

Size Exclusion Chromatography



Sepax Technologies

Zenix™



Better Surface Chemistry for Better Separation

Sepax Technologies, Inc.

Sepax Technologies, Inc. develops and manufactures products in the area of chemical and biological separations, biosurfaces and proteomics. Sepax product portfolio includes 1) liquid chromatography columns and media, 2) SPE and Flash chromatography columns and tubes, 3) bulk resin for preparative separation and process chromatography, and 4) natural product and Chinese traditional medicine separation and purification.



Leader in Biological Separations

Sepax develops and manufactures 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 from non-porous to 2000 Å. Unique and proprietary resin synthesis and surface technologies have been developed for solving separation challenges in biological area.



Bioseparation Products

Size Exclusion

SRT[®], SRT[®]-C

Nanofilm[®]

ZenixTM, ZenixTM-C

Ion-exchange

Proteomix[®]

GlycomixTM

Antibody Separation

AntibodixTM

Carbohydrate Separation

Carbomix[®]

Analytical, Semi-prep and Preparative

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 the particle size of 3 µm. Zenix SEC packings 3 µm particle size combined with large pore volume achieve the highest separation efficiency and resolution. The well-controlled surface chemistry results in excellent lot-to-lot reproducibility. Our unique bonding chemistry, coupled with the maximized bonding density, allows Zenix SEC to provide high stability and negligible non-specific interactions. 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.

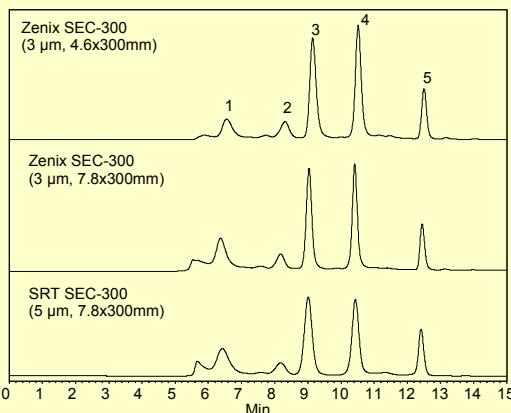
Featured Characteristics

- Particle size of 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 virus
- Ideal for separation and analysis of natural polymers, e.g. polysaccharides, synthetic polymers, and nanomaterials, e.g. nanoparticles

High Separation Efficiency

The advantages of developing small particle size 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. 1 and Table 1, the plate numbers of BSA dimer, BSA, and ribonuclease A increased from 2720 to 4600, 6590 to 13090, 11160 to 22000 when the particle size decreased from 5 µm to 3 µm. Fig. 2 and Fig. 3 further show that high efficiency has been achieved by 3 µm Zenix columns with various proteins. The efficiency of p-aminobenzoic acid reached the plate number of 40,000 for a 30 cm long Zenix column.

Figure 1. Separation of protein mixture A by Zenix SEC-300 and SRT SEC-300 columns.



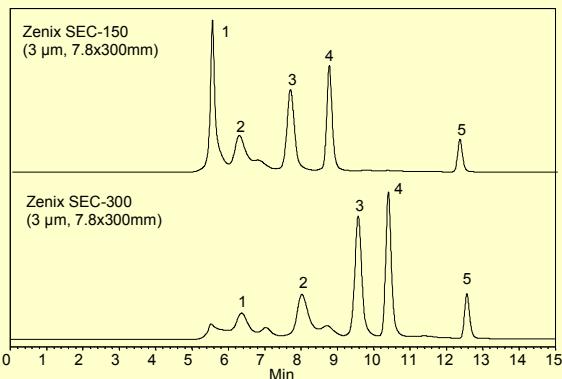
Column:	Zenix SEC-300 and SRT SEC-300
Mobile phase:	150 mM PBS, pH 7
Flow rate:	1.0 mL/min for 7.8x300 mm 0.35 mL/min for 4.6x300 mm
Temperature:	Ambient (~23° C)
Detection:	UV 214nm
Injection:	10 µL (3 µL for 4.6x300 mm)
Sample:	1) Thyroglobulin (1.0 mg/mL), 670 kD; 2) BSA dimer, 132 kD; 3) BSA (1.0 mg/mL), 66 kD; 4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and 5) Uracil (2.5 µg/mL), 120D.

Table 1. Efficiency of Zenix SEC-300 and SRT SEC-300 columns

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 2. Separation of protein mixture B by Zenix SEC-150 and 300 columns with 7.8 mm ID.

