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

Instruction Manual

For technical service call your local Bio-Rad office or in the U.S. Call **1-800-4BIORAD** (1-800-424-6723)

Table of Contents

Section 1		**Introduction to Resin-Based HPLC Columns**	**1**
Section 2		**Column Set-Up**	**2**
	2.1	Unpacking	2
	2.2	Preparing the Eluant	2
Section 3		**Guard Columns**	**2**
	3.1	Installing Guard Columns	3
	3.2	Purging the Guard Columns	3
Section 4		**Attaching the Column**	**4**
Section 5		**Operating Parameters**	**5**
	5.1	Flow Rate	5
	5.2	Sample Preparation	6
	5.3	Pressure Checks	6
	5.4	Column Testing	7
Section 6		**Regeneration Procedures**	**14**
	6.1	Fluffing the Resin Bed	14
	6.2	Cleaning a Contaminated Column	14
	6.3	Reconverting a Column to its Original Ionic Form	15
	6.4	Topping Off a Column Bed	15
	6.5	Rectifying Instrument/Connecting Tubing Problems	16
Section 7		**Heated Column Shut-Down**	**16**
Section 8		**Column Storage**	**17**
	8.1	Long Term Storage	17
	8.2	Short Term Storage	17
Section 9		**Troubleshooting**	**17**

Section 1
Introduction to Resin-Based HPLC Columns

Resin-based HPLC columns use the mechanisms of ion exclusion, ion exchange, ligand exchange, size exclusion, reversed phase, and normal phase partitioning. These multiple modes of interaction offer a unique ability 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. With resin-based HPLC columns, 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, they simplify sample preparation methods, and they 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 high performance liquid chromatographic system. The success or failure of the 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.

The 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 acts as a filter. The bonded resin column packings are suitable for separating certain solutes, but are also capable of retaining other components of the sample indefinitely. These retained compounds may significantly decrease column efficiency and selectivity. If proper care of the column is not taken, then time and money are wasted, since 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 the proper set-up, 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 Set-Up

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 1-800-4BIORAD, or your local Bio-Rad office.

2.2 Preparing the Eluant

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

The best method for degassing solvent is a method which uses both vacuum and ultrasonics. Vacuum degassing alone will work, but requires a longer time. A stirring bar in the vacuum flask helps to facilitate the release of gas from the solvent. Filtering alone will not remove all the gas. The flask used to degas the solvent could also be used as the reservoir. A 1 liter vacuum filtering flask works well. 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. Micro-Guard® cartridges not only extend the lifetime of the analytical column, but also provide a convenient method for in-line sample preparation. Contaminants which interfere with analytical separations, as well as compounds which foul the analytical columns, can be removed with these cartridges.

Interferences caused by anions, cations, organics, salts, insolubles, and particulates can be reduced or eliminated using Micro-Guard cartridges. Micro-Guard cartridges are strongly recommended for use 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 which use water as a mobile phase.

The guard column must be replaced before contamination extends to the main column. The replacement frequency cannot be standardized, since 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 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 the direction of the used cartridge may cause particulate matter to flow off the cartridge and contaminate the analytical column. Attach a short piece of tubing leading from the cartridge holder to the main HPLC column in a fashion similar to the inlet tubing (see instructions for cartridge holder). Place the guard column into the holder and secure the holder finger tight, using both end nuts. Further tightening is unnecessary and may damage the seal or holder. Never use tools to over tighten this seal.

To store a cartridge out of its holder between uses, protect its Kel-F frit assemblies from dirt and scratches. Store it 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 Column

Flush approximately 10–15 ml of mobile phase through a new guard column at a flow rate of 0.2–0.3 ml/minute. 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 now may 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 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 been 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 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 (0.010 inch ID) should be used between the injector and the column, and also between the column and detector. Be sure to make provision for collecting and properly disposing of waste solvent. Place the column in Bio-Rad's Column Heater (catalog number 125-0425), 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 the 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 insure minimum dead volumes, tighten the assembly of tubing, ferrule, and nut finger tight. Push the tubing in until it bottoms firmly. Using a 1/4'' wrench, tighten 1/4 turn. The fitting only needs to be tight enough to seal; its lifetime will be diminished by over-tightening.

