Size Exclusion Chromatography for Small Biomolecule Separation

Zenix SEC-80
Zenix-C SEC-80

(3 µm, 80 Å)

Sepax Technologies

Content

Introduction

Zenix™ SEC phase

Developed based on innovative surface coating technology comprised of an uniform, hydrophilic, and neutral nanometer thick film chemically bonded on high purity and mechanically stabilized silica. The coating chemistry of Zenix™ SEC, with its stand-up monolayer bonded on porous silica, offers an ideal phase chemistry for high performance sized based separations. The 3 µm based Zenix™ provides a powerful solution for robust, reproducible, and highest resolution size based separations of biological molecules on the market.

Zenix™-C SEC phase

Developed based on innovative surface coating technology comprised of an uniform, hydrophilic, and neutral nanometer thick film chemically bonded on high purity and mechanically stabilized silica. The coating chemistry of Zenix™-C SEC, with its lay-down monolayer bonded on porous silica, offers an ideal phase chemistry for high performance sized based separations. The 3 µm based Zenix™-C provides a powerful solution for robust, reproducible, and highest resolution size based separations of biological molecules on the market.

Stationary Phase Structures

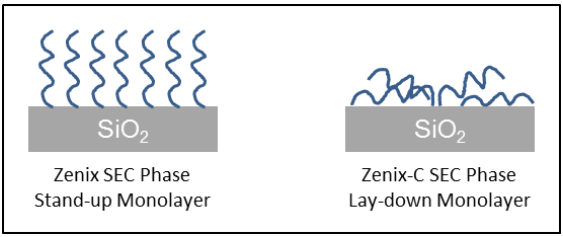


Figure 1. Phase structures for both the surface of Zenix™ SEC and Zenix™-C SEC.

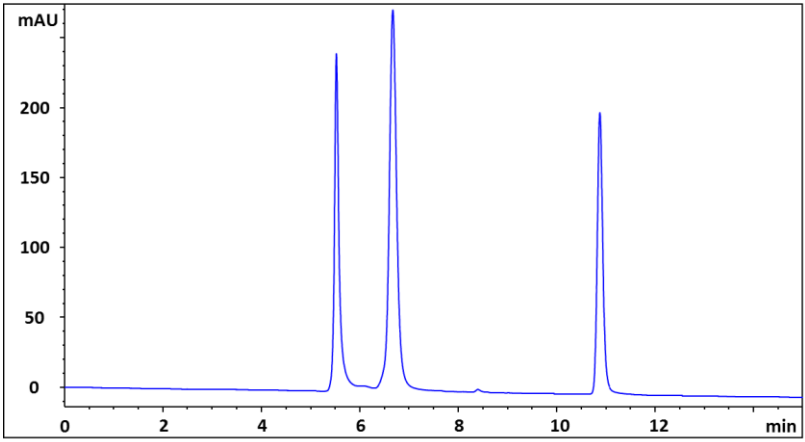
Key features of Zenix™ and Zenix™-C phases

Characteristics	Zenix™ SEC-80	Zenix™-C SEC-80
Particle size	3 µm	3 µm
Pore size (Å)	80	80
Surface structure	Chemically bonded stand-up monolayer	Chemically bonded lay-down monolayer

Technical specifications of Zenix™ SEC-80 and Zenix™-C SEC-80

Phase	Zenix™ SEC-80	Zenix™-C SEC-80
Material	Neutral, hydrophilic film bonded silica	Neutral, hydrophilic film bonded silica
Particle size	3 µm	3 µm
Pore size (Å)	~ 80	~ 80
pH stability	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)	2 – 8.5 (pH 8.5-9.5 can be tolerated temporarily.)
Maximum backpressure	~ 4,500 psi	~ 4,500 psi
Maximum temperature (°C)	~ 80	~ 80
Mobile phase compatibility	Aqueous and organic	Aqueous and organic
MW Range (native)	100 - 50,000 Da	100 - 50,000 Da

