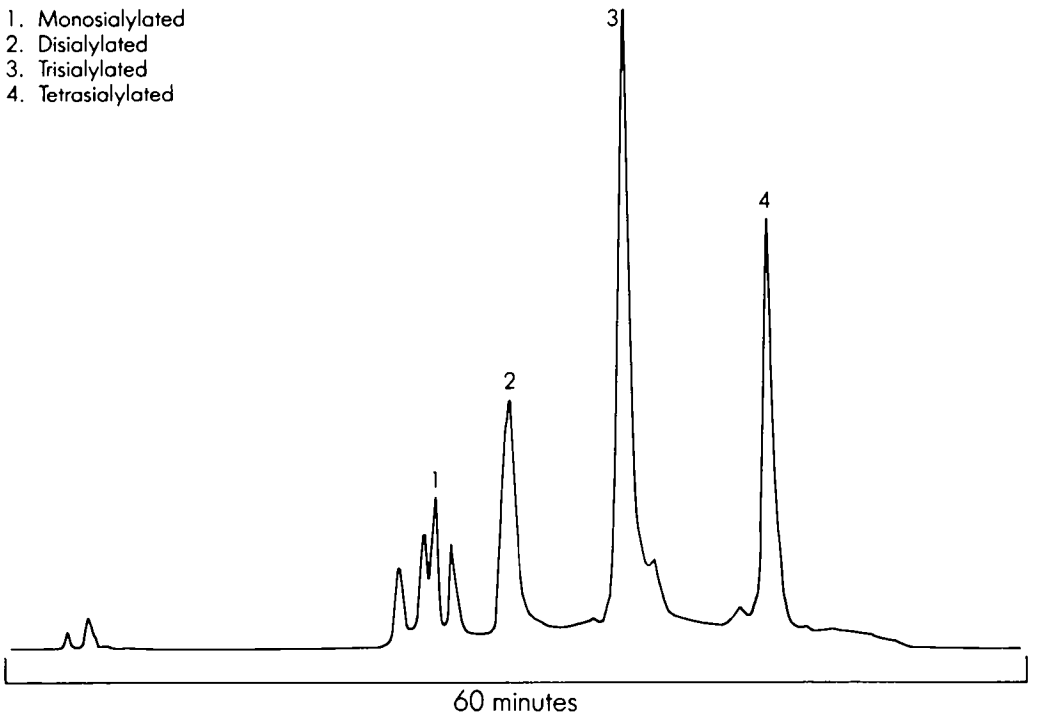
High performance liquid chromatography for the isolation and purification of oligosaccharides from glycoconjugates.

Waters Glyco-Pak™ DEAE and N
Obtain high purity carbohydrates for NMR and MS. Prepare carbohydrates for use as substrates or to determine biological activity.

Fast and easy separation of acidic and neutral carbohydrates
Polymeric columns permit recovery of carbohydrates without interference and provide high stability for consistent chromatography.

Non-destructive purification
Methods provide unaltered and intact carbohydrates ideal for structural analysis or biological activity elucidation.

Fetuin oligosaccharides

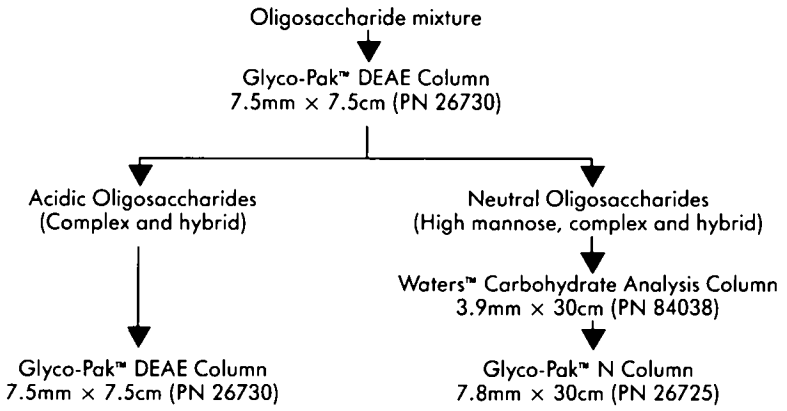


Glyco-Pak chemistries for the isolation and purification of oligosaccharides

The oligosaccharides (polymers of 2–50 monosaccharides) derived from glycoconjugates are one of the most challenging groups of biological molecules to separate and purify due to the large number of possible structures and linkages. High purity oligosaccharides are required for structure determination by NMR or MS as well as for use as substrates to determine their biological activity (i.e., enzymology of oligosaccharide biosynthesis, glycoconjugate modification, etc.). Single mode chromatography frequently is inadequate to purify these compounds to homogeneity.

