GL SCIENCES

HPLC COLUMNS

COLUMN USE AND CARE

Basic Operating Instructions and Suggested Care of INERTSIL® HPLC Columns

Inertsil® HPLC packings are subject to a rigorous array of QC tests in a ISO 9001 compliant facility, with special emphasis on reagent purity, raw material traceability and consistency in raw materials, and final products. A detailed analysis of all of the physical and chemical properties of Inertsil®, combined with tests for chromatographic selectivity and column packing efficiency, ensure that each lot of Inertsil® is identical to all previous lots and column-to-column reproducibility is of the highest order. To maintain and maximize peak performance of Inertsil® HPLC columns, and ensure long life and stability of columns please read the following instructions before use.

UNPACKING

Inspect the column upon arrival and contact GL Sciences (info@glsciencesinc.com or 310-265-4424) immediately if you see signs of damage to product or product packaging that may have occured during shipping.

SHIPPING SOLVENTS

Inertsil® columns are shipped with the solvent used for the final QC test of the column, as detailed on the test chromatogram delivered with the column.

When preparing to introduce your desired mobile phase into a new column, be aware of the miscibility of the solvents being introduced to the column and the solvent inside the column. If the new eluent being introduced contains buffer sales, it is recommended that the column is flushed with a highly aqueous eluent (such as 90:10 Water:MeCN) before introducing buffer, to avoid precipitation of salts on the column. For extra precaution, introduce new buffered eluents WITHOUT the buffer component for 5-10 column volumes, and then switch to the fully buffered eluent composition. Precipitation of buffer salts on the columns is essentially irreversible and destroys the column! 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 at a reduced flow rate (approximately 50% of normal). Flushing with a minimum of 5 column volumes is recommended (e.g. 10 mL for a 150 x 4.6mm I.D. column).

Note: With Inertsil® SIL-100A, it is time consuming to flush the buffer or water in the pores of the packing after using reversed-phase eluent. In fact, it is nearly impossible to remove water from the surface of a silica column and once water adsorbs on the surface, the selectivity of the column is subsequently altered. (If a subsequent analysis in the normal phase mode is performed, and the flush has not been thoroughly completed, the elution order and retention time may vary significantly). Therefore this type of column should be used solely for one type of analysis (basically, normal phase. If you want to use reverse phase on a polar column, we recommend CN, Diol, or Amino.

COLUMN CARE

Do not drop or bump columns, as this can fracture the uniformity of the bed and result in tailing or split peaks.
Eluent pH - GL Sciences' InertSustain® HPLC columns are designed for use between pH 1.0 and 10.0, and have known to provide good stability even at pH approaching 11. Other Inertsil® columns, such as ODS-2, ODS-3, and ODS-4 provide optimum lifetime when used with eluents within the pH range of 2.0 to < 8. At pH above 8, silica gels begin to dissolve; at acidic pH below 2.0 certain bonded phases (particularly CN) become hydrolyzed and gradual loss of bonded phase can occur. While many customers use the columns outside both sides of the pH spectrum with excellent results and good column lifetime, the best lifetimes are usually obtained at intermediate pH conditions. One benefit of Inertsil's extreme base deactivation is the ability to analyze highly polar compounds at their pKa without peak tailing.

Pressure - To maximize column life operate at pressures up to 20 MPa (~ 3000 psi) for standard HPLC phases (UHPLC columns can be used at higher pressures, as indicated on the test chromatogram).
Column Temperature - To maximize column lifetime, limit column temperature to **60C**.
Sample Dissolution - Samples should be dissolved in the eluent or solvent weaker than the eluent, which helps avoid sample precipitation at the column head and inconsistent retention values. Filter sample with 0.45µ membrane to remove particulate matter before injection. Solvents - Use HPLC or spectroscopy grade solvents that have been filtered through a 0.45µ filter. Filter all buffer solutions before use. Avoid introduction of particulates onto the column at all costs.

COLUMN PROTECTION

Installation of a GL Sciences' Preclean ORG guard cartridge between the pump and the injector removes organic contamination from aqueous eluent. Guard columns also help protect more valuable analytical columns by binding non-eluting sample components before the analytical column becomes contaminated.

STORAGE OF COLUMNS

After using the column with eluent containing buffer or ion-pair reagent, wash the column completely with a salt-free eluent before storing. When storing the column for a long period, store it in 100% organic solvent. MeCN is the most commonly used solvent, though isopropyl alcohol and MeOH are also commonly used. Seal the column with the plugs provided and store it in a location with a stable temperature. Inertsil® columns are manufactured, inspected, packaged and shipped under strict standards of quality control. Should you find any defect in performance, please contact your GL Sciences' provider, who will ensure your complete satisfaction. 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 or as a result of normal aging.

COMMON PROBLEMS TO AVOID AT INSTALLATION

If there are tailing peaks early in the elution, the cause is probably dead volume, most commonly caused by a pore fitting connection between the injector and column. Check that the connecting supply line is properly inserted all the way into the column joint. Piping to the injector and to the detector should be as short and have as small an inside diameter as possible. Sub-optimum connections and plumbing have increasing negative effects as the column diameter is decreased.

Baseline drift and noise can be caused by defective pump operation due to air bubbles, reduction of light intensity when using a UV detector. Bubbles often occur during high temperature analysis or the when there are problems with solvent purity.

The most common cause of column back pressure increases or double peaks is blockage of the inlet frit by sample particulates - either from the analyte/matrix or particles created by aging pump seals - or large quantities of lipophilic compounds adsorbing to the head of the column. Pass the eluent through a 0.45µ membrane filter before use. The column can also be protected from contamination by using a guard column / cartridge.

Type	Principal Columns	Shipping Solvnet
Normal-Phase	CN-3, NH2, Diol, Silica	n-hexane:Ethanol
Reverse-Phase	ODS, C8, Phenyl, C4,	MeCN:Water or MeOH:Water
Ion-Exchange	SAX, SCX	Methanol (100%)