



## SRT SEC Column Manual

### Column Information

Utilizing proprietary surface technologies, Sepax SRT SEC phases are made of the uniform, hydrophilic, and neutral nanometer thick films chemically bonded on the high purity and enhanced mechanical stability silica. The Sepax proprietary surface technologies allow the chemistry of thin film formation well controlled, which results in high column-to-column reproducibility. The nature of the chemical bonding and the maximum bonding density of the thin film benefit SRT SEC phases with high stability. The uniform surface coating enables high efficiency separation. The narrow dispersed, spherical silica particles of the SRT packings for SEC-100, SEC-150, SEC-300, SEC-500, SEC-1000 and SEC-2000 have nominal pore sizes at 100 Å, 150 Å, 300 Å, 500 Å, 1,000 Å, and 2,000 Å, respectively. Their specially designed large pore volume (ca. 1.35 mL/g for SRT SEC-150, 300 and 500, and ca. 1.0 mL/g for SRT SEC-100, 1000 and 2000) enables high separation capacity, leading to high separation resolution. SRT SEC columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency.

Combining the innovative surface chemistry with their widest selection of pore size from 100Å to 2,000 Å, SRT SEC phases are designed to ensure highest resolution and maximum recovery for a very broad range of separation applications. The separation applications cover large biological molecules, such as proteins and nucleic acids, small biological molecules, such as peptides and oligonucleotides, natural polymers, such as polysaccharides, synthetic polymers, biological cells, such as bacteria and virus, and nanomaterials, such as nanoparticles. Typical applications for SRT SEC columns are the separation and detection in aqueous buffer mobile phases.

### Column Stability and Performance

The SRT SEC columns use full coverage bonded silica packing, which allows exceptional high stability. They are compatible with most aqueous buffers, such as ammonium acetate, phosphate, tris and so on. When 150 mM phosphate buffer at pH 7.0 was used as the mobile phase to run SRT SEC columns, 100 injections or 1 month of usage has negligible deterioration for the SRT SEC columns.

The neutral and hydrophilic SRT stationary phases have negligible nonspecific interactions with biological molecules, especially proteins. Combined with their high capacity, the SRT SEC columns enable high efficiency and high recovery separations. A typical test chromatogram for quality control is shown in Figure 1 for 4.6x300mm SRT SEC columns.

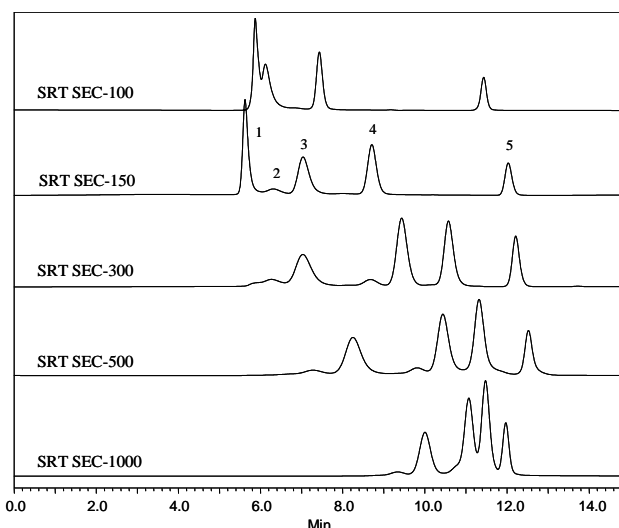


Figure 1. Elution profiles of a protein mixture by SRT SEC-100, SRT SEC-150, SRT SEC-300, SRT SEC-500 and SRT SEC-1000 columns (5µm, 4.6x300mm). The separation conditions: mobile phase, 150 mM Phosphate buffer, pH 7.0; flow rate, 0.35 mL/min; detection: UV 214 nm; temperature, ambient (23 °C); and injection volume, 3 µL. The protein mixture: (1) Thyroglobulin (1.0 mg/mL), 670 kD; (2) BSA dimer, 132 kD; (3) BSA (1.0 mg/mL), 66 kD; (4) Ribonuclease A (1.0 mg/mL), 13.7 kD, and (5) Uracil (2.5 µg/mL), 120.

