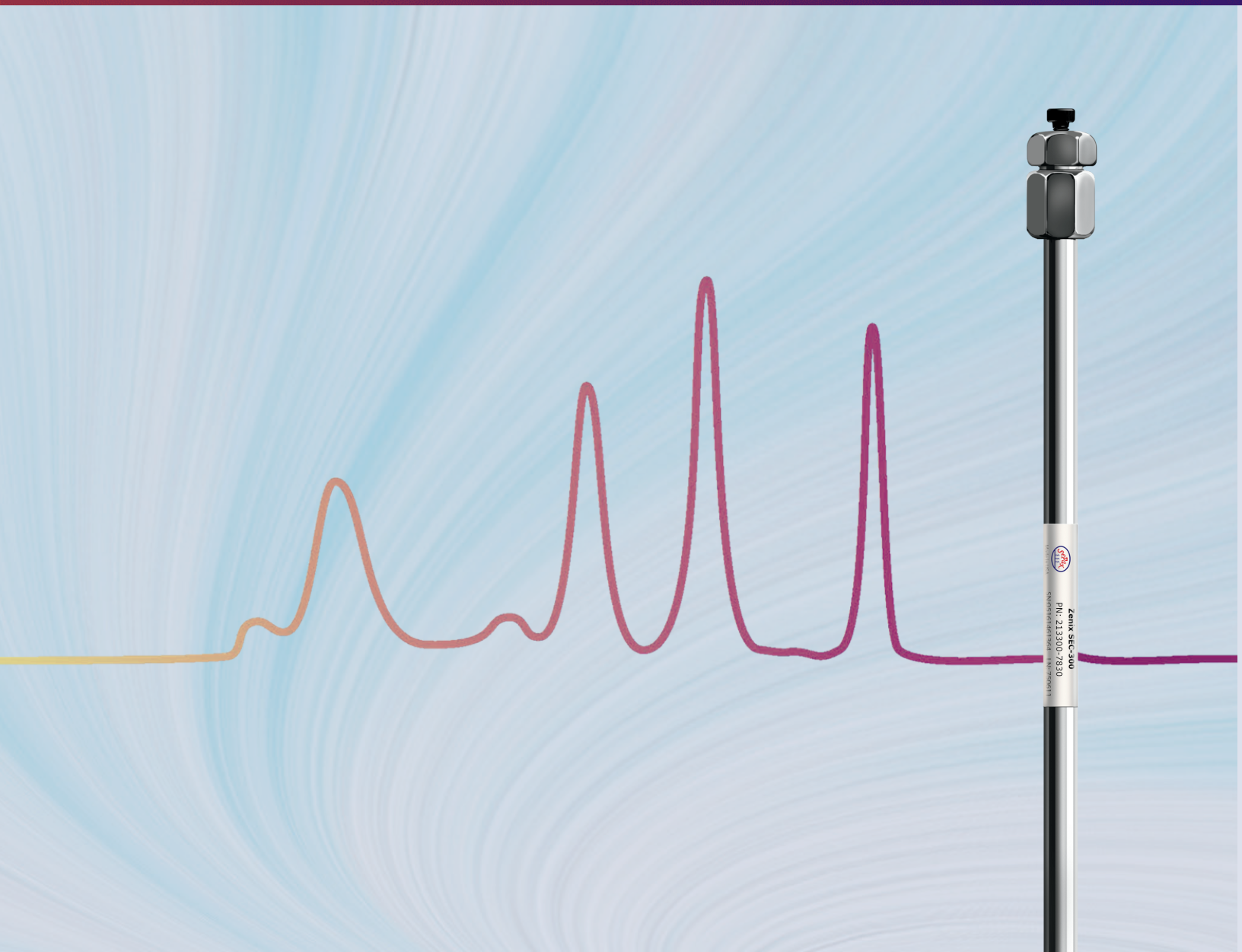
Sepax Technologies

SEPAX ZENIX® COLUMNS

Size Exclusion Chromatography

High Resolution SEC Separation for HPLC and UHPLC



Sepax Technologies, Inc.

Better Surface Chemistry for Better Separation

Sepax Technologies, Inc. is a Delaware, USA based leading chromatography product manufacturer and service provider specializing in biological separation areas. We offer unique ranges of HPLC columns for bioanalytical characterization and testing, as well as process media for downstream process and purification of MAb, BsMAb, ADC, Proteins, AAV, VLP, DNA, RNA, and various biologic samples. Sepax has a full portfolio of bead technology platforms, linker and organic synthesis chemistry, as well as protein chemistry in different stages of R&D and production pipelines to support the various needs of the biopharmaceutical industry and our customers. Certified to ISO 9001:2015 standards, Sepax focuses on customer & market needs, and is continuing to expand its presence and supply chain around the globe.

Leader in Biological Separations

Sepax develops and manufactures a wide range of biological separation products using both silica and polymeric resins as the support. The selection of particle size is from 1 µm to 100 µm and pore size is from non-porous to 2000 Å. Unique and proprietary resin synthesis and surface technologies have been developed for solving separation challenges in the area of biologics.

Bioseparation Products

Size Exclusion
SRT, SRT -C
Nanofilm
Zenix, Zenix-C
Biomix

Ion-exchange
Proteomix
Antibodix
Glycomix

Hydrophobic Interaction
Proteomix HIC

Reversed Phase
Proteomix RP
Bio-C4
Bio-C8
Bio-C18

Affinity
ProAqa Excel Protein A
Monomix dT20

Analytical, Semi-prep and Preparative

Column Dimension Availability

Available Zenix SEC column dimensions are 2.1, 4.6, 7.8, 10, 21.2 and 30 mm I.D., and 50, 100, 150, 250, and 300 mm length. Sepax also offers custom-made columns. Both stainless steel and PEEK tubing are available.

Part Number	Phase	Particle Size, Pore Size, ID×Length
213080-4605	Zenix SEC-80	3 µm, 80 Å, 4.6 × 50 mm
213080-4615	Zenix SEC-80	3 µm, 80 Å, 4.6 × 150 mm
213080-4630	Zenix SEC-80	3 µm, 80 Å, 4.6 × 300 mm
213080-7805	Zenix SEC-80	3 µm, 80 Å, 7.8 × 50 mm
213080-7815	Zenix SEC-80	3 µm, 80 Å, 7.8 × 150 mm
213080-7820	Zenix SEC-80	3 µm, 80 Å, 7.8 × 200 mm
213080-7830	Zenix SEC-80	3 µm, 80 Å, 7.8 × 300 mm
213100-4605	Zenix SEC-100	3 µm, 100 Å, 4.6 × 50 mm
213100P-4605	Zenix SEC-100	3 µm, 100 Å, 4.6 × 50 mm
213100-4615	Zenix SEC-100	3 µm, 100 Å, 4.6 × 150 mm
213100-4630	Zenix SEC-100	3 µm, 100 Å, 4.6 × 300 mm
213100P-4630	Zenix SEC-100	3 µm, 100 Å, 4.6 × 300 mm
213100-7805	Zenix SEC-100	3 µm, 100 Å, 7.8 × 50 mm
213100-7815	Zenix SEC-100	3 µm, 100 Å, 7.8 × 150 mm
213100-7820	Zenix SEC-100	3 µm, 100 Å, 7.8 × 200 mm
213100-7830	Zenix SEC-100	3 µm, 100 Å, 7.8 × 300 mm
213100-10005	Zenix SEC-100	3 µm, 100 Å, 10.0 × 50 mm
213100-10015	Zenix SEC-100	3 µm, 100 Å, 10.0 × 150 mm
213100-10030	Zenix SEC-100	3 µm, 100 Å, 10.0 × 300 mm
213100-21205	Zenix SEC-100	3 µm, 100 Å, 21.2 × 50 mm
213100-21230	ZenixSEC-100	3 µm, 100 Å, 21.2 × 300 mm
213150-4605	Zenix SEC-150	3 µm, 150 Å, 4.6 × 50 mm
213150P-4605	Zenix SEC-150	3 µm, 150 Å, 4.6 × 50 mm
213150-4615	Zenix SEC-150	3 µm, 150 Å, 4.6 × 150 mm
213150-4630	Zenix SEC-150	3 µm, 150 Å, 4.6 × 300 mm
213150P-4630	Zenix SEC-150	3 µm, 150 Å, 4.6 × 300 mm
213150-7805	Zenix SEC-150	3 µm, 150 Å, 7.8 × 50 mm
213150-7815	Zenix SEC-150	3 µm, 150 Å, 7.8 × 150 mm
213150-7820	Zenix SEC-150	3 µm, 150 Å, 7.8 × 200 mm
213150-7830	Zenix SEC-150	3 µm, 150 Å, 7.8 × 300 mm
213150-10005	Zenix SEC-150	3 µm, 150 Å, 10.0 × 50 mm
213150-10015	Zenix SEC-150	3 µm, 150 Å, 10.0 × 150 mm
213150-10030	Zenix SEC-150	3 µm, 150 Å, 10.0 × 300 mm
213150-21205	Zenix SEC-150	3 µm, 150 Å, 21.2 × 50 mm
213150-21230	Zenix SEC-150	3 µm, 150 Å, 21.2 × 300 mm
213300-4605	Zenix SEC-300	3 µm, 300 Å, 4.6 × 50 mm
213300P-4605	Zenix SEC-300	3 µm, 300 Å, 4.6 × 50 mm
213300-4615	Zenix SEC-300	3 µm, 300 Å, 4.6 × 150 mm
213300-4625	Zenix SEC-300	3 µm, 300 Å, 4.6×250mm
213300-4630	Zenix SEC-300	3 µm, 300 Å, 4.6 × 300 mm
213300P-4630	Zenix SEC-300	3 µm, 300 Å, 4.6 × 300 mm
213300-7805	Zenix SEC-300	3 µm, 300 Å, 7.8 × 50 mm
213300-7815	Zenix SEC-300	3 µm, 300 Å, 7.8 × 150 mm
213300-7820	Zenix SEC-300	3 µm, 300 Å, 7.8 × 200 mm
213300-7830	Zenix SEC-300	3 µm, 300 Å, 7.8 × 300 mm
213300-10005	Zenix SEC-300	3 µm, 300 Å, 10.0 × 50 mm
213300-10015	Zenix SEC-300	3 µm, 300 Å, 10.0 × 150 mm
213300-10030	Zenix SEC-300	3 µm, 300 Å, 10.0 × 300 mm
213300-21205	Zenix SEC-300	3 µm, 300 Å, 21.2 × 50 mm
213300-21230	Zenix SEC-300	3 µm, 300 Å, 21.2 × 300 mm

