GLYCO-PAK DEAE COLUMN

I. INTRODUCTION

Waters Glyco-Pak™ DEAE column is a polymeric column designed for the fractionation of acidic oligosaccharides released from glycoconjugates.

CONTENTS

I. INTRODUCTION
- a. Mode of Action
- b. Typical Chromatogram

II. INSTALLATION
- a. Column Installation
- b. Solvent and Sample Preparation

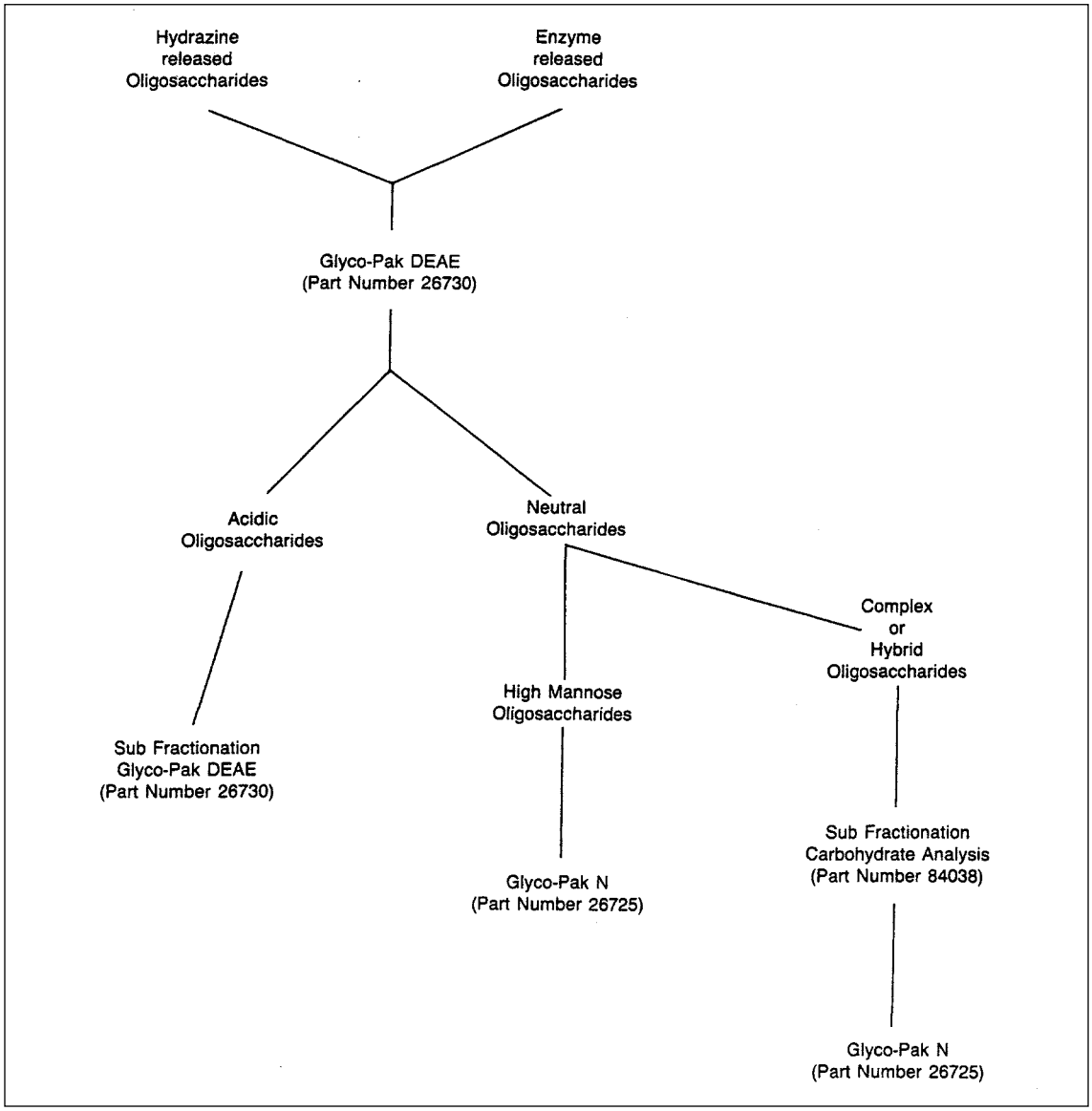
III. OPERATION
- a. Flow Rate
- b. Operating Precautions
- c. Typical Operating Conditions
- d. Column Efficiency
- e. Column Testing

