Guidlines for Use and Care of Aminex® Resin-Based Columns

Instruction Manual

For technical support, call your local Bio-Rad office, or in the U.S., call 1-800-424-6723.

BIO-RAD

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Section 1: Introduction to Resin-Based HPLC Columns

Resin-based high performance liquid chromatography (HPLC) columns can use the mechanisms of ion exclusion, ion exchange, ligand exchange, size exclusion, and reversed phase and normal phase partitioning to separate compounds. The charge on the resin provides the capability for ion exclusion, while the polystyrene backbone allows hydrophobic interaction to take place. The extent of the interactions depends on the compounds being analyzed and the degree of selectivity required.

Reversed phase and ion pairing HPLC techniques require complex eluant conditions for effective separations. These methods work on the principle of modifying the compound to be analyzed until it is compatible with the column. Resin-based HPLC columns turn that around: instead of modifying the compound to be analyzed, the column packing material is modified and chromatographic conditions are optimized to be compatible with the compound structure. Therefore, resin-based columns often allow the use of an isocratic HPLC system, simplify sample preparation methods, and require no sample derivatization. By cutting down sample preparation time, resin-based columns greatly reduce total analysis time. Filtration is the only sample preparation necessary in most separations.

The column is the heart of the HPLC system. The success or failure of an analysis often depends on selecting proper operating conditions and on maintenance of the column. No matter how good the HPLC system performance and the sample preparation are, successful separations may not result if the column is not functioning properly.

A packed bed is a depth filter, and thus it is an excellent collection device for particulate matter. The smaller the packing media, the better it filters. Bonded resin column packings are suitable for separating certain solutes, but they are also capable of retaining other components of the sample indefinitely.

These retained compounds may significantly decrease column efficiency and selectivity. If the column is not properly cared for, time and money are wasted, as a good column may be ruined in a short amount of time. It is extremely important to take the time required to do any column maintenance, which will keep problems to a minimum. With proper setup, proper maintenance, and good laboratory technique, the column will not lose efficiency. This guide for the care of resin-based columns will help to provide higher resolution, longer column life, and better reproducibility.

Section 2: Column Setup

2.1 Unpacking

While unpacking the column, check it carefully for evidence of shipping damage, rough handling, or leaking solvent. Save the shipping container to store the column. If there is evidence of damage, immediately notify the carrier and contact Bio-Rad Technical Service at 1-800-424-6723 or your local Bio-Rad office.

2.2 Preparing the Eluant

Only freshly distilled and deionized water, analytical-grade reagents, and high-quality organic solvents should be used for eluant preparation. The prepared eluant should be run through a 0.45 µm filter before use to eliminate insoluble particles that could clog the system inlet filter. Poor baseline stability is often caused by a dirty mobile phase. Thoroughly degas the prepared eluant prior to use.

The best way to degas solvent uses both vacuum and ultrasonic techniques. Vacuum degassing alone will work, but it takes longer; filtering alone will not remove all the gas. A stir bar in the vacuum flask facilitates the release of gas from the solvent. A 1 liter vacuum filtering flask works well.

The flask used to degas the solvent should also be used as the reservoir, as pouring degassed solvent into another reservoir will only add gas to the eluant. Switching between aqueous and organic solvents is especially likely to cause outgassing. Be sure that both solvents are thoroughly degassed.

Section 3: Guard Columns

Guard columns have been an accepted part of HPLC technology for a number of years because of the role they play in protecting both the analytical column and the HPLC system. Bio-Rad's Micro-Guard cartridges not only extend the lifetime of the analytical column, they also provide a convenient method for inline sample preparation. Contaminants that interfere with analytical separations, as well as compounds that foul the analytical columns, can be removed with these cartridges.

Interference caused by anions, cations, organics, salts, insoluble particles, and particulates can be reduced or eliminated using Micro-Guard cartridges. We strongly recommend the use of Micro-Guard cartridges with Bio-Rad's HPLC columns.

The Micro-Guard HPLC column protection system consists of a disposable guard cartridge in a standard guard cartridge holder, or an anion and a cation cartridge in a double deashing holder. The deashing holder is used with the Aminex HPX-42A silver form column. The deashing format can also be used with other columns that use water as a mobile phase.

The guard column must be replaced before any contamination reaches the main column. Replacement frequency cannot be standardized because it depends on sample preparation conditions. In general, the column should be checked periodically with a standard sample. When some change in the measured data is observed, the guard column should be replaced immediately.