Zenix® SEC Phases

Highest Efficiency and Resolution Size Exclusion Separation

General Description

Utilizing proprietary surface technologies, Zenix SEC phases are made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded on high purity and mechanically stabilized silica with a particle size of 3 µm. The Zenix SEC 3 µm particle size, combined with large pore volumes, allows for the highest separation efficiency and resolution. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. Zenix SEC columns provide high stability and negligible non-specific interactions through unique bonding chemistry, coupled with maximized bonding density. The available pore sizes of Zenix packings are 80, 100, 150, and 300 Å. Typical applications for Zenix SEC columns include separation and analysis of biological molecules and water soluble polymers in aqueous buffers.

Featured Characteristics

- Particle size: 3 µm
- Selection of pore size: 80, 100, 150, and 300 Å
- Highest separation efficiency and resolution
- High capacity
- High stability over low and high concentration salt
- Lot-to-lot reproducibility
- High protein recovery with intact biological activity
- Negligible non-specific interactions
- Ideal for separation and analysis of biological molecules: proteins, nucleic acids, oligonucleotides, peptides, and viruses
- Ideal for separation and analysis of natural and synthetic polymers, polysaccharides, and nanomaterials (nanoparticles)

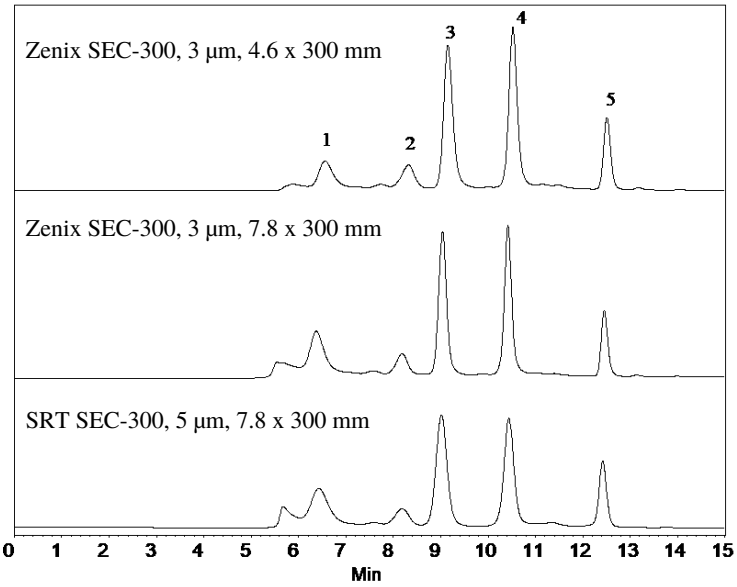
High Separation Efficiency

The advantages of developing smaller particle sizes are higher efficiency and higher resolution. When particle size is decreased to 3 µm from 5 µm, the column efficiency is almost doubled. As shown in Fig. 2, the plate numbers of BSA dimmer, BSA, and ribonuclease A increased from 2720 to 4600, 6590 to 13090, and 11160 to 22000 when the particle size decreased from 5 µm to 3 µm. Figure 3 further shows that high efficiency has been achieved by 3 µm Zenix columns with various pore sizes.

Figure 1. Pore Size vs. MW Exclusion Limit

Phase (3 µm)	Pore Size	Protein MW Exclusion Limit
Zenix SEC-80	80 Å	50,000
Zenix SEC-100	100 Å	100,000
Zenix SEC-150	150 Å	150,000
Zenix SEC-300	300 Å	1,250,000

Figure 2. Separation of Protein Mixture A by Zenix SEC-300 and SRT SEC-300

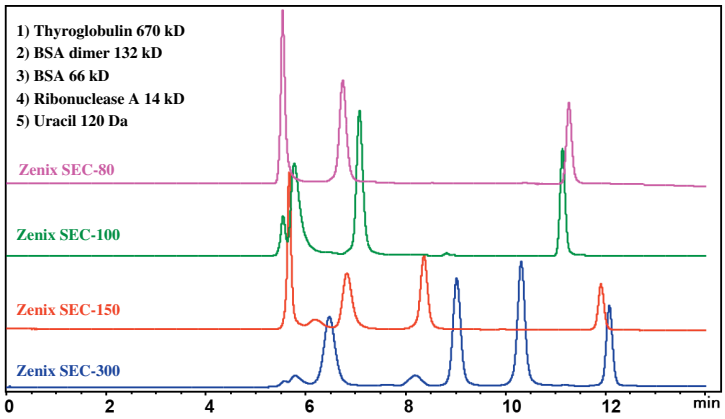


Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1.0 mL/min for 7.8 x 300 mm; 0.35 mL/min for 4.6 x 300 mm; Temperature: RT; Detection: UV 214 nm; Injection Volume: 10 µL (3 uL for 4.6 x 300 mm); Sample: 1. Thyroglobulin (1.0 mg/mL), 670 kD; 2. BSA dimer, 132 kD; 3. BSA (1.0 mg/mL), 66 kD; 4. Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5. Uracil (2.5 g/mL), 120 D

Efficiency of Zenix SEC-300 and SRT SEC-300

Peak	Protein	Zenix 300 (4.6x300)	Zenix 300 (7.8x300)	SRT 300 (7.8x300)
1	Thyroglobulin	2180	1730	1120
2	BSA Dimer	4390	4600	2720
3	BSA	10280	13090	6590
4	Ribonuclease A	16490	22000	11160
5	Uracil	33640	38500	27860

Figure 3. Protein Standard Separation on Zenix SEC-80 - 300 Å

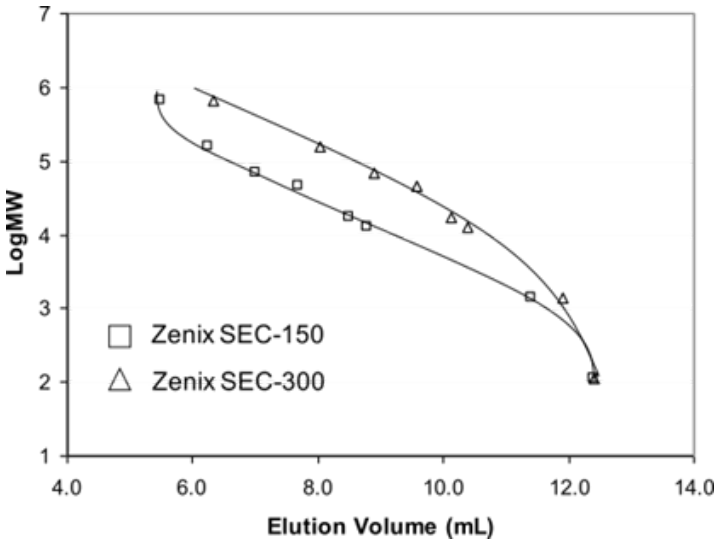


Columns: Zenix SEC, 3 µm, 80, 100, 150, 300 Å, 7.8 x 300 mm each; Mobile Phase: 150 mM phosphate buffer, pH 7.0; Flow Rate: 1 mL/min; Detection: UV 214 nm; Temperature: RT; Injection Volume: 5 µL

