CAPSULES
notes and applications from Bioanalytical Systems, Inc.

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Use and Maintenance of BAS Standard-Bore Columns

Note: even experts should heed the following advice:

WARNING

When using your column for the first time or after storage, you must first flush it for 10 min. with 100% filtered degassed, LC-grade acetonitrile to ensure "wetting" of the packing material. This should be followed by 10 min. of acetonitrile:water (40:60, v/v). Flush at the normal flow rate for the column. After these two steps, the analytical mobile phase can be equilibrated. This protocol is imperative for proper retention when using BAS UniJet™, Phase II™ or Biophase™ columns.

When not in use, columns should be flushed for 10 min. with acetonitrile:water (40:60, v/v) and the ends capped.

This short guide is intended to provide the user with a reference for column use and maintenance and for troubleshooting column problems. With proper attention to mobile phase and sample preparation, BAS columns should provide efficient separations for hundreds of samples. All columns, however, have a finite lifetime and must be replaced whenever the efficiency of separation becomes unacceptable.

Column Installation

All columns are individually packed and tested before shipment. Each column is shipped with a test report which contains the actual column testing conditions along with the calculated efficiency and skew. This report should be saved for future reference.

UniJet™ PEEK columns. These columns can be recognized by their tan or black plastic bodies. Observe the flow-direction arrow on the label when installing. Always use plastic fingertight fittings (MR-4409) to connect these columns, as steel fittings can mangle their plastic threads.

Conventional steel columns (permanent end fittings). When installing the column, either end may be used as the "inlet." These columns are reversible and one-way flow through the column is not necessary. Two compression nuts and ferrules are provided for making the connections. Fingertight fittings (eg., MR-4409) may be used, provided care is taken to fully insert the tubing when making the connection. Substitution of other fittings may cause leaks or poor sample dispersion due to dead volume formation.

Cartridge columns. To install a cartridge, unscrew the holder and carefully insert the new cartridge. Reseal the holder to finger tightness using both end caps. Center the holder body by tightening the end caps equal amounts. Further tightening is unnecessary and may damage the seal or holder.
Never use tools to tighten the holder!

To remove the cartridge, unscrew the holder to expose the end of the cartridge. Using the thumb and forefinger (not pliers!), pull the cartridge out. When not in use, store the cartridge in a protective environment, such as a glass test tube with stopper. This will protect its Tefzel frit assemblies from dirt and scratches. Do not add liquid to the tube. An alternative storage method is to cap the entire assembly with solid plugs.

Connect the assembled column/holder to the chromatograph with either the metal fittings provided or fingertight fittings (MR-4409). The preferred flow through the cartridge is from left to right as you read the label. Avoid backflushing. If the cartridge leaks at less than its rated pressure limit, do not try to tighten it with tools. A leak indicates that the sealing surface must be either dirty, scratched, or deformed. Replace the cartridge with another cartridge and check again for leaks. If it still leaks, the high pressure seals must be replaced. Use the Seal Replacement Kit, p/n MF-6028.

Equilibration. It is recommended that columns be flushed with a stronger eluting solvent than the mobile-phase solvent before starting mobile-phase flow. The solvent must thoroughly "wet" the column packing material. (Note: BAS columns are shipped with a 40:60 acetonitrile:water mixture.) Follow the instructions in the box at the top of this capsule. After flushing, allow a minimum of 1 hour to equilibrate to the initial mobile-phase conditions. Mobile phases containing an ion-pair reagent take several hours to equilibrate.

Mobile Phase and Sample Preparation

To obtain the maximum lifetime of efficiency from any LC column, the mobile phase should be prepared using high-purity buffers and deionized, reagent-grade water. The prepared mobile phase should be filtered through a 0.2 µm membrane before use. Samples should have any proteinaceous material removed, when possible, by precipitation or solvent extraction, and the final solution filtered through a 0.2 µm membrane before injection onto the column. BAS Microfilters™ are ideal for the filtration of small volume samples prior to liquid chromatography. (Table 1).

General Column Care

Whenever mobile phase is not going to be flowing through the column for an extended period, the column should be flushed for 10 min. with 40:60 acetonitrile:water to remove the buffer salts from the chromatographic system.

Column Problems

The most commonly encountered problems are loss of efficiency or high back pressure.