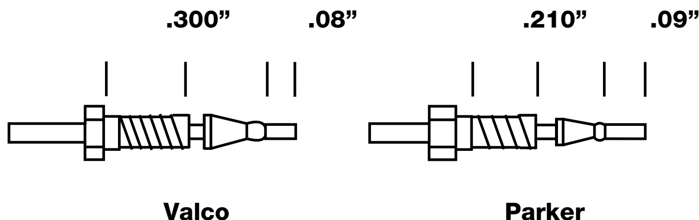

Fig. 1. Bio-Rad’s resin-based columns are manufactured using Parker end-fittings. Reverse nuts and ferrules are supplied with the columns and must be used to attach the column to the HPLC system if the system is not already equipped with Parker 1/16” reverse nuts or the equivalent.

Section 5 Operating Parameters

At this time the HPLC system should 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 slowly on Aminex columns. 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 (Aminex HPX-87C, Aminex HPX-87P, Aminex HPX-87N, or Aminex HPX-87K columns) above 0.3 ml/min while at ambient temperatures. First, allow the column heater to heat the column while maintaining slower flow rates; then 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, but even then, columns should not be operated at maximum flow rates for extended periods. Optimum flow rates are specified in published methods, and also flow rates can be selected based on the quality of the resolution. Many times, slower flow rates improve resolution. Typically, 300 mm long (30 cm) Aminex columns operate best at 0.5 to 0.7 ml/min, when back pressure on the column is below maximum levels. Back pressures may increase with column age, so let back pressure be the primary determinant of flow rate. Standard flow rate for the Aminex HPX-87C, Aminex HPX-87H, and other Aminex HPX-87 series columns 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 over-pressure. On some HPLC pumps, the pressure sensor cannot be adjusted to this close a tolerance at the low pressure required. In this event, extra care must be taken to insure that the pressure limit is not exceeded.

Always pressure-up slowly to normal flow rates (ca. 1–2 min). This method is gentle on the column, and provides maximum longevity and retention of resolution and efficiency. Starting the column at full flow rate may compress the packing and create an inlet void, which will reduce performance.

When first connecting the column to an HPLC system, check the pressure while operating at normal flow rates, after the slow start-up. The total back pressure of the system will be approximately 700–1,100 psi. (**Note:** some HPLC pumps do not give accurate pressure readings at low pressures; use of a 0–1,000 psi gauge placed upstream of the injector may be necessary.) To determine the back pressure of the column, read the total system operating pressure, 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 a 1–2 min period.)

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

compounds adsorbing to the column matrix over a period of many injections. Occasionally, back pressure increases are caused by clogged frits. In any case, following the cleaning procedures and/or reversing the column flow may help to reduce the back pressure. If the guard system is in place and if the cartridge is changed according to directions, fewer contaminants adsorb to the matrix and higher column back pressures are delayed over the life of the column. Do not open the column to resolve back pressure problems. The packing material of an opened column may partially extrude from the opening and ruin the column.

When using an organic modifier, run 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 possible over pressure.

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) must both meet minimum requirements.

The performance of the HPLC system should be verified independent 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, the 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 the various resin-based analysis columns and provide operating guidelines. Table 1 provides column specifications and typical operating parameters. Table 2 provides data which 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	Lead
Support	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer
Particle size	9 µm	25 µm	9 µm
Maximum pressure	1,500 psi	800 psi	1,500 psi
Maximum flow rate at temperature max.	1.0 ml/min	0.7 ml/min	1.0 ml/min
Maximum temperature	85°C	85°C	85°C
Typical mobile phase	H_2O	H_2O	H_2O
pH range	5–9	5–9	5–9
Guard cartridge*	125-0128	125-0128	125-0119

