

# CARE AND USE OF SILICA-BASED COLUMNS

AXXIOM-CHROMATOGRAPHY columns use the highest quality raw materials available for reliable, high-resolution chromatographic separations. In-process and finished product quality control procedures insure that each AXXIOM-CHROMATOGRAPHY column will meet your most demanding expectations. Every column is rigorously tested, and the test chromatogram is included for each column along with a sample of test mixture. Periodic evaluation of the column with this test mix can help you to solve most problems that might occur. Following these guidelines will maximize your AXXIOM-CHROMATOGRAPHY column performance and longevity.

## Recommended Column Usage

- Keep dead volume in the sample flow path to a minimum. Use 0.010" or 0.007" ID tubing, and minimize tubing length between the injector, column and detector. (When using 2.1mm ID columns, use 0.007" or 0.005" ID tubing.)
- Use solvents with pH values between 2 and 7.5. Siloxane bonds, although stable in most environments, can hydrolyze in high and low pH mobile phases.
- Flow the eluant through the column in the direction indicated by the flow arrow on the column. Use only HPLC-grade solvents, and filter them before use. This is especially important when using buffers. If your samples contain particulates, filter them or use solid phase sample preparation before injection into your HPLC.
- We highly recommend that you use a guard column. Contact your AXXIOM-CHROMATOGRAPHY representative or Cole Scientific for the appropriate guard column for your application and column. Use of a silica presaturator between the pump and injector can also substantially prolong column lifetimes, especially if your mobile phase contains a high concentration buffer or is at a high or low pH.
- Avoid pressures above 4500 psi, Silica is a form of glass, and like glass it can fracture: Also avoid any mechanical shock to the column.
- Whenever possible, avoid use of halogen salts in the mobile phase, as they can induce corrosion of the stainless steel surfaces if allowed to remain in the column with no flow: If halogen salts are used, make sure that the column is thoroughly flushed with distilled water and then a suitable organic solvent before storage. Degassing the mobile phase to remove oxygen is also helpful.
- Thoroughly equilibrate the column with the mobile phase before beginning a separation. Bonded phase columns require approximately 20 column volumes [50ml] for complete equilibration; unbonded silica columns require approximately 60 column volumes [150ml].
- Elevating the temperature can, in many cases, improve reproducibility, selectivity, and resolution. However, if you use a column heater or oven, temperatures less than 70° C are recommended. Higher temperatures can promote hydrolysis of bonded phases and shorten the column lifetime.

# CARE AND USE OF SILICA-BASED COLUMNS

## Storing Your Column

Reverse phase columns (ODS, OCTYL, CN). If your mobile phase contains buffers, wash the column first with a mixture of 1% glacial acetic acid in 50:50 methanol/water. Then store with 70:30 methanol/water, and store in a cool place. (CN columns should be also washed with and finally stored in acetonitrile). 50ml of each wash solvent is usually sufficient. Normal phase columns (silica, CN). Wash with 50:50 2-propanol/heptane (or hexane) and store in 100% heptane (hexane). Always cap the column inlet and outlet tightly before storage, using the column plugs provided. This prevents the column from drying out, which can result in bed collapse upon re-use. A good universal storage solvent for all bonded phase columns (except AMINO) is acetone.

## Troubleshooting Column Problems

We recommend that you re-run the test mix under the QC data sheet included with the column before putting your new column into service and keep these results on file. Should you subsequently encounter a problem with your separation and you suspect the column, you will have a "reference" chromatogram run on your HPLC system. You can then again run the test mix using the same condition, and compare the backpressure, retention times, and efficiency with your original results and the column release data. If your problem is high backpressure, first remove the column from the HPLC and verify that the problem is not due to plugged tubing or a plugged valve. Also run the column without detector connected to see if the detector is causing the problem. If the backpressure is found to be from the column, the inlet frit may be plugged. You can replace the inlet column frit with the frit included with the column, per the following procedure:

- First, remove the column from the HPLC. Using appropriate wrenches, remove the inlet end fitting. [Never remove the outlet fitting, since this can disturb the packed column bed.]
- Remove the old frit and check the column for a void (empty space at the top of the column bed)
- Check for heavy discoloration of the packing at the top of the column. Some discoloration is normal, depending on the type of samples you are running. If the discoloration is heavy the Column may need to be washed or replaced.
- Carefully fill the void with a methanol slurry of new material available from your AXIOM -CHROMATOGRAPHY representative. Make Sure that the new packing material is suitable for the type of column you are using.
- Place the new frit on top of the column. The Side of the frit With the two "dimples" should face the packing material.
- Replace the end fitting, tightening 1/8 turn past finger tight. Avoid getting packing material on the ferrule or fitting threads, or leaks may result. The column is now ready for further use.

If replacing the frit does not reduce the backpressure, there may be particulate contamination of the column bed. As a last resort, the column may be back flushed. See back flushing instructions on page 3.

# CARE AND USE OF SILICA-BASED COLUMNS

## Column Back Flushing

CAUTION: When back flushing a column, use low-viscosity strong solvent (reverse phase - Methanol or Acetonitrile; normal phase - Chloroform or Methylene Chloride) and low flow rates, typically 0.5ml/minute, with a volume of 200ml. If your problem is loss of efficiency or poor peak shape (tailing), it is typically caused by bad connections and/or a change in the column chemistry. This can be irreversible or (hopefully) reversible. If the situation is reversible, you can probably remedy it with the following procedure:

- Check all connections for possible leaks or excessive contributions to dead volume.

If this does not solve the problem...

- Thoroughly wash the column Reverse phase columns may be washed by pumping 50ml each of the following solvents through the column:
  - 50%Methanol/50% Water/1% Acetic Acid
  - Chloroform
  - Heptane
  - Methylene Chloride
  - Methanol
- Normal phase columns may be washed by pumping 50ml Isopropanol and then 50ml Acetone through the column.
- If this fails to restore column performance, the column should be replaced.

## When Problems Persist

Please call first! Your AXXIOM-CHROMATOGRAPHY representative and the AXXIOM-CHROMATOGRAPHY Support staff are here to help - don't hesitate to call us directly, we have a staff of chromatographers with extensive HPLC experience. Chances are that one of them has come across a similar problem to yours. But before calling us, please run the test mix included with your column; it can provide important information in helping us diagnose and solve your problem. Please also have handy your column's serial number, as well as information about your separation.

Cole Scientific is dedicated to helping you improve your chromatography. We can provide assistance in column selection, as well as equipment selection and optimization. We welcome the opportunity to assist you!