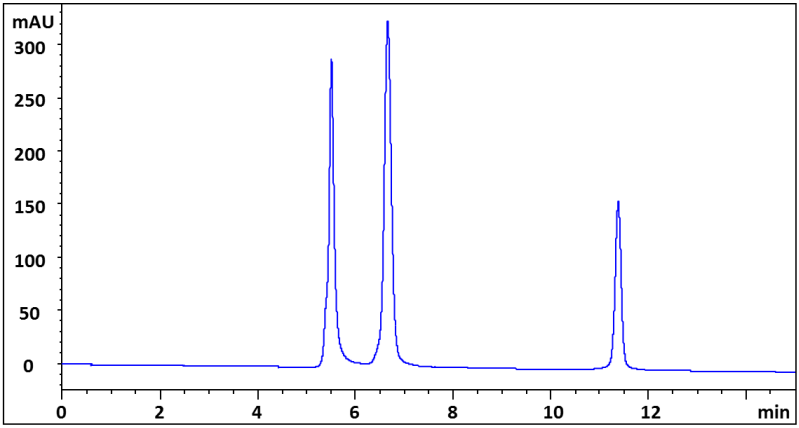
Quality Control Test for Zenix™ SEC-80 7.8 x 300 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
BSA	5.52	1676	22493	1.49	---
Ribonuclease A	6.67	3056	10253	1.05	5.57
Uracil	10.87	1630	46129	1.19	18.03

Figure 2. A standard quality control test on a Zenix™ SEC-80 7.8 x 300 mm. Mobile phase was 150 mM sodium phosphate buffer, pH 7.0. Flow rate was 1.0 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of BSA, Ribonuclease A and Uracil (1 mg/mL each). The pressure for this run was 101 bar.

Quality Control Test for Zenix™-C SEC-80 7.8 x 300 mm



Compound Name	RT (min)	Area	Plates	Tailing	Resolution
BSA	5.51	2546	15999	1.12	---
Ribonuclease A	6.66	3572	10452	0.95	5.29
Uracil	11.38	1441	43021	0.97	19.64

Figure 3. A standard quality control test on a Zenix™-C SEC-80 7.8 x 300 mm. Mobile phase was 150 mM sodium phosphate buffer, pH 7.0. Flow rate was 1.0 mL/min. UV detection was set at 214 nm. 5 µL of sample was injected and the sample is a mixture of BSA, Ribonuclease A and Uracil (1 mg/mL each).

Difference between Zenix™ SEC-80 and Zenix™-C SEC-80 Phases

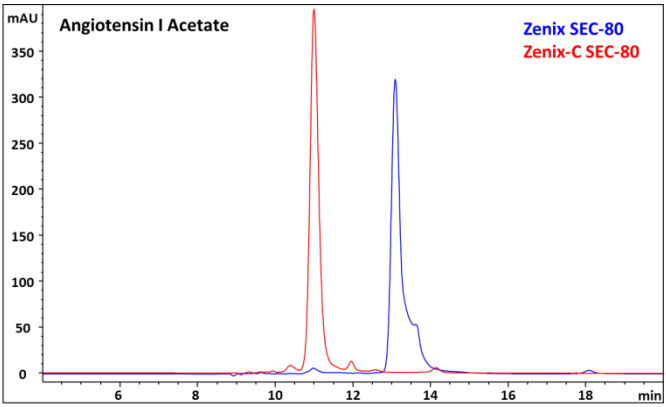


Figure 4. Overlay of angiotensin I acetate analyzed on both Zenix™ SEC-80 and Zenix™-C SEC-80 7.8 x 300 mm. Mobile phase was 150 mM sodium phosphate buffer, pH 7.0. Flow rate was 1.0 mL/min. UV detection was set at 214 nm. 5 µL of Angiotensin I acetate (1,297 Da) at 1 mg/mL was injected.

