SEC SCREENING KIT

Zenix® -300 & Zenix® -C 300 SEC

P/N SECKIT-7830

QUALITY SYSTEM CERTIFICATION
ISO 9001:2008
DNV

Sepax

Sepax Technologies

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	Ion-exchange	Antibody Separation
SRT®, SRT®-C	Proteomix®	Antibodix®
Nanofilm®	Glycomix™	**Carbohydrate Separation**
Zenix® , Zenix® -C		Carbomix®

Analytical, Semi-prep and Preparative

Introduction

Zenix® , Zenix® -C SEC phases

These Phases are developed based on innovative surface coating technology comprised of uniform, hydrophilic, and neutral nanometer thick films chemically bonded to high purity and mechanically stabilized silica. The two different types of coatings, Zenix (stand-up monolayer bonded on porous silica) and Zenix-C (lay-down monolayer bonded on porous silica) offer ideal phase morphologies for specific sample type separation. The 3 µm based Zenix and Zenix-C allow for high resolution and performance separation. The combination of SEC phases provides a powerful total solution for robust, reproducible, and highest resolution size based separation of biological molecules in the market.

Stationary Phase Structure

Zenix ® Phase
Stand-up Monolayer

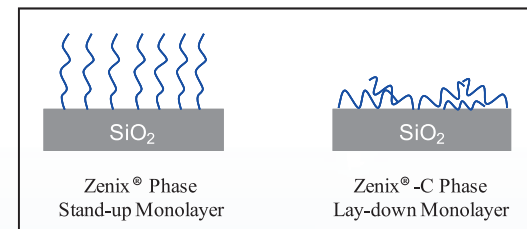Zenix ® -C Phase
Lay-down Monolayer

Figure 1. Phase structure difference: monolayer stands up on the surface for Zenix, and monolayer lays down on the surface for Zenix-C.

Key features of Zenix® ang Zenix®-C phases

Characteristics	Zenix	Zenix-C
Particle size	3 µm	3 µm
Pore size (Å)	300	300
Surface structure	Chemically bonded stand-up monolayer	Chemically bonded lay-down monolayer

Technical specifications of Zenix ® and Zenix ®-C 7.8x300 mm

Phase	Zenix SEC-300, Zenix-C SEC-300
Material	Neutral, hydrophilic film bonded silica
Particle size	3 µm
Pore size (Å)	~ 300
Protein MW range (native)	*5,000 – 1,250,000* Dalton
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)
Standard flow rate	1 mL/min
Backpressure for 7.8x300 mm (1.0 mL/min)	~ 1,100 psi
Maximum back pressure (psi)	~ 3,500
Salt concentration range	20 mM - 2.0 M
Maximum temperature (°C)	~ 80
Mobile phase compatibility	Aqueous and organic

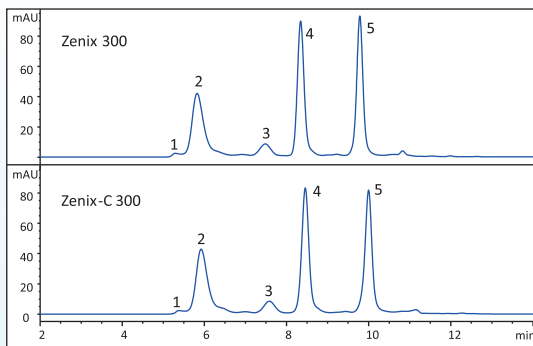### Protein separations on Zenix ® SEC-300 and Zenix ® -C SEC-300

Figure 2. Proteins Thyroglobulin, BSA and Ribonuclease A (2 mg/mL) separation on Zenix SEC-300 and Zenix -C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is 1 mL/min with 20 µL injection and UV 280 nm detection. Elution profile is as follows: peak 1- thyroglobulin aggregates; peak 2- thyroglobulin; peak 3-BSA dimer; peak 4-BSA monomer; peak 5-ribonuclease A.