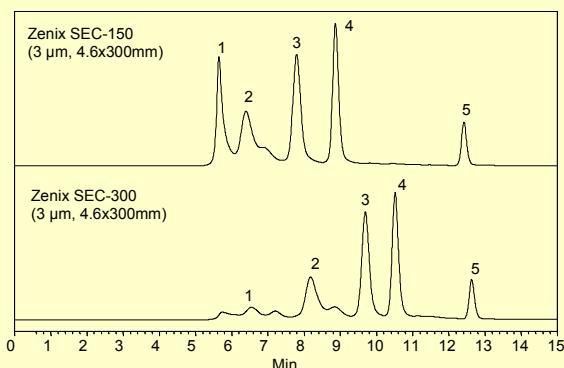


Column: 7.8x300 mm, 3 μ m
 Mobile phase: 150 mM PBS, pH 7
 Flow rate: 1.0 mL/min
 Temperature: Ambient (~23° C)
 Detection: UV 214nm
 Injection volume: 10 μ L
 Sample: 1) Thyroglobulin, 670 kD; 2) γ -Globulin, 158 kD;
 3) Ovalbumin, 44 kD; 4) Ribonuclease A, 13.7 kD;
 5) p-Aminobenzoic acid, 137 D.

Table 2. Efficiency of 7.8x300 mm Zenix SEC-150 and 300 columns.

Peak	Protein	Zenix 150	Zenix 300
1	Thyroglobulin	12420	1295
2	γ -Globulin	2860	3650
3	Ovalbumin	6620	11760
4	Ribonuclease A	16450	21690
5	p-Aminobenzoic acid	40550	39400

Figure 3. Separation of protein mixture B by Zenix SEC-150 and 300 columns with 4.6 mm ID.



Column: 4.6x300 mm, 3 μ m
 Mobile phase: 150 mM PBS, pH 7
 Flow rate: 0.35 mL/min
 Temperature: Ambient (~23° C)
 Detection: UV 214nm
 Injection volume: 5 μ L
 Sample: 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.

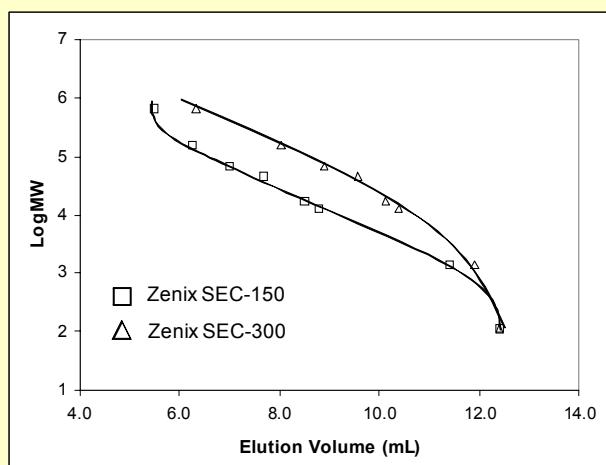
Table 3. Efficiency of 4.6x300 mm Zenix SEC-150 and 300 columns.

Peak	Protein	Zenix 150	Zenix 300
1	Thyroglobulin	6020	2410
2	γ -Globulin	2560	3000
3	Ovalbumin	6030	10260
4	Ribonuclease A	13350	17020
5	p-Aminobenzoic acid	35500	33480

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 for Zenix SEC-150 and 300.

Figure 4. Molecular weight calibration curve on Zenix SEC column.



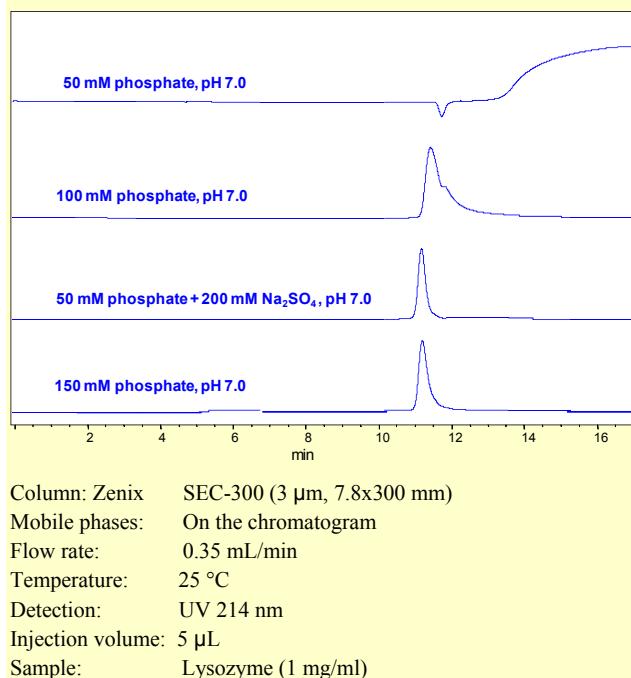
Column: 3 μ m, 7.8x300 mm
 Mobile phase: 150 mM phosphate buffer, pH 7.0
 Flow rate: 1.0 mL/min
 Detection: UV 214 nm
 Injection volume: 10 μ L
 Samples: 1) Thyroglobulin 67 kD, 2) γ -Globulin 158 kD,
 3) BSA 66kD, 4) Ovalbumin 44 kD,
 5) Myoglobin 17.6 kD, 6) Ribonuclease 13.7 kD,
 7) Vitamin B12 1.35 kD, 8) Uracil 120 kD

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 methanol, ethanol, THF, DMF, DMSO, etc., and the mixture of water and organic solvents.