Similarity between Zenix™ SEC-80 and Zenix™-C SEC-80 Phases

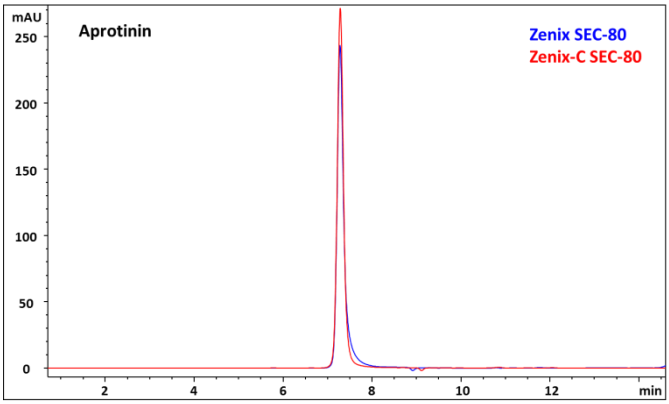


Figure 5. Overlay of aprotinin analyzed on both Zenix™ SEC-80 and Zenix™-C SEC-80 7.8 x 300 mm. Mobile phase was 150 mM sodium phosphate buffer, pH 7.0. Flow rate was 1.0 mL/min. UV detection was set at 214 nm. 5 µL of Aprotinin (6,500 Da) at 1 mg/mL was injected.

Mobile Phase Comparison for the Analysis of Insulin on Zenix™ SEC-80

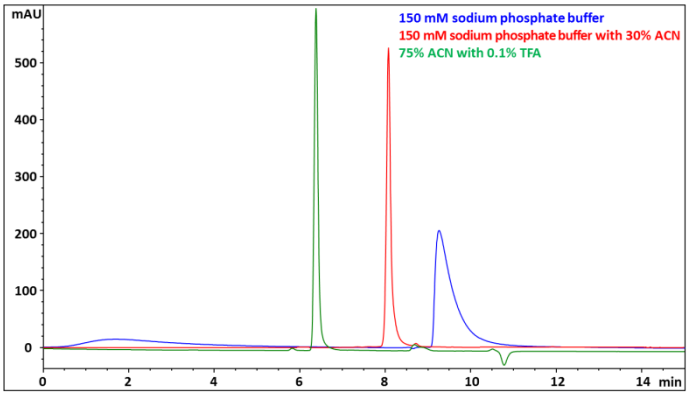


Figure 6. Overlay of different mobile phases used for the analysis of insulin on Zenix™ SEC-80 7.8 x 300 mm. Mobile phases were 150 mM sodium phosphate buffer pH 7.0, 150 mM sodium phosphate buffer with 30% acetonitrile, and 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of insulin (5,778 Da) at 1 mg/mL was injected each run.

Mobile Phase Comparison for the Analysis of Insulin on Zenix™-C SEC-80

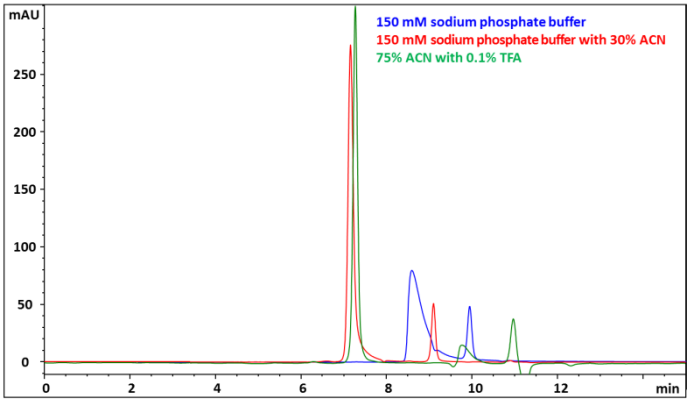


Figure 7. Overlay of different mobile phases used for the analysis of insulin on Zenix™-C SEC-80 7.8 x 300 mm. Mobile phases were 150 mM sodium phosphate buffer pH 7.0, 150 mM sodium phosphate buffer with 30% acetonitrile, and 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of insulin (5,778 Da) at 1 mg/mL was injected each run.