MW Calibration for Protein Separation

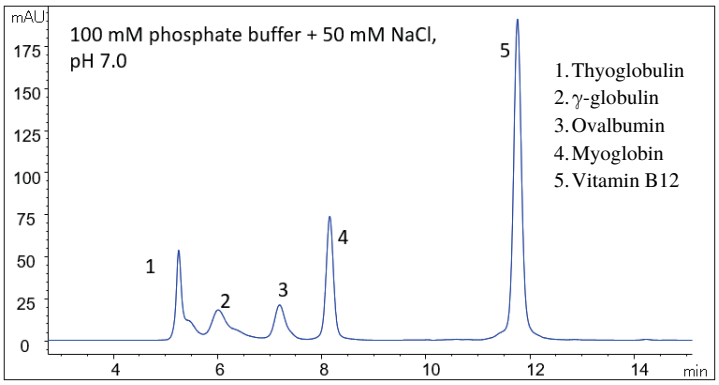
For size exclusion chromatography, individual pore size of packings determines the range of molecular weight for separation, while the pore volume controls the separation capacity and resolution. Figure 4 shows the protein calibration curves and Fig. 5 and Fig. 6 presents the separation profile of Bio-Rad Protein Standards on Zenix SEC-150 and 300 columns.

Figure 4. Molecular Weight Calibration on Zenix SEC Column



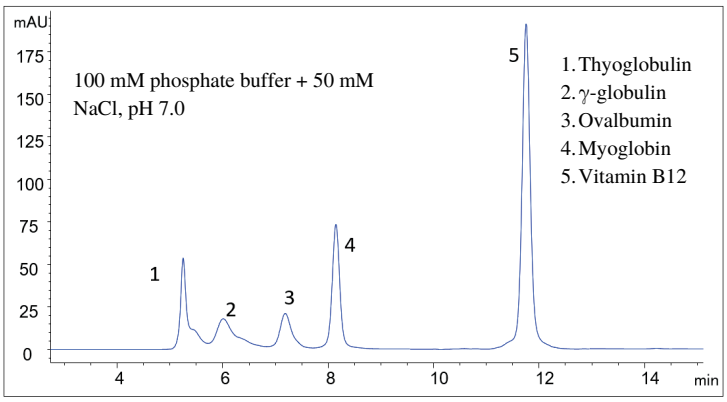
Columns: Zenix SEC-150, 3 µm, 150 Å, 7.8 x 300 mm and Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate; pH 7.0; Flow Rate: 1 mL/min; Detection: UV 214 nm; Injection Volume: 10 µL; Samples: 1. Thyroglobulin, 670 kD; 2. Globulin 158 kD; 3. BSA 66 kD; 4. Ovalbumin 44 kD; 5. Myoglobin 17.6 kD, 6. Ribonuclease 13.7 kD; 7. Vitamin B12 1.35 kD; 8. Uracil 120 Da

Figure 5. Zenix SEC-150 with Bio-Rad Protein Standard



Column: Zenix SEC-150, 3 µm, 150 Å, 7.8 x 300 mm; Flow Rate: 1 mL/min; Detection: UV 280 nm; Injection Volume: 10 µL

Figure 6. Zenix SEC-300 with Bio-Rad Protein Standard



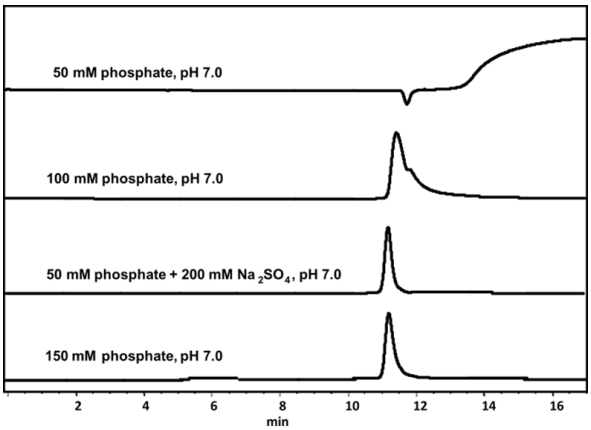
Column: Zenix SEC-300, 3 µm, 150 Å, 7.8 x 300 mm; Flow Rate: 1 mL/min; Detection: UV 280 nm; Injection Volume: 10 µL

Mobile Phase Compatibility

Zenix SEC phases are compatible with most aqueous buffers, such as ammonium acetate, phosphate, trizma and so on. Zenix SEC phases can tolerate a high concentration of salts, such as 2.0 M. Furthermore, Zenix SEC columns are stable in both organic solvents, such as Acetonitrile (ACN), ethanol (EtOH), and isopropyl alcohol (IPA), as well as the mixture of water and organic solvents.

Mobile Phase Optimization

Figure 7. Analysis of Lysozyme on Zenix SEC-300



Column: Zenix SEC-300, 3 µm, 300 Å, 4.6 x 300 mm; Flow Rate: 0.35 mL/min; Detection: UV 214 nm; Injection Volume: 5 µL; Sample: Lysozyme (1 mg/mL)

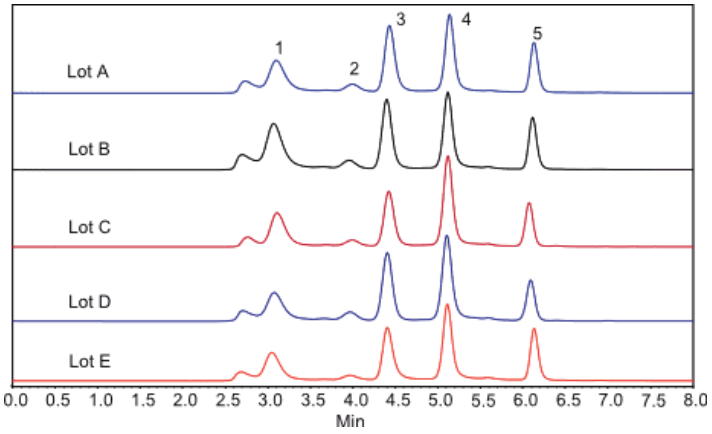
High Stability

The proprietary stationary phases of Zenix SEC packings utilize densely bonded chemistry on the silica surface, which greatly hinders the diffusion of the molecules that would attack the bonds of the silica-stationary phase layer, thus enabling high stability over a wide range of pH from 2 to 8.5.

Lot-to-Lot Reproducibility

The controlled surface chemistry used to synthesize Zenix SEC phases makes the surface coating highly reproducible, leading to consistent column manufacturing.

Figure 8. Lot-to-Lot Reproducibility of Protein Mixture on Zenix SEC-300



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 150 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detection: UV 214 nm; Temperature: RT; Injection Volume: 10 µL; Samples: 1.Thyroglobulin, 2. BSA dimer, 3. BSA monomer, 4. Ribonuclease A, 5. Uracil

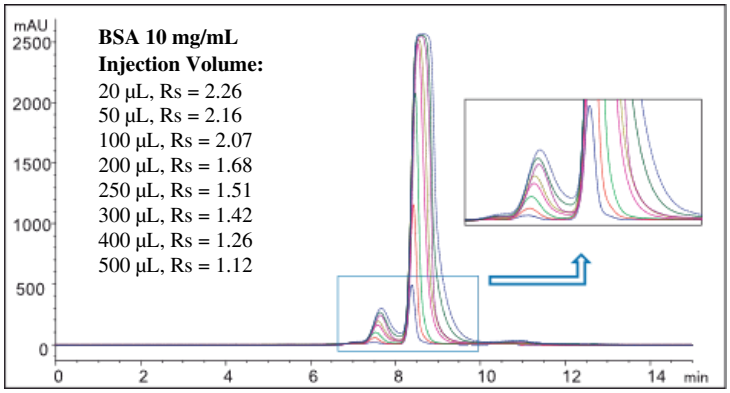
High Protein Recovery

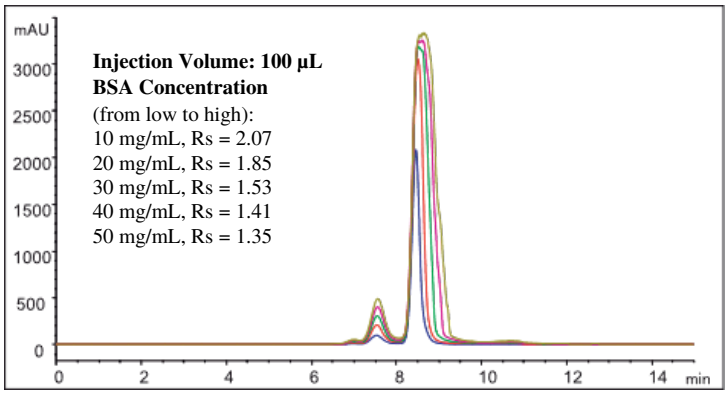
Zenix SEC phases are hydrophilic and neutral. Proteins and other biological molecules have negligible nonspecific interactions with Zenix stationary phases. The protein adsorption to the silica surface is suppressed, leading to high recovery of intact proteins, maintaining the protein activity after separation. More than 95% recovery is achieved for BSA and lysozyme, the representatives for acidic and basic proteins, respectively.

