

## COLUMN USE AND CARE INSTRUCTION

Please read carefully before using this column

Thank you for purchasing an Antec column. Inspect the column immediately upon its arrival. If there are any signs of damage, notify your local Antec representative at once. To insure that the column provides you with reliable chromatography, please follow the guidelines below.

**Column care:** Handle the column with care. Do not drop or shock the column as this may damage the packed bed, resulting in decreased column performance. The Antec C18-3 columns are designed for use within the pH range of 2.0 to 7.5. Higher pHs will dissolve the silicagel and lower pHs can strip away some of the bonded phase. To maximize column life operate at pressures up to 20 MPa.

**Samples:** Prepare in mobile phase or a very similar solvent (preferably a weaker solvent than the mobile phase). This will prevent the sample precipitation at the column inlet. If gradient elution is to be performed, prepare the sample in a solvent mix that is the same or similar to the  $T_0$  composition. Ensure the final, sample solution is particulate free, preferably by filtration through a 0.2  $\mu\text{m}$  filter.

**Eluents:** All buffers, if required, should be prepared on the day of analysis. If buffers are not prepared freshly, ensure that their pH has not changed and that there is no microbial growth present. The pH of the buffer used is dependent on the application and the packing media in this column. We recommend use of buffers between pH 2 and 7.5 be used with all columns. All solvents should preferably be filtered through a 0.2  $\mu\text{m}$  filter and thoroughly degassed before use.

**Shipping solvent:** All columns are shipped with the solvents used for the final test of the column. The Antec C18-3 columns are delivered with either Water:Acetonitril or Water:Methanol as shipping solution. For detailed specification see individual test chromatogram.

**Column Installation & Equilibration:** Ensure that the seating and depth of fittings and ferrules is optimized to reduce the risk of leaks and dead volume. In case of use of buffered or salt containing aqueous mobile phases with a small amount of modifier (MeOH or ACN): to prevent precipitation flush the column first with water:methanol solution (80:20 v/v%) with a minimum of 5 column volumes prior to flushing with the

mobile phase. Equilibrate your column with a minimum of 20 column volumes of mobile phase prior to starting of a first analysis.

In case of unbuffered solvents, when switching between solvents with vastly different polarities, it may be necessary to first purge the column with a mutually miscible solvent such as Isopropyl Alcohol or Dioxane under reduced flow by half. Flushing with a minimum of 5 column volumes is recommended. If transferring between normal and reversed phase, ensure that all the normal phase solvents are removed from the column before any aqueous solvents are introduced and vice versa.

**Column Protection:** For "dirty" samples or those whose purity is not known, we recommend the use of a column pre-filter and/or guard column. Their use will extend the lifetime of your analytical column by trapping any particulates and contaminants in your sample.

**Troubleshooting:** If there are tailing peaks early in the elution, the cause is probably dead volume. Check if the tubing between the injector and column has nicely cut tubing ends and is properly inserted all the way into the column joint and injector port. The tubing between the injector, column and detector should be as short and have as small an inside diameter as possible. Particularly when operating semi-micro columns with a low flow these considerations have a large effect. The cause of column back pressure increase or double peaks are probably dirt or blockages in the column inlet filter.

**Column Aftercare:** Prior to storage, flush the column with a wash/storage solvent (containing no buffers) to remove all traces of organic and inorganic compounds from the packed bed. In case salt-containing buffered aqueous solutions were used, first flush with a water:modifier solution with a moderate concentration of modifier (20%v) to prevent precipitation of salts etc. For reversed phase columns, the wash/storage solvent used should contain at least 30 %v/v organic solvent (preferably acetonitrile). For storage periods in excess of 7 days, store it filled with a 100% organic solvents (Acetonitril). Seal the column with the plugs provided and store it in a location with a stable temperature.

Antec C18-3 columns are manufactured, inspected, packaged and shipped under strict standards of quality control. Should you find any defect in performance, please contact our representative. We regret, however, that we cannot guarantee the lifetime of columns, nor can we accept any claim when their performance has deteriorated due to no-compliance with the above operating instructions.

FAILURE TO FOLLOW THESE PRECAUTIONS  
MAY VOID THE COLUMN WARRANTY