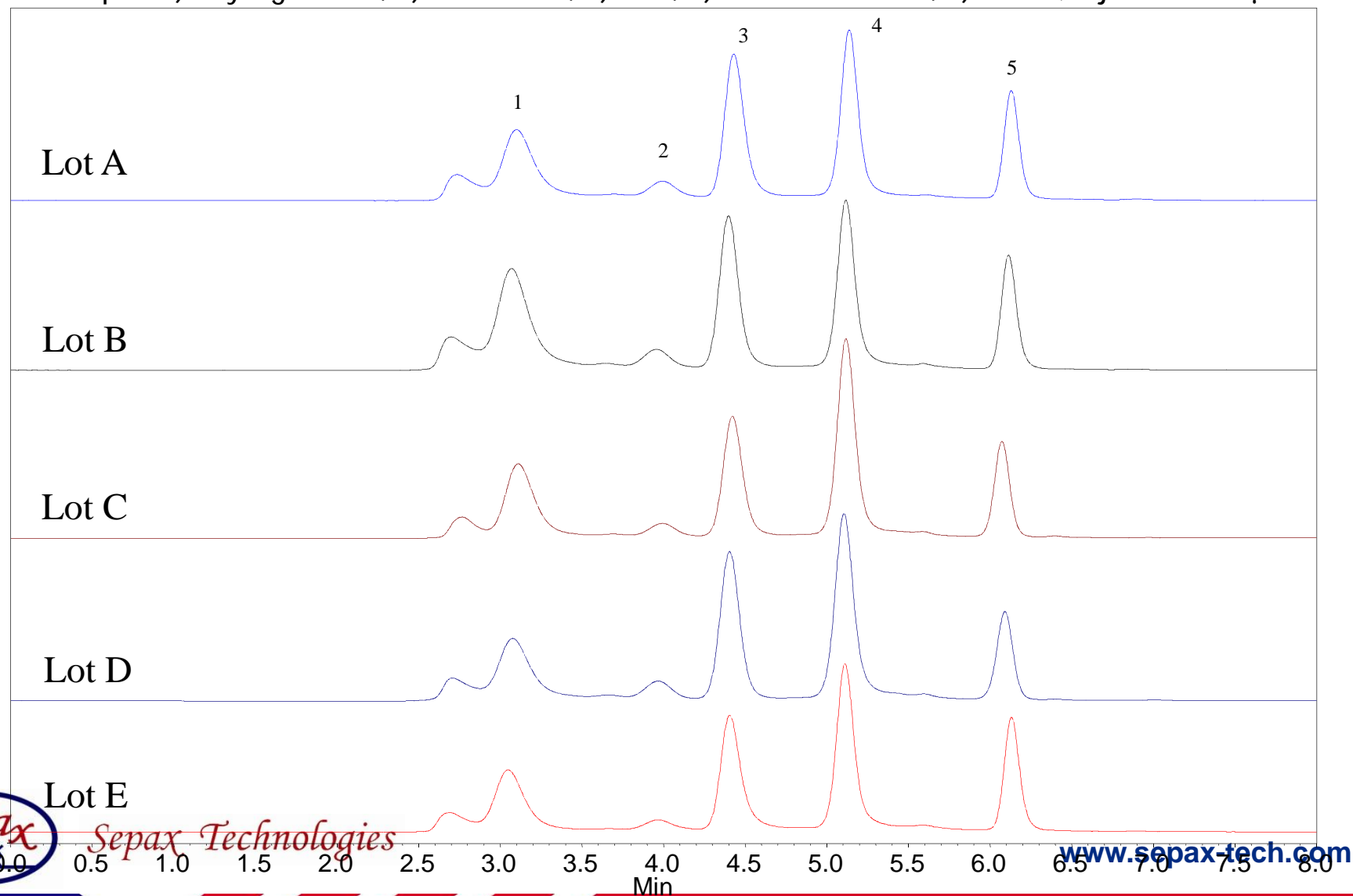
www.sepax-tech.com

Lot-to-Lot Reproducibility: Zenix SEC-300 (3 μ m, 7.8x150mm)

Mobile phase: 150 mM Sodium Phosphate, pH 7;