Sample Overlays on Zenix™ SEC-80 7.8 x 300 mm

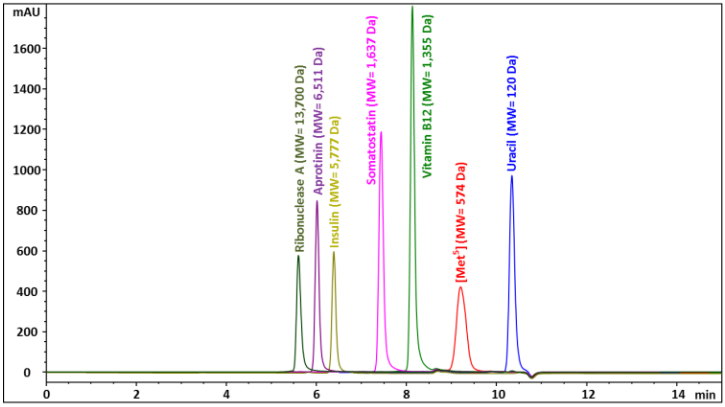


Figure 8. Overlay of different sample injections on Zenix™ SEC-80 7.8 x 300 mm. Mobile phase was 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of sample (1 mg/mL each) was injected for every run.

Sample Overlays on Zenix™-C SEC-80 7.8 x 300 mm

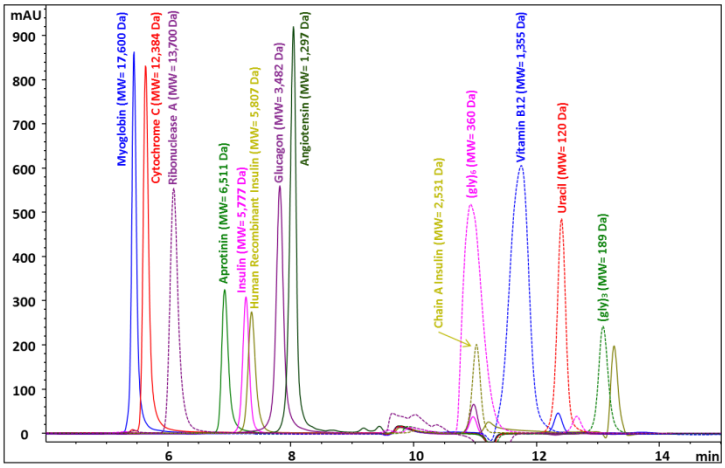


Figure 9. Overlay of different sample injections on Zenix™-C SEC-80 7.8 x 300 mm. Mobile phase was 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of sample (1 mg/mL each) was injected for every run.

Calibration Curve for Zenix™ and Zenix™-C SEC-80

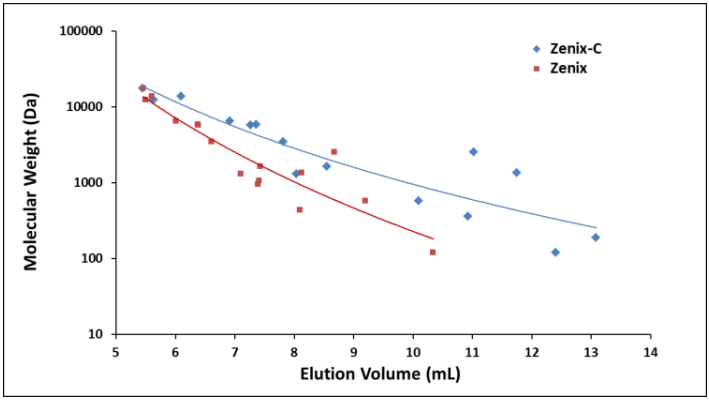


Figure 10. Calibration curves for Zenix™ SEC-80 and Zenix™-C SEC-80 7.8 x 300 mm. Mobile phase was 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of sample (1 mg/mL each) was injected for every run.

