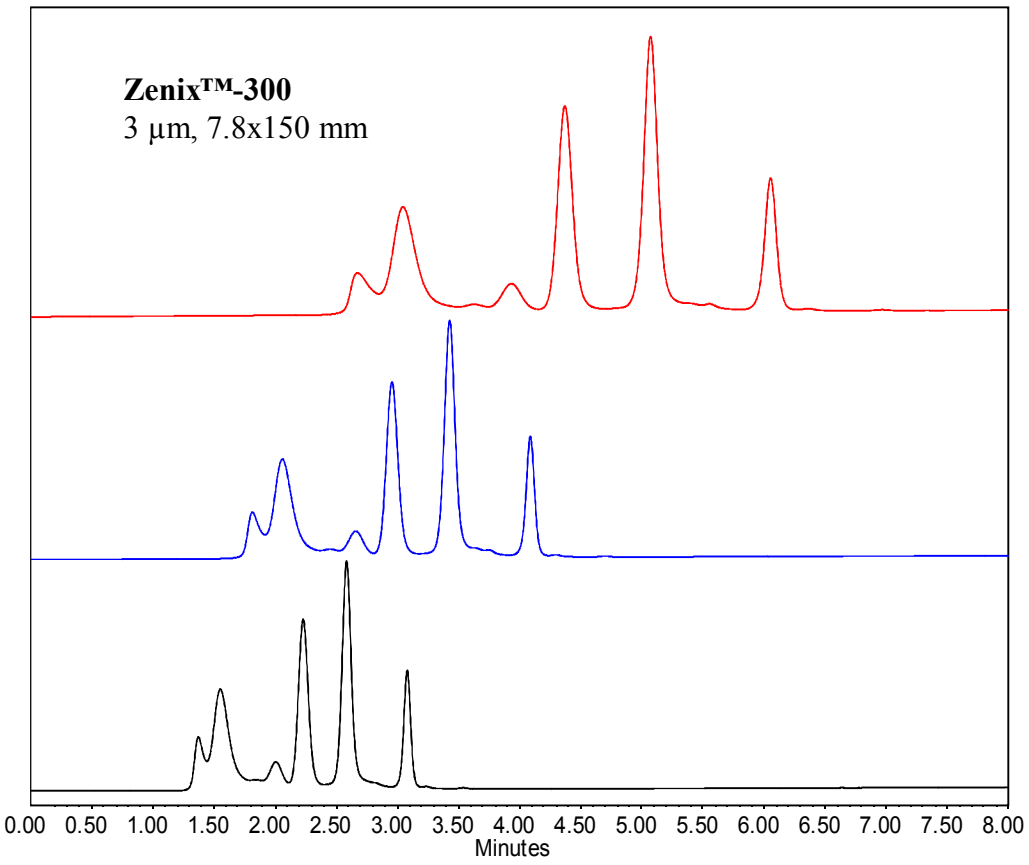
Fast Assay Application
Zenix™ SEC-300

ZP1007



7 min assay
1.0 mL/min
Plate count: 17617
Back pressure: 850 psi

4.5 min assay
1.5 mL/min
Plate count: 16215
Back pressure: 1350 psi

3.5 min assay
2.0 mL/min
Plate count: 13541
Back pressure: 1600 psi

Mobile Phase: 150 mm phosphate buffer, pH 7

Detection: UV 214 nm

Samples: Thyroglobulin, BSA, Ribonuclease A, Uracil

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Tel: (302) 366-1101 | Fax: (302) 366-1151
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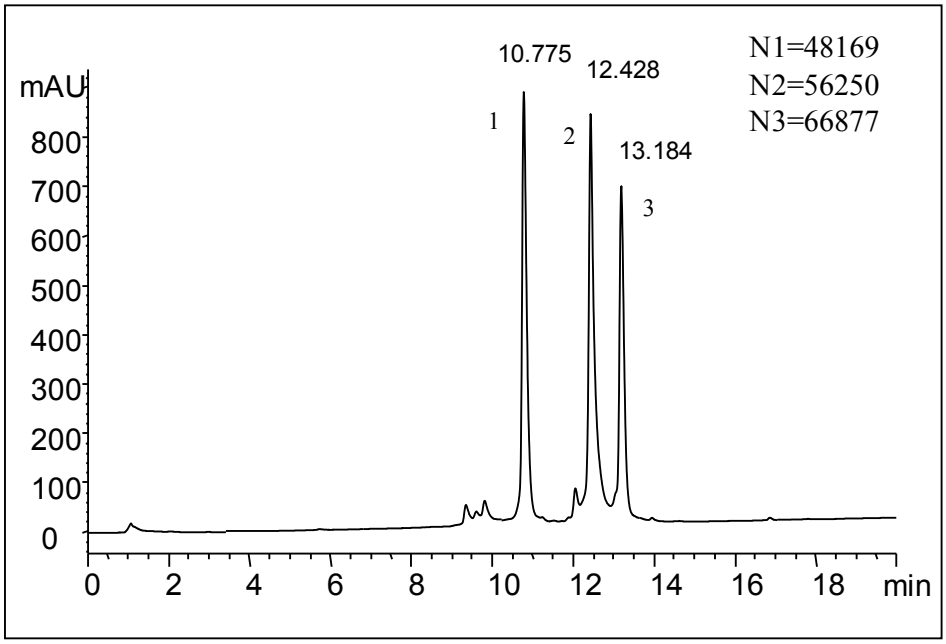
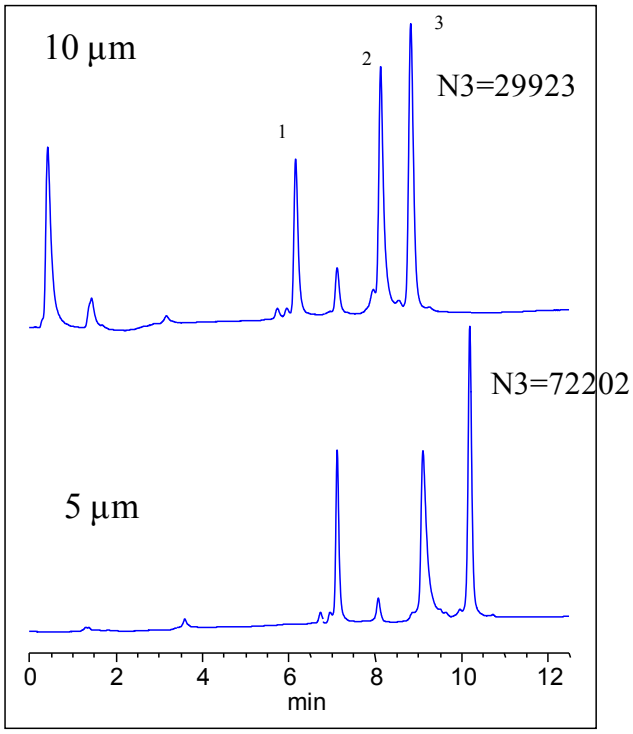
Separation of Proteins on Antibodix™ NP1.7 (1.7 μm, 4.6x50 mm)



Column:	Antibodix™ NP1.7 (1.7 µm, 4.6x50 mm)
Mobile Phases:	A, 10 mM phosphate buffer, pH 6.0; B, A + 1.0 M NaCl
Gradient:	10-100% B (25 min)
Flow Rate:	0.3 mL/min
Temperature:	Ambient
Detection:	UV 214 nm
Concentration:	1 mg/mL for each
Injection Volume:	5 µL
Samples:	1. Cytochrome C, 2. Lysozyme, 3. Ribonuclease A

Keywords: Weak cation exchange, Antibodix, protein, high resolution

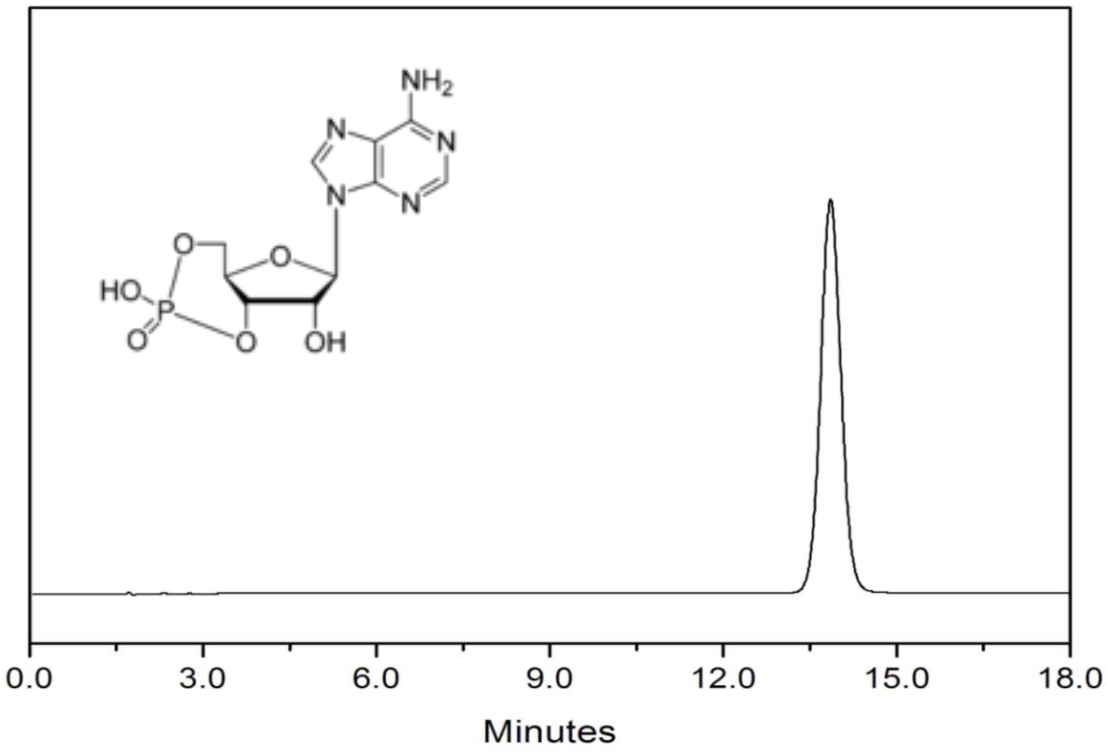
Separation of Proteins on Antibodix™ (7.8x75 mm) - Particle Size Impact



Columns:	Antibodix™ NP5, NP10 (5 µm and 10 µm, 7.8x75 mm)
Mobile Phases:	A. 10 mM phosphate buffer, pH 6; B. A + 1.0 M NaCl
Gradient:	10-100% B (25min)
Flow Rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV 214 nm
Samples:	1. Cytochrome C 2. Lysozyme 3. Ribonuclease A

Keywords: Weak cation exchange, Antibodix, protein, particle size

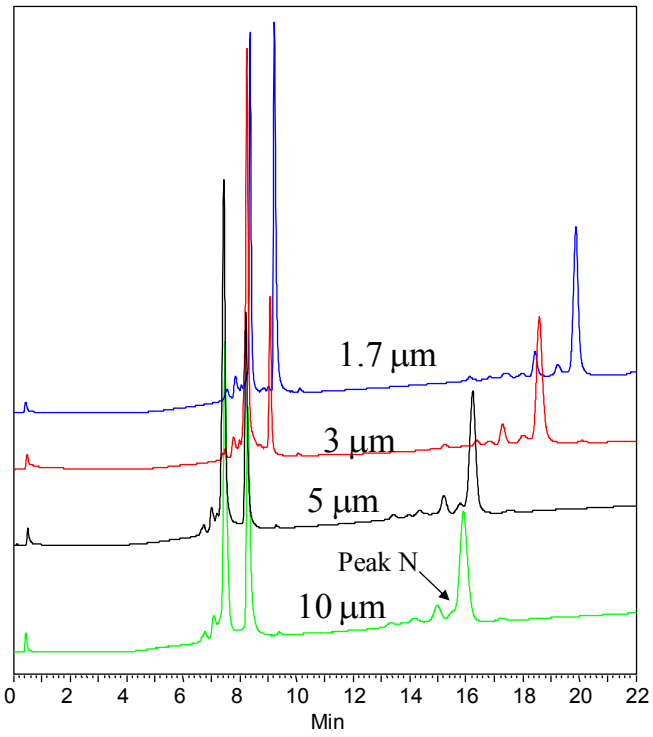
Analysis of Cyclic Adenosine Monophosphate (cAMP) on HP-C18



Column:	HP-C18, 5 µm, 120 Å, 4.6×150 mm
Mobile phase:	200 mmol/L PB: CH_3OH =85: 15 (v/v)
Flow rate:	1.0 mL/min
Temperature:	35 °C
Detection:	250 nm
Injection Volume:	20 µL
Sample:	Cyclic Adenosine Monophosphate (cAMP)

Keywords: HP-C18, reverse phase, Cyclic Adenosine Monophosphate, Alkylating antineoplastic, cAMP, pharmaceutical

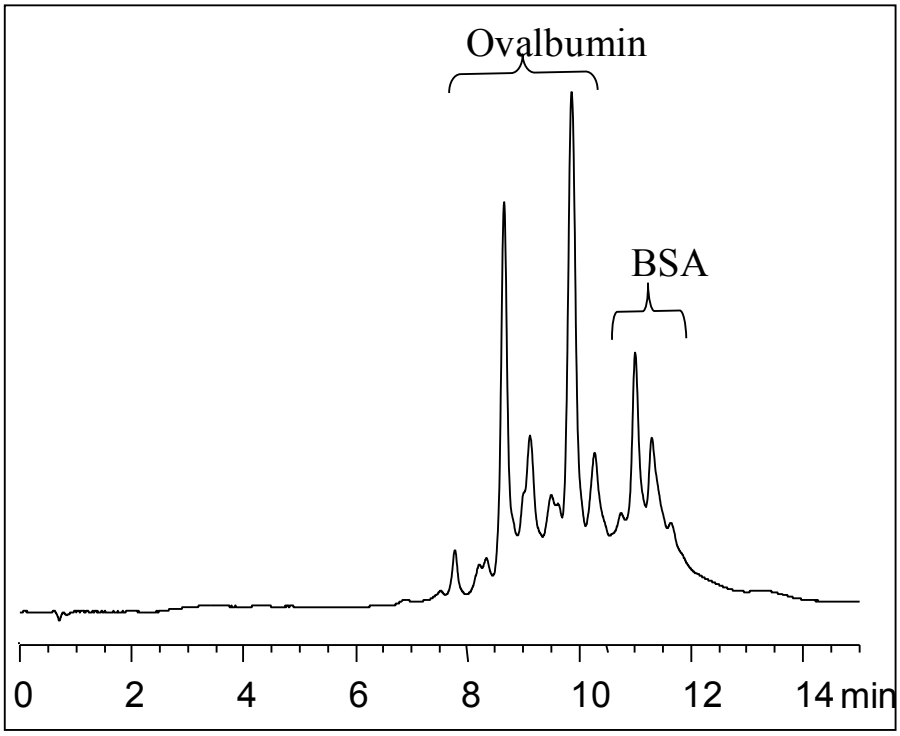
Separation of Proteins on Proteomix® WCX – Effect of Particle Size



Column:	Proteomix® WCX-NP (4.6x50 mm)
Mobile Phases:	A: 20 mM phosphate; B: A+1.0 M NaCl
Gradient:	0-100%B (20 min)
Flow Rates:	1.0 mL/min (WCX-NP3, WCX-NP5, WCX-NP10); 0.75 mL/min (WCX-NP1.7)
Detection:	UV 280 nm
Samples:	1. Ribonuclease A 2. Cytochrome C 3. Lysozyme

Keywords: Ion exchange, Proteomix, weak cation exchange, protein, particle size

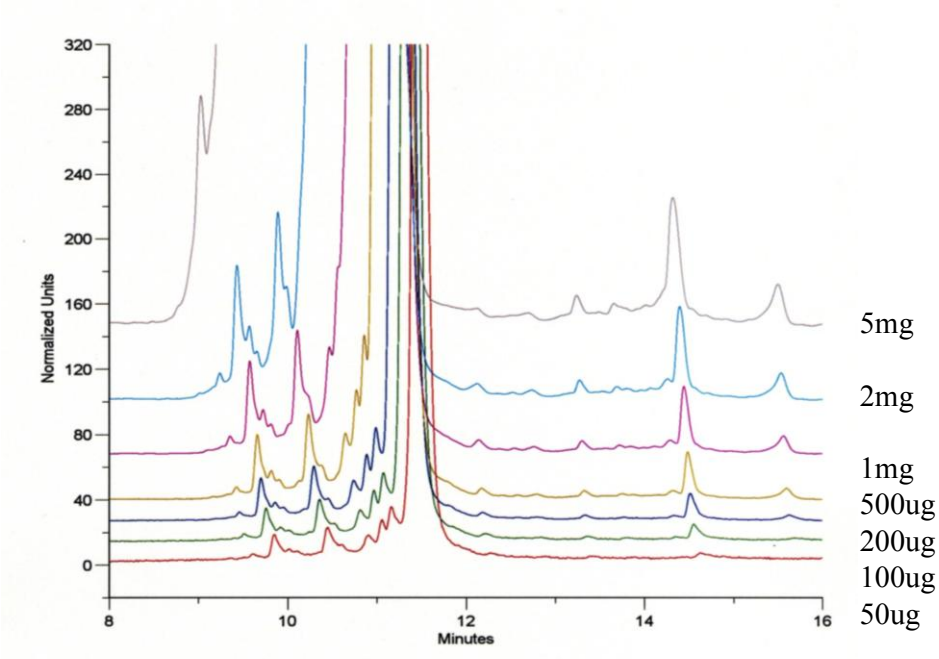
Separation of Proteins (Ovalbumin and BSA) on Proteomix® SAX-NP3 (3 μm, 4.6x50 mm)



Column:	Proteomix® SAX-NP3 (3μm, 4.6x50mm)
Mobile Phase:	20 mM Tris buffer, pH 8.0
Gradient:	0-0.3 M NaCl (15 min)
Flow Rate:	0.5 mL/min
Back Pressure:	1,600 psi
Detection:	UV 214 nm

Keywords: Ion exchange, Proteomix, strong anion exchange, protein, Ovalbumin, BSA

Loading Capacity of RNaseA on Proteomix® SCX-NP3 (3 µm, 4.6x50 mm)

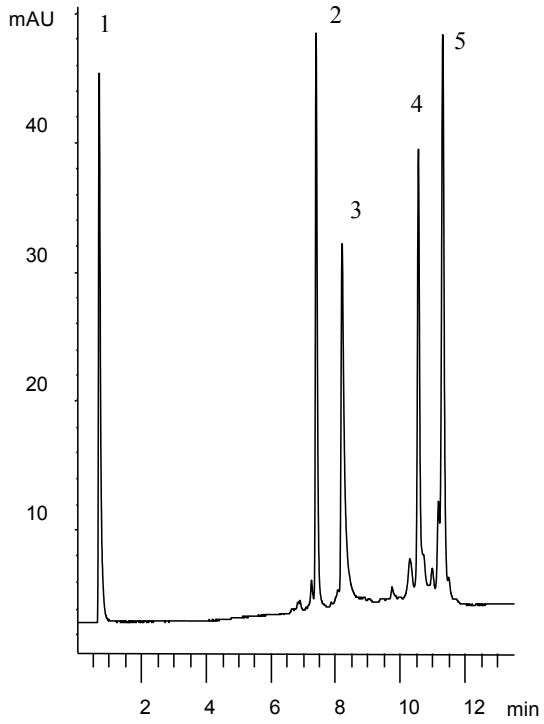


Column:	Proteomix® SCX-NP3 (3 µm, 4.6x50 mm)
Mobile Phase:	10 mM phosphate buffer, pH 6.0
Gradient:	0-1.0M NaCl (20 min)
Flow Rate:	1.0 mL/min
Temperature:	25 ºC
Detection:	UV 280 nm
Injection Volume:	100 µL

(Courtesy of Miyako Kawakatsu, M&S Instruments, Inc.)

Keywords: Ion exchange, Proteomix, strong cation exchange, loading capacity, Ribonuclease A

Separation of Proteins with different pI on Proteomix® SCX-NP3 (3 μm, 4.6 x 50 mm)

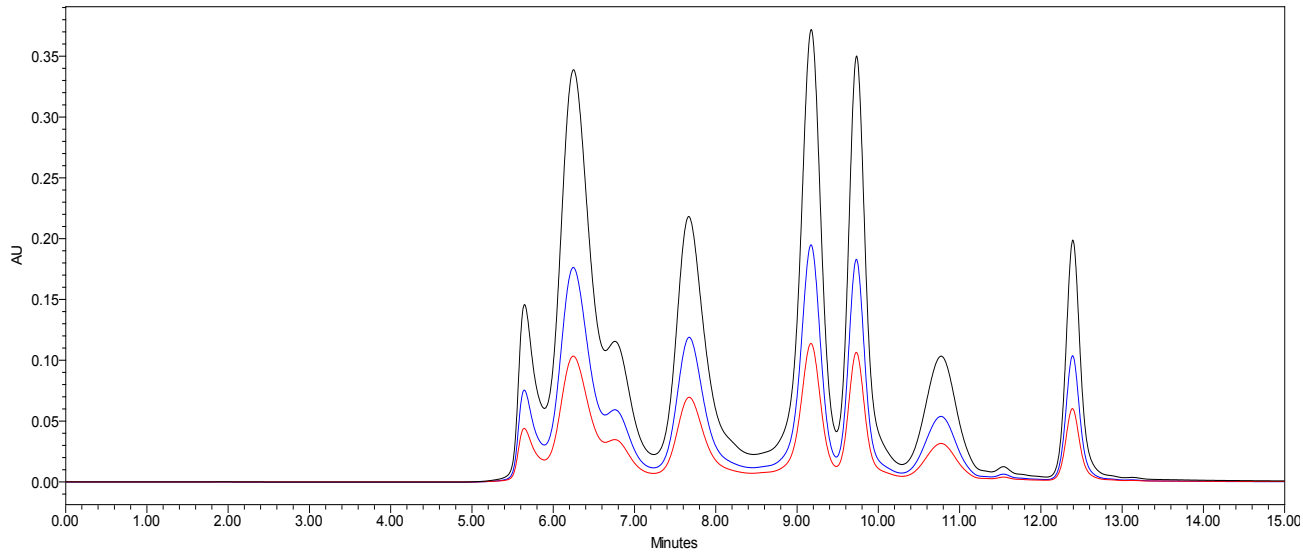


Column:	Proteomix® SCX-NP3 (3µm, 4.6x50mm)
Mobile Phase:	10 mM phosphate buffer, pH 6.0
Gradient:	0-1.0 M NaCl, 15 min
Flow Rate:	0.5 mL/min
Detection:	UV 280 nm

Samples:	PI:
1. Ovalbumin	4.6
2. Ribonuclease A	8.7
3. Cytochrome C	9.6
4. Aprotinin	10.0
5. Lysozyme	11.0

Keywords: Ion exchange, Proteomix, strong cation exchange, protein

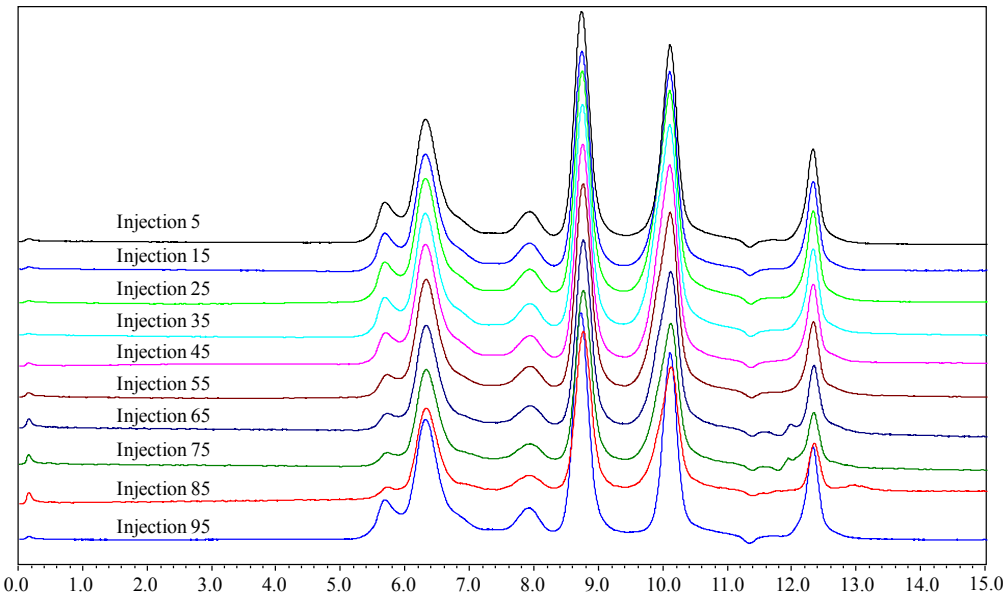
Protein Loading Capacity Study on SRT®-C 300



