Errata

Product Manual for Dionex CarboPac™ MA1, PA1, PA10 and PA100 Columns
031824-08

For new orders of the following parts discussed in this manual, please use the updated part numbers listed below.

Part	Old Part Number in this manual	Updated Part Number to use for new orders
PROD,ICS3,SP,QUARTERANARY,+DGAS	*061706*	*079819*
PROD,ICS3,DP,QUAT/QUAT,+DGAS	*061712*	*079825*
PROD,EG,DUAL,ICS3	*061714*	*079827*
PROD,EC DETECTOR,ICS3	*061718*	*079830*
PROD,EC CELL,ICS3000	*061756*	*AAA-061756*

PRODUCT MANUAL

for

CarboPac® MA1
CarboPac® PA1
CarboPac® PA10
CarboPac® PA100

Now sold under the
Thermo Scientific brand

Thermo
SCIENTIFIC

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DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

PRODUCT MANUAL

for the

CarboPac® MA1

4x250 mm (P/N 044066)

CarboPac® MA1 GUARD

4x50 mm (P/N 044067)

CarboPac® PA1

2x250 mm (P/N 057178)
4x250 mm (P/N 035391)
9x250 mm (P/N 039686)
22x250 mm (P/N SP2866)

CarboPac® PA1 GUARD

2x50 mm (P/N 057179)
4x50 mm (P/N 043096)

CarboPac® PA10

2x250 mm (P/N 057180)
4x250 mm (P/N 046110)
9x250 mm (P/N SP4216)

CarboPac® PA10 GUARD

2x50 mm (P/N 057181)
4x50 mm (P/N 046115)

CarboPac® PA100

2x250 mm (P/N 057182)
4x250 mm (P/N 043055)
9x250 mm (P/N SP2089)
22x250 mm (P/N SP2667)

CarboPac® PA100 GUARD

2x50 mm (P/N 057183)
4x50 mm (P/N 043054)

Document No. 031824
Revision 08
May 2010

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SECTION 1 – INTRODUCTION TO THE CARBOPAC COLUMNS

The CarboPac family of columns is designed to address the analytical requirements of a wide range of carbohydrate chemists. Underivatized carbohydrates are separated at high pH, in an approach that is unique to Dionex, simple to reproduce, and made possible by the pH stability of the CarboPac columns. The CarboPac MA1 (4-mm) is optimized for weakly ionizable compounds such as sugar alcohols. The resin technology in the CarboPac MA1 column is an efficient tool for the analysis of mono- and disaccharide alditols since they are oxy anions at pH values above 12.5. Because of its higher capacity, the macroporous CarboPac MA1 resolves many carbohydrates that are poorly retained on pellicular anion-exchange columns, and successfully separates alditols such as sorbitol, glycerol, and mannitol found in food products.

The non-porous pellicular resins used in the CarboPac PA1, PA10 and PA100 provide higher efficiencies than the macroporous CarboPac MA1. The CarboPac PA1 (2-mm and 4-mm) is a general-purpose column for the separation of mono-, di-, and some oligosaccharides by high pH anion-exchange chromatography, coupled with pulsed amperometric detection (PAD). It can be used for baseline resolution of large linear maltodextrins, which are present in many tableting pharmaceuticals and calorie nutritive supplements.

The CarboPac PA10 (2-mm and 4-mm) analytical columns are recommended for monosaccharide composition analysis. It is the best column to combine with the AminoTrap® column for glycoprotein monosaccharide composition analysis, before the introduction of the CarboPac PA20 column. For detailed information please see the product manual for the CarboPac PA20 column (P/N 031884). The AminoTrap column moves the problematic amino acids out of the analysis, thereby eliminating working electrode poisoning. The CarboPac PA10 also works well in combination with the AminoTrap and EG40 eluent generator for analysis of “wood” sugars.

The CarboPac PA100 (2-mm and 4-mm) is a high-resolution strong anion-exchange column developed for enhanced chromatography of oligosaccharides. It uses an advanced, high-performance, pellicular anion exchange resin to offer higher efficiency than the CarboPac PA1 for the separation of neutral and anionic oligosaccharide mixtures. If desalting of collected oligosaccharides is desired, the CarboPac PA100 column is the column of choice because lower salt concentrations are required for elution compared with the CarboPac PA1.

High-pH anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) has been demonstrated to be effective in the separation and detection of carbohydrates ranging from small monosaccharides to branched oligosaccharides, and large linear polysaccharides. HPAE is proven to be capable of separating monosaccharides, positional, linkage and branch isomers of branched oligosaccharides, and homopolymer oligosaccharides that differ only in length. Each class of carbohydrates has different separation characteristics requiring different column chemistries.

Table 1, “Comparison of the CarboPac MA1, PA1, PA10 and PA100,” provides a brief side-by-side comparison of the four columns, in terms of the resin, column capacity, etc.

Assistance is available for any problem during the shipment or operation of Dionex instrumentation and columns through the Dionex North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or through any of the Dionex Offices listed in “Dionex Worldwide Offices” on the Dionex Reference Library CD-ROM.

Table 1
Comparison of the CarboPac MA1, PA1, PA10 and PA100

Characteristic	CarboPac MA1	CarboPac PA1	CarboPac PA10	CarboPac PA100
Recommended Applications	Mono- and disaccharide alchols in foods, physiological fluids, tissues, and reduced glycoconjugate saccharides	Monosaccharide compositional analysis, linear homopolymer separations, saccharide purification	Mono- and disaccharide analysis	Oligosaccharide mapping and analysis
Resin Composition	Macroporous 7.5 µm diameter vinylbenzyl-chloride/divinyl benzene macroporous substrate fully functionalized with an alkyl quaternary ammonium group	Pellicular 10 µm diameter polystyrene/divinyl benzene substrate agglomerated with 580 nm MicroBead quaternary ammonium functionalized latex	Pellicular 10 µm diameter polystyrene/divinyl benzene substrate agglomerated with 460 nm MicroBead difunctional quaternary ion	Pellicular 8.5 µm diameter ethylvinylbenzene/ divinyl benzene substrate agglomerated with 275 nm MicroBead quaternary ammonium functionalized latex
Substance Cross-linking	15%	2%	55%	55%
MicroBead Latex Cross-Linking	N/A, no latex	5 % Cross-linked	5 % Cross-linked	6 % Cross-linked
Anion Exchange Capacity	1450 µeq per 4 x 250 mm column	100 µeq per 4 x 250 mm column	100 µeq per 4 x 250 mm column	90 µeq per 4 x 250 mm column
Recommended Flow Rate	0.4 mL/min (4 mm)	1 mL/min (4 mm)	1 mL/min (4 mm)	1 mL/min (4 mm)
pH Compatibility	pH 0 - 14	pH 0 - 14	pH 0 - 14	pH 0 - 14
Organic Solvent Compatibility	0%	0 - < 2 %	0 - 90 %	0 - 100 %
Maximum Backpreasure	2000 psi (14 MPa)	4000 psi (28 MPa)	3500 psi (25 MPa)	4000 psi (28 MPa)

1.1 Guidelines for CarboPac Column Selection

Table 2 provides a comparison of the target applications for the CarboPac columns. The following guidelines are useful in selecting the best CarboPac column for a specific application. The CarboPac PA1 and the CarboPac PA10 are used for similar applications, but the PA1 is recommended for routine applications while the PA10 is recommended for high sensitivity applications.

Table 2
CarboPac Column Selection Guide

Column	Target Application
CarboPac MA1	A column specifically designed for reduced mono- and disaccharide alditol analysis. Also well suited for analysis of exoglycosidase released neutral monosaccharides due to baseline resolution of fucose, N-acetyl-(D)-glucosamine, N-acetylgalactosamine, mannose, glucose, and galactose. Monosaccharides are moved away from the column void, enabling immediate evaluation of contaminating exoglycosidase activities. **WARNING** This column is NOT recommended for the separation of charged oligosaccharides or amino sugars which are poorly recovered. Do NOT use sodium acetate gradients. Do NOT exceed 0.4 mL/min.
CarboPac PA1	A general purpose column for mono-, di- and some oligosaccharide analyses. Sialic acid separations have been demonstrated. Not as high sensitivity as the CarboPac PA10 for monosaccharide analysis due to the possible interference of the oxygen dip. Oligosaccharide peak efficiencies may not be as good as with the CarboPac PA100. CarboPac PA1 can also be used for separations of aminoglycosides and antibiotics.
CarboPac PA10	A column specifically designed for mono- and disaccharide analysis. Best choice for high sensitivity monosaccharide composition analysis because the oxygen dip has been moved out of the analysis allowing low picomole determinations of e.g. fucose, glucosamine, galactosamine, galactose, glucose, and mannose. It is the best choice for glycoprotein monosaccharide composition analysis when used in combination with the AminoTrap column. It is a good choice for sialic acid analysis and works well with the EG40/EG eluent generator for the analysis of "wood" sugars (mannitol, fucose, arabinose, rhamnose, galactose, glucose, sucrose, xylose, mannose, fructose and ribose). **WARNING** This column is NOT recommended for complex oligosaccharide analysis.
CarboPac PA100	This column is a good choice for oligosaccharide profiling, linear polysaccharide profiling and fraction collection. **WARNING** This column is NOT recommended for monosaccharide or sialic acid analysis.
Amino Trap	Use with the CarboPac PA10 to eliminate amino acid fouling problems that may occur when glycoprotein acid hydrolysates are analyzed for monosaccharides. Also required for selectivity control with the CarboPac PA10 and EG40 for wood sugars, as described for CarboPac PA10.
Borate Trap	Install the BorateTrap™ column between the pump and the injection valve to remove borate from the eluent. The BorateTrap column improves peak symmetry of alditols, fructose, and mannose.

1.1.1 Monosaccharides

The CarboPac PA10 column is used for separation of reducing mono- and disaccharides. The CarboPac PA20 can provide better resolution than PA10, but also requires a slower flow rate and has less loading capacity. For more information please see CarboPac PA20 manual (P/N 031884). The CarboPac MA1 is recommended for neutral sugar alcohols and also for the separation of *N*-acetamido sugars from hexose sugars.

1.1.2 Neutral Oligosaccharides

The CarboPac PA100 is the most appropriate column for the oligosaccharide mixtures characteristic of glycoprotein-derived oligosaccharides, although these compounds are only slightly less well resolved on the CarboPac PA1 column. Oligosaccharides cleaved by reductive β-elimination from glycoproteins contain a reduced terminal and are eluted earlier than the same oligosaccharide with a reducing terminal. Reduced, neutral di- and trisaccharides may be poorly retained on the CarboPac PA columns, but are separated readily on the CarboPac MA1.

The empirical relationships between oligosaccharide structure and chromatographic retention are well documented (Rohrer, J., Glycobiology, 1995, 5, 359-360).

1.1.3 Charged Oligosaccharides

Charged oligosaccharides (e.g. sialylated, phosphorylated, sulfated, or containing carboxyl groups) are separated based on their composition, linkage, and level of formal negative charge. They can be separated at both high (pH 13) and low (pH 4.6) pH. At low pH, the separations are largely dependent on the charge-to-mass ratio of the oligosaccharide but may also be influenced by linkage. Selectivity for sialylated oligosaccharides will change with pH as a result of oxyanion formation. The CarboPac PA100 is recommended for sialylated oligosaccharides, although in many cases the PA1 performs adequate separations.

1.1.4 Glycosaminoglycans

Oligosaccharides derived from glycosaminoglycans, such as non-sulfated chondroitin disaccharides (Shibata S., Midura R.J. and Hascall V.C., *J. Biol. Chem*., 267, 1992, 6548-6555), are separated on the CarboPac PA1. Sulfated disaccharides can be separated and detected using either the CarboPac PA1 or CarboPac PA100.

1.1.5 Linear Polysaccharides

Linear polysaccharides can be separated on the basis of length on any of the CarboPac PA columns. The CarboPac PA1 has a slightly higher capacity than the PA100 and is the better column to use for linear homopolymers. Elution of carbohydrates on the CarboPac PA100 requires a lower sodium acetate concentration than the PA1, and is the preferred column if fraction collection with desalting is required.

NOTE ***Please familiarize yourself with the installation, operation, and various system components of the Dionex IC System before beginning an analysis.***

SECTION 2 – INSTALLATION

CarboPac application methods have been optimized for use on the DIONEX ICS-2500 and ICS-3000 systems, although any high quality HPLC system for high performance anion exchange chromatography may be used. Ideally, the system should be metal free and capable of producing ternary or quaternary gradients. Metal systems will often leach metals that will degrade column performance. The length of liquid lines between the pump, injection valve, column and detector should be minimized. DIONEX recommends the use of 0.010" ID PEEK tubing for 4 x 250 mm systems and 0.005" ID PEEK tubing for 2 x 250 mm systems.

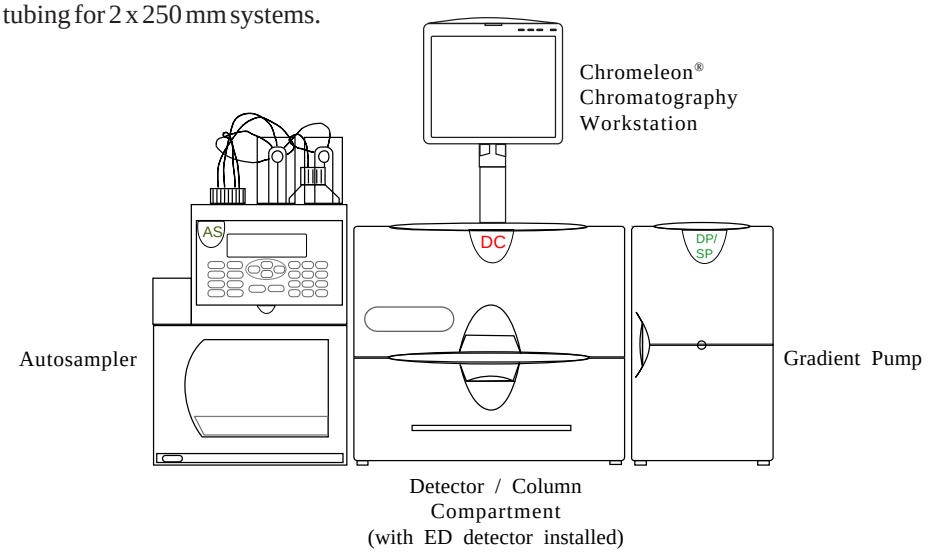


Figure 1
Carbohydrate System Configuration

2.1 System Requirements

The following systems are recommended for supported applications on the CarboPac columns (see Table 3).

Table 3
Recommended Carbohydrate System and Optional Modules for 4mm and 2mm columns

Recommended Gradient System (ICS-3000 chromatography system)	Optional Modules
ICS-3000 DP or SP gradient pump (PN 061712 or 061706)	ICS-3000 EG Eluent Generator (PN 061714)
ICS-3000 AS Autosampler (PN 063102)	EluGen II NaOH Cartridge (PN 058900)
ICS-3000 DC detector/chromatography module (PN 063773)	Vacuum pump and degas kit for EG (PN 063353)
ICS-3000 ED electrochemical detector (PN 061718 and 061756)	
ICS-3000 eluent organizer (PN 062628)	

The EG eluent generator (P/N 061714) can be included for CarboPac PA10 applications to deliver carbonate-free, high-purity hydroxide eluent on-line. This requires the EG vacuum degas conversion kit (P/N 063353) to ensure the degas unit in the pump sufficiently removes H_2 gas formed electrolytically by the EG.

