

## Column care and use - Silicia based preparative columns

Each KNAUER column is individually packed and tested to ensure a reliable performance. The enclosed test certificate includes the performance data, serial number and batch number of the column material.

### Column installation

Columns should be handled with care, as every drop or shock can damage the column or the column bed. Remove safety plugs before installation and use only compatible solvents. The flow direction is indicated by an arrow at the outside of the column. Flush the system and the installed column at low flow rates and gradually increase the flow rate up to the desired value.



**Note:** Before installation, make sure to have the right fittings available. Columns with 1/8" can only be connected with UNF 5/16-24 fittings (A7226 and A7227).

### pH stability

Silica based columns are stable between pH 2-8. Special endcapped column materials can be operated outside these values. Refer to the KNAUER website or your supplier for more information.

### Mechanical stability

The mechanical stability depends on the particle size and other conditions. Refer to the vendor or the KNAUER [website](#) for further information about optimal working conditions and maximal pressure.

### Mobile phases (eluent)

Silica based columns are compatible with all common organic solvents. Prepared buffers should be filtered through a 0.45 µm filter to avoid blockage of the column.

Precolumns provide a good measure to increase the column longevity, as any potential harmful particles are stuck in the precolumn first.

### Proper storage of silica based HPLC columns

Columns should be stored in aprotic solvents. Best storage solvent for RP columns (C18, C8, C4, C1, C30, CN and Phenyl) is MeOH/water (50:50, v:v). Water content should generally be below 50 %. Best storage conditions for NP columns (Silica, Diol, Cyano and Amino) is heptane/isopropanol (90:10, v:v). HILIC columns (HILIC, Amino, Silica) should be stored in MeOH/water (90:10, v:v).



**Note:** If you are using HILIC columns, the methanol content should always be equal or greater than 90 %.

Before storage all buffer solutions should be thoroughly flushed out of the column with the above mentioned solvents. The openings of the columns need to be closed with blind nuts to avoid any evaporation.

### Equilibration time

Equilibration time is dependend on the former solvent. During a change from one organic solvent to another, we recommend flushing the column overnight in a circular flow. If the column is stored in a similar solvent, flush the column for at least 15 min before use.

## Regeneration of the columns

When a sudden change in peakform, retention time, resolution or backpressure is observed, a column regeneration is recommended. Refer to the table below for suitable regeneration schemes. After regeneration re-equilibrate the column with the desired mobile phase before starting the analysis.



**Note:** In case of a sudden increase in backpressure, carefully backflush the column under low flow and low pressure to remove build up particles from the column filter and/or exchange the precolumn. This works only for Eurospher and Eurospher II columns.

| Regeneration scheme for RP columns<br>(C18, C8, C4, C1, C30, CN and Phenyl stationary phases) | Regeneration scheme for NP columns<br>(Silica, Diol, Nitro, Cyano and Amino phases) | Regeneration scheme for columns used in HILIC mode<br>(HILIC and Silica stationary phases) |
|---|---|--|
| 20 min, water   | 20 min, heptane   | 20 min, water  |
| 20 min, acetonitrile  | 5 min, isopropanol  | 30 min, 0.5 M ammonium acetate   |
| 5 min, isopropanol  | 20 min, acetonitrile  | 30 min, water  |
| 20 min, heptane   | 20 min, water   | 20 min, acetonitrile-water (50:50 v/v)   |
| 5 min, isopropanol  | 20 min, acetonitrile  | 20 min, acetonitrile   |
| 20 min, acetonitrile  | 5 min, isopropanol  | 20 min. acetonitrile-water (50:50 v/v)   |
|   | 20 min, heptane   |  |



**Note:** After the regeneration procedure, re-equilibrate the column with the mobile phase before starting the analysis.

Please note that any failure to follow these precautions may void the column warranty. Technical data can be subject of change without notice.

If there are any further questions do not hesitate to contact our technical customer support:

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