Mobile Phase Optimization

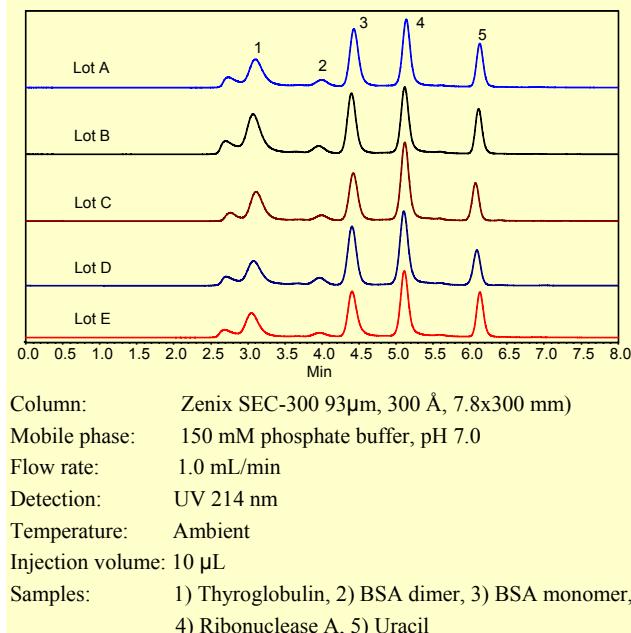
Figure 5. Analysis of Lysozyme on Zenix SEC-300 column.



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. Separation variation from batch to batch is controlled to be within 5% for retention time.

Figure 6. Lot-to-Lot reproducibility of protein mixture on Zenix SEC-300 column.



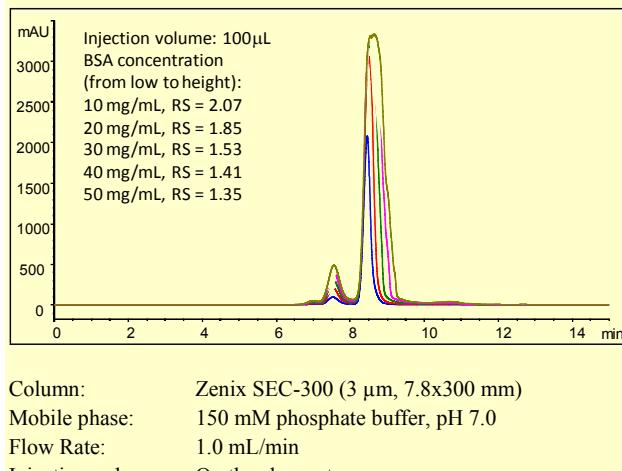
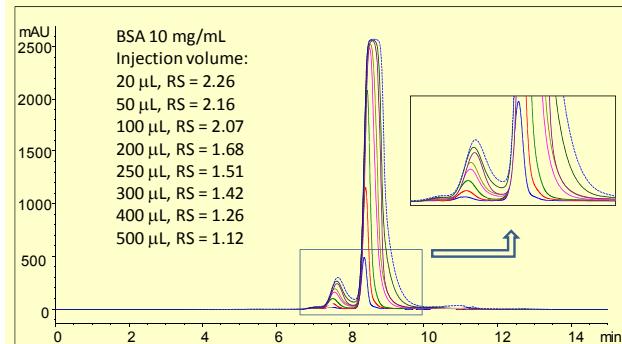
High Stability

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 bond of silica-stationary phase layer, thus enabling high stability over a wide range of pH from 2 to 8.5.

High Loading Capacity

Loading capacity is critical for size exclusion separation and purification. Figure 4 shows high loading capacity for BSA as one example (>2.5 mg for an analytical column).

Figure 6. BSA loading test on a Zenix SEC-300 column.



Column: Zenix SEC-300 (3 μ m, 7.8x300 mm)
Mobile phase: 150 mM phosphate buffer, pH 7.0
Flow Rate: 1.0 mL/min
Injection volume: On the chromatogram
Detection: UV 280 nm

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.

Pore size vs. MW exclusion limit

Phases (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

Column Dimension Availability

Available Zenix SEC column dimensions are 0.75, 1.0, 2.1, 3.0, 4.6, 7.8, 10, 21.2 and 30 mm I.D., and 20, 30, 50, 100, 150, 250, 300 and 600 mm length. Sepax also offers custom-made columns. Both stainless steel and PEEK tubes are available.