	Fermentation Monitoring Column, 150 x 7.8 mm	**Aminex HPX-72-O column, 300 x 7.8 mm**	**Aminex HPX-72S column, 300 x 7.8 mm**
Catalog number	125-0115	125-0141	125-0146
Resin ionic form	Hydrogen	Hydroxide	Sulfate
Support	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer	Quaternized divinyl benzene-styrene copolymer
Particle size	9 µm	11 µm	11 µm
Maximum pressure	1,500 psi	1,200 psi	1,200 psi
Maximum flow rate at temperature max.	1.0 ml/min	1.0 ml/min	1.0 ml/min
Maximum temperature	65°C	35°C	65°C
Typical mobile phase	H_2O	H_2O	H_2O
pH range	1–3	9–14	4–9
Guard cartridge*	125-0129	125-0133	125-0132

Table 1. Specifications and Operating Guidelines for Aminex Columns (*continued)*

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	Fast Acid Analysis Column, 100 x 7.8 mm
125-0105	125-0097	125-0140	125-0100
Lead	Silver	Hydrogen	Hydrogen
Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer
9 µm	25 µm	9 µm	9 µm
1,500 psi	800 psi	1,500 psi	1,500 psi
85°C	85°C	65°C	65°C
H_2O	H_2O	0.005 M H_2SO_4	0.005 M H_2SO_4
5–9	6–8	1–3	1–3
125-0119	125-0118	125-0129	125-0129

Aminex HPX-87N column, 300 x 7.8 mm	Aminex HPX-87K column, 300 x 7.8 mm	Aminex HPX-87C column, 250 x 4 mm	Aminex Glyphosate Analysis Columns, 100 x 4.6 mm, 250 x 4.6 mm, 300 x 4.6 mm
125-0143	125-0142	125-0094	125-0108, 125-0106 125-0104
Sodium	Potassium	Calcium	Potassium
Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer	Sulfonated divinyl benzene-styrene copolymer
9 µm	9 µm	9 µm	9 µm
1,500 psi	1,500 psi	1,500 psi	1,500 psi
1.0 ml/min	1.0 ml/min	1.0 ml/min	1.0 ml/min
85°C	85°C	85°C	50°C
H_2O	H_2O	H_2O	0.005 M KH_2PO_4 + 4% MeOH
5–9	5–9	5–9	pH 2.0
125-0508	125-0507	125-0128	None required

*Requires cartridge holder.

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, other salts	MeOH, acids, bases, other salts	MeOH, acids, other salts, anions that form insoluble ppts with lead
Cleaning Solvent	30% CH_3CN in water	30% CH_3CN in water	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 $Ca(NO_3)_2$	30% acetonitrile; 0.1 M $Pb(NO_3)_2$, pH 4.0*
Flow rate	0.2 ml/min	0.2 ml/min	0.2 ml/min
Temperature	85°C	85°C	60°C
Duration	4-16 hr	4-16 hr	16 hr
Shipping/storage Solvent	Water	Water	Water

*Adjust $Pb(NO_3)_2$ to pH 4.0 with 0.1 M HNO_3

Table 2. Cleaning, Regeneration, and Storage Guidelines (*continued)*

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	Fast Acid Analysis Column, 100 x 7.8 mm
Acetonitrile 30%; EtOH, IPA, 5%	Acetonitrile 30%; EtOH, IPA, 5%	CH_3CN 40%; EtOH, IPA, 5%	CH_3CN 40%; EtOH, EtOH, IPA, 5%
Lead nitrate	None	Phosphoric acid, nitric acid (<5%), HCl	Phosphoric acid, nitric acid (<5%), HCl
MeOH, acids, bases, other salts	MeOH, acids, bases, all salts,	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
30% CH_3CN in water	30% CH_3CN in water	(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
0.2 ml/min	0.2 ml/min	0.2 ml/min	0.2 ml/min
25°C	25°C	65°C	65°C
4 hr	4 hr	(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
30 % acetonitrile; 0.1 M $Pb(NO_3)_2$, pH 4.0*	Not recommended	0.025 M H_2SO_4	0.025 M H_2SO_4
0.2 ml/min	—	0.2 ml/min	0.2 ml/min
60°C	—	65°C	65°C
16 hr	—	4–16 hr	4–16 hr
Water	Water	0.005 M H_2SO_4	0.005 M H_2SO_4