3.1 Installing the Guard Columns

To install a cartridge, unscrew the end nut from one end of the holder. Attach the solvent tubing from the injector to the Micro-Guard holder with the Parker-style nut and ferrule included with each new cartridge holder. While the cartridge may be used initially in either direction, changing direction once a cartridge has been used may cause particulate matter to flow off the cartridge and contaminate the analytical column. Attach a short piece of tubing from the cartridge holder to the main HPLC column in a fashion similar to the way inlet tubing is set up (see instructions for cartridge holder). Place the guard column in the holder and secure the holder fingertight using both end nuts. Further tightening is unnecessary and may damage the seal or holder. Never use tools to overtighten this seal.

If storing a cartridge out of its holder between uses, protect its PCTFE frit assemblies from dirt and scratches. Store the cartridge in its zip-locking bag with a few drops of recommended storage solvent to keep the cartridge from drying out.

3.2 Purging the Guard Columns

Flush approximately 10–15 ml of mobile phase through a new guard column at a flow rate of 0.2–0.3 ml/min. With resin-based HPLC columns, it is not unusual to find yellow eluant coming off the column initially for a short period. This material is polysulfonate formed during column storage. Repeat this procedure each time a new guard column is installed. The analytical column may now be attached to the Micro-Guard column.

Section 4: Connecting the Column

Reduce the flow rate to 0.2 ml/min. Remove the end screws from the analytical column and attach the outlet end of the guard column tubing to the inlet end of the analytical column. Connect the analytical column with the pump running at a slow flow rate to exclude any air from the column inlet. Pass approximately 20 ml of degassed solvent through the column. When a new column is initially placed on an LC system for use or testing, the column should be attached only at the inlet end when introducing the mobile phase. This prevents particulates of packing (should the frit have broken in shipment) or air bubbles (if the column dried during storage) from getting into the detector flow cell. When clear, bubble-free solvent is flowing from the outlet end of the analytical column, the column outlet may be attached to the detector.

It is important that the tubing between the column and the detector be as short as possible. Tubing with a small inner diameter (0.01" ID) should be used between the injector and the column and between the column and the detector. Be sure to make provision for collecting and properly disposing of waste solvent. Place the column into an HPLC column heater, equipped with the appropriate inserts, after checking for leaks in guard column and analytical column connections. Turn on the column heater and adjust the temperature. **Never heat a column without flow**. Increase the flow rate only after the column has reached the set temperature. Equilibrate the column with your eluant. With gradient systems, use the starting eluant.

Be sure air does not get into the column. If there is reason to believe that it has, reduce the column temperature, reverse the column direction, and allow the solvent to flow slowly through the column until the air is eliminated. Be sure to remove air from all system piping. Then reconnect the column correctly.

Note that all metal tube connections are of the compression screw (reverse nut) type. A ferrule is compressed permanently against the tubing. To ensure minimum dead volumes, tighten the assembly of tubing, ferrule, and nut fingertight. Push the tubing in until it bottoms firmly. Using a 1/4” wrench, tighten 1/4 turn. The fitting needs to be only tight enough to seal; its lifetime will be diminished by overtightening.

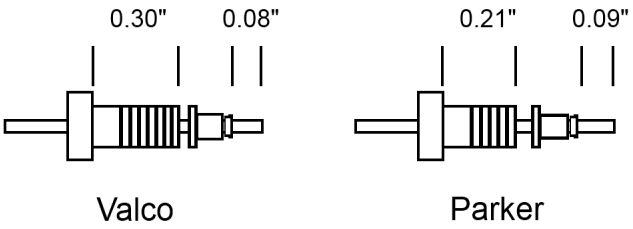

Figure 1. Bio-Rad's resin-based columns are manufactured using Parker end-fittings. Reverse nuts and ferrules are supplied with the columns and must be used to attach the column to the HPLC system if the system is not already equipped with Parker 1/16" reverse nuts or the equivalent.

Section 5: Operating Parameters

The HPLC system should now have been purged with priming solvent followed by the proper mobile phase, the guard column should have been equilibrated, and the analytical column should be attached. The flow rate should be at 0.2 ml/min.

5.1 Flow Rate

Ramp up the flow rate on Aminex columns slowly. Increase the flow rate of a heated column only after the column has come up to operating temperature. Do not operate high-temperature Aminex carbohydrate columns (HPX-87C, HPX-87P, HPX-87N, or HPX-87K columns) above 0.3 ml/min while at ambient temperatures.

