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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 resist high pressure.

### Characteristics

Support: spherical, PS/DVB particles  
Pore size: non-porous, 100, 300 and 500 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  
High pressure capability (>8,000 psi for non-porous and 100 Å)

### Column Stability and Performance

PolyRP columns are highly stable over variety of operation conditions. They are stable to resist high temperature up to 200 °C. They are compatible with nearly all organic solvents and aqueous buffers. Solvents can be changed without damaging the column. PolyRP columns have long life time – negligible deterioration after 3 months of standard usage. With well controlled polymer resin manufacturing process and column packing process, PolyRP 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 in using. Compared to silica based reversed phases, PolyRP phases have advantages over applications at extreme pH (1-14) with the similar separation efficiency and better selectivity. A typical test chromatogram for

quality control is shown in Figure 1 for a 7.8x300mm PolyRP-300 (10 µm) column.

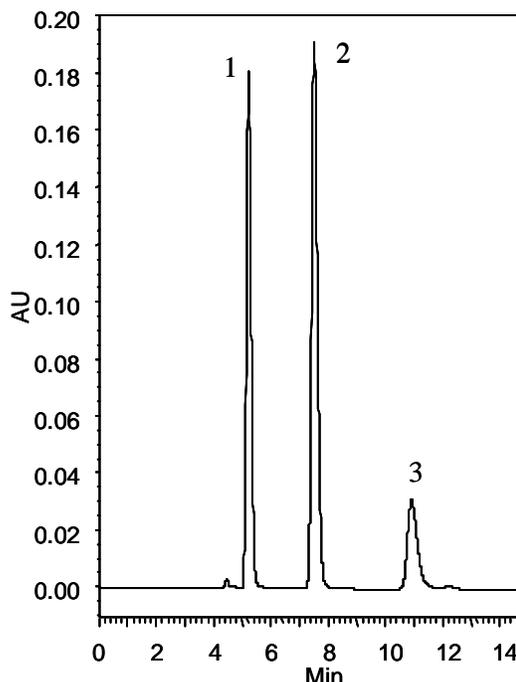


Figure 1. A test chromatogram of a PolyRP-300 column (10µm, 300Å, 7.8x300mm)  
Column Temperature: ambient  
Mobile Phase: 55% Acetonitrile/45% H<sub>2</sub>O/0.1% TFA  
Flow Rate: 2.0 mL/min  
Detection (UV): 254 nm  
Injection volume: 10 µL  
Sample: 1. p-Amino benzoic acid  
2. p-Cyanophenol  
3. 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 leaked 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 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) 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<sub>2</sub>O/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 use. The extreme pH would damage the stainless steel column tube and frits for long time storage.

**Pressure** Even though PolyRP columns can operate at pressure up to 6,000 psi, the normal operation is usually under 3,000 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 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 200 °C. Continuous use of the column at higher temperature (>200 °C) 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 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 PolyRP 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. In like manner, 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<sub>3</sub>CN, CH<sub>3</sub>OH, or 2-propanol.

## PolyRP Column Products

5 µm Phases	250x4.6	250x7.8
PolyRP-100 (100 Å)	260100-4625	260100-7825
PolyRP-300 (300 Å)	260300-4625	260300-7825
10 µm Phases	250x4.6	250x7.8
PolyRP-100 (100 Å)	261100-4625	261100-7825
PolyRP-300 (300 Å)	261300-4625	261300-7825
Non-porous	250x4.6	250x7.8
PolyRP-NP1.7 (non-porous)	262002-4625	262002-7825
PolyRP-NP3 (non-porous)	262003-4625	262003-7825
PolyRP-NP5 (non-porous)	262005-4625	262005-7825