Column:	SRT® C-300 (5 µm, 300 Å, 7.8x300 mm)
Mobile Phase:	150 mM sodium phosphate buffer, pH 7
Temperature:	Ambient (~23° C)
Flow Rate:	1.0 mL/min
Detection:	UV 214 nm
Injection Volume:	3, 5, 10 µL
Samples:	Protein mixture: 1) Thyroglobulin aggregate, 2) Thyroglobulin, 670kD; 3) γ-Globulin dimer; 4) γ-Globulin, 158 kD; 5) Ovalbumin, 44kD; 6) Myoglobin, 17.6 kD; 7) Poly-DL-alanine (1-5 kD), 8) Uracil, 120D

Keywords: Size exclusion, SRT-C, protein, peptide

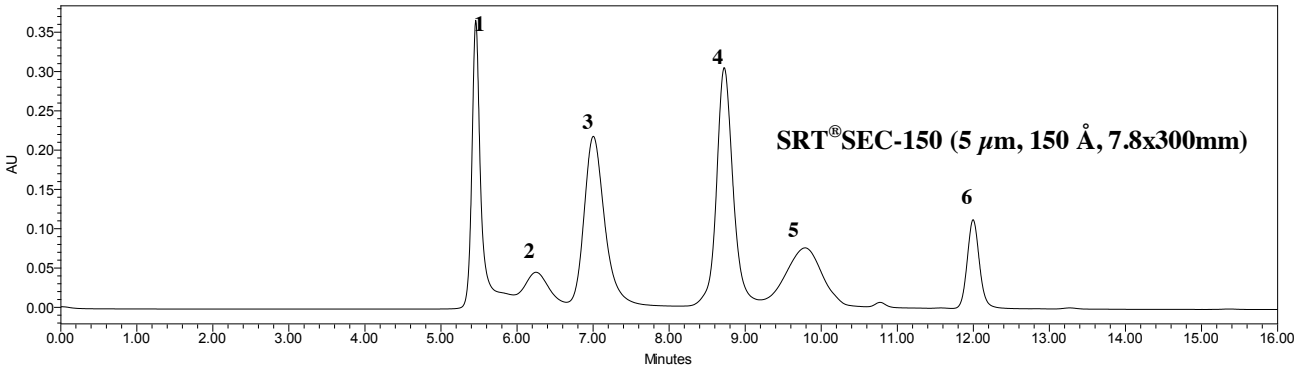
Analysis of Protein on SRT®-C - Column Stability Test



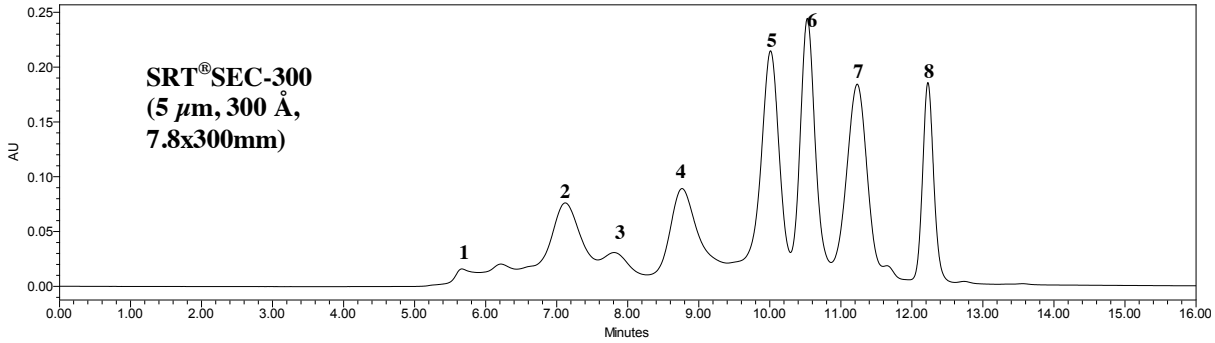
Column:	SRT®-C 300 (5 µm, 300 Å, 7.8x300 mm)
Mobile Phase:	150mM phosphate buffer, pH 7.0
Temperature:	Ambient
Flow Rate:	1.0 mL/min
Detection:	UV 214 nm
Injection Volume:	2 µL
Samples:	1) thyroglobulin (0.5 mg/mL), 2) BSA(0.5 mg/mL), 3) RNase A(0.5 mg/mL), 4) uracil (0.1 mg/mL

Keywords: Size exclusion, SRT-C, protein, stability

Separation of Peptide in protein mixture



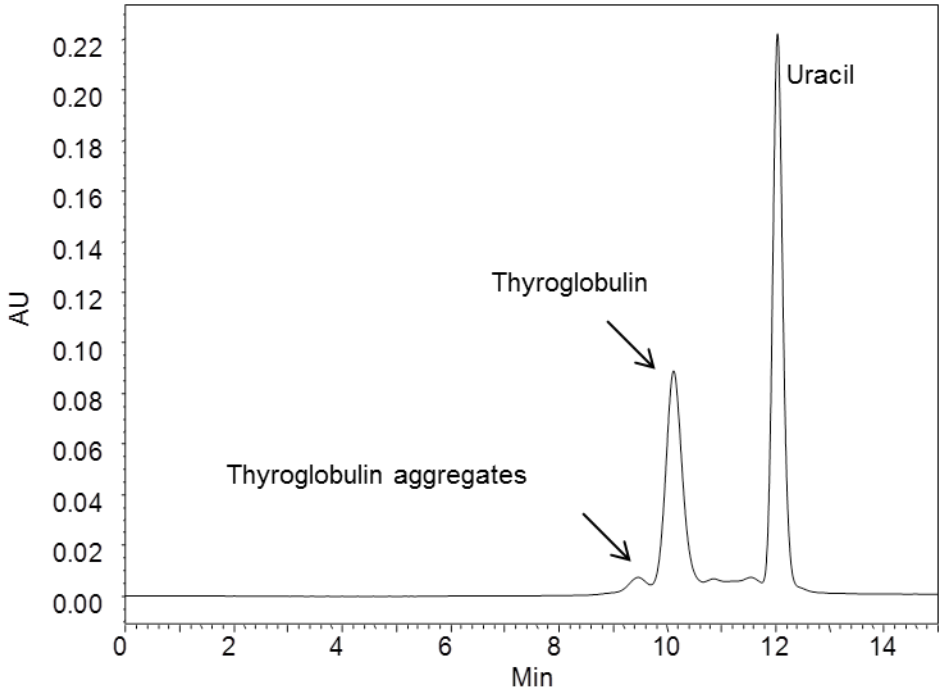
Mobile Phase:	150 mM phosphate buffer, pH 7.0
Flow Rate:	1.0 mL/min
Temperature:	22°C
Detection:	UV 214 nm
Samples:	1. Thyroglobulin; 5.46 min; 2. BSA Dimer; 6.25 min; 3. BSA; 7.00 min; 4. Ribonuclease A; 8.72 min; 5. Poly-DL-alanine (from Sigma 25281-63-4, MW 1,000-5,000); 9.78 min; 6. Uracil; 12.00 min



Mobile Phase:	150 mM phosphate buffer, pH 7.0
Flow Rate:	1.0 mL/min
Temperature:	22°C
Detection:	UV 214 nm
Samples:	1. Thyroglobulin; 5.46 min; 2. BSA Dimer; 6.25 min; 3. BSA; 7.00 min; 4. Ribonuclease A; 8.72 min; 5. Poly-DL-alanine (from Sigma 25281-63-4, MW 1,000-5,000); 9.78 min; 6. Uracil; 12.00 min

Keywords: size exclusion, SRT, peptides, proteins, poly-alanine

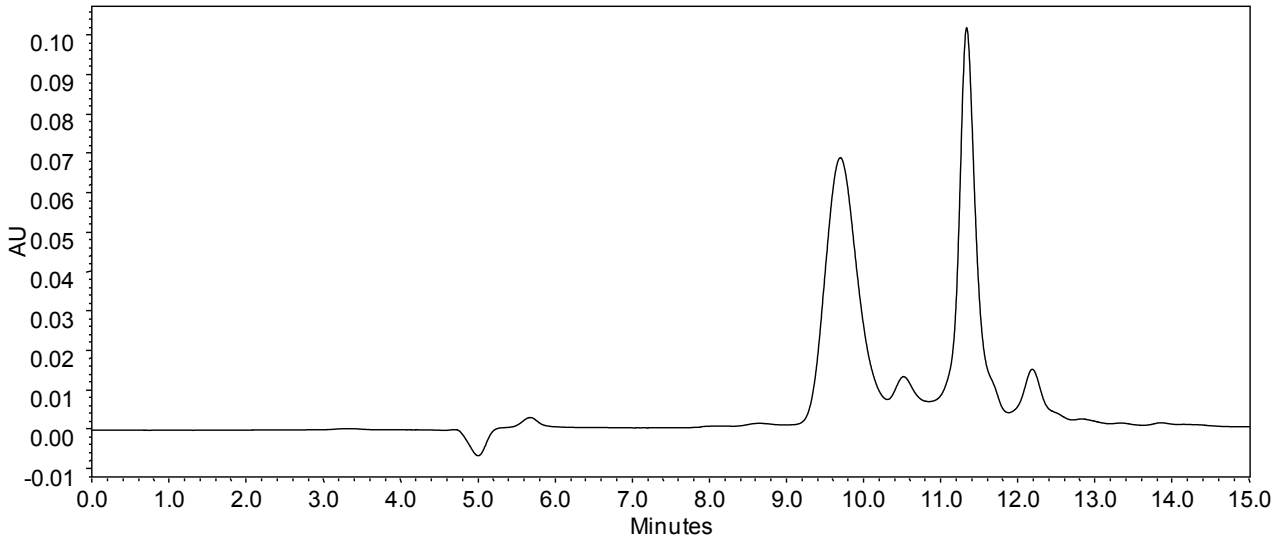
Separation of Thyroglobulin on SRT®-1000



Column:	SRT® SEC-1000 (5 µm, 1000 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM phosphate buffer, pH 7.0
Flow Rate:	0.35 mL/min
Injection vol:	3 µL
Temperature:	Ambient
Detection:	UV 214 nm
Samples:	1. Thyroglobulin (670kD) 1 mg/mL; 2. Uracil (120D), 0.1 mg/mL

Keywords: size exclusion, SRT, SRT 1000, protein, thyroglobulin, aggregate

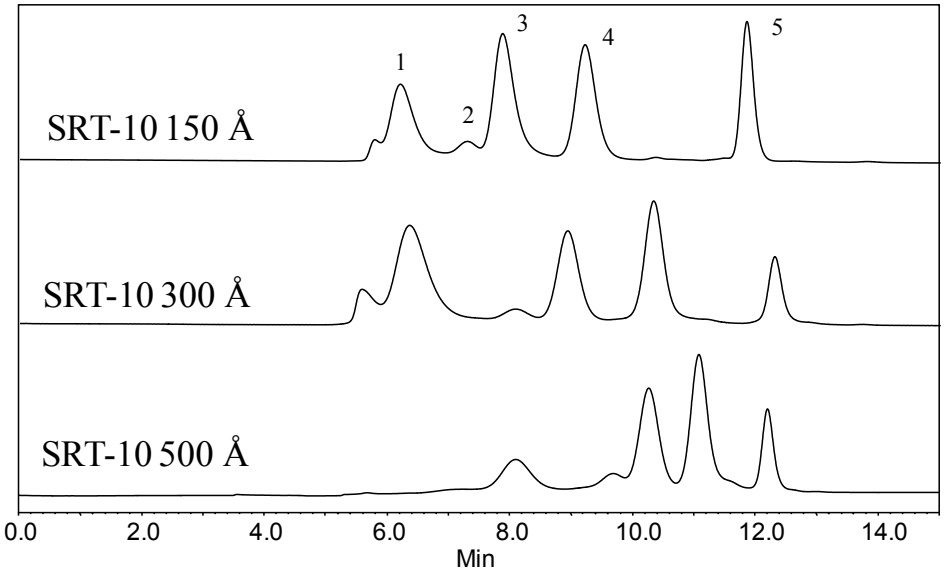
Separation of Human Recombinant Protein on SRT®-1000



Column:	SRT® SEC-1000 (5 µm, 1000 Å, 4.6 x 300 mm)
Mobile Phase:	150 mM phosphate buffer, pH 7
Flow Rate:	0.35 mL/min
Temperature:	Ambient
Sample:	rhIL-10 sR
Concentration:	1 mg/mL
Injection Volume:	3 µL

Keywords: size exclusion, SRT, SRT 1000, human recombinant protein

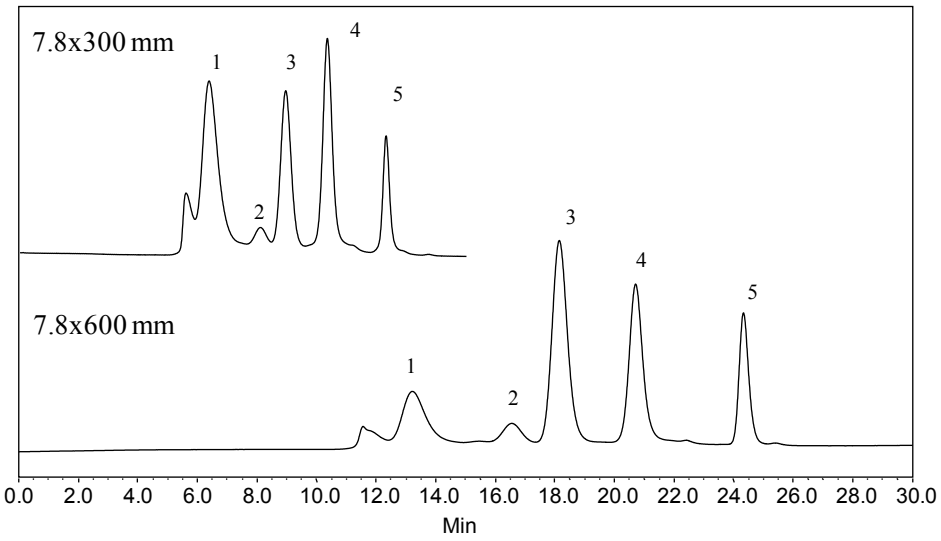
Comparison of the Separation Profiles with Various SRT®-10 Phases



Column:	SRT® (10 µm, 7.8x300 mm)
Mobile Phase:	150 mM phosphate buffer, pH 7.0
Flow Rate:	1.0 mL/min
Detection:	UV 214 nm
Temperature:	Ambient
Injection Volume:	5.0 µL
Samples:	1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5 g/mL), 120.

Keywords: size exclusion, SRT, proteins, pore size

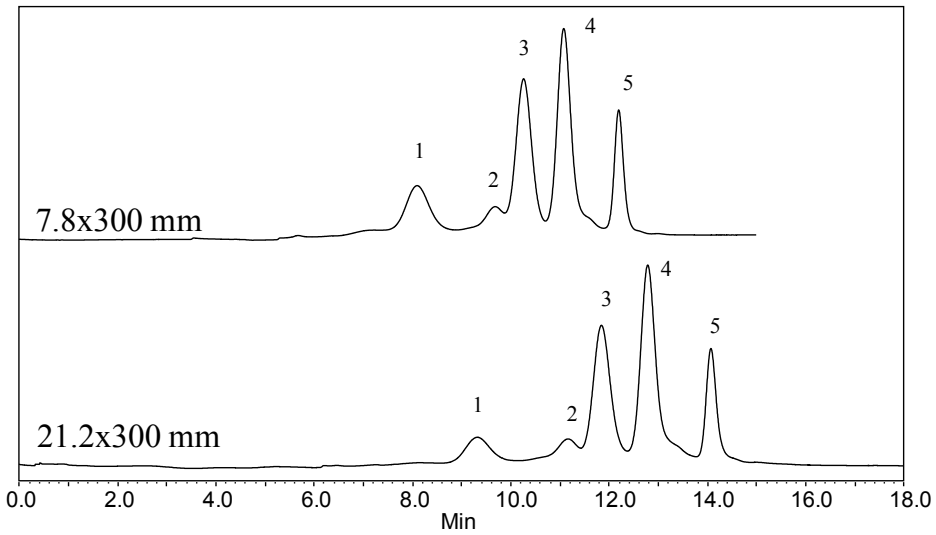
The Column Length Impact on Separation Efficiency



Column:	SRT®-10 300 (10 µm, 300 Å)
Mobile Phase:	150 mM phosphate buffer, pH 7.0
Flow Rate:	1.0 mL/min
Detection:	UV 214 nm
Temperature:	Ambient
Injection Volume:	5.0 µL
Samples:	1) Thyroglobulin (670 kD), 2) BSA dimmer (132 kD), 3) BSA (66 kD), 4) Ribonuclease A (13.7 kD), and 5) Uracil (120D).

Keywords: size exclusion, SRT, SRT 300, proteins, column length

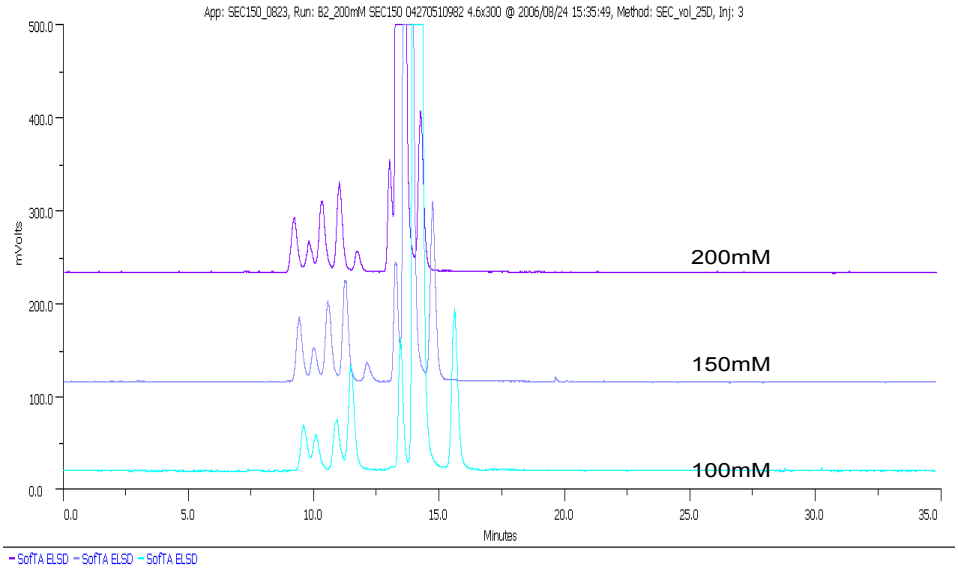
The Column ID Impact on Separation Efficiency



Column:	SRT®-10 500 (10 µm, 500 Å)
Mobile Phase:	150 mM phosphate buffer, pH 7.0
Flow Rates:	1.0 mL/min for 7.8x300 mm and 7.0 mL/min for 21.2x300mm
Detection:	UV 214 nm
Temperature:	Ambient
Injection Volumes:	20 and 5.0 µL for 7.8 and 21.2 mm ID
Samples:	1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5 µg/mL), 120.

Keywords: size exclusion, SRT, SRT 500, proteins, column ID

Protein Separation in Organic Buffer

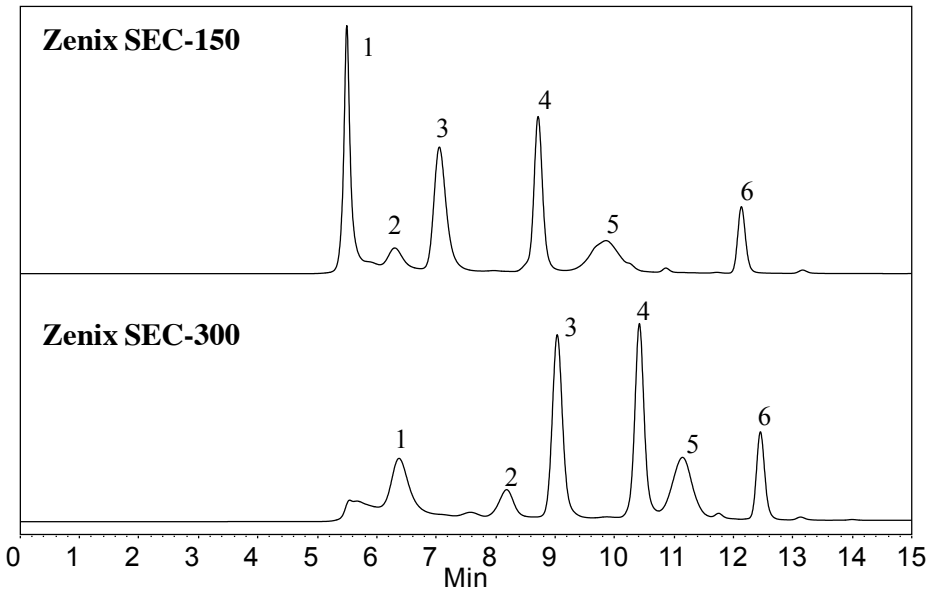


Column:	SRT® SEC-150 (5 µm, 150 Å, 4.6 x 300 mm)
Mobile Phase:	100 – 200 mM CH_3COONH_4/CH_3CN, pH 6.3
Flow Rate:	0.25 mL/min
Detection:	SofTA ELSD
Sample:	Molecular weight marker proteins, horse serum

(Courtesy of Miyako Kawakatsu, M&S Instruments Inc)

Keywords: size exclusion, SRT, proteins, volatile buffer, organic solvent

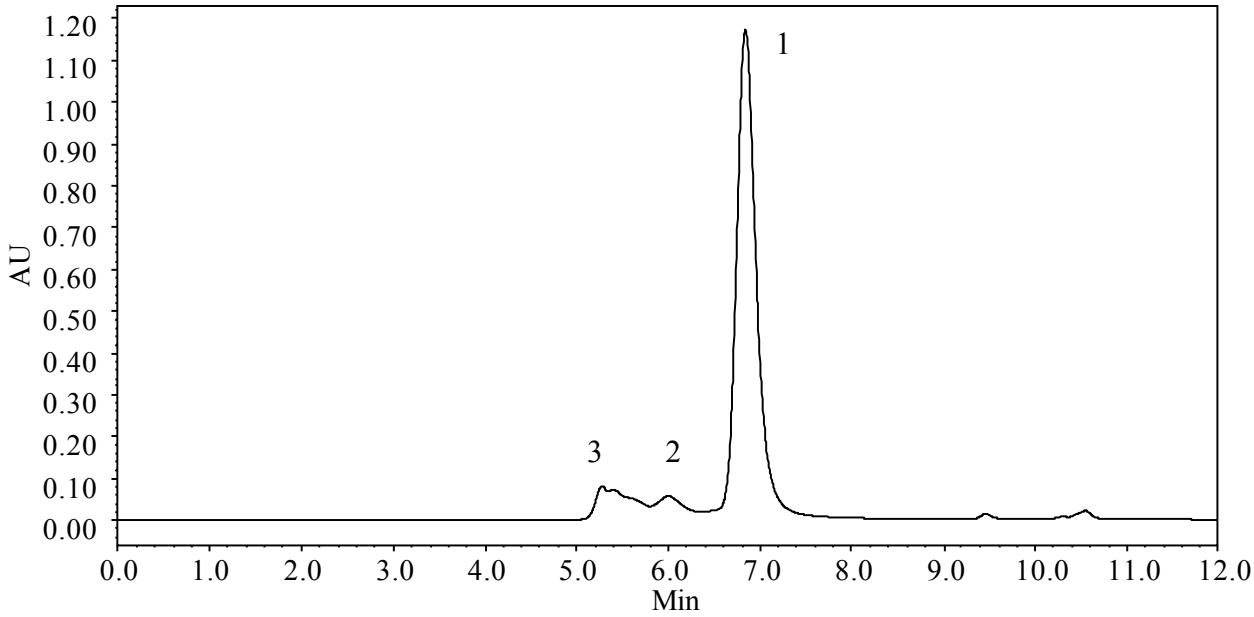
Separation of a mixture of proteins and peptide



Column:	Zenix™ SEC (3 µm, 7.8x300 mm)
Mobile Phase:	150 mM Phosphate Buffer, pH 7
Flow Rate:	1.0 mL/min
Temperature:	Ambient (~23° C)
Detection:	UV 214nm
Injection Volume:	10 µL
Samples:	1) Thyroglobulin, 670kD; 2) BSA monomer, 66kD; 3) Ribonuclease A, 13.7kD; 4) poly-DL-alanine, 1-5 kD; 5) Uracil, 120D.