Maintain slower flow rates while the column heater heats the column. Increase to the operating flow rate when the column has arrived at the recommended operating temperature. Do not allow the flow rate to exceed the maximum rate specified for the column. Even at the recommended temperature, columns should not be operated at maximum flow rates for extended periods.

Optimum flow rates are specified in published methods. Also, flow rates can be selected based on the quality of the resolution. Slower flow rates often improve resolution. Typically, 300 mm long (30 cm) Aminex columns operate best at 0.5 to 0.7 ml/min, when backpressure on the column is below maximum levels. Backpressures may increase with column age, so let backpressure be the primary determinant of flow rate. Standard flow rate for columns in the Aminex HPX-87 series is 0.6 ml/min.

5.2 Sample Preparation

Some of the sample components may not be soluble in some solvents. To prevent any problems of this sort, always dissolve the sample in the mobile phase. Filter the sample solution through a 0.45 µm filter to remove particulates.

5.3 Pressure Checks

The HPLC pump pressure limit device should be adjusted so that a pressure increase (10–20% above the standard operating pressure) will cause the pumps to turn off. This will protect the system from accidental overpressure. On some HPLC pumps, the pressure sensor cannot be adjusted to this close a tolerance at the low pressure required for these columns. In that case, take extra care to ensure that the pressure limit is not exceeded.

Always increase pressure slowly to normal flow rates (~1–2 min). This method is gentle on the column and provides maximum longevity while retaining resolution and efficiency. Starting the column at full flow rate may compress the packing and create an inlet void, which would reduce performance.

When first connecting the column to an HPLC system, check the pressure while the system is operating at normal flow rates, after the slow start-up. The total backpressure of the system will be approximately 700–1,100 psi. (**Note**: some HPLC pumps do not give accurate pressure readings at low pressures; it may be necessary to use a 0–1,000 psi gauge placed upstream of the injector.) To determine the backpressure of the column, read the total system operating pressure, then disconnect the tubing between the guard column and the analytical column and note the pressure drop. The decrease should be approximately 100–200 psi. (Note: remember to reduce the flow rate of the pump before reconnecting the column, then slowly return to the full flow rate over 1–2 min.)

If the total backpressure of the system increases during use (other than the normal increase caused by the higher-concentration solvents that may be used during elution), repeat the above procedure to determine the cause. If the backpressure increase is small and is due to the guard column, the system can be operated normally. If the increase in pressure is greater than 150% of the pressure when the guard column was new, the guard column should be replaced.

If the system pressure increase is caused by the column, the column may be partially clogged, and flow rates should be reduced to stay within the operating limits. Sometimes column backpressure increases are caused by nonpolar compounds adsorbing to the column matrix over many injections. Occasionally, backpressure increases are caused by clogged frits. In any case, following the cleaning procedures and/or reversing the column flow may help to reduce the backpressure. If a guard system is in place and if the cartridge is changed according to directions, fewer contaminants adsorb to the matrix and higher column backpressures are delayed over the life of the column.
Do not open the column to resolve backpressure problems. The packing material of an opened column may partially extrude from the opening, which would ruin the column.

When using an organic modifier, run the column at 0.1 ml/min at 5% solution until a stable baseline is obtained, then increase the organic modifier to the desired concentration. Observe maximums for each column. A first step using a 5% solution reduces immediate swelling of the column material and the possibility of overpressure.

Avoid sudden pressure surges on the column. The packing may compress, which will result in tailing and decreased column efficiency.

5.4 Column Testing

All new columns should be tested to verify proper performance before they are used for analysis. Column performance can change after extended use, and retesting is useful to evaluate the changes. For successful analysis, the efficiency (number of theoretical plates) and selectivity (resolution) of a column must both meet minimum requirements.

The performance of the HPLC system should be verified independently of the column. Since each column is checked just prior to shipping, failure to reproduce the supplied chromatogram may reflect problems with the HPLC hardware or the preparation of the mobile phase. Be sure to keep all extra-column volumes to a minimum. Loss of efficiency is often due to tubing or injector problems. If the peak separation differs dramatically from the chromatogram supplied, the mobile phase should be prepared again with careful consideration of each of its components.

If the peak elution order and peak shapes resemble the test chromatogram but the compounds are not fully resolved, minor changes in mobile phase and other chromatographic variables may be made to achieve optimal separation or to improve resolution. When all components of the standard are resolved sufficiently, analysis of samples may begin.