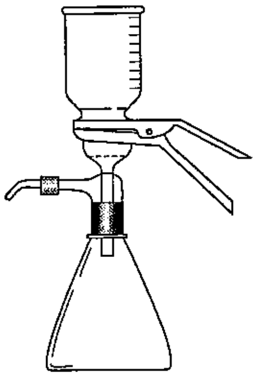
Loss of efficiency. If the column has shown a gradual loss of efficiency, a possible cause is strongly retained compounds on the column packing. In most cases, BAS columns can be sufficiently regenerated by flushing for 10 min. with a strong eluting solvent such as acetonitrile. If this flushing procedure does not have the desired effect, replace the column.

High Back-pressure. If high system back-pressure is encountered, it may be due to pumping a solution of high viscosity. Aqueous mixtures of short-chain alcohols are extremely viscous and can cause very high back-pressures even at low flow rates. If the solution being pumped is known not to be the problem, stop solvent flow, disconnect the column, and restart flow. If **any** back-pressure is present, the problem is due to flow restriction in some part of the plumbing. It may be a particle in the injection valve, a crimped piece of tubing, an improperly swaged fitting, or a clogged filter. Isolate the area of flow restriction by opening connections along the flow path and checking the back-pressure. Replace fittings or tubing as

Table 1. Recommended Membranes and Hardware for Mobile Phase and Sample Filtration

Source	Item	Part No.	Quantity
BAS	Mobile Phase Filtration Kit MF-5520 (47mm) membranes	MF-6126	1 each
BAS	Regenerated Cellulose Membranes 0.2 µm pore size, 47 mm	MF-5520	50/pkg.
BAS	Nylon 66 Membranes, 0.2 µm pore size, 47 mm (Universal applications)	MF-5621	100/pkg.
BAS	Microfilters for sample filtration	MF-5500	12/pkg.
BAS	Microfilter membranes, regenerated cellulose, 0.2 µm	MF-5658	100/pkg.

MF-6126

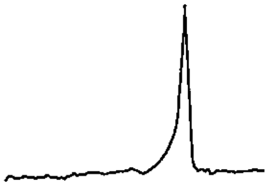
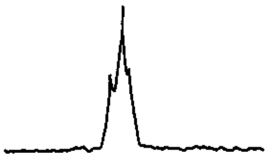
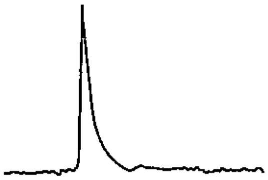


necessary. Refer to valve manufacturer's instructions if valve must be disassembled.

In some cases, high back-pressure can be caused by particulates blocking the inlet frit of the column. Particulates may be removed by carefully back-flushing the column. Reverse the column assembly, and pump 50 mL acetonitrile:water (40:60, v:v) through the column to waste. Then pump 200 mL neat acetonitrile through the column to waste. Turn the column assembly around to the normal flow direction and pump another 50 mL 40:60 acetonitrile:water through it. Then re-equilibrate with mobile phase.

Other Column Problems

There are many other chromatographic problems which can be traced to the analytical column, though these problems may often have other, less obvious causes. The following troubleshooting guide is a table of some of these problems and their solutions.

SYMPTOM	CAUSE	REMEDY
Peak "Fronting" 	A. Void in column B. "Overloading" column C. Sample in solution of significantly different composition from mobile phase	A. Replace column B. Reduce Sample load C. Dilute or reconstitute sample in mobile phase
Peak "Splitting"*† 	A. Void in column B. Sample in solution of significantly different composition from mobile phase C. Mobile phase pH too close to pk value of analyte	A. Replace column B. Dilute or reconstitute sample in mobile phase C. Adjust mobile phase pH so sample is in non-ionic form*
Peak "Tailing"*† 	A. Ionic strength of mobile phase too low B. Incorrect mobile phase pH C. Old column, lost efficiency	A. Increase ionic strength B. Adjust mobile phase pH so sample is in non-ionic form* C. Flush. If no improvement, replace column
Decrease in retention time	A. Lost efficiency	A. See 'Column Problems'
Increase in retention time	A. Organic modifier evaporated from mobile phase	A. Make fresh mobile phase
No retention	A. Sample ionized at mobile phase pH B. "Channeling" in column C. Silica backbone being dissolved by inappropriate mobile phase pH D. Too much organic solvent E. Packing material not wet	A. Adjust mobile phase so that sample is in non-ionic form* B. Replace column C. Observe pH limits of silica gel (between pH 2 and pH 8) D. Decrease organic solvent E. Follow recommended flush procedure

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*This is assuming that the ion-suppression mode is being used. Ion-exchange and ion-pair chromatography utilize ionization. This is not strictly true for all compounds (aromatic amines, for example).

† Only if all peaks do this. A single split or tailing peak may indicate a co-eluting compound.