Protein MW Calibration

Protein molecular weight vs. elution volume is plotted in Figure 3, indicating that Zenix and Zenix -C SEC-300 have large linear elution regions.
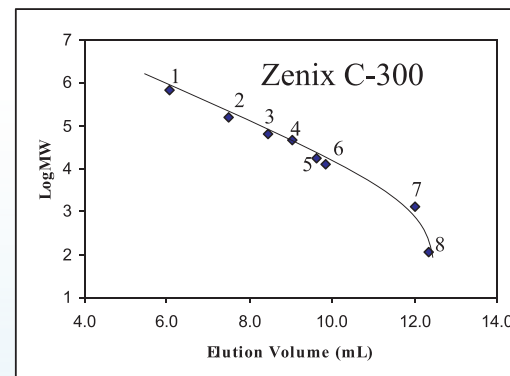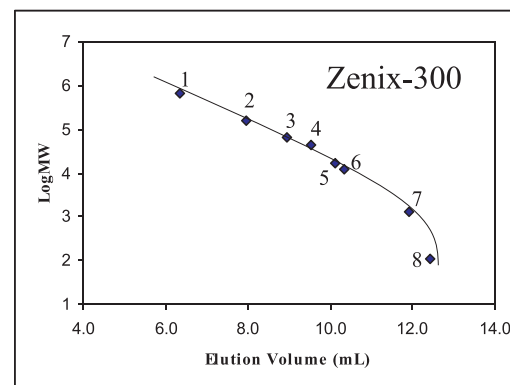

Figure 3. Protein MW calibration with elution volume for Zenix SEC-300 and Zenix - C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0, flow rate is 1.0 mL/min with detection at UV 214 nm. Samples: 1. Thyroglobulin, 670 kD; 2. γ-globulin, 158 kD; 3. BSA, 66 kD; 4. Ovalbumin, 44 kD; 5. Myoglobin, 17.6 kD; 6. Ribonuclease A, 13.7 kD; 7. Vitamin B12, 1.35 kD; 8. Uracil, 120 Da.

Applications

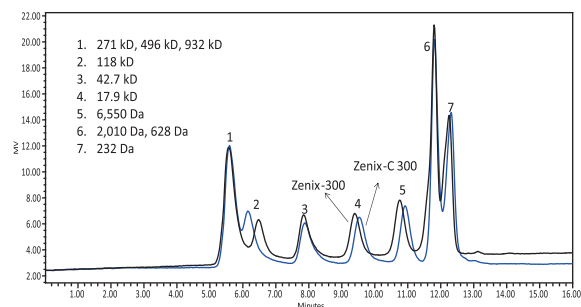## SEC screening for PEG standards

Figure 4. PEG standards separation on Zenix SEC-300 and Zenix -C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is a 1.0 mL/min with reflective index detection. Concentrations of all standards are 1mg/mL, except 932 kD standard which is 0.5 mg/mL in the mobile phase. Injection volume is 20 µL. There is no difference in separation resolution of PEG standards between the columns.

SEC screening for traditional monoclonal antibody

Figure 5. Monoclonal antibody separation on Zenix SEC-300 and Zenix -C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is 1.0 mL/min with UV 280 nm detection. MAb concentration is 1 mg/mL, injection volume is 10 µL. There is no difference in separation resolution for traditional monoclonal antibody between the columns.

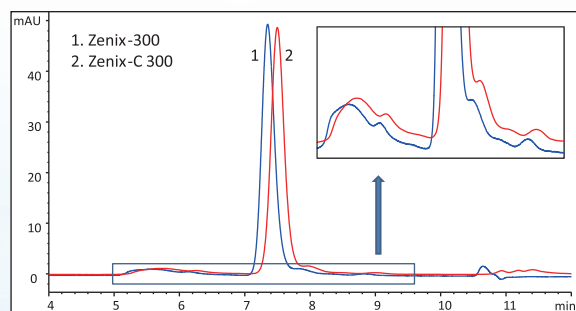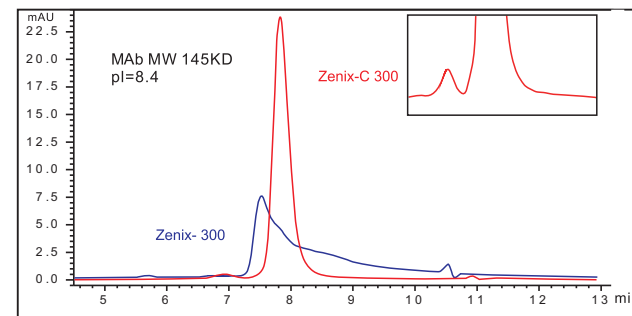## SEC screening for hydrophobic monoclonal antibody

Figure 6. Monoclonal antibody F separation on Zenix SEC-300 and Zenix-C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is 1.0 mL/min with UV 280 nm detection. MAb F concentration is 1.23 mg/mL in 10 mM sodium succinate, pH 5.0. Injection volume is 10 µL. Zenix-C SEC-300 minimizes the secondary interaction between the hydrophobic protein and column resin, thus improves the separation over traditional SEC column phase.
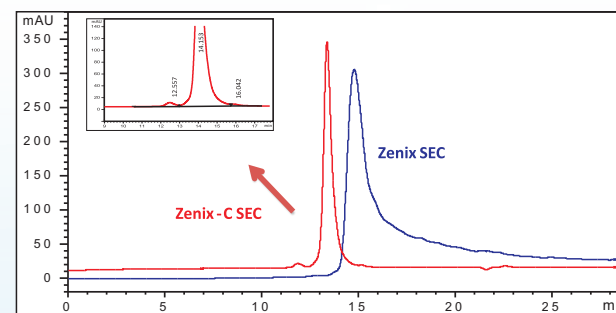
Fusion protein analysis