Bio-Rad's columns are tailored for specific applications by optimizing several parameters, including resin ionic form and column configuration. The following tables compare Bio-Rad's various resin-based analysis columns and provide operating guidelines. Table 1 provides column specifications and typical operating parameters. Table 2 provides data that can be useful for resin modification, as well as cleaning, regeneration, and storage information.

Table 1. Specifications and Operating Guidelines for Aminex Columns.

	Aminex HPX-87C Column, 300 x 7.8 mm	Aminex HPX-42C Column, 300 x 7.8 mm	Aminex HPX-87P Column, 300 x 7.8 mm
Catalog number	125-0095	125-0096	125-0098
Resin ionic form	Calcium	Calcium	Calcium
Support	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene
Particle size	9 µm	25 µm	9 µm
Maximum pressure	1,500 psi	800 psi	1,500 psi
Maximum flow rate at temperature max	0.6 ml/min	0.6 ml/min	0.6 ml/min
Maximum temperature	85°C	85°C	85°C
Maximum injection volume	20 µl	20 µl	20 µl
Typical mobile phase	H_2O	H_2O	H_2O
pH range	5–9	5–9	5–9
Guard cartridge	125-0128	125-0128	125-0118 and/or 125-0119

	Fermentation Monitoring Column, 150 x 7.8 mm	Fast Carbohydrate Analysis Column, 100 x 7.8 mm	Aminex HPX-42A Column, 300 x 7.8 mm
Catalog number	125-0115	125-0105	125-0097
Resin ionic form	Hydrogen	Lead	Silver
Support	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene
Particle size	9 µm	9 µm	25 µm
Maximum pressure	1,500 psi	1,500 psi	800 psi
Maximum flow rate at temperature max	1.0 ml/min	1.0 ml/min	0.6 ml/min
Maximum temperature	Ambient/65°C	85°C	85°C
Maximum injection volume	20 µl	20 µl	20 µl
Typical mobile phase	H_2SO_4	H_2O	H_2O
pH range	1–3	5–9	6–8
Guard cartridge	125-0129	125-0119	125-0118

Table 1. Specifications and Operating Guidelines for Aminex Columns, cont.

	Aminex HPX-87H Column, 300 x 7.8 mm	Fast Acid Analysis Column, 100 x 7.8 mm	Aminex HPX-87N Column, 300 x 7.8 mm
Catalog number	125-0140	125-0100	125-0143
Resin ionic form	Hydrogen	Hydrogen	Sodium
Support	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene
Particle size	9 µm	25 µm	9 µm
Maximum pressure	1,500 psi	800 psi	1,500 psi
Maximum flow rate at temperature max	0.6 ml/min	0.6 ml/min	0.6 ml/min
Maximum temperature	Ambient/65°C	Ambient/65°C	85°C
Maximum injection volume	20 µl	20 µl	20 µl
Typical mobile phase	0.005 M H_2SO_4	0.005 M H_2SO_4	H_2O
pH range	1–3	1–3	5–9
Guard cartridge	125-0129	125-0129	125-0508

	Aminex HPX-87K Column, 300 x 7.8 mm	Aminex HPX-87C Column, 300 x 7.8 mm
Catalog number	125-0142	125-0094
Resin ionic form	Potassium	Calcium
Support	Sulfonated divinyl benzene-styrene	Sulfonated divinyl benzene-styrene
Particle size	9 µm	9 µm
Maximum pressure	1,500 psi	1,500 psi
Maximum flow rate at temperature max	0.6 ml/min	0.6 ml/min
Maximum temperature	85°C	85°C
Maximum injection volume	20 µl	20 µl
Typical mobile phase	H_2O	M H_2O
pH range	5–9	5–9
Guard cartridge	125-0507	125-0128

Table 2. Cleaning, Regeneration, and Storage Guidelines.

