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Protein-Pak SEC Columns

CONTENTS

I. INTRODUCTION

This Care and Use Manual contains information pertaining to the use of the following Protein-Pak™ SEC Columns:

Protein-Pak 60 7.8 mm (i.d.) x 30 cm, Steel (P/N WAT085250)

Recommended for use with biopolymers such as peptides, polypeptides, steroids and small proteins. The pore size distribution provides excellent separation in the following molecular weight ranges: Native Globular 1000–20,000 and Random Coil 600–8000. Shipped in 100% methanol.

Protein-Pak 125 7.8 mm (i.d.) x 30 cm, Steel (P/N WAT084601)

Especially suited for separation of biopolymers such as proteins and enzymes in the following molecular weight ranges: Native Globular 2000–80,000 and Random Coil 1000–30,000. Shipped in 100% methanol.

Protein-Pak 300 SW 7.5 mm (i.d.) x 30 cm, Steel (P/N WAT080013)
Protein-Pak 200 SW 8.0 mm (i.d.) x 30 cm, Glass (P/N WAT011786)
Protein-Pak 300 SW 8.0 mm (i.d.) x 30 cm, Glass (P/N WAT011787)

The columns of choice for use with larger biological active molecules in the molecular weight range of Native Globular 10,000–400,000 and Random Coil 2000–150,000. Shipped in 0.05% sodium azide in water solution.

II. PREPARATION FOR OPERATION

a. Steel Column Installation

Remove the end plugs from your column with a 5/18" wrench and save them for storage when the column is removed from the system. The column outlet is indicated by an arrow on the label (showing the direction solvent should flow). Tighten the fittings 1/4-to-1/2 turn. DO NOT OVERTIGHTEN — THIS WILL DAMAGE THE FITTING SEAT. A properly prepared and assembled compression fitting in good conditions is all that is required.

Follow the next four steps of this procedure if tubing cutting is required to connect a new steel column or to improve the end connections on your existing fittings.

1. Using a file with a cutting edge, escribe the circumference of the tubing at the desired break.
2. Grasp the tubing on both sides of the scribe mark with cloth covered pliers (to prevent marring the tube surface), and gently work the tube back and forth until it separates.
3. File the ends smooth and assemble as shown.

Note: Proper positioning of the ferrule in the fitting seat will prevent formation of unwanted dead volume which could result in sample mixing.

4. Slide the compression fitting, followed by the ferrule (large end of the taper first) over the tube. Be certain to bottom the tube in the fitting seat for which its use is intended to assure a leak-free connection.

Note: Attach a union in place of the column and flush the lines free of microparticulates before attaching the column.

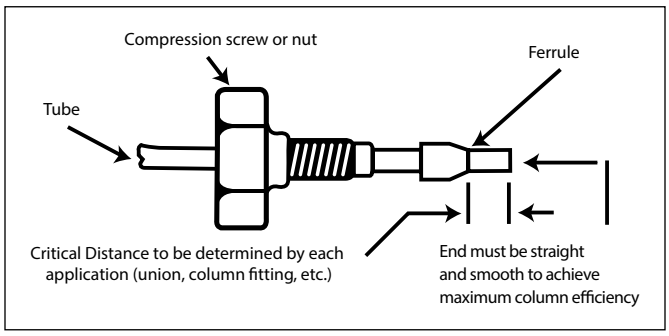

Figure 1. Ferrule and Compression Screw Assembly

b. Glass Column Installation

Waters Protein-Pak 200 or 300 SW glass columns can be connected to any HPLC or medium-pressure system remembering not to exceed 300 psi backpressure with the installed glass SEC columns. The following parts can be used to adapt these columns to any of our standard Waters systems:

Product Description	Part No.
WAZ TO 1/4 UNF Adapter	WAT089472
Compression Screw 1/8 o.d. 1/4 – 28	WAT088467
Reverse Ferrule 1/8 o.d.	WAT088466
Tube Cut 0.125 x 0.009 x 120	WAT088431

c. Solvent Requirements

Protein-Pak 60 and 125 columns are shipped containing an organic solvent compatible with water-miscible solvents (such as methanol, acetonitrile, etc.) The Protein-Pak 200 and 300 SW columns are shipped in water containing 0.05% sodium azide, added as a bacteriostat. If an immiscible solvent is to be used for analysis, make the changeover gradually using at least one intermediate solvent (similar to changing over solvents during analysis). When changing the organic concentration in the mobile phase to reduce the flow rate. When adding organic solvents to aqueous buffer solutions avoid salt precipitation.