Keywords: Size exclusion, Zenix, protein, peptide

Size Exclusion Separation of Protein and PEGylated Proteins

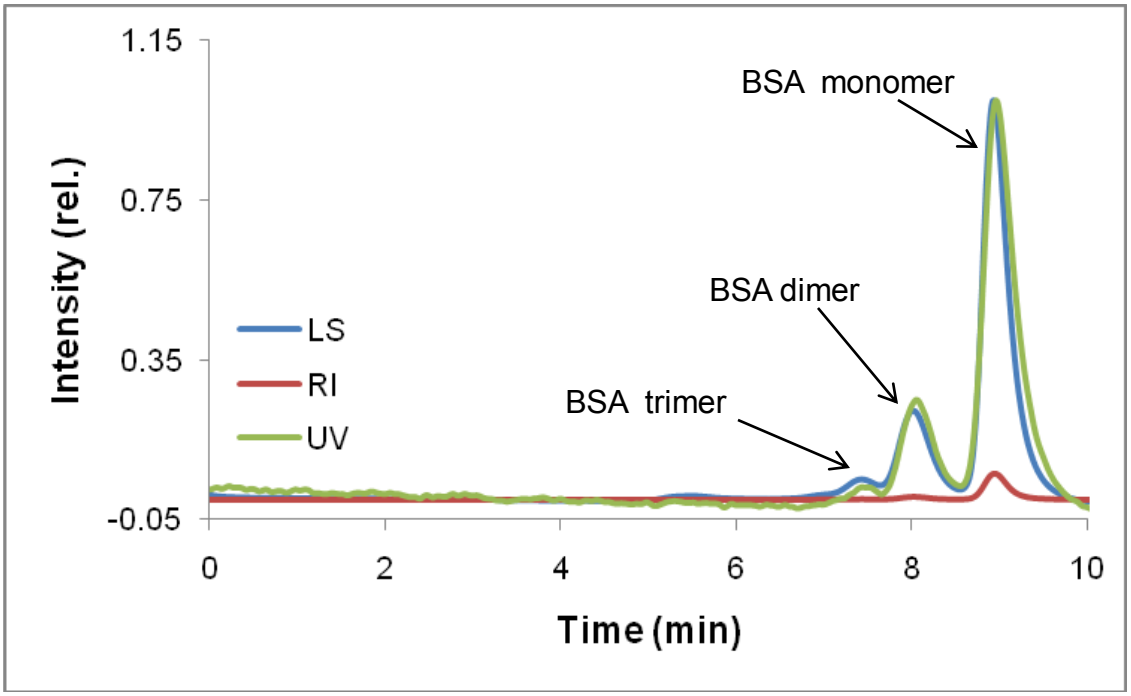


Column:	Zenix™ SEC-150 (3 µm, 150 Å, 7.8x300 mm)
Mobile Phase:	150 mM Phosphate Buffer, pH 7.0;
Flow Rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV 214 nm
Concentration:	2.0 mg/mL
Injection Volume:	20 µL
Samples:	1, Protein with MW 70KD (from an undisclosed drug development company) 2, Protein 1 attached with 1 PEG (MW 40KD) molecule 3, Protein 1 attached with 2 PEG (MW 40KD) molecules

Keywords: Size exclusion, Zenix, PEGlyated protein, protein

Light Scattering Detection of BSA on Zenix SEC-300 (3µm, 300Å, 7.8x300mm)

The chromatogram shows an overlay of the ultraviolet (UV), differential refractive index (RI) and multi angle light scattering data (MALS) for a sample of bovine serum albumin; showing the monomer, dimer and trimer. The chromatogram illustrates the power of light scattering used with Sepax's Zenix SEC in protein characterization. The light scattering data makes it possible to determine the molecular weight of the monomer, dimer and trimer.

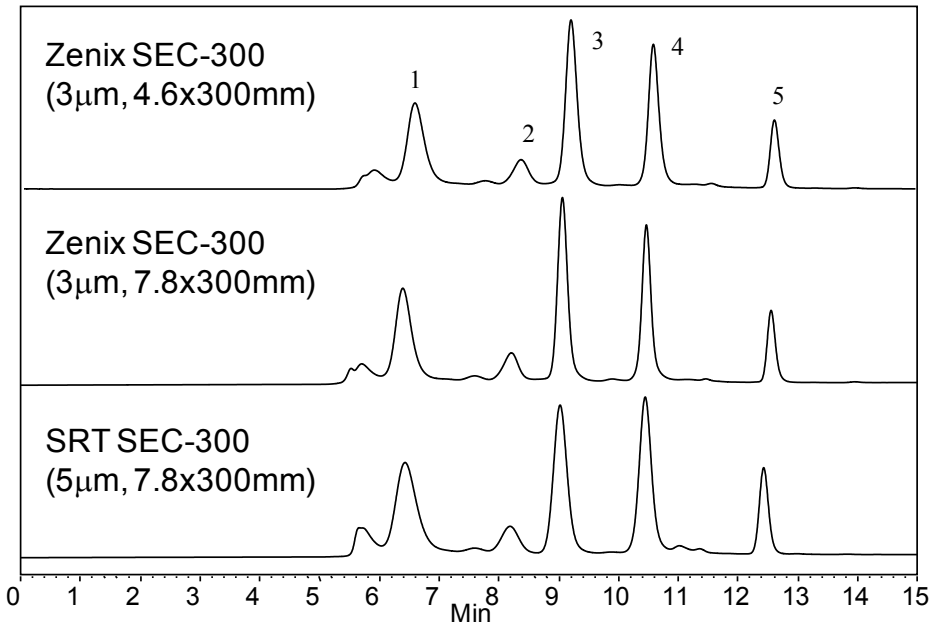


Column:	Zenix SEC-300 (3µm, 300Å, 7.8x300mm)
Mobile Phase:	0.1M Phosphate Buffer
Flow Rate:	1.0 mL/min
Temperature:	25 °C
Triple Detection:	Hitachi-Elite LaChrom L-2130 pump equipped with a multi-angle light scattering detector (DAWN-HELIOS: Wyatt Technology), a refractive index detector (Hitachi L-2490) and a UV-Vis detector (Hitachi 2420)
Injection Volume:	20 µL
Samples:	BSA (20mg/mL)

Acknowledgements and thanks to Matthew Thompson for this data.

Postdoctoral Researcher - Gianneschi Lab
Department of Chemistry & Biochemistry
University of California, San Diego

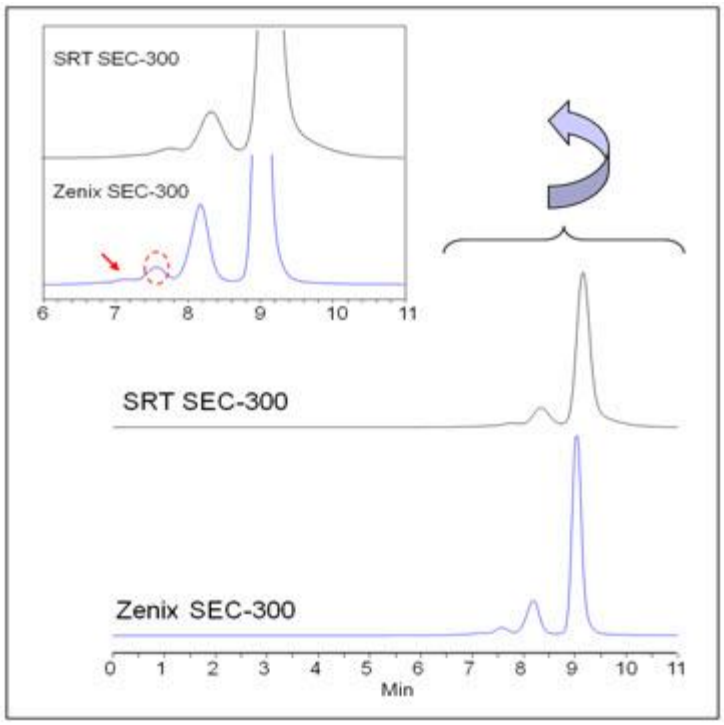
SEC Efficiency Comparison – Zenix™ (3μm) vs SRT® (5 μm)



Columns:	Zenix™ 300 (3 µm, 300 Å) and SRT® 300 (5 µm, 300 Å)
Mobile Phase:	150 mM Phosphate Buffer, pH 7
Flow Rates:	1.0 mL/min for 7.8x300 mm; 0.35 mL/min for 4.6x300 mm
Temperature:	Ambient (~23° C)
Detection:	UV 214nm
Injection Volume:	10 µL (3 µL for 4.6x300 mm)
Samples:	1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5 µg/mL), 120D.

Keywords: Size exclusion, Zenix, SRT, protein, particle size, comparison, column screening

High Resolution Difference – Zenix™ (3μm) vs. SRT® (5 μm)

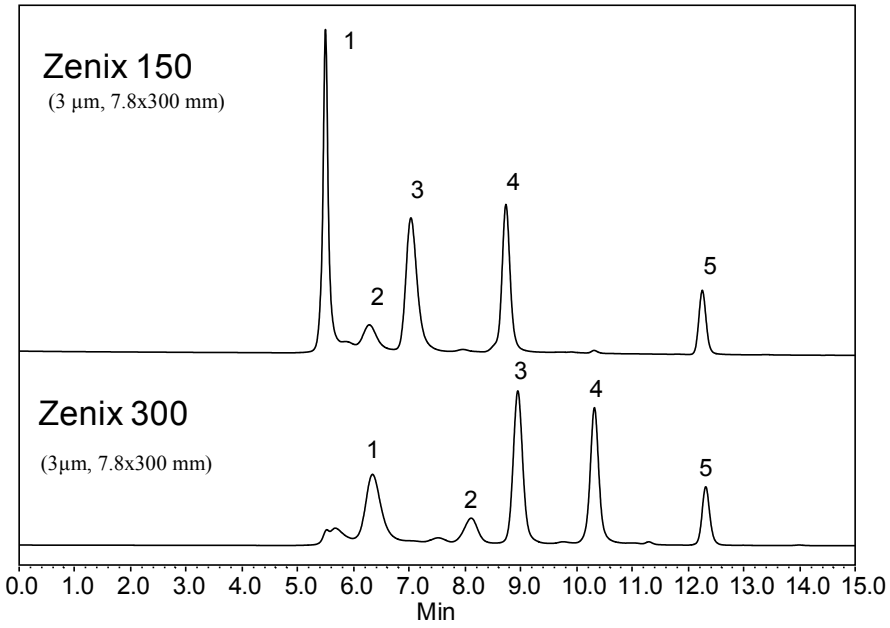


Columns:	SRT®-300 (5 µm, 300 Å), Zenix™ (3 µm, 300 Å), 7.8x300mm
Mobile Phase:	150 mM Phosphate Buffer, pH 7
Flow Rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV 214nm
Concentration:	5 mg/ml
Injection Volume:	10 µL
Sample:	BSA

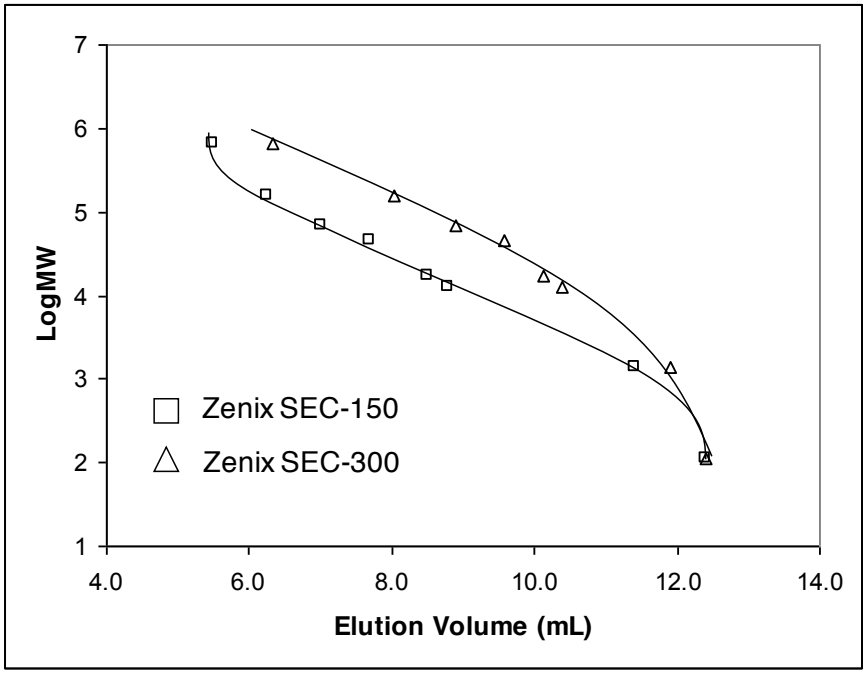
Keywords: Size exclusion, Zenix, SRT, protein, particle size, comparison

ZP1003

High SEC Efficiency for Protein Separation and MW Calibration Curve – Zenix™ (7.8 x 300 mm)



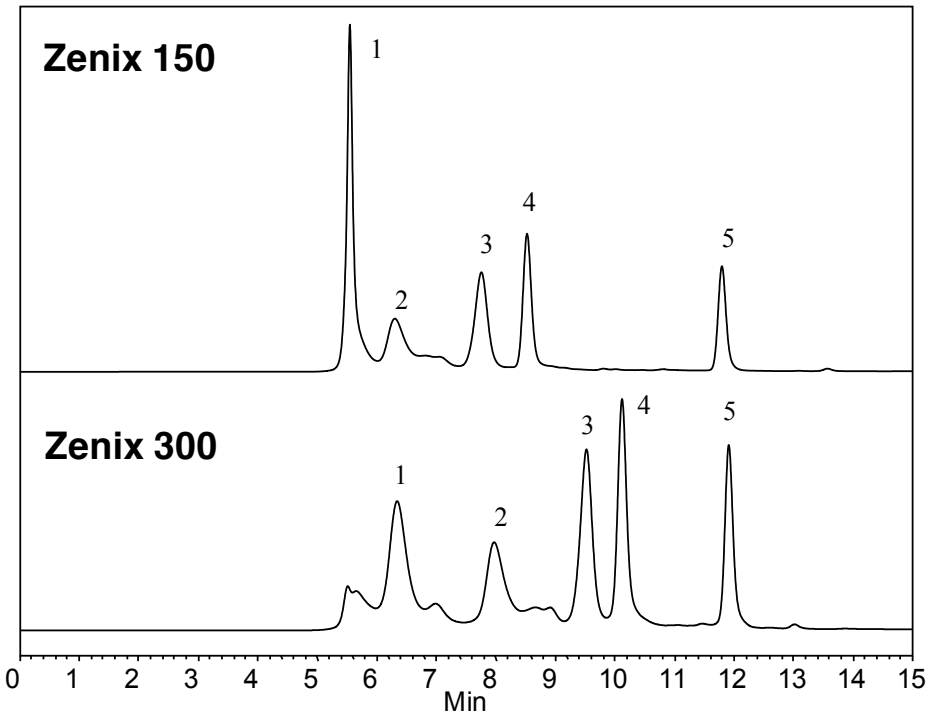
Mobile Phase: 150 mM phosphate buffer, pH 7
Flow Rate: 1.0 mL/min
Detection: UV 214 nm
Injection Volume: 10 µL
Samples: 1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5 µg/mL), 120D



Column: Zenix™ (3µm, 7.8 x 300 mm)
Mobile Phase: 150 mM phosphate buffer, pH 7
Flow Rate: 1.0 mL/min
Detection: UV 214 nm
Injection Volume: 10 µL
Samples:
1) Thyroglobulin 670 kD
2) γ-Globulin 158kD
3) BSA 66kD
4) Ovalbumin 44 kD
5) Myoglobin 17.6 kD
6) Ribonuclease 13.7 kD
7) B12 1.35 kD
8) Uracil 120D

Keywords: Size exclusion, Zenix, proteins, pore size, calibration curve

Separation of Bio-Rad Protein Mixture by Zenix™ SEC Phases

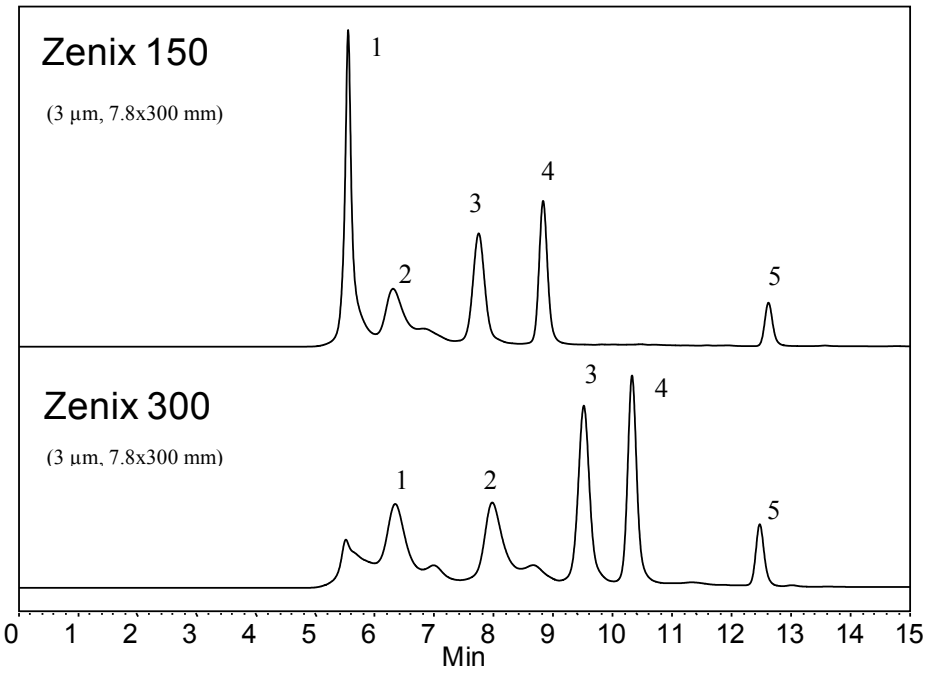


Column:	Zenix™ (3 µm, 7.8x300 mm)
Mobile Phase:	150 mM Phosphate Buffer, pH 7
Flow Rate:	1.0 mL/min
Detection:	UV 214nm
Injection Volume:	10 µL
Samples:	1) Thyroglobulin, 670 kD; 2) γ-Globulin, 158 kD; 3) Ovalbumin, 44 kD; 4) Myoglobulin, 16.9 kD; 5) Vitamin B12, 1355 D

Keywords: Size exclusion, Zenix, protein, pore size

ZP1005

Separation of Protein Mixture by Zenix™ SEC Phases

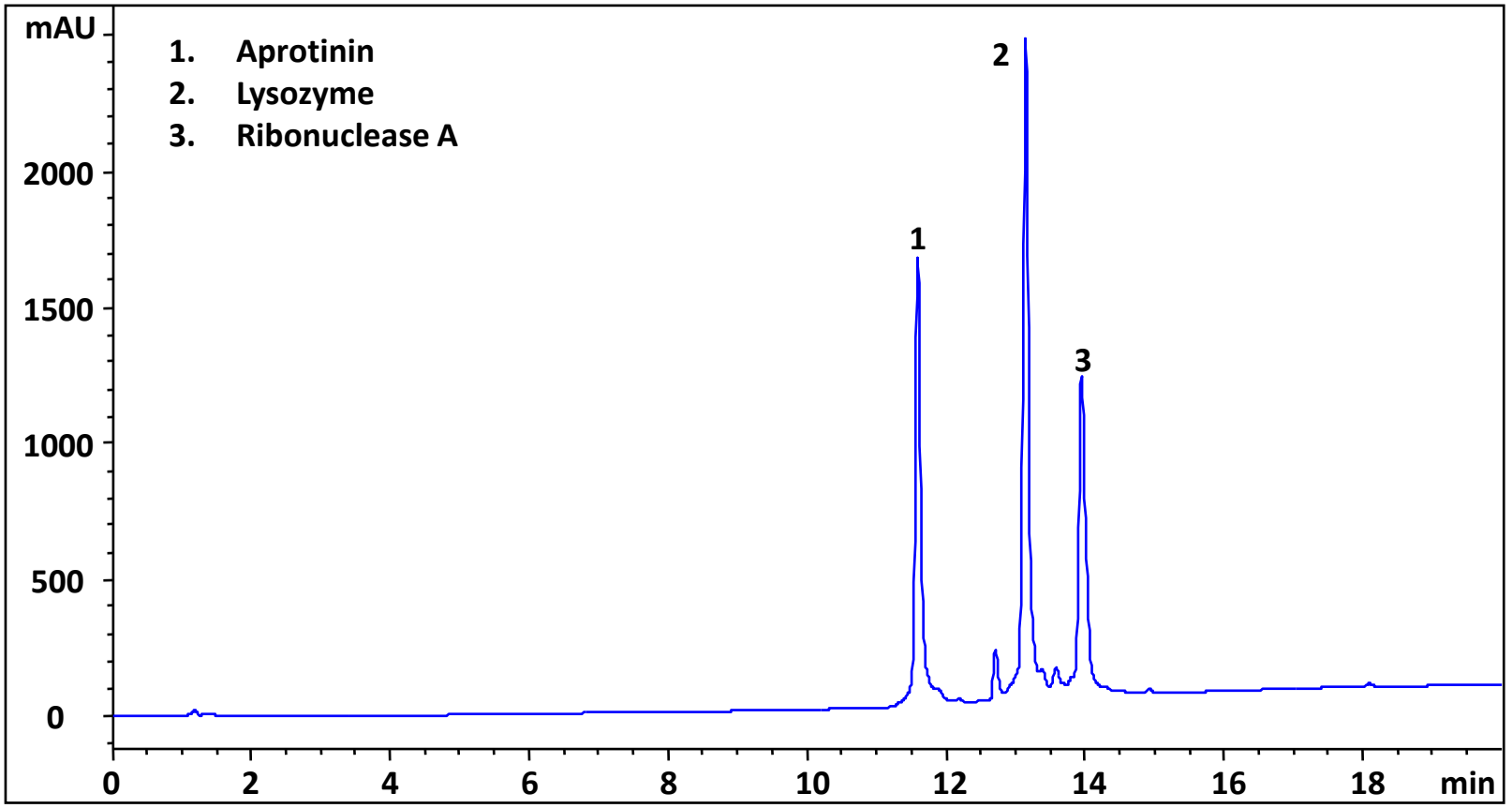


Mobile Phase:	150 mM phosphate buffer, pH 7
Flow Rate:	1.0 mL/min
Temperature:	Ambient
Detection:	UV 214 nm
Injection Volume:	10 µL
Samples:	1) Thyroglobulin, 670 kD; 2) γ-Globulin, 158 kD; 3) Ovalbumin, 44 kD; 4) Ribonuclease A, 13.7 kD; 5) p-Aminobenzoic acid, 137 D

Keywords: Size exclusion, Zenix, proteins, pore size

Antibodix WCX NP1.7 4.6 x 50 mm

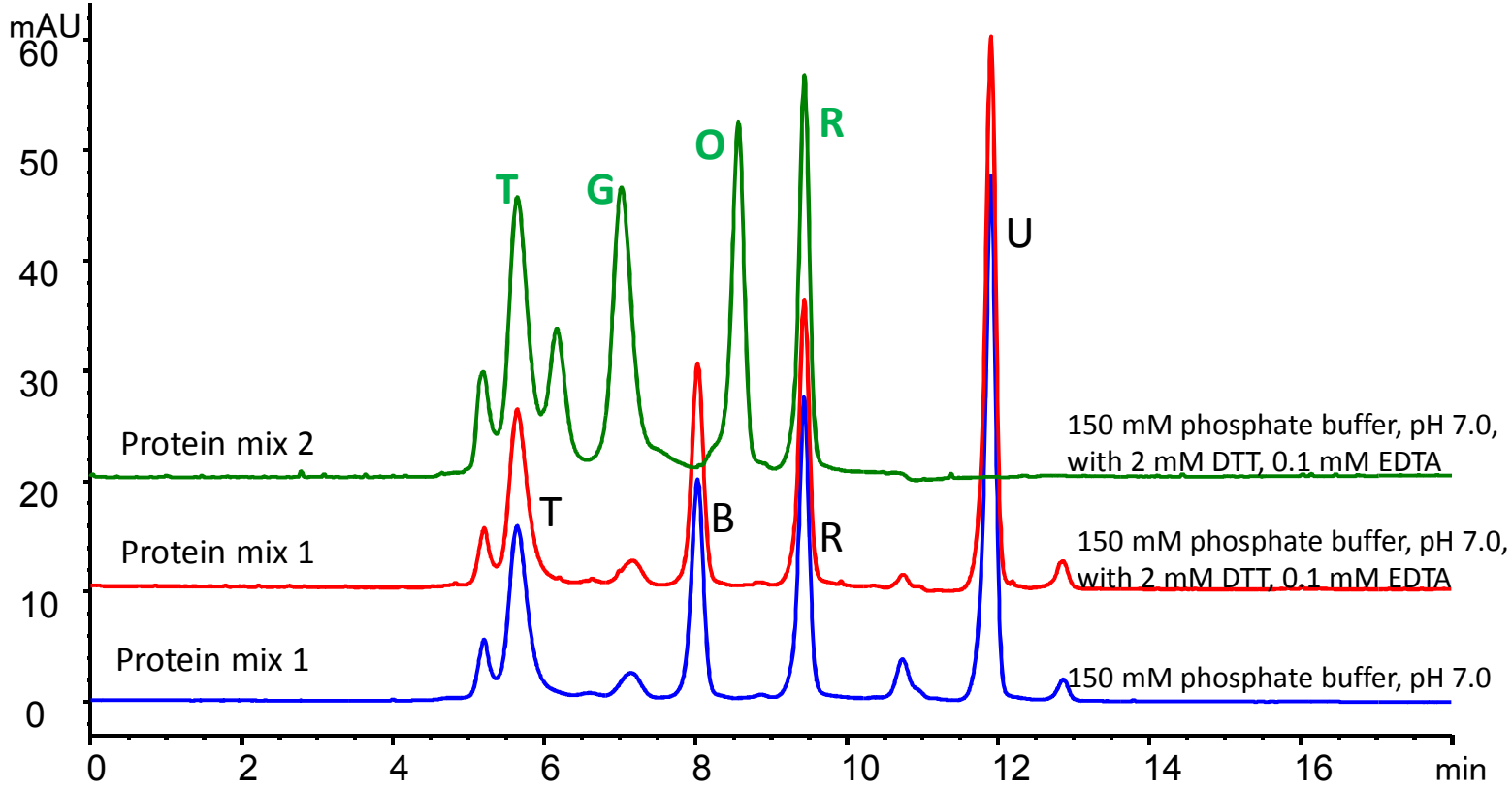
Mobile Phase A: 10 mM sodium phosphate buffer, pH 6.0, Mobile Phase B: A + 1.0 M NaCl, Flow Rate: 0.3 mL/min, Temperature: Ambient, Gradient: 0-25 min from 10-100% B, 15 min prewash, Samples: 5 µL injection Aprotinin, Lysozyme and Ribonuclease A (1 mg/mL), Detection: 214 nm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	9.94	9489	85527	1.44	---
Cytochrome C	11.50	14976	101466	1.02	11.14
Lysozyme	12.04	8625	51440	1.03	3.03

DTT and EDTA effect on SEC protein separation

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 ℃, Injection volume: 10 µL,
Sample: protein mix 1 (thyroglobulin "T", BSA "B", ribonuclease A "R"1mg/mL each, uracil "U" 0.1 mg/mL)
protein mix 2 (thyroglobulin "T", γ-globulin "G", ovalbumin "O", ribonuclease A "R", 1.25 mg/mL each)



DTT and EDTA addition in SEC mobile phase will not reduce separation efficiency.