	Aminex HPX-87C Column, 300 x 7.8 mm	Aminex HPX-42C Column, 300 x 7.8 mm	Aminex HPX-87P Column, 300 x 7.8 mm
Organic modifier (maximum)	Acetonitrile 30%; EtOH, IPA, 5%	Acetonitrile 30%; EtOH, IPA, 5%	Acetonitrile 30%; EtOH, IPA, 5%
Inorganic modifier	Calcium sulfate or nitrate	Calcium salts	Lead nitrate
Avoid	MeOH, acids, bases, Na azide, other salts	MeOH, acids, bases, Na azide, other salts	MeOH, acids, NaN_3, other salts, anions that form insoluble precipitates with lead
Cleaning solvent	Start w/ 5% CH_3CN in water. Then increase to 30% CH_3CN in water	Start w/ 5% CH_3CN in water. Then increase to 30% CH_3CN in water	Start w/ 5% CH_3CN in water. Then increase to 30% CH_3CN in water
Flow rate	0.2 ml/min	0.2 ml/min	0.2 ml/min
Temperature	25°C	25°C	25°C
Duration	4 hr	4 hr	4 hr
Regeneration solvent	0.1 M $Ca(NO_3)_2$	0.1 M $Ca(NO_3)_2$	Not recommended
Flow rate	0.2 ml/min	0.2 ml/min	N/A
Temperature	85°C	85°C	N/A
Duration	4–16 hr	4–16 hr	N/A
Shipping/storage solvent	Water	Water	Water

Table 2. Cleaning, Regeneration, and Storage Guidelines, cont.

	Fast Carbohydrate Analysis Column, 100 x 7.8 mm	Aminex HPX-42A Column, 300 x 7.8 mm	Aminex HPX-87H Column, 300 x 7.8 mm
Organic modifier (maximum)	Acetonitrile 30%; EtOH, IPA, 5%	Acetonitrile 30%; EtOH, IPA, 5%	CH_3CN 40%; EtOH, IPA, 5%
Inorganic modifier	Lead nitrate	None	Phosphoric acid, nitric acid (<5%)
Avoid	MeOH, acids, bases, all salts	MeOH, acids, bases, all salts	MeOH, salts, bases, metal ions, amines, other organic solvents, H_2O >pH 3
Cleaning solvent	5% CH_3CN in water followed by 30% CH_3CN in water	5% CH_3CN in water followed by 30% CH_3CN in water	(1) 5% CH_3CN in 0.005 M H_2SO4; (2) 30% CH_3CN in 0.005 M H_2SO_4
Flow rate	0.2 ml/min	0.2 ml/min	0.2 ml/min
Temperature	25°C	25°C	65°C
Duration	4 hr	4 hr	(1) 4 hr; (2) 12 hr; (3) run mobile phase until steady baseline
Regeneration solvent	Not recommended	Not recommended	0.025 M H_2SO_4
Flow rate	—	—	0.2 ml/min
Temperature	—	—	65°C
Duration	—	—	4–16 hr
Shipping/storage solvent	Water	Water	0.005 M H_2SO_4

Table 2. Cleaning, Regeneration, and Storage Guidelines, cont.

	Fast Acid Analysis Column, 100 x 7.8 mm	Fermentation Monitoring Column, 300 x 7.8 mm	Aminex HPX-87N Column, 300 x 7.8 mm
Organic modifier (maximum)	CH_3CN 40%; EtOH, EtOH, IPA, 5%	CH_3CN 40%; EtOH, EtOH, IPA, 5%	CH_3CN 30%; EtOH, IPA, 5%
Inorganic modifier	Phosphoric acid, nitric acid (<5%)	Phosphoric acid, nitric acid (<5%)	Na_2SO_4
Avoid	MeOH, salts, bases, metal ions, amines, other organic solvents, H_2O >pH 3	MeOH, salts, bases, metal ions, amines, other organic solvents, H_2O >pH 3	MeOH, acids, bases, sodium azide
Cleaning solvent	(1) 5% CH_3CN in 0.005 M H_2SO_4; (2) 30% CH_3CN in 0.005 M H_2SO_4	1) 5% CH_3CN in 0.005 M H_2SO_4; (2) 30% CH_3CN in 0.005 M H_2SO_4	5% CH_3CN in 0.01M Na_2HPO_4 followed by 30% CH_3CN 0.01 M Na_2HPO_4 30% CH_3CN in 0.01 M Na_2HPO_4
Flow rate	0.2 ml/min	0.2 ml/min	0.2 ml/min
Temperature	65°C	65°C	25°C
Duration	(1) 4 hr; (2) 12 hr; (3) run mobile phase until steady baseline	(1) 4 hr; (2) 12 hr; (3) run mobile phase until steady baseline	4 hr
Regeneration solvent	0.025 M H_2SO_4	0.025 M H_2SO_4	0.025 M H_2SO_4
Flow rate	0.2 ml/min	0.2 ml/min	0.2 ml/min
Temperature	65°C	65°C	85°C
Duration	4–16 hr	4–16 hr	4–16 hr
Shipping/storage solvent	0.005 M H_2SO_4	0.005 M H_2SO_4	0.01 M Na_2HPO_4

Table 2. Cleaning, Regeneration, and Storage Guidelines, cont.

