

Column care and use - Silica based phases

Each KNAUER column is individually packed and tested to ensure a reliable performance. The enclosed test certificate includes the performance data, serial number and the batch number of the column material. The serial number of your column is noted on the column certificate as well as on the column label. Please retain this information. To ensure that your column provides you with reliable chromatography results, please adhere to the guidelines below.

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 compatible solvents. The flow direction is indicated on the column flag. Flush the system and a new column at low flow, before gradually increasing the the flow rate to the desired value.

pH stability

Silica based columns are stable between pH 2-8. Special endcapped columnn material can exceed these limites. Refer to the KNAUER website or your regional supplier for more information.

Mechanical stability

The mechanical stability depends on the particle size, temperature, flow, solvents etc. Refer to our [website](#) or your regional supplier for further information about optimal and maximum working condition of your column.

Mobile phases (eluents)

Silica based columns are compatible with all common organic solvents. Prepared buffer should be filtered through a 0.45 µm filter to avoid blockage of the column. Integrated and external precolumns provide a good measure to increase column longevity, as all potentially harmful residues are first trapped in the precolumn.



Note: Columns with small ID (such as 2 mm) should not be used with integrated precolumns as increased deadvolume can lead to peak broadening. For these applications, KNAUER offers a UHPLC precolumn filter (B2/B2002) as an alternative.

Proper storage of silica based HPLC columns

Columns should be stored in aprotic solvents. Best storage for RP columns (C18,C8, C4, C1, C30, CN and Phenyl) is MeOH: water (50:50, v:v). Water content should generally be equal or lower than 50%.

Best storage conditions for NP columns (silica, diol, cyano and amino) is heptane/isopropanol (90:10, v:v). HILIC columns (HILIC, amino, silicy) 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 colum 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 of the former solvent and the column volume. Refer to the table below for optimal equilibration time.

Column dimension [length x ID]	Column volume [ml]	Typical flow rate [ml/min]	Equilibration time [min]
250 x 0.8 mm	12.56	4.0	43
250 x 4.6 mm	4.15	1.0	56
150 x 4.6 mm	2.49	1.0	34
250 x 4.0 mm	3.14	1.0	43
150 x 4.0 mm	1.88	1.0	26
150 x 3.0 mm	1.06	0.6	24
100 x 3.0 mm	0.71	0.6	16
100 x 2.0 mm	0.31	0.5	9
50 x 2.0 mm	0.15	0.5	5

The equilibration time can be reduced by increasing the flow. Make sure that the back pressure does not exceed the pressure limited by the columns.

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 sudden increase in backpressure, carefully backflush the column under low flow and low pressure to remove settled particles from the column filter and/or exchange the precolumn. This applies to Eurospher and Eurospher II columns.

Regeneration scheme for RP columns (C18, C8, C4, C1, C30, CN and Phenyl stationary phases)	Regeneration scheme for NP columns (Silica, Diol, Nitro, Cyano and Amino phases)	Regeneration scheme for columns used in HILIC mode (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:

Telefon: +49 30 809727-111 (9-17 h, Central European Time)

Fax: +49 30 8015010

E-Mail: support@knauer.net

Languages: German, English

For more information visit our website: www.knauer.net