Flow rate: 1.0 mL/min; Detection: 214 nm; T=Ambient

Sample: 1) Thyroglobulin, 2) BSA dimer, 3) BSA, 4) Ribonuclease A, 5) Uracil; Injection: 10 μ L



Sepax Technologies

www.sepax-tech.com

Tween 80 or Tween 20 Has **no Impact** on MAb Separation

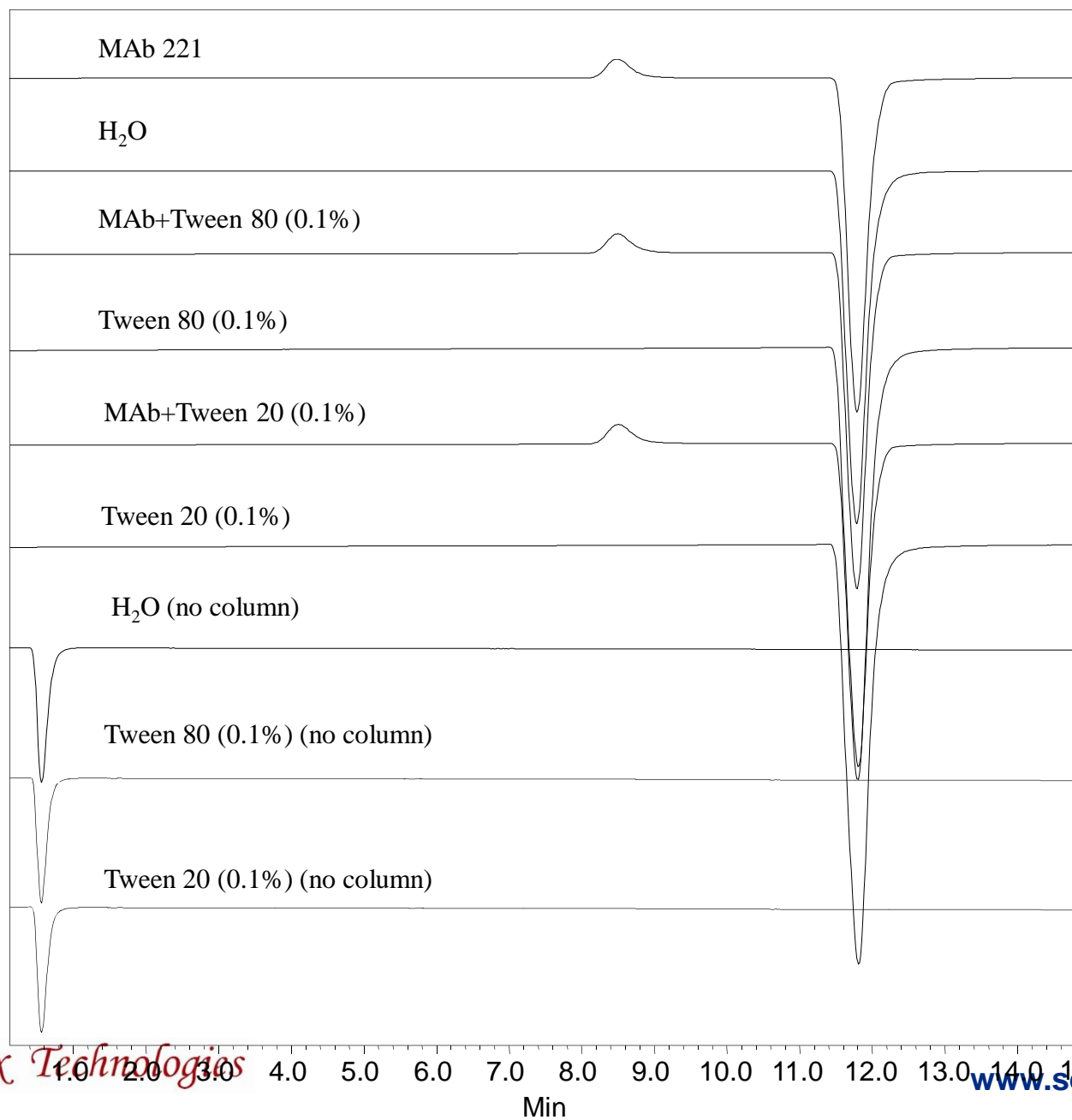
Column: SRT SEC-300 (5 μ m, 300Å, 4.6x300mm)
Mobile phase: 150 mM Phosphate Buffer, pH=7.0
Flow rate: 0.35 mL/min
Detector: Refractive Index
Temperature: RT
Sample: MAb 221 (1.4 mg/mL)
Injection Volume: 3 μ L

Directly inject H₂O, Tween 80 or Tween 20 into the HPLC system without column connected.