	Aminex HPX-87K Column, 300 x 7.8 mm	Aminex HPX-87C Column, 300 x 7.8 mm
Organic modifier (maximum)	CH_3CN 40%; EtOH, EtOH, IPA, 5%	CH_3CN 40%; EtOH, EtOH, IPA, 5%
Inorganic modifier	K_2SO_4	Calcium sulfate or nitrate
Avoid	MeOH, acids, bases, sodium azide	MeOH, acids, bases, sodium azide
Cleaning solvent	5% CH_3CN in 0.01M Na_2HPO_4 followed by 30% CH_3CN 0.01 M Na_2HPO_4 30% CH_3CN in 0.01 M Na_2HPO_4	5% CH_3CN in water followed by 30% CH_3CN in water
Flow rate	0.2 ml/min	0.2 ml/min
Temperature	25°C	25°C
Duration	4 hr	4 hr
Regeneration solvent	0.020 M K_2HPO_4	0.1 M Ca $(NO_3)_2$
Flow rate	—	0.2 ml/min
Temperature	85°C	85°C
Duration	4–16 hr	4–16 hr
Shipping/storage solvent	0.020 M K_2HPO_4	Water

Section 6: Regeneration Procedures

6.1 Fluffing the Resin Bed

Ion exchange resins are resilient. Gentle backwashing can fluff a collapsed bed back to its original configuration or allow entrained air spaces to redissolve. If bed compression is severe, the column may not return to original performance.

1. Turn off the pump; let the column bed relax for ~15 min.
2. Reverse the flow direction and backwash the column at 0.1 ml/min with the running solvent for at least 4 hr. If necessary, run overnight.
3. Return the column to original operating conditions.
4. Observe the suggested maximum flow rate.

6.2 Cleaning a Contaminated Column

Most particulate contamination from solvent, pump, injector, or sample collects on the inlet frit of the column and can easily be removed by briefly reversing the flow direction. This procedure is sometimes effective for removing microbial contamination as well.

1. Reverse the flow direction and backwash the column at 0.1 ml/min for at least 4 hr, using the appropriate cleaning solvent.
2. Continue the backwash with the appropriate regenerating solvent for an additional 4 hours.
3. Return the column to original operating conditions.

If necessary, replace the end fitting. Very small particles, ion precipitation from sample/column ionic interaction (i.e., calcium carbonate), and strongly retained chemical contaminants that reach the bed can often be removed from the plugged outlet frit by a more extensive cleanup. Inlet and outlet end fittings should be removed one at a time according to the procedure in Section 6.4. Substitute step 4 of the procedure in Section 6.4 with sonication of the end fitting in pure water. Note that this is a procedure of last resort and should be used only if all other attempts to repair the column have failed and nothing else can be done to rejuvenate it.

To avoid contaminating the detector flow cell when cleaning or regenerating the column, disconnect the tubing between the column and the detector. Allow the appropriate solvent to pass directly to waste.

6.3 Reconverting a Column to Its Original Ionic Form

Ionic exchange resins shrink and swell with changes in ionic form. If a new buffer with the wrong counterion is used, the column may be converted to a new form, with accompanying resolution and backpressure problems. In most, but not all, cases, the column may be reconverted to the original form by the following procedure.

1. Reverse the flow direction and backwash the column at 0.1 ml/min for at least 4 hr in the appropriate regenerating solvent.
2. Return the column to its original operating conditions.

6.4 Topping Off a Column Bed

In rare cases, some bed settling may occur while a column is in use. More frequently, serious overpressure may cause irreversible bed collapse. The resulting void space at the column head can often be topped off with material from an old column.

To be sure that the column problem is due to a void in the inlet side of the column, run an unretained compound (sample), and look for tailing on the resulting peak. If you are unsure, consult Technical Services at 1-800-424-6723, or contact your local Bio-Rad office.