IV. MAINTENANCE AND TROUBLESHOOTING
- a. Column Storage
- b. Troubleshooting

V. WARRANTY AND SERVICE INFORMATION
- a. Service Information
- b. Warranty

VI. ORDERING INFORMATION

Figure 1: Typical Isolation Scheme



Oligosaccharides released by hydrazinolysis or enzyme treatment may be simultaneously separated into neutral and multiple acidic fractions (depending on the number of sialic acids or degree of sulfation) on the Glyco-Pak DEAE column (P/N 26730). Acidic fractions may be further purified on the Glyco-Pak DEAE by changing the mobile phase and/or gradient conditions.

High mannose and simple mixtures of complex or hybrid oligosaccharides can then be fractionated directly on the Glyco-Pak N column (P/N 26725). For more complex mixtures a subfractionation on a different column (Carbohydrate Analysis column and others) may be of value before a final purification on a Glyco-Pak N column. Use these procedures to ensure that you obtain quality results and take full advantage of the features your Waters column offers.

a. Mode of Action

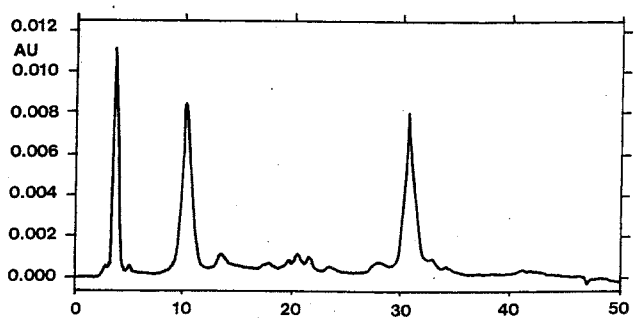
The Glyco-Pak DEAE column operates in an ion-exchange mode. Retention times and resolution are very sensitive to small changes in the ionic strength and or pH of the mobile phase.

b. Typical Chromatograms

Conditions

Sample:	Hydrazine released oligosaccharides from fibrinogen
Mobile Phase:	A: 1 mM sodium phosphate pH 6.0
	B: 10 mM sodium phosphate pH 6.0
Gradient:	Isocratic A for 10 min then Linear gradient 100% A to 100% B over 20 min then hold 100%B for 10 min
Flow Rate:	0.8 ml/min
Detectors:	490 detector 214 nm with baseline subtraction

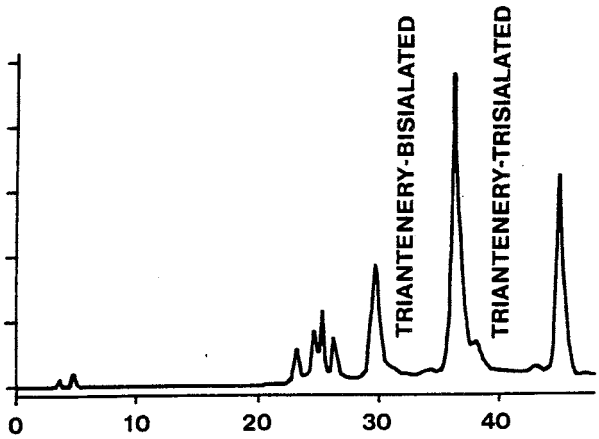
Figure 2: Hydrazine Released Fibrinogen Oligosacchrides



Conditions

Sample:	Hydrazine released fetuin oligosaccharides
Mobile Phase:	A: water
	B: 100 mM NaC1
Gradient:	0-50 % B, Curve 6, 30 min
Flow Rate:	0.6 ml/min
Detection:	490 detector 205 nm with baseline subtraction