2.2 System Void Volume

Minimize the number of unions and the length of all the liquid lines. Tubing between the injection valve and the detector should be 0.010" ID PEEK tubing (P/N 042690). The use of larger tubing will decrease peak resolution. When the 2 mm column set is used, replace the 0.010" ID PEEK tubing with 0.005" ID PEEK tubing.

2.3 Optimal Settings for Pulsed Amperometric Detection of Carbohydrates

Dionex recommends quadruple potential waveform (Waveform A) for carbohydrate analysis. Quadruple potential waveform provides the best reproducibility of absolute electrochemical response.

While Dionex realizes that some customers have adopted the triple potential waveform (Waveform B) in their organizations' approved methods, Dionex does not recommend this waveform. Waveform B uses oxidative cleaning and can cause electrode wear and a gradual decrease in carbohydrate peak area over time. Technical Note 21 describes how to choose the optimal waveform.

Table 4
Waveform A

Waveform A (Quadruple Potential Waveform)		
Time (sec.)	**Potential (V) vs. Ag/AgCl**	**Integration**
0.00	+ 0.1	
0.20	+ 0.1	Begin
0.40	+ 0.1	End
0.41	- 2.0	
0.42	- 2.0	
0.43	+ 0.6	
0.44	- 0.1	
0.50	- 0.1	


NOTE

Waveforms for older Dionex Amperometric detectors are listed in Section 9.

SECTION 3 – OPERATION

3.1 General Operating Conditions

The CarboPac columns have been designed for isocratic or gradient separations utilizing sodium hydroxide eluents up to a concentration of 1 M. A comparison of general conditions for the various 4-mm columns are listed in Table 6.

Table 5
General Operating Conditions (4-mm)

Standard Condition	CarboPac MA1	CarboPac PA1	CarboPac PA10	CarboPac PA100
Flow Rate	0.4 mL/min	1.0 mL/min	1.0 mL/min	1.0 mL/min
Storage Solution	600 mM NaOH	200 mM NaOH	18 mM NaOH	100 mM NaOH/ 100 mM NaOAc
Injection Volume	10 - 100 µL	10 - 50 µL	10 - 25 µL	< 20 µL
Back Preasure	1,100 psi	1,100 psi	2,300 psi	2,000 psi
Temperature	Ambient	Ambient	Ambient	Ambient

3.2 Operating Limitations

Table 6
Operating Recommendations and Limitations (4-mm)

Condition	CarboPac MA1	CarboPac PA1	CarboPac PA10	CarboPac PA100
pH Range	0 - 14	0 - 14	0 - 14	0 - 14
Flow Rate	0.2 - 0.5 mL/min	1.0 - 1.5 mL/min	1.0 - 1.5 mL/min	1.0 - 2.0 mL/min
Maxinum Presure	2,000 psi	4,000 psi	3,500 psi	< 5,000 psi
Eluent Ionic From	Hydroxide Only	Acetate or Hydroxide Only	Acetate or Hydroxide Only	Acetate or Hydroxide Only
Organic Eluents	Aviod Exposure	< 2%	0 - 90% for cleaning only	100% compatible for cleaning only
Detergents	Avoid Anionic Detergents	Avoid Anionic Detergents	Avoid Anionic Detergents	Avoid Anionic Detergents
Temperature	4 - 50 °C	4 - 55 °C	4 - 55 °C	4 - 55 °C

Aviod running deionized water at a low flow rate through CarboPac columns when the column is not in use because start-up conditions will generate excessive backpressure which can damage the system.

3.3 Chemical Purity Requirements

Obtaining reliable, consistent, and accurate results requires eluents that are free from ionic and spectrophotometric impurities. Chemicals and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also helps to protect ion exchange columns and system components. DIONEX cannot guarantee proper column or detector performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

3.3.1 Inorganic Chemicals

Reagent grade chemicals should always be used to prepare ionic eluents. Whenever possible, chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. Chemicals that meet or exceed these standards state the chemical purity on the label.

3.3.2 Deionized Water

The purity of the deionized water used to prepare eluents is critical for proper performance. It must be degassed Type I Reagent Grade Water with a specific resistance of 18.2 megohm-cm. The deionized water must be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water (with the exception of Burdick and Jackson) should not be used because most bottled water contains an unacceptable level of ionic impurities. Peak efficiencies will be diminished (especially for mannose and alditols) if the eluent water contains >10 µg/L (10 ppb) borate.

3.4 Eluent Preparation

We recommend reading Dionex Technical Note 71.

NOTE

3.4.1 Degassing Deionized Water

A. Vacuum degas water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir for 5-10 minutes while sonicating. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere.

B. On-line degassing is supported through the use of the ICS-3000 DP / SP, GP40, GP50, and GS50 gradient pumping systems and the IS20 and IS25 isocratic pumping systems.

C. Use the degassed, Type I Reagent Grade deionized water for the preparation of the specific eluents required to run applications.

3.4.2 Sodium Hydroxide Eluent Preparation

To make 1 M NaOH, dilute 80 g (52.3 mL) of 50% (w/w) NaOH with degassed Type 1 deionized Reagent Grade Water (having a specific resistance of 18.2 megohm-cm) to a final volume of 1,000 mL. Avoid the introduction of carbon dioxide from the air into the 50% NaOH or the deionized water being used to make the eluent. Do not shake the stock 50% (w/w) NaOH. Pipette the required aliquot from the middle of the stock solution where sodium carbonate is least likely to have formed. Do not pipette from the bottom where sodium carbonate precipitate may have fallen. Regardless of the method employed, always use the same methodology to ensure consistency.

DO NOT prepare NaOH eluents from sodium hydroxide pellets. The pellets are coated with a layer of carbonate.

WARNING

Always degas water used to make NaOH eluents and store NaOH eluents in eluent bottles blanketed with helium or nitrogen to avoid carbon dioxide contamination from the air.

NOTE

WARNING

The sodium hydroxide eluents used with the CarboPac columns will readily absorb carbon dioxide and produce carbonate. The presence of variable amounts of carbonate will lead to inconsistent retention times. Take precautions during eluent preparation to minimize contamination with carbon dioxide from the air.

NOTE

Use Certified Sodium hydroxide solution, 50% w/w. Use fresh bottles and discard if sodium carbonate precipitate is evident. Usually about 2/3 of the bottle can be used before a fresh bottle is needed.

NOTE

The eluents can be prepared by either weight or volume. Using a volumetric pipette is more effective in preventing contamination than weighing, but is less precise. For applications requiring less than or equal to 100 mM hydroxide, an on-line eluent generator may be used. Thus, the preparation of caustic eluents may be avoided altogether.

1. Weight Method

Pipette (do not pour) the 50% w/w sodium hydroxide into the weighing dish. Minimize the time that the solution is exposed to air. Weigh out the required amount of 50% sodium hydroxide.

Example: To make 1 L of 0.6 M NaOH, use 48.0 g of 50% w/w sodium hydroxide.

$$g = \frac{(M) \times (V) \times (MW)}{r}$$

where, g = mass (in g) of 50% NaOH used, V = volume (L), MW = molecular weight of NaOH, M = final molarity and r = fractional purity of concentrated solution.

$$48\ g = \frac{(0.6\ mol/L) \times (1\ L) \times (40.01\ g/mol)}{0.5}$$

2. Volume Method

Pipette (do not pour) the 50% w/w sodium hydroxide into the volumetric flask. Minimize the time that the solution is exposed to air.

Example: To make 1 L of 0.6 M NaOH, use 31.4 mL of 50% w/w sodium hydroxide.

$$v = \frac{(M) \times (V) \times (MW)}{dr}$$

where, v = volume (mL) of 50% NaOH used, V = volume of final solution (L), MW = molecular weight of NaOH, M = final molarity, d = density of the concentrated solution (g/mL) and r = fractional purity of concentrated solution.

$$31.4\ mL = \frac{(0.6\ mol/L) \times (1\ L) \times (40.01\ g/mol)}{(1.53\ g/mL) \times 0.50}$$

NOTE

DIONEX recommends using the weight method for preparing NaOH eluents.

The following table lists the mass, or volume, of NaOH (50% w/w) required in 1 L to make the listed concentrations.

Table 7
Mass or Volume of NaOH Required to Make 1 L of Common Eluents

Eluent Concentration	50% (w/w) NaOH solution (g)	50% (w/w) NaOH solution (mL)
0.1 M	8	5.2
0.2 M	16	10.5
0.3 M	24	15.7
0.4 M	32	20.9
0.5 M	40	26.1
0.6 M	48	31.4
0.7 M	56	36.6
0.8 M	64	41.8
0.9 M	72	47.1
1.0 M	80	52.3

3.4.3 Sodium Hydroxide/Sodium Acetate Eluent Preparation

To help maintain baseline stability, keep the sodium hydroxide concentration constant during the sodium acetate gradient, because acetate has no buffering capacity at high pH. This is achieved by making the following eluents:

Eluent A: (*x* mM NaOH)
Eluent B: (*x* mM NaOH) *and* (*y* mM NaOAc)

To make one (1) liter of Eluent B (0.1 M Sodium Hydroxide/0.5 M Sodium Acetate):

a) Dispense approximately 800 mL of water into a 1 L volumetric flask.
b) Add a stir bar and begin stirring.
c) Weigh out 41.0 g anhydrous crystalline sodium acetate.
d) Add the solid sodium acetate steadily to the briskly stirring water to avoid the formation of clumps which are slow to dissolve.
e) After the salt dissolves, remove the stir bar with a magnetic retriever.
f) Using a plastic pipette, measure 5.2 mL of 50% (w/w) sodium hydroxide and add it to the acetate solution.
g) Rinse the pipette by drawing up the acetate solution into the pipette and dispensing it back into the graduated cylinder several times.
h) Add water to the volumetric flask until the 1 L line.
i) Replace the stir bar and stir briefly to mix.
j) Vacuum filter through a 0.2 µm nylon filter. This may take a while, as the filter may clog with insolubles from the sodium acetate.
k) Keep blanketed under helium at 34 to 55 kPa (5-8 psi) at all times, and the eluent will last about one week.

NOTE

Use Dionex certified sodium acetate salt (P/N 059326) to assure eluent quality.

3.5 Column Temperature Requirements

CarboPac columns are normally operated at ambient temperature (22 °C). The operational temperature range for the columns is 4 – 55 °C. Resolution typically decreases with increasing temperature.

3.6 Column Start-Up Requirements

Each CarboPac column is shipped in a special solution and requires a unique start-up procedure.

NOTE ***When following the start-up procedures detailed below, the column should be disconnected from the cell.***

3.6.1 The CarboPac MA1

The CarboPac MA1 is shipped in 0.6 M NaOH. To prepare the column for the alditol standard analysis do the following:

a) Wash the CarboPac MA1 for at least 20 minutes at 0.4 mL/min using 0.6 M NaOH.
b) Equilibrate the column under starting conditions for 15-20 minutes.
c) Switch your injection valve to LOAD (the loop now contains eluent equivalent to the initial conditions).
d) Connect the column to the cell.

NOTE ***When using low NaOH concentrations, column regeneration between analyses is recommended to remove carbonate. Analyses at higher NaOH concentrations may not require column regeneration.***

3.6.2 The CarboPac PA1

The CarboPac PA1 is shipped in 200 mM NaOH. To prepare the column for standard analysis:

a) Wash for at least 20 minutes at 1.0 mL/min using 200 mM NaOH.
b) Equilibrate the column at 16 mM NaOH for at least 20 minutes.
c) Switch your injection valve to LOAD (the loop now contains eluent equivalent to the initial conditions).
d) Connect the column to the cell.

NOTE ***When using 16 mM NaOH, column washing with 200 mM NaOH for at least 10 minutes between analyses is recommended to remove carbonate. Analyses using higher NaOH concentrations may not require column washing.***

3.6.3 The CarboPac PA10

The CarboPac PA10 column is shipped in 18 mM NaOH. To prepare the column for standard analysis do the following:

a) Wash for at least 20 minutes at 1.0 - 1.5 mL/min using 200 mM NaOH.
b) Equilibrate the column at 18 mM NaOH for 15 – 25 minutes.
c) Switch your injection valve to LOAD (the loop now contains eluent equivalent to the initial conditions).
d) Connect the column to the cell.

NOTE ***When using 18 mM NaOH, column washing with 200 mM NaOH for at least 10 minutes between analyses is recommended to remove carbonate. Analyses using higher NaOH concentrations may not require column washing.***

3.6.4 The CarboPac PA100

The CarboPac PA100 is shipped in 100 mM NaOH/100 mM NaOAc. To prepare the column for standard analysis do the following:

a) Wash for at least 20 minutes at 1.0 mL/min using 500 mM NaOAc in 100 mM NaOH.
b) Equilibrate the column using your starting conditions for 15-20 minutes.
c) Switch your injection valve to LOAD (the loop now contains eluent equivalent to the initial conditions).
d) Connect the column to the cell.

3.7 System Start-Up

3.7.1 Installation of Disposable Electrodes

WARNING

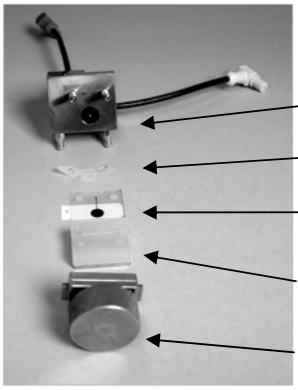
Disposable working electrodes may offer the carbohydrate user quicker startups because equilibrating the electrode after sanding and polishing is not required. Another advantage of a disposable electrodes is improved peak area response reproducibility because electrode recession does not contribute to lower response.

SAFETY

Disposable electrodes can only be used with waveform A (the quadruple potential waveform). The recommended waveforms and Teflon gaskets included in each package must be used, or the product warranty is void. Always wear gloves when handling electrodes. Never touch the electrode surface.