High Loading Capacity

Loading capacity is critical for size exclusion separation and purification. Figure 9 shows high loading capacity for BSA as one example (>2.5 mg for an analytical column).

Figure 9. BSA Loading Test on a Zenix SEC-300





Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detection: UV 280 nm; Temperature: RT

Figure 10. Sample Loading Recommendations (Zenix-300 with HPLC)

ID	**2.1 x 300 mm**	**4.6 x 300 mm**	**7.8 x 300 mm**	**10 x 300 mm**	**21.2 x 300 mm**	**30 x 300 mm**
Type	Nano	Narrow-bone	Regular	Semi prep	Prep	Process
V-Injection	0.1-18 µL	0.5-85 µL	1-250 µL	1- 420 µL	0.01- 2 mL	0.1-4 mL
Maximum Mass (BSA)	200 µg	1 mg	3 mg	5 mg	22 mg	45 mg
Standard Flow Rate (Maximum)	0.067 mL/min	0.35 mL/min	1.0 mL/min	1.5 mL/min (2.0 mL/min)	7 mL/min (10 mL/min)	15 mL/min (25 mL/min)
Sensitivity	Highest	Higher	High	N/A	N/A	N/A
Back Pressure	~1,200 psi	~1,200 psi	~1,200 psi	700-900 psi	700-900 psi	700-900 psi
Instrument Type	Capillary	Regular	Regular	Prep	Prep	Process

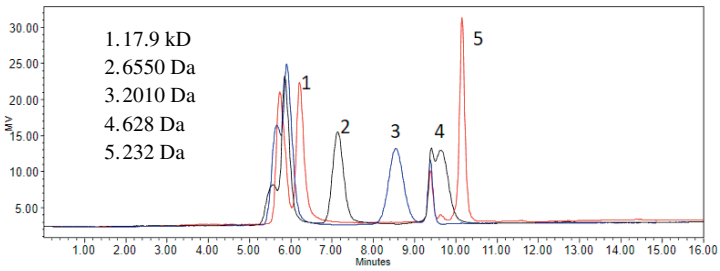
Applications

Protein Separation
Antibody Separation
Mab Fragment Separation
DNA Separation
Peptide Separation
Method Development Techniques - Non-Denaturing
Method Development - Volatile Buffer

Zenix phases have wide applications for separation, identification and purification of proteins, protein variants, peptide fragments, phosphorylated, sialylated, pegylated, and other derivatized proteins. They are well suited for studies such as molecular weight estimation, purification, and analysis of biological molecules.

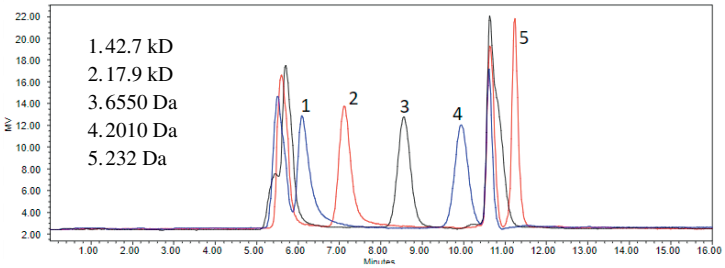
PEGylated Protein

Figure 11. PEG Separation on Zenix SEC-100



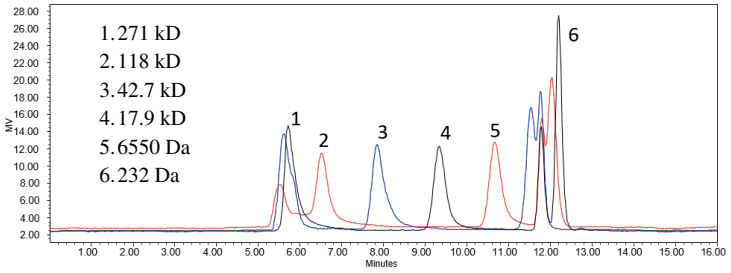
Column: Zenix SEC-100, 3 µm, 100 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Temperature: RT; Detection: RI (30 °C); Injection Volume: 20 µL; Samples: PEG1: 932 kD, 118 kD, 6.55 kD, 0.628 kD; PEG2: 496 kD, 42.7 kD, 2.01 kD; PEG3: 271 kD, 17.9 kD, 0.232 kD

Figure 12. PEG Separation on Zenix SEC-150



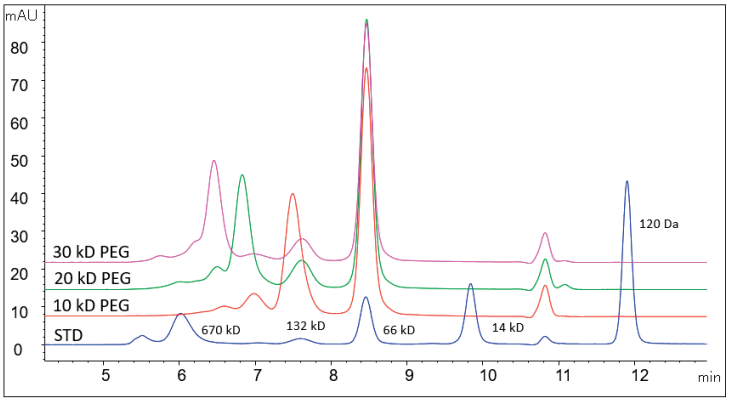
Column: Zenix SEC-150, 3 µm, 150 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Temperature: RT; Detection: RI (30 °C); Injection Volume: 20 µL; Samples: PEG1: 932 kD, 118 kD, 6.55 kD, 0.628 kD; PEG2: 496 kD, 42.7 kD, 2.01 kD; PEG3: 271 kD, 17.9 kD, 0.232 kD

Figure 13. PEG Separation on Zenix SEC-300



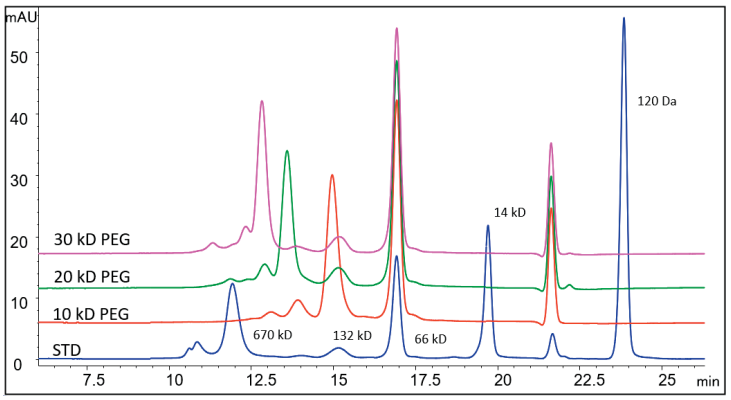
Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer, pH 7.0; Flow Rate: 1 mL/min; Temperature: RT; Detection: RI (30 °C); Injection Volume: 20 µL; Samples: PEG1: 932 kD, 118 kD, 6.55 kD, 0.628 kD; PEG2: 496 kD, 42.7 kD,2.01 kD; PEG3: 271 kD, 17.9 kD, 0.232 kD

Figure 14. PEGylated BSA



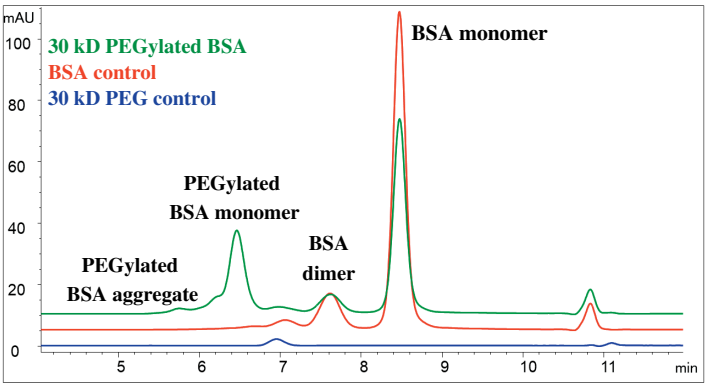
Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detector: UV 280 nm; Temperature: RT; Samples: Protein Standards: Thyroglobulin (670 kD), BSA (66 kD), Ribonuclease A (14 kD), Uracil (120 Da), and 10, 20, 30 kD PEGylated BSA (50 µg each)