Figure 7. Fusion protein analysis on Zenix SEC-300 and Zenix-C SEC-300. Mobile phase is 150 mM phosphate buffer (pH 7.0) with 200 mM NaCl. Flow rate is 0.5 mL/min with UV 214 nm detection. Column temperature is 25 ℃. Injection volume is 10 µL. The sample is a fusion protein with 170 kD molecular weight and pI 6.8-7.0.Zenix-C SEC minimizesminimizes the sample secondary interaction and improves the separation with less tailing and better resolution between the aggregate and monomer of the fusion protein.
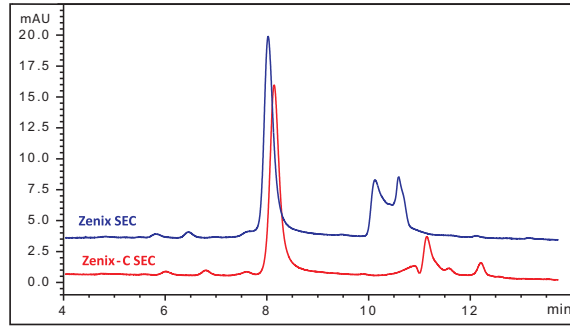
Glycoprotein analysis: β2 Glycoprotein I

Figure 8. β2 Glycoprotein I analysis on Zenix\ SEC-300 and Zenix-C SEC-300. Mobile phase is 150 mM Sodium phosphate buffer, pH 7.0. Flow rate is 1.0 mL/min. Sample β2 glycoprotein I has a concentration of 0.5 mg/mL in 1 M glycine, 750 mM NaCl, pH 7.4. Injection volume is 20 mL with UV 280 nm detection.

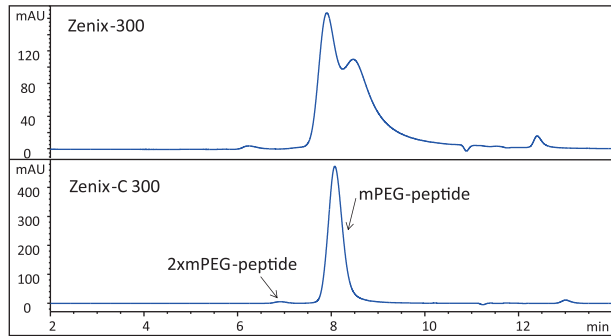## SEC screening for mPEG-peptide (20 kD PEG + 4 kD peptide)

Figure 9. MPEG Pegylated peptide (20 kD mPEG and 4 kD peptide) separation on Zenix SEC-300 and Zenix-C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is 1.0 mL/min with UV 214 nm detection. MPEG-Peptide concentration is 6 mg/mL, injection volume is 10 µL. The pegylated peptide has a stronger retention on Zenix SEC-300 which leads to a broad elution with two split peaks. Zenix-C SEC-300 shows a superior performance in this specific mPEG-peptide separation.

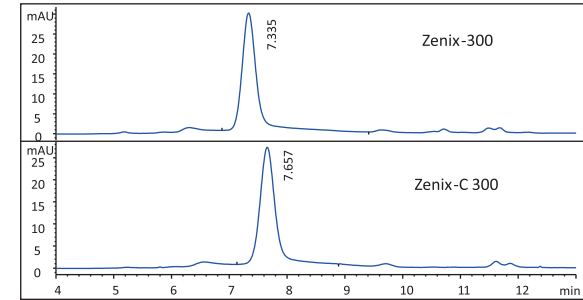## SEC screening for pegylated recombinant human growth hormone (mPEG-rhGH)