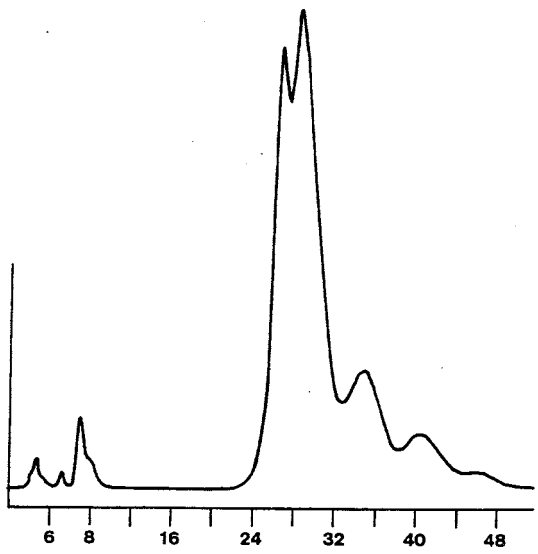
Figure 3: Initial Fractionation of Fetuin Oligosaccharides



Conditions

Sample:	Trisialyl-triantennary fetuin oligosaccharides
Eluent:	15 mM Mono-basic sodium phosphate adjusted to pH 5.4 with sodium hydroxide
Gradient:	Isocratic
Flow Rate:	0.8 ml/min
Detection:	490 at 200 nm

Figure 4: Sub-fractionation of the Trisialylated-triantennary Fraction of Fetuin



II. INSTALLATION

a. Column Installation

Remove the end-cap fittings from your Glyco-Pak DEAE column with a 5/16-inch wrench. Save the end-cap fittings to recap the column when it is removed from the system.

Mobile phase flow direction for the Glyco-Pak DEAE column is indicated by an arrow on the column label.

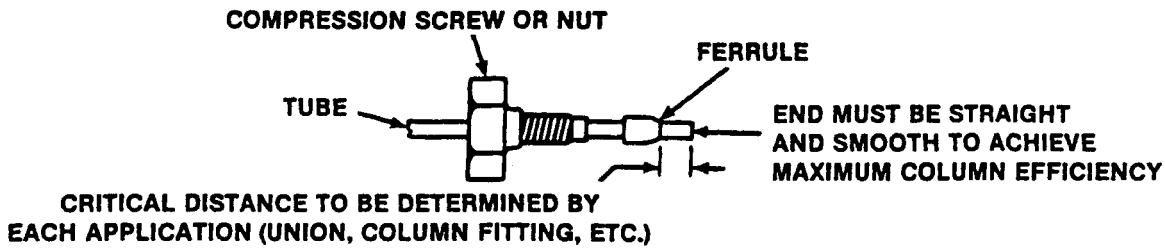
Note: When changing columns, always replace the ferrules. Carefully reseat the ferrule to avoid creating dead volume.

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

1. Using a three-cornered file with a cutting edge, scribe 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. Slide the compression fitting, followed by the ferrule (large end of the taperr first) over the tube. Be certain to bottom the tube in the fitting seat to assure a leak-free connection.
4. Use a 5/16-inch wrench to install the column in the system.

Note: Attach a union in place of the column and flush the lines before installing the column. This flushes previous, possibly incompatible mobile phases from the system.

Figure 5: Ferrule and Compression Assembly



b. Mobile Phase and Sample Preparation

Refer to the following list for mobile phase and sample considerations:

- Use LC grade salts filtered through a Durapore® membrane, with a Waters Solvent Clarification Kit (P/N 85113) or other suitable membrane to remove microparticulate matter above 0.45 µm. A particulate-free mobile phase reduces the problem of plugged filters and column beds and preserves column life.
- Use vacuum filtration or sonication to remove dissolved gasses which could affect your mobile phase delivery system.
- Filter particulate matter from samples to prevent excessive pressure buildup. Use Millex®-HV Filters (P/N 83996) or the Waters Sample Clarification Kit (P/N 26865) to filter samples.
- Use a Waters In-line Precolumn Filter (P/N 84560) to obtain maximum column life.
- Anionic species, such as protein denaturing agents, should be removed from samples prior to fractionation.
- Buffers typically used with other DEAE columns can be used with the Glyco-Pak DEAE column. These include sodium, potasium, amonium and iris salts of phosphate, acetate and chloride.