Figure 15. PEGylated BSA Analysis with Two Columns in Tandem



Columns: 2 x Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detector: UV 280 nm; Temperature: RT; Samples: QC standards, 10, 20, 30 kD PEGylated BSA (50 µg each)

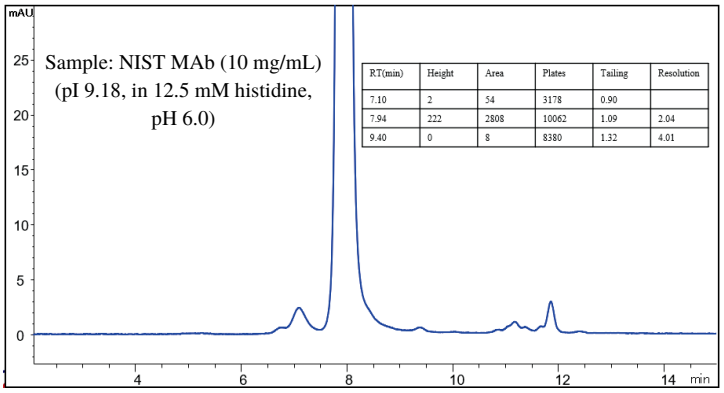
Figure 16. PEGylated BSA Analysis on Zenix SEC-300



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detector: UV 280 nm; Temperature: RT; Samples: BSA control (5 mg/mL in sodium acetate, 10 µL), 30 kD PEG (10 mg/mL, 5 µL), 30 kD PEGylated BSA (3.93 mg/mL, 12.7 µL)

Antibody Separation

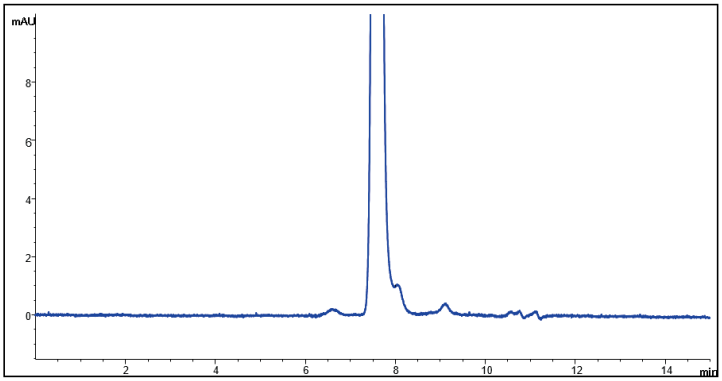
Figure 17. NIST MAb SEC Analysis on Zenix SEC-300 – Zoom In



RT(min)	Height	Area	Plates	Tailing	Resolution
7.10	2	54	3178	0.90	
7.94	222	2808	10062	1.09	2.04
9.40	0	8	8380	1.32	4.01

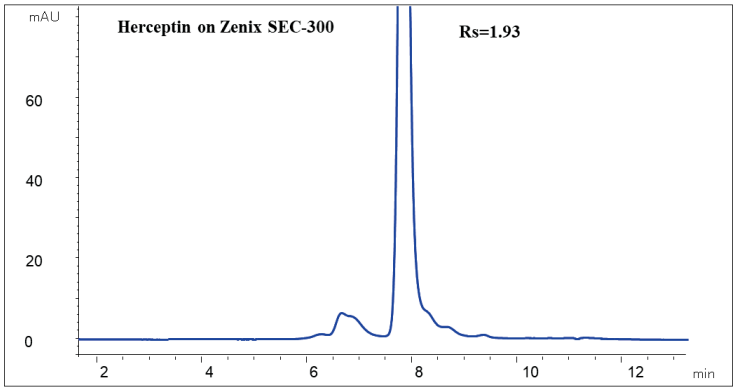
Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detector: UV 280 nm; Temperature: RT; Injection Volume: 3 µL

Figure 18. Adalimumab (Humira) Test on Zenix SEC-300



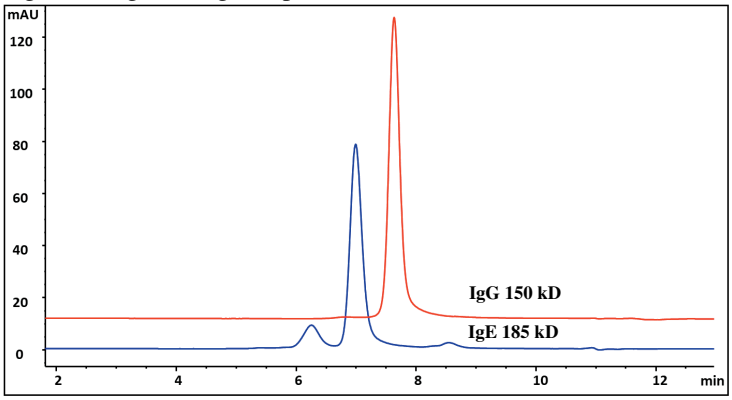
Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer, 300 mM NaCl, pH 7.0; Flow Rate: 1 mL/min; Detector: 280 nm; Temperature: 23 °C; Sample: 2 mg/mL Adalimumab; Injection Volume: 5 µL

Figure 19. Herceptin Analysis on Zenix SEC-300



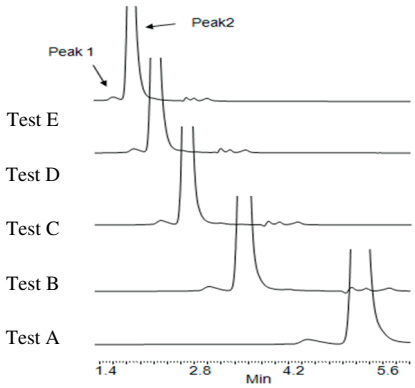
Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detector: UV 280 nm; Temperature: RT; Sample: 20 µL Herceptin (2.3 mg/mL)

Figure 20. IgE and IgG Separation on Zenix SEC-300



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase:150 mM Sodium Phosphate Buffer; pH 7.0; Temperature: RT; Flow Rate: 1 mL/min; Sample Injection: Human IgE (from Abcam), Mouse IgG (20 µg each)

Figure 21. FAST MAb Separation – Zenix SEC-300, 7.8 x 200 mm



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
E	3.0	2450	1.62	1.89	1.26	96.92	1.3

MAb Fragment Separartion

Figure 22. MAb fragment Analysis

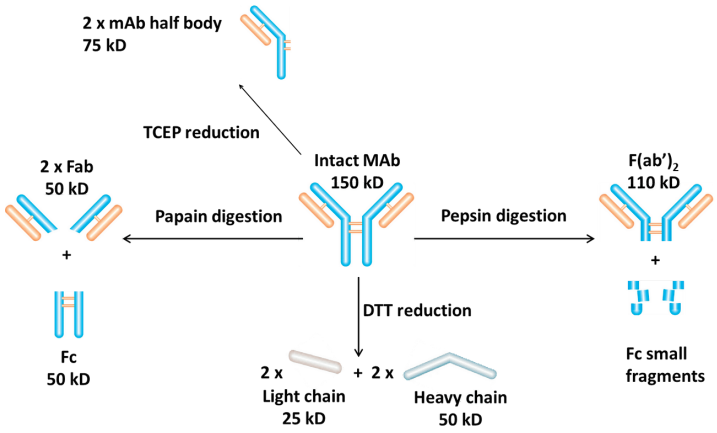
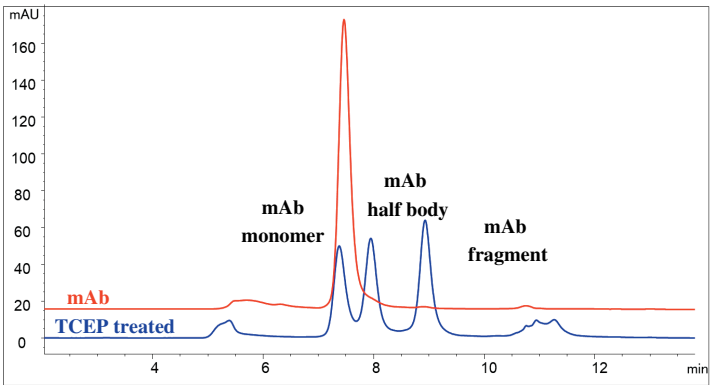
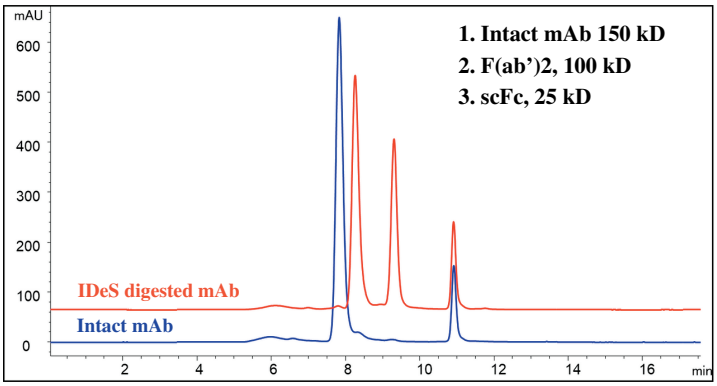