ED (ICS3000) **ED40 / ED50 / ED50A**

STEP 1
Check availability of all parts



Cell body with reference electrode installed

Teflon gasket for disposable electrodes

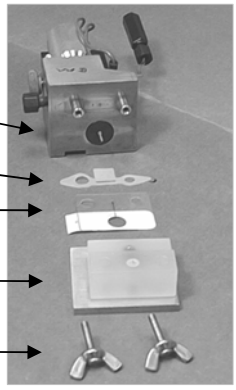
Disposable Electrode

Spacer block (P/N 062158)

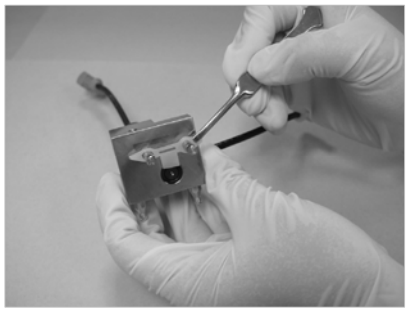
Yoke-knob Assembly

Spacer block (P/N 060297)

Wing nuts

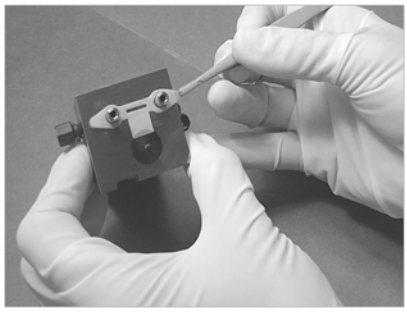


STEP 2
Install the Teflon gasket

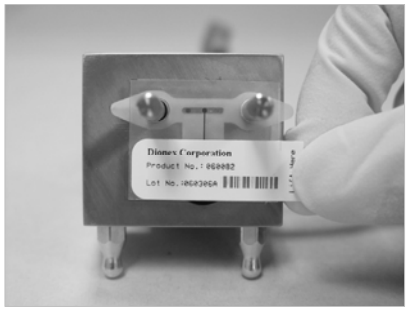


Check for correct gasket orientation.

Avoid any wrinkles inside the sealing area of the gasket.

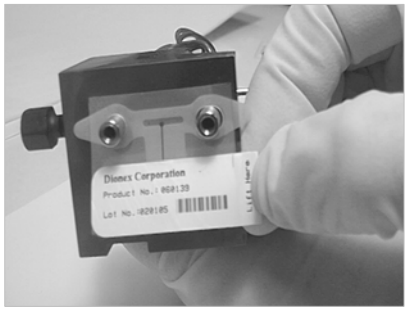


STEP 3
Install the disposable electrode

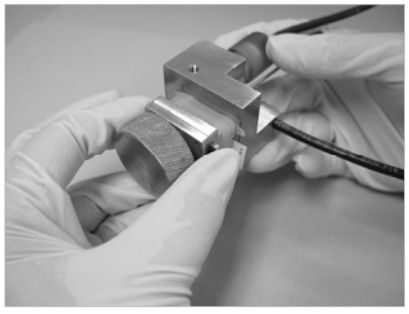


Make sure the disposable electrode is oriented correctly.

The gold electrode surface must face ED cell body



STEP 4
Place spacer block over the disposable electrode

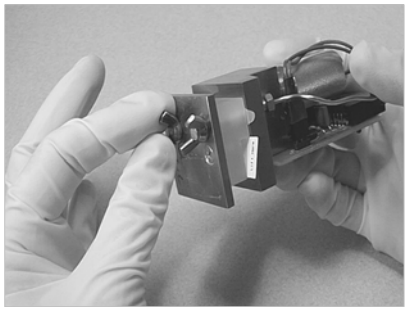


Tighten the yoke-knob till it clicks.

Tighten the wing nuts evenly.

Finger Tight only.

Do not use tools such as pliers.



3.7.2 Selecting the Optimal Electrodes and Detector Settings

The triple potential waveform has been widely used since its introduction with the ED40 electrochemical detector. However, because this waveform uses oxidative electrode cleaning (positive cleaning potential), the working electrode becomes eroded over time. This is accompanied by a gradual decrease in carbohydrate peak response. Quantitative analysis is still possible by using internal standards and regularly spaced injections of external standards.

The quadruple potential waveform was developed to minimize electrode wear and to optimize long term reproducibility. Minor disadvantages of using this waveform include a slightly noisier signal and an increased sensitivity to dissolved oxygen. Oxygen causes a dip in the baseline as it passes through the detector. With the CarboPac PA1 this occurs between galactose and mannose. This effect is noticeable at low detection levels.

Dionex recommends using the quadruple waveform in order to take advantage of its long-term detection stability. The waveform is pre-programmed into the ED50 and ED detector. A more extensive discussion of waveforms for carbohydrate detection can be found in Technical Note 21, "Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector," on the Dionex Reference Library CD-ROM.

Table 8
Reorder Information

Part Number	Description
060139	Carbohydrate Disposable Working Electrodes, Pack of 6 and two 2.0 mil gaskets
060216	Carbohydrate Disposable Working Electrodes, 4 Bundled Packages of 6 and eight 2.0 mil gaskets
060141	Gasket for Disposable Electrode, Pack of 4, ED40/ED50/ED50A Amperometry Cell, 2.0 mil
066480	Carboyhdrate Disposable Working Electrodes, Gold on PTFE, Pack of 6

NOTE ***Do not exceed 100 mM hydroxide concentration when using these polyester-based disposable electrodes (Table 8). However, 2-5 minute-long rinsing intervals with 200-500 mM hydroxide can be tolerated within 20-40 minute eluent programs. There are no limitations on the concentration of sodium acetate within the recommended range for CarboPac columns (0-1 M).***

NOTE ***Use the PTFE-based disposable gold electrodes (Table 8) for any concentration of sodium hydroxide required for CarbaPac columns. Extensive testing confirmed useful lifetimes of these electrodes of more than 4 weeks in 0.65 M sodium hydroxide. Any concentration of sodium acetate within the common range for CarboPac column can be tolerated.***

3.8 Guard Column Use

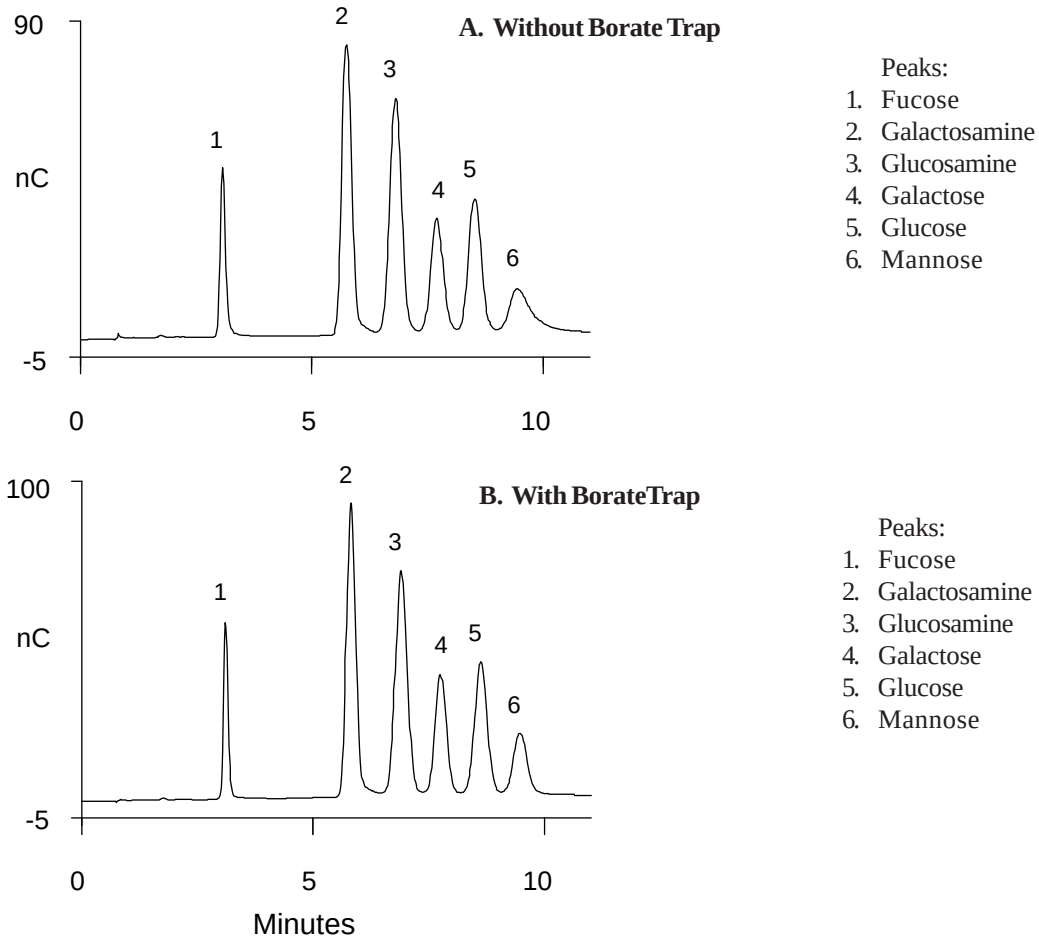
The appropriate CarboPac guard column is normally used in conjunction with the analytical column. A guard column is usually placed before the analytical column to prevent sample contaminants from eluting onto the analytical column. It is less expensive to clean or replace a guard column than an analytical column. The addition of the guard to the analytical also increases the column capacity by approximately 20%, which translates into a increase of approximately 20% in the retention times for isocratic runs. If a guard is added to a system running a gradient method that was initially developed for analytical columns only, the gradient schedules should be increased by approximately 20%, to ensure similar resolution between the eluting peaks.

If the samples are glycoprotein hydrolysates that have a high ratio of amino acids to carbohydrates, the AminoTrap column is the guard column of choice. This scenario will arise most commonly with the CarboPac PA10 column. The AminoTrap column will remove problematic amino acids from the carbohydrate elution window, reduce the working electrode poisoning, give cleaner chromatography for monosaccharides, and greatly reduce the need for correction factors.

3.9 BorateTrap

Borate can affect monosaccharide peak symmetry, even when present in the low part-per-billion concentration range. Borate is one of the first ions to break through a water de-ionization system. Its presence in eluent water for carbohydrate analysis can cause a significant loss of peak efficiency, especially for mannose and reduced monosaccharides. The BorateTrap is used immediately before the injection valve and serves to remove borate from the eluent just before chromatography. A borate trap is not needed for systems with an eluent generator and CR-ATC (for more details, see Dionex Technical Note 40).

Sample Volume:	10 µL
Column:	CarboPac PA10 (4 mm)
Eluent:	18 mM NaOH, 10 ng/mL borate
Flow Rate:	1.5 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold



Peaks:
1. Fucose
2. Galactosamine
3. Glucosamine
4. Galactose
5. Glucose
6. Mannose

Peaks:
1. Fucose
2. Galactosamine
3. Glucosamine
4. Galactose
5. Glucose
6. Mannose

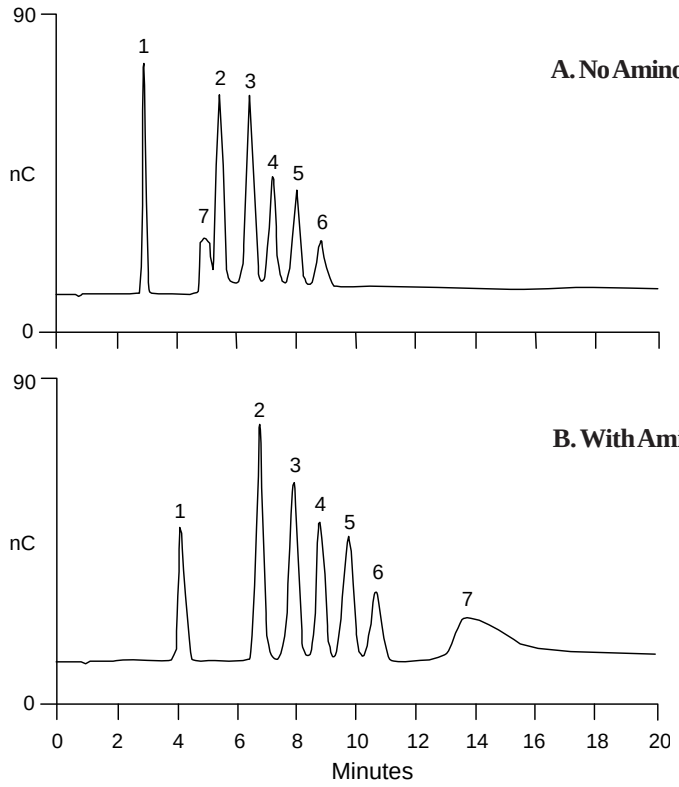
Figure 2
Effect of Borate and the BorateTrap on Monosaccharide Peak Symmetry

3.10 AminoTrap

The AminoTrap, placed before the CarboPac PA1 or PA10, improves quantitation of monosaccharides in matrices containing amino acids. Specifically designed to retain amino acids with minimal retention of carbohydrates, the AminoTrap allows monosaccharides to elute well before interfering amino acids such as lysine.

Monosaccharide detection can be compromised by amino acids in the working electrode. This is especially apparent with amine-containing glycoconjugates with low levels of glycosylation. Lysine, which is eluted before galactosamine when the AminoTrap is not employed, tails on the gold electrode. The slow release of lysine's oxidation products inhibits detector response for later eluting monosaccharides. The AminoTrap resolves the quantitation problem by retaining lysine until after the monosaccharides have been eluted (see Figure 3).

Sample Volume:	10 µL
Analytical Column:	CarboPac PA10 (4 mm)
Eluent:	18 mM NaOH
Flow rate:	1.5 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold
Storage solution:	Eluent, 18 mM NaOH



Peaks:	nmol
1. Fucose	1
2. Galactosamine	1
3. Glucosamine	1
4. Galactose	1
5. Glucose	1
6. Mannose	1
7. Lysine	100

Peaks:	nmol
1. Fucose	1
2. Galactosamine	1
3. Glucosamine	1
4. Galactose	1
5. Glucose	1
6. Mannose	1
7. Lysine	100

Figure 3
Effect of AminoTrap on the Separation of Monosaccharides in the Presence of Lysine

SECTION 4 – METHOD DEVELOPMENT

4.1 Sample Preparation

The CarboPac columns are strong anion exchangers, and the normal caveats applicable to ion exchange chromatography apply. High salt concentrations in the samples should be avoided wherever possible. Special care should be taken with samples containing high concentrations of anions, which are strong eluents for the CarboPac columns (e.g. chloride, carbonate, phosphate, etc.). It is best to avoid extremes of sample pH (especially extremely acid samples). The presence of anionic detergents (e.g. Sodium Dodecyl Sulfate) in samples should be avoided entirely. Nonionic or cationic detergents may be acceptable in low concentrations.

CAUTION ***Never pump pure water through the CarboPac PA10.***

When using PAD for detection, beware of high concentrations of electrochemically-active components (e.g. TRIS buffer, alcohols, and other hydroxylated compounds). Small amounts of organic solvents in the sample will not harm the column, although the organics may interfere with the chromatography or detection of the analytes of interest. If necessary, samples may be treated with reversed phase or ion exchange cartridges (such as the DIONEX OnGuard cartridges) before analysis. However, because the CarboPac columns are extremely rugged, it is often worthwhile to analyze an aliquot of the sample directly, without any pre-column cleanup.