Sepax Technologies

www.sepax-tech.com



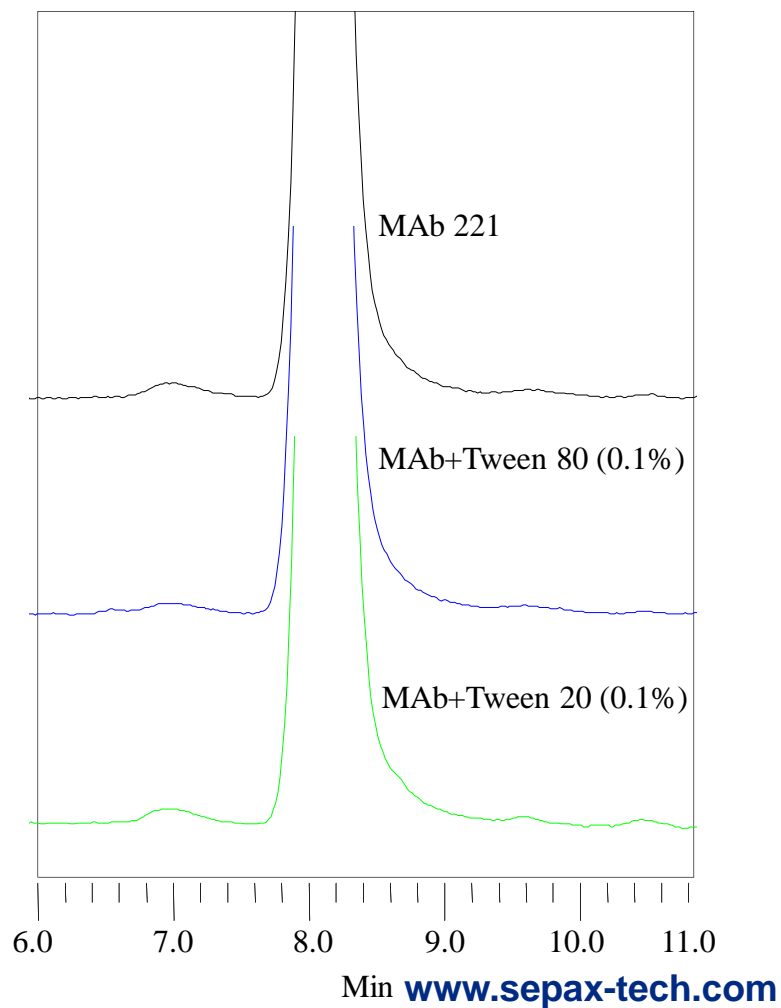
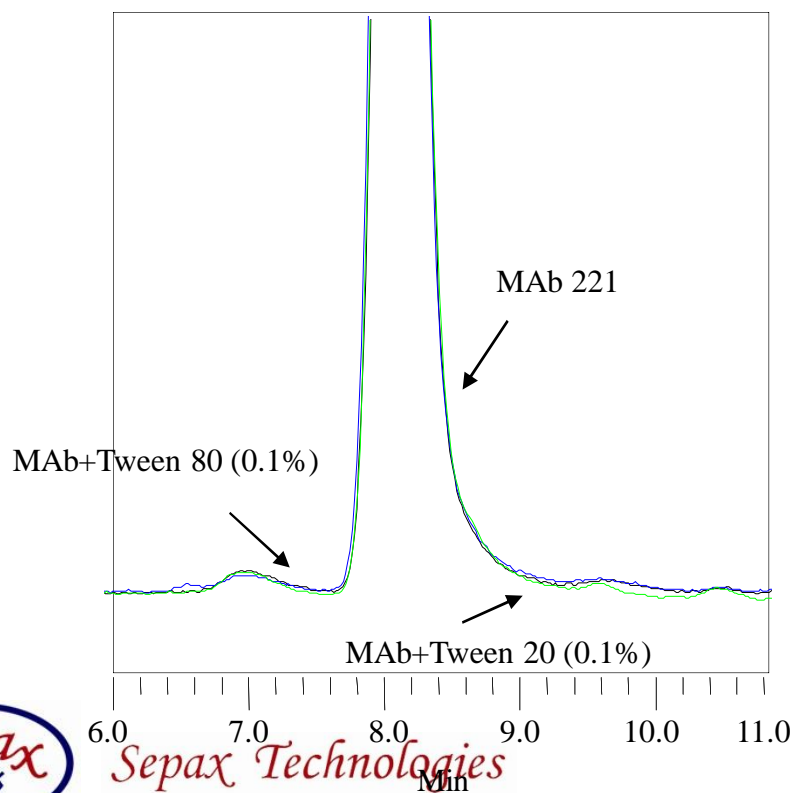
Sepax Technologies