Figure 23. MAb Half Body SEC Analysis on Zenix SEC-300



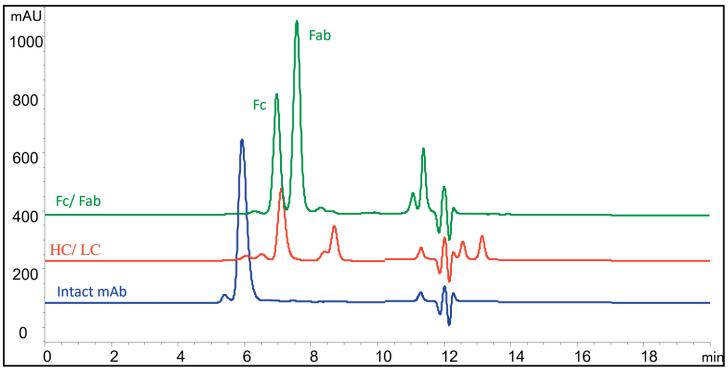
Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; mAb half body generation: 150 mM bond breaker TCEP, neutral pH, 500 mM stock, 30 mL TCEP + 70 mL (5 mg/mL) mAb 32; Flow Rate: 1 mL/min; Detector: UV 280 nm; Temperature: RT; Injection: 50 µg mAb and TCEP treated mAb each

Figure 24. Mab Fragment Analysis: IDeS Digestion



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; MAb Digestion: 5 mg/mL of mAb sample was incubated with FragIT IDeS resin at 37°C for 1 hour; Flow Rate: 1 mL/min; Detector: UV 214 nm; Temperature: RT; Injection: 15 µg IDeS digested mAb and 12 µg undigested mAb

Figure 25. Intact MAb and MAb Fragment Separation

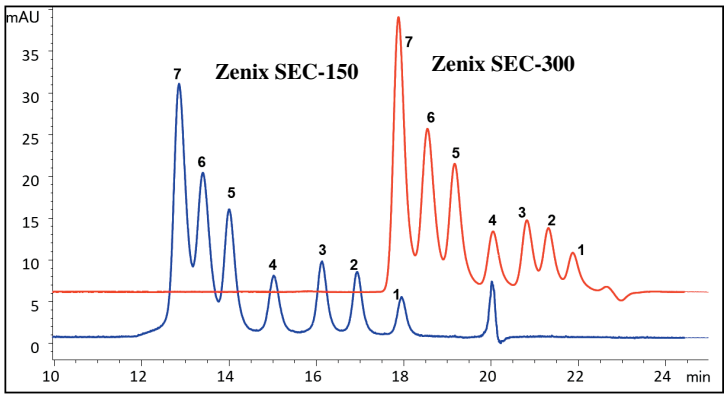


Column: Zenix SEC-300 PEEK, 3 µm, 300 Å, 4.6 x 300 mm; Mobile Phase: 20% ACN, 0.1% TFA, 0.1% Formic Acid; Flow Rate: 0.35 mL/min; Detector: UV 214 nm; Temperature: RT; Injection Volume: 5 µL (1 mg/mL); Sample: Fc/Fab (papain digested mAb), Reduced mAb (20 mM DTT at 65 °C for 20 minutes)

DNA Separation

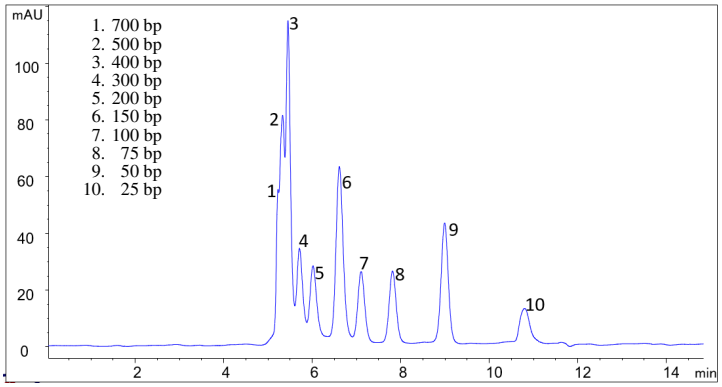
Zenix SEC-300 300 Å columns give better resolution for longer oligos (>30 nt), and Zenix SEC-150 150 Å columns separate poly dAs with baseline resolution for shorter oligos (<35 nt).

Figure 26. Zenix SEC-150 and Zenix SEC-300 Comparison



Columns: Zenix SEC- 300, 3 µm, 300 Å, 7.8 x 300 mm and Zenix SEC-150, 3 µm, 150 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 0.5 mL/min; Detector: UV 260 nm; Temperature: RT; Injection Volume: 30 µL; Pressure: 41 bar; Samples: 1. dA10, 2. dA15, 3. dA20, 5. dA40, 6. dA50, 7. dA60, 0.1 µM each in water, 4. 5' ATATCTACACGGCTACCCGTACCAATGCTGCTTCC-3' (35 nt)

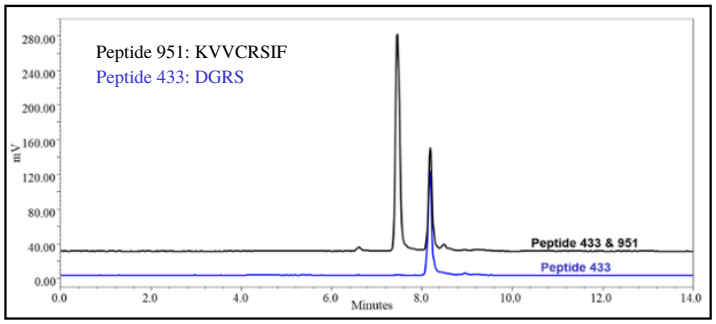
Figure 27. DNA Separation on Zenix SEC-300



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Flow Rate: 1 mL/min; Detector: UV 260 nm; Temperature: RT; Sample: DNA standards 0.5 mg/mL each 700, 500, 400, 300, 200, 150, 100, 75, 50, 25 bp

Peptide Separation

Figure 28. High Resolution of Close MW Small Peptides



Column: Zenix SEC-80, 3 µm, 80 Å, 7.8 x 300 mm; Mobile Phase: Acetonitrile : Water : TFA = 75 : 25 : 0.1 (v/v); Flow Rate: 1 mL/min; Detector: ELSD (temperature: 65 °C, gas flow: 2 L/min, gain: 1); Temperature: RT; Pressure: 960 psi; Injection Volume: 10 µL; Samples: Peptide 433, Peptide 951: 0.5 mg/mL each in water

Precolumn Filter for Analytical Columns

For Analytical Columns with Particle Size 5 µm and 10 µm

PN: 102000-P355 PEEK Precolumn Filter & Frit (2 µm)

PN: 102001-P355 PEEK Refill Frits (2 µm), 5 units/pk

with Particle Size below 3 µm

PN: 102000-P356 PEEK Precolumn Filter & Frit (0.5 µm)

PN: 102001-P356 PEEK Refill Frits (0.5 µm), 5 units/pk

PN: 102000-P346 PEEK Precolumn Filter SS Frit (0.5 µm)

PN: 102001-P346 SS Refill Frits (0.5 µm), 5 units/pk

Cartridge & Holder

Packed with Resin, Part Number is dependent to Phase

Replacement Cartridge Holder

PN: 102000-2001, Holder for 2.1mm ID column

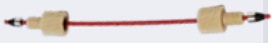
PN: 102000-4001, Holder for 4.6 mm ID column

