TechnicalNote

Use and Care of BAKERBOND HPLC Columns and Bonded Phases

HPLC Columns Solvents and Mobile Phase Components

To maximize column lifetime, use only solvents and reagents which are specially purified for HPLC (such as Avantor's J.T.Baker brand HPLC grade or Ultrapure Bioreagents). For maximum column lifetime, prepared mobile phases should be filtered through 0.45 micron filters before use.

Special Precautions

BAKERBOND HPLC columns are silica-based and should generally be used in the pH range 2.0–8.0.[1] Columns are shipped containing the mobile phase as indicated in the Certificate of Analysis. Special use conditions will be found on an accompanying package insert, if applicable.

If salt buffers are to be used, replace the shipping solvent with water, pumping for 10–15 minutes, then displace the water with the initial buffer until the column is equilibrated. Care should be taken not to exceed the solubility of the salt in the solvent, as buffer precipitation in the column may be irreversible. Use flow rates of 1 mL/min for analytical columns and 4 mL/min for preparative columns.

1 All BAKERBOND Wide-Pore bonded phases for ion-exchange and hydrophobic interaction chromatography can be operated in the pH range 2–10. The polymer coating that covers the silica in these packings stabilizes them against hydrolysis over this pH range.

Column Installation

All columns are furnished with nuts and ferrules for 1/16" capillary tubing. To form a seal in the inverted end-fitting, the compression nut and ferrule must be installed as shown in **FIGURE 1**. Due to the design of the fittings, high-pressure seals can be made without high torque. Do not over tighten compression nuts. BAKERBOND HPLC columns may be used with any high performance liquid chromatographic equipment. Adaptors are needed for medium pressure systems such as the FPLC. Some high pressure systems may require unions available from the HPLC manufacturer.

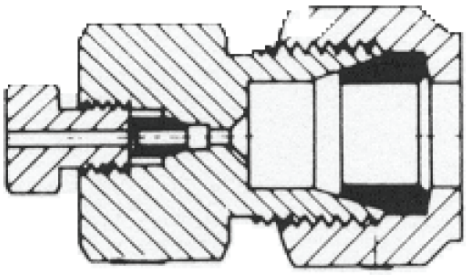

FIGURE 1 *Cross-section view of compression nut and ferrule for 1/16" capillary tubing.*

Column Operation

BAKERBOND columns have certain limitations with respect to operating parameters. Exceeding these limitations may cause deterioration in column performance. **TABLE 1** indicates the limiting values of these parameters.

Table 1 Operational Limits

Parameter	Value
Pressure Maximum	5000 psi
Flow Rate Maximum	none
Temperature	70°C

Table 2 Typical Backpressure of 5 Micron BAKERBOND HPLC Columns at Standard Flow Rates with Water

Column Configuration	Flow (mL/min)	Pressure Range[1] (psi)	Column Volume (mL) (Empty)
4.6 × 50 mm	1.0	200–450	0.83
4.6 × 100 mm	1.0	200–450	1.65
4.6 × 250 mm	1.0	1000–1500	4.16
7.75 × 100 mm	1.0	200–450	4.72
10.0 × 250 mm	4.0	1000–1500	19.64
Guard Columns	1.0	500	0.10

1 Higher pressures should be expected with high salt concentrations or more viscous organic solvents.

The use of a high-pressure-rated in-line filter, column pre-filter or guard column is recommended to protect

the frit and column from accumulating particulates or strongly retained contaminants. Avoid subjecting the column to sudden pressure changes, which may disrupt the homogeneity of the bed.

Bulk Bonded Phase Packing

Most BAKERBOND bonded phases are available in bulk form for repairing pre-packed columns, packing guard columns or packing your own columns. In general, only 40 micron BAKERBOND bonded phases can be simply and reproducibly packed into columns without special equipment. The technique for this is as follows:

Dry-Packing—Assemble the column using 20 micron frits leaving one end open. Place the assembly vertically in a fume hood, resting one set of end-fittings on a hard surface. While simultaneously tapping the end of the column and rotating it by hand, add the bonded phase slowly, but continuously through a funnel placed at the open end of the column. When the column is filled, assemble the inlet end-fitting, ensuring at this stage that there is no void at the column inlet. Pump approximately 100 column volumes over the column at the highest flow rate possible (< 5,000 psi) using the recommended A buffer, and tapping the column in order to remove trapped air or bubbles. Open the inlet end of the column and "top it off" with more bonded phase. Close the column end-fittings and pump additional A buffer over the column.

Slurry Packing—Assemble the column as above. Prepare a slurry of the BAKERBOND bonded phase in a solvent (methanol is generally suitable). Use between 4 and 12 grams per 100 mL of solvent. Thoroughly mix the slurry and sonicate for several minutes in an ultrasound bath. Pour the homogeneous slurry into the column, while applying a slight vacuum to draw off solvent. Pack the column continuously until nearly filled. DO NOT ALLOW THE COLUMN PACKING TO DRY OUT AT ANY STAGE. Apply enough thick slurry to completely fill the column. Assemble the inlet end-fitting ensuring at this stage that there is no void at the column inlet.