d. Buffers

Protein-Pak columns are stable in normal salt buffer solutions such as sodium sulfate, ammonium acetate, ammonium formate, phosphate buffers, tris acetate and acetate buffers. Ionic strength equivalent to 0.1 M minimizes the ionic interaction between the protein sample and the silica stationary phase. Generally the salt concentrations in aqueous solutions should be maintained below 0.5 M.

e. Protein Denaturants

Aqueous solutions of SDS, guanidine hydrochloride and urea are compatible with the Protein-Pak columns although there is a tendency to display shorter column life when compared to non-detergent aqueous solutions. We recommend dedicating columns used with these solutions rather than changing back to other aqueous solutions with the same column.

f. Equilibration

A necessary step to successful use of your new column is the initial solvation (or wetting) of the packing. Purge the column with 5–10 column volumes of the stronger eluting component of the mobile phase alone before a final purge with the actual mobile phase. Equilibration between the mobile phase and packing is established when a stable baseline can be produced. If your result is unsatisfactory repeat the equilibration process.

g. 10X Column Purge Volume

Column Size (mm x cm)	Approx 10X Purge Volume (mL)
7.5, 7.8, or 8.0 x 30	100

II. CARE AND USE

Liquid chromatography columns have a finite life influenced by their care and use, number of injections, sample and solvent cleanliness, frequency of solvent changeover and handling and storage procedures among other factors. If a change is observed in the:

- Retention of a particular compound
- Resolution between two compounds
- Peak shape

Take immediate steps to determine the reason for the changes, and until the determination is made, the results of any separations using the column must not be relied upon. Follow generally accepted procedures for quality control and methods development when using these columns.

Important Note: Before running the first analysis on your new column perform the test sample separation given in the test conditions section.

a. Sample Preparation and Filtration

Use HPLC grade solvents, filtered to remove microparticulate matter above the 0.45 µm. This reduces the problem of plugged filters and preserves column life. Vacuum filtration or sonification may be used to remove dissolved gasses which could affect your solvent delivery system.

Always filter prepared samples to prevent excessive pressure buildup due to particulate matter. Adequate sample cleanup (using Sep-Pak® cartridges designed for this purpose) prevents alteration of the column chemistry by strongly adsorbing or precipitating sample components.

b. Precautions

Normal recommended pressure should not exceed 3500 psi for the Protein-Pak SEC steel columns and no greater than 300 psi for glass SEC columns. Maximum flow rate should not exceed: 1 mL/min

- For all silica-based packing materials, stay within a pH range of 2–8 (i.e. avoid using concentrated acids or bases). For maximum column life stay within the pH range: 3.5–6.5.
- Use Sentry™ Universal Guard Column Holder (WAT046910) and Protein-Pak 125 Sentry Guard 2/pkg. (186000926) to protect your column from contaminants and extend column life.
- Try to dedicate more columns to specific applications. Constant switching of samples and solvents will cause a more rapid column contamination and loss of resolution.
- Filter all aqueous buffers. Avoid using turbid or cloudy buffers. Be sure that any solutions containing buffers, salts, etc. are compatible with the wetted surfaces of the column and equipment.
- Protect column from vibration, mechanical shock, and rapid changes in pressure. Column packings are based on a highly porous and delicate silica gel alignment. Any thermal, physical or chemical shock (such as changing solvents rapidly or at high flow rates) can cause the particles to shift and may result in a loss of efficiency.
- When using water, distill or treat with a Milli-Q® or equivalent system. De-ionized water is not acceptable because it contains organic compounds which alter column selectivity.

- Avoid precipitation. DO NOT inject sample directly into the mobile phase whenever possible. Dissolve (or dilute) samples in an appropriate volume of the mobile phase first. If other solvents must be used, be sure that no precipitation occurs when they are injected into the mobile phase.
- Protect the column from rapid changes in solvent composition. DO NOT change the flow rate faster than 0.5 mL/min. increments.

c. Cleaning and Regeneration

Changes in peak shape, such as increased tailing, shoulders on the peak, shifts in retention, change in resolution, ghost peaks, or increased backpressure may indicate contamination of the column. Choose a cleaning option that may be expected to dissolve the suspected contaminant.