Fast Analysis using a Zenix™ SEC-80 2.1 x 50 mm for Mass Spec

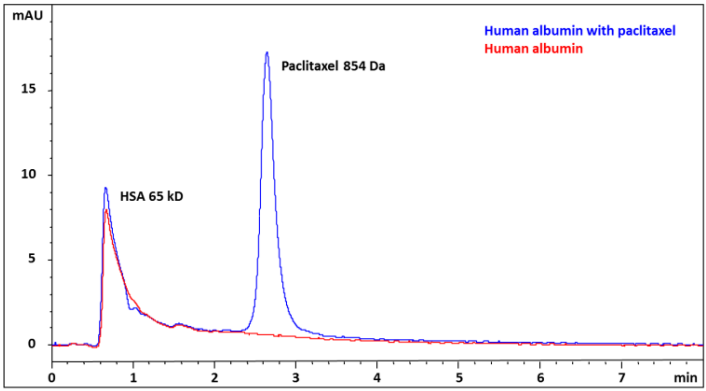


Figure 11. Fast Analysis using a Zenix™ SEC-80 2.1 x 50 mm for mass spec. Flow rate was 0.2 mL/min and detection was set at UV 228 nm. The mobile phase was 50 mM ammonium acetate with 30% acetonitrile. 0.1 µL of each sample was injected for analysis.

Percentage of Organic Additive for Peptide Separation on Zenix™ SEC-80

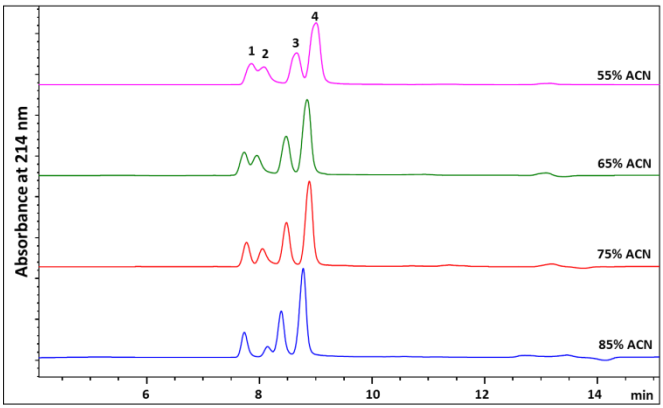
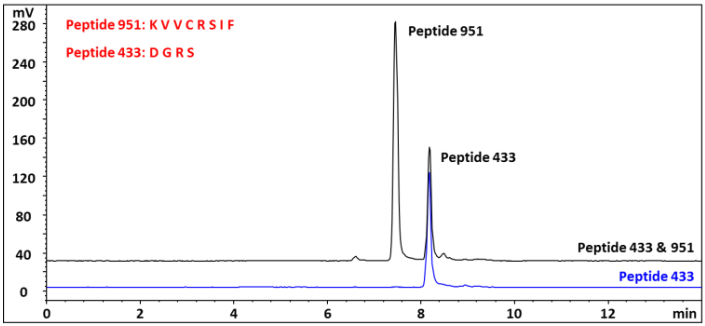


Figure 12. Varying amounts of acetonitrile added into the mobile phase for the separation of a peptide mixture on Zenix™ SEC-80 7.8 x 300 mm. Mobile phases were the indicated amount of acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 0.8 mL/min. UV detection was set at 214 nm and 5 µL of sample was injected for every run. The sample was a mixture of Insulin (5,778 Da), Glucagon (3,483 Da), Angiotensin I acetate (1,297 Da) and Bradykinin (1,060 Da) at 0.5 mg/mL each.