III. OPERATION

a. Flow Rate

Maintain Glyco-Pak DEAE flow rate at less than 1.2 ml/min. In general, flow rates of 0.5-1.0 ml/min are recommended.

a. Operating Precautions

Refer to the following list for operation precautions:

- Glyco-Pak DEAE columns are compatible with buffers in the pH range of 2 -12.
- Flush system after use with HPLC grade or Milli-Q® water when using buffers containing halide ions.
- Filter all aqueous buffers. Avoid using turbid or cloudy mobile phases. Be sure that any solutions containing buffers, salts, etcetera are compatible with the wetted surfaces of the column and equipment.
- DO NOT exceed 20 % organic content in the mobile phase.
- DO NOT expose columns to freezing temperatures.
- Protect the column from vibration, mechanical shock and rapid changes in pressure. Column packings are based on a porous rigid polymer alignment. Any thermal, physical or chemical shock (such as changing mobile phases rapidly or high flow rates) can cause the particles to shift and may result in a loss of efficiency.
- Treat water with a Milli-Q or equivalent system. De-ionized water is not acceptable because it contains organic compounds which alter column selectivity.
- Protect the column from rapid changes in mobile phase composition. DO NOT change the flow rate faster than 0.5 ml/min increments.
- Do not use sodium azide, sodium dodecylsulfate (SDS) or anionic detergents.

c. Typical Operating Conditions

Mixtures of acidic oligosaccharides typically are separated with a linear gradient from A to B over 30 minutes at a flow rate of 0.8 ml/min with the following mobile phases:

A		B
Water	→	100 mM NaCl
1.0 mM NaH_2PO_4 pH 5-6 with NaOH	→	100 mM NaH_2PO_4 pH 5-6 with NaOH

Volatile buffers such as ammonium acetate may be used for preparative work. Final conditions must be appropriately adjusted for the individual sample.

For sub-fractionation of peaks collected from a gradient run, phosphate buffers of appropriate pH and molar strength can be used isocratically for enhanced resolution.

d. Column Efficiency

Liquid chromatography columns have a finite life which is directly related to the care and use they receive. Column life is influenced by the number of injections, sample and solvent cleanliness, frequency of solvent changeover, and handling and storage procedures.

If you observe a change in the (1) retention of a particular compound, (2) resolution between two compounds, or (3) peak shape, take immediate steps to determine the reason for the changes. Until the cause of the change is determined, the results of any separation using the column must not be relied upon.

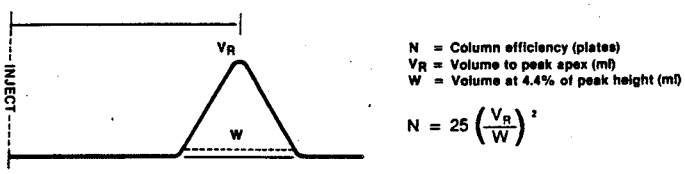
Waters columns are thoroughly tested in our quality control laboratories for adherence to our specifications. Variations in your results can occur depending on the equipment used, test sample makeup, mobile phase, equipment settings and conditions.

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

e. Column Testing

Waters uses the 5-sigma method shown in Figure 6 to measure column efficiency. Unlike the tangent method used to determine system efficiency, this stringent method considers natural peak asymmetry.

Figure 6: 5-Sigma Test Method

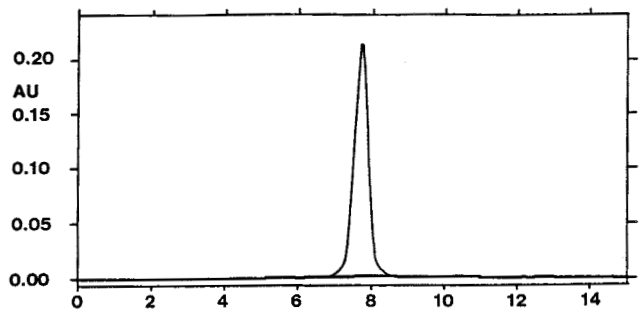


To aid in diagnosing potential column problems:

- Equilibrate the column in 15 mM sodium acetate adjusted to a pH of 5.0 with acetic acid at a flow rate of 0.8 ml/min for 1 and 1/2 hours
- Inject 10 µl of a 10 mg/ml solution of N-acetylneuraminic acid in 15 mlVl sodium acetate buffer at pH 5.0
- Set detector to 220 nm.