It may be useful to conduct cleaning procedures at one-half the flow rate typically used with that column. In this way the possibility of high pressure events is reduced.

Recommended cleaning solvents:

a. A concentrated salt solution at low pH (e.g. 0.5 M Na SO, pH 2.7).

b. A low concentration of methanol (e.g., 20%) in HPLC-grade water.

c. Use of ionic detergents and other surfactants should be avoided if the SEC column is to be subsequently used to analyze native proteins.

Note: Choose a cleaning solvent based on sample properties, e.g. use (a) to remove basic protein and (b) to remove hydrophobic proteins. Chaotrophic agents can solvate strongly adsorbed proteins via hydrogen bond disruption.

As a last resort, flow reversal or back flushing can be tried at a low flow rate (e.g., 0.1 mL/min). However, this approach may further damage the column or only provide short-lived improvement in performance.

d. Storage Considerations

- DO NOT store column in water alone for more than three days, this practice will promote microbial contamination. Store this series of columns in a 0.05% sodium azide in water solution.
- DO NOT allow buffers or other potentially harmful materials to remain in the system overnight when not being used. Flush and replace with 0.05% sodium azide in water solution.
- Return the column to its box with the end caps firmly in place for storage. Allowing steel columns to dry out can result in poor chromatographic performance.

IV. TROUBLESHOOTING

a. Service and Applications Information

The Waters staff of trained and experienced Service Specialists provides maintenance for Waters instruments on preventative and/or corrective levels. Contact Waters Office at 1-800-252-HPLC or your local Waters Representative for answers to specific chromatography questions in methods development, applications, quality control, and service related matters.

Problem	Cause	Solution
Excess pressure buildup	■ Filters plugged with particulates ■ Sample precipitates on column (sample not soluble in mobile phase)	■ Clean in an ultrasonic bath or replace. ■ Always alter mobile phases and samples. ■ Slowly purge with a strong mobile phase that is both appropriate to dissolve the contaminate and compatible with the column.
Loss of resolution, broad peaks, low plate counts	■ Filters partially plugged ■ Contaminated column, insufficient equilibration ■ Column collapse and void formation	■ Replace or clean inlet and outlet filters in an ultrasonic bath.

b. Column Efficiency

Waters measures the efficiency of the Protein-Pak 60 and 125 columns by using the 5 sigma method. Plate count as an expression of efficiency, is determined by measuring the peak width at 4.4% of the peak height method.