1. Disconnect the column from the HPLC system.
2. Store the column at 4°C for 3 hr. Do not freeze.
3. Loosen the nut on the column inlet fitting, which is located at the column end opposite to the flow arrow.
4. Using a clean spatula, carefully pack resin of the same type and ionic form (obtained from an old column) into any apparent void space. Resin should be packed into a shallow cone 1 mm or so above the column end.
5. Replace the end fitting and tighten the nut leak-tight.
6. Return the column to original operating conditions.

6.5 Rectifying Instrument/Connecting Tube Problems

Many instances of poor resolution are not the result of column problems, but of hardware problems elsewhere in the system. The following list indicates some troublesome areas and provides ideal specifications.

1. Injector

Sample volume	100 µl maximum
Loop volume	500 µl maximum

2. Connecting Tubing

Fittings	not deteriorated
Tube end	cut square
Tube bottoms	in fitting
Tube ID	0.013" maximum
Tube length	10" maximum

3. Detector

Cells	clean
Cell inlet tubing	0.013" ID maximum
Column	connected to sample side, not reference side
Temperature	stable

Section 7: Heated Column Shutdown

After a high-temperature separation, reduce the flow rate to 0.2 ml/min and turn off the column heater. Continue to pump solvent until the column returns to ambient temperature. Removing a column from the system while it's hot will cause the solvent to contract as it cools, which will pull air into the column.

If a fast change between two columns is required, the hot column can be immersed in filtered, freshly distilled and deionized water to cool rapidly. Remember to replace the end screws tightly to prevent the resin from drying out.

Section 8: Column Storage

Be certain to always exclude air when closing column end fittings. For prolonged storage, the columns may be refrigerated to prevent drying out. However, they should never be frozen. If the column will not be used for several days, it should be stored according to one of these procedures.

8.1 Long-Term Storage

1. Replace the mobile phase with the appropriate storage/ shipping solvent as shown in Table 2. The flow rate during the mobile phase change should be kept lower than normal flow rate.
2. Keep the ends of the column tightly capped. Use the nuts originally furnished with the column. This minimizes evaporation of solvent and keeps the resin fully hydrated. The detector cells should be flushed as well.
3. Store the column in the original shipping container when not in use. To help prevent evaporation and microbial growth, resin columns can be stored at 4°C (not frozen).

8.2 Short-Term Storage

If the column is used daily, the eluant may be left in the column overnight. Keep a low flow passing through the column to prevent buffer salt precipitation and to allow faster equilibration the next day. If a halide salt is present in the mobile phase, slowly rinse and store the column in distilled, deionized water or appropriate storage solvent prior to storage.

Section 9: Troubleshooting

Table 3 is a troubleshooting guide. It also refers to regeneration processes, listed as Procedures 6.1–6.5. These procedures are given in detail in Section 6. Operating the column within the guidelines given in Table 1 and Table 2 will help ensure a long column life. Any deviation from these guidelines can reduce column efficiency and shorten column life.

Table 3. Troubleshooting Guide.

Problem	Cause	Characteristics	Solution	Regeneration Procedure
High backpressure	Bed collapse from excessive flow rate	Sudden catastrophic pressure increase to a flow rate above the recommended maximum	Turn off pump. Allow 15 min relaxation, then operate at suitable flow rate	6.1
	Chemical contamination	Gradual increase in pressure during use	Reverse flow direction and backwash	6.2
	Trapped air	Column returned to use after storage	—	6.1
	Microbial contamination	Column returned to use after storage	Reverse flow direction and backwash	6.2
	Change in ionic form	Rapid pressure increase after buffer change	Regenerate column	6.3
	Bed degradation from wrong organic solvent	Sudden catastrophic pressure increase with solvent change	—	6.1

Table 3. Troubleshooting Guide, cont.

Problem	Cause	Characteristics	Solution	Regeneration Procedure
Loss of resolution	Particulate contamination	Gradual loss in efficiency or changing retention	Reverse flow direction and backwash	6.2
	Change in ionic form	Changing retention times. Sudden change, which may be accompanied by poor efficiency	Regenerate column	6.3
	Bed collapse from excessive flow rate	Retention times constant; poor efficiency; skewed peaks. Sudden change	Shut off pump. Allow 15 min relaxation before operating at suitable flow	6.1, 6.4
	Bed compression	Retention times constant; lowered efficiency; tailing skewed peaks. Gradual change	—	6.1, 6.4
	Bed degradation from wrong organic solvent	Changing retention times accompanied by poor efficiency	—	6.2
	Excessive dead volume	Inability to match test chromatogram	Check all instrument fittings	6.5

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