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Unix 300 SEC Column Manual

Column Information

Utilizing proprietary surface technologies and 1.8 µm particle size, Unix SEC phases are made of uniform, hydrophilic, and neutral nanometer thick films chemically bonded on the high purity and enhanced mechanical stability silica. The proprietary surface technologies allow the chemistry of thin film formation to be 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 Unix SEC phases with high stability. The uniform surface coating enables high efficiency separation. The narrowly dispersed, spherical silica particles of the Unix packing have nominal pore size of 300 Å. With a small particle size of 1.8 µm and specially designed large pore volume, Unix phases have achieved unprecedented high separation efficiency and resolution. Unix SEC columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency.

Unix SEC phases are designed to ensure highest resolution and maximum recovery for a broad range of separation applications. Typical applications for Unix SEC columns are separation and detection in aqueous buffer mobile phases.

Column Stability and Performance

Unix SEC columns use full coverage bonded silica packing, which allows exceptionally high stability. They are compatible with most aqueous buffers, such as ammonium acetate, phosphate, tris, etc.

The neutral and hydrophilic Unix stationary phases have negligible nonspecific interactions with biological molecules, especially proteins. Combined with their high capacity, Unix SEC columns enable high efficiency and high recovery separations. A typical quality control chromatogram is shown in Figure 1 for a 4.6 x 150 mm Unix SEC column.

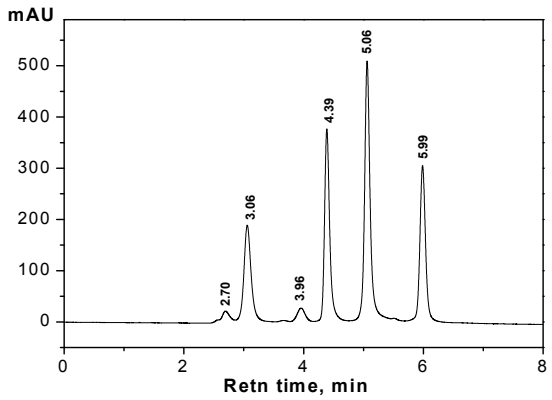
Column Characteristics

Silica: *Spherical, high purity (<10 ppm metals)*
Particle size: *1.8 µm*
Pore size: *300 Å*

Safety Precaution

The columns are normally operated under moderate pressure. Loose connections will cause leaking of buffers and injected samples, all of which should be considered as hazards. In the case of leaking, proper gloves should be worn while handling the columns. When opening the columns, proper protections should be used to avoid inhalation of the small silica particles.

Figure 1. Elution profiles of a protein mixture by Unix SEC-300 phase.



Column: 1.8 µm, 4.6 x 150 mm
Mobile phase: 150 mM Sodium Phosphate Buffer, pH 7.0
Flow rate: 0.35 mL/min
Temperature: Ambient (~23° C)
Detection: UV 214 nm
Injection: 3 µL
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 (0.1 mg/mL), 120 D.

Column Installation and Operation

The column should always be capped at both ends when it is not in use. When installing the column to the system, first remove the end caps. Unless a user has special purpose to reverse the flow direction, for example, removal of the inlet blockage, follow the flow direction as marked on the column. 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" outer diameter 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 finger-tighten 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 should be filtered through 0.45 μm or 0.2 μm filters before use. Unix SEC columns are compatible with aqueous mobile phases or a mixture of organic solvent 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 150 mM sodium phosphate buffer, pH 7.0. During stocking and shipping, the silica packing may become dried out. It is recommended that 10-20 column volumes of 150 mM sodium phosphate buffer at pH 7.0 be purged to activate the column.

Column Installation Flush the column with your mobile phase while gradually increasing the flow rate from 0.1 mL/min to your operating flow rate allowing the column to equilibrate the pressure until the baseline is stable at each step. If the column back pressure and baseline fluctuate, this might be due to air bubbles trapped inside the column. Flush the column for longer time until the back pressure and base line are stable.

Pressure Even though the columns can operate at a pressure up to 4,500 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 back pressure. It is expected that the back pressure might gradually increase with its service. A sudden increase in back pressure suggests that the column inlet frit might be clogged. In this case it is recommended that the column be flushed with reverse flow in an appropriate solvent.

Flow Rate Standard operating flow rate is 0.1 - 0.35 mL/min.

pH For optimum performance and lifetime keep pH between 2.0 and 8.5.

Temperature The maximum operating temperature is 80 °C. The optimum operating temperature for the longest lifetime is 10 – 30 °C. Continuous use of the column at higher temperatures (>30 °C) can damage the column, especially under high pH (>8.0).

Storage When the column is not in use for an extended time, the column should be stored in water (H_2O) with 0.02% sodium azide or 10% methanol. Each column is shipped with two removable end plugs. To prevent 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 an increase in back pressure and a broader peak. When this occurs, it is time to clean your column. The general procedure for column cleaning is as follows:

1. Disconnect the column from the detector.
2. Clean the column in the reverse flow direction.
3. Run the column at below 50% of the maximum recommended flow rate. Monitor the back pressure. 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 nanopure water between each solution.

Cleaning Solutions Low pH salt solutions help remove basic proteins. Organics are useful when removing hydrophobic proteins. Chaotropic agents help to remove strongly adsorbed materials (via hydrogen bonding). Only use chaotropic agents when neutral salts or organics have not improved resolution. Two cleaning solutions are recommended for general cleaning:

1. Concentrated neutral salt (e.g., 0.5 M Na_2SO_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 sodium phosphate, pH 7.0)

If both solutions fail to clean the column, use 6 M urea (filter before use).

a. 2 cv 6 M urea at 0.35 mL/min (monitor pressure)
b. 3 cv nanopure water at 0.35 mL/min
c. 7 cv mobile phase at 0.35 mL/min

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/UHPLC 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/UPLC system.