General HPLC Column Care

The correct use of an HPLC column is extremely important for the life time of a column and therefore for the benefit of your HPLC analysis. The following pages will give you some guidelines for the use, cleaning and storage of HPLC columns. These guidelines depend on the one hand on the nature of the chromatographic support (silica, polymers or others) and on the other hand on the surface chemistry of the corresponding stationary phase.

Silica based columns

General guidelines

Silica is the ideal support for HPLC columns. It offers a large mechanical stability, excellent physicochemical surface properties, a wide range of bonding chemistries and is compatible with a broad range of organic solvents. However, the following points are extremely important to know when working with silica based HPLC columns.

- ***pH stability***

  In general, HPLC columns are stable within a pH range of 2 to 8. If you are measuring a pH value, the measurement must be done in the aqueous media before mixing the eluent with organic solvents. Modern HPLC columns can be used outside that pH range. The new bonding chemistries allow use down to pH 1 for some stationary phases. However, please check vendor's product information before using silica based column outside the pH range of 2 to 8. However, best lifetimes are obtained between pH 2.0 and pH 6.8.

  Stationary phases based on ultra pure silica gel can also be used at higher pH ranges, up to pH 11, depending on the chemical nature of the modifier used in the mobile phase. Large bases (like Pyrolidine) are not able to attack the surface of the silica and therefore can be used at higher pH values. If you are working at pH values above 8 with small bases as modifier (like Ammonia), we highly recommend using stationary phases based on Polymers or Zirconiadioxide.

- ***Mechanical stability***

  Stationary phases based on silica are mechanically very stable. The packed columns show no pressure limit and can be used at more than 40 MPa (6000 psi) without any

problem. However, please avoid pressure shocks on the column. Pressure shocks lead to channeling in the column, which results in peak splitting in the corresponding chromatogram.

- ***Mobile phases (Eluents)***

Silica based stationary phases are compatible with all organic solvents in the above mentioned pH range. Please use the highest quality solvents available (HPLC grade). Also, please filter all prepared buffer through a 0.5 µm filter before using them in your HPLC system. Always keep in mind; your column is the best filter!

The use of non pure solvents in HPLC causes irreversible adsorption of impurities on the column head. These impurities block adsorption sites, change the selectivity of the column and lead to peak splitting in the chromatogram. In gradient elution, impurities cause so called "Ghost Peaks". Ghost peaks are peaks that always appear in the same position on the chromatogram. Their origin is not the sample, but the impurities from the solvents or solvent additives. Therefore, it is highly recommended to run a gradient without injection in the beginning of each method to determine the ghost peaks.

To avoid irreversible adsorption at the column head, you should always use a pre-column. The use of a pre-column increases the life time of a column dramatically. In addition, a pre-column can filter solid parts stemming from pump seals or injection rotors. An alternative to a pre-column is an in-line filter. These filters are attached directly to the column. These filters get rid of solid parts in the eluent but will not avoid irreversible adsorption of organic impurities.

Proper storage of HPLC columns

- For short term storage, i.e. over night, columns can be stored in the eluent used in last analysis.
- For middle term storage, i.e. 2 days or over the weekend, columns should be flushed with pure water to prevent microbial growth.
- For long term storage, silica based columns should be stored in an aprotic solvent. The water content should not be higher than 50%. The best storing solvent is Acetonitrile.

- ***Caution!!!*** Please make sure that all buffers are washed out of the column before flushing with Acetonitrile. Buffer salts are mainly not soluble in Acetonitrile and can block the capillaries and the column.

Equilibration time

The equilibration time of a column depends on the column dimensions. In general, a column is equilibrated after flushing with 20 column volumes. The equilibration time for the most important column dimensions is summarized in the following table.

Column dimension	Column volume [ml]*	Flow rate [ml/min]	Equilibration time [min]
250 x 4.6 mm	2,91	1,00	58
150 x 4.6 mm	1,74	1,00	35
100 x 4.6 mm	1,16	1,00	23
50 x 4.6 mm	0,58	1,00	12
250 x 4.0 mm	2,20	1,00	44
125 x 4.0 mm	1,10	1,00	22
250 x 2.0 mm	0,55	0,25	44
150 x 2.0 mm	0,33	0,25	26
50 x 2.0 mm	0,11	0,25	9

*$_T$ = 0,7

Shorter equilibration times are possible if you simply increase the flow rate. It is no problem to do that if no chromatography is done. However, 20 column volumes are necessary to ensure a 100% equilibration.

Regeneration of a column

Irreversible adsorption of impurities stemming from the matrix on the column head can cause changes in selectivity or peak splitting. Often those “dirty columns” can be regenerated by applying the following protocols.

Regeneration of RP packings

RP- packings are C18, C8, C4, C1, C30, CN or Phenyl stationary phases.

- Flush the column with 20 column volumes Water
- Flush the column with 20 column volumes Acetonitrile
- Flush the column with 5 column volumes Isopropanol
- Flush the column with 20 column volumes Heptane
- Flush the column with 5 column volumes Isopropanol
- Flush the column with 20 column volumes Acetonitrile

Regeneration of NP (Normal Phase) packings

NP-packings are Silica, Diol, Nitro and Amino stationary phases.

- Flush the column with 20 column volumes Heptane
- Flush the column with 5 column volumes Isopropanol
- Flush the column with 20 column volumes Acetonitrile
- Flush the column with 20 column volumes Water
- Flush the column with 20 column volumes Acetonitrile
- Flush the column with 5 column volumes Isopropanol
- Flush the column with 20 column volumes Heptane

Regeneration of Ion Exchange Packings

Ion exchange packings are Anion or Cation exchangers (WCX, SCX, WAX and SAX)

- Flush the column with 20 column volumes of the same eluent, but double the buffer concentration
- Follow the regeneration protocol for RP packings (see above)
- Flush with 20 column volumes of Water
- Equilibrate the column now to the original conditions.

Polymer based columns

Polymer based stationary phases show higher pH stability but lower mechanical stability, compared to silica based columns. Also, polymer based packings are not compatible with all organic solvents. They swell or shrink in some organic solvents. Unfortunately, the pressure

stability and solvent compatibility are different for the different nature of polymers and from manufacturer to manufacturer. Therefore, no general rules for the column care of polymer based materials can be given. Always read the instructions for the use of those columns. In case of doubt please contact the corresponding manufacturer.