Attach the packed column to a source of solvent or buffer. Displace the storage/shipping solvent using an appropriate miscible solvent, such as isopropanol (IPA) for nonpolar mobile phases or water for aqueous phases. For column use, follow procedures outlined above in HPLC column section.

Equilibration/Reequilibration

For isocratic analysis and purification, columns should be equilibrated with mobile phase until reproducible chromatography is obtained with standards or a small amount of sample.

For ion-exchange chromatography, the effluent pH should be the same as that of the inlet mobile phase. This equilibration typically requires 10–30 column volumes of the highest ionic strength buffer to elute strongly bound material followed by 10–30 column volumes of A buffer mobile phase. In any reequilibration procedure, the flow rate may be increased to double the normal rate to reduce analysis time

In gradient elution, column equilibration is very important. A gradient run involving a change in ionic strength, pH or both, may be equilibrated with 30–50 column volumes of starting buffer.

VERY IMPORTANT!

PLEASE READ CAREFULLY!

Column Conditioning Procedure

(Wide-Pore Columns and Packings)

The following column conditioning procedure is recommended for all new wide-pore ion exchange and HIC media columns **in order to avoid the loss of small amounts of valuable protein on the frit material** in the initial chromatographic run. New columns should be flushed with 10 column volumes of distilled water (HPLC-grade) in order to remove the shipping solvent. Next, the column should be washed with 20 column volumes of the appropriate A (binding) buffer as noted in **TABLES 3 AND 4**. Dilute bovine serum albumin (BSA) or any solution containing a purified protein or a mixture of proteins in the A buffer to a final concentration of 5 mg/mL. Inject 1.0 mL of this solution for every 5 mL of empty column volume (approximalely 1.0 mL onto a 7.75 × 100 mm column, 1.0 mL on a 4.6 × 250 mm column, 200 µL on a 4.6 × 50 mm column). Wash with 5 column volumes of A buffer and then elute with 20 mL of the appropriate B (elution) buffer, as noted in **TABLE 3**. For especially valuable samples, reequilibrate and run the gradient again without reinjecting the protein. Finally, equilibrate the column in buffer A and inject your sample. This procedure is also recommended with columns packed with PREPSCALE (40-µm) low pressure bonded phase.

Table 3 Recommended Buffers for Protein Chromatography on BAKERBOND Matrices

Matrix	A (Binding) Buffer	B (Elution) Buffer
ABx	25 mM MES, pH 5.6	500 mM $(NH_4)_2SO_4$, pH 7.0 or NaOAc*, pH 7.
CBX, CARBOXY-SULFON	25 mM MES pH 5.6 or 10 mM KH_2PO_4, pH 5.6	500 mM $(NH_4)_2SO$, pH 7.0 or 1M NaOAc, pH 7.0 or 500 mM KH_2PO_4, pH 6.0
PEI	10 mM KH_2PO_4, pH 5.6 or 25 mM Tris-Oac, pH 6.5	500 mM KH_2PO_4. pH 6.0 or 2M NaOAc, pH 6.0
QUAT	20 mM piperazine HCl, pH 6.0	20 mM piperazine HCl, 1M NaCl, pH 6.0
HI-Propyl	2M$(NH_4)_2SO_4$ plus 25 mM KH_2PO_4, pH 7.0	25 mM KH_2PO_4, pH 7.0
C_4, C_8, C_{18}	0.1% TFA in Water	0.1% TFA in Acetonitrile

* Sodium acetate

Table 4 Suggested A (Binding) Buffer pHfor Use with BAKERBOND Ion Exchangers

	Approximate A Buffer pH			
Approximate pI of Target Proteins	Abx,[1] CBX	CARBOXY-SULFON	PEI[3]	QUAT
<4		2.0–4.0	≤5.0	≤5.0
5	4.0	4.5–5.0	6.0	6.0
6	5.0	5.5–6.0	7.0	7.0
7	6.0	6.5–7.0		8.0
8	7.0	7.5–8.0		9.0
9	8.0	8.5–9.0		10.0[2]
10	9.0	9.5–10.0		
>11	10.0[2]	10.0[2]		

1 ABx and CBX lose their capacity at pH values below 4.0.
2 pH values above 10.0 are not recommended.
3 PEI loses most of its capacity at pH values above 7.5; for basic proteins (pI > 7) cation exchange typically provides better results or alternatively, use QUAT.

Regeneration, Cleaning and Sterilization

Depending on the nature of the samples and bonded phase, the column may, over a period of time, accumulate strongly binding impurities, causing increased pressure and changing chromatography. These impurities may be removed with solvents chosen to dissolve them without harming the column packing. **TABLE 5** lists cleaning solvents that can be used for various bonded phases.