Figure 10. MPEG-rhGH (22 kD rhGH and 20 kD mPEG) separation on Zenix SEC-300 and Zenix-C SEC-300   (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is 1.0 mL/min with UV 280 nm detection. MPEG-rhGH concentration is 24 mg/mL, injection volume is 1 µL. Both columns show almost identical separation profiles.
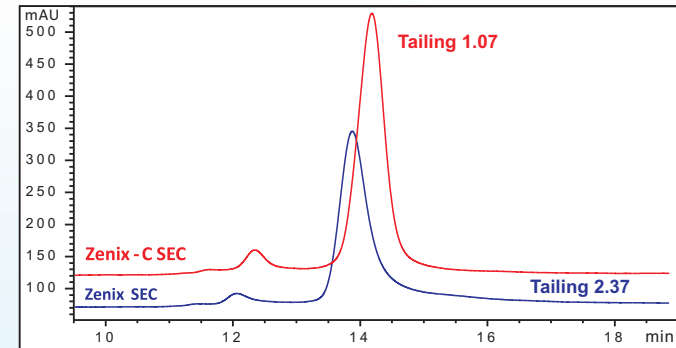
Pegylated exenatide separation

Figure 11. Pegylated exenatide analysis on Zenix SEC-300 and Zenix-C SEC-300 (7.8 x 300 mm). Mobile phase is 1:9 (v/v) mixture of acetonitrile and 50 mM ammonium acetate. Flow rate is 0.5 mL/min with UV 214 nm detection. Column temperature is 25 ℃. Injection volume is 15 µL. Sample is 3.3 mg/mL pegylated exenatide in water (PEG 23 kD).
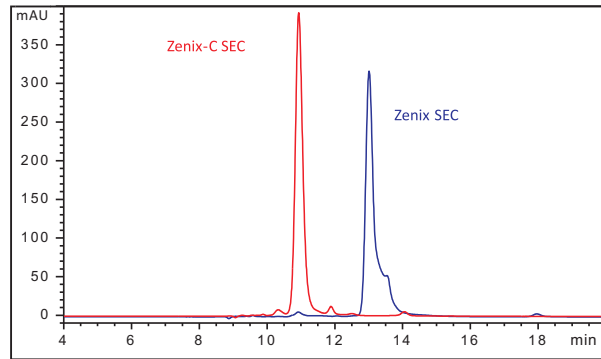
Angiotensin I acetate separation

Figure 12. Peptide angiotensin I acetate (1,297 Da) separation on Zenix SEC-80 and Zenix-C SEC-80 (7.8 x 300 mm). Flow rate is 1 mL/min with UV 214 detection. Mobile phase is 150 mM sodium phosphate buffer, pH 7.0. Injection volume is 5 µL.

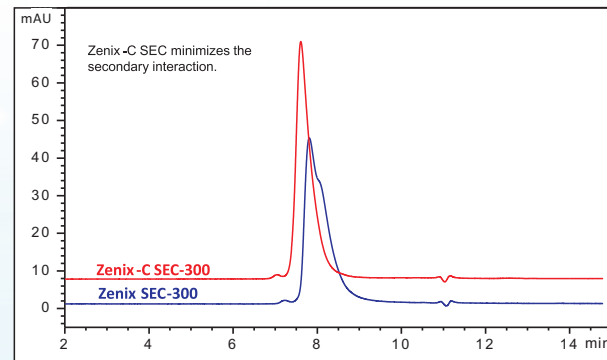## Human serum albumin conjugated peptide - SEC phase screening

Figure 13. HSA conjugated peptide analysis on Zenix SEC-300 and Zenix -C SEC-300 (7.8 x 300 mm). Mobile phase is 150 mM phosphate buffer, pH 7.0. Flow rate is 1.0 mL/min with UV 280 nm detection. Column temperature is 25 ℃, injection volume is 10 µL. HSA fusion peptide has a concentration of 5 mg/mL with MW 75 kD and pI 5.0.

Installation and Operation of Zenix®SEC-300 and Zenix®-C SEC-300 7.8 x 300 mm Column

1. Filter all samples and mobile phases through 0.45 µm or 0.2 µm filters before use.
2. Attach the column to your HPLC system following the flow direction as marked.
3. New columns are shipped in 50 mM sodium phosphate buffer, pH 7.0, 0.02% sodium azide. Run 10-20 column volumes of 50 mM sodium phosphate buffer at pH 7.0 to activate the column. Equilibrate the column with desired mobile phase until detection signal reaches baseline.
4. Inject desired amount of sample and run the column with desired flow rate.
5. Store columns in 50 mM sodium phosphate buffer, pH 7.0, w/ 0.02% sodium azide for long term storage.

Note: Solvent compatibility

　　Columns are compatible with aqueous buffers, such as phosphate, acetate, Tris, etc; and hydrophilic organic solvents, such as Methanol, ethanol, isopropanol, acetonitrile, THF, etc. When switching from an aqueous buffer to an organic solvent, the column should be washed with nanopure water for at least 30-column volume, then ethanol for 20-column volume. When switching from an organic solvent to an aqueous buffer, the column should be washed with ethanol for at least 30-column volume, then nanopure water for 20-column volume, and finally 20-column volume aqueous buffer. After washing, it is recommended that the column be stored in the aqueous buffer for 48 hours to get well equilibrated for satisfactory performance.