Waters has developed a multiple column approach for oligosaccharide purification using two new column chemistries. The Waters Glyco-Pak DEAE and Glyco-Pak N are highly stable polymeric columns. They have been specifically developed and tested to provide consistent chromatography and yield carbohydrates without interferences due to the stationary phase. The Glyco-Pak DEAE column is designed for the purification of acidic oligosaccharides. The Glyco-Pak N column is a hydrophilic polymer column with distinctly different chemistry from the traditional silica based amino columns. This column is for the purification of neutral oligosaccharides.

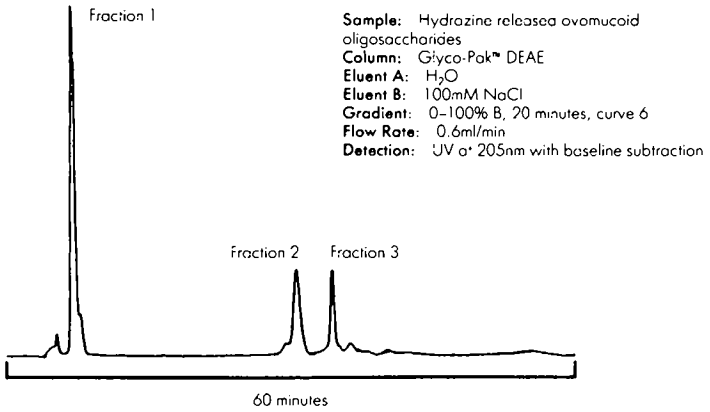
A multi-column separation approach for the purification of oligosaccharides.



Oligosaccharides can be released chemically or enzymatically from glycoconjugates. After the removal of the sugars, the mixture of oligosaccharides is initially fractionated by gradient elution on the Glyco-Pak DEAE column. On this column, the neutral oligosaccharides are not retained and elute in the column void volume. Acidic oligosaccharides are eluted from the column according to the number of sialic acids or degree of sulphation. The acidic fractions can be further purified by an isocratic run on the Glyco-Pak DEAE column. The neutral fraction is then run on the Glyco-Pak N column. For more complex neutral mixtures, a subfractionation may be performed using a variety of Waters column chemistries. In the approach outlined above, the Waters Carbohydrate Analysis column is used to fractionate the neutral carbohydrates. Fractions from the Waters Carbohydrate Analysis column are then further purified on the Glyco-Pak N column. Used with the Waters 600E System Controller, which features a multisolvent delivery system and unique dual piston design, highly reproducible gradients are achieved ensuring consistent chromatography.

When isolating oligosaccharides for analysis by NMR, MS or for use as substrates, a minimum of 100μg of material is generally required. It is important that the mobile phase and detection be non-destructive. Ultraviolet and Refractive Index detection meet these requirements (detection limits of about 750ng for UV and 100ng for RI). Waters offers a full range of detection options from single or multi-channel UV/Visible to full spectral analysis as well as high sensitivity RI. Many of these detectors come with additional capabilities such as baseline correction for subtracting gradient shifts or spectral libraries for directly comparing peaks to previously acquired spectra.

