

## Care and Use of Hotsep® micro-HPLC columns

### Installation and testing

Remove the column from its container and retain the container for storing the column when not in use. The flow direction during the column packing process is indicated on the column tag. Operate the column with the mobile phase flowing in this direction. Before connecting the column outlet to the detector, flush the column with mobile phase, this will prevent small particles, settled on the column frits during shipping and handling, from being washed into the detector. With high performance columns, significant efficiency will be lost if long lengths of large I.D. tubing are employed. For optimum performance, we recommend the following connecting tubing dimensions:

Column ID (mm)	Flow rate (µL/min)	Tubing ID (µm)
0.075 / 0.1 *	0.2 / 0.3	25
0.3	3	< 75
0.5	10	< 125
1.0	40	< 200

\* nano-LC columns require a dedicated nano-injector even when using column-switching for sample loading (max. 0.1 mm bore).

Filters and guard columns can further reduce column performance if not properly selected and maintained. After connecting to the HPLC system, begin to pump an appropriate mobile phase to equilibrate the column. Enclosed along with your new HotSep® micro-HPLC column you will find a performance test chromatogram (Certificate of Analysis) generated on your column. The mobile phase used for the separation of the test mixture is the shipping solvent.

### Care and maintenance

The following guidelines will be helpful for most columns prepared with rigid silica-based packings:

**Pressure:** The column backpressure depends on the packing material and the mobile phase used. With extended use a gradual increase in pressure is usually seen, however, a sudden increase in pressure signals a plugging problem that should be corrected (see column cleaning). However, do not exceed a maximum pressure of 500 bars on your column. Usually, this is not a relevant problem for most users, as most HPLC pumps have a maximum pressure below this limit. Exceptions are packings with pore size of 300Å and larger.

### Temperature / column oven

The hardware of HotSep® micro-HPLC columns tolerates temperatures up to 150°C. However, at high temperatures bonded phases can be lost over time and a decrease in efficiency and peak symmetry might be observed due to dissolution of the silica particles. Column temperatures above 60°C are not recommended for silica-based particles. We recommend using a column oven to assure reproducible retention times.

### Filters and guard columns

Column life is improved with in-line filters or guard columns. Contact our technical personnel for help in choosing guard protection.

### Mobile phase solvents

All common HPLC grade organic solvents can be used with your HotSep® micro-HPLC column. Buffers made from acetate, formate, citrate and phosphate salts can be used up to 0.2 M without adverse effects. As long as the appropriate pH range is not exceeded, organic modifiers and ion pair reagents can be used. However, some ion-pair reagents could be difficult to flush from the column, and columns used with these reagents should be dedicated to the particular analysis involved. Limit the use of strong bases, and avoid strong acids. Do not mix solutions that might precipitate or gel in the column or in the system. The pH range for your column should usually not exceed 1.5-9.5.

### Column lifetime

The lifetime of your HotSep® micro-HPLC column is highly dependent on the sample and the employed conditions and cannot be generalized. Maximize column lifetime by making sure that samples and mobile phases are clean and particle free, and by using a guard column and/or filters.

### Column storage

When storing your HotSep® micro-HPLC column, flush it with acetonitrile/methanol after cleaning and seal it. Do not store columns containing buffers, salt solutions, acidic mobile phases or tetrahydrofuran.