Table 9
Matrix Interferent, Effect, and Possible Removal

Matrix Interferent	Effect	Possible Removal
Hydroxylated compounds (e.g. Tris buffers, alcohols)	PAD-active (interferes with carbohydrate detection)	Dialysis or dilution
Halides	Will bind to column, may affect retention time of analytes and interact with the gold electrode.	Dialysis, dilution, or solid-phase extraction using OnGuard-Ag (silver) cartridge.
Amine-containing compounds (including proteins, peptides and free amino acids)	PAD active	Solid-phase extraction using OnGuard-A (anion-exchange). For inline use, the AminoTrap column is used for proteins, peptides, and amino acids
Lipids	May damage column	Liquid-liquid extraction.
Organic Solvents	May affect analyte retention and cause diminished electrode response	Solid phase extraction using OnGuard RP (reversed phase)
Anionic detergents (such as SDS)	Will bind irreversibly to the column	Solid phase extraction using OnGuard RP

NOTE ***Sample matrices in glycoprotein analysis can be greatly simplified by performing a Western blot and selectively removing the carbohydrates from the PVDF membrane-bound proteins. Refer to DIONEX Technical Note 30, "Monosaccharide and Oligosaccharide Analysis of Glycoproteins Electrotransferred onto Polyvinylidene Fluoride (PVDF) Membranes" on the Dionex Reference Library CD-ROM.***

4.2 Analytical Conditions for Unknown Samples

Analyte separations are highly dependent on hydroxide concentration in HPAE. Many separations require only an isocratic separation. However, some groups of analytes will require a step or gradient elution. Retention of carbohydrates can be varied with eluent concentration, in some cases changing the elution order as the sodium hydroxide concentration increases.

4.2.1 CarboPac MA1

The following table can be used as a guide for selecting initial conditions for a separation.

Table 10
Selecting Initial Conditions Guide

Groups	Analytes	Run Conditions
Sugar alcohols and monosaccharides commonly found in food products	myo-inositol, glycerol, arabitol, sorbitol, dulcitol, mannitol, mannose, glucose, galactose, fructose, sucrose	480 mM NaOH isocratic, 0.4 mL/min
Disaccharide alcohols found in sweeteners	Isomaltitol, lactitol, Gp-mannitol, maltitol	250 mM NaOH isocratic, then step to 600 mM NaOH
Alditols found in physiological fluids	Glycerol, myo-inositol, scyllo-inositol, erythritol, arabitol, sorbitol, dulcitol	80 mM NaOH isocratic, then gradient from 80 mM to 700 mM NaOH
Alditols released by direct β-elimination from glycoproteins	Fucitol, GalNAcol, GlcNAcol, mannitol	100 mM NaOH, then gradient from 100 mM to 850 mM NaOH

4.2.2 CarboPac PA1

The following table can be used as a guide for selecting initial conditions for a separation.

Table 11
Selecting Initial Conditions Guide

Groups	Analytes	Run Conditions
Common monosaccharides from mammalian glycoproteins	Fucose, D-galactosamine, Dglucosamine, D-galactose, D-glucose, D-mannose	16 mM NaOH isocratic, 1.0 mL/min
Sugars in Molasses	Glucose, fructose, lactose, sucrose	150 mM NaOH, isocratic
Sialic Acids	N-acetylneuraminic acid, Nglycolylneuraminic acid	100 mM NaOH/50 mM NaOAc gradient to 100 mM NaOH/180 mM NaOAc
Polysaccharides	Maltodextrins, such as maltrin. Inulin, colominic acid	100 mM NaOH, then gradient from 100 mM NaOH to 100 mM NaOH and 1.00 M NaOAc
Aminoglycosides / Antibiotics	Tobramycin, Neomycin, Paromomycin Streptomycin, etc.	2 - 3 mM KOH 70 mM and 200 mM NaOH

4.2.3 CarboPac PA10

The following table can be used as a guide for selecting initial conditions for a separation.

Table 12
Selecting Initial Conditions Guide

Group	Analytes	Run Conditions	Section
Common monosaccharides from mammalian glycoproteins	Fucose, D-galactosamine, D-glucosamine, D-galactose, D-glucose, D-mannose	18 mM NaOH isocratic, 1.5 mL/min	4.6.1
Mono-, di- and trisaccharides	Dulcitol, sucrose, D-raffinose, stachyose, maltose	90 mM NaOH, isocratic, 1.5 mL/min	4.6.3
Sialic Acids	N-acetylneuraminic acid, N-glycolylneuraminic acid	100 mM NaOH/70 mM NaOAc gradient to 100 mM NaOH/300 mM NaOAc	4.6.2
Food sugars and sugar alcohols	Glycerol, xylitol, sorbitol, mannitol, glucose, fructose, sucrose, lactose	52 mM NaOH, isocratic, 1.5 mL/min with borate trap	4.6.4

4.2.4 CarboPac PA100

The following table can be used as a guide for selecting initial conditions for a separation.

Table 13
Selecting Initial Conditions Guide

Group	Analytes	Run Conditions	Section
Neutral and sialylated N-linked oligosaccharides from glycoproteins	Neutral and sialylated N-linked oligosaccharides	100 mM NaOH with 0 mM-250 mM NaOAc gradient in 110 min, 1 mL/min	4.7.1
Oligosaccharides with monosaccharide linkage isomerism	α-2,3 and α-1,6 NANA-Gal linkage	100 mM NaOH with 0 mM-250 mM NaOAc gradient in 110 min, 1 mL/min	4.7.2
Oligosaccharide and polysaccharides in food products	Oligosaccharide and polysaccharides	100 mM NaOH with NaOAc gradient, 1 mL/min	4.7.3
Oligosaccharides profiling during beer production	Ethonol, glucose, maltose, maltotriose, maltose oligosaccharides	100 mM NaOH with NaOAc gradient, 1 mL/min	4.7.4

The elution order of oligosaccharides is somewhat predictable, and well documented (Rohrer J., Glycobiology, **1995**, 5, 359-360). They can be summarized by the following rules:

1. The larger the oligosaccharide, the later it elutes (e.g. the greater the number of mannose residules in a high-mannose oligosaccharide, the later it elutes).
2. Increased negative charge (sialylation, phosphorylation, sulphonation), increases retention.
3. Addition of fucose to an oligosaccharide reduces its retention.
4. An oligosaccharide with a Neu5A*ca*2→3Gal elutes later than the same oligosaccharide with a Neu5Ac*a*2→6Gal.
5. An oligosaccharide with a Galβ1→3GlcNAc elutes later than the same oligosaccharide with Galβ1→4GlcNAc.
6. Addition of a bisecting GlcNAc to an oligosaccharide increases its retention.
7. Replacement of Neu5Ac with Neu5Gc increases oligosaccharide retention.
8. Addition of lactosamine repeat to a neutral oligosaccharide increases its retention.
9. Addition of lactosamine repeat to a sialylated oligosaccharide decreases its retention.
10. An oligosaccharide elutes earlier after reduction of its reducing terminal GlcNAc.
11. An oligosaccharide with a complete chitobiose elutes earlier than an oligosaccharide with half its chitobiose.
12. Sialylated oligosaccharide with all Neu5Ac*a*2→3Gal are better resolved at pH 5.

4.3 CarboPac MA1 Example Chromatograms

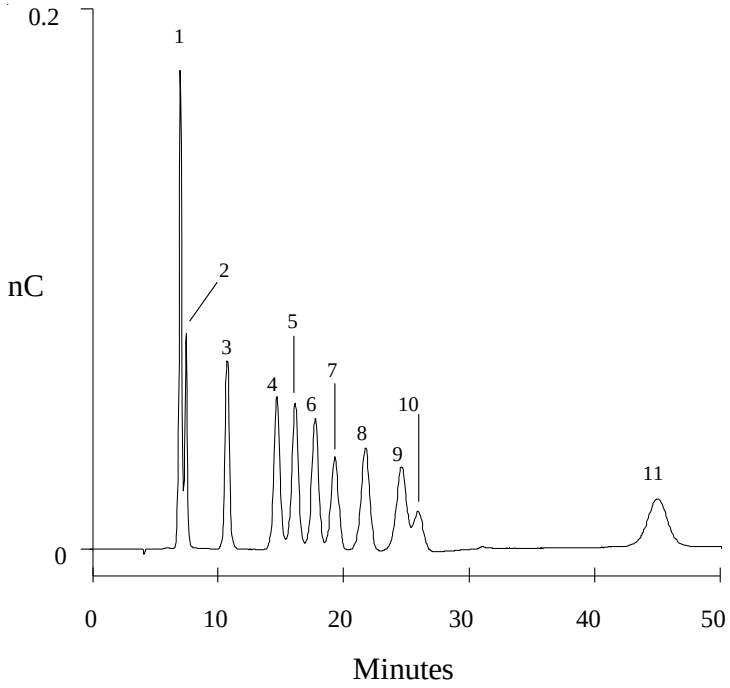
The following section provides examples of the types of applications for which the CarboPac MA1 has been designed.

4.3.1 Separation of Sugar Alcohols and Monosaccharides Commonly Found in Food Products

These analytes, found in fruits and vegetables, are separated isocratically using 480 mM NaOH and detected using pulsed amperometric detection. Mannose and galactose have been added to show that aldoses exhibit greater retention than this group of alditols.

The glycerol/inositol resolution can be improved at the expense of either run time or mannitol/mannose resolution. A 300 mM NaOH eluent concentration will elute glycerol before inositol. However, mannose, glucose and galactose will be eluted much later than shown here. A 550 mM NaOH eluent concentration will cause glycerol to be eluted later, with a resulting improvement in inositol/glycerol resolution. However, at this higher NaOH concentration, mannose will begin to co-elute with mannitol.

Sample Volume:	30 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent:	480 mM NaOH
Flow rate:	0.4 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold



Peaks:	mg/L
1. *myo*-inositol	18
2. Glycerol	19
3. Arabitol	15
4. Sorbitol	18
5. Dulcitol	18
6. Mannitol	18
7. Mannose	18
8. Glucose	18
9. Galactose	18
10. Fructose	18
11. Sucrose	34

Figure 4
Separation of Common Food Alditols

Vanillin is the most widely used flavoring in food products. Adulteration of natural vanillin by synthetic products is a major concern. Vanillin is an aromatic aldehyde and can be detected by pulsed amperometric detection with high sensitivity and specificity. This is particularly advantageous when determinations are made in complex food matrices because clean up procedures can be minimized. This chromatogram shows the determination of vanillin in a sample of yogurt. The sample was diluted 1:10 and filtered through a 0.45 µm filter prior to injection.

Sample Volume:	25 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent:	750 mM NaOH
Flow rate:	0.3 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

A: Vanillin standard 0.46 µg/L
B: Vanillin in sample 0.45 µg/L

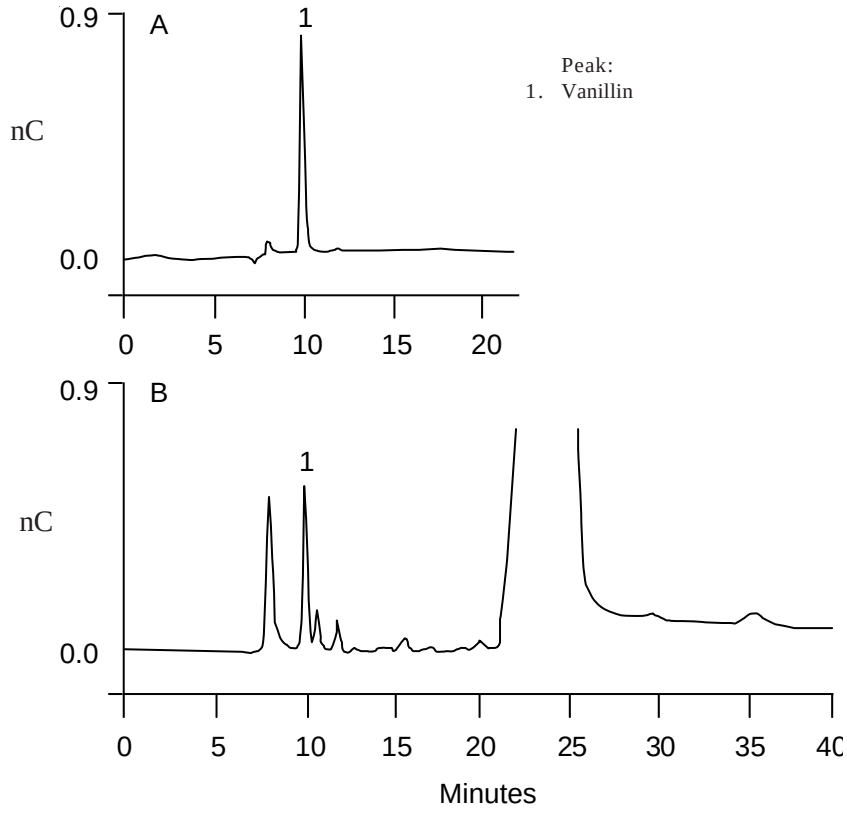


Figure 5
Vanillin in Yogurt

4.3.2 Analysis of Sweeteners

The two chromatograms below show the use of HPAE for the analysis of sweeteners and the components of sweeteners. The first chromatogram shows the separation of isomaltitol and glucopyranosyl-mannitol (GpM) which are found as components in some commercial sweeteners.

Sample Volume:	20 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent:	612 mM NaOH
Flow rate:	0.4 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Peaks:	mg/L
1. Isomaltitol	34
2. Lactitol	34
3. Gp-Mannitol	34
4. Maltitol	34

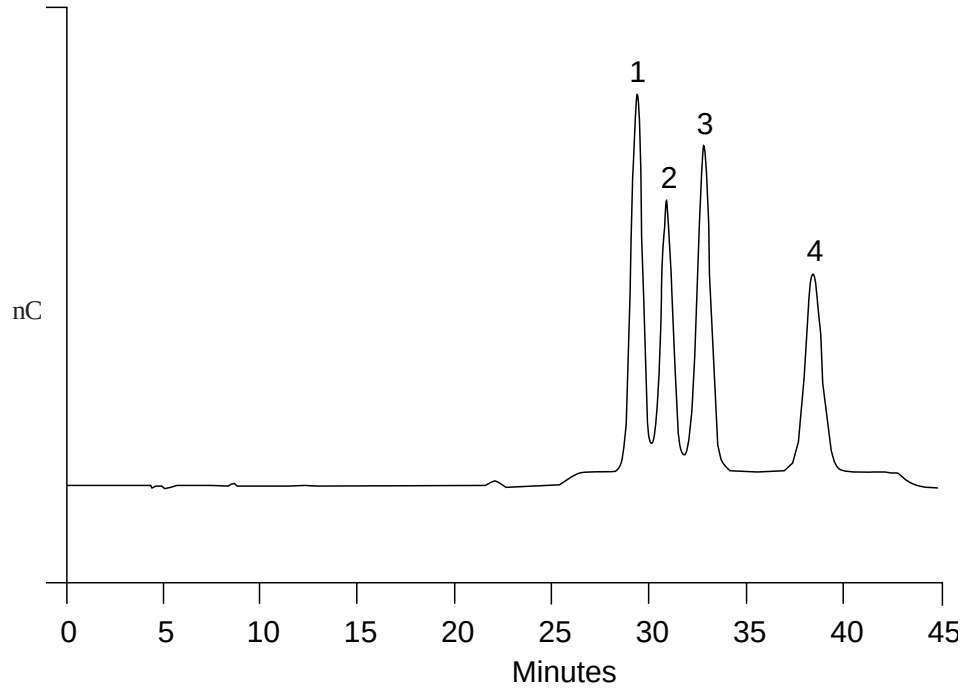


Figure 6
Separation of Disaccharide Alcohols in Sweeteners

This chromatogram shows the analysis of Sucralose, which is a high intensity sweetener with 400-800 times the sweetness of sucrose. It is manufactured by selective chlorination of sucrose and is currently the only non-nutritive sweetener that is based on sucrose.