2.1 mm ID Size Exclusion chromatography for Protein Separation

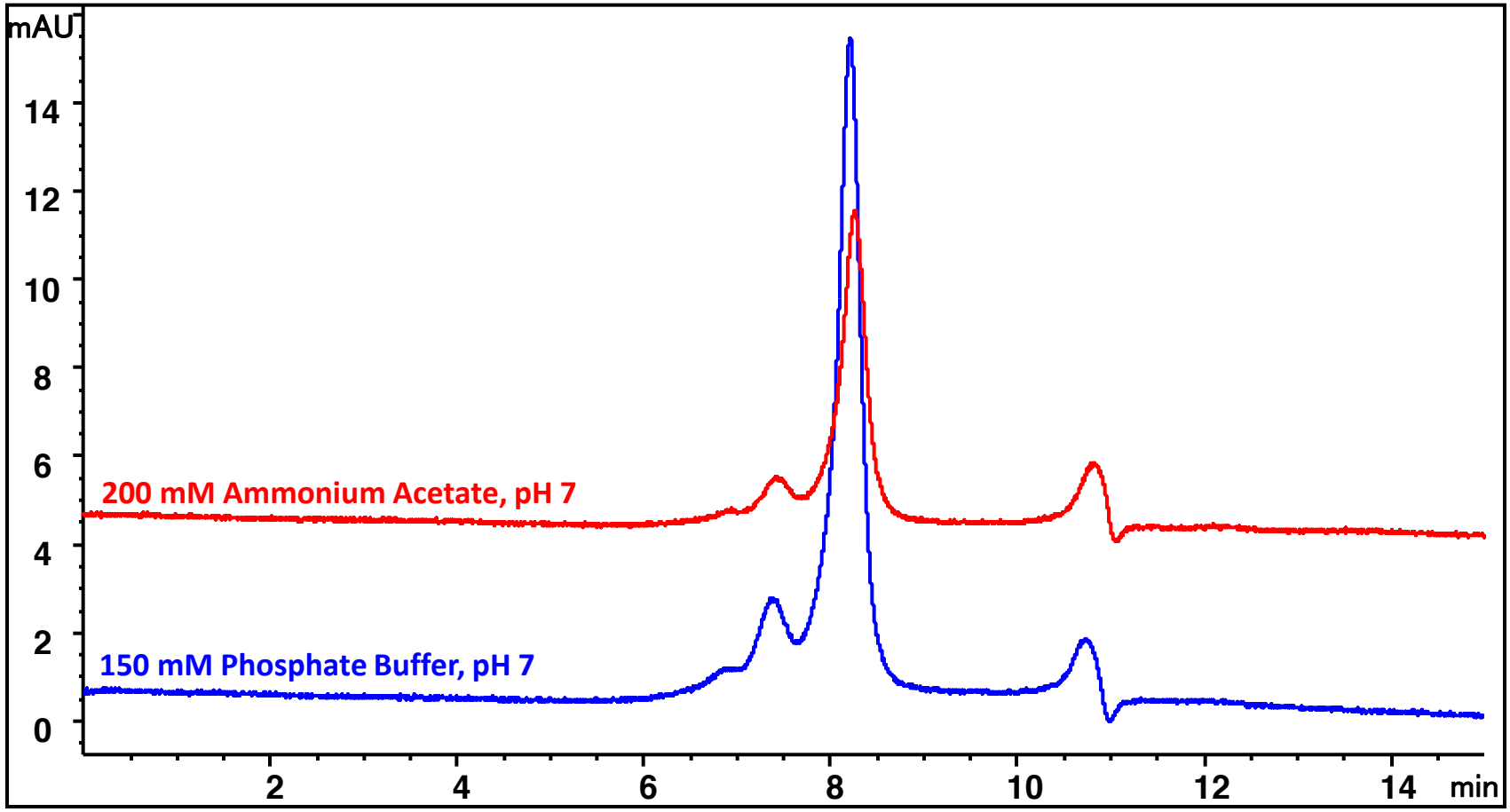
Native volatile buffer
200 mM ammonium acetate, pH 7.0
Vs.
Native salt buffer
150 mM sodium phosphate, pH 7.0

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

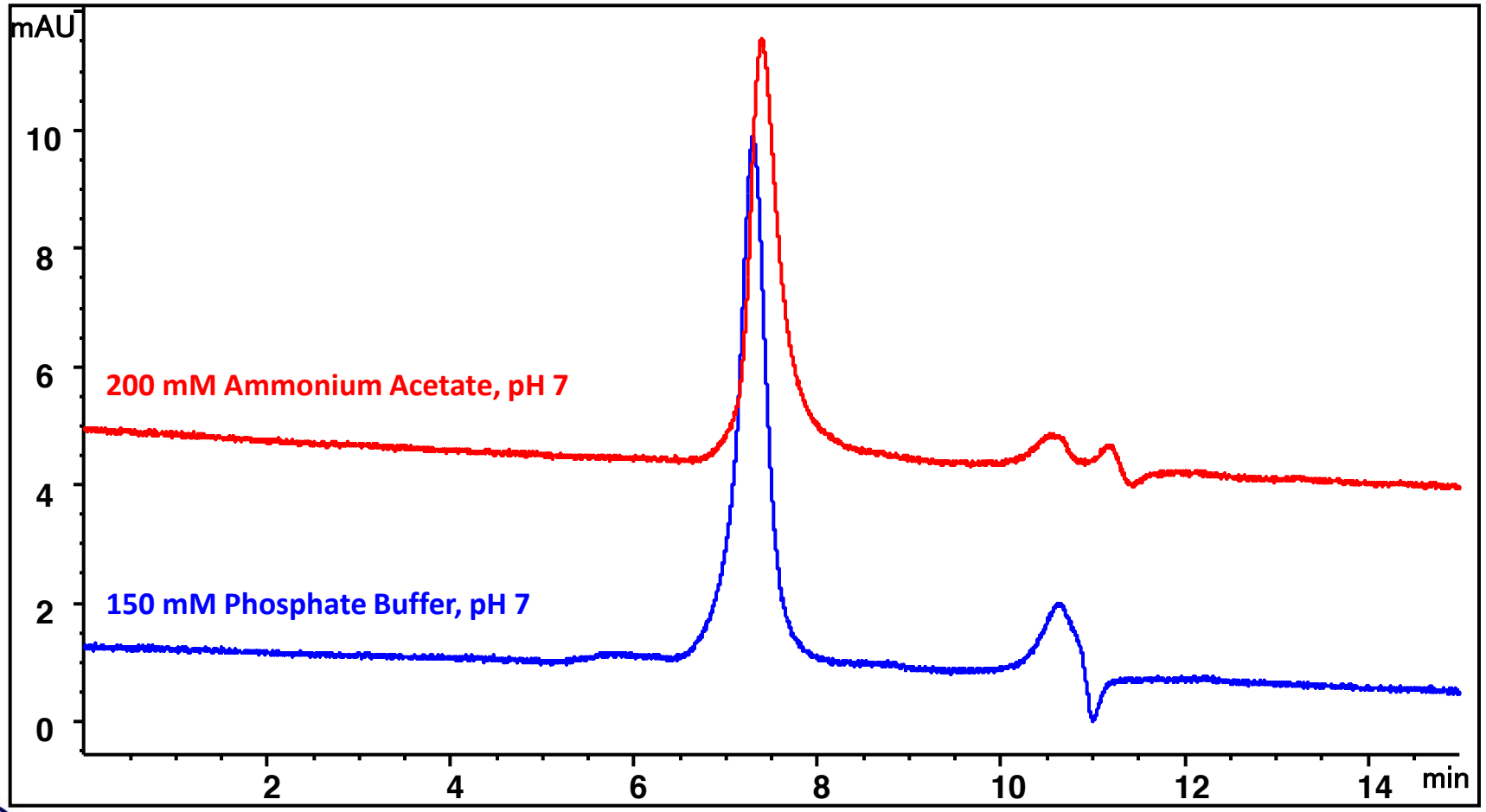
BSA SEC Analysis

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



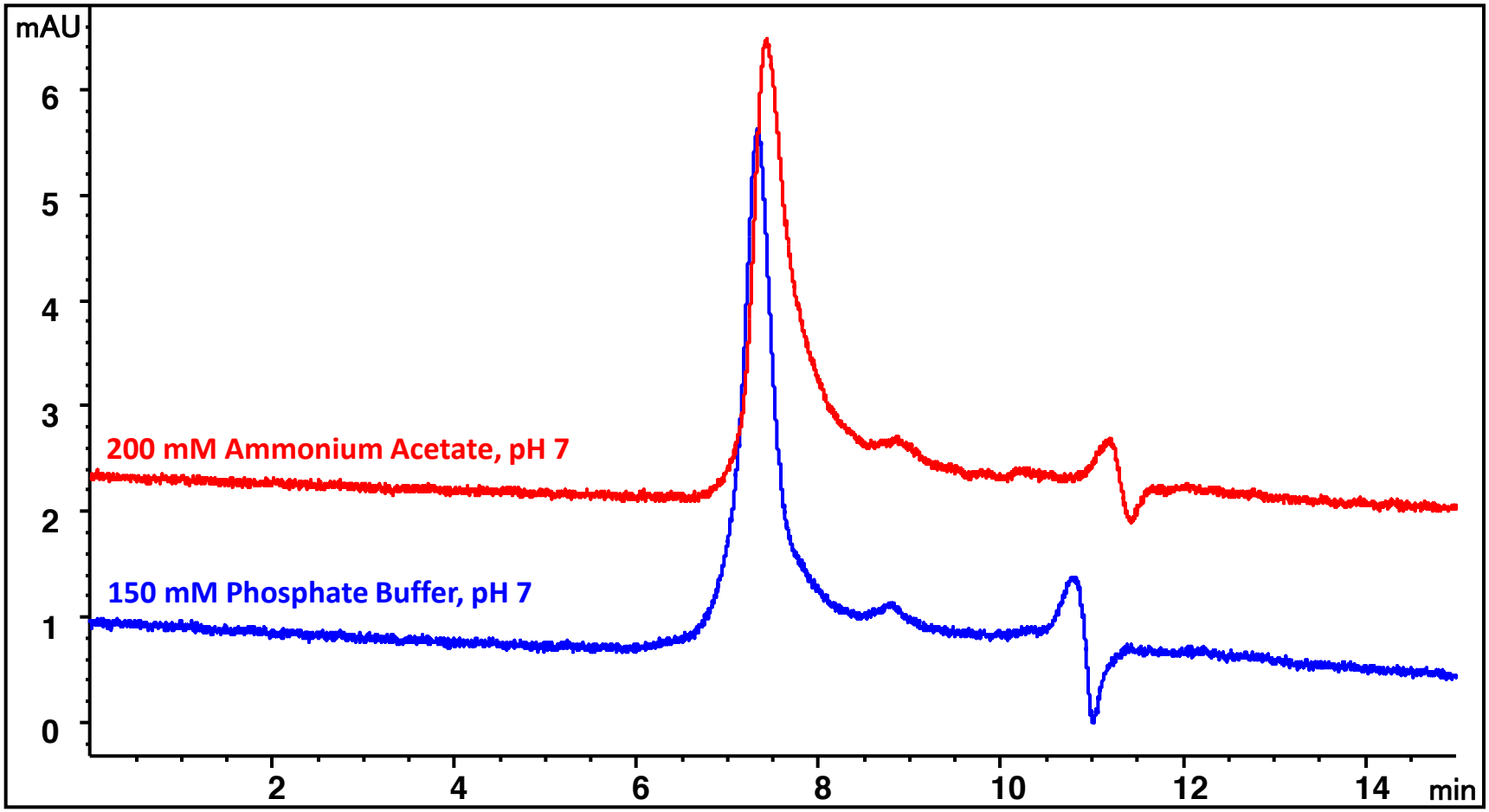
Monoclonal antibody analysis

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



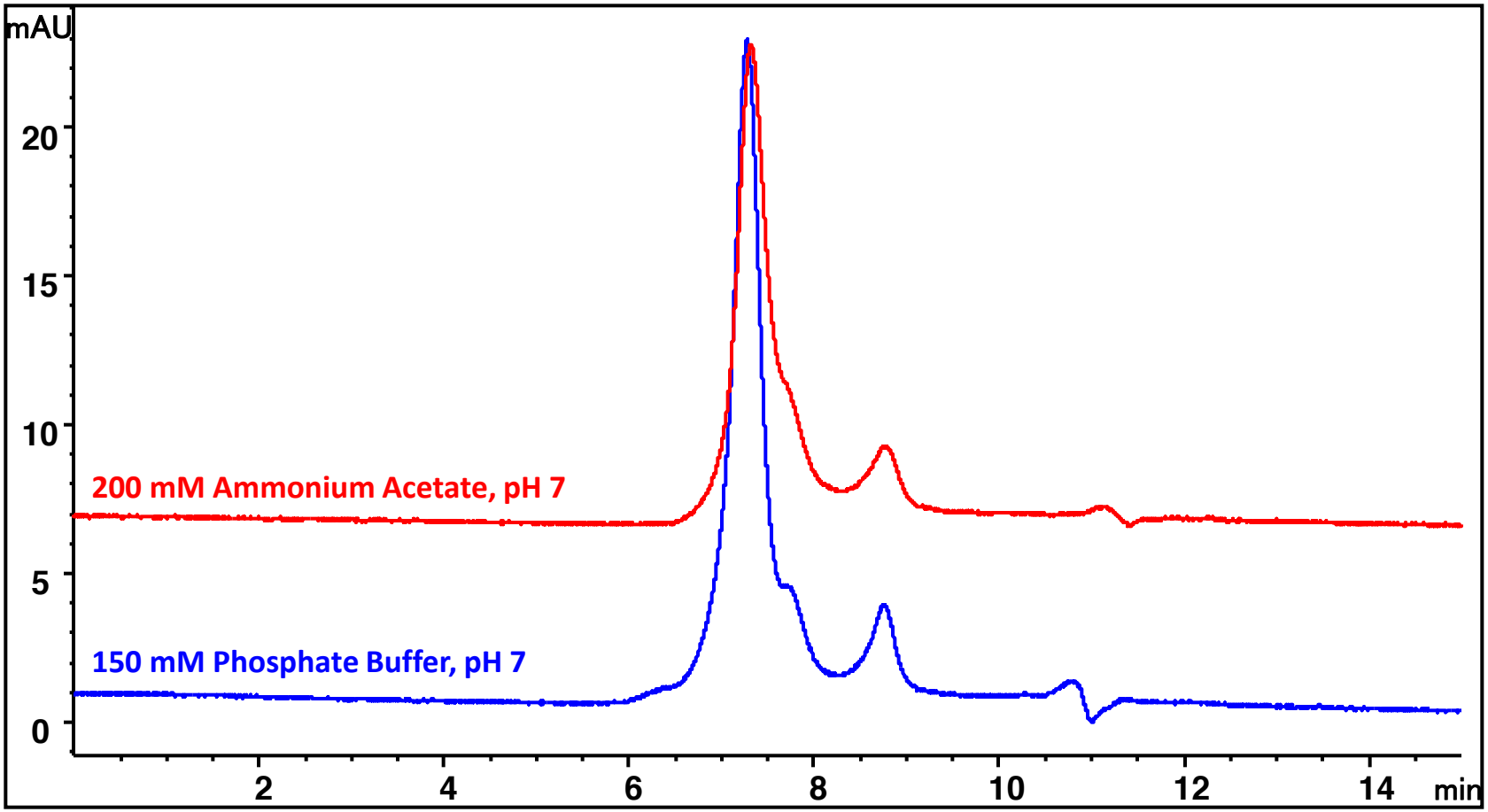
Antibody drug conjugate #1-cysteine conjugate with cleavable linker

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



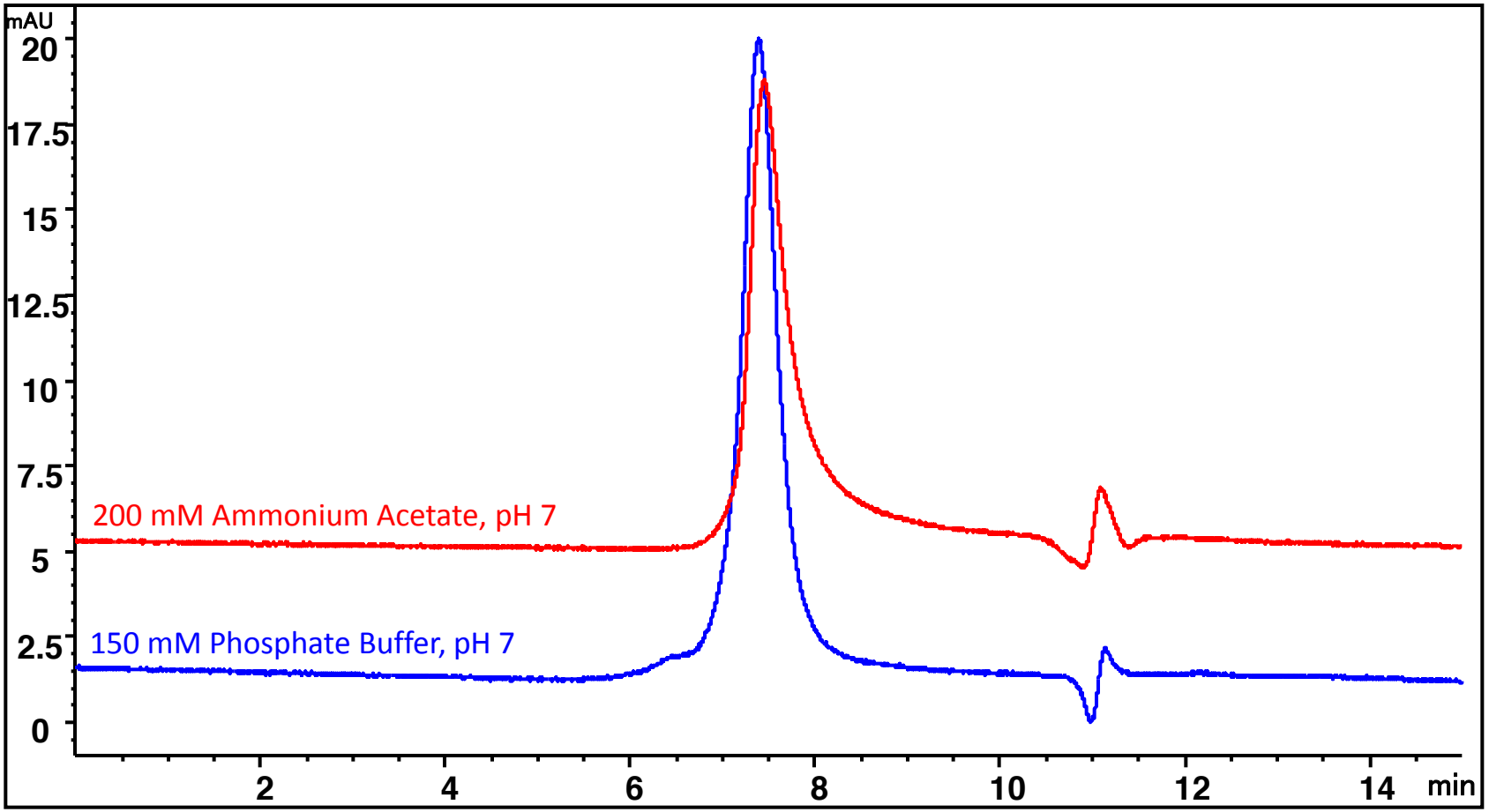
Antibody drug conjugate #2-cysteine conjugate with non-cleavable linker

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 ℃
Injection volume: 1 µL, Sample: ADC #2 1mg/mL



Antibody drug conjugate #3 - Lysine conjugate

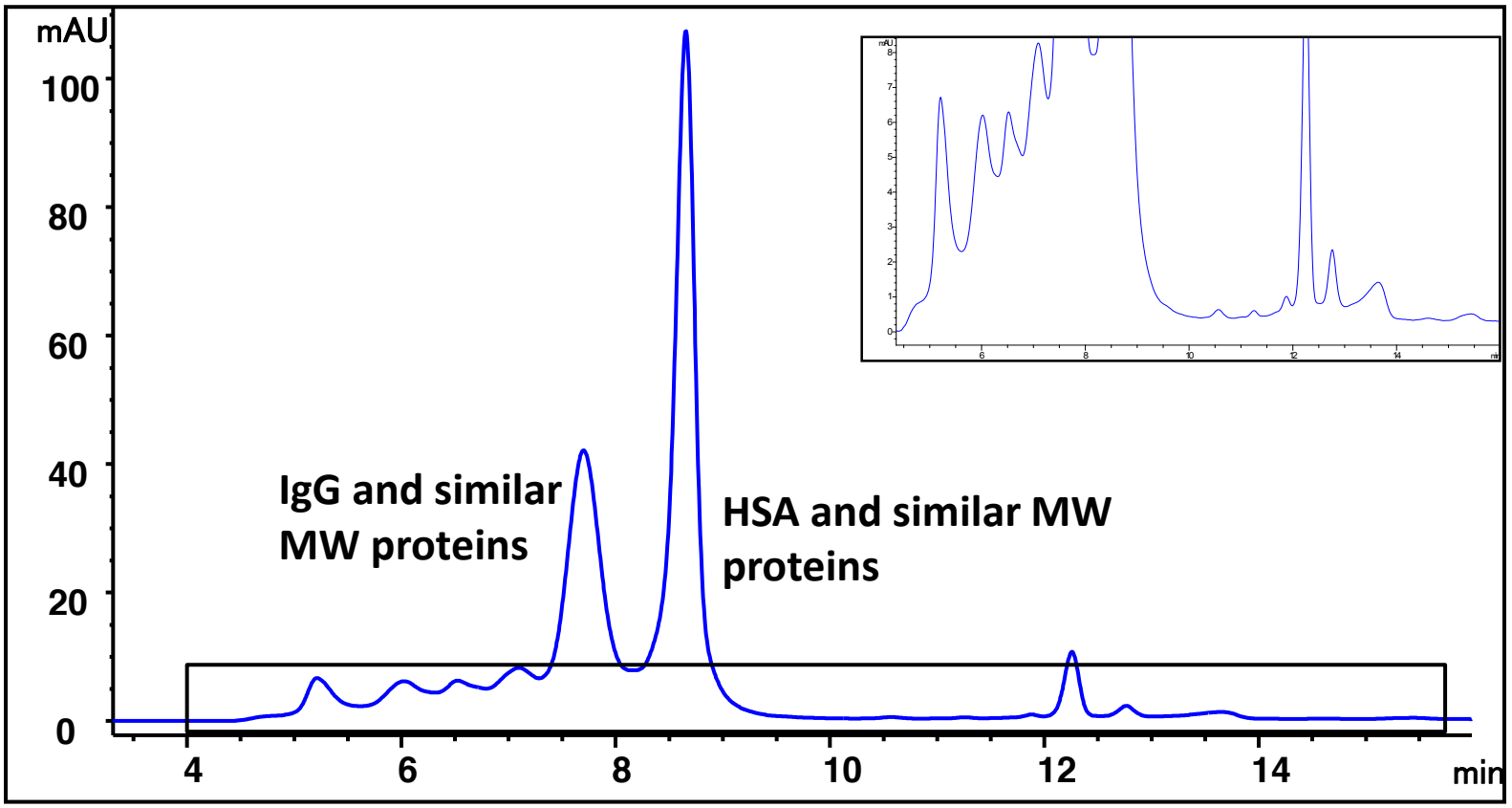
Column: Zenix-C SEC-300 (3 µm, 300 Å, 2.1 x 300 mm)
Mobile phase: See chromatogram
Flow rate: 0.08 mL/min; Detector: UV 280 nm; Column temperature: 25 ℃
Injection volume: 1 µL, Sample: ADC #3 1mg/mL



