CARE & USE
OF
HICHROM HPLC COLUMNS

PLEASE READ THIS CARE AND USE INFORMATION BEFORE USING THE COLUMN.

All Hichrom columns are individually manufactured and tested to meet strict specifications. We guarantee to replace any column which does not give full customer satisfaction. The following measures will enhance their performance and lifetime.

1) COLUMN INSTALLATION

SYSTEM DEAD VOLUME

Reduce dead volume in the system to a minimum by using connection tubing with an internal diameter of 0.010" or less. Connections between injector, column and detector should be kept as short as possible.

COLUMN CONNECTION

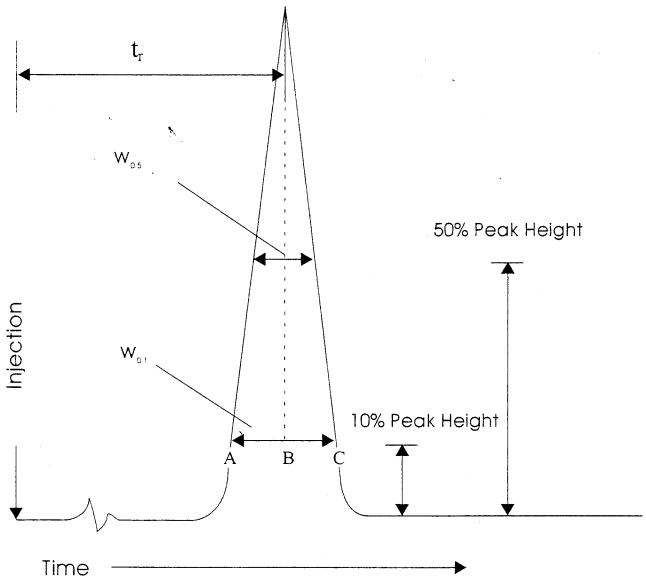
For optimum performance, it is important that the tubing used to connect the column to the injector or detector is positioned such that it abuts the internal shoulder of the fitting. We recommend the use of fingertight fittings for these connections.

EQUILIBRATION

The storage solvent in a new column is the mobile phase used to evaluate the column, unless otherwise specified on the chromatogram. Initially, care should be taken not to pass any material through the column that may precipitate in the storage solvent. Ensure that the column is fully equilibrated with the mobile phase prior to starting analysis. A normal phase silica column usually requires more conditioning than a reversed-phase column. The direction of flow is marked on the column.

PERFORMANCE TESTING

It is recommended that the performance of columns is tested on arrival and at periodic intervals during use. The performance parameters measured are defined below:



Hichrom use two asymmetry factors based on the ratios:-

$$As_1 = \frac{N_{0.1}}{N_{0.5}} \qquad \text{and} \qquad As_2 = \frac{BC}{AB}$$

using the efficiencies,

$$N_{0.1} = 18.55\left(\frac{t_r}{w_{0.1}}\right)^2 \qquad \text{and} \qquad N_{0.5} = 5.54\left(\frac{t_r}{w_{0.5}}\right)^2$$

where

$N_{0.1}$ = Efficiency measured at 10% of peak height
$N_{0.5}$ = Efficiency measured at 50% of peak height

As_1 is more sensitive to the negative effects of tailing and fronting than the conventional measurement As_2.

For an ideal Gaussian peak As_1 will be 1.00.

As_2 indicates relative 'fronting' & 'tailing'.

As_2=1.00 for symmetrical peaks
As_2<1.00 indicates fronting peak As_2>1.00 indicates tailing peak.

2) OPERATIONAL GUIDELINES

HPLC SOLVENTS

Use only HPLC grade solvents and freshly prepared aqueous buffer solutions in order to minimise bacterial growth. A slip-on pump inlet filter will remove extraneous particles.

MOBILE PHASE pH

The recommended mobile phase pH for silica columns is between 2 and 7.5. However, use of a pH between 3.5 and 6.5 will ensure maximum column life.

COLUMN PROTECTION

Silica HPLC packing materials gradually dissolve in aqueous solution. Wherever possible, use high levels of organic solvents in the mobile phase to minimise silica dissolution.

When high levels of aqueous solvent are required, the use of a scavenger or pre-column, placed between the pump and the injector is recommended. These columns help to saturate the mobile phase with silica and minimise analytical column dissolution problems. They also protect the column from particulate matter and pump seal wear particles.

A guard column placed between the injector and the analytical column will further protect the latter from sample contaminants, highly retained solutes and wear particles from the injection valve.

PRESSURE

Exposure of a column to rapid changes in back pressure or to pressures greater than 4000 psi (276 bar) may reduce column life.

STORAGE

Wash out all water and buffers from bonded silica columns and store in the storage solvent listed overleaf. Unbonded silica and Chiral columns may be stored in heptane or similar organic solvents. Keep in a cool area and replace end-caps to prevent the packing bed drying out.

MECHANICAL DAMAGE

Protect the column from mechanical shock. Dropping or striking a column can impair its performance.

FITTINGS - TORQUE

Excessive tightening of the column end fittings will result in damage to the column tubing or fittings. Removal of an end fitting to replace a frit or top-up the packing material should be regarded as a last resort to prolong column life.

3) SAFETY & DISPOSAL

☒ This column contains amorphous silica which may be hazardous to health if the column is unpacked and the silica dried. The occupational exposure standard for amorphous silica (8 hr time weighted average) is:

6 mg/m^3 - Total inhalation dust
3 mg/m^3 - Respirable dust

Exposure should be controlled to only a fraction of these limits, especially for bonded silicas.

MAXIMUM OPERATING PRESSURE = 5000 psi (345 bar)
MAXIMUM TEMPERATURE = 60°C

Provided the silica is contained within the column it presents no hazard to health.

BS EN ISO 14001 ENVIRONMENTAL STANDARD

For advice or assistance on the safe disposal of used Hichrom columns please contact our Technical Department.

All columns returned to Hichrom MUST be accompanied by a decontamination certificate, which is available upon request.

4) INFORMATION

- Hichrom Quality Control test samples are available on request.
- A comprehensive range of columns & accessories, including Guard Cartridges are available from the Hichrom catalogue.