Table 5 Regeneration/Solvents forBAKERBOND HPLC Columns Suggested Order of Priority for Use

Reversed Phase or Hydrophobic Interaction Packings
1. 0.1% Aqueous TFA in isopropanol (40/60)
2. DMSO/water (50/50)
3. Chaotropic agents, such as 6–10M urea, 5–6M guanidine HCl, if necessary
Normal Phase (except BAKERBOND Chiral Phase DNBPG (Ionic))
1. Isopropanol or tetrahydrofuran
2. Acetic acid (10% in isopropanol)
3. Hexane: IPA 80:20 (20%) (for the Ionic Chiral Phase only)
Ion Exchange
1. Sodium acetate (2M, pH 7–8)
2. Acetic acid (10%)
3. Water then DMSO/water (50/50)
4. Chaotropic agents, such as 6–10M urea, 5–6M guanidine HCl, if necessary.

A suggested protocol for using the above solvents is as follows:

1. Wash the column with ten (10) column volumes of water. For normal phase columns wash with isopropanol or tetrahydrofuran.
2. For reversed phase and hydrophobic interaction packings, run several gradients and retrogradients with the 0.1% TFA/IPA solvent until no ghost peaks are observed. Then pump the DMSO/water through the column (five column volumes). If necessary, wash with urea or guanidine, after washing out the DMSO with water (10 column volumes).
3. For ion exchange (includes BAKERBOND ABx columns) run a gradient with 2M sodium acetate pH 7–8 (ten volumes). Follow this with water (five volumes); then acetic acid (10%, ten volumes)

If the above treatments do not result in satisfactory drops in pressure or do not restore the original chromatography, the following steps may be taken:

Frit Removal

Remove the body of the inlet column end-fitting and test it for backpressure. Do this by attaching the end-fitting body to the pump and running flow rates at 1 mL/min to check tor a clogged frit. At 1 mL/min there should be little or no pressure. If the frit is clogged, it should be removed using high flow rates or compressed air, and replaced with a new one. If this procedure is not sufficient to dislodge the frit, the end-fitting should be sonicated in 20% (6N) nitric acid for 10–20 minutes and then in distilled water for two (2) minutes. This is generally effective in removing adsorbed impurities and reducing backpressure in the end-fitting.

Backflushing

As a last resort, the reassembled column may be backfushed by reversing the flow in the column. Very strongly adsorbed impurities are more likely to be removed under these conditions. The general protocols described above should be followed.

Sterilization

Sterilization of BAKERBOND columns or bonded phases may be accomplished by washing with any bactericidal organic solvent, such as chlorhexidine, ethanol, methanol, or acetonitrile, or with a 0.1% solution of sodium azide. The bonded phases are unaffected by and totally resistant to these treatments.

Storage

At the end of each day, it is advisable to wash ion exchange columns with high salt buffers (1M KH_2PO_4, 2M NaOAc, or 2M $(NH_4)_2SO_4$, pH 6.0) to clean off contaminants and to prevent the growth of microorganisms. Unlike most polysaccharide-based soft gels, J.T.Baker silica-based bonded phases do not act as growth media for microorganisms. However, moderate to low ionic strength mobile phases do allow microbial growth. Therefore, if the column is to be stored for long periods (more than a week), the use of a preservative such as chlorhexidine, 0.1% sodium azide in high ionic strength buffer (1M KH_2PO_4 or 2M NaOAc, pH 6.0) or pure acetonitrile is recommended. Ensure that the two Delrin plugs are well secured to avoid evaporation. Storage of your column in a refrigerator in less than 1M salt will also reduce the chances of microbial growth.

TABLE 6 lists solvents that can be used to store BAKERBOND HPLC columns for short or long periods.

Table 6 Storage Solvents for BAKERBOND HPLC Columns

Column Type	Overnight Storage	Several Days or More
Reversed Phase	Methanol	Methanol
Hydrophobic Interaction	Water (Distilled)	Methanol or 0.1% Azide
Normal Phase (Except DNBPG Ionic)	Isopropanol	Isopropanol
DNBPG (Ionic)	Hexane: IPA (80:20)	Hexane: IPA (80:20)
Ion Exchangers, including ABx	1 M KH_2PO_4, or 2M NaOAc. or 2M $(NH_4)_2SO_4$ all pH 6.0, or methanol	Acetonitrile or 0.1% sodium azide in 1M KH_2PO_4 or 2M sodium acetate, pH 6.0

References

1. C. Timothy Wehr, Methods in Enzymology, Vol. 104, 1984, pp 133- 154.

ISO Phillipsburg, NJ 9001: 2008 & 14001: 2004
Paris, KY 9001: 2008
Mexico City, Mexico 9001: 2008
Deventer, Holland 9001: 2008 & 14001: 2004
Selangor, Malaysia 9001: 2008

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