Sample Loading Recommendation (Zenix-300 with HPLC)

ID	2.1x300 mm	4.6x300 mm	7.8x300 mm	10x300 mm	21.2x300 mm	30x300 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

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 (Å)	~80	~100	~150	~300
Protein MW range (native)	100-50,000	100 - 100,000	500 - 150,000	5,000 – 1,250,000
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)			
Backpressure for 7.8x300 mm (1.0 mL/min)	~1,500 psi	~1,500 psi	~1,375 psi	~1,100 psi
Backpressure for 4.6x300 mm(0.35 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			

Applications

Separation and Analysis
<i>Proteins</i>
<i>Monoclonal antibodies</i>
<i>Cell lysates</i>
<i>Nucleic acids</i>
<i>Nucleotides</i>
<i>Peptides</i>
<i>Water soluble polymers</i>
<i>Nanoparticles</i>
<i>Nanotubes</i>

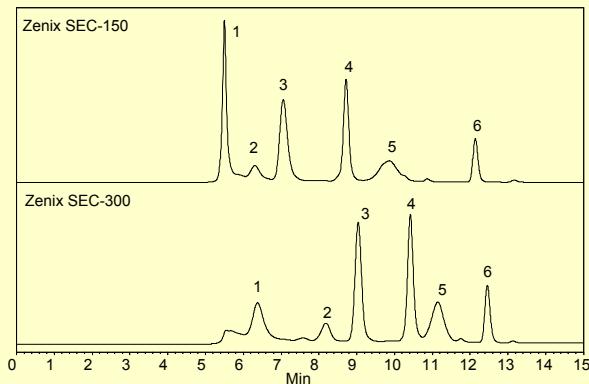
Zenix phases have wide applications for separation, identification and purification of proteins, protein variants, peptide fragments, phosphorylated, sialylated, pegylated, and other derivatized proteins. They are well suited for studies such as molecular weight estimation, purification and analysis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

Separation of protein and peptide mixture

Zenix SEC columns offer a number of benefits. First, Zenix offers higher capacity, 6.7 mL and 6.9 mL for Zenix SEC-150 and 300, respectively, calculated from the total permeation peak (uracil) to total exclusion peak (thyroglobulin). Secondly Zenix offers higher resolution. Poly-DL-alanine (from Sigma) is a peptide with the MW of 1-5 kD. For size exclusion chromatography, an empirical rule is that a baseline separation can be achieved for two compounds if their MWs difference is twofold (2x). Both Zenix SEC-150 and 300 columns well separated ribonuclease A (13.7kD) and poly-DL-alanine (1-5 kDa), as shown in Fig. 6. Thirdly the Zenix column shows a good separation profile of Poly-DL-alanine, indicating Zenix packing does not have non-specific interactions with Poly-DL-alanine.



Figure 7. Separation of a mixture of proteins and peptide by using Zenix SEC-150 and 300 columns.

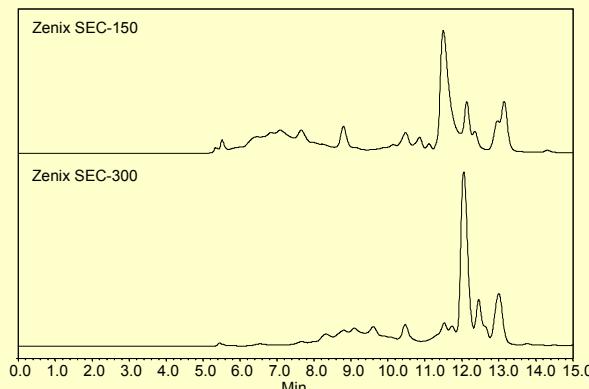


Column: Zenix SEC (3 μ m, 7.8x300 mm)
 Mobile phase: 150 mM PBS, pH 7
 Flow rate: 1.0 mL/min
 Temperature: Ambient (\sim 23 °C)
 Detection: UV 214nm
 Injection volume: 10 μ L
 Sample: 1) Thyroglobulin, 670kD; 2) BSA dimer,
 3) BSA monomer, 66kD; 4) Ribonuclease A,
 13.7kD; 5) poly-DL-alanine, 1-5 kD;
 6) Uracil, 120D.

Separation of *E. coli* Lysate

Zenix SEC-150 and 300 columns are used to separate the *E. coli* lysate. The elution profiles in Fig. 7 show both columns achieved high resolution separation. However, Zenix SEC-150 is more suitable for separation of *E. coli* lysate due to the small molecule weight of the lysate.

Figure 8. Separation of *E. coli* lysate with various pore size Zenix columns.