Sample Volume:	25 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent:	150 mM Sodium acetate, 0.2% (v/v) Acetic acid pH 5.5
Flow rate:	0.4 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Peaks:	mg/L
1. 4-Cl-Galactose	7.8
2. 1,6-di-Cl-Fructose	2.4
3. Sucralose	

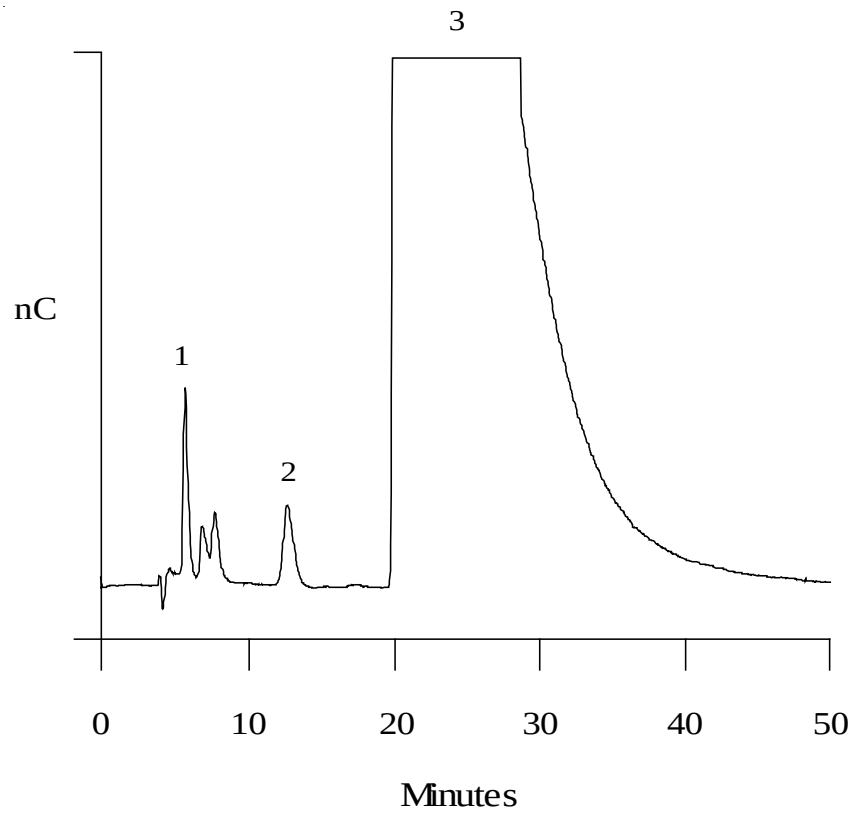


Figure 7
Analysis of Sucralose

4.3.3 Alditols found in Physiological Fluids

In this separation, the initial NaOH concentration of 80 mM is used to optimize the separation of glycerol, *myo*-inositol and *scyllo*-inositol early in the chromatogram. The gradient that follows allows the resolution of sorbitol and dulcitol. The latter two alditols are poorly resolved at 80 mM NaOH.

Sample Volume:	20 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent A:	Water
EluentB:	1.0 M NaOH
Flow rate:	0.4 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
0.0	92	8	80 mM NaOH, Inject
4.0	92	8	80 mM NaOH for 4 minutes
15.1	30	70	80-700 mM NaOH over 11.1 minutes
25.2	30	70	700 mM NaOH for 10.1 minutes
26.3	92	8	700-80 mM NaOH in 1.1 minutes
46.3	92	8	Re-equilibrate prior to next injection

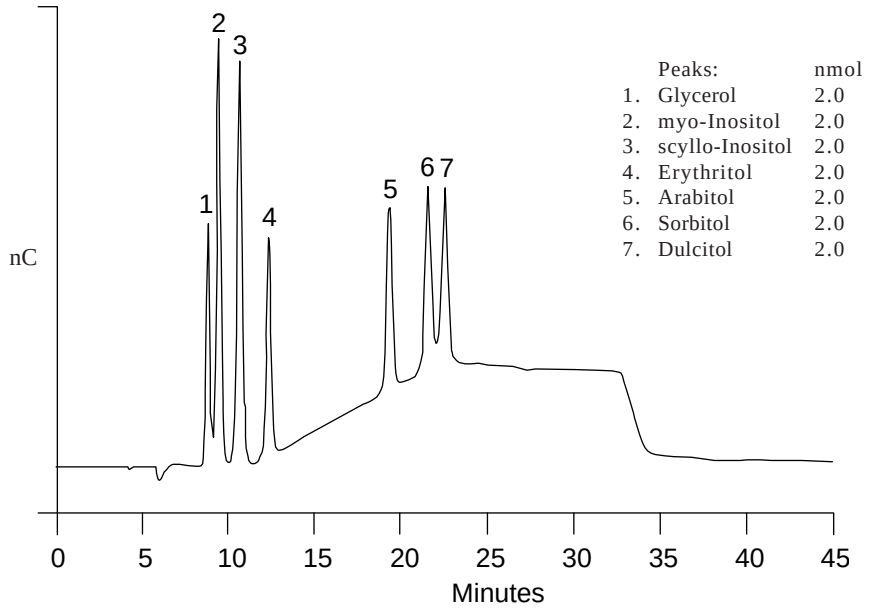


Figure 8
Alditols Found in Physiological Fluids

4.3.4 Separation of Monosaccharide Alditols Released by β-elimination from Glycoproteins

Many glycoproteins contain one or more sugars linked through serine or threonine residues. Upon β-elimination, these are released to form alditols (e.g. N-acetyl galactosaminitol (GalNAcol), fucitol) or oligosaccharides having the sugar moiety previously linked to serine or threonine reduced to the alditol form. An initial isocratic step at 100 mM NaOH separated N-acetyl glucosaminitol (GlcNAcol) and GalNAcol from one another and from fucitol. The gradient that follows this isocratic separation accelerates the elution of mannitol as well as that of any oligosaccharide alditols that may have been released during the β-elimination process.

Sample Volume:	20 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent A:	Water
EluentB:	1.0 M NaOH
Flow rate:	0.4 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
0.0	90	10	100 mM NaOH, Inject
4.7	90	10	100 mM NaOH for 4.7 mins
15.8	30	70	100-700 mM NaOH over 11.0 minutes
25.9	30	70	700 mM NaOH for 9.0 minutes
27.0	90	10	700-100 mM NaOH in 1.1 minutes

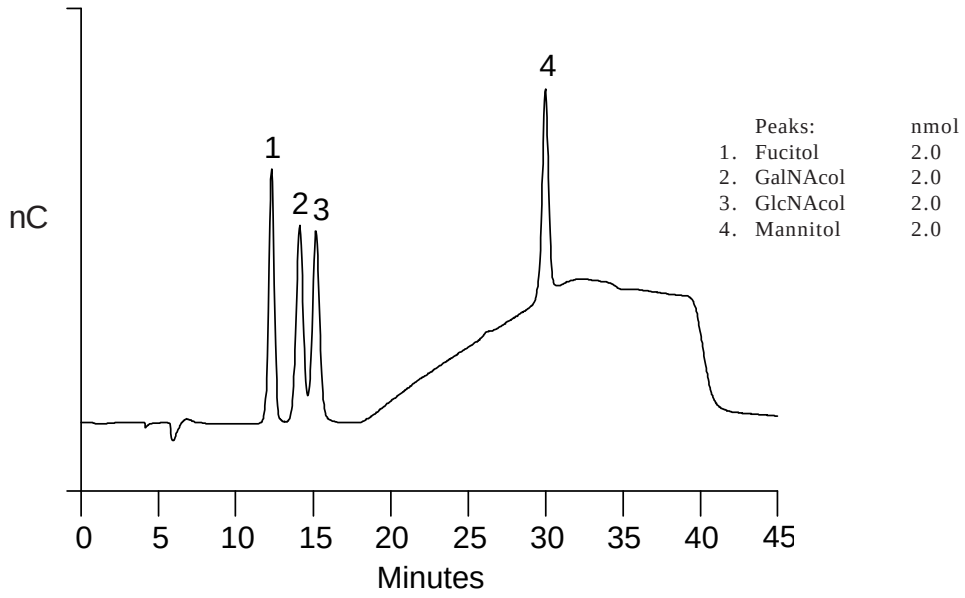


Figure 9
Separation of Monosaccharide Alditols Released by Direct β-elimination from Glycoproteins

4.3.5 Separation of Monosaccharide Alditols Released by Hydrolysis of β-eliminated Glycoconjugates

This example chromatogram shows the separation of monosaccharides derived from glycoprotein oligosaccharides. Oligosaccharides are released by reductive β-elimination which converts only the terminal peptide-linked sugar to the alditol form. The terminally-reduced oligosaccharide is chemically or enzymatically digested into a mixture containing one alditol (Peaks 1, 2, 3, 4, 7, 8 and 10) and one or more non-reducing monosaccharides. Arabitol (Peak 5) serves as an internal standard. An initial isocratic step at 100 mM NaOH separates N-acetyl glucosaminitol (GlcNAcol) and N-acetyl galactosaminitol (GalNAcol) from one another and from fucitol. The gradient that follows this isocratic separation accelerates the elution of mannitol as well as any oligosaccharide alditols that may have been released during this β-elimination reaction and positions the elution of several aldoses potentially present so they do not interfere with the other analytes.

Sample Volume:	20 µL
Analytical Column:	CarboPac MA1 (4 x 250 mm)
Eluent A:	Water
EluentB:	1.0 M NaOH
Flow rate:	0.4 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
0.0	94	6	60 mM NaOH, Inject
5.0	94	6	60 mM NaOH for 5.0 mins
35.0	34	66	60-660 mM NaOH over 30.0 minutes, curve 4
40.0	34	66	660 mM NaOH for 5.0 minutes
41.0	94	6	Initial conditions over 1 minute
50.0	94	6	Re-equilibrate at initial conditions for 5 minutes

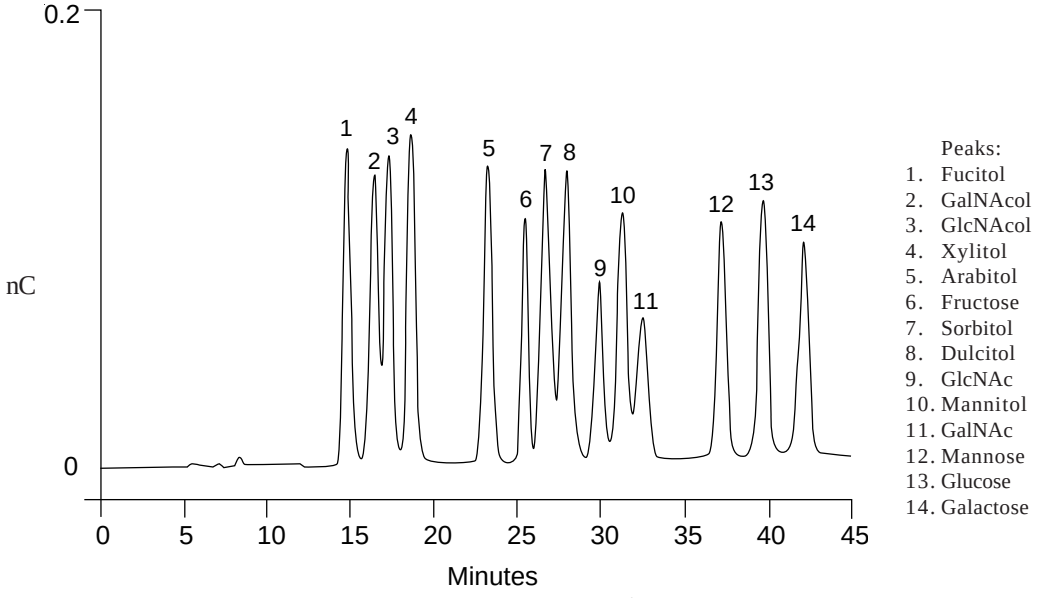


Figure 10
Gradient Separation of Alditols and Aldoses Released by Hydrolysis of β-eliminated Oligosaccharides with Arabitol as Internal Standard

4.4 CarboPac PA1 Example Chromatograms

The following section provides examples of the types of applications for which the CarboPac PA1 is designed.

4.4.1 Isocratic Methods to Determine Sugars in Molasses

This method is the official method of the International Commission for Uniform Methods of Sugar Analysis (ICUMSA) and was approved in 1994. Approval of this method was based on an international collaborative study involving 11 laboratories. Excellent reproducibility was obtained, and the results were in close agreement with a parallel GC collaborative study.

Sample Volume:	50 µL
Analytical Column:	CarboPac PA1 (4 x 250 mm) and Guard
Eluent A:	150 mM NaOH
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Peaks:	
1. Glucose	4.39%
2. Fructose	6.67%
3. Lactose	(internal std)
4. Sucrose	30.8%

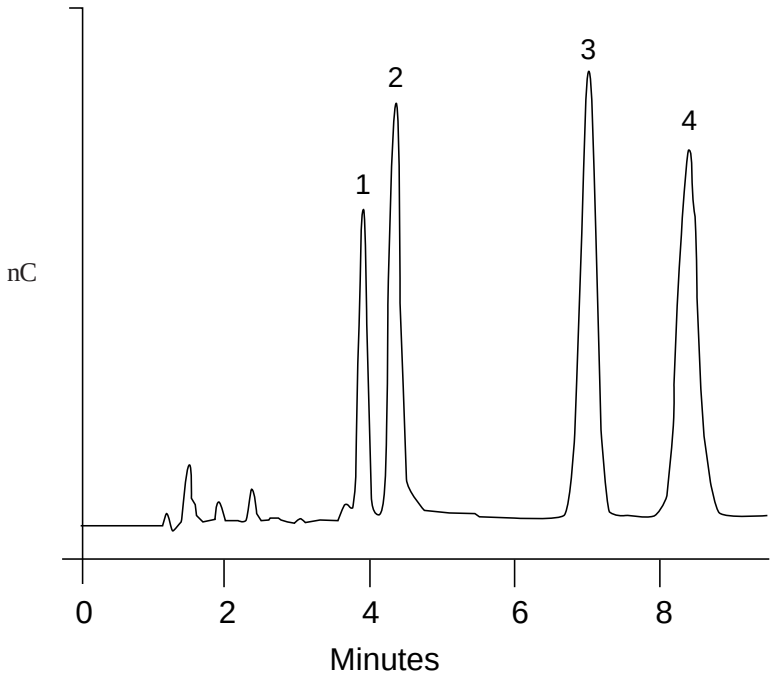


Figure 11
Sugars in Molasses

The HPAE-PAD technique also provides a useful approach to characterization of molasses from different geographical locations. The next two chromatograms show a comparison of the sugar and oligosaccharide profiles for beet sugar molasses samples from Britain and the United States.