### Column Characteristics

Silica: *Spherical, high purity (<10 ppm metals)*

Particle size: 5 µm

Pore size for protein separation:

100 Å, MW range 100 ~ 100,000

150 Å, MW range 500 ~ 150,000

300 Å, MW range 5,000 ~ 1,250,000

500 Å, MW range 15,000 ~ 5,000,000

1000 Å, MW range 50,000 ~ 7,500,000

2000 Å, MW range >10,000,000

### Safety Precaution

The columns are normally operated under moderate pressure. Loose connections will cause leaking of organic solvents and injected samples, all of which should be considered as the hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When open the columns, proper

protections should be used to avoid inhalation of the small silica particles.

## Column Installation and Operation

When column is shipped or not in use, it is always capped at both ends. When install the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has special purpose to reverse the flow direction, for example, removal of the inlet pluggage, follow the flow direction as labeled. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

(a) Place the male nut and ferrule, in order, onto a 1/16" o.d. piece of tubing. Be certain that the wider end of the ferrule is against the nut.

(b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.

(c) Repeat this coupling procedure for the other end of the column.

## Samples and Mobile Phases

To avoid clogging the column, all samples and solvents including buffers should be filtered through 0.45  $\mu\text{m}$  or 0.2  $\mu\text{m}$  filters before use. The *SRT SEC* columns are compatible with aqueous mobile phase or a mixture of organic and water, such as methanol or acetonitrile and water. Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

## Column Care

**Shipping Solvent** New columns are shipped in 0.15 M sodium phosphate buffer, pH 7.0. During stocking and shipping, the silica packing could be dried out. It is recommended that 10-20 column volumes of 0.15 M sodium phosphate buffer at pH 7.0 be purged to activate the column. Flush the column with your mobile phase with gradual increasing the flow rate from 0.1 mL/min to your operation condition, until the baseline is stable. If the column backpressure and baseline fluctuate, this might be due to the air bubbles trapped inside the column. Flush the column with higher flow rate for 2-5 minutes, for example 0.5 mL/min for a 4.6x300 mm column.

**PH** The optimum performance and operation for longest lifetime are at pH 2 - 8.5.

**Pressure** Even though the columns can operate at pressure up to 3,500 psi, the normal operation is usually under 2,000 psi. Continuous use at high pressure may eventually damage the column. Since the pressure is generated by the flow rate. The maximum flow rate is limited by the backpressure. It is expected that the backpressure might gradually increase with its service. A sudden increase in backpressure suggests that the column inlet frit might be plugged. In this case it is recommended that the column be flushed with reverse flow in an appropriate solvent.

**Temperature** The maximum operating temperature is 80°C. The optimum temperature operation for longest lifetime is 10 -

30°C. Continuous use of the column at higher temperature (>80°C) can damage the column, especially under high pH (>8).

**Flow rate Range** Normal operation is 0.1 - 0.4 and 0.1 - 1.25 mL/min for 4.6 mm and 7.8 mm I.D. columns, respectively.

**Storage** When not in use for extended time, store the column in 0.15 M sodium phosphate buffer, pH 7.0, w/ 0.02% sodium azide. Each column is shipped with two removable end plugs. To prevent the drying of the column bed, seal both ends of the column with the end plugs provided.

**Cleaning** From time to time, some samples could get adsorbed onto the inlet frit or the packing material. When the adsorption accumulates to a certain level, it is usually indicated by that the backpressure is increased and the peak becomes broader. When this occurs, it is time to clean your column. The general guidelines for column cleaning are the followings.

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. If you see the pressure is much higher than the normal operating conditions, you need to lower the flow rate or change the washing buffer as the cleaning solutions may be of different viscosities.
4. Typically, 10-15 column volumes of cleaning solution are sufficient. Rinse well with 3-5 column volume of distilled, deionized water between each solution.

**Cleaning solutions** Some general guidelines are recommended for choosing cleaning solutions here. A low pH solution will help to remove basic proteins. Organics will help to remove hydrophobic proteins. Chaotropic agents will help to remove strongly adsorbed materials (e.g., via hydrogen bonding). Only use chaotropic agents when neutral salt or organic has not improved resolution. Two cleaning solutions are recommended for general cleaning as following.

1. Concentrated neutral salt (e.g., 0.5 M  $\text{Na}_2\text{SO}_4$ ) at low pH (e.g., pH 3.0)
2. Water soluble organic (MeOH, ACN, EtOH, 10 %-20 %) in aqueous buffer (e.g., 50 mM phosphate, pH 7.0)

## 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 in the sample or the mobile phase, or leached from HPLC system, such as pump and injector seals. However, a guard column is highly recommended because it will more effectively trap highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system.