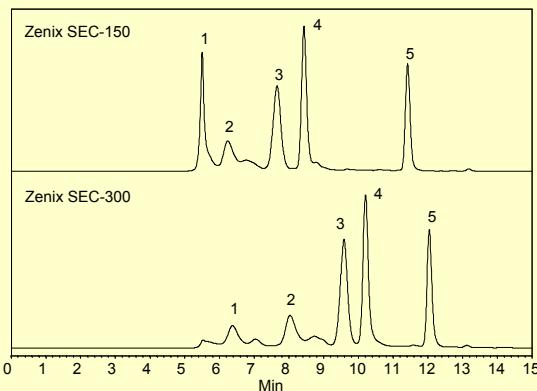


Column: Zenix SEC (3 μ m, 7.8x300 mm)
 Mobile phase: 0.15 M PBS, pH 7.0
 Flow rate: 1.0 mL/min
 Detection: UV 214 nm
 Injection: 10 μ L
 Sample: *E. coli* lysate (2.5 mg/mL)

Separation of Biorad standard protein sample

Thyroglobulin, γ -globulin, ovalbumin, myoglobin and vitamin B12 have the molecular weight in the range of 660,000 – 1,355. The peak efficiency is shown in Table 4. The plate number of myoglobin is more than 20,000 with Zenix SEC-300 column, which was not achieved by any other SEC columns.

Figure 9. Separation of a protein mixture by Zenix SEC-150, 300 and 500 columns.



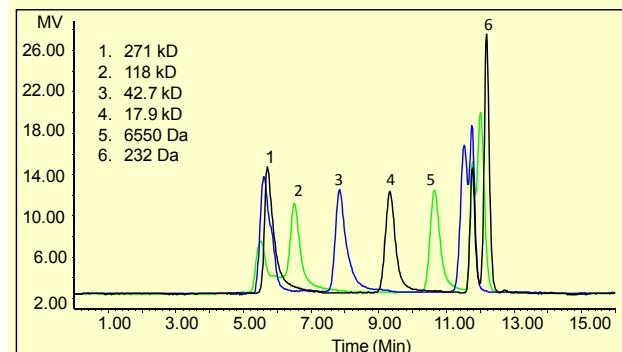
Column:	Zenix SEC (3 μ m, 7.8x300 mm)
Mobile phase:	0.15 M PBS, pH 7.0
Flow rate:	1.0 mL/min
Detection:	UV214 nm
Injection:	10 μ L
Sample:	1) Thyroglobulin, 670 kD; 2) γ -Globulin, 158kD; 3) Ovalbumin, 44 kD; 4) Myoglobin,16.9 kD; 5) Vitamin B12, 1355 D.

Table 4. Efficiency of Zenix SEC-150 and 300 columns

Peak	Protein	Zenix 150 7.8x300mm	Zenix 300 7.8x300mm
1	Thyroglobulin	12420	1760
2	γ -Globulin	2860	3650
3	Ovalbumin	6620	11760
4	Myoglobin	15020	20810
5	Vitamin B12	34370	35460

Separation of PEGs

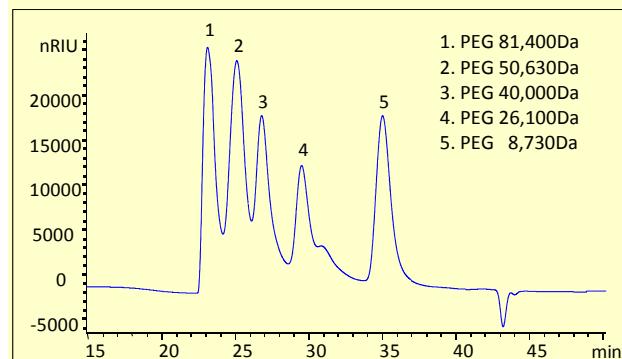
Figure 10. Separation of PEGs by Zenix SEC-300 column.



Column: Zenix SEC-300 (3 μ m, 7.8x300mm)
 Mobile phase: 150 mM Sodium phosphate buffer, pH 7.0
 Flow rate: 1.0 mL/min
 Temperature: Ambient
 Detection: RI 30 °C
 Injection volume: 20 μ L
 Samples: PEG1: 932kD, 118kD, 6.55kD, 0.628kD
 PEG2: 496kD, 42.7kD, 2.01kD
 PEG3: 271kD, 17.9kD, 0.232kD

Separation of PEGs by two Zenix columns in tandem

Figure 11. Separation of PEGs by 2 Zenix SEC-300 columns.



Column: 2 Zenix SEC-300 (3 μ m, 300 Å, 7.8x300 mm connected in tandem)

Mobile phase: 150mM Sodium Phosphate Buffer, pH 7.0

Flow rate: 0.5 mL/min

Temperature: 25 °C

Detection: RI 30 °C

Injection volume: 30 °C

Samples: 1. PEG 81,400Da (2mg/mL)

2. PEG 50,630Da (2mg/mL)

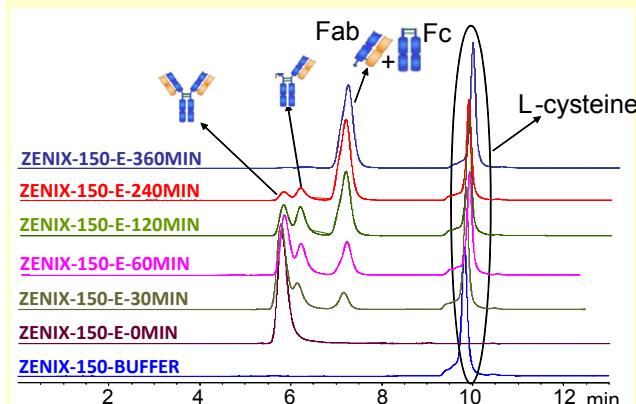
3. PEG 40,000Da (2mg/mL)

4. PEG 26,100Da (2mg/mL)

5. PEG 8,730Da (2mg/mL)

Analysis of Antibody Fragments

Figure 12. Analysis of antibody fragments from papain digestions on Zenix SEC-150 column.