Retention time will vary with absolute concentration of the buffer as well as instrument configuration, however, the peak shape and retention should be similar to that seen in the chromatogram below.

Figure 8: Test Chromatogram, N-Acetylneuraminic Acid



Performing this test prior to discussing the problem with your Waters representative will help in diagnosing the problem.

a. Column Storage

- Store columns in distilled water and refrigerate at 4 - 6 °C. DO NOT FREEZE.
- DO NOT allow buffers or other potentially harmful materials to remain in the system overnight when not being used. Run buffers with a slow flow rate (0.1 ml/min) overnight if necessary.
- Return the column to its box with the end caps firmly in place for storage. Allowing columns to dry out can result in poor chromatographic performance.

b. Troubleshooting

Use Table 2 to troubleshoot problems with your column.

Table 2: Column Problems and Solutions

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	• Replace or clean inlet and outlet filters in an ultrasonic bath.

V. WARRANTY AND SERVICE INFORMATION

a. Service Information

Waters Corporation staff of experienced service specialists provide maintenance assistance on both preventative and/or corrective levels. For complete information and assistance, please call Waters Service Department at 1-800-252-HPLC. For solutions to particular applications questions Waters team of technical support personnel are available to help you with specialized support. They may be contacted at 1-800-252-HPLC in Milford, MA.

Waters Corporation staff of experienced service specialists provide maintenance assistance on both preventative and/or corrective levels. For complete information and assistance, please call Waters Service Department at 1-800-252-HPLC. For solutions to particular applications questions Waters team of technical support personnel are available to help you with specialized support. They may be contacted at 1-800-252-HPLC in Milford.

b. Warranty

Waters Corporation warrants its high performance liquid chromatography columns in accordance with the following terms and conditions:

Waters will replace without cost any steel column that fails to perform satisfactorily if notified within 90 days from your receipt. Any column returned must have a Return Authorization Number granted by the Waters Customer Service Department. Approval is subject to the following exclusions:

- Physical damage to the column because of misuse or abuse.
- Chemical damage to the packing material because of use with incompatible mobile phases or buffers, or at an incorrect pH.
- Physical damage to the packing material because of operation at incorrect temperatures or pressures.
- Particulate buildup or precipitation in the column or end fittings causing high internal pressure which has occurred because of improper mobile phase or sample filtration practices.

VI. ORDERING INFORMATION

Item	Part Number
Glyco-Pak N Column	WAT026725
Glyco-Pak DEAE Column	WAT026730
Sample Clarification Kit	WAT026865
Solvent Clarification Kit	WAT085113
In-Line Precolumn Filter	WAT084560

Sales Offices:

Austria and European Export (Central South Eastern Europe, CIS and Middle East) 431 877 18 07

Australia 2 9933 1777

Belgium 32 2 726 1000

Brazil 55 11 5094 3788

Canada 800 252 4752

China 8621 6495 6999

CIS/Russia +7 495 3367000

Czech Republic 42 02 617 11384

Denmark 45 46 59 8080

Finland +358 9 5659 6288

France (33) 1 30 48 72 00

Germany 49 6196 400600

Hong Kong 852 29 64 1800

Hungary 36 1 350 5086

India and India Subcontinent 91 80 2 837 1900

Ireland 353 1 448 1500

Italy 39 02 274 211

Japan (81) 3 3471 7191

Korea (82) 2 820 2700

Mexico 5255 5200 1860

The Netherlands +31 (0)76-50 87 200

Norway 47 63 84 60 50

Poland (48) 22 833 4400

Puerto Rico 787 747 8445

Singapore 65 6273 1221

Spain 34 93 600 93 00

Sweden 46 8 555 11500

Switzerland 41 62 889 2030

Taiwan 886 2 2543 1898

United Kingdom 44 208 238 6100

September 2007 WAT026735 Rev 2 VW-PDF

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com