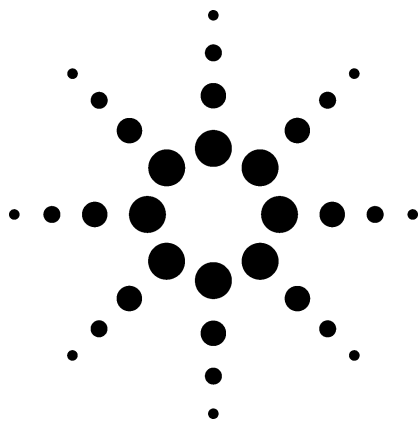




Introduction

Poly F is a proprietary polymeric fluorocarbon HPLC packing material for the reversed-phase separation biological macromolecules.¹ The packing has a 20-micron particle size with 300Å pores and has several unique properties described in the next section which should be reviewed carefully before the column is used.

¹R.C. Williams, J. Vasta-Russell, J. Glajch, K. Golebiewska, J. Of Chromatogr. 371 (1986), 63-70.

Column Performance & Conditions For Use

- The Poly F packing is very hydrophobic and must be washed with a water-miscible organic alcohol, such as methanol, before it can be wet with aqueous solvents. Using a Poly F column without appropriate wetting can damage the column due to high back pressure. It is necessary to condition a new Poly F column by washing with 20 column volumes (20 ml) of methanol before use. The column can then be used for months without reconditioning as long as the packing is not allowed to dry. However, as a precaution to avoid accidental damage to the column, we suggest washing with 10 column volumes (10 ml) of methanol each day before use.

Note: A Poly F column will not be damaged if allowed to dry as long as it is properly reconditioned with methanol before reuse.

- The direction of mobile phase flow through the column must always be in the direction of the arrow on the label. Reversal of the flow voids the column warranty and could adversely affect column stability and column efficiency.
- The pressure limit of the column is 135 bars (2000 psi).

Note: We advise setting 135-bar maximum pressure limit on your HPLC in order to avoid accidental overpressuring of the column.

- The mobile phase flow rate limit is 6 mL/min. Mobile phase flow rates up to this value can be used as long as pressure does not exceed 135 bars.
- The Poly F packing is chemically stable and can be used with aqueous and organic mobile phases without pH limitation. However, the stainless steel column can be attacked by halide acids and halide buffers and these should be avoided. If it is necessary to use halide buffers, the column should be washed out after use. Fluorinated solvents should also not be used as a major (over 10%) component of the mobile phase since they may affect the stability of the packing.
- The column can be used up to 100° C. In general, narrower peak widths can be obtained at elevated column temperatures, e.g., 50° C. Temperatures above the boiling point of the mobile phase should be avoided.
- The column should be stored at room temperature in methanol. The column end fittings should be sealed with the protective screw plugs during shipping. Methanol is the shipping solvent in the column.

Agilent Bio Series

Poly F datasheet

Guard Columns

The Poly F column packing is available in 2-gram quantities (P/N 820957-901). This is a large-particle (60-micron) version of the packing which can be easily dry-packed into guard columns or used for retopping HPLC columns. The Poly F resin can be used with any commercial guard column assemblies.

Note: After dry-packing, a Poly F guard column must be wetted with 20 column volumes of methanol before use with aqueous solvents.

Applications - Proteins and Peptides

Since the Poly F column has no pH limitations, it can be used with either acidic or basic mobile phases. Reversed phase separations of peptides and proteins are best done with mobile phases between pH 1 and 3 or pH 11 and 13. In these pH ranges a wide variety of peptides and proteins, up to 70,000 daltons and above, can be efficiently separated by gradient elution. Only small peptides can usually be eluted from reversed-phase columns in the pH range of 3 to 11. The chromatograms in Figure 1 show the separations of a standard mixture with the same gradient conditions but different mobile phase pH. At pH 1.8 and 12.5, all of the components of the mixture are eluted from the column but with distinctly different selectivity. Reversed-phase columns containing bonded silica packings are usually stable only in the pH range of 2 - 8 and cannot be used for separation of proteins in basic pH. However, the chemically stable Poly F can be used to optimize a separation in either acidic or basic mobile phases. A number of different mobile phase pH modifiers can be used with Poly F, such as the inorganic H_3PO_4 and NaOH or the volatile organics such as trifluoroacetic acid (TFA), triethylamine (TEA), and triethylamine acetate (TEAA).

The recovery of a variety of proteins on the column has been measured to be between 95 and 100% in both acidic and basic pH (Table 1) for sample sizes between 10 and 500 micrograms. The dynamic capacity of the column for an individual protein (amount which causes doubling of peak width) is about 1 to 2 milligrams and absolute capacity (maximum amount retained) of the column is 10 to 20 milligrams of protein.

Since all samples should be separated with gradient elution, it is possible to inject large aqueous samples (up to 1 mL) on the Poly F column without degradation of column performance.