Analytical Column:	CarboPac PA1 (4 x 250 mm)
Eluent:	100 mM NaOH, 20 mM NaOAc
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

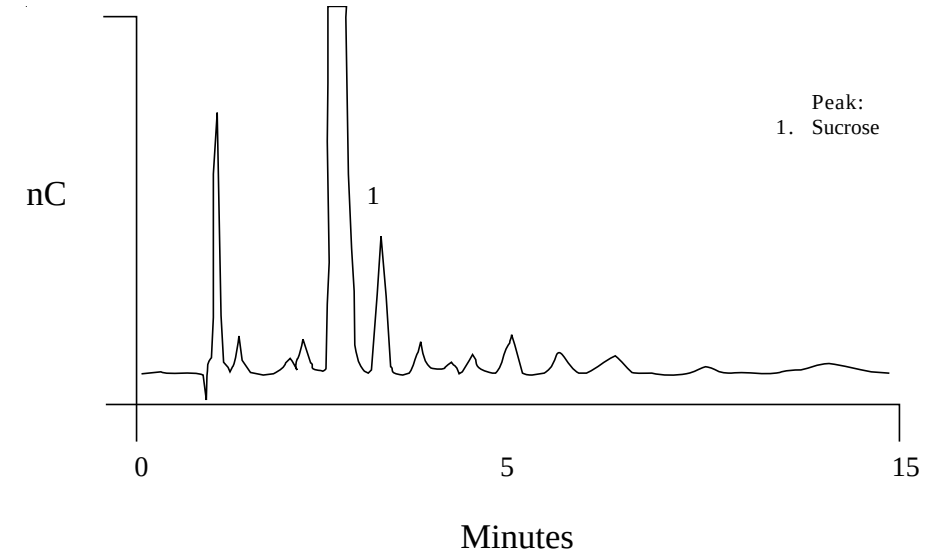


Figure 12
Beet Sugar Molasses from Britain

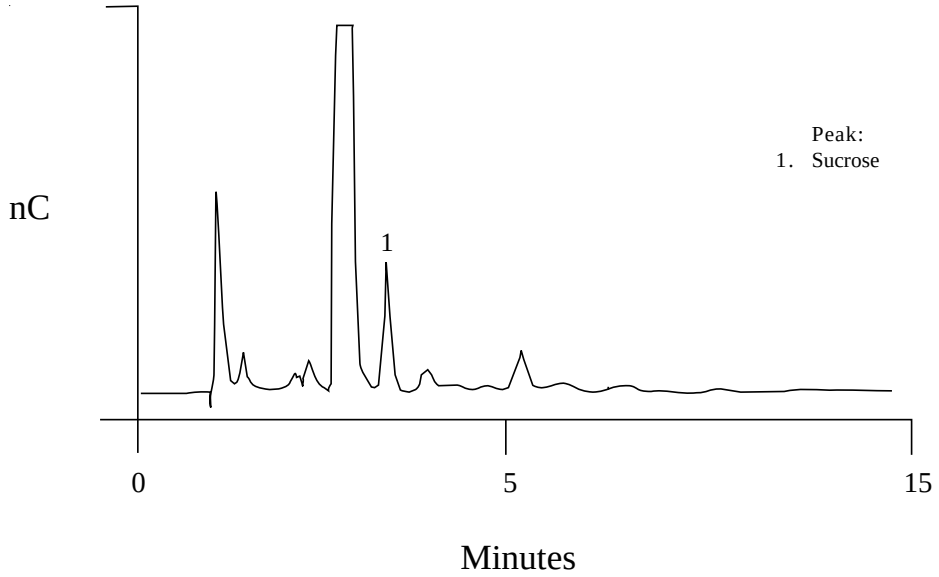


Figure 12
Beet Sugar Molasses from the United States

4.4.2 Gradient Separation of Sialic Acids

Sialic acids commonly found in glycoproteins can be separated using a sodium acetate gradient in 100 mM sodium hydroxide. The following conditions retain N-acetylneuraminic acid sufficiently to keep it away from glycoprotein hydrolysis products while still eluting N-glycolylneuraminic acid in just over 20 minutes.

Sample Volume:	20 µL
Analytical Column:	CarboPac PA1 (4 x 250 mm)
Eluent:	50-180 mM NaOAc in 100 mM NaOH from 0-20 minutes
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Peaks:	pmol
1. N-acetylneuraminic acid	500
2. N-glycolylneuraminic acid	500

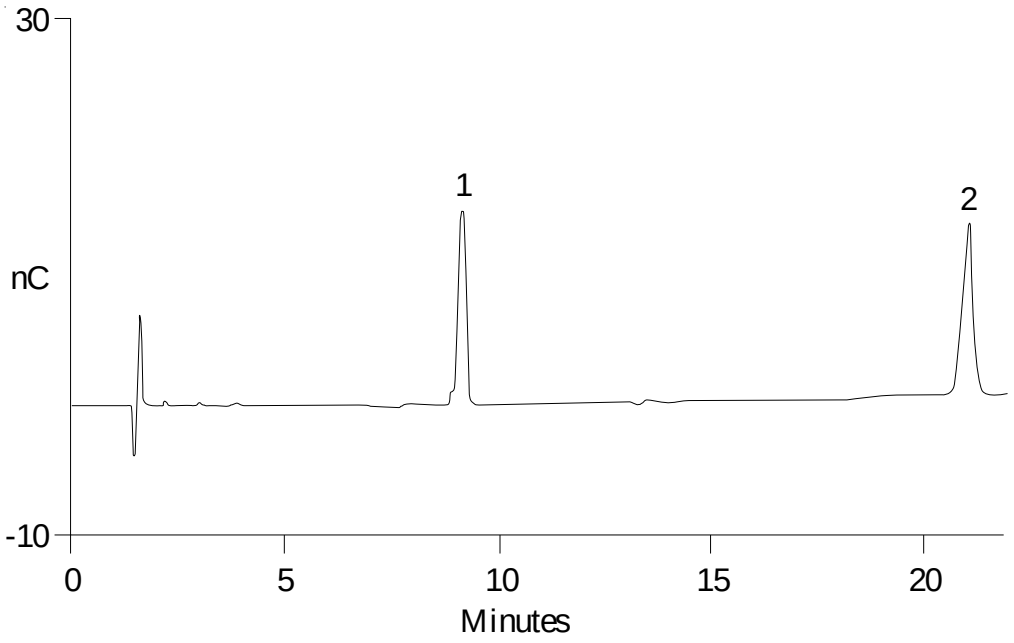


Figure 13
Gradient Separation of Sialic Acids

4.4.3 Gradient Separation of Linear Polysaccharides

Many commercial low-calorie bulk sweeteners, bulking agents, and fat substitutes are polysaccharide or polyol materials derived from various types of starch. Determination of the distribution of polysaccharide chain lengths in these products is important because it has a direct bearing in the product functionality. HPAE-PAD is the method of choice because other approaches cannot separate the higher DP oligosaccharide chains. Linear polysaccharides can be separated up to a degree of polymerization (DP) of 60 at single residue resolution using a sodium acetate gradient in sodium hydroxide. The following examples show separations of Maltrin and a sample of inulin.

Sample Volume:	25 µL
Analytical Column:	CarboPac PA1 (4 x 250 mm)
Eluent A:	0-300 mM NaOAc in 100 mM NaOH from 0-30 minutes
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

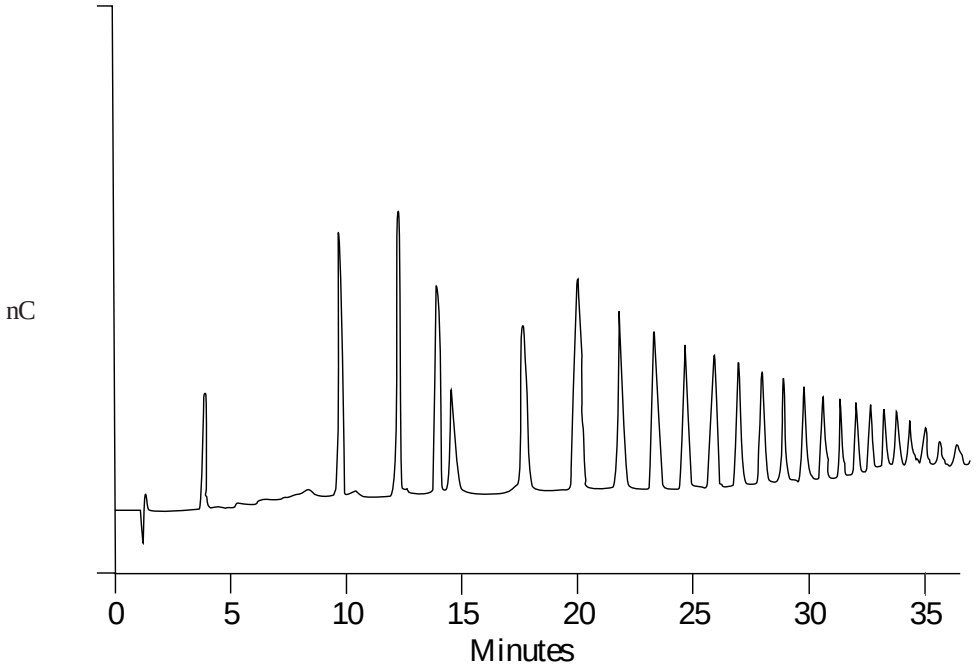


Figure 14
Gradient Separation of Maltrin®, a Linear Polysaccharide

Inulin-based products are marketed as fat replacers, and for adding dietary fiber content to food formulations. They are extracted commercially from crops such as chicory root and Jerusalem artichoke. Inulins are mixtures of linear polyfructose chains and linear polyfructose chains incorporating terminal glucose units.

Sample:	0.3% water washed inulin (polyfructose) in 0.1M NaOH
Analytical Column:	CarboPac PA1 (4 x 250 mm)
Eluent A:	200-600 mM NaOAc in 100 mM NaOH from 0-40 minutes
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

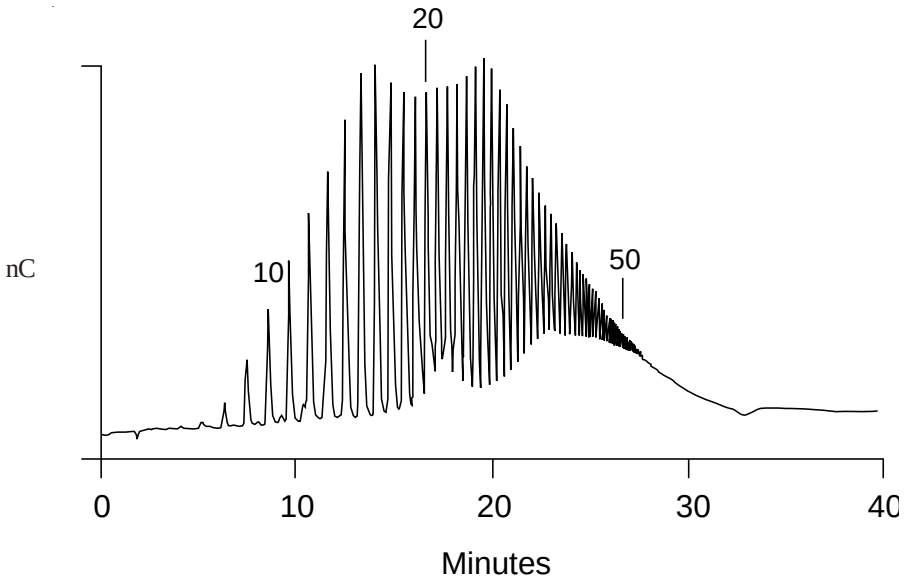


Figure 15
Gradient Separation of Inulin Polymers

Commercial inulin products have degrees of polymerization (DP) which have been tailored for a particular end use. Therefore, it is important to determine the chain length distribution during product development, production and for quality control of the end product. The following chromatogram shows the excellent resolution, which can be achieved for DP values up to 50 for inulin derived from chicory.

Sample Volume:	10 µL, 1mg Chicory inulin
Analytical Column:	CarboPac PA1 (2 x 250 mm) and guard (2 x 50 mm)
Eluent A:	DI Water
Eluent B:	200 mM NaOH
Eluent C:	100 mM NaOH, 500 mM NaOAc
Flow rate:	0.25 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	%C	Comments
0.0	35	35	30	Inject
90.0	5	5	90	NaOAc gradient
90.1	35	35	30	Back to initial conditions

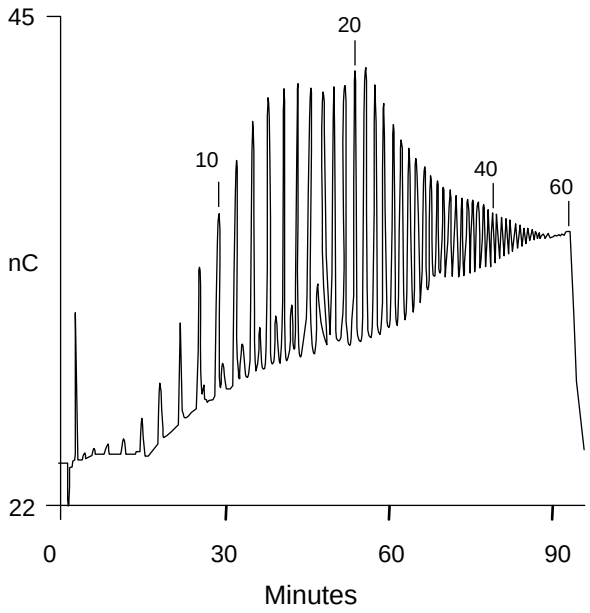


Figure 16
Gradient Separation of Chicory Inulin Polymers

4.4.4 Analysis of Oligosaccharides in Beer

Non-fermented oligosaccharides in the final product contribute to the sweet flavor and also the caloric content. Dry beers are made with a lower residual sugar content for a less sweet flavor and reduced calorie content. Oligosaccharide profiles can be determined in less than 30 minutes.