Analysis of two Peptides on Zenix™ SEC-80 7.8 x 300 mm



Name	Retention Time (min)	Area	Resolution	Tailing	Plate Count
Peptide 951	7.460	1373189		1.121	30778
Peptide 433	8.187	741998	4.4	1.062	43397

Figure 13. Analysis of two peptides on Zenix™ SEC-80 7.8 x 300 mm. Flow rate was 1.0 mL/min and detection was done using an ELSD (temperature at 65 ℃, gas flow was 2 L/min and gain was 1). The mobile phase was 75% acetonitrile with 0.1% TFA. 10 µL of sample was injected for each run. The samples were Peptide 951 (951 Da) and Peptide 433 (433 Da) each at a concentration on 0.5 mg/mL in water.

Analysis of E.coli Tryptic Digest on Zenix™ SEC-80

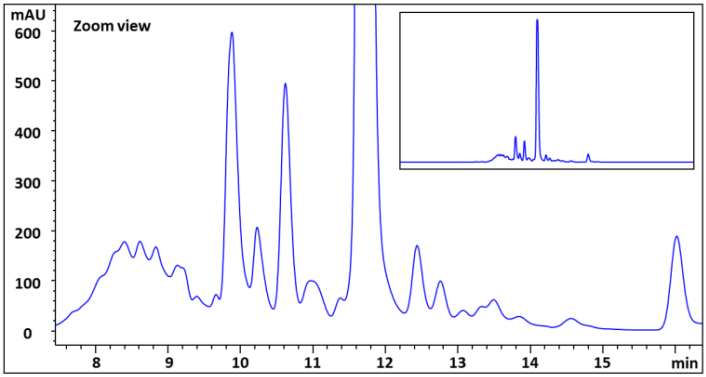


Figure 14. Analysis of E.coli Tryptic Digest on Zenix™ SEC-80 7.8 x 300 mm. Mobile phase was 25 mM Sodium Acetate with 300 mM NaCl pH 4.5. Flow rate was 0.8 mL/min. UV detection was set at 214 nm and 40 µL of sample (20 µg of digested protein) was injected for every run.

Column Lifetime Test on Zenix™ SEC-80 7.8 x 300 mm

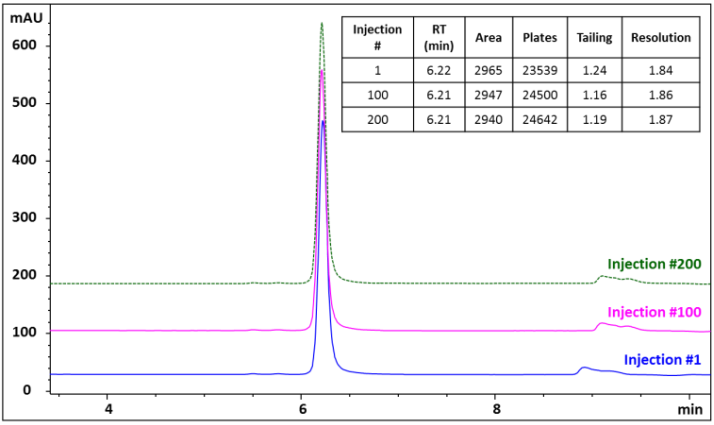
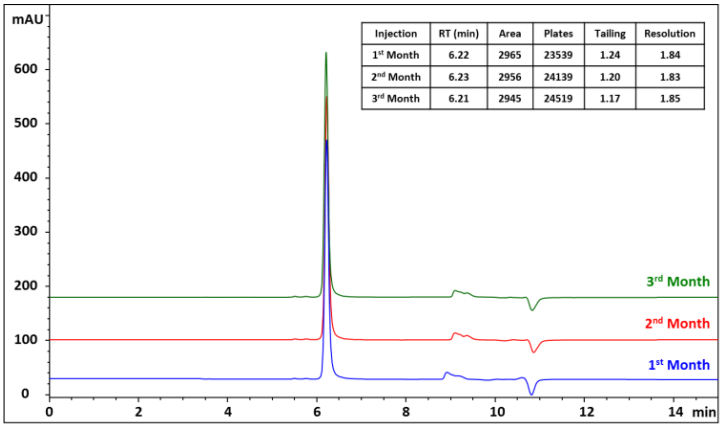


Figure 15. Overlay of aprotinin injections on Zenix™ SEC-80 7.8 x 300 mm. Mobile phase was 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of aprotinin (6,500 Da) at 1 mg/mL was injected each run.