Papain degradation conditions-

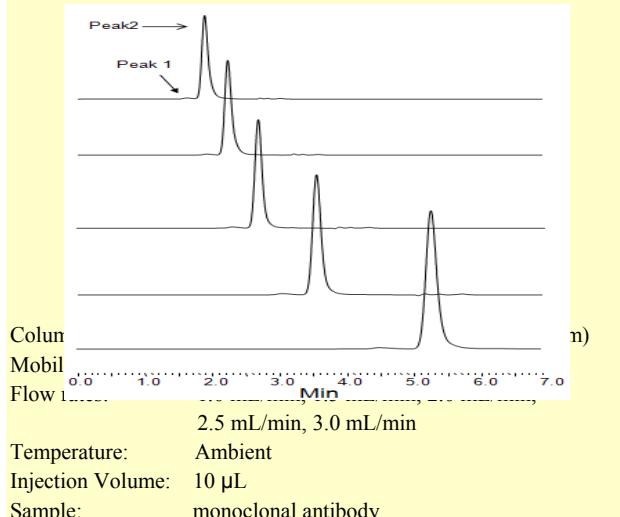
Buffer: 5.0 mM L-Cysteine,
2.0 mM EDTA and 300 nM NaCl

Enzyme: 80 µg/mL papain
Temperature: 37 °C
Time: 30 min, 60 min, 120 min, 240 min, 360 min

Column: Zenix SEC-150 (3 µm, 150 Å, 7.8x300mm)
Mobile phase: 20 mM phosphate buffer, pH 7.0, 150mM NaCl
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV 280 nm
Injection Volume: 10 µL
Samples: Erbitux digested in papain

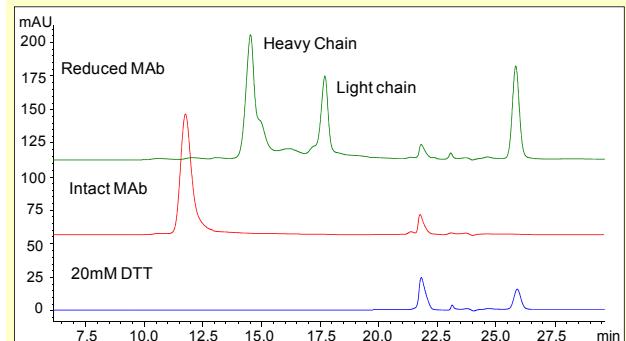
Fast MAb Separation

Figure 13. Fast MAb separation on Zenix SEC-300 column.



Separation of MAb Heavy and Light Chains

Figure 16. Separation of MAb heavy and light chains on Zenix SEC-300 column.



Column: Zenix SEC-300 (3 µm, 300 Å, 7.8x300 mm)
Mobile phase: 0.1% TFA, 0.1% formic acid and 20% ACN
Flow rate: 0.5 mL/min
Detection: UV 280 nm
Injection volume: 20 µL
Sample: Reduced MAb (1mg/mL) with 20 mM DTT

Separation of Peptides by Zenix SEC-80

Zenix SEC-80 can be applied to separations of small proteins and peptides. Figure 9 showed a high resolution separation of *E. coli* tryptic digest on Zenix SEC-80. A four peptide mixture whose molecular weights range from 1 kD to 6 kD was separated on Zenix SEC-80 with good resolution, as shown in figure 10.

Figure 14. Separation of *E. coli* tryptic digest by Zenix SEC-80 column.

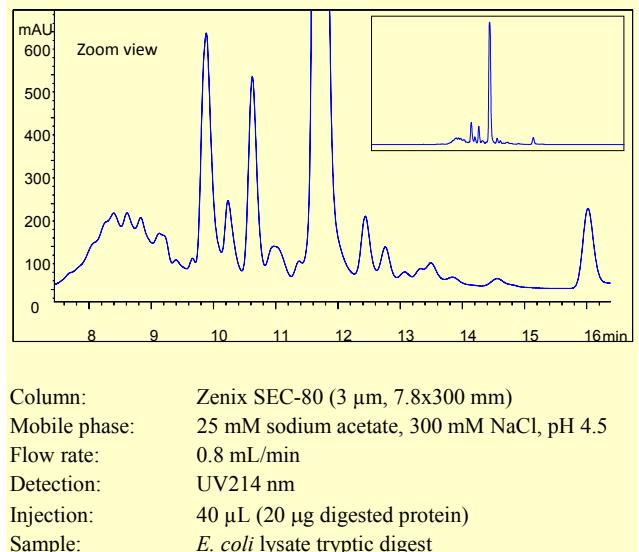
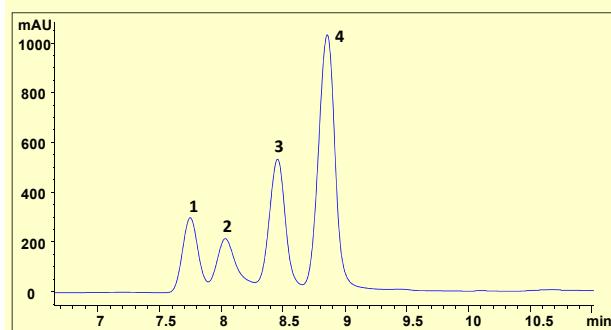