Sample:	Beer (Dry Style)
Analytical Column:	CarboPac PA1 (4 x 250 mm) and guard
Eluent A:	100 mM NaOH
Eluent B:	100 mM NaOH, 100 mM NaOAc
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
0.0	95	5	Inject
15.0	0	100	NaOAc gradient
16.0	95	5	Back to initial conditions

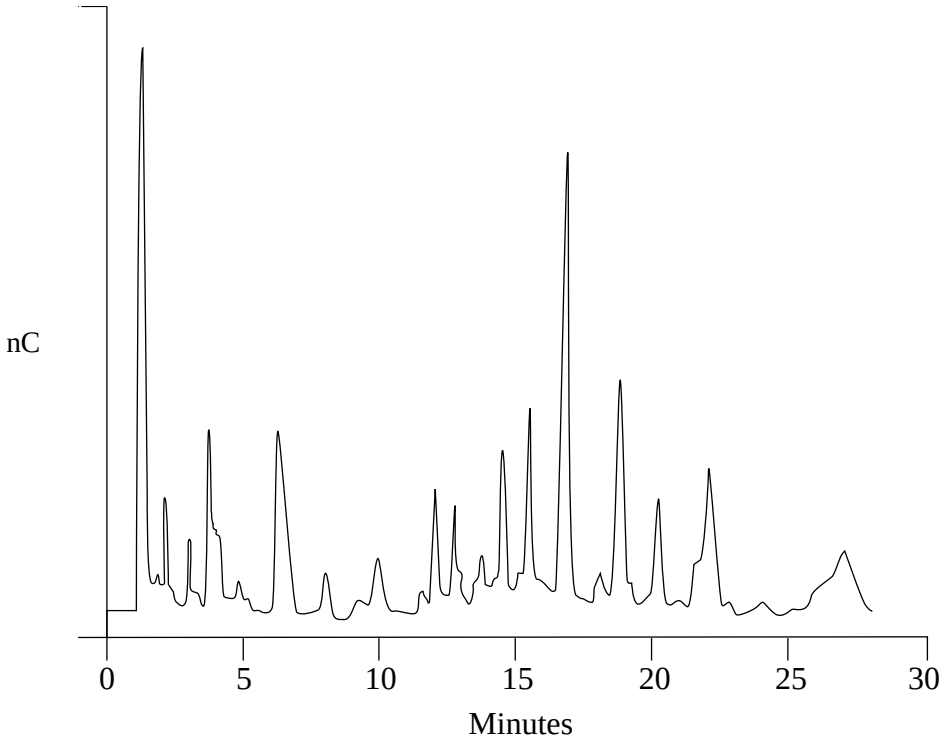


Figure 17
Analysis of Oligosaccharides in Dry Beer

The unique mix of residual non-fermentable sugars and oligosaccharides in dry beers contribute to each beer's characteristic flavor. Residual sugars and oligosaccharide profiles for different manufacturer's low calorie beers can be easily compared as shown below.

Sample Volume:	6 µL
Analytical Column:	CarboPac PA1 (4 x 250 mm) and guard
Eluent A:	150 mM NaOH
Eluent B:	150 mM NaOH, 100 mM NaOAc
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
0.0	95	5	Inject
15.0	0	100	NaOAc gradient
16.0	95	5	Back to initial conditions

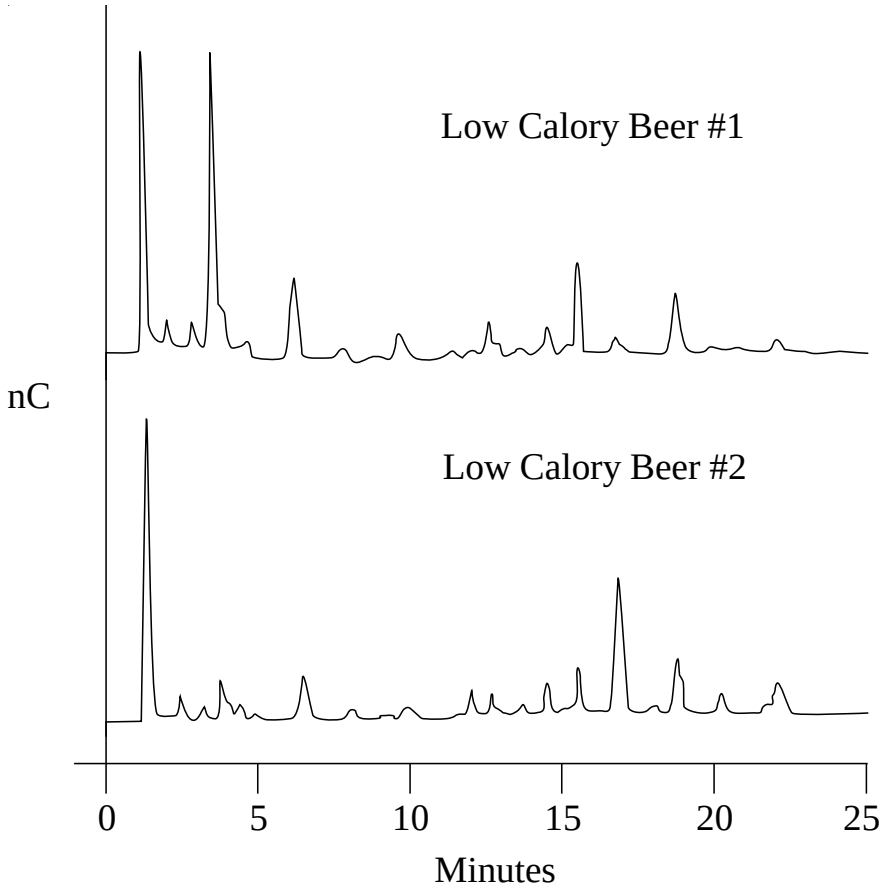


Figure 18
Comparative Analysis of Low Calory Beers

4.4.5 Analysis of Oligouronides from Citrus Pectins

Pectins have been used for years to thicken gel products such as jams and jellies, but more recently, new applications are being discovered. For example, in late 1991, Hercules introduced Slendid, a specialty pectin product consisting of partially methylated polygalacturonic acid extracted from citrus peel for use as a fat substitute. Characterization of these specialty materials for distribution of polymer chain lengths (DP) is essential in maintaining consistent product quality and performance. Another potential application for this type of procedure is in detection of pulp wash and peel extract adulteration of pure fruit juices.

Sample Volume:	25 µL
Analytical Column:	CarboPac PA1 (4 x 250 mm)
Eluent A:	400-900 mM NaOAc in 100 mM NaOH from 0-45 minutes
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

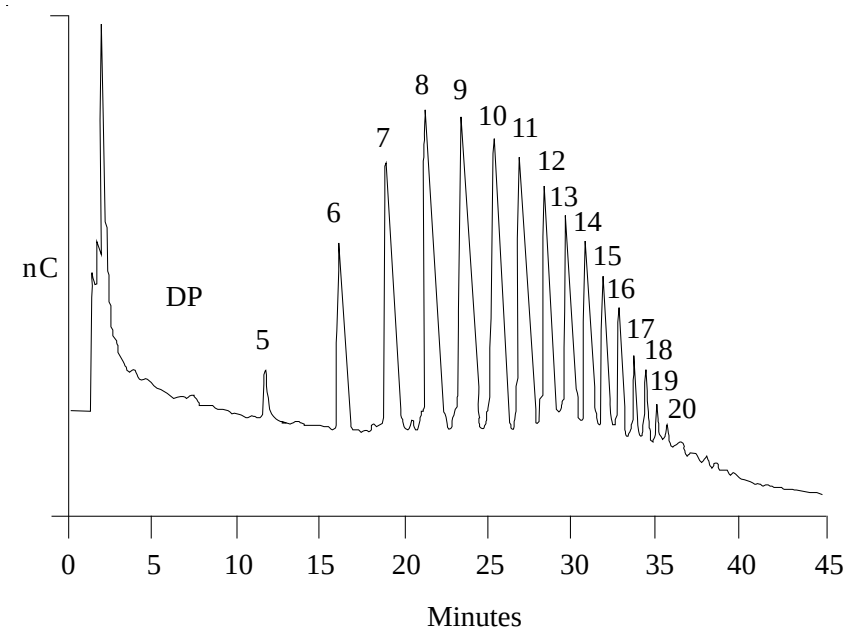


Figure 19
Analysis of Oligouronides from Citrus Pectins

4.5 CarboPac PA10 Example Chromatograms

The following section provides an example of the types of applications for which the CarboPac PA10 is designed.

CAUTION
Pumping pure water through the CarboPac PA10 will cause irreversable damage to the bed.

4.5.1 CarboPac PA10 Fast Run

The CarboPac PA10 is capable of withstanding backpressures up to 3,500 psi. The following chromatogram shows the separation of six carbohydrates in less than 12 minutes.

Sample Volume:	10 µL
Analytical Column:	CarboPac PA10 (4 x 250 mm) and Guard
Eluent A:	18 mM NaOH
Flow rate:	1.5 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

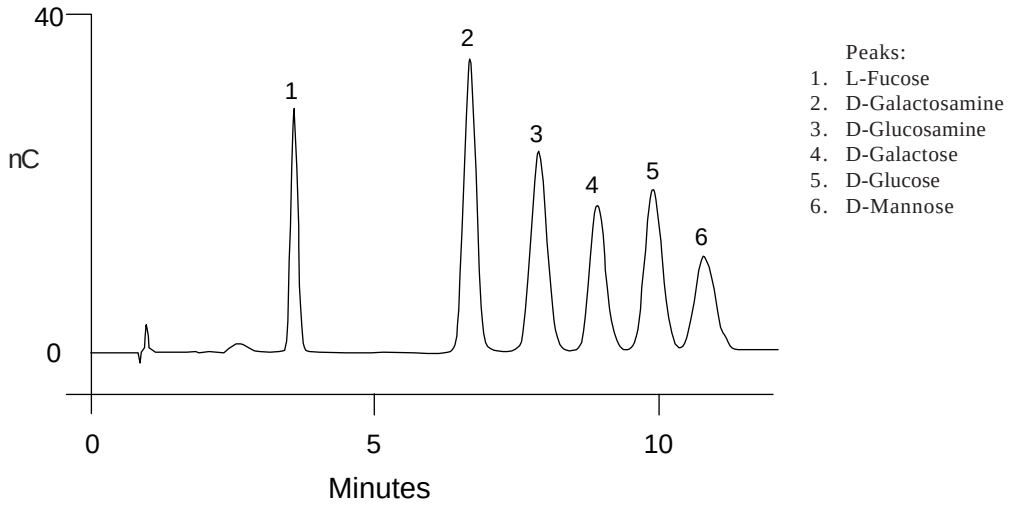


Figure 20
CarboPac PA10 Fast Run

4.5.2 Sialic Acid Analysis

Sialic acids comprise a large family of N- and O- substituted neuraminic acids. The amino group of neuraminic acid is linked to either an N-acetyl or N-glycolyl group, which yields N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc), respectively. The hydroxyl of both sialic acids are often modified. Sialic acids occupy terminal positions on many mammalian glycoprotein and glycolipid oligosaccharides. When a glycoprotein loses sialic acid residues, it has a reduced serum half-life, and in some cases reduced activity. Therefore, it is important to know the sialic acid content of a glycoprotein when assaying its function or its efficacy as a pharmaceutical therapeutic. HPAE-PAD is an effective way to determine Neu5Ac and Neu5Gc without derivatization.

The following chromatogram shows the separation of Neu5Ac and Neu5Gc with 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) as an internal standard. Although there are many conditions that can be used to separate these three sugar acids, the conditions below allow a relatively fast separation, with Neu5Ac well retained beyond the void.

Sample Volume:	25 µL
Analytical Column:	CarboPac PA10 (4 x 250 mm) and Guard
Eluent A:	100 mM NaOH
Eluent B:	100 mM NaOH with 1 M NaOAc
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
Initial	93	7	Initial conditions
0.0	93	7	Inject, NaOAc gradient starts
10.0	70	30	NaOAc gradient ends, isocratic
11.0	70	30	End isocratic, gradient back to initial conditions
12.0	93	7	Start re-equilibration at initial conditions

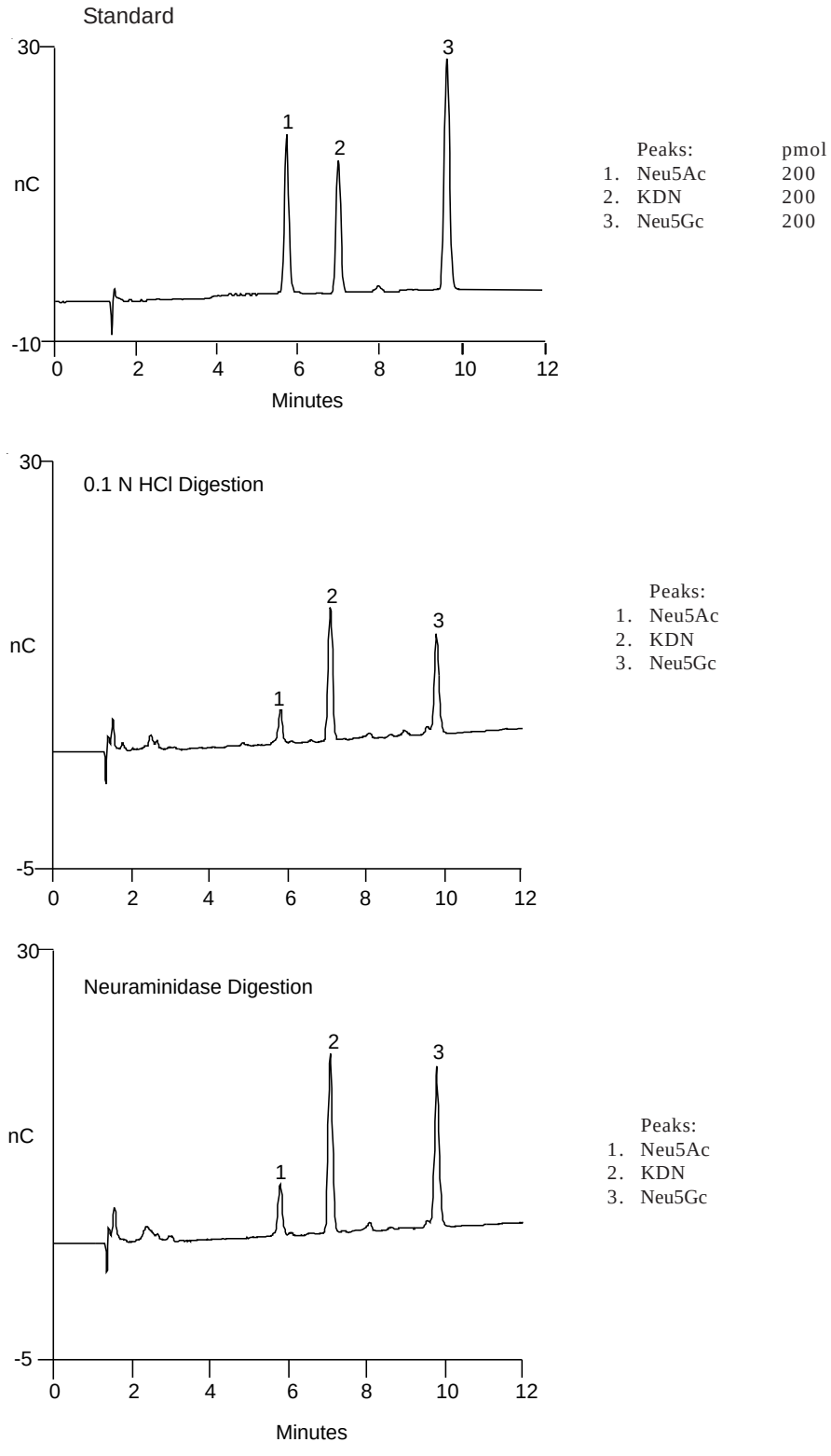


Figure 21

Neuraminidase Digestion of Bovine Transferrin

4.5.3 Small Oligosaccharides Analysis

This separation of dulcitol, sucrose, raffinose, stachyose and maltose shows the ability of the CarboPac PA10 to separate low to high carbohydrates isocratically in less than 20 minutes. The particular group of analytes is from a seed hull application. If maltose is not present in the sample, the run time can be shortened to 12 minutes, using 140 mM NaOH as the eluent. Under these conditions, if maltose is present, stachyose and maltose are not resolved.