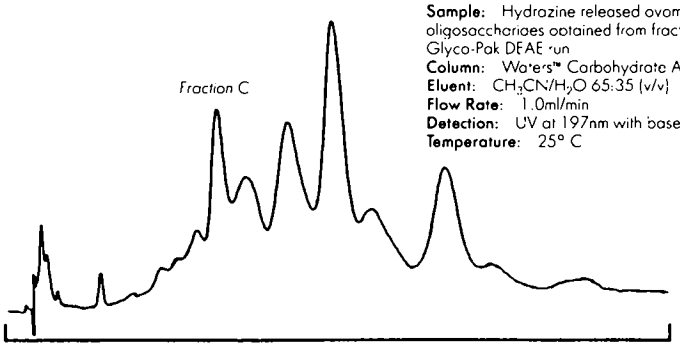
Ovomucoid oligosaccharides



Sample: Hydrazine released ovomucoid oligosaccharides
Column: Glyco-Pak™ DEAE
Eluent A: H_2O
Eluent B: 100mM NaCl
Gradient: 0–100% B, 20 minutes, curve 6
Flow Rate: 0.6ml/min
Detection: UV at 205nm with baseline subtraction

The initial fraction of oligosaccharides derived from ovomucoid is performed on the Waters Glyco-Pak DEAE column. Fraction 1 contains neutral carbohydrates and fractions 2 and 3 contain acidic carbohydrates.

Purification of neutral oligosaccharides

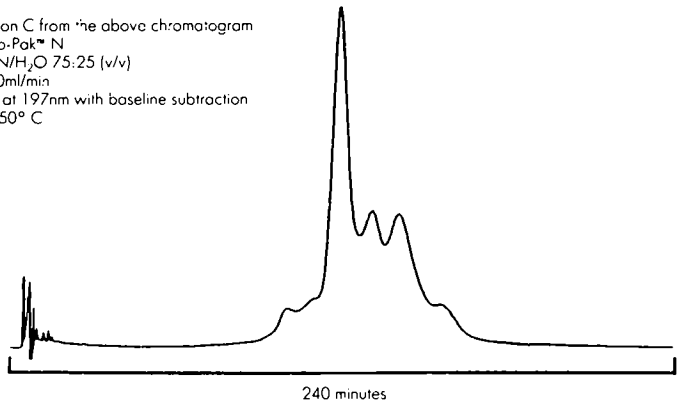


Sample: Hydrazine released ovomucoid neutral oligosaccharides obtained from fraction 1 of the Glyco-Pak DEAE run
Column: Waters™ Carbohydrate Analysis
Eluent: CH_3CN/H_2O 65:35 (v/v)
Flow Rate: 1.0ml/min
Detection: UV at 197nm with baseline subtraction
Temperature: 25° C

The neutral oligosaccharides obtained from fraction 1 of the DEAE run are now fractionated on the Waters Carbohydrate Analysis column.

Final purification of the neutral oligosaccharides

Sample: Fraction C from the above chromatogram
Column: Glyco-Pak™ N
Eluent: CH_3CN/H_2O 75:25 (v/v)
Flow Rate: 1.0ml/min
Detection: UV at 197nm with baseline subtraction
Temperature: 50° C



Fraction C collected from the Waters Carbohydrate Analysis column is now fractionated on the Glyco-Pak N column. This column provides an additional mode of separation with selectivity that is distinctly different from traditional NH_2 columns.

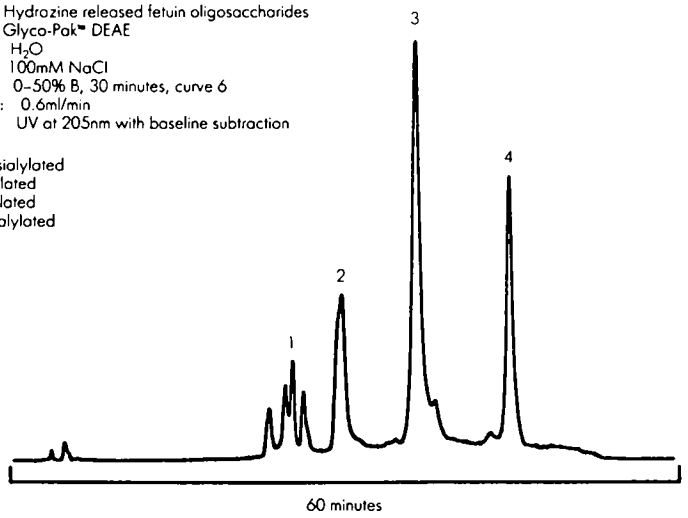
The Waters HPLC systems for complex carbohydrate analysis are complete gradient HPLC systems, featuring multisolvent inlet capability which permits the blending of up to four solvents or buffers. The microprocessor based control unit of the 600E can be programmed to store up to 15 complete gradient protocols allowing for quick conversion between modes of chromatography when performing multi-column carbohydrate separations.

