# SHARC COLUMN OPERATION AND STORAGE

Good column care requires little additional time yet will significantly extend the lifetime of your SHARC1 HPLC columns. Column care for SHARC columns involves use of a suitable mobile phase and storage using an appropriate solvent system.

# Mobile Phases for Column Operation and Storage

Typical mobile phases for SHARC columns include acetonitrile (ACN), alcohols, and in some cases water. Additives to the mobile phase include organic acids (formic, acetic, etc.), inorganic acids (sulfuric, phosphoric, methanesulphonic, etc.), and simple bases (triethylamine, non-aqueous ammonia, etc.).

## **General Guidelines**

1. The SHARC 1 column is operated in organic mode with different combinations of ACN and alcohols. Other solvents, such as tetrahydrofuran (THF) or certain hydrocarbons, may be used to enhance separation. Ensure that your solvents are miscible and that your additives are soluble within all your solvent ratios.

2. If the mobile phase requires addition of acetic acid, formic acid, or corresponding buffers, these will be dissolved in the ACN component of the solvent.

3. It is recommended that you create stock solutions of buffers and additives with concentrations 5-20 times higher than desired concentrations. These stock solutions may be delivered from a separate channel or used to pre-mix mobile phases.

4. Acid must first be dissolved in ACN prior to dissolving the corresponding salts (e.g. ammonium formate or ammonium acetate).

5. Regular 5 um SHARC columns are designed to be used with PEEK tubing and finger-tight PEEK fittings. NEVER USE PLIERS OR WRENCHES to attach the column to your system, as this can destroy PEEK seals inside the column and destroy the column. Columns that are 3 um may be used with SS tubing and fittings.

# General Guidelines for storing SHARC columns

1. Do not store SHARC HPLC columns in aqueous mobile phases with pH outside of the recommended storage range (pH=1.5-7.5).

2. Do not store SHARC HPLC columns in solvents that degrade easily - tetrahydrofuran (THF), triethylamine (TEA), and trifluoroacetic acid (TFA). Unstabilized THF can form peroxides, which may degrade the column.

### Short Term Storage

Flush the column with your mobile phase minus any additives (acetic or trifluoroacetic acids, triethylamine) for short term storage.
e.g. Mobile Phase: ACN/MeOH/Formic acid/ammonium formate=50/50/0.2/0.02

Flush: ACN/MeOH-50/50

This will eliminate the risk of stationary phase degradation over prolonged storage time and will minimize re-equilibration time when the column is next used.

2. For overnight storage, keep the mobile phase flowing at 0.05-0.10 mL/min to reduce re-equilibration time the following day. This slow purge will consume less than 100 mL of the mobile phase.

### Long Term Storage

For long-term storage of SHARC columns, we recommend 100% ACN. If you use a mobile phase with a modifier (acids, bases, acid-salt additives, base salt additives, etc.), first flush the column with the mobile phase minus modifier, then with 100% ACN.

# Other Operational Guidelines

1. Operate SHARC columns below the specified maximum temperature limits to maximize lifetime. Columns degrade faster at high temperatures due to loss of bonded phase or silica dissolution, depending on the pH of the mobile phase. It is important to control temperature for the most reproducible results, but not to exceed the maximum recommended operating temperature for any mobile phase condition. The operating temperature for SHARC columns is 0-50°C.

2. Operate SHARC columns below the specified maximum pressure limits (5000 psi). Note the column pressure when you begin using a column with your mobile phase. If you are running a gradient, the column pressure will change during the gradient. In many cases, if the starting pressure increases during column operation, the column inlet frit may be plugged and the column might require a back flash.

3. Use only fresh reagents or reagents stored in the refrigerator, especially if you work with TEA and TFA because these are more likely to become contaminated from the lab environment. Contamination may interfere with the chromatographic process or alter the column, thus negatively impacting results. Note any quality issues with your mobile phase components before you use them for your chromatographic analyses.