Table 2. Cleaning, Regeneration, and Storage Guidelines (continued)

	Fermentation Monitoring Column, 300 x 7.8 mm	Aminex HPX-72-O Column, 300 x 7.8 mm	Aminex HPX-72S Column, 300 x 7.8 mm
Organic modifier (maximum)	CH_3CN 40%; EtOH, IPA, 5%	CH_3CN 30%; EtOH, IPA, 5%	CH_3CN 40%; EtOH, IPA, 5%
Inorganic modifier	Phosphoric acid, nitric acid (<5%)	NaOH, KOH	Sulfate salts
Avoid	MeOH, salts, bases, metal ions, amines, other organic solvents, H_2O >pH 3	MeOH, anions different than ionic form	MeOH, anions different than ionic form
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	(1) 5% CH_3CN in 0.005 M H_2SO_4; (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	65°C	25°C	25°C
Duration	(1) 4 hr; (2) 12 hr; (3) run mobile phase until steady baseline	(1) 4 hr; (2) 6 hr	(1) 4 hr; (2) 6 hr
Regeneration Solvent	0.025 M H_2SO_4	0.2 M NaOH	0.5 M $(NH_4)_2SO_4$, pH 9
Flow rate	0.2 ml/min	0.2 ml/min	0.2 ml/min
Temperature	65°C	25°C	25°C
Duration	4–16 hr	4–16 hr	4–16 hr
Shipping/storage Solvent	0.005 M H_2SO_4	0.05 M NaOH	0.1 M $(NH_4)_2SO_4$

Table 2. Cleaning, Regeneration, and Storage Guidelines (continued)

Aminex HPX-87N Column, 300 x 7.8 mm	**Aminex HPX-87K Column, 300 x 7.8 mm**	**Aminex HPX-87C Column, 250 x 4 mm**	**Glyphosate Analysis Columns, 100-300 x 7.8 mm**
CH_3CN 30%; EtOH, IPA, 5%	CH_3CN 30%; EtOH, IPA, 5%	CH_3CN 40%; EtOH, IPA, 5%	CH_3CN 30%; EtOH, IPA, 5%
Na_2SO_4	K_2SO_4	Calsium sulfate or nitrate	KH_2PO_4
MeOH, acids, bases,	MeOH, acids, bases,	MeOH, acids, bases,	Bases, metal ions
30% CH_3CN in 0.01 M Na_2HPO_4	30% CH_3CN in 0.01 M K_2HPO_4	30% CH_3CN in water	0.005 M Trisodium citrate; 0.005 M Tetrasodium ethylene diaminetetraacetate; 4% MeOH, pH 10.0 (NaOH)
0.2 ml/min	0.2 ml/min	0.2 ml/min	0.2 ml/min
25°C	25°C	25°C	50°C
4 hr	4 hr	4 hr (3) run mobile phase	4 hr
0.020 M Na_2HPO_4, pH 9	0.020 M K_2HPO_4,	0.1 M Ca $(NO_3)_2$	0.005 M K_2HPO_4, 4% MeOH
0.2 ml/min	—	0.2 ml/min	0.2 ml/min
85°C	85°C	85°C	50°C
4–16 hr	4–16 hr	4–16 hr	16 hr
0.01 M Na_2HPO_4	0.01 M K_2HPO_4	Water	0.005 M KH_2PO_4 + 4% MeOH

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 and let the column bed relax for about 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 be easily removed by briefly reversing the flow direction. This procedure is sometimes effective for removal of 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 which reach the bed can often be removed from the plugged outlet fit by a more extensive cleanup. Inlet and outlet end fittings should be removed one at a time according to the procedure given 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 only be used if all other attempts at repairing the column have failed and nothing else can be done to rejuvenate the column.

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 back pressure 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 original operating conditions.