High resolution separation of acidic oligosaccharides with anion exchange HPLC.

Fractionation of fetuin oligosaccharides on the Waters Glyco-Pak DEAE column

Sample: Hydrozine released fetuin oligosaccharides
Column: Glyco-Pak™ DEAE
Eluent A: H_2O
Eluent B: 100mM NaCl
Gradient: 0–50% B, 30 minutes, curve 6
Flow Rate: 0.6ml/min
Detection: UV at 205nm with baseline subtraction

1. Monosialylated
2. Disialylated
3. Trisialylated
4. Tetrasialylated



Acidic oligosaccharides are purified using the Glyco-Pak DEAE column in an anion exchange mode. This separation is based on degree of sialylation. Fractions can be collected and further purified isocratically on the Glyco-Pak DEAE column.

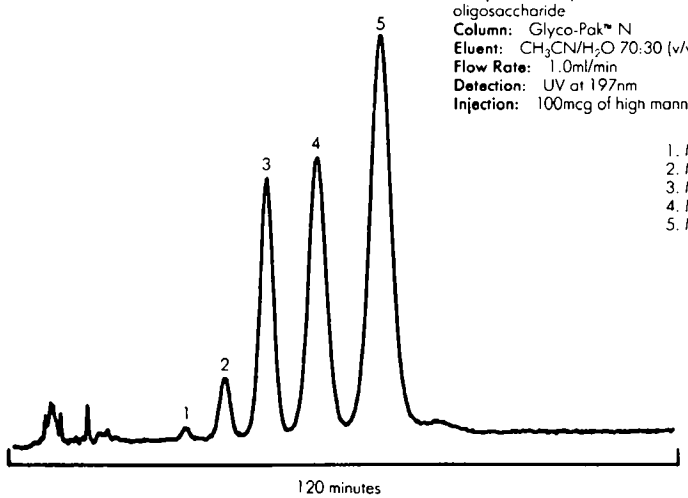
Oligosaccharides released chemically or enzymatically can be purified on the Glyco-Pak columns. Samples such as the Genzyme/Waters high mannose oligosaccharide standard can be purified in a single step.

Neutral oligosaccharide separations through partition HPLC.

High mannose oligosaccharides

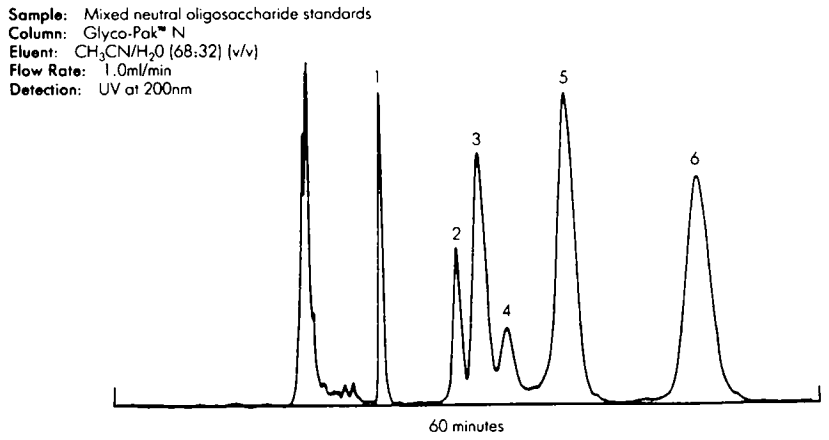
Sample: Genzyme Endo H released high mannose oligosaccharide
Column: Glyco-Pak™ N
Eluent: CH_3CN/H_2O 70:30 (v/v)
Flow Rate: 1.0ml/min
Detection: UV at 197nm
Injection: 100mcg of high mannose standard

1. Man_4Gn
2. Man_5Gn
3. Man_6Gn
4. Man_7Gn
5. Man_8Gn



The Genzyme/Waters high mannose oligosaccharide standard separated on the Glyco-Pak N column.