Human Serum on Zenix-C 300

EP1006

Column: Zenix-C 300, 3 µm, 7.8 x 300 mm, S/N 35771
Mobile phase: 150 mM phosphate buffer, pH 7.0,
Flow rate: 1 mL/min, Detection: UV 280 nm, Injection Volume: 1 µL Sigma human serum S7023



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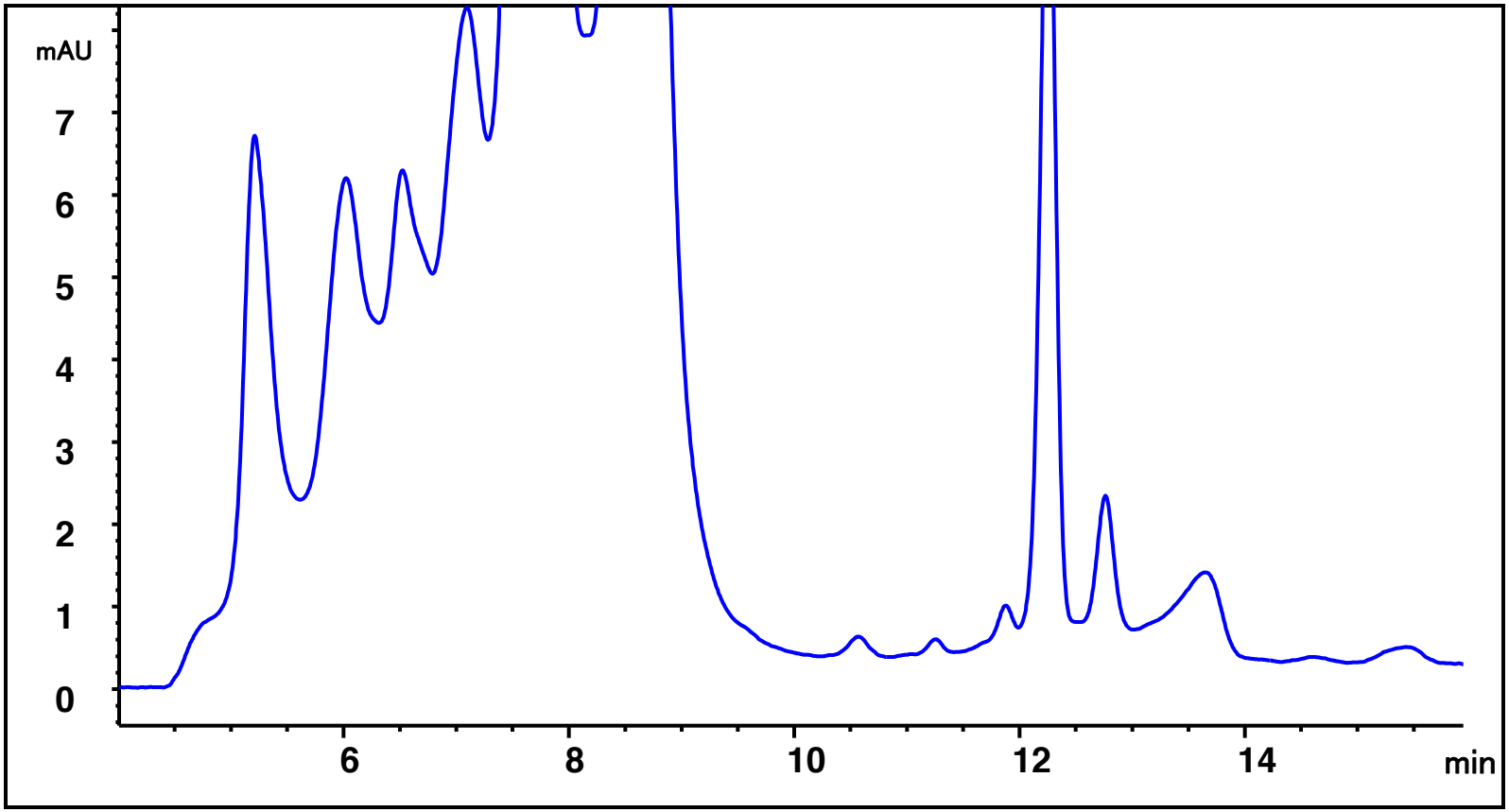
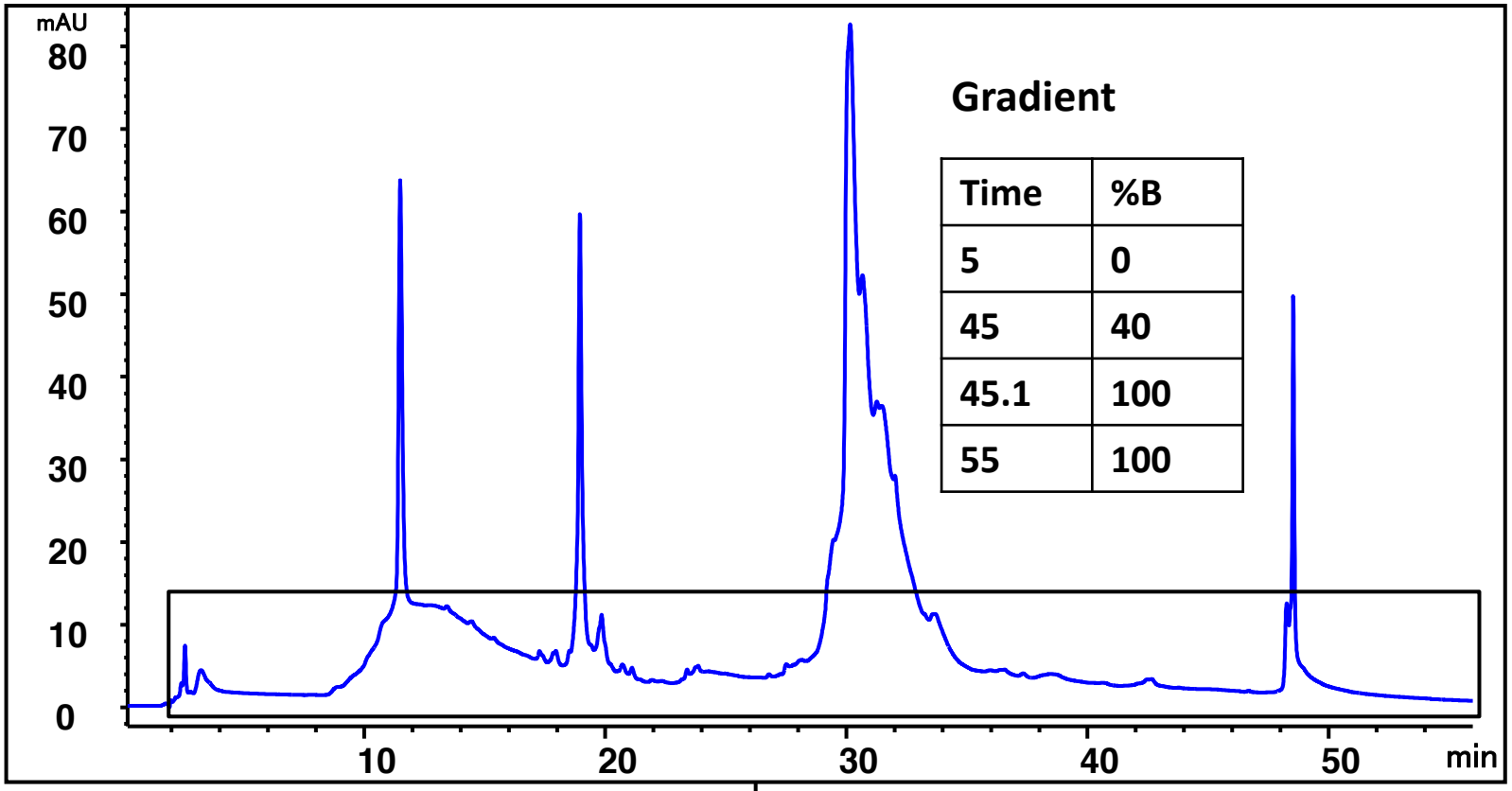
Zenix-C Zoom in Area



Human Serum on Proteomix SAX

EP1006

Column: Proteomix SAX, 3 µm, 7.8 x 100 mm
Mobile phase: A: 20 mM Tris, pH 8.0, B: A+1M NaCl,
Flow rate: 0.8 mL/min, Detection: UV 280 nm,
Injection Volume: 5 µL Sigma human serum S7023 diluted 10 fold with buffer A



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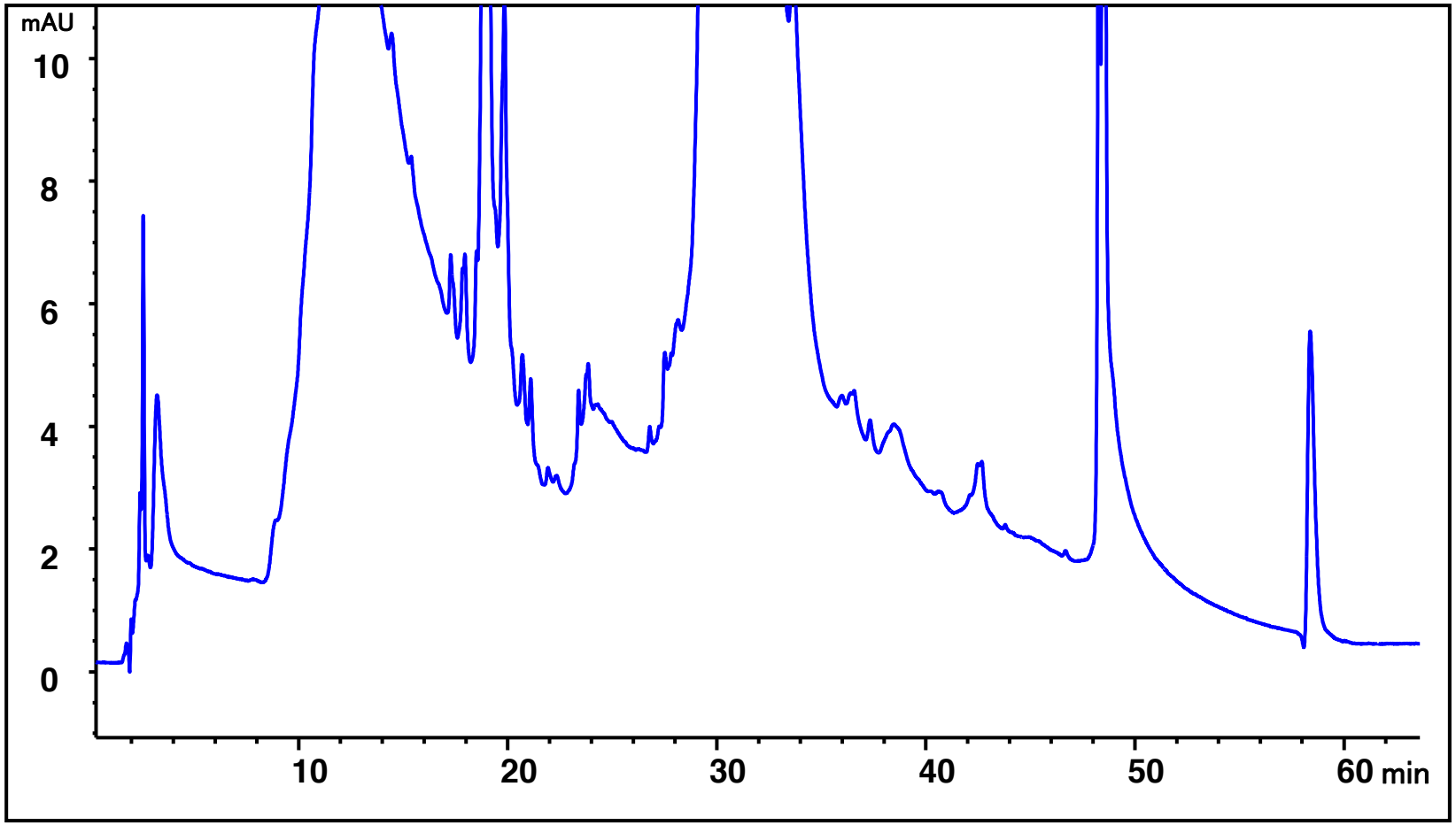
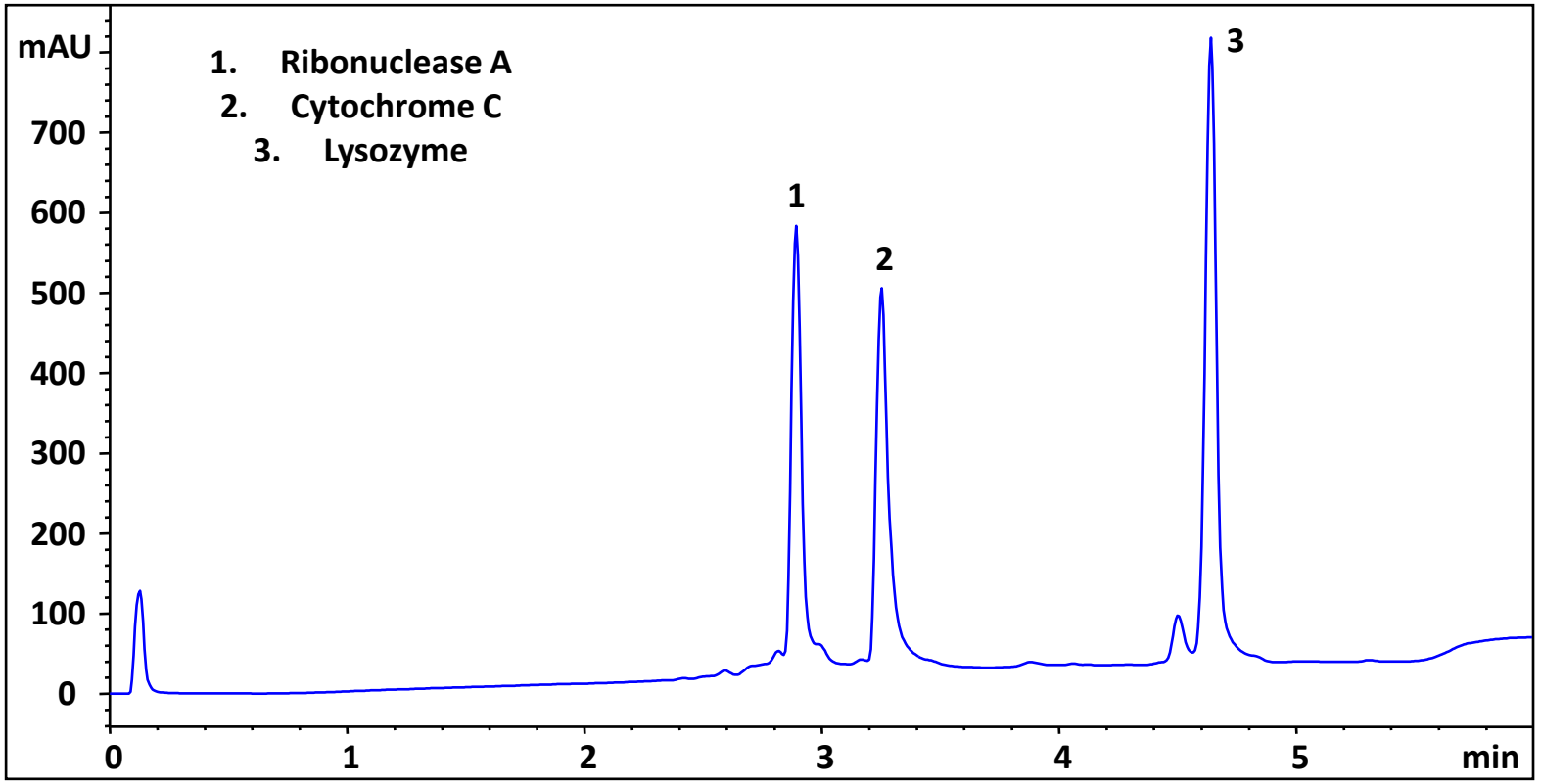
SAX Zoom in Area



Proteomix SCX NP1.7 4.6 x 30 mm

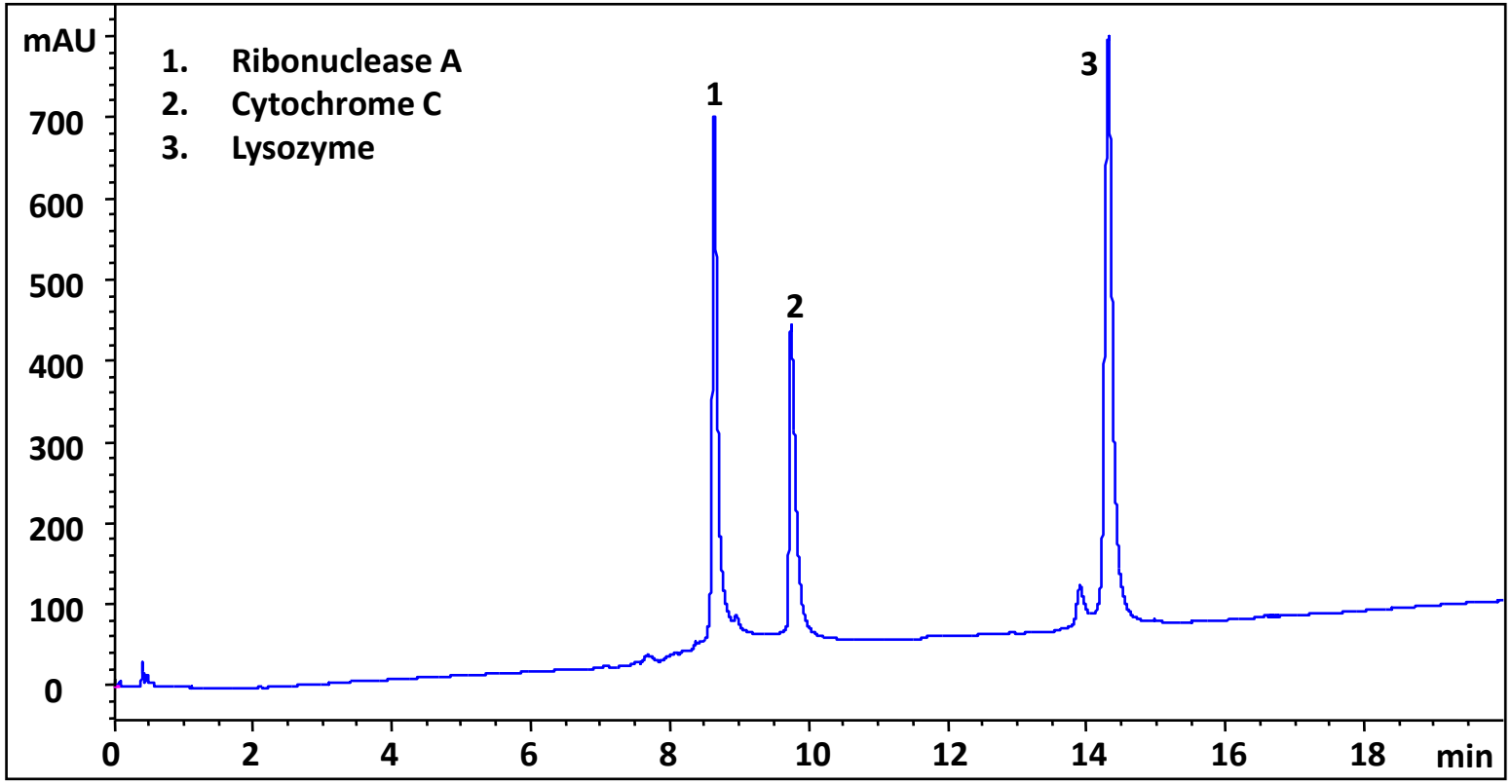
Mobile Phase A: 20 mM sodium phosphate buffer, pH 6.0, Mobile Phase B: A + 1.0 M NaCl, Flow Rate: 2 mL/min, Temperature: Ambient, Gradient: 0-5 min from 0-50% B, 15 min prewash, Samples: 5 µL injection of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL), Detection: 214 nm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	2.89	1867	19185	1.71	---
Cytochrome C	3.25	2049	17838	1.64	3.96
Lysozyme	4.64	3151	41932	0.97	14.74

Proteomix SCX NP1.7 4.6 x 50 mm

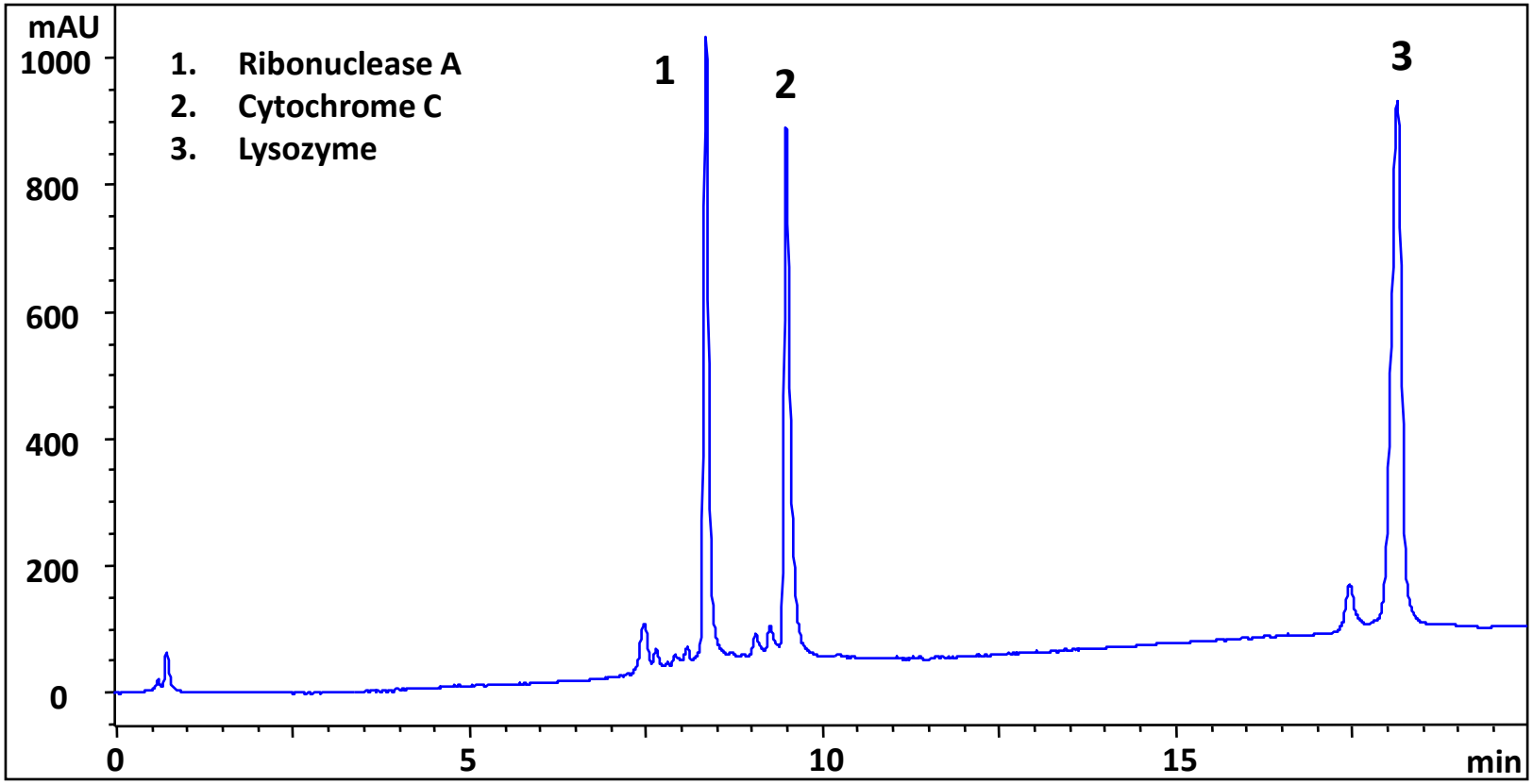
Mobile Phase A: 20 mM sodium phosphate buffer, pH 6.0, Mobile Phase B: A + 1.0 M NaCl, Flow Rate: 0.75 mL/min, Temperature: Ambient, Gradient: 0-25 min from 0-75% B, 15 min prewash, Samples: 5 µl injection Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL), Detection: 214 nm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	8.64	3875	71970	2.13	---
Cytochrome C	9.75	2549	59402	2.31	7.65
Lysozyme	14.32	5585	92428	1.36	26.17