6.4 Topping Off a Column Bed

In rare cases, some bed settling may occur in use. More frequently, serious over pressure may cause irreversible bed collapse. The resulting void space at the column head can often be topped off with material from an old column or with material obtained from Bio-Rad Laboratories. In order to be assured 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, then consult Technical Services at 1-800-424-6723, or contact your 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 or directly from Bio-Rad) 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 Tubing Problems

Many instances of poor resolution are not the result of column problems, but rather the result of hardware problems elsewhere in the system. The following list can help to point out troublesome areas and provide 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**
   Clean cells
   Cell inlet tubing 0.013” ID maximum
   Column connected to sample side, not reference side
   Temperature stable

Section 7
Heated Column Shut-Down

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 hot column from the system will cause the solvent to contract while cooling and air will be pulled into the column.

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

Section 8
Column Storage

Always be certain to 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 to be 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, which also refers to regeneration procedures, 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 maintain a long column lifetime. Any deviation from these guidelines can reduce column efficiency and shorten column life.

Table 3. Troubleshooting Guide

Problem	Cause	Characteristics
High back pressure	1. Bed collapse from excessive flow rate	Sudden catastrophic pressure increase at flow above recommended maximum
	2. Chemical contamination	Gradual increase in pressure during use
	3. Trapped air	Column returned to use after storage
	4. Microbial contamination	Column returned to use after storage
	5. Change in ionic form	Rapid pressure increase after buffer change
	6. Bed degradation from wrong organic solvent	Sudden catastrophic pressure increase with solvent change
Loss of resolution	1. Particulate contamination	Gradual loss in efficiency or changing retention
	2. Change in ionic form	Changing retention times; Sudden change which may be accompanied by poor efficiency
	3. Bed collapse from excessive flow rate	Retention times constant; poor efficiency; skewed peaks. Sudden change.
	4. Bed compression	Retention times constant; lowered efficiency; tailing skewed peaks. Gradual change.
`	5. Bed degradation from wrong organic solvent	Changing retention times accompanied by poor efficiency
	6. Excessive dead volume	Inability to match test chromatogram

Table 3. Troubleshooting Guide (*continued*)

Solution	Regeneration Procedure
1. Turn off pump. Allow 15 min relaxation, then operate at suitable flow rate	6.1
2. Reverse flow direction and backwash	6.2
3. —	6.1
4. Reverse flow direction and backwash	6.2
5. —	6.3
6. —	6.1
1. —	6.2
2. —	6.3
3. Shut off pump. Allow 15 min relaxation before operating at suitable flow.	6.1, 6.4
4. —	6.1, 6.4
5. —	6.2
6. Check all instrument fittings	6.5

BIO-RAD

Bio-Rad
Laboratories, Inc.

Life Science
Group

Web site *www.bio-rad.com* ***USA*** *(800) 4BIORAD* ***Australia*** *02 9914 2800*
Austria *(01)-877 89 01* ***Belgium*** *09-385 55 11* ***Brazil*** *55 21 2527 3454*
Canada *(905) 712-2771* ***China*** *(86 21) 6426 0808*
Czech Republic *+ 420 2 41 43 05 32* ***Denmark*** *44 52 10 00*
Finland *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84-0*
Greece *30 210 777 4396* ***Hong Kong*** *(852) 2789 3300*
Hungary *36 1 455 8800* ***India*** *(91-124)-2398112/3/4, 5018111, 6450092/93*
Israel *03 951 4127* ***Italy*** *39 02 216091* ***Japan*** *03-5811-6270*
Korea *82-2-3473-4460* ***Latin America*** *305-894-5950*
Mexico *55-52-00-05-20* ***The Netherlands*** *0318-540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30*
Poland *+ 48 22 331 99 99* ***Portugal*** *351-21-472-7700*
Russia *7 095 721 1404* ***Singapore*** *65-64153188*
South Africa *00 27 11 4428508* ***Spain*** *34 91 590 52 00*
Sweden *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *(886 2) 2578 7189/2578 7241* ***United Kingdom*** *020 8328 2000*

LIT42 Rev D