Neutral oligosaccharide standards



Separation of mixed neutral oligosaccharide standard on the Glyco-Pak N column.

Neutral oligosaccharides are separated on the Glyco-Pak N column with a mobile phase consisting of water and 65 to 75% acetonitrile. Structures as diverse as monosaccharides, Man 9 high mannose and multiantennary complex carbohydrates can be separated isocratically free of contamination that is found with silica based amino columns. These columns provide consistent chromatography and because of nondestructive detection and mobile phase, yields carbohydrates without altering their structure.

Neutral oligosaccharide standard structures†

(1)
GlcNAc (α, β)

(2)
Manα1–6
Manβ1–4GlcNAc (α, β)
3
GlcNAcβ1–2Manα1

(3)
Manα1–6 Fucα1–6
Manβ1–4GlcNAcβ1–4GlcNAc (α, β)
3
GlcNAcβ1–2Manα1

(4)
GlcNAcβ1–2Manα1–6
GlcNAcβ1–4Manβ1–4GlcNAcβ1–4GlcNAc (α, β)
3
GlcNAcβ1–2Manα1

(5)
Galβ1–4GlcNAcβ1–2Manα1–6
4Manα1–4GlcNAcβ1–4GlcNAc (α, β)
3
Galβ1–4GlcNAcβ1–2Manα1

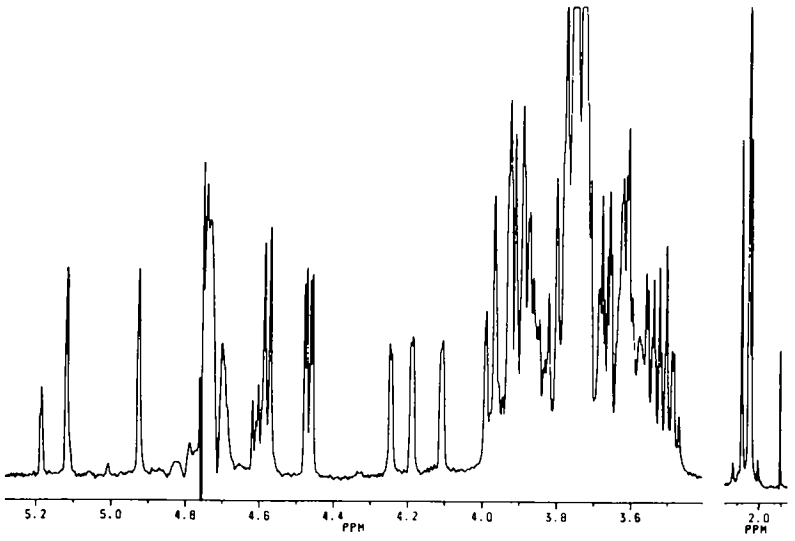
(6)
Manα1–2Manα1–6
Manα1–6
Manβ1–4GlcNAcβ1–4GlcNAc (α, β)
3
Manα1–2Manα1
3
Manα1–2Manα1–2Manα1

The above structures are of the neutral oligosaccharide standard separated on the Glyco-Pak N column.

† Data courtesy of Brad Bendiak, Jeanne Orr, Inka Brockhausen, George Vella, and Chuck Phoebe, Analytical Biochemistry, 174 (1988).

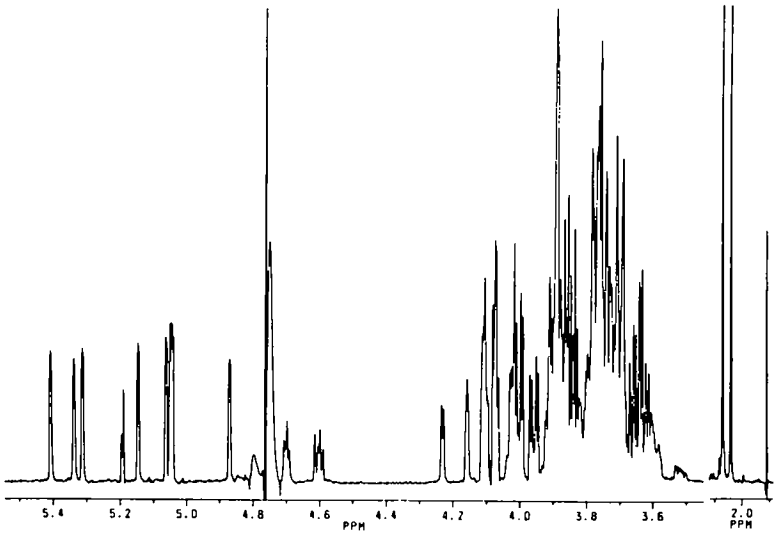
NMR of peak 5 of neutral oligosaccharide standard