Aprotinin Analysis on Zenix™ SEC-80 after Storage in 20% Acetonitrile



Injection	RT (min)	Area	Plates	Tailing	Resolution
1st Month	6.22	2965	23539	1.24	1.84
2nd Month	6.23	2956	24139	1.20	1.83
3rd Month	6.21	2945	24519	1.17	1.85

Figure 16. Overlay of each month's aprotinin injections on Zenix™ SEC-80 7.8 x 300 mm. Mobile phase was 75% acetonitrile with 0.1% trifluoroacetic acid. Flow rate was 1.0 mL/min. UV detection was set at 214 nm and 5 µL of aprotinin (6,500 Da) at 1 mg/mL was injected for each run.

Column Installation and Operation for Zenix™ SEC-80 and Zenix™-C SEC-80 7.8 x 300 mm

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 150 mM sodium phosphate buffer, pH 7.0. 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 150 mM sodium phosphate buffer, pH 7.0 for long term storage.

Note: Solvent compatibility

Zenix™ SEC and Zenix™-C SEC columns are compatible with aqueous buffers such as phosphate, acetate, Tris, etc.; and water miscible organic solvents, such as MeOH, ethanol, isopropanol, acetonitrile, THF, etc. When switching from an aqueous buffer to an organic solvent, the column should be washed with nanopure water for about 2-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 2-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 for Zenix™ SEC-300 and Zenix™-C SEC

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 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 blocked. 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 with filtration.

Poor resolution

1. Column may be overloaded. Reduce sample injection.
2. Make sure the sample's molecular weight range falls in the separation range of the column. Both columns have a pore size of 80Å with a separation range between 100 Da and 50,000 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 if native protein conformation needs to be maintained).

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 and Zenix™-C SEC 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. 10-15 column volumes of cleaning solution are sufficient. Run 2 column volumes of nanopure water between each solution.

The following cleaning solutions are recommended:

1. Concentrated neutral 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).
    a. 2 cv 6 M urea at 0.5 mL/min
    b. 2 cv nanopure water at 0.5 mL/min
    c. 7-10 cv 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 separation 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™ SEC-80 (3 µm, 80 Å)

P/N	ID x Length (mm)
213080-4605	4.6 x 50 (guard)
213080-4615	4.6 x 150
213080-4625	4.6 x 250
213080-4630	4.6 x 300
213080-7805	7.8 x 50 (guard)
213080-7815	7.8 x 150
213080-7825	7.8 x 250
213080-7830	7.8 x 300
213080-10005	10 x 50 (guard)
213080-10015	10 x 150
213080-10025	10 x 250
213080-10030	10 x 300
213080-21205	21.2 x 50 (guard)
213080-21215	21.2 x 150
213080-21225	21.2 x 250
213080-21230	21.2 x 300

Zenix™-C SEC-80 (3 µm, 80 Å)

P/N	ID x Length (mm)
233080-4605	4.6 x 50 (guard)
233080-4615	4.6 x 150
233080-4625	4.6 x 250
233080-4630	4.6 x 300
233080-7805	7.8 x 50 (guard)
233080-7815	7.8 x 150
233080-7825	7.8 x 250
233080-7830	7.8 x 300
233080-10005	10 x 50 (guard)
233080-10015	10 x 150
233080-10025	10 x 250
233080-10030	10 x 300
233080-21205	21.2 x 50 (guard)
233080-21215	21.2 x 150
233080-21225	21.2 x 250
233080-21230	21.2 x 300

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