Proteomix WCX NP1.7 4.6 x 50 mm

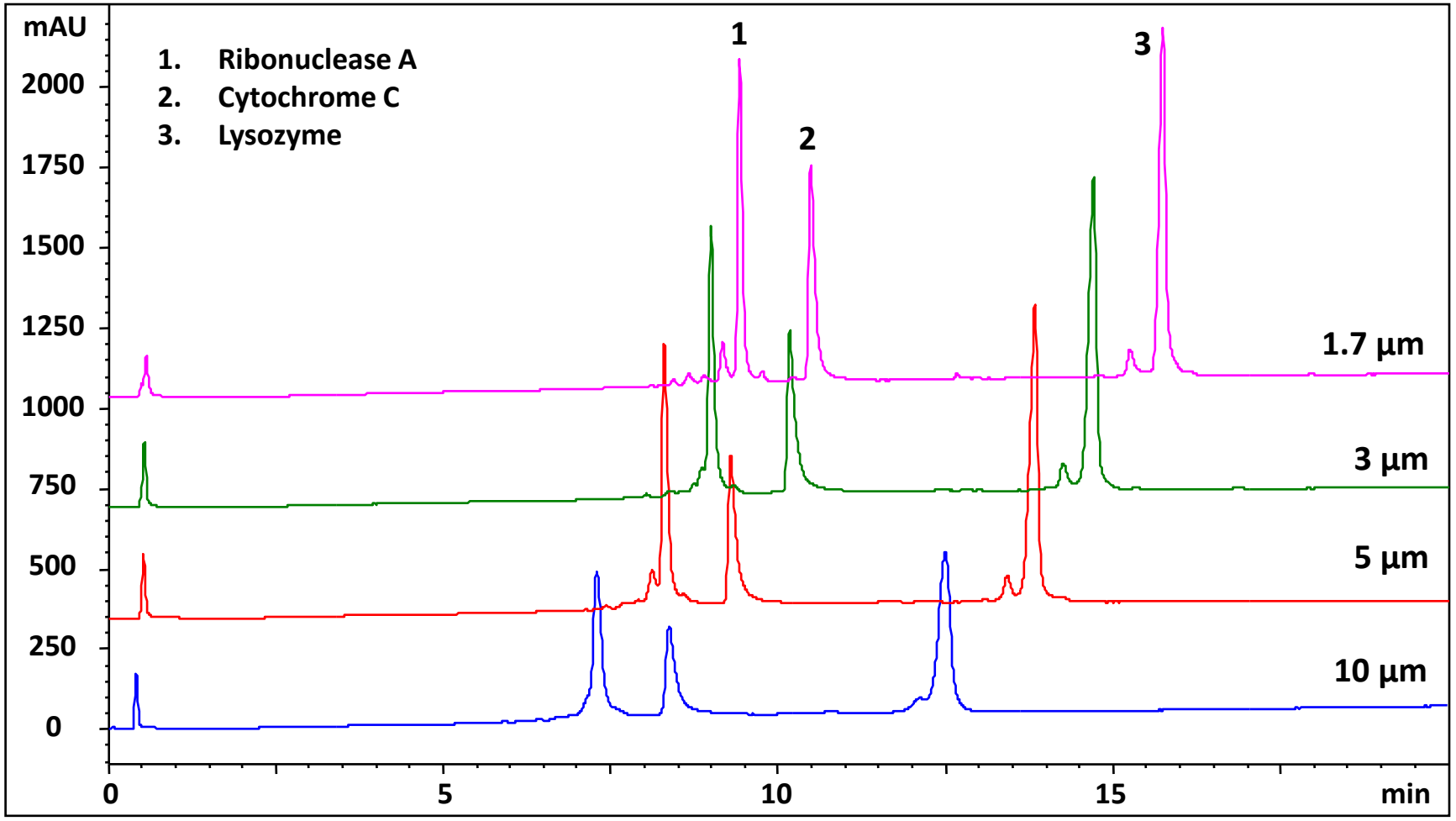
Column: Proteomix SCX NP 1.7 mm 4.6 x 50 mm, Mobile Phase A: 20 mM sodium phosphate buffer, pH 6.5, Mobile Phase B: A + 1.0 M NaCl, Flow Rate: 0.5 mL/min, Temperature: Ambient, Gradient: 0-25 min from 0-100% B, 15 min prewash, Detection: 214 nm, Samples: 5 µl injection Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL)



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
Ribonuclease A	7.31	6052	71850	3.05	---
Cytochrome C	8.42	4212	78343	2.44	9.72
Lysozyme	16.36	9283	83449	0.99	45.70

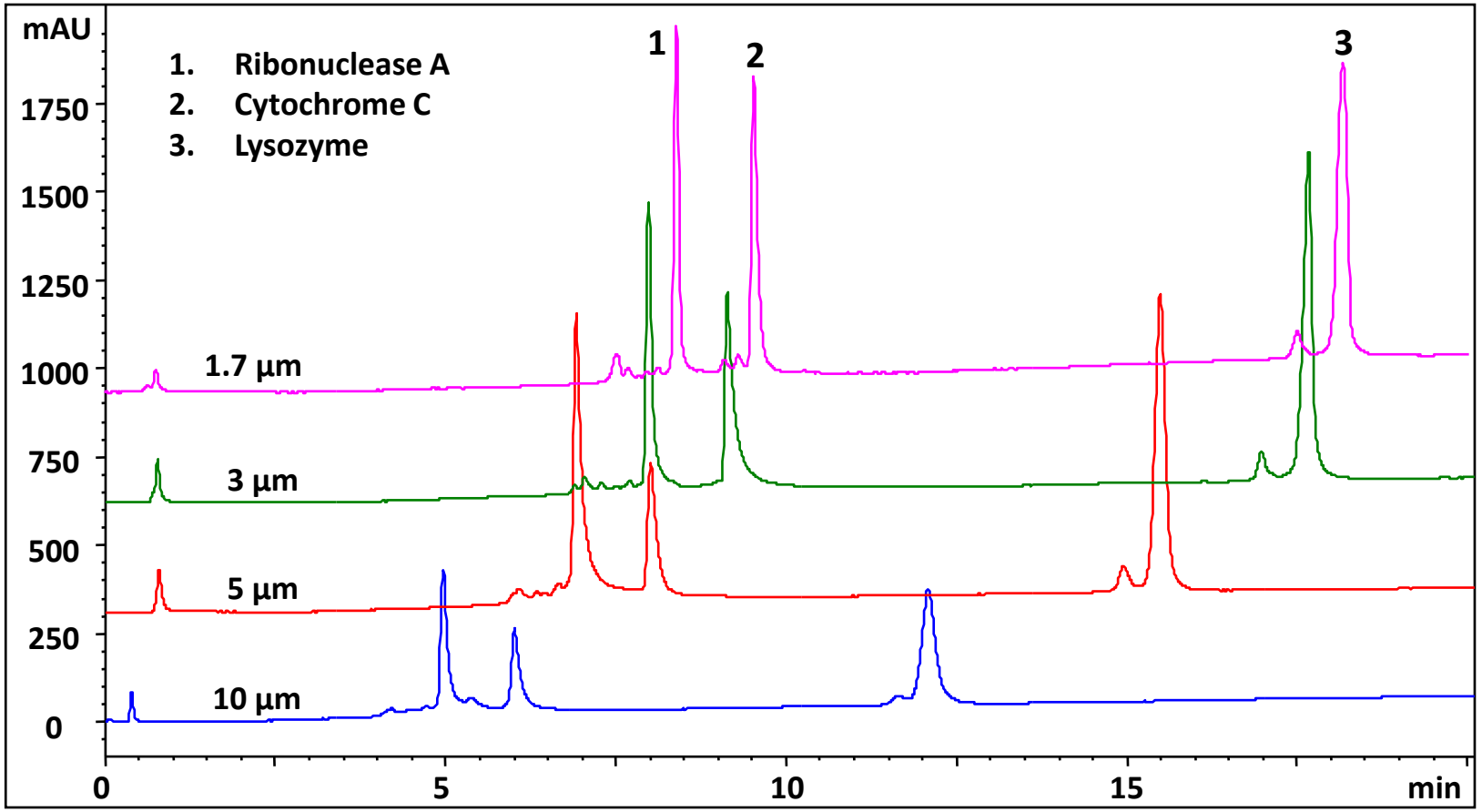
Comparison of Different Particle Sizes for Proteomix SCX

Column: Proteomix SCX NP 4.6 x 50 mm, Mobile Phase A: 20 mM sodium phosphate buffer, pH 6.0, Mobile Phase B: A + 1.0 M NaCl, Flow Rate: 0.75 mL/min, Temperature: Ambient, Gradient: 0-25 min from 0-75% B, 15 min prewash, Detection: 214 nm, Samples: 5 µL injection of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL)



Comparison of Different Particle Sizes for Proteomix WCX

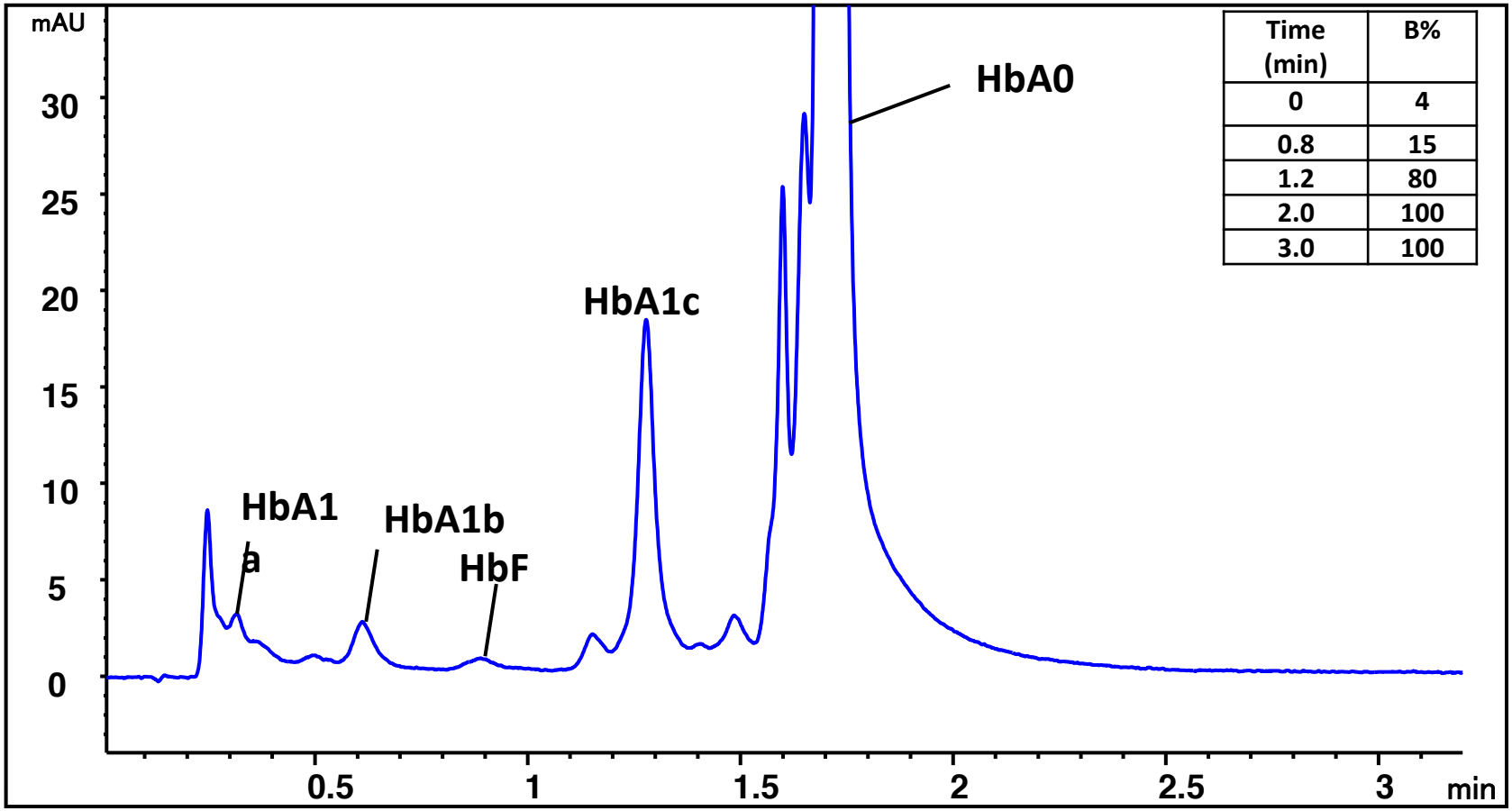
Column: Proteomix WCX NP 4.6 x 50 mm, Mobile Phase A: 20 mM sodium phosphate buffer, pH 6.5, Mobile Phase B: A + 1.0 M NaCl, Flow Rate: 0.5 mL/min, Temperature: Ambient, Gradient: 0-25 min from 0-100% B, 15 min prewash, Detection: 214 nm, Samples: 5 µL injection of Ribonuclease A, Cytochrome C and Lysozyme (1 mg/mL)



Hemoglobin variants separation on Proteomix SCX NP5-4.6 x 50 mm

PP1022

Column: Proteomix® SCX NP5 (5 μm, 4.6 x 50 mm)
Mobile phase: A:50 mM phosphate buffer (pH 6.0)
B: 50 mM phosphate buffer (pH 6.0)+ 0.5 M NaCl, Flow rate: 1.5 mL/min; Detector: Vis 415 nm
Column temperature: 40 ℃ ; Injection: 10 μL
Sample: blood sample 200 fold dilution in water

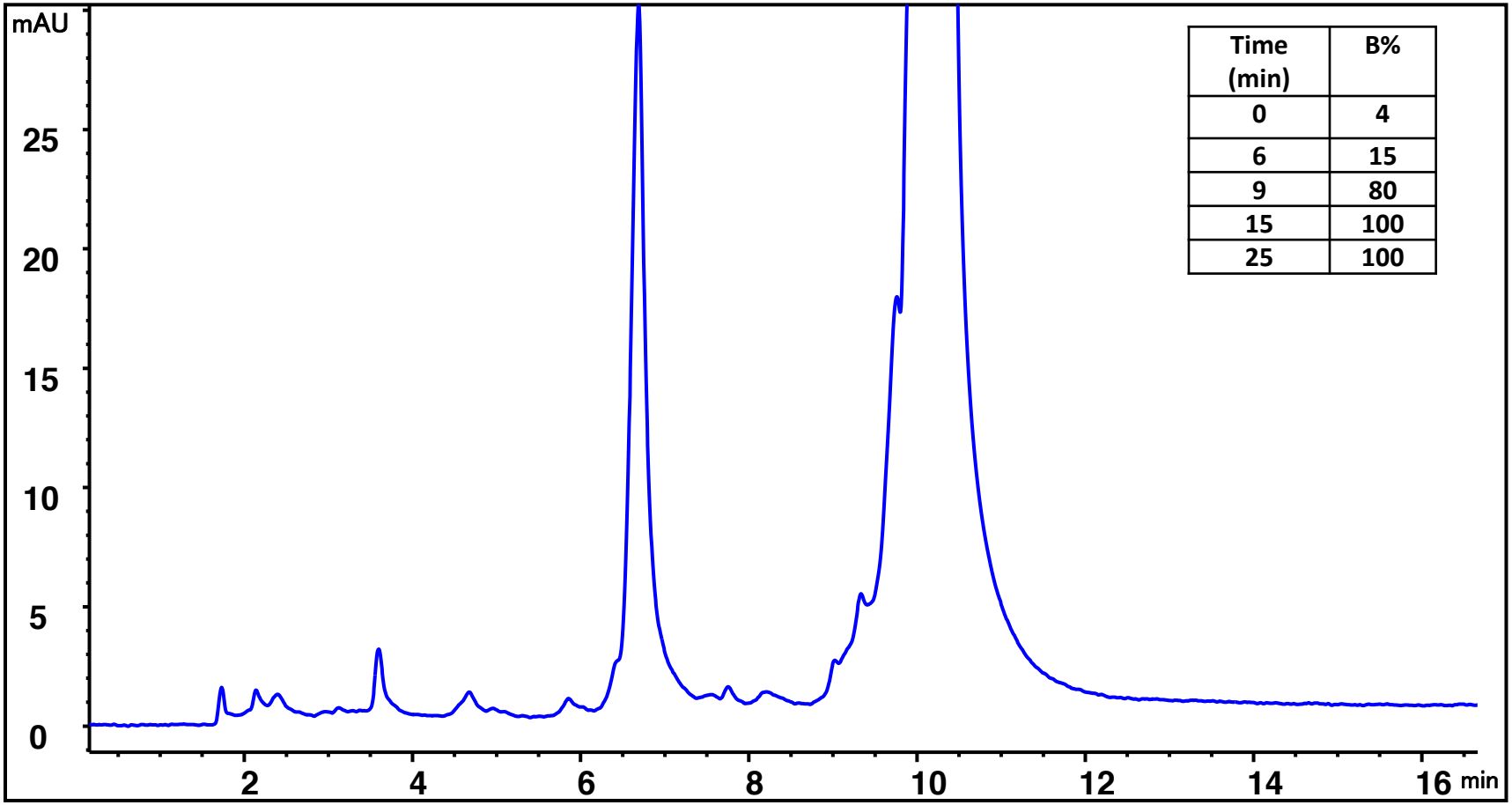


Time (min)	B%
0	4
0.8	15
1.2	80
2.0	100
3.0	100

Hemoglobin variants separation on Proteomix SCX NP5-4.6 x 250 mm

PP1022

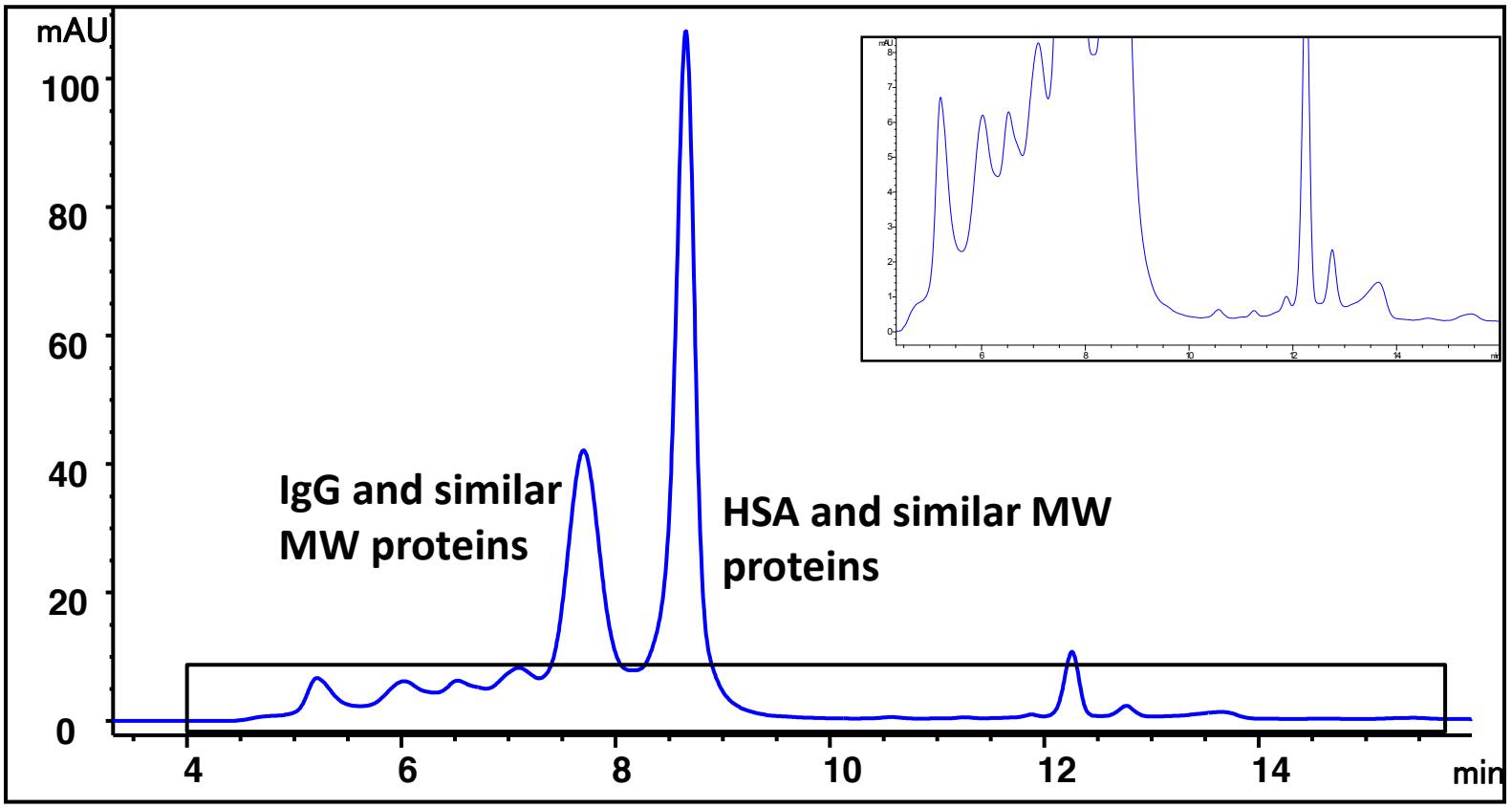
Column: Proteomix® SCX NP5 (5 μm, 4.6 x 250 mm)
Mobile phase: A:50 mM phosphate buffer (pH 6.0)
B: 50 mM phosphate buffer (pH 6.0)+ 0.5 M NaCl, Flow rate: 1.0 mL/min; Detector: Vis 415 nm
Column temperature: 40 ℃ ; Injection: 20 μL
Sample: blood sample 200 fold dilution in water



Time (min)	B%
0	4
6	15
9	80
15	100
25	100

Human Serum on Zenix-C 300

Column: Zenix-C 300, 3 µm, 7.8 x 300 mm, S/N 35771
Mobile phase: 150 mM phosphate buffer, pH 7.0,
Flow rate: 1 mL/min, Detection: UV 280 nm, Injection Volume: 1 µL Sigma human serum S7023