www.sepax-tech.com

Min

Separation of MAb with Tween 20 or 80

Column: SRT SEC-300 (5 μ m, 300A, 4.6x300mm)
Mobile phase: 150 mM Phosphate Buffer, pH=7.0
Flow rate: 0.35 mL/min
Detector: Refractive Index
Temperature: RT
Sample: MAb 221 (1.4 mg/mL)
Injection Volume: 3 μ L

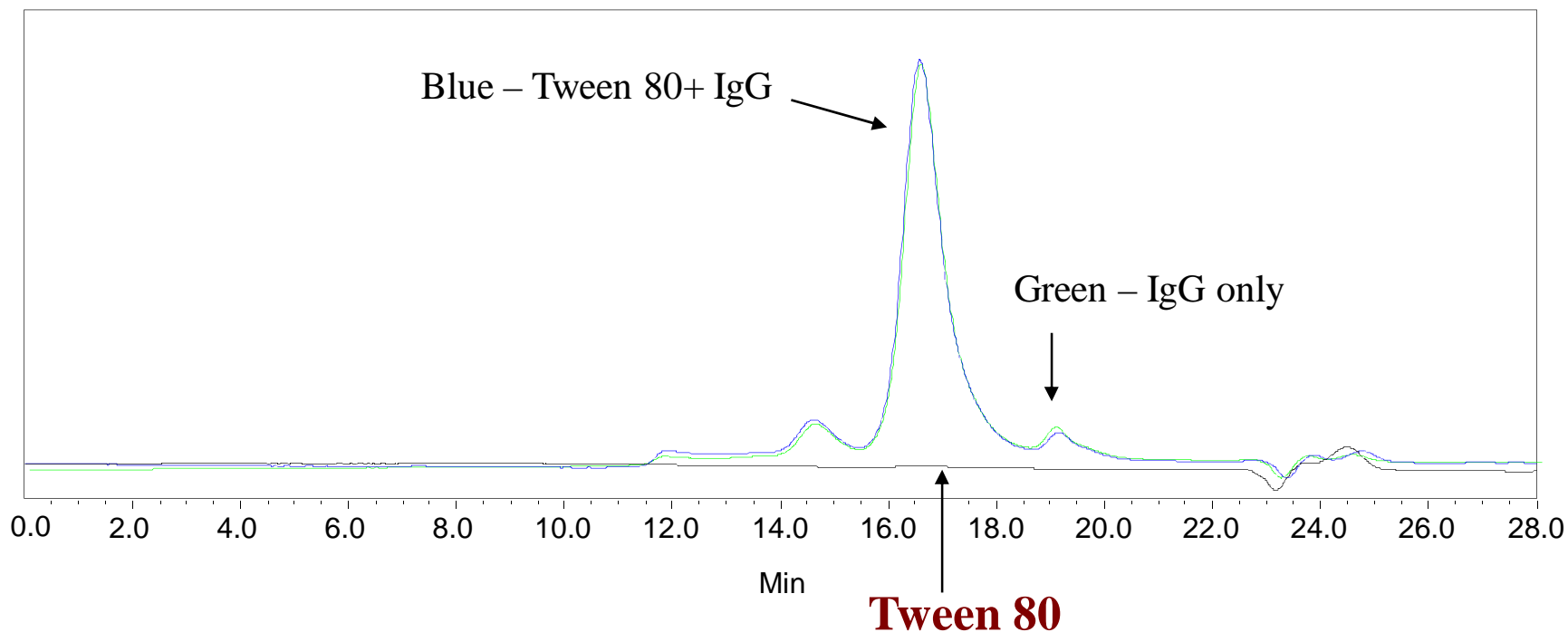


Sepax Technologies

Min www.sepax-tech.com

Separation of IgG Sample in Tween 80 with High Loading Volume

Tween 80 has no impact on the separation



Column:	SRT-300 (3 μ m, 7.8x300mm)
Mobile phase:	150 mM sodium phosphate, pH 7.0
Injection volume:	100 μL
Detection:	UV 214 nm
Temperature:	Ambient (~23 $^{\circ}$ C)
Flow rate:	0.5 mL/min
Sample:	1) IgG (0.1 mg/mL), 2) Tween 80 (0.05%); 3) IgG + Tween 80

Sepax Technologies (0.1 mg/mL in 0.05% Tween 80)



www.sepax-tech.com

Fast Separation of MAb and its Aggregates in Less Than 5 Minutes

Column: Zenix SEC-300 (3 μ m, 7.8x200 mm)
Mobile phase: 150mM sodium phosphate, pH 7.0
Flow rate: A/B/C/D/E = 1.0/1.5/2.0/2.5/3.0 mL/min
Injection volume: 10 μ L
Detection: UV214 nm
Temperature: Ambient (~23 °C)
Sample (1.5 mg/mL):
1. Aggregate of MAb 211
2. MAb 211 (from a biotech company)

