HPLC · SMB · Osmometry

BlueShell Column Operating Guidelines

KNAUER BlueShell columns are based on high purity core shell silica with 2.6 µm particle size for fast liquid chromatography. BlueShell particles were developed to provide improved speed, resolution and lower eluent consumption by keeping the backpressure as low as for classical HPLC applications. Each column is individually packed and tested to ensure reliable performance. The enclosed test certificate includes a test chromatogram and specific column data concerning performance. 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 Connections – It is highly recommended that only capillaries with accurately cut straight ends be used for column installation to avoid excessive dead volume. Micro and narrow bore columns require HPLC equipment specifically designed with low volume components. We recommend that capillaries with an inner diameter of ≤ 0.12 mm ID be used and that the connections between the injector and column, as well as the column and detector, be kept as short as possible. The end fittings on BlueShell columns are compatible with all UHPLC a swell as HPLC systems, however we recommend using the KNAUER PEEK compression screw if using flexible stainless steel capillaries from KNAUER (0.5 mm OD).

Column Installation – Please handle the column with care, every drop or shock to the column can damage the packed column bed. The column is shipped with PEEK end plugs. Please loosen and remove the plugs before installation. Flush all capillaries with compatible eluent before use with the column. When the column is shipped it contains the solvent listed on the column test certificate (the column is also safely stored in this solvent.) Be sure that your mobile phase is compatible with this storage solvent. If not, flush the column with an intermediate solvent which is compatible with both solvents. We recommend isopropanol. The flow direction is given by an arrow on the column label. Firstly, connect the column only at the injector, flush the system and column at low flow rates and gradually increase the flow rate up to the optimum value. Finally after about 5 minutes, connect the column to your detector. This procedure helps to avoid air bubbles from being introduced into the flow cell. Before starting any analysis, check for leak tightness by observing the backpressure or using a flow control unit.

Equilibration, Regeneration and Storage

Equilibration

The period of equilibration depends on the flow rate; we recommend using a minimum of 10-20 column volumes. Due to the core particles the equilibration time is much shorter comparing to spherical porous column materials

Column dimensions (length x ID)	Column volume	Equilibration time at 250 µl/min	Equilibration time at 500 µl/min
50 x 2 mm	157 µl	3 min	1.5 min
100 x 2 mm	314 µl	6 min	3 min
150 x 2 mm	471 µl	9 min	4.5 min

Regeneration

We recommend that the column be regenerated, if changes in peak form, retention time, resolution or an increase in back pressure are observed. If the system pressure begins to rise, remove the column and check the system to find whether the pressure increase is being caused by the system or the column.

Pressure increase caused by system: flush system, exchange eluent filters, frits and/or blocked capillaries. Pressure increase caused by column: backflush the column carefully to remove particle buildup from the inlet frit (connect the column outlet to the pump/injector and flush). Do not connect the column to the detector. If the column still has a high backpressure, flush the column according to the following regeneration scheme.

Regeneration scheme for RP and HILIC columns (C18, C18A, HILIC)
20 column volumes water
20 column volumes acetonitrile
5 column volumes isopropanol
20 column volumes heptane
5 column volumes isopropanol
20 column volumes acetonitrile

After the regeneration procedure, re-equilibrate the column with the mobile phase before analyses.

Storage – Reversed phase columns can be safely stored for longer terms in at least 50% organic solvent (e.g. acetonitrile or methanol). For short-term or overnight storage, use a mixture of water with acetonitrile or methanol (not buffer).

Additional recommendations – We highly recommend that you keep a record of the column's operating pressure. Sudden changes in pressure (from either direction) can damage the column, so please avoid rapid increases or drops in pressure. Although working pressures up to 1000 bar (15000 psi) are possible, we advise that you work at pressures < 800 bar for a longer column lifetime. If using buffered eluents, the pH of the eluent used will be dependent on the application and packing material. We recommend using eluents between pH 2 and 8 (pH values at range limits reduce column lifetime). All eluents should be filtered through a 0.45 µm filter and degassed. Use only HPLC-grade eluent (high quality/high purity). Filter all samples before injection (≤ 0.45 µm membrane filter unit) to prevent blocking the column. For dirty samples or those with unknown purity, we recommend the use of a column pre-filter and/or precolumn. If possible, dilute samples in the same eluent which is to be used for the analysis start conditions. We recommend a temperature limit up to max. 60°C.

Because every UHPLC system is unique, especially in regards to the dwell volume, your results may vary from those obtained in our laboratory. Please don't hesitate to call our column specialists to assist you in optimizing your separation.

Failure to follow these precautions may void the column warranty. Technical data are subject to change without notice.

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