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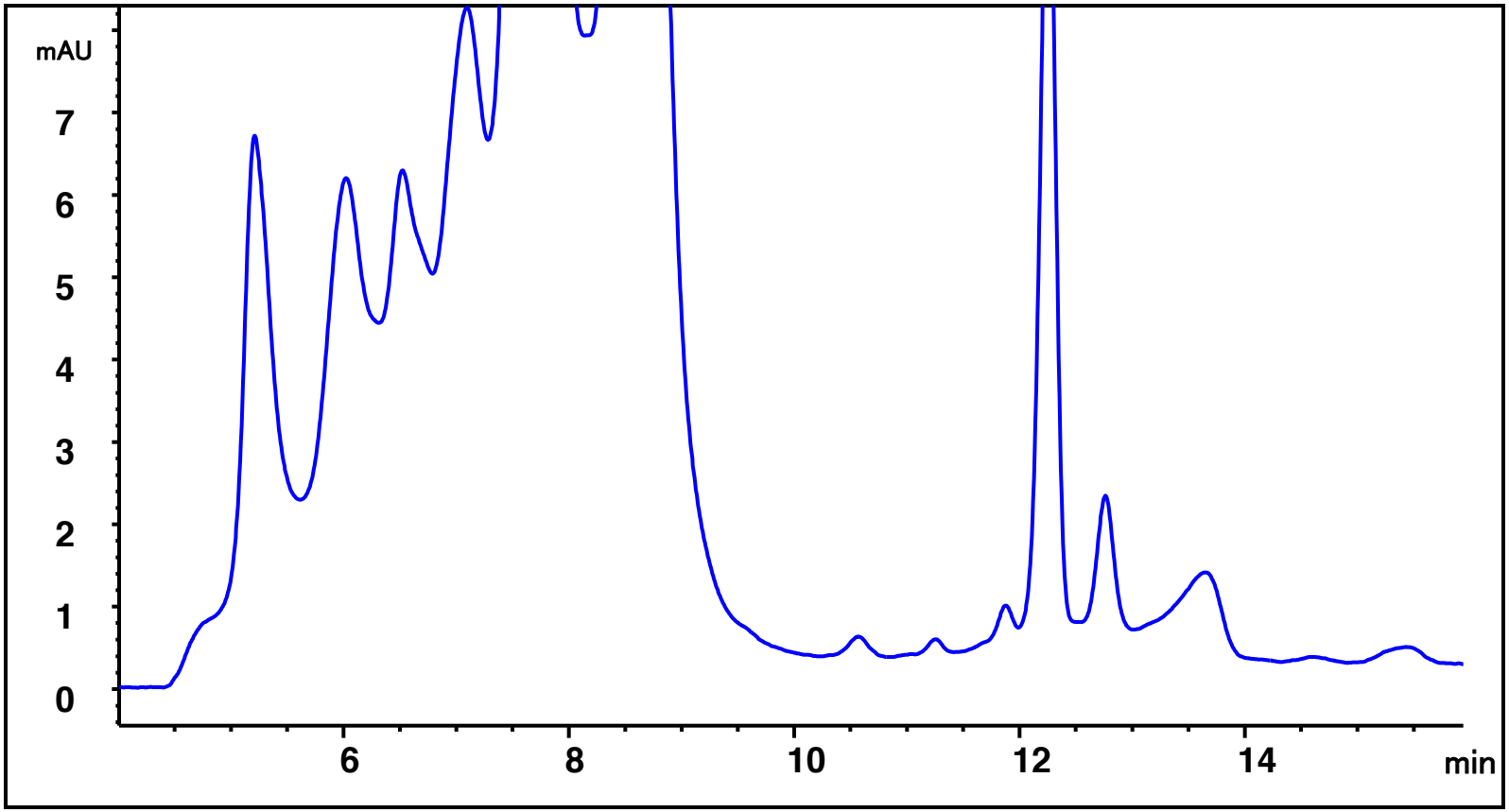
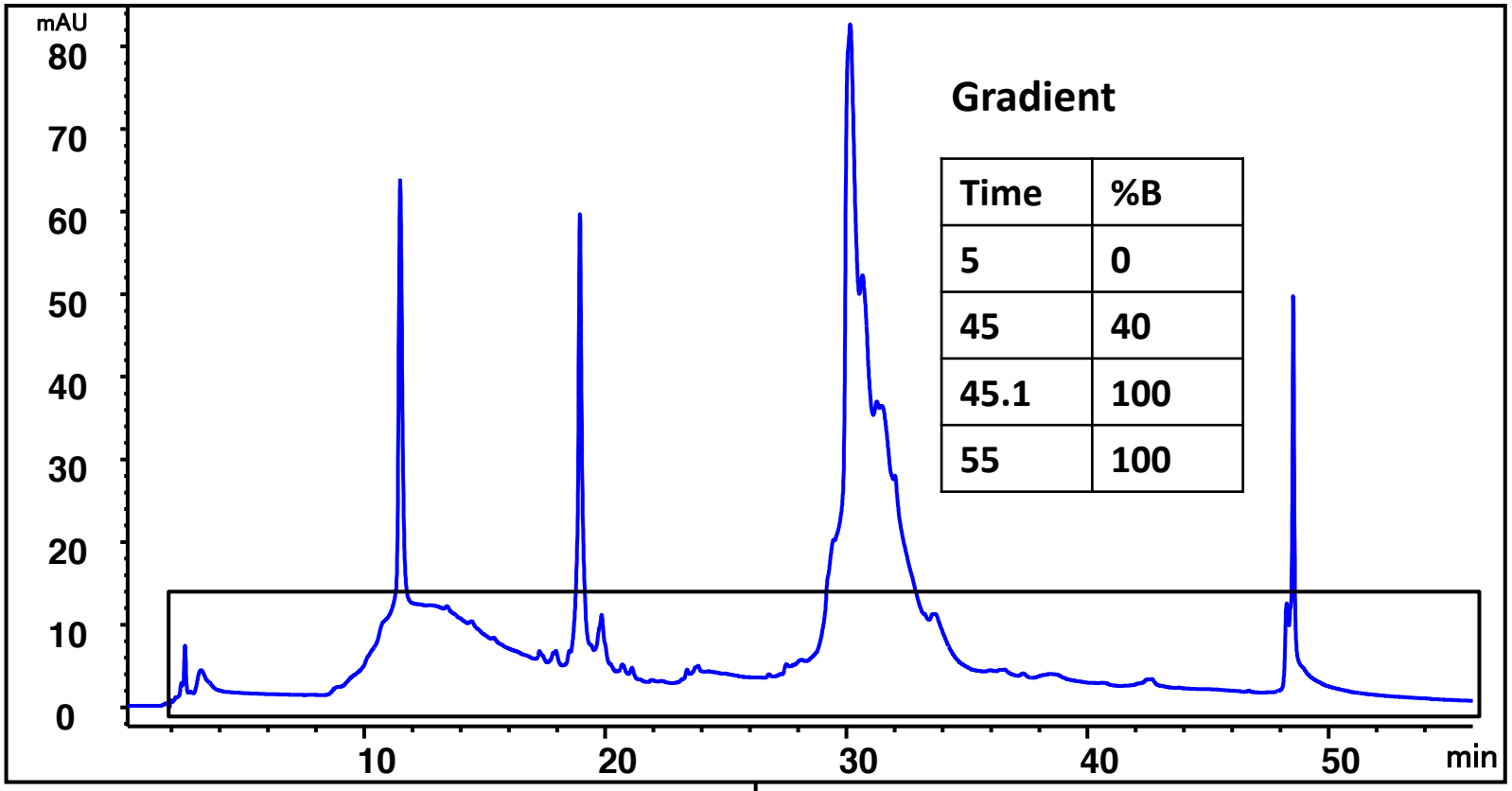
Zenix-C Zoom in Area



Human Serum on Proteomix SAX

Column: Proteomix SAX, 3 µm, 7.8 x 100 mm
Mobile phase: A: 20 mM Tris, pH 8.0, B: A+1M NaCl,
Flow rate: 0.8 mL/min, Detection: UV 280 nm,
Injection Volume: 5 µL Sigma human serum S7023 diluted 10 fold with buffer A



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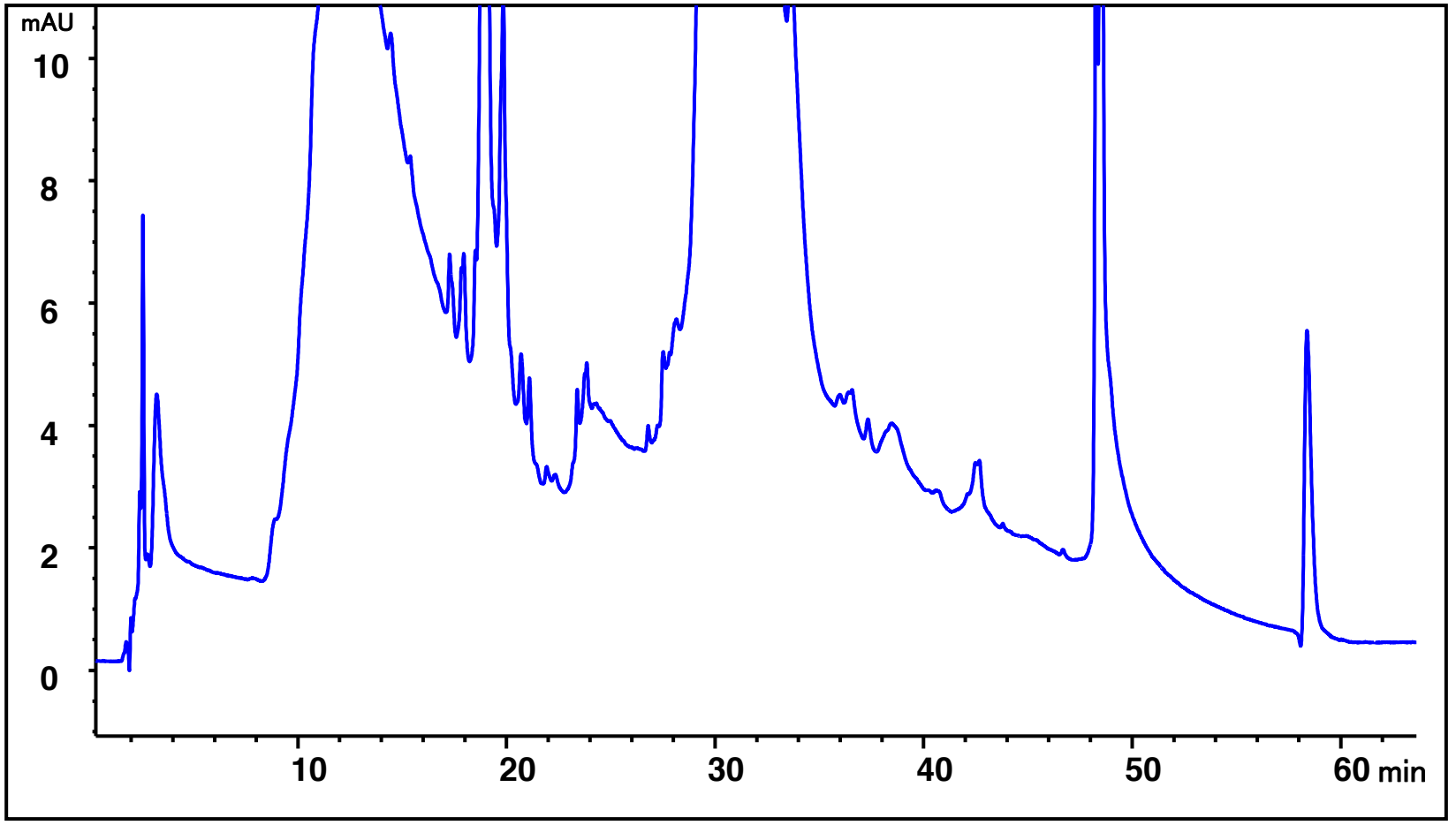
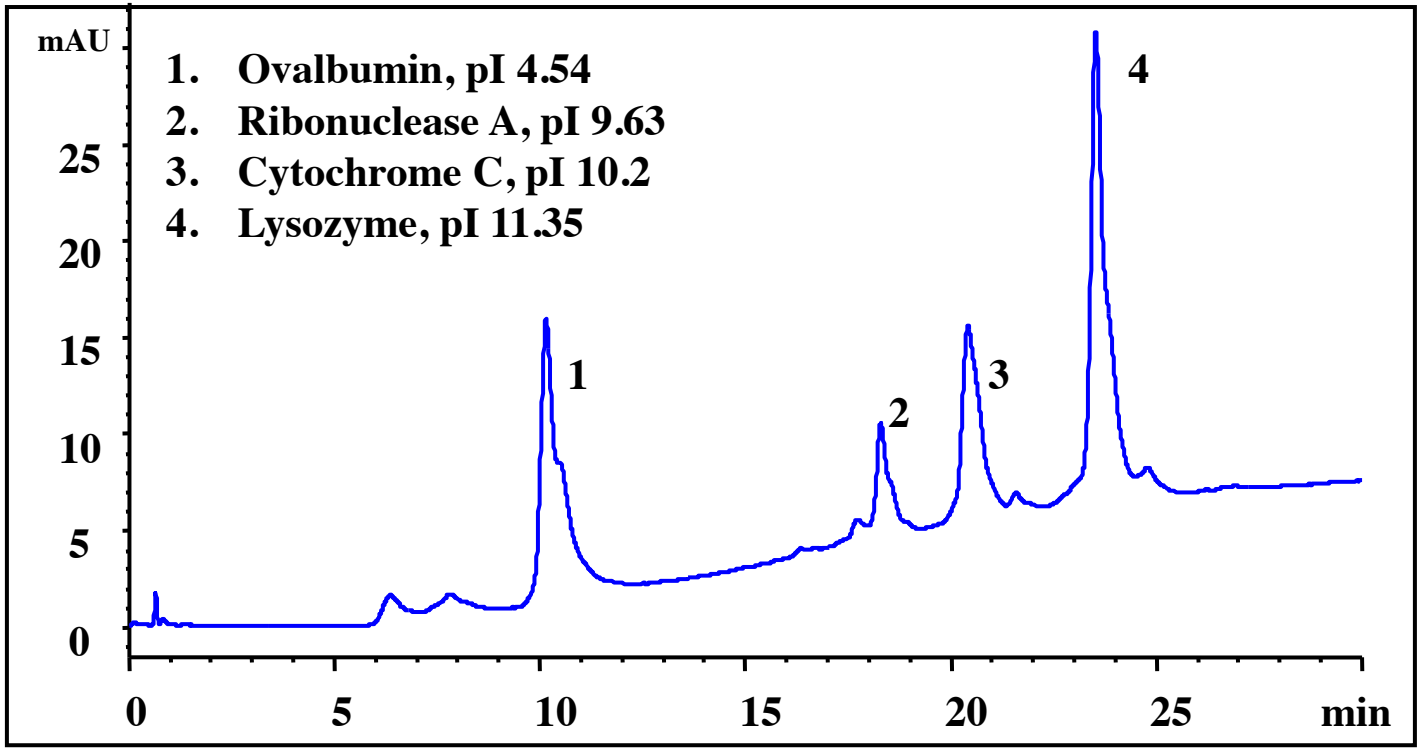
SAX Zoom in Area



Proteomix SCX NP3_2110

Column: Proteomix® SCX-NP3 (3 µm, 2.1 x 100 mm),
Mobile phase: A: 0.5% Formic acid, B: 1M ammonium formate in A
Flow rate: 0.3 mL/min, 163 bar;
Detector: UV 280 nm, Column temperature: 25 ℃ ,Samples: 2 µL Ovalbumin (4mg/mL), Ribonuclease A (1mg/mL), Cytochrome C (1mg/ml) and Lysozyme (1mg/mL)

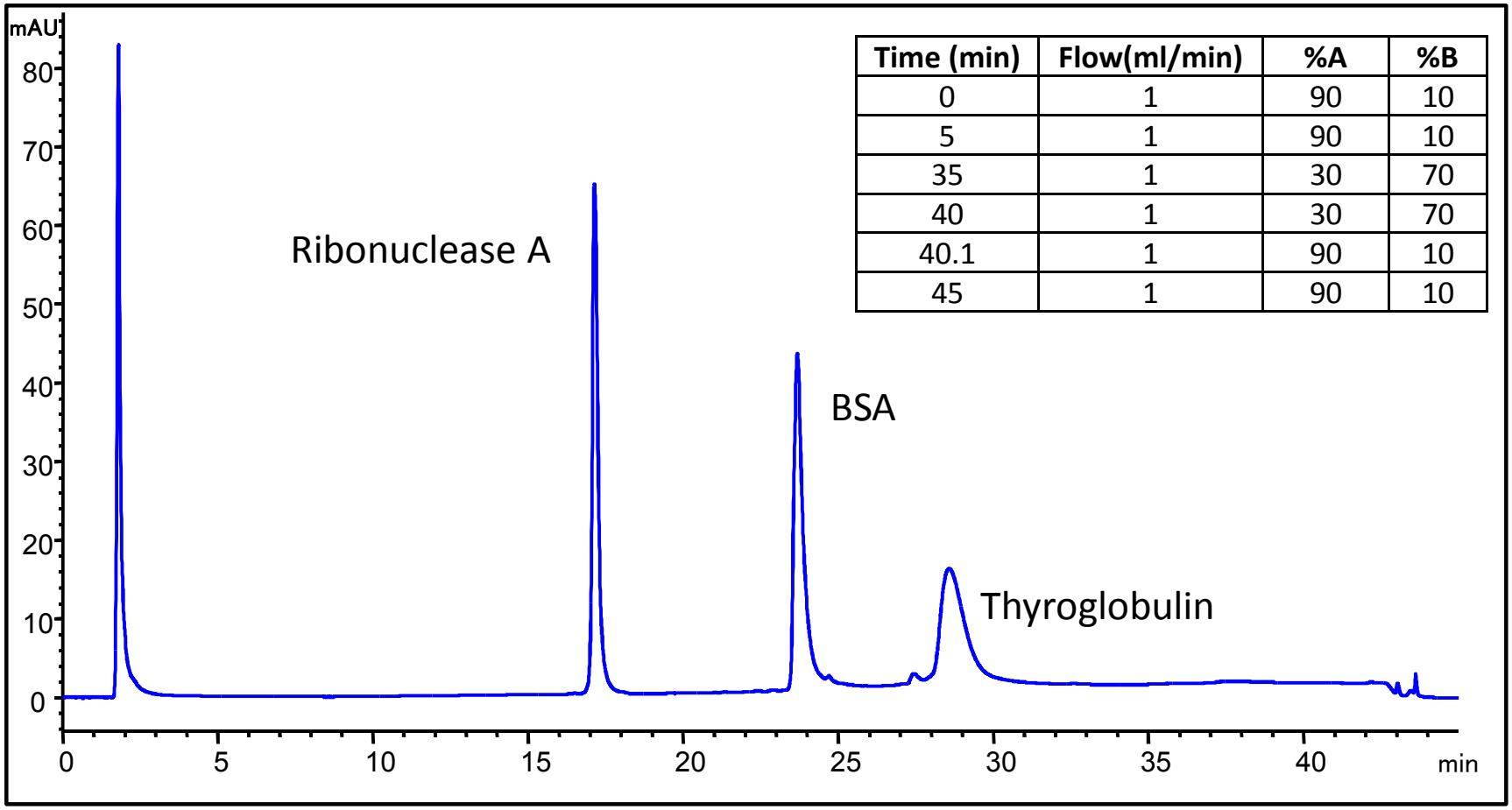
Time (min)	B%
0	20
2	20
26	100
30	100
30.1	20
60	20



QP1001

Protein Separation on PolyRP Reversed Phase Column

Column: PolyRP 1000Å, 5 µm, 4.6 x 150 mm
Mobile Phase A: 0.1% TFA in water, Mobile Phase B: 0.1% TFA in acetonitrile
Detector: UV 280 nm, Column temperature: 30 ℃ ,
Samples: 5 µL Protein mixture (Thyroglobulin 5.46 mg/ml, BSA 6.3 mg/ml, Ribonuclease A 5.9 mg/ml)



Time (min)	Flow(ml/min)	%A	%B
0	1	90	10
5	1	90	10
35	1	30	70
40	1	30	70
40.1	1	90	10
45	1	90	10

Preparative Size Exclusion Chromatography for Protein Separation

SRT-10 SEC-300 Prep Column

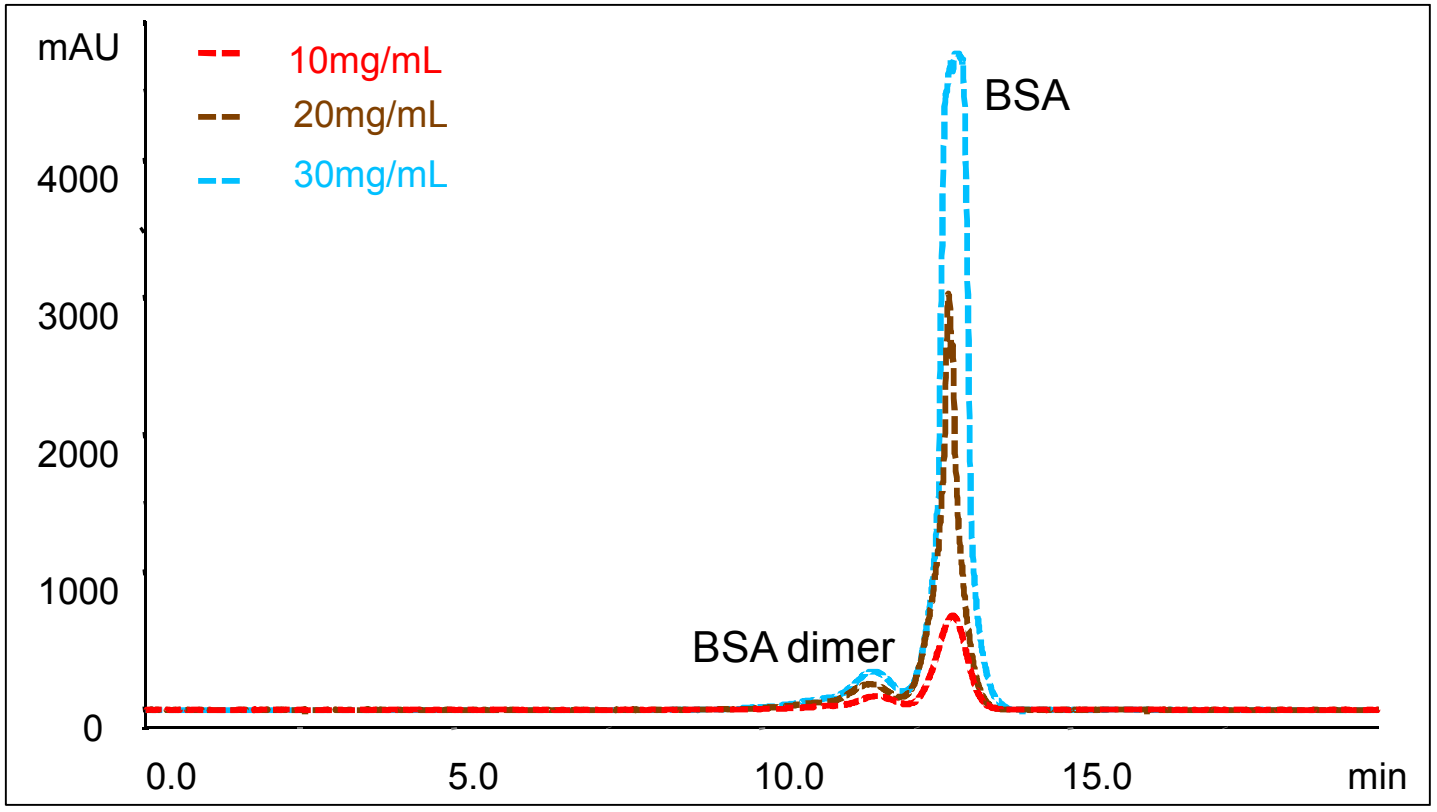
21.2 x 400 mm (141 mL)

SRT-10 SEC-300 Prep Column

- Column size 21.2 x 400 mm (21.2 x 100mm P/N 225300-21210 + 21.2 x 300 mm P/N 225300-21230)
- Columns are connected with PEEK column coupler (Sepax part # 102002-coupler)
- Instrument GE AKTA explorer
- Pressure: 17 bar at 7 mL/min, 25 bar at 10 mL/min, well below the pressure limit for the AKTA system 100 bar or 250 bar depending on the model.