Separation results at various flow rates

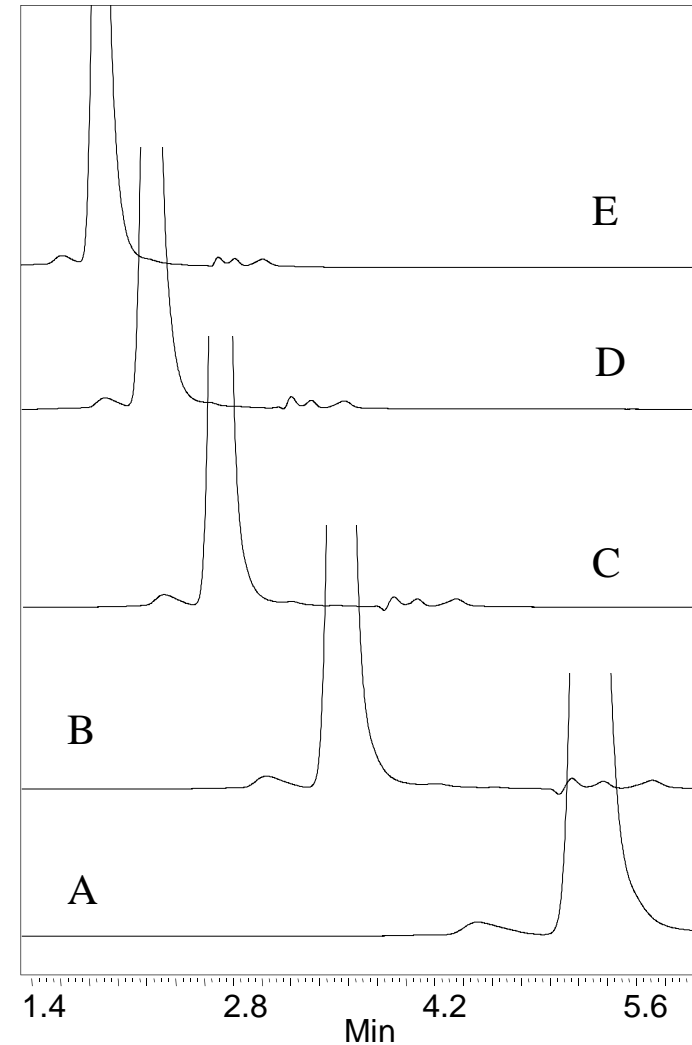
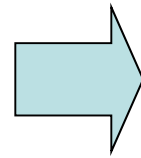
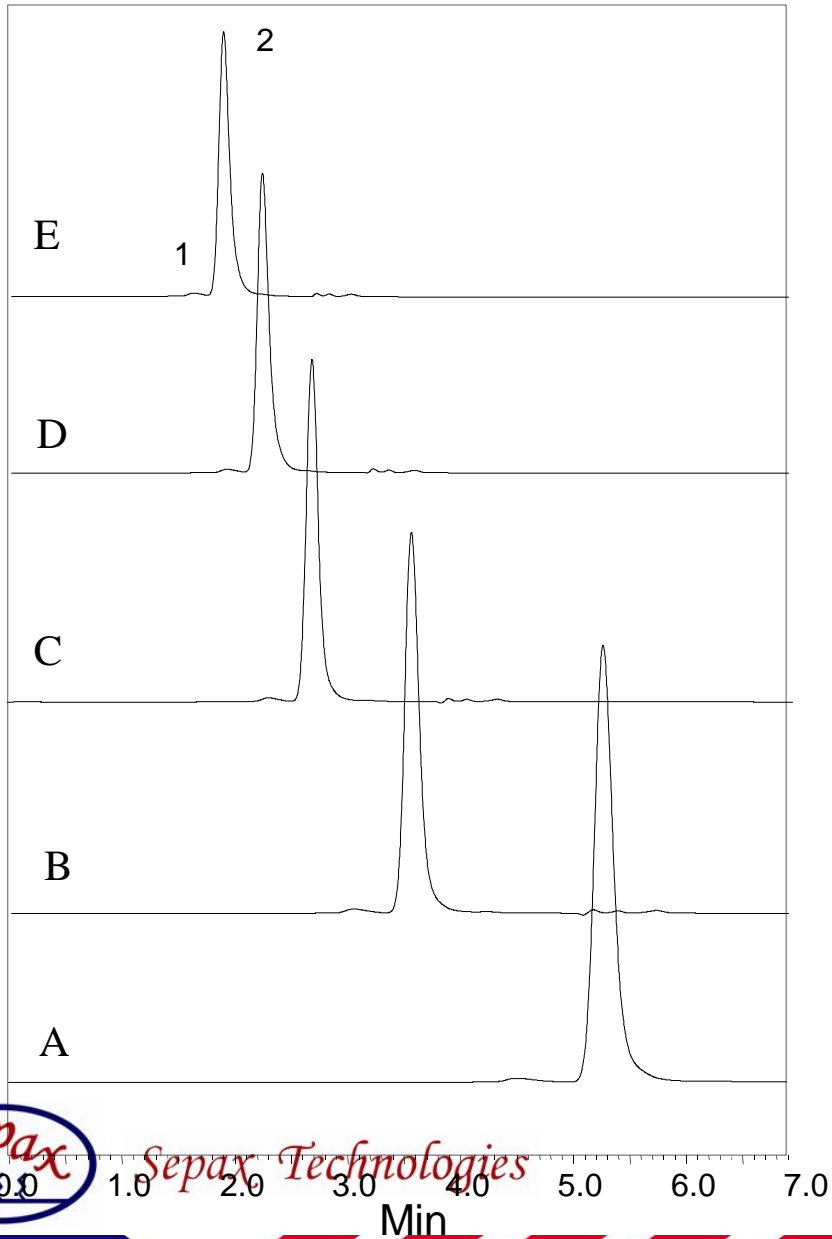
Test	Flow rate (mL/min)	Backpressure (psi)	Retention time (min)		% Area		USP Resolution
			Peak 1	Peak 2	Peak 1	Peak 2	
A	1.0	550	4.51	5.28	1.38	97.92	1.9
B	1.5	1020	3.04	3.55	1.36	97.84	1.6
C	2.0	1550	2.23	2.71	1.31	97.20	1.6
D	2.5	2050	1.92	2.23	1.38	97.02	1.5
F	3.0	2450	1.62	1.89	1.26	96.92	1.3