Oligosaccharides purified on the Glyco-Pak N column can be taken directly to NMR or mass spectrometry without additional purification steps to remove contaminants. The Glyco-Pak N column has been found to yield cleaner NMRs without loss of the carbohydrate due to additional sample clean up.



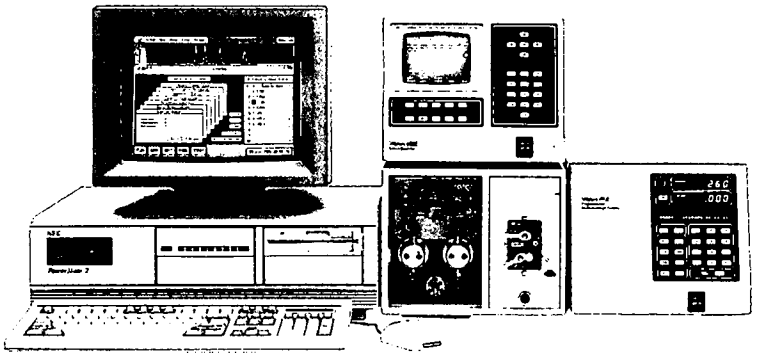
Structural reporter regions of 500-MH_7 proton NMR of peak 5 of neutral oligosaccharide standard, a biantennary structure with terminal galactose (from fibrinogen) after purification on the Glyco-Pak N column.

NMR of peak 6 of neutral oligosaccharide standard



Structural reporter regions of 500-MH_2 proton NMR of peak 6 of neutral oligosaccharide standard, (Man 9 from soybean agglutinin) after purification on the Glyco-Pak N column.

Complex carbohydrate separation systems



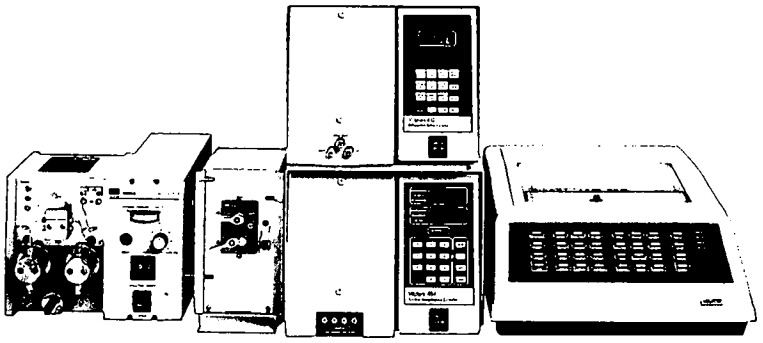
The Waters family of complex carbohydrate separation systems are complete gradient HPLC systems, featuring precise gradient control, variable volume injection, sensitive and stable absorbance detection, data reduction, and full automation. They are modular in design and can be configured for either high pressure or low pressure chromatography.

Ordering information

Glyco-Pak DEAE Column	7.5mm × 7.5cm	PN 26730
Glyco-Pak N Column	7.8mm × 30cm	PN 26725
Waters Carbohydrate Analysis Column	3.9mm × 30cm	PN 84038

Options

Waters 600E Gradient Controller	PN 62710
Waters 484 Tunable UV/Visible Absorbance Detector	PN 80560
Waters 490 Programmable Multiwavelength Detector	PN 46000
Waters 410 Differential Refractometer	PN 70390



Waters HPLC systems for complex carbohydrates are modular in design and can be optimally configured to the needs of your laboratory. As your needs expand, your Waters complex carbohydrate system can grow with you.

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