Troubleshooting

It is the user's responsibility to determine the optimum sample loading and running conditions to best utilize Zenix SEC-300 and Zenix-C SEC-300 columns. The following information is provided for reference to troubleshoot your experiments.

High back pressure

A sudden increase in backpressure suggests that the column inlet frit might be clogged. In this case it is recommended that the column be flushed in reverse flow with an appropriate solvent. To prevent the clogging, remove the particulates from samples and mobile phases by filtration.

Poor resolution

1. Column may be overloaded. Reduce sample injection amount.
2. Make sure the sample's molecular weight range falls in the separation range of the column. Both columns have a pore size of 300 Å with a separation range between 5,000 Da and 1,250,000 Da.
3. Two of the same columns in tandem may improve the resolution of close molecular weight separations.

Peak tailing

This may indicate secondary hydrophobic interaction between the sample and column matrix. To minimize the interaction, increase ionic strength of the mobile phase or add organic solvents (low percentage that do not change the protein conformation).

Samples with surfactants

Surfactants may irreversibly bind to the column matrix, which changes the matrix surface. This can result in column performance changes, such as retention time shift and altered peak shape for proteins with non-detergent mobile phases. Columns should be dedicated to the same surfactant application.

Column cleaning and regeneration

Zenix SEC-300 and Zenix-C SEC-300 columns may be contaminated by strongly adsorbed samples, which results in decreasing column performance. It is usually indicated by an increase in backpressure and a broader peak. When this happens, the general procedure for column cleaning is as follows:

1. Disconnect the column from the detector.
2. Clean your column in the reverse flow direction.
3. Run the column at less than 50% of the maximum recommended flow rate. Monitor the backpressure.
4. Run10-15 column volumes of cleaning solution. Run 3-5 cv Nanopure water between each solution.

The following cleaning solutions are recommended:

1. Concentrated salt (e.g., 0.5 M Na_2SO_4) at low pH (e.g., pH 3.0) to remove basic proteins.
2. Water soluble organic (MeOH, ACN, EtOH, 10%-20%) in aqueous buffer (e.g., 50 mM phosphate, pH 7.0) to remove hydrophobic proteins.
3. If both solutions fail to clean the column, use 6 M urea (filter before use) as follows:
    a. 2cv 6 M urea at 0.5 mL/min
    b. 20cv nanopure water at 0.5 mL/min
    c. 7-10cv mobile phase at 1 mL/min

For information regarding mobile phase optimization please visit the FAQ section of our website.

Column Protection

In addition to filtering the sample and the mobile phase, the best way to protect the column is to install a guard column or a pre-column filter in front of it. In most cases a pre-column filter helps to remove the residual particulates that are in the sample, the mobile phase, or leached from the HPLC system, such as pump and injector seals. However, a guard column is highly recommended because it is more effective in trapping highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system.

Ordering Information

Zenix ®

P/N	ID x Lenth (mm)	Pore Size (Å)
213080-2130	2.1x300	80
213080-4630	4.6x300	80
213080-7830	7.8x300	80
218030-10030	10.0x300	80
213080-21230	21.2x300	80
213100-2130	2.1x300	100
213100-4630	4.6x300	100
213100-7830	7.8x300	100
218030-10030	10.0x300	100
218030-21230	21.2x300	100
213150-2130	2.1x300	150
213150-4630	4.6x300	150
213150-7830	7.8x300	150
213150-10030	10.0x300	150
213150-21230	21.2x300	150
213300-2130	2.1x300	300
213300-4630	4.6x300	300
213300-7830	7.8x300	300
213300-10030	10.0x300	300
213300-21230	21.2x300	300

Zenix® -C

P/N	ID x Lenth (mm)	Pore Size (Å)
233100-4630	4.6x300	100
233100-7830	7.8x300	100
233100-10030	10.0x300	100
233100-21230	21.2x300	100
233150-4630	4.6x300	150
233150-7830	7.8x300	150
233150-10030	10.0x300	150
233150-21230	21.2x300	150
233300-4630	4.6x300	300
233300-7830	7.8x300	300
233300-10030	10.0x300	300
233300-21230	21.2x300	300

SEC Screening Kit (P/N SECKIT-7830) Includes:

P/N	ID x Length (mm)	Pore Size (Å)
213300-7830	7.8x300	300
233300-7830	7.8x300	300

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