# HPLC COLUMN INSTRUCTIONS FOR USE

**COLUMN INSTALLATION**

Remove the plugs from the column and connect the column with the flow arrow aligned in the direction of the eluent flow (towards the detector). Use 10-32 CPI type male fittings to connect Hamilton HPLC columns

**COLUMN OPERATION**

Hamilton silica-based HPLC columns are pH stable from 2 to 7.5. Using eluents outside of this range will shorten column life. Organic solvents are compatible as long as miscibilty is maintained with the buffer. Buffers up to 0.2 N are compatible. This includes phosphate, acetate, citrate, formate and trifluoroacetic acid buffers within the 2 to 7.5 pH range. When switching from one buffer to another it is always a good idea to flush the old buffer from the column with at least 10 column volumes of water. Column Equilibration usually requires 10 column volumes of mobile phase. A good indication of column equilibration is a stable baseline and reproducible sample retention.

**COLUMN VALIDATION**

Evaluate column efficiency and retention using the test conditions shown on the front of this page. Periodically repeat the test to monitor column performance. Please note that slight variations in retention and performance will be observed on different HPLC systems and with different batches of mobile phase. Additional lot testing is done for each batch of functionalized silica. The test results are shown on the lot certificate of analysis.

**COLUMN MAINTENANCE**

**Routine Care**

* Guard Columns - The use of a Hamilton guard column packed with the same support is recommended to prevent contaminants from reaching the analytical column. Guard columns are available for stainless steel and PEEK analytical columns and stainless steel semi-prep and preparative columns. If system backpressure increases with usage replace the guard column.

* Mobile Phase Filtration - Use only HPLC grade chemicals and deionized water to prepare mobile phases. Then filter through a 0.2 um membrane before use. Prepare new mobile phase every few days to prevent microbial growth.

* Sample Preparation - Filter samples through a 0.2 um membrane as this will prolong column life.

* Solvent Miscibility - When changing mobile phases always check to ensure the new mobile phase is miscible with that in the column and will not cause a precipitate to form. If they are not miscible use an intermediate solvent and flush for at least 20 column volumes.

* Back Pressure - Column back pressure should not exceed 6,000 psi. If the column back pressure exceeds 6,000 psi, it may be plugged with a contaminant that needs to be dissolved. Pumping a sequence of progressively more nonpolar solvents will frequently remove the contaminants. For example, switch from water to tetrahydrofuran to methylene chloride. Return to the operating mobile phase by reversing the sequence. Proteinaceous materials can frequently be removed with repeated 100 uL injections of dimethylsulfoxide.

**COLUMN STORAGE**

Short term storage (1-3 days) does not require a special mobile phase. Longer storage (beyond 3 days) should be done using a mobile phase that will inhibit microbial growth. The storage mobile phase listed on the HPLC Column Performance Report can be used. Columns that used with an ion pairing mobile phases are an exception to the above guideline and can stored in the mobile phase if acetonitrile is present. It is advisable to dedicate columns to a particular analysis as it is difficult to remove ion pairing agents.

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