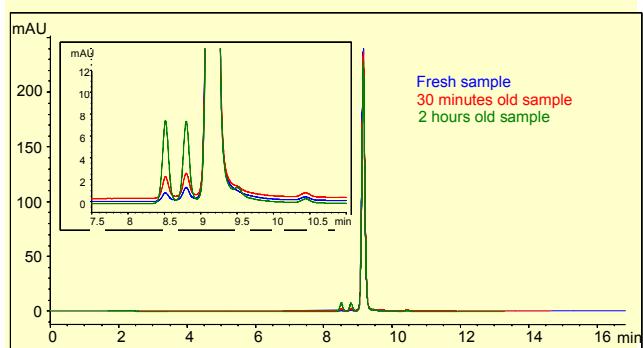
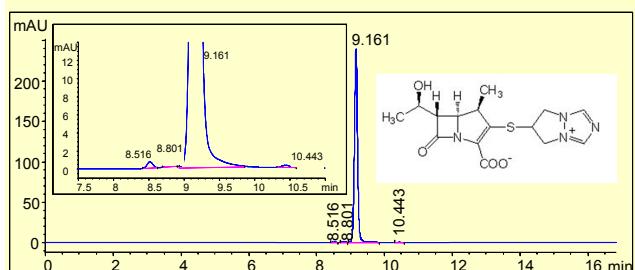
Figure 15. Separation of a four peptide mixture by Zenix SEC-80 column.



Column: Zenix SEC-80 (3 μ m, 7.8x300 mm)
 Mobile phase: 0.1% TFA/75% acetonitrile/H₂O
 Flow rate: 0.8 mL/min
 Detection: UV214 nm
 Injection: 5 μ L (0.5 mg/mL each peptide)
 Sample:
 1) insulin from porcine pancreas (7.75 min, MW 5,778),
 2) glucagon (8.03 min, MW 3,483),
 3) angiotensin I (8.45 min, MW 1,297),
 4) bradykinin (8.86 min, MW 1,060)

Separation of Antibiotic Biapenem

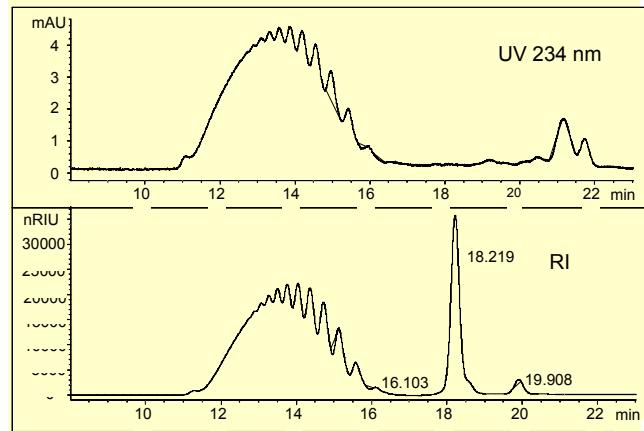
Figure 17. Separation of Antibiotic Biapenem on Zenix SEC-100 column.



Column: Zenix SEC-100 (3 μ m, 100 Å, 7.8x300 mm)
 Mobile phase: 150 mM sodium phosphate buffer, pH 7.0
 Flow rate: 1.0 mL/min
 Temperature: Ambient
 Detection: UV 215 nm
 Injection volume: 20 μ L
 Samples: Biapenem (0.2 mg/mL in mobile phase)

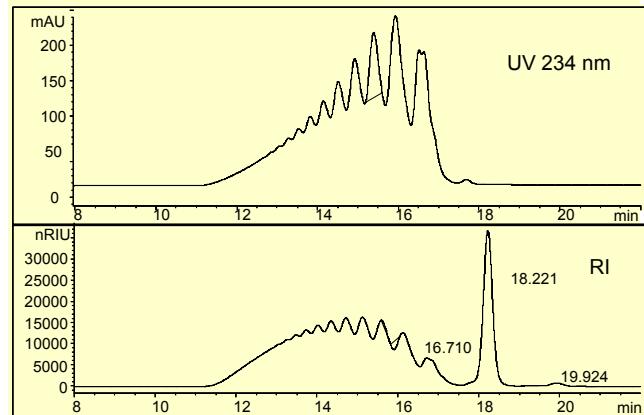
Analysis of Low Molecular Weight Samples

Figure 18. Analysis of low molecular weight heparin-dalteparin on Zenix SEC-100 column.



