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Sepax Proteomix® RP Column Manual

Column Information

Proteomix® RP packings are porous polystyrene divinylbenzene (PS/DVB) polymer resins with narrow-dispersed particle size distribution. The base matrix of the resin is made of highly cross-linked PS/DVB which functions as reversed phase for chromatographic separation. The phase structure is phenyl and substituted phenyl functional groups that enable hydrophobic interaction. These highly rigid resins are provided with the particle size selection of 5, and 10 µm. In comparison to silica based reversed phase media, Proteomix® RP phases have advantages for biomolecule separations with wide pH range (1-14) and high thermal stability. At various column temperatures, the Proteomix® RP phases offer special selectivity, high resolution separation with online mass spec analysis capability for proteins such as mAb (monoclonal antibody), ADC (antibody drug conjugate) and the related protein fragments.

Characteristics

Support: spherical, PS/DVB particles
Phase structure: phenyl and substituted phenyl
Pore size: non-porous, 500 and 1000 Å
Particle size: 5 and 10 µm for porous
Pore volume: ~1.0 mL/g for porous resins
Application pH range: 1-14
Operating temperature: up to 80 ˚C
Operating pressure: up to 200 bar
Mobile Phase Compatibility: compatible with aqueous solution, a mixture of water and acetonitrile, acetone, methanol, or THF

Column Stability and Performance

Proteomix® RP columns are highly stable over a variety of operational conditions. They are stable to resist high temperatures up to 200 ˚C. They are compatible with many commonly used organic solvents and aqueous buffers. Solvents can be changed without damaging the column. Proteomix® RP columns have a long life time-negligible deterioration after 3 months of standard usage. With a well-controlled polymer resin manufacturing process and column packing process, Proteomix® RP columns are very reproducible from batch to batch. Good cleaning procedure has been used to remove the residual monomers and surfactants, resulting in highly pure reversed phase surface without leaching during use. Compared to silica based reversed phases, Proteomix® RP phase is more stable at extreme pH (1-14) with the similar separation efficiency and better selectivity. An example of protein separation is shown in Figure 1 for a 4.6 x 150 mm Proteomix® RP-1000 (5 µm) column.

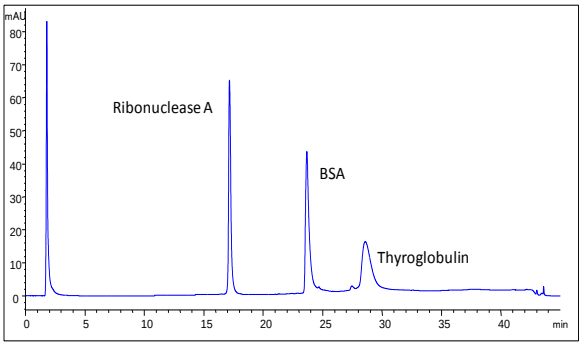

Figure 1. A test chromatogram of a Proteomix® RP-1000 column (5µm, 1000Å, 4.6 x 150 mm)
Column Temperature: 30 ˚C
Mobile Phase: A: 0.1% TFA in water, Mobile Phase,
B: 0.1% TFA in acetonitrile
Flow Rate: 1.0 mL/min
Detection (UV): 280 nm
Injection Volume: 5 µL
Sample: Ribonuclease A 5.9 mg/mL, BSA 6.3 mg/mL Thyroglobulin 5.46 mg/mL
Gradient: 5-35 min 10%-70% B

Safety Precaution

Proteomix® RP columns are normally operated under relatively high pressure. Loose connections will cause leaking of organic solvents and injected samples, all of which should be considered as hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When opening the columns, proper protection should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When a column is shipped or not in use, it is always capped at both ends. When installing the column to the system, first remove the end caps. Follow the flow direction as marked on the column. Unless a user has a specific reason for reversing 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) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to tighten the nut 90 degrees past fingertightness.

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

New Proteomix® RP columns are shipped in 55% acetonitrile/45%H_2O/0.1%TFA. During stocking and shipping, the polymer packing could be dried out. It is recommended that 10-20 column volumes of pure organic solvents, such as acetonitrile be purged to activate the column. Flush the column with your mobile phase with gradually increasing 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 1.0 mL/min for 4.6 x 50 mm.

Samples and Mobile Phases

To avoid clogging the column, all samples and solvents including buffers should be filtered through 0.45 µm or 0.2 µm filters before use. Proteomix® RP columns are compatible with nearly all organic solvents. Typical solvent systems include acetonitrile, tetrahydrofuran (THF), methanol and toluene. Solvents can be changed without damaging the column. Always purge your column into a new solvent until 10-20 full column volumes of solvents have passed through the column.

Column Care

PH Wide pH range from 1 to 14. Avoid storing the column below pH 2 or above 12 when not use. The extreme pH will damage the stainless steel column tube and frits during long term storage.

Pressure Proteomix® RP columns can operate at pressure up to 200 bar. Continuous use at high pressure may eventually damage the column as well as the pump. 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 usage. 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. Continuous use of the column at higher temperature can damage the column, especially in pure organic solvents.

Storage When not in use for extended time, it is recommended to store the column in solution with 50% or higher organic solvents, such as ethanol methanol or acetonitrile.

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Avoiding Tailing and/or Adsorption Based on styrene/divinylbenzene, Proteomix® RP resins have a large number of aromatic rings inherent in the packing's structure that will give unique responses to certain types of samples that contain aromatic rings or atoms such as O or N with unshared electron pairs. Those samples have the potential to be strongly retained and/or tail on the Proteomix® RP columns unless there is a competing electron-rich solvent in the mobile phase. Thus, to obtain sharper peaks with less tailing and good resolution, you can "adjust" the surface chemistry with a competing electron-rich solvent like acetonitrile or use a mobile phase additive such as triethylamine (TEA) or n-butylamine which can coordinate with the aromatic rings of the packing material creating a less electron-dense surface chemistry. For certain separations it is also possible to use sodium acetate to modify peak shape and retention intensity. Similarly, using low percentages of glycerol, 2-propanol, or other similarly hydrophilic hydroxylated solvents reduces the net effective surface hydrophobicity. It is recommended to use quantities of 0.5-2.0% of TEA or ethylene glycol, or 0.01M Na Acetate, and anywhere from 2.0-100% of solvents such as CH_3CN, CH_3OH, or 2-propanol.

Proteomix® RP Column Order Information

PN#	Column Size	Particle Size	Pore Size
465500-4605	4.6x50mm	5 µm	500 Å
465500-4610	4.6x100mm	5 µm	500 Å
465500-2105	2.1x50mm	5 µm	500 Å
465950-4605	4.6x50mm	5 µm	1000 Å
465950-4610	4.6x100mm	5 µm	1000 Å
465950-2105	2.1x50mm	5 µm	1000 Å
469500-4605	4.6x50mm	10 µm	500 Å
469500-4610	4.6x100mm	10 µm	500 Å
469500-2105	2.1x50mm	10 µm	500 Å
469950-4605	4.6x50mm	10 µm	1000 Å
469950-4610	4.6x100mm	10 µm	1000 Å
469950-2105	2.1x50mm	10 µm	1000 Å

Different package sizes also available