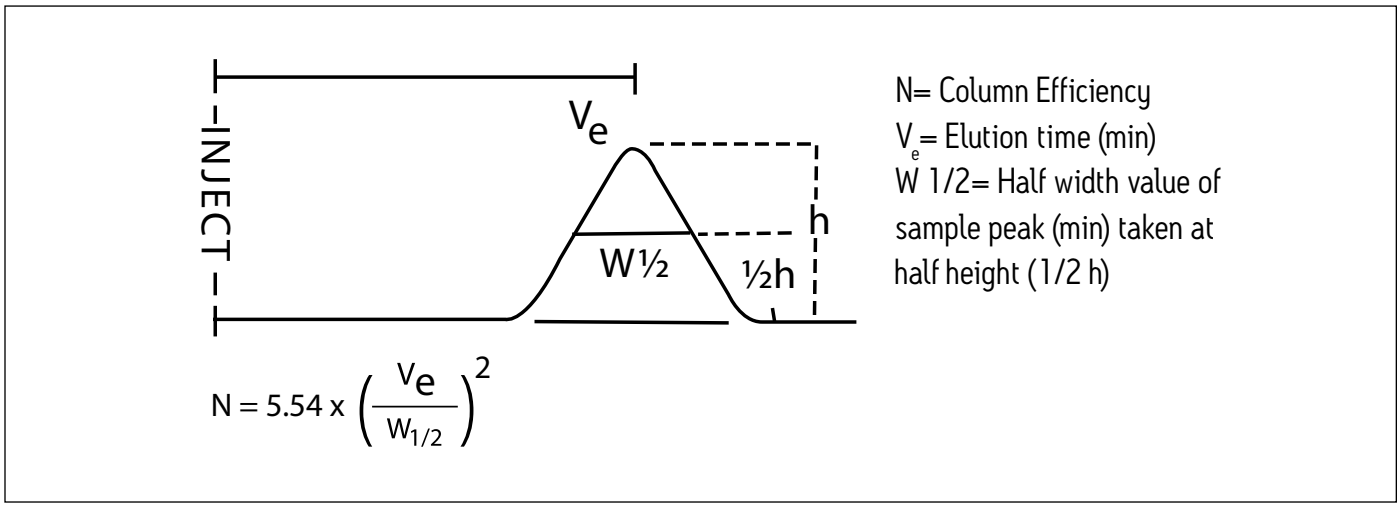

Figure 2. Half Height Method

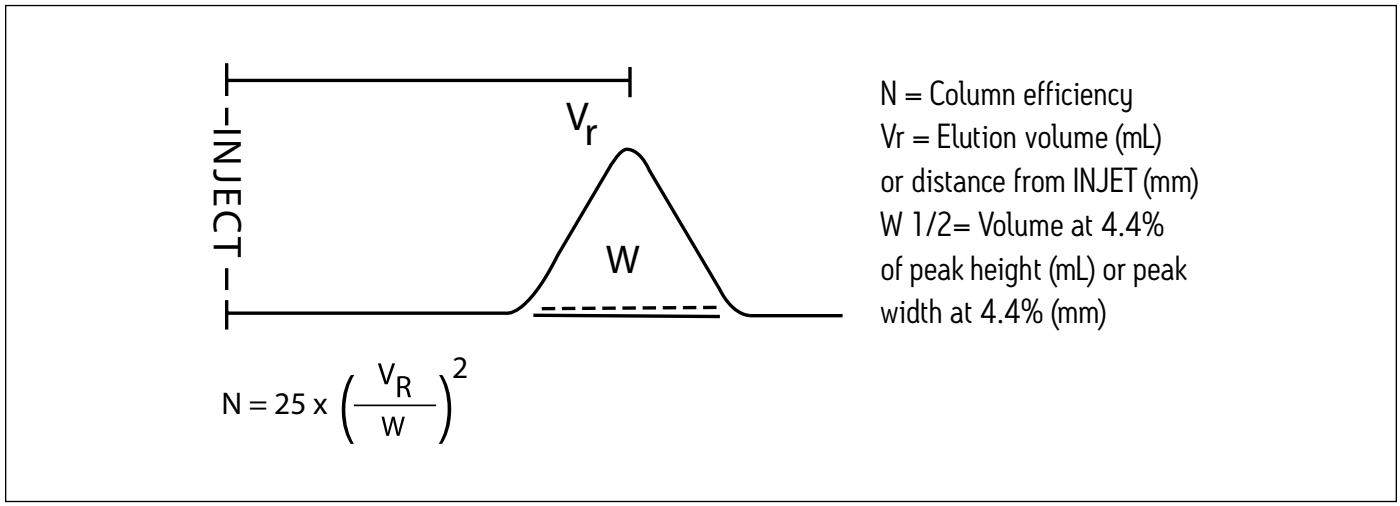

Figure 3. 5 Sigma Method

c. Test Conditions

Columns are thoroughly testing in our quality control laboratories for adherence to our specifications. Since slight variations in your results will occur depending on the equipment used, test your sample makeup and equipment settings and condition, perform the test sample run given here for your new column and record the results (retention time and the settings used) before attempting the first analysis. Use these results for comparison throughout the life of your column.

Detectors should run at 254 nm or 280 nm [UV]; otherwise, use instrument settings that produce an acceptable test peak [this may mean using a lower sensitivity on UV detectors than normally practiced for a specific analysis].

Note: Be sure to record results and instrument settings (and configurations) to allow exact reproduction and comparison in the future.

Key

Mobile Phase	Test Sample Peak
1 100% Methanol	A 1,2 Dichlorobenzene
2 0.05% Sodium Azide	B Ethylene glycol in water

Column name	Part Number	Mobile Phase	Optimal Flow Rate (mL/min)	Test Sample Peak
Protein-Pak 60 7.8 mm x 30 cm	WAT085250	1	0.5	A
Protein-Pak 125 7.8 mm x 30 cm	WAT084601	1	0.5	A
Protein-Pak 300 SW 7.5 mm (i.d.) x 30 cm, Steel	WAT080013	2	0.5	B
Protein-Pak 200 SW 8.0 mm (i.d.) x 30 cm, Glass	WAT011786	2	0.5	B
Protein-Pak 300 SW 8.0 mm (i.d.) x 30 cm, Glass	WAT011787	2	0.5	B

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