SEC prep column- BSA loading study

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 ℃
Samples: 3 mL BSA (10, 20, or 30 mg/mL)



SEC prep column-BSA loading

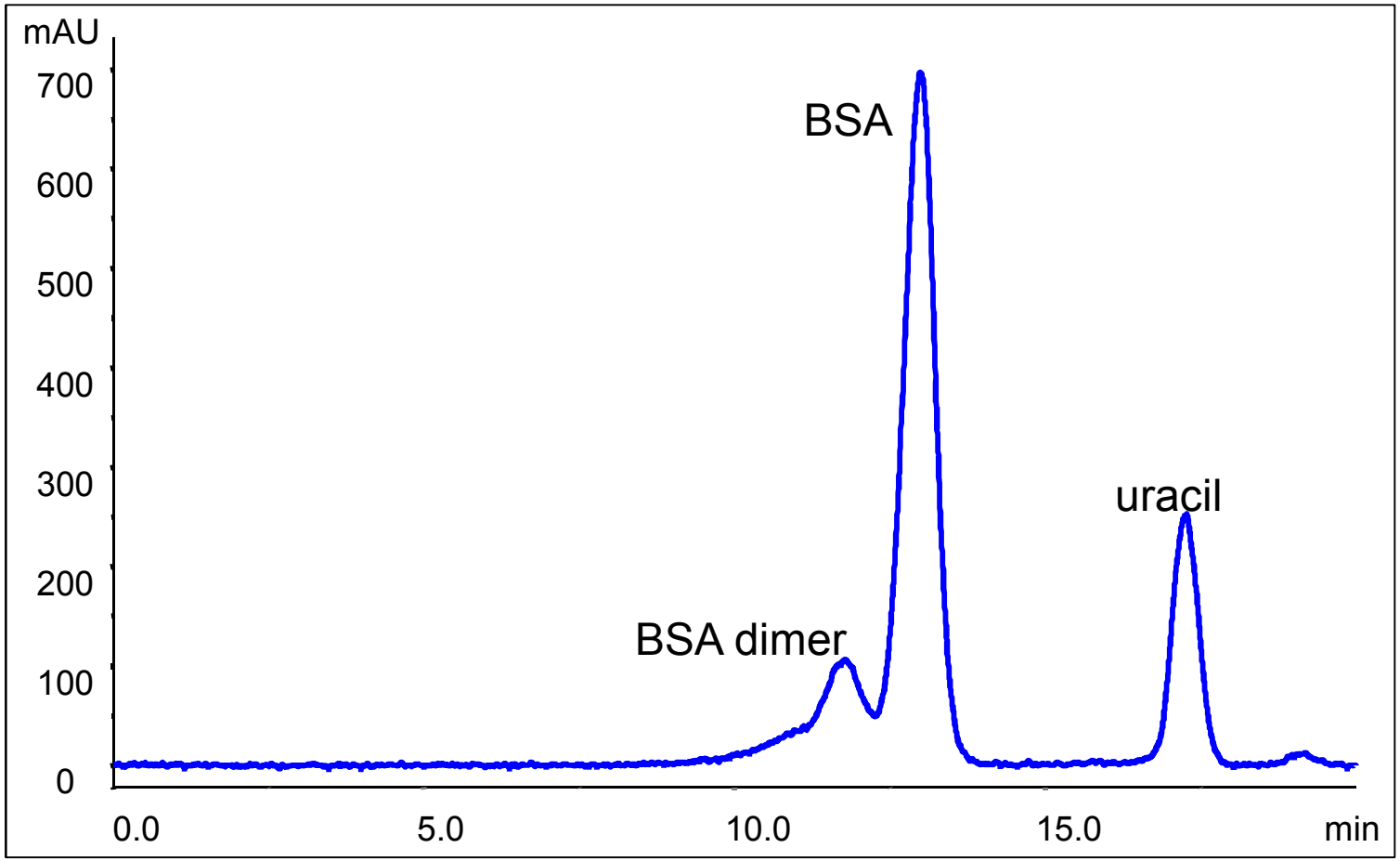
Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 °C;
Samples: 3 mL BSA (10, 20, or 30 mg/mL)

BSA30mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter (N / m	Asymmetry
BSA dimer	11.85	101.6082	102.906	0.8		3069	0.34
BSA	13.07	417.1044	690.324	0.55	1.06	7720	0.88
BSA60mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter (N / m	Asymmetry
BSA dimer	11.76	195.445	190.721	0.87		2535	0.34
BSA	13.02	1163.3833	3033.566	0.26	1.31	35524	0.86
BSA90mg							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
BSA dimer	11.82	284.065	280.617	0.86		2640	0.33
BSA	13.19	2629.0897	4781.054	0.47	1.22	11005	0.64

SEC prep column –BSA/Uracil-7 mL/min

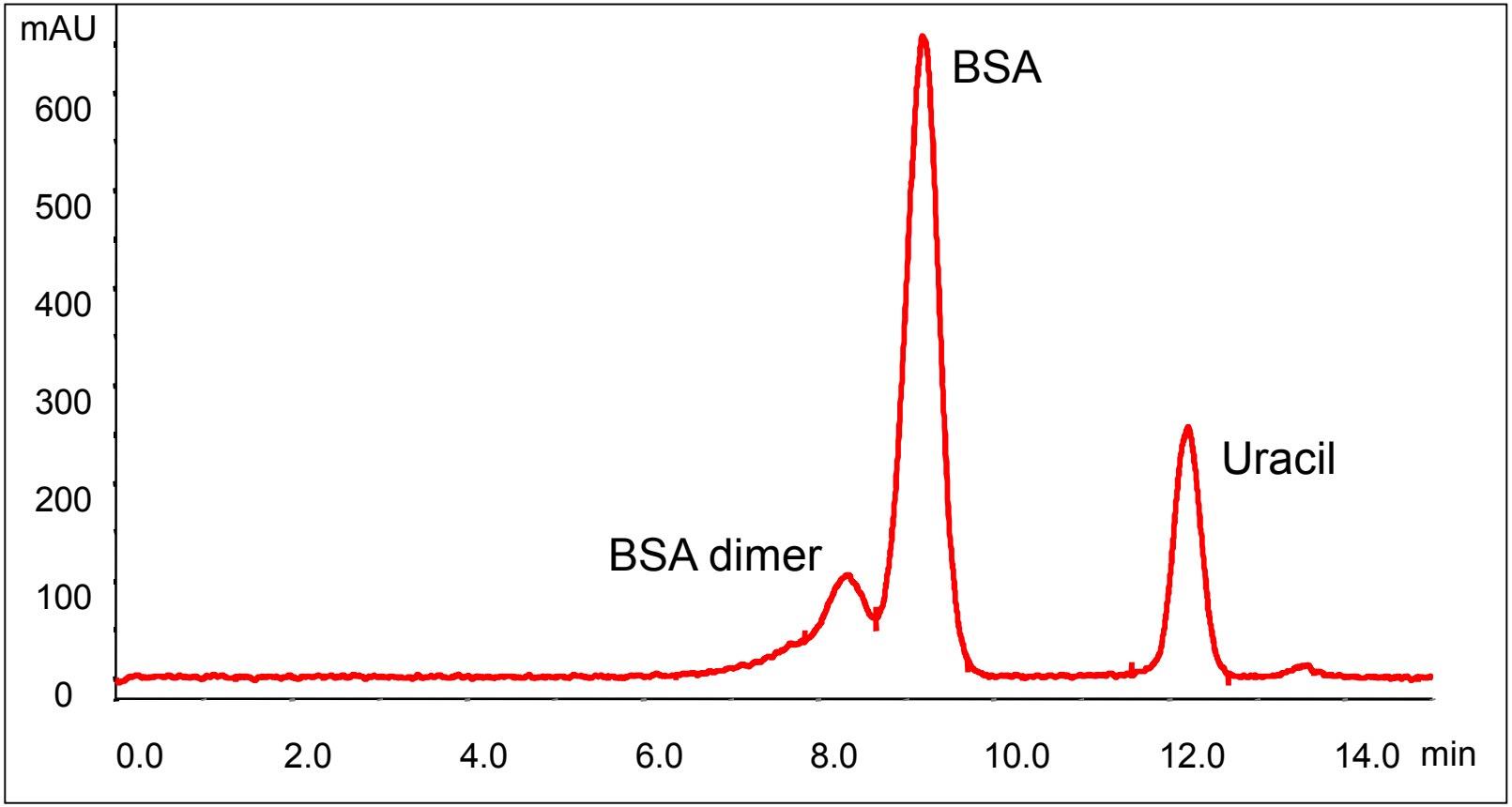
SP1019

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: **7 mL/min**, 17 bar; Detector: UV 280 nm; Column temperature: 23 ℃
Samples: 3 mL BSA(10 mg/mL), Uracil (0.17 mg/mL)



SEC prep column –BSA/Uracil-10 mL/min

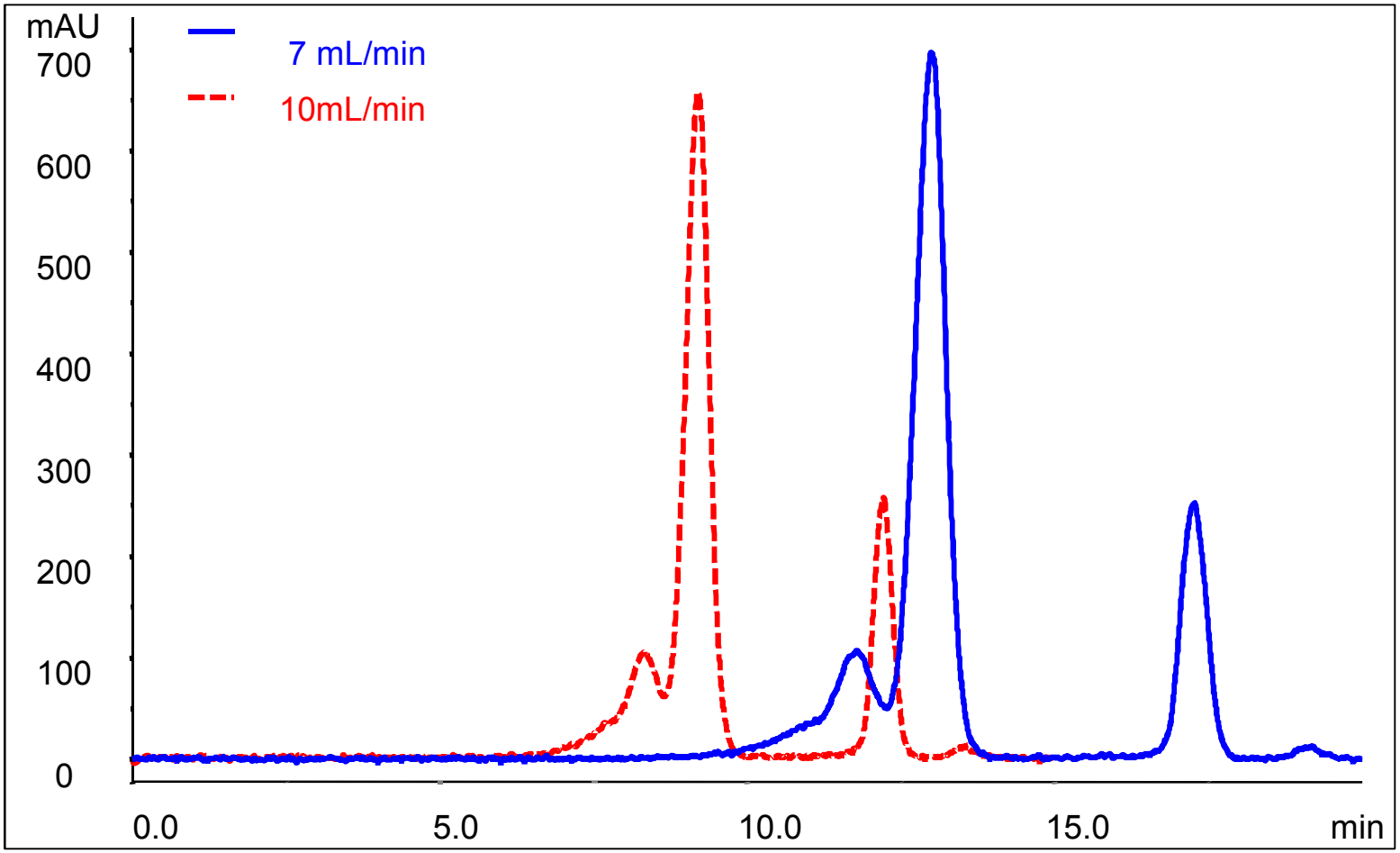
Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: **10 mL/min**, 25 bar; Detector: UV 280 nm; Column temperature: 23 ℃
Samples: 3 mL BSA (10 mg/mL), Uracil (0.17 mg/mL)



BSA/uracil- overlay 7 and 10 mL/min

SP1019

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 or 10 mL/min; Detector: UV 280 nm, Column temperature: 23 ℃ ,
Samples: 3 mL BSA(10mg/mL), Uracil (0.17mg/mL)



Higher flow rate shortens the separation time while maintaining the resolution.

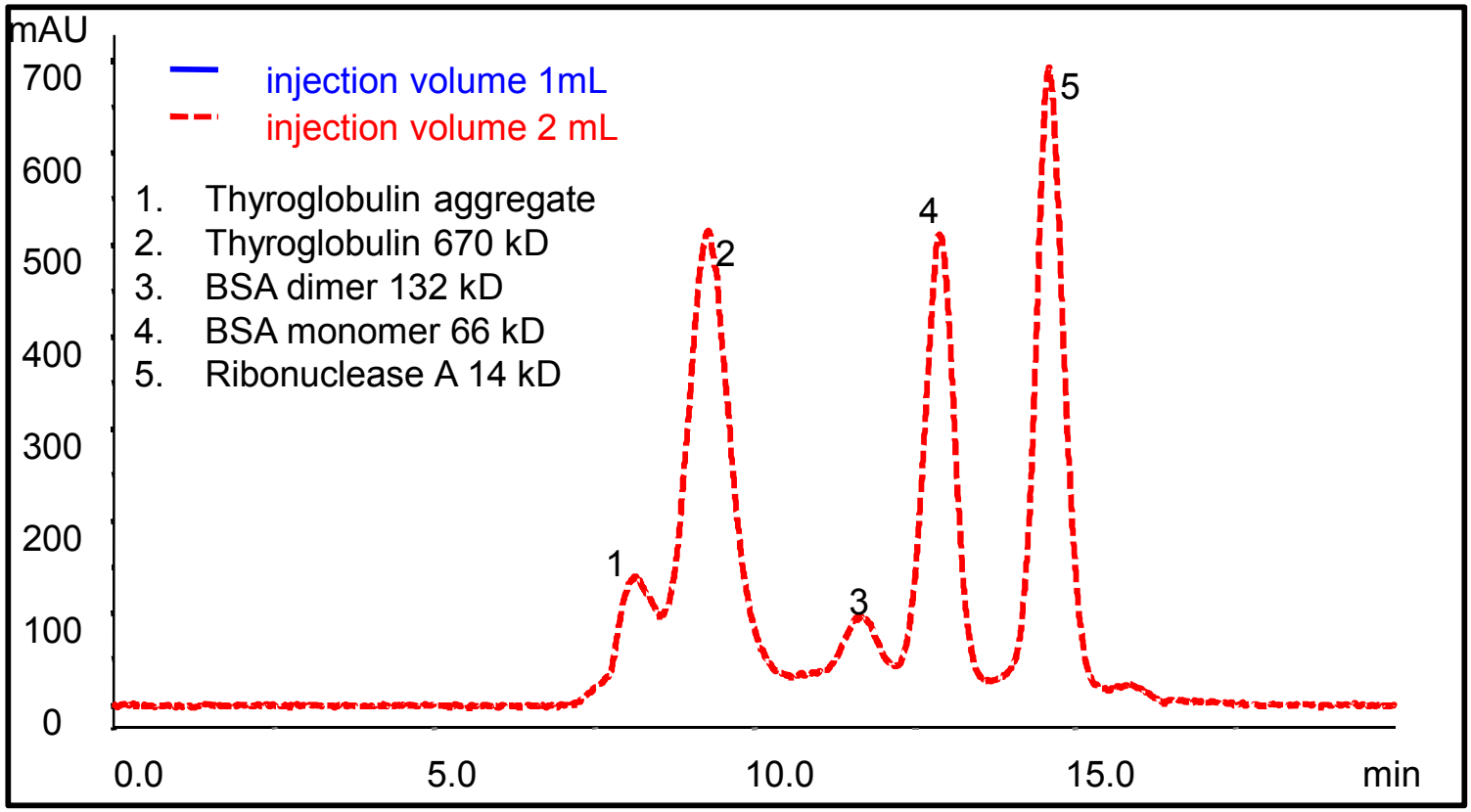
SEC prep column-different flow rate

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 or 10 mL/min; Detector: UV 280 nm, Column temperature: 23 ℃
Samples: 3 mL BSA(10mg/mL), Uracil (0.17mg/mL)

flow rate 7mL/min							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
BSA dimer	11.78	114.8196	107.195	0.87		2562	0.3
BSA	12.98	431.4186	697.461	0.57	0.99	7240	0.85
Uracil	17.28	129.0461	253.549	0.48	4.83	18101	0.96
flow rate 10mL/min							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
BSA dimer	8.33	85.0802	106.557	0.66		2186	0.25
BSA	9.19	315.8112	658.445	0.44	0.91	6058	0.8
Uracil	12.21	99.1489	257.95	0.35	4.5	16609	0.98

SEC prep – QC standard mixture

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 ℃
Samples: 1 or 2 mL Thyroglobulin, BSA, Ribonuclease A (10mg/mL each)



QC standard separation parameters

Column: SRT-10 SEC-300 (10 µm, 300Å, 21.2 x 400 mm),
Mobile phase: phosphate buffer, 150mM, pH7.0
Flow rate: 7 mL/min, 17 bar; Detector: UV 280 nm; Column temperature: 23 ℃
Samples: 1 or 2 mL Thyroglobulinm, BSA, Ribonuclease A (10mg/mL each)

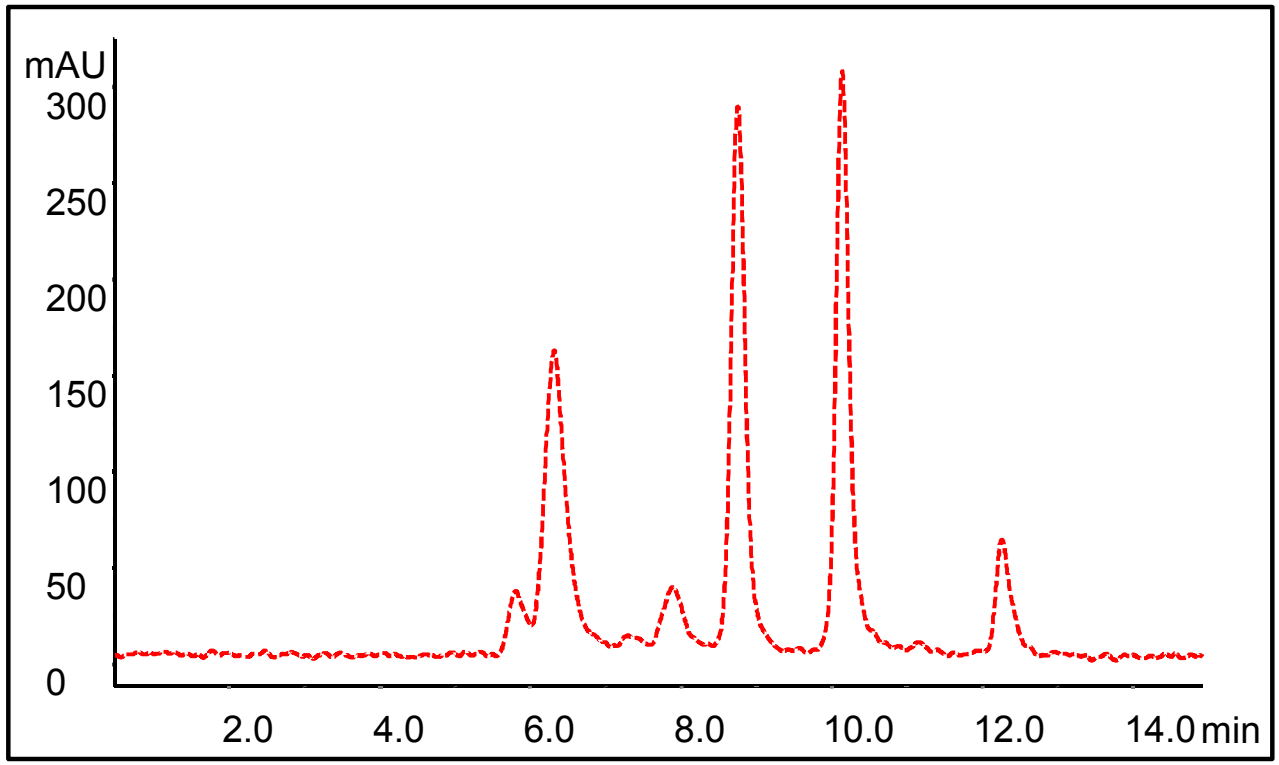
Injection volume 1mL							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
Thyroglobulin aggr	8.19	42.7311	64.826	0.72		1793	1.4
Thyroglobulin	9.33	196.6968	246.72	0.72	0.93	2352	1.46
BSA dimer	11.8	37.4138	46.508	0.76	1.98	3380	0.67
BSA	12.98	137.3646	248.467	0.51	1.1	9092	0.92
Ribonuclease A	14.71	176.2706	334.151	0.46	2.1	13873	1.13

Injection volume 2mL							
Peak	RT(min)	Area (mAU*min)	Height (mAU)	W1/2 (min)	Rs	Plates/meter(N / m)	Asymmetry
Thyroglobulin aggr	8.13	99.9927	140.34	0.74		1689	0.65
Thyroglobulin	9.28	430.9852	516.172	0.75	0.91	2108	1.26
BSA dimer	11.66	78.8725	96.316	0.84	1.77	2690	0.77
BSA	12.89	303.9722	511.408	0.53	1.05	8078	0.89
Ribonuclease A	14.59	377.8125	693.522	0.48	1.97	12584	1.14

Zenix™ SEC-300 on AKTA/FPLC

ZP1019

Column: Zenix™ SEC-300 (3 µm, 300Å, 7.8 x 300 mm),
Mobile phase: 150mM sodium phosphate buffer, pH7.0
Flow rate: 1 mL/min, 57 bar, Detector: UV 214nm,
Column temperature: 23 ℃ ,Samples: 20 µL Thyroglobulin, BSA, Ribonuclease A and Uracil (1mg/mL each)

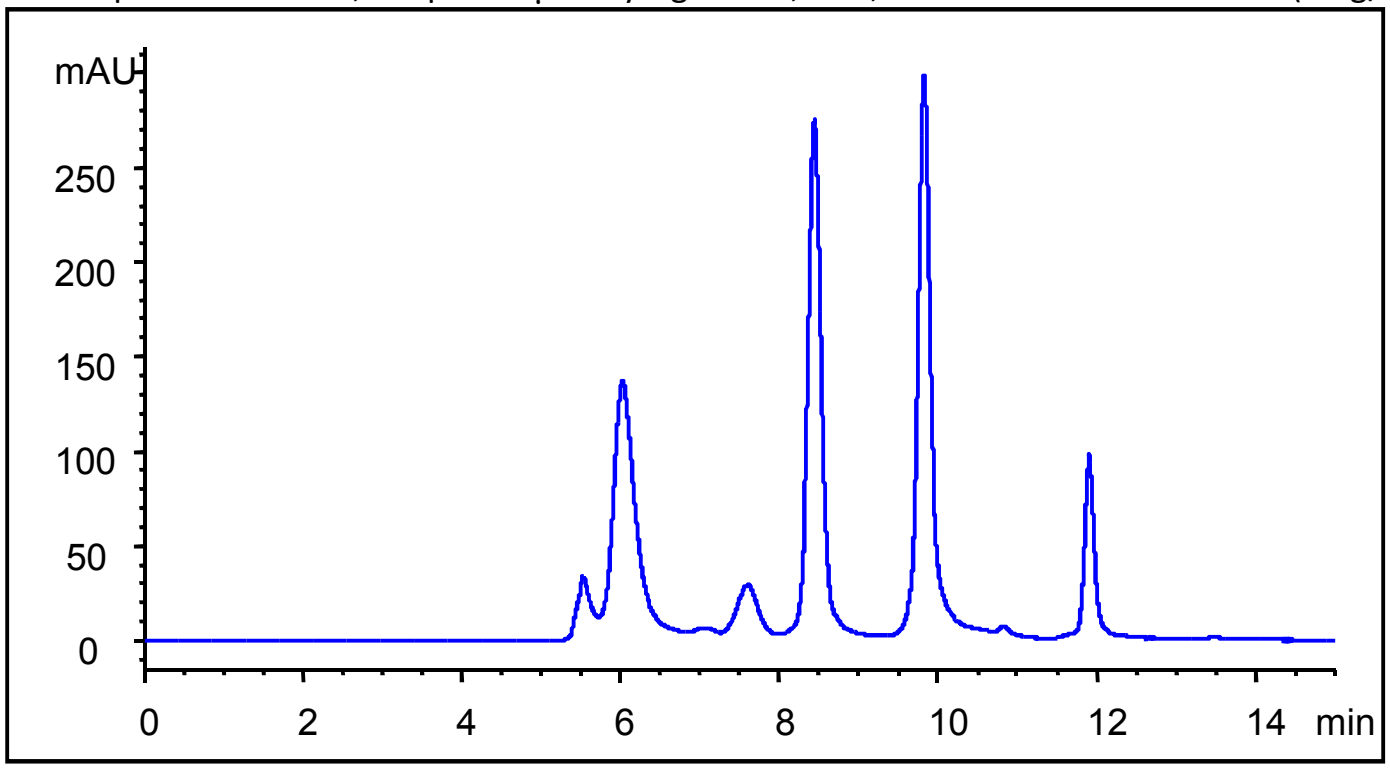


Peak	RT(min)	Area (mAU*min)	Height (mAU)	Resolution	Plates
Thyro. Agg.	5.8	7.53	30.61		8792
Thyroglobulin	6.32	52.72	154.89	1.06	8146
BSA dimer	7.89	9.83	31	3.07	12536
BSA	8.76	64.77	282.47	2.01	34312
Ribonuclease A	10.14	67.25	301.87	4.19	54732
Uracil	12.26	15.22	60.5	6.24	60542

Zenix™ SEC-300 on HPLC

ZP1019

Column: Zenix™ SEC-300 (3 µm, 300Å, 7.8 x 300 mm),
Mobile phase: 150mM sodium phosphate buffer, pH7.0
Flow rate: 1 mL/min, 88bar, Detector: UV 214nm,
Column temperature: 23 ℃ ,Samples: 5 µL Thyroglobulin, BSA, Ribonuclease A and Uracil (1mg/mL each)



Compound Name	RT [min]	Height	Area	Plates	Tailing	Resolution
Thyroglobulin aggr	5.53	32	369	5000	1.12	
Thyroglobulin	6.03	133	2469	2583	1.22	1.26
BSA dimer	7.61	25	409	4317	1.02	3.36
BSA	8.45	269	3047	13278	1.09	2.22
Riobonuclease A	9.82	291	3130	22256	1.20	4.95
Uracil	11.91	95	800	47688	1.13	8.64