Sepax Technologies

www.sepax-tech.com

Fast Separation of MAb and its Aggregates in Less Than 5 Minutes



www.sepax-tech.com

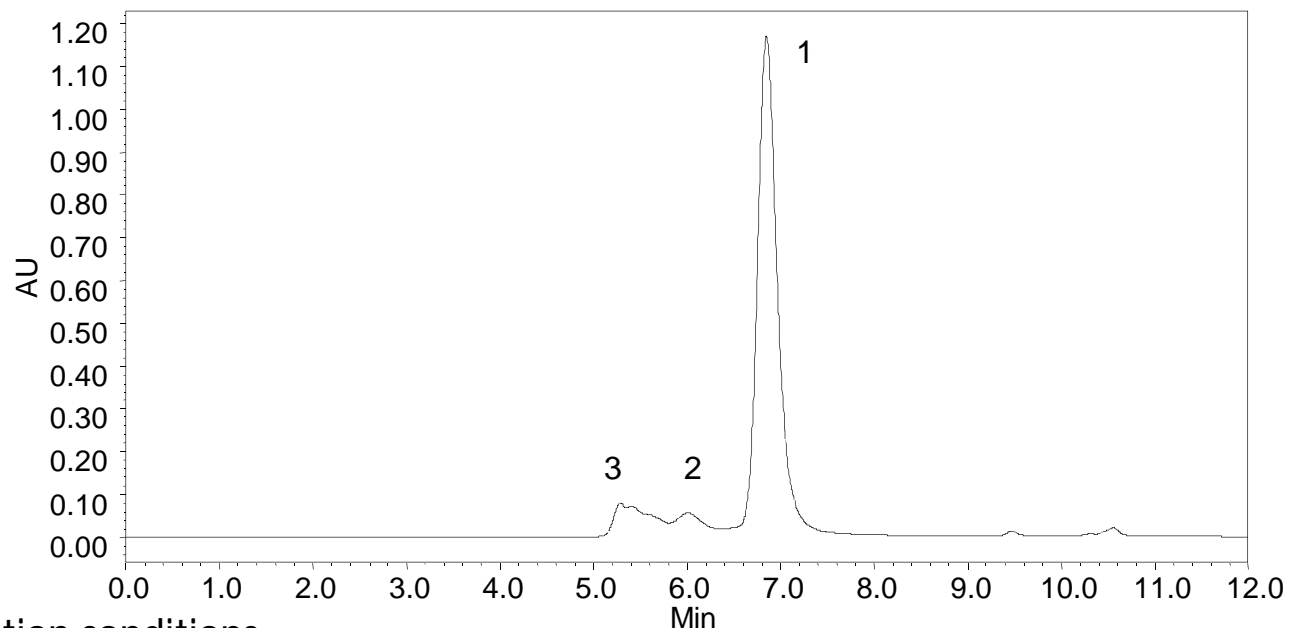
Separation of Protein and PEGylated Proteins by Zenix

Column: Zenix SEC-150 (3 μ m, 7.8x300mm)

Sample: 1, Protein with MW 70KD (from an undisclosed drug development company)

2, Protein 1 attached with 1 PEG (MW 40KD) molecule (MW 110KD)

3, Protein 1 attached with 2 PEG (MW 40KD) molecules (MW 150KD)



Separation conditions:

Mobile phase: 150 mM Phosphate pH=7.0;

Flow rate: 1.0 mL/min

Wavelength: 214 nm; Temperature: Ambient

Sample concentration: 2.0 mg/mL;

Injection volume: 20 μ L

Column backpressure: 1615-1640 psi

Conclusion:

- 1) Zenix SEC-150 offers high resolution of PEGylated proteins.
- 2) The major component of this sample is the non-PEGylated protein and PEGylation yield is low.