Column: Zenix SEC-100 (3 μ m, 100 Å, 7.8x300 mm)
 Flow rate: 0.5 mL/min
 Mobile phase: 2.84% Na₂SO₄, pH 5.0
 Detection: UV 234 nm and RI
 Injection volume: 25 μ L
 Samples: 10 mg/mL Dalteparin in water, pH 6.0, MW 3,000 – 8,000 Da

Figure 19. Analysis of low molecular weight enoxaparin on Zenix SEC-100 column.



Column: Zenix SEC-100 (3 μ m, 100 Å, 7.8x300 mm)
 Flow rate: 0.5 mL/min
 Mobile phase: 2.84% Na₂SO₄, pH 5.0
 Temperature: 35 °C
 Detection: UV 234 nm and RI
 Injection volume: 25 μ L
 Samples: 10 mg/mL Enoxaparin in mobile phase, MW 3,000 – 8,000 Da

Ordering Information

Zenix SEC-80 (3 µm, 80 Å)

Length x ID (mm)	P/N
21.2x300	SEP213080-21230
21.2x250	SEP213080-21225
21.2x150	SEP213080-21215
21.2x100	SEP213080-21210
21.2x50 (Guard)	SEP213080-21205
10x300	SEP213080-10030
10x250	SEP213080-10025
10x150	SEP213080-10015
10x100	SEP213080-10010
10x50 (Guard)	SEP213080-10005
7.8x300	SEP213080-7830
7.8x250	SEP213080-7825
7.8x150	SEP213080-7815
7.8x50 (Guard)	SEP213080-7805
4.6x300	SEP213080-4630
4.6x250	SEP213080-4625
4.6x150	SEP213080-4615
4.6x50 (Guard)	SEP213080-4605

Zenix SEC-300 (3 µm, 300 Å)

Length x ID (mm)	P/N
21.2x300	SEP213300-21230
21.2x250	SEP213300-21225
21.2x150	SEP213300-21215
21.2x100	SEP213300-21210
21.2x50 (Guard)	SEP213300-21205
10x300	SEP213300-10030
10x250	SEP213300-10025
10x150	SEP213300-10015
10x100	SEP213300-10010
10x50 (Guard)	SEP213300-10005
7.8x300	SEP213300-7830
7.8x250	SEP213300-7825
7.8x150	SEP213300-7815
7.8x50 (Guard)	SEP213300-7805
4.6x300	SEP213300-4630
4.6x250	SEP213300-4625
4.6x150	SEP213300-4615
4.6x50 (Guard)	SEP213300-4605

Zenix SEC-100 (3 µm, 100 Å)

Length x ID (mm)	P/N
21.2x300	SEP213100-21230
21.2x250	SEP213100-21225
21.2x150	SEP213100-21215
21.2x100	SEP213100-21210
21.2x50 (Guard)	SEP213100-21205
10x300	SEP213100-10030
10x250	SEP213100-10025
10x150	SEP213100-10015
10x100	SEP213100-10010
10x50 (Guard)	SEP213100-10005
7.8x300	SEP213100-7830
7.8x250	SEP213100-7825
7.8x150	SEP213100-7815
7.8x50 (Guard)	SEP213100-7805
4.6x300	SEP213100-4630
4.6x250	SEP213100-4625
4.6x150	SEP213100-4615
4.6x50 (Guard)	SEP213100-4605

Zenix SEC-150 (3 µm, 150 Å)

Length x ID (mm)	P/N
21.2x300	SEP213150-21230
21.2x250	SEP213150-21225
21.2x150	SEP213150-21215
21.2x100	SEP213150-21210
21.2x50 (Guard)	SEP213150-21205
10x300	SEP213150-10030
10x250	SEP213150-10025
10x150	SEP213150-10015
10x100	SEP213150-10010
10x50 (Guard)	SEP213150-10005
7.8x300	SEP213150-7830
7.8x250	SEP213150-7825
7.8x150	SEP213150-7815
7.8x50 (Guard)	SEP213150-7805
4.6x300	SEP213150-4630
4.6x250	SEP213150-4625
4.6x150	SEP213150-4615
4.6x50 (Guard)	SEP213150-4605

**Precolumn Filter 0.5 µm PEEK SEP102000-P356



Precolumn Filter

How to Order

www.dichrom.com

dichrom GmbH
Gräwenkolkstr. 66
45770 Marl
Germany
Tel. +49-2365-69926-0
Fax +49-2365-69926-11

E-Mail: info@dichrom.com
Internet: www.dichrom.com

