



*Sepax Technologies, Inc.*

Delaware Technology Park  
5 Innovation Way, Newark, DE 19711, USA  
Phone: (302) 366-1101; Fax: (302) 366-1151  
Toll Free: 1-877-SEPAX-US; www.sepax-tech.com

## Sepax Mono-GPC Column Manual

### Column Information

Mono GPC resins have been specifically designed for high resolution gel permeation separation of synthetic polymers. These novel packing materials are based on highly cross-linked polystyrene / divinylbenzene (PS/DVB) particles with very narrow particle size and pore size distributions. Their uniform pore size distribution offers near linear calibration curves covering wide molecular weight range. The highly cross-linked porous particles provide excellent chemical and physical stability and permit easy transfer across the full range of organic solvents with little change in the shape of the calibration curve or the efficiency of the columns. Their high pore volume enables the high resolution of polymer separation. Mono GPC column packings can be the resins with an individual pore size or a mixture of a series of pore sizes.

### Characteristics

Support: Spherical, PS/DVB particles  
Pore size: 100, 300, 500 and 1,000 Å  
Particle size: 5 and 10 µm  
Pore volume: ~1.0 mL/g  
Phase structure: hydrophobic  
Chemical composition: polystyrene/divinylbenzene

### Column Stability and Performance

Mono GPC columns are highly stable over variety of operation conditions. They are stable to resist high temperature up to 145° C in nearly all organic solvents. Solvents can be changed without damaging the column. Mono GPC columns have long life time – negligible deterioration after 3 month of standard usage. The narrow distributed pore size enables linear calibrations that can maximize precision of molecular weight calculations. Mono GPC offers a wide range of porosities from 100Å to 1000Å as well as mixed beds. Mono-dispersed particles achieve high efficient separation and their high pore volume results in greater resolution. A typical test chromatogram for quality control is shown in Figure 1 for a 4.6x300mm Mono GPC-300 column.

### Safety Precaution

Mono GPC 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.

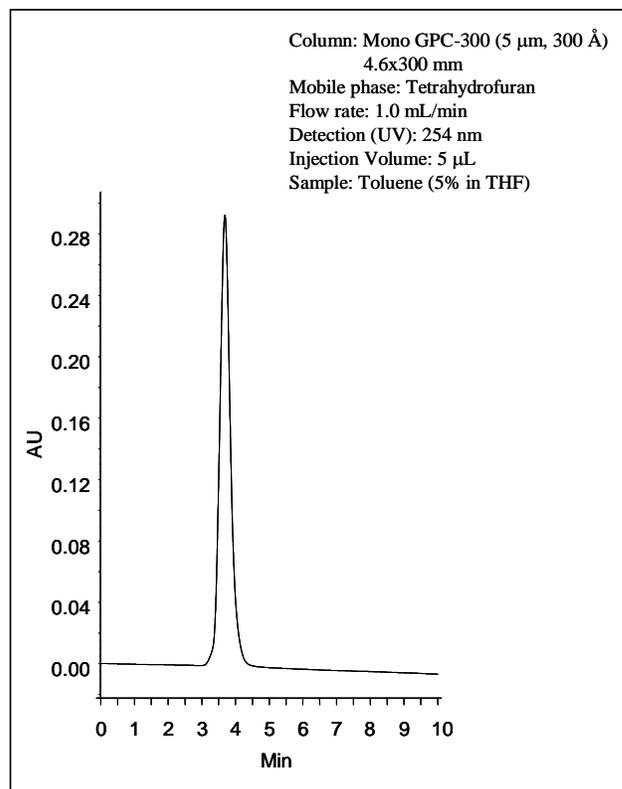


Figure 1. Toluene is used to test the column for quality control.

### 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 Mono GPC columns are shipped in tetrahydrofuran (THF). 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 THF 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. They are stable to resist high temperature up to 145° C in nearly all organic solvents. Typical solvent systems include tetrahydrofuran (THF), chloroform, dimethylacetamide (DMAC), dimethylformamide (DMF), trichlorobenzene (TCB), N-methylpyrrolidone (NMP), Hexafluoroisopropanol (HFIP) and Toluene. Solvents can be changed without damaging the column. Before changing solvents please confirm that your column is compatible with your new mobile phase. See our solvent compatibility chart for help or call us for technical assistance. Always purge your column into a new solvent at 0.2ml/min until two full column volumes have passed through the column.

### Column Care

**PH** Wide pH range - mobile phase pH for use can be used from 0 to 14. Avoid storing the column below pH 1 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 Mono GPC columns can operate at pressure up to 5,000 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 145°C. Continuous use of the column at higher temperature (>145°C) can damage the column, especially under 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, Mono GPC resins have a large number of aromatic rings inherent in the packing's structure 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 Mono GPC 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.

**Frit Fouling** If you notice a calibration change after significant use, you may need a clean frit particularly on the column inlet. If it the original inlet frit clogs, it will contribute to shearing of high MW polymer.

### Mono GPC Products

5 µm Phases	300x4.6	300x7.8
Mono GPC-100 (100 Å)	230100-4630	230100-7830
Mono GPC-300 (300 Å)	230300-4630	230300-7830
Mono GPC-500 (500 Å)	230500-4630	230500-7830
Mono GPC-1000 (1000 Å)	231000-4630	231000-7830
Mono GPC-5MP (Mixed Bed 100-1000 Å)	231135-4630	231135-7830
10 µm Phases	300x4.6	300x7.8
Mono GPC-100 (100 Å)	240100-4630	240100-7830
Mono GPC-300 (300 Å)	240300-4630	240300-7830
Mono GPC-500 (500 Å)	240500-4630	240500-7830
Mono GPC-1000 (1000 Å)	241000-4630	241000-7830
Mono GPC-10MP (Mixed Bed 100-1000 Å)	241135-4630	241135-7830