Troubleshooting

1. Poor Peak Shape

- This could be caused by a partially plugged column inlet frit, which should be replaced.
- A void on the top of the column bed could also cause peak tailing. Column voids can be retopped with the same Poly F packing used to fill guard columns.
- Alternatively, poor peak shape could be caused by contamination on the column from previous samples; the column should be washed off with organic solvents such as acetonitrile or toluene or strong acids or bases, such as formic acid or sodium hydroxide.

2. High Backpressure

- This could be caused by plugged column inlet frit, which should be replaced. Sample filtration or a guard column should be used with complex samples that may contain particulate matter.
- High back pressure may also be caused by a collapsed packing bed if pressure has been allowed to exceed 135 bars. A void on top of packing bed is indicative of this.

Safety Considerations

Some important points to keep in mind for safe operation with HPLC components are:

- All points connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the potential hazards from such leaks, due to the flammability and chemical and biological toxicity of the chosen mobile phases.
- Because of its small particle size, dry Poly F packing is respirable and should be handled in a well-ventilated area.
- These columns have not been approved for use in processing products for human use.
- Although the Poly F packing material can withstand extreme mobile phase conditions, care must be taken to avoid mixing mobile phase components which are incompatible and could cause explosions or fires.

Maximize Column Life

- Condition columns with methanol before using with aqueous solvents.
- Never reverse the direction of flow. Mobile phase must always flow in direction of arrows marked on the column.
- Use a guard column or filter samples before injection or both.

- Filter the mobile phases before use.
- Do not open the column unless necessary to change frit.
- Do not allow the column to dry out.
- Do not autoclave the column.
- Do not freeze the column.

Ordering Information

Bio series Poly F Column

6.2 mm ID x 80 mm
Poly F packing
2 grams for guard column
Replacement Frits

Agilent Part No.

884984-901
820957-901

280959-003

Figure 1

Separation of Proteins on Poly F at Different pH

Chromatographic Conditions

Column: Bio Series Poly F

Mobile Phase: A = Aqueous Solution

B = CH₃CN

Linear Gradient: 0-70% B in 20 min

Flow: 2 mL/min

Temp: Ambient

Detector: UV 220 nm, 1.28 auFS

Sample: 25 Micrograms each

PEAK	MW
1. Methionine Enkephalin	634
2. Bradykinin	1,060
3. Insulin Chain A Oxidized	2,530
4. Ribonuclease A	13,700
5. Insulin	5,700
6. Cytochrome C	13,000
7. Melittin	2,846
8. Carbonic Anhydrase	29,000

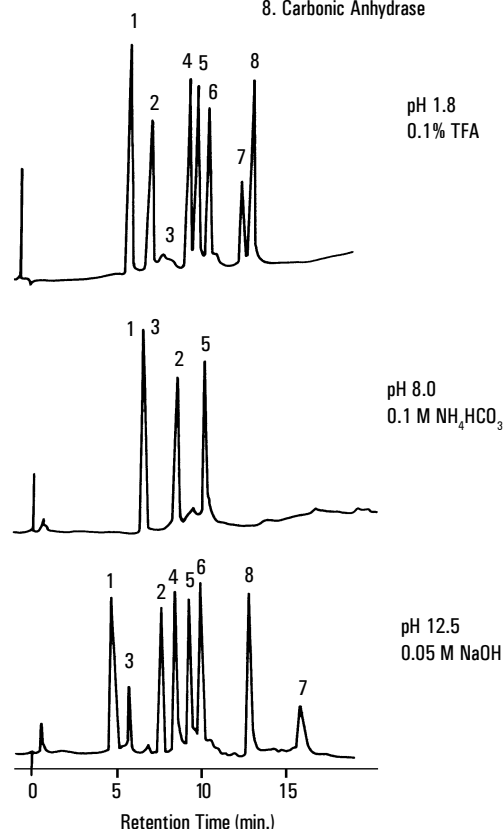


Table 1
Sample Recovery* From Poly F Column

Sample	MW	% Recovery		
		pH2 0.1% TFA	pH7 0.1 M TEAA	pH12 0.05 M NaOH
Angiotensin II	1055	95	100	99
Insulin Chain A	2530	90	87	100
Insulin (Bovine Pancreas)	5700	96	98	100
Ribonuclease A	13700	100	0	96
Cytochrome C	13000	100	0	100
Lysozyme	14400	100	0	93
Carbonic Anhydrase	29000	100	0	91
Oligonucleotide (15 bases)			99	
t-RNA (from E. coli, Strain W)			93	

Chromatographic Conditions:

Mobile phase A = Aqueous

B = CH₃CN

Linear Gradient 0-70% B in 20 min

Flow: 2 mL/min

Temp.: Ambient

Detection and measurement of trapped peaks and standard samples by Bradford test or by UV.



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