PEEK Column Coupler

PN: 102002-COUPLER

PEEK Coupler with Flexible Tubing

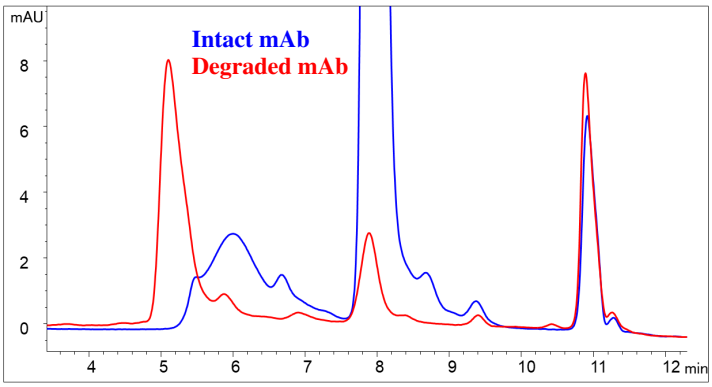
PN: 102006-COUPLER



Method Development Techniques - Non-Denaturing

Figure 29 through Figure 33 Conditions: Column: Zenix-300, 3 µm, 300 Å, 7.8 x 300 mm and Flow Rate: 1 mL/min

Figure 29. SEC Analysis of MAb-Forced Degration



Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Detection: UV 280 nm; Injection Volume: 10 µL; Samples: Intact mAb (5 mg/mL) and degraded mAb (2.5 mg/mL). Degraded mAb was treated with 178 mM citric acid, pH 3.5, and incubated at 60 °C for 1 hour

MAb Aggregate Analysis: Sample Concentration

Figure 30. Sample Concentration Affect: High Concentration

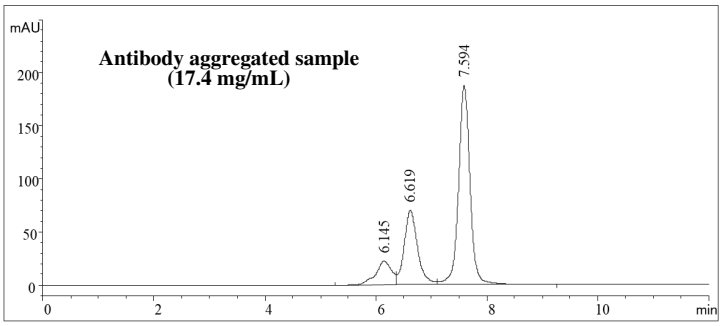
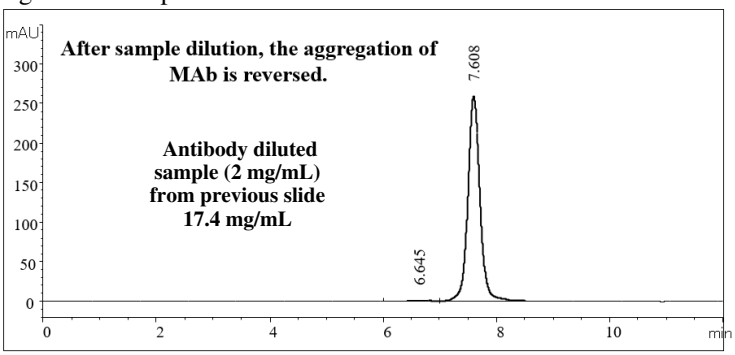
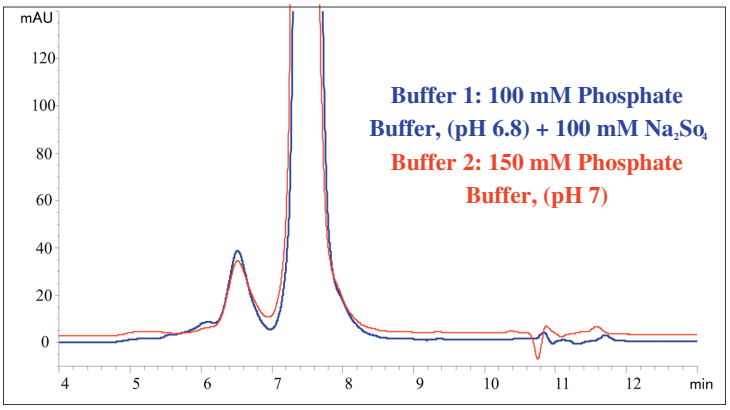


Figure 31. Sample Concentration Affect: Low Concentration



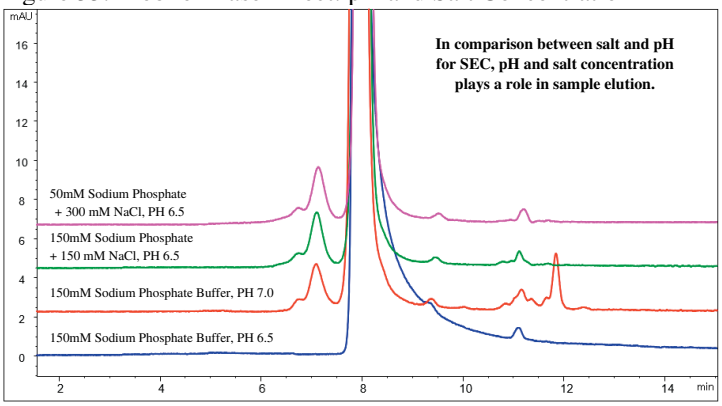
Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0; Detector: UV 280 nm; Temperature: RT; Injection Volume: Fresh mAb 25 µL (2 mg/mL), Aggregated mAb 2.9 µL (17.4 mg/mL)

Figure 32. Mobile Phase Effect: Salt Addition to Running Buffer



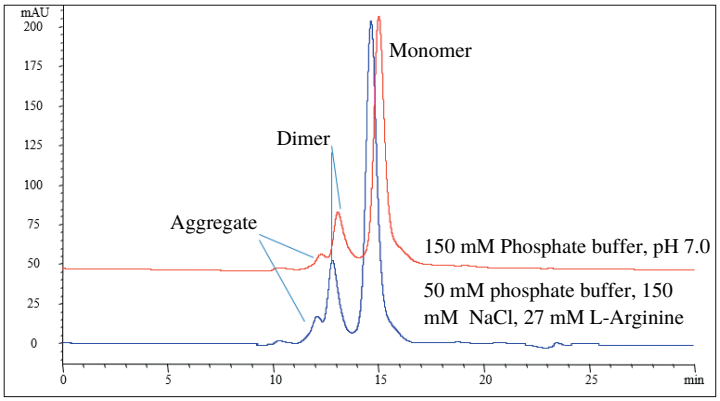
Detector: UV 214 nm; Injection Volume: 20 µL (1 mg/mL); Temperature: RT; Pressure: 77 bar; Sample: mAb

Figure 33. Mobile Phase Effect: pH and Salt Concentration



Detector: UV 280 nm; Temperature: RT; Injection Volume: 3 µL; Sample: NIST mAb (10 mg/mL) (pI 9.18, in 12.5 mM histidine, pH 6.0)

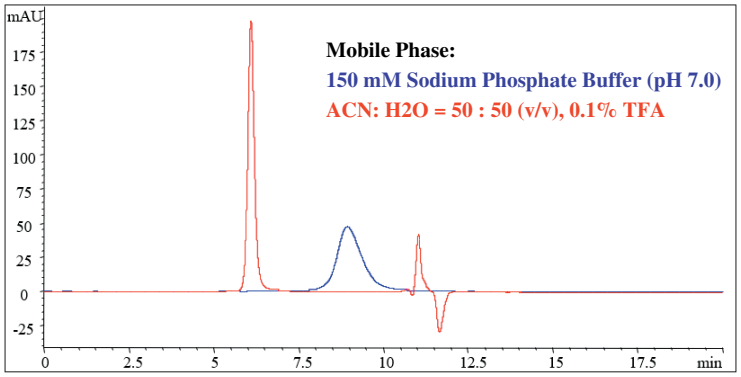
Figure 34. Mobile Phase Effect of Arginine Addition to Reduce Electrostatic Interactions



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Flow Rate: 0.5 mL/min; Detector: UV 280 nm; Temperature: RT; Injection Volume: 50 µL; Pressure: 50 bar; Sample: CHO cell expressed Fc-fusion protein 160 kD (2.0 mg/mL)