Sepax Technologies

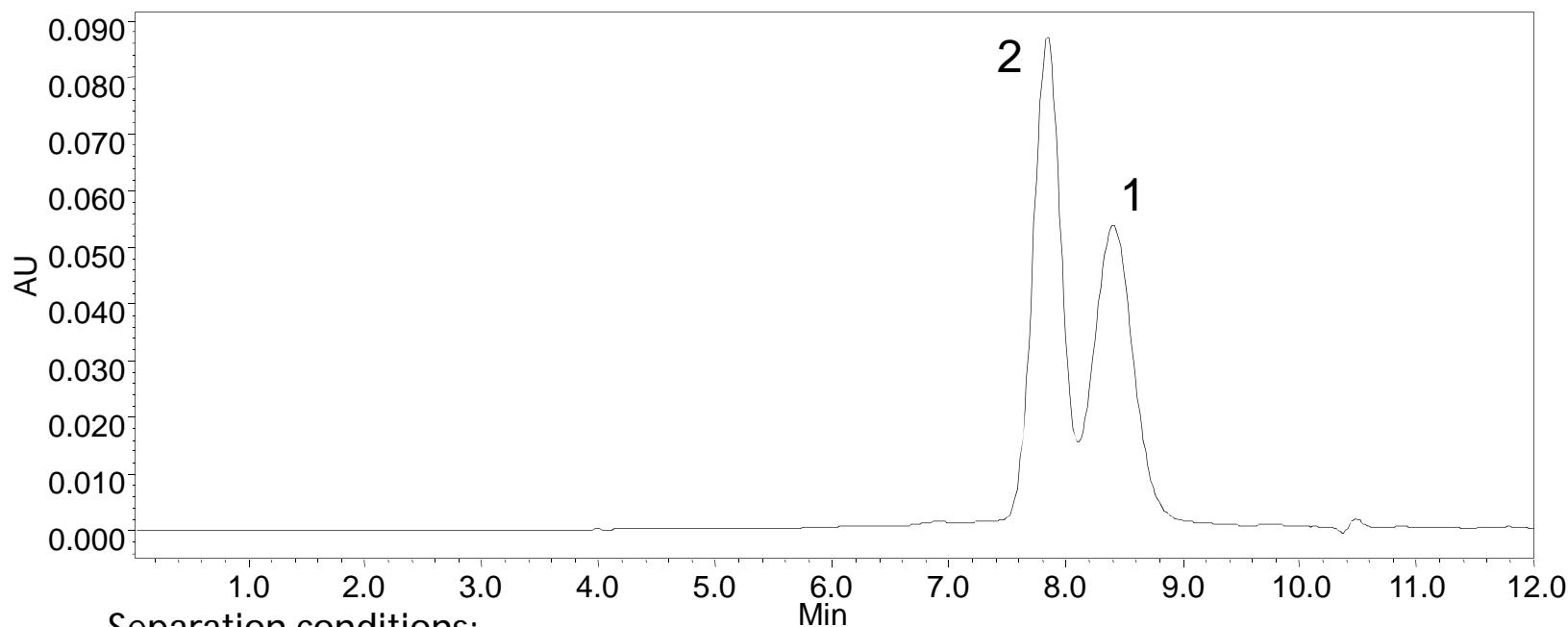
www.sepax-tech.com

High Resolution of Peptide and PEGylated Peptide Conjugate

Column: Zenix SEC-150 (3 μ m, 7.8x300mm)

Sample: 1, PEG with MW 5KD

2, PEG attached salcatonin (a peptide with MW 3KD) (8KD)
(from an undisclosed drug development company)



Separation conditions:

Mobile phase: 150 mM Phosphate pH=7.0;

Flow rate: 1.0 mL/min

Wavelength: 214 nm; Temperature: Ambient

Sample concentration: 2.0 mg/mL;

Injection volume: 20 μ L

Sepax Technologies

Conclusion: Zenix SEC-150 offers high resolution of PEGylated peptide and free PEG with close MWs, even though the baseline separation is not achieved.



www.sepax-tech.com

Conclusions

With uniquely designed surface chemistries, SRT, Zenix, SRT-C, Zenix-C, and Nanofilm phases cover most comprehensive samples with great compatibility. 3 μ m Zenix and Zenix-C packings achieve highest resolution and efficiency. With the pore size from 100 to 2000 Å, a combination of those SEC phases offer a total solution for size exclusion of biomolecules and water soluble polymers.

Typical applications include:

- *Proper elution of positively charged proteins*
- *High stability*
- *Low salt concentration*
- *Suitable for LC/MS applications*
- *Multi-dimension separation*
- *High resolution and selectivity*
- *Applications from low MW polysaccharides to large biological entities, such as virus*



Sepax Technologies

www.sepax-tech.com