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Sepax PolyRP Column Manual

Column Information

PolyRP resins have been specifically designed for hydrophobic interaction separation of small organic molecules, peptides, oligonucleotides and proteins. As reversed phase, PolyRP media are based on highly cross-linked polystyrene / divinylbenzene (PS/DVB) resins with very narrow particle size and pore size distributions. PolyRP resins are manufactured with both porous and non-porous structures. Their uniform particle size distribution offers high efficiency separation. PolyRP resins are inherently hydrophobic and reproducible, and do not require a bonded alkyl chain, e.g. C8 and C18 to confer hydrophobicity. With the high cross-linking structure, PolyRP resins provide excellent chemical and physical stability, as well as high rigidity to tolerate high pressure.

Characteristics

Support: spherical, PS/DVB particles
Pore size: non-porous, 100, 300, 500 Å and 1000 Å
Particle size: 5 and 10 µm for porous
Particle size: 1.7, 3, 5 and 10 µm for non-porous
Pore volume: ~1.0 mL/g for porous resins
Phase structure: hydrophobic
Chemical composition: polystyrene/divinylbenzene
Application pH range: 1-14

Column Stability and Performance

PolyRP columns are highly stable over variety of operation conditions such as high temperatures up to 200 ˚C. They are compatible with nearly all organic solvents and aqueous buffers. Solvents can be changed on a same column without damaging the column. PolyRP columns have long life time – negligible deterioration after 3 months of standard usage. With well controlled polymer resin manufacturing and column packing processes, PolyRP columns are very reproducible from batch to batch. Good resin cleaning procedure has been applied to remove the residual monomers and surfactants, resulting in highly pure reversed phase surface. Compared to silica based reversed phases, PolyRP phases have advantages for applications at extreme pH (1-14) with the similar separation efficiency and better selectivity. A test chromatogram for quality control is shown in Figure 1 for a 7.8 x 300mm PolyRP-300 (10 µm) column.

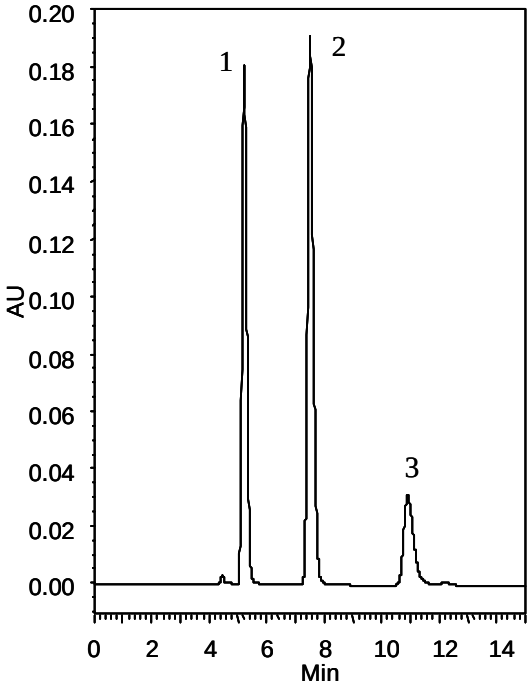

Figure 1. A test chromatogram of a PolyRP-300 column (10 µm, 300 Å, 7.8x300mm)
Column Temperature: ambient
Mobile Phase: 55% Acetonitrile/45%H_2O/0.1%TFA
Flow Rate: 2.0 mL/min
Detection (UV): 254 nm
Injection volume: 10 µL
Sample: 1. p-Amino benzoic acid
2. p-Cyanophenol
3. p-Nitroaniline

Safety Precaution

PolyRP columns are normally operated under high 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 leaking columns. When open the columns, proper protections should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When 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. 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 blockage, 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 PolyRP 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 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 1.0 mL/min for 4.6x300mm.

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. PolyRP 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 two full column volumes 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 in use. The extreme pH would damage the stainless steel column tube and frits for long time storage.

Pressure PolyRP columns should be operated at pressures as follows.

Column	Optimum pressure	Maximum pressure
PolyRP-NP	< 2,000 psi	4,000 psi
PolyRP-100	< 2,000 psi	3,500 psi
PolyRP-300	< 2,000 psi	3,000 psi
PolyRP-500	< 2,000 psi	3,000 psi
PolyRP-1000	< 1,500 psi	2,000 psi

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 service. 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 with reverse flow in an appropriate solvent.

Temperature The maximum operating temperature is 200 ^oC. Continuous use of the column at higher temperature (>200 ^oC) 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 pure THF solvent.

Avoiding Tailing and/or Adsorption Based on styrene/divinylbenzene, PolyRP resins have a large number of inherent aromatic rings in the packing's structure. They 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 PolyRP columns unless there is a competing electron-rich solvent in the mobile phase. In order to obtain sharper peaks with less tailing and good resolution, you can create a less electron-dense surface chemistry with a competing electron-rich solvent like acetonitrile or a mobile phase additive such as triethylamine (TEA) or n-butylamine which can coordinate with the aromatic rings of the packing material. For certain separations it is also possible to use sodium acetate to modify peak shape and retention intensity. In a likely manner, low percentages of glycerol, 2-propanol, or other similarly hydrophilic hydroxylated solvents can reduce the net 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.