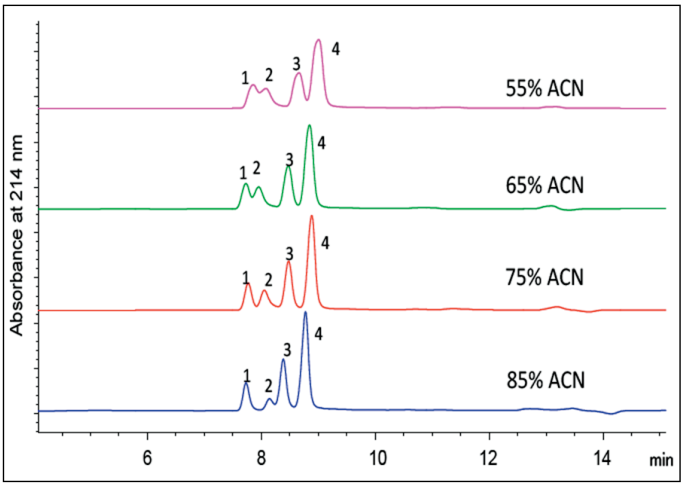
Method Development - Volatile Buffer

Figure 35. Mobile Phase Effect: Salt vs Organic



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 150 mM Sodium Phosphate Buffer; pH 7.0, ACN : H2O = 50 : 50 (v/v), 0.1% TFA; Flow Rate: 1 mL/min; Detector: UV 220 nm; Temperature: RT; Injection Volume: 5 µL (4 mg/mL) PEGylated peptide (40 kD PEG and 5,000 Da peptide, pI about 4.5); Pressure: 91 bar

Figure 36. Mobile Phase Effect: ACN Concentration



Peak	Protein	MW (Da)	Retention Time (min)	Resolution	Plate Count
1	Insulin (porcine)	5778	7.75		16711
2	Glucagon	3483	8.03	1.07	12132
3	Angiotensin I	1297	8.46	1.58	19741
4	Bradykinin	1060	8.86	1.65	

Precolumn Filter for Prep Columns

For Prep Column (≥ 21.2mm ID) Particle Size 5 µm or Above

PN: 102020-21200 Stainless Steel Filter & Frits (2 µm)

PN: 102020-00001 Stainless Steel Refill Frits (2 µm) (5 units/pk)

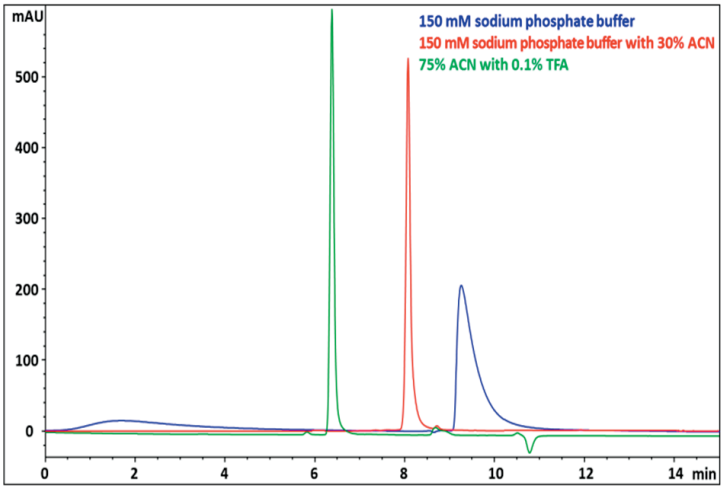
Cartridge & Holder

Packed with Resin, Part Number dependent to Phase

PEEK/SS Coupler

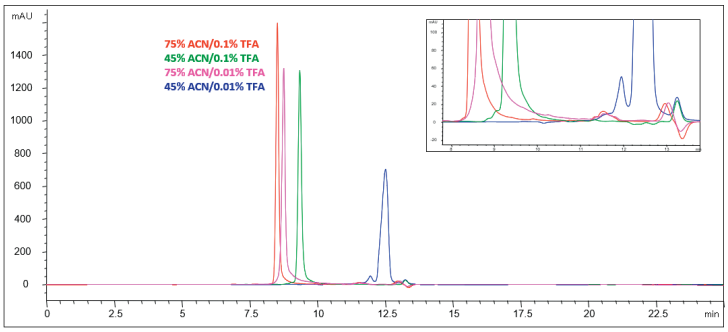
PN: 102003-COUPLER

Figure 37. Mobile Phase Comparison: Aqueous vs. Organic



Overlay of different mobile phases used for the analysis of insulin on Zenix SEC-80, 3 µm, 80 Å, 7.8 x 300 mm. UV detection was set at 214 nm and 5 µL of insulin (1 mg/mL) was injected each run.

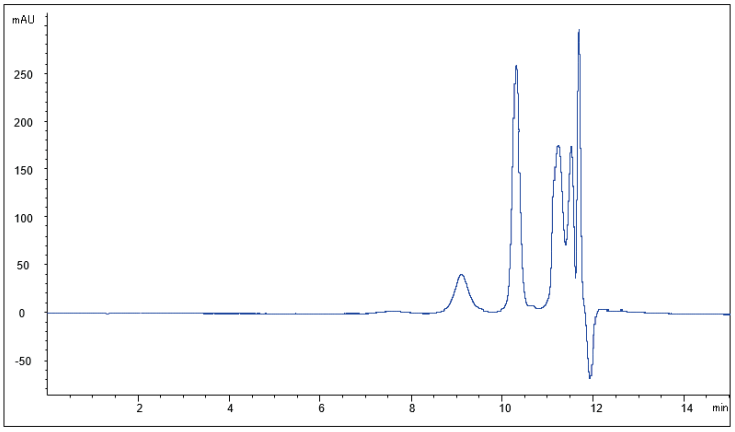
Figure 38. Mobile Phase Effect: Change in ACN Percent for Glucagon Separation



Sample Buffer

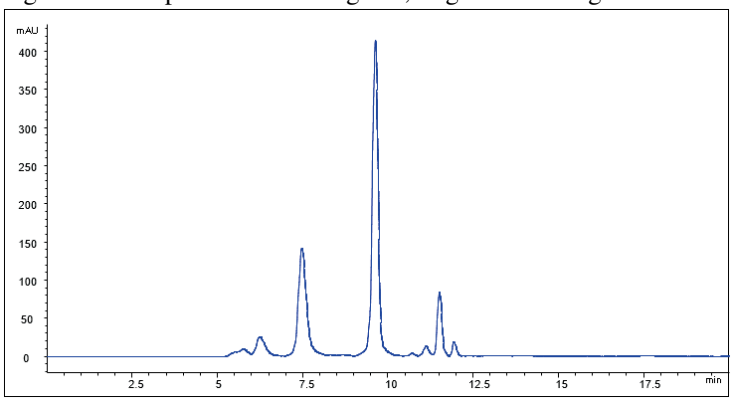
The importance of having equivalent sample buffers and runner buffers under organic conditions is seen below in Figure 39 and Fig. 40.

Figure 39. Sample Diluted in Phosphate, Organic Running Conditions



Column: Zenix SEC-300, 3 µm, 300 Å ,7.8 x 300 mm; Mobile Phase: 20% Acetonitrile + 0.1% TFA + 0.1% FA; Flow Rate: 1 mL/min; Detector: UV 214 nm; Temperature: RT; Samples: 5 µL, Protein Standards: 1. Thyroglobulin 670 kD, 2. BSA dimer 132 kD, 3. BSA 66 kD, 4. Ribonuclease A 14 kD, 5. Uracil 120 Da

Figure 40. Sample Diluted in Organic, Organic Running Conditions



Column: Zenix SEC-300, 3 µm, 300 Å, 7.8 x 300 mm; Mobile Phase: 20% Acetonitrile + 0.1% TFA + 0.1% FA; Flow Rate: 1 mL/min; Detector: UV 214 nm; Temperature: RT; Samples: 5 µL, Protein Standards: 1. Thyroglobulin 670 kD, 2. BSA dimer 132 kD, 3. BSA 66 kD, 4. Ribonuclease A 14 kD, 5. Uracil 120 Da

Zenix SEC Technical Specifications

Phase	**Zenix SEC-80**	**Zenix SEC-100**	**Zenix SEC-150**	**Zenix SEC-300**
Material	Neutral, hydrophilic film bonded silica			
Particle Size	3 µm			
Pore Size	~80 Å	~100 Å	~150 Å	~300 Å
Protein MW Range (Native)	100-50,000 Da	100-100,000 Da	500-150,000 Da	5,000-1.250,000 Da
pH Stabilty	2 - 8.5 (pH 8.5 - 9.5 can be tolerated temporarily)			
Backpressure for 7.8 x 300 mm (1 mL/min)	~1,500 psi	~1,5000 psi	~1,375 psi	~1,100 psi
Backpressure for 4.6 x 300 mm (.35 mL/min)	~1,400 psi	~1,250 psi	~1,000 psi	~1,000 psi
Maximum Backpressure	~4,500 psi	~4,500 psi	~4,500 psi	~3,500 psi
Salt Concentration Range	20 mM - 2.0 M			
Maximum Temperature	~80 °C			
Mobile Phase Compatibility	Aqueous and Organic			

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