Sample Volume:	10 µL
Analytical Column:	CarboPac PA10 (4 x 250 mm) and Guard
Eluent A:	90 mM NaOH
Flow rate:	1.5 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

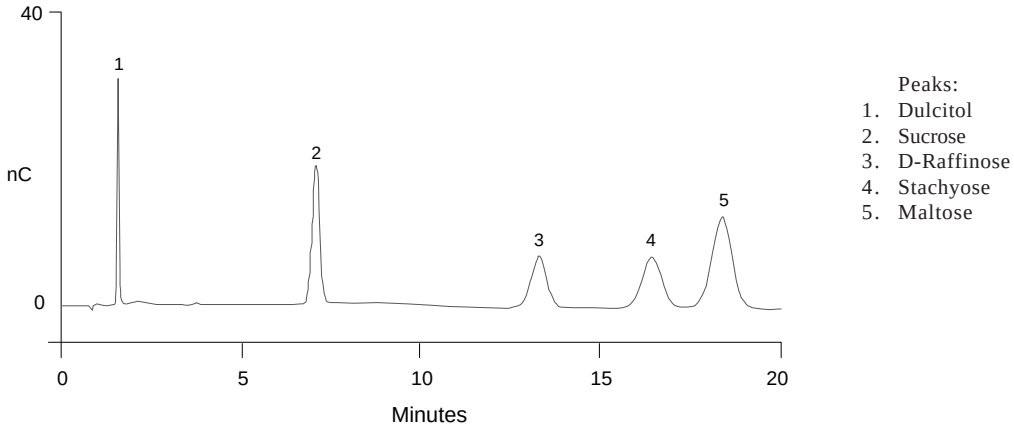


Figure 22
Mono-, Di- and Trisaccharide Analysis

4.5.4 Isocratic Separation of Food Sugars and Food Alcohols

This application uses an isocratic eluent to separate alditols, monosaccharides and disaccharides commonly found in food samples, in a single run. Alditol retention is only slightly affected by sodium hydroxide concentration between 18 mM and 50 mM. This allows for optimization of monosaccharide and disaccharide separations by increasing the hydroxide concentration while still maintaining adequate alditol retention. Use of the BorateTrap column greatly improves the peak shape of the alditols.

Sample Volume:	10 µL
Analytical Column:	CarboPac PA10 (4 x 250 mm) and Guard, BorateTrap
Eluent A:	52 mM NaOH
Flow rate:	1.5 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

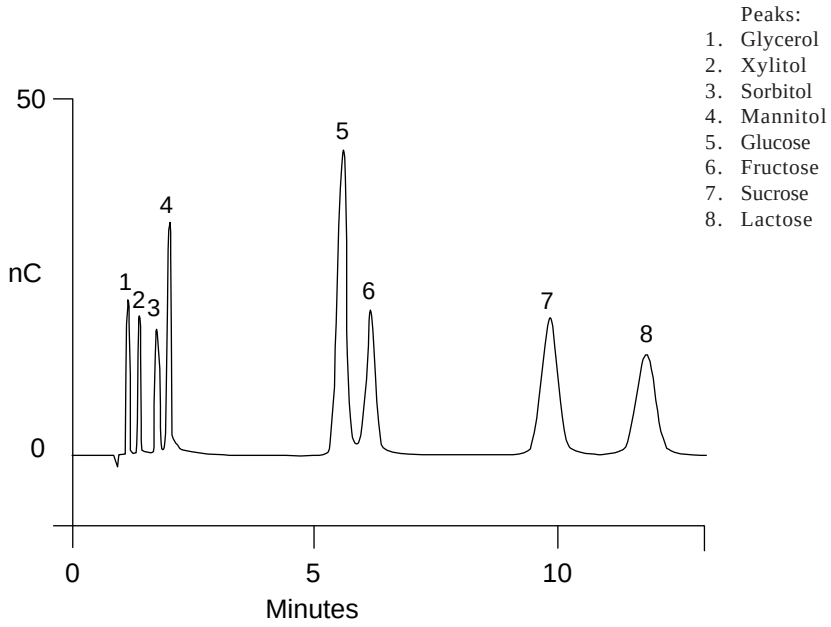


Figure 23
Separation of Food Sugars and Food Alcohols

4.5.5 Carbohydrate Separations with On-line Eluent Generation

The Dionex EG40 is an on-line eluent generator which can be used for the production of carbonate-free hydroxide eluent. The pump is used to pump degassed deionized water through the EG40 and the eluent is electrolytically generated (see the EG40 manual for details). The EG40 minimizes the concentration of carbonate in alkaline eluents and eliminates carbonate selectivity problems.

Two ATC-3 trap columns in series are recommended to remove trace anions from the deionized water source prior to use with the EG40. The hydrogen gas generated by electrolysis must be removed to avoid interference with PAD detection. A vacuum degas kit is recommend for this application. The following chromatogram shows four consecutive injections at 18 mM hydroxide, each followed by a quick hydroxide rinse at 80 mM hydroxide to remove late eluting components of protein hydrolysates. The total run time is approximately 30 minutes. An AminoTrap column is used in line to remove basic amino acids and peptides produced from the protein hydrolysis.

Sample Volume:	10 µL
Analytical Column:	CarboPac PA10 (4 x 250 mm) and AminoTrap
Eluent A:	18 megohm deionized, degassed water
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

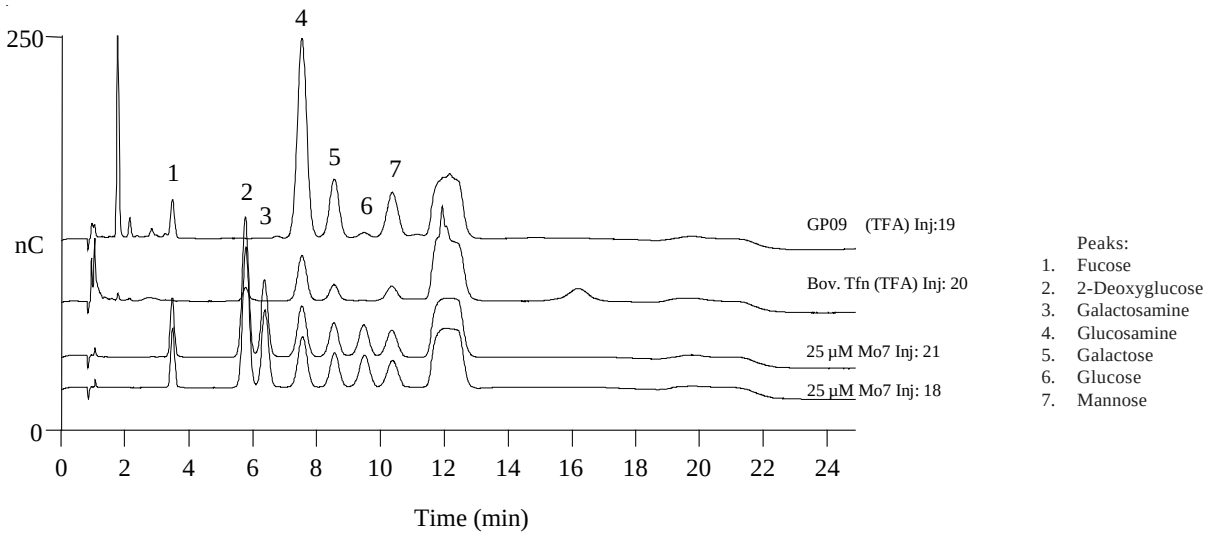


Figure 24
Hydrolysis Product Elution with Quick KOH Step

The following table shows the retention time stability over numerous injections of standards and hydrolysates without any regeneration of the column. The retention time Relative Standard Deviations for these monosaccharides are less than 0.5%.

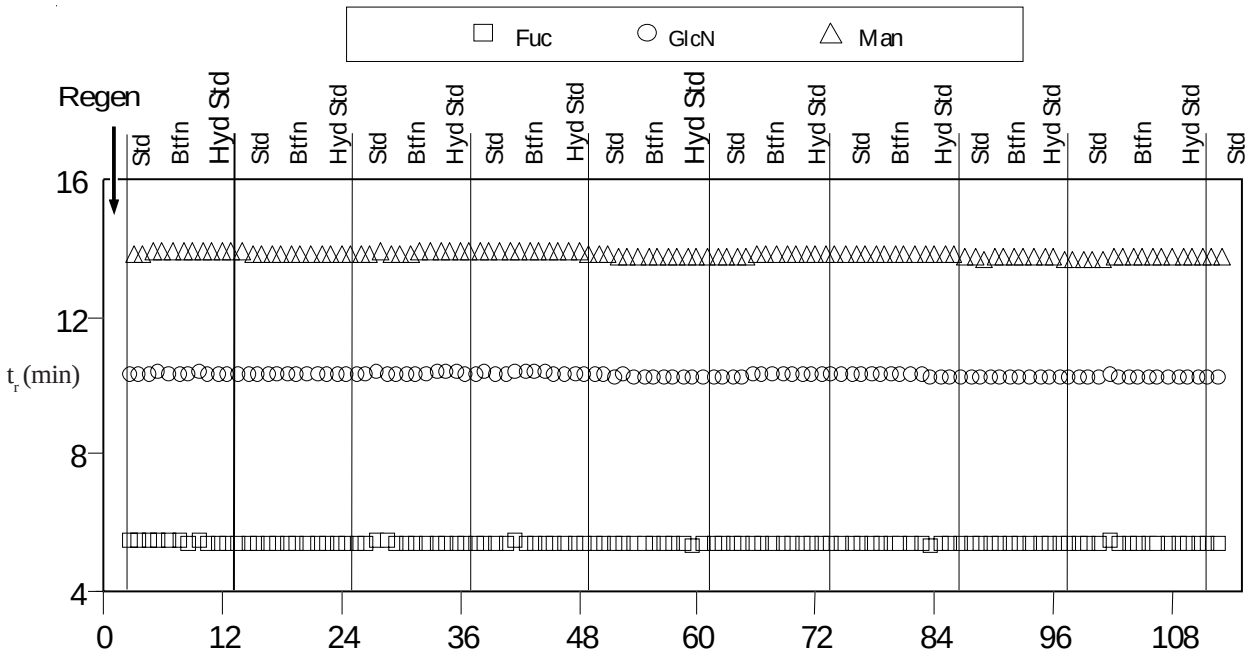


Figure 25
Monosaccharide Retention Time Stability:
Isocratic On-line Eluent Generation (30 min/sample)

4.6 CarboPac PA100 Example Chromatograms

The following section provides an example of the types of applications for which the CarboPac PA100 is designed. There are several factors affecting the elution of oligosaccharides using the CarboPac PA100 column: (a) fucosylated oligosaccharides elute earlier than their afucosylated analogs, (b) as the number of mannose residues in a high mannose oligosaccharide increases, its retention time also increases, (c) as the degree of branching increases, the retention time of the oligosaccharide increases, and (d) removal of the terminal galactose residues from a complex oligosaccharide reduces its retention time.

Separations of oligosaccharides are based on their fine structural differences such as the composition and the sequence of the oligosaccharides, linkage isomerism, degree of sialylation, and degree of branching.

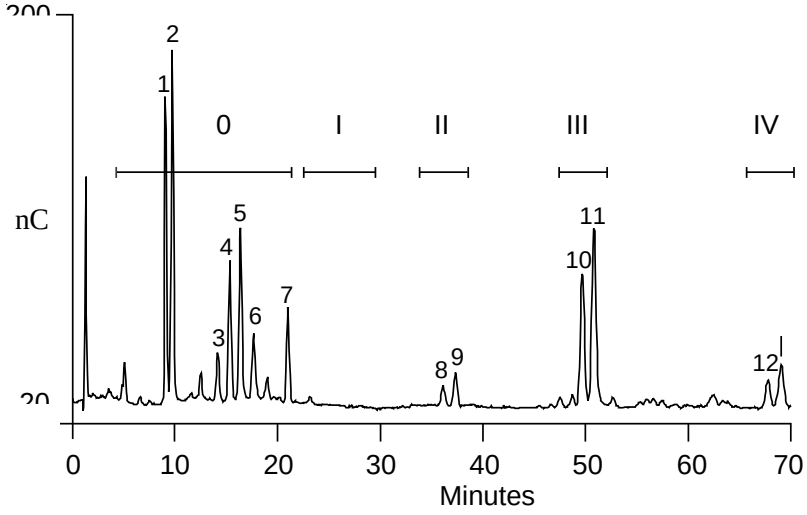
4.6.1 Separation of Neutral and Sialylated Oligosaccharides

The elution of acidic sugars from the CarboPac PA100 column requires stronger eluents than those used for the elution of neutral sugars. This is usually accomplished by the addition of sodium acetate to the sodium hydroxide eluent. Sodium acetate accelerates the elution of strongly bound species without compromising selectivity or interfering with pulsed amperometric detection.

This chromatogram shows the separation of neutral and sialylated oligosaccharides in a single run, using a sodium acetate gradient in 100 mM NaOH. The neutral sugars are eluted in a group at the beginning of the profile, followed by the disialylated, the trisialylated, and finally the tetrasialylated oligosaccharides.

Sample Volume:	10 µL
Analytical Column:	CarboPac PA100 (4 x 250 mm)
Eluent A:	500 mM NaOAc in 100 mM NaOH
Eluent B:	100 mM NaOH
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Time (min)	% A	% B	Comments
0.00	0	100	Starting conditions, inject, start NaOAc gradient
110	50	50	NaOAc gradient ends
111	0	100	Back to initial conditions



Peaks:

1.	Fucosylated Man3GlcNAc2
2.	Man3GlcNAc2
3.	Asialo, agalacto bi, core fuc
4.	Asialo, agalacto bi
5.	Asialo bi, core fuc
6.	Asialo bi
7.	Man9GlcNAc2
8, 9	Disialylated tri (reduced)
10, 11	Trisialylated tri (reduced)
12, 13	Tetrasialylated tri (reduced)

Figure 26
Separation of Neutral and Sialylated Oligosaccharide Standards

4.6.2 Separation of Fetuin N-linked Oligosaccharide Alditols

The separation of bovine fetuin oligosaccharides by HPAE-PAD is shown below. The structures for Peaks 1-6 are shown in Figure 28 below. Peak 7 is a trisialylated triantennary complex oligosaccharide. Under alkaline conditions, the technique resolves these species not only by sialic acid content, but also according to the combination of α (2,3)- and α (2,6)-linked sialic acids within each charge class. Oligosaccharides with the greatest proportion of α (2,6)- to α (2,3)-linked sialic acids are the least-retained. The neutral component of the oligosaccharides also influence separation. Of the oligosaccharides studied, those containing a Galβ(1,3)GlcNAc sequence are retained more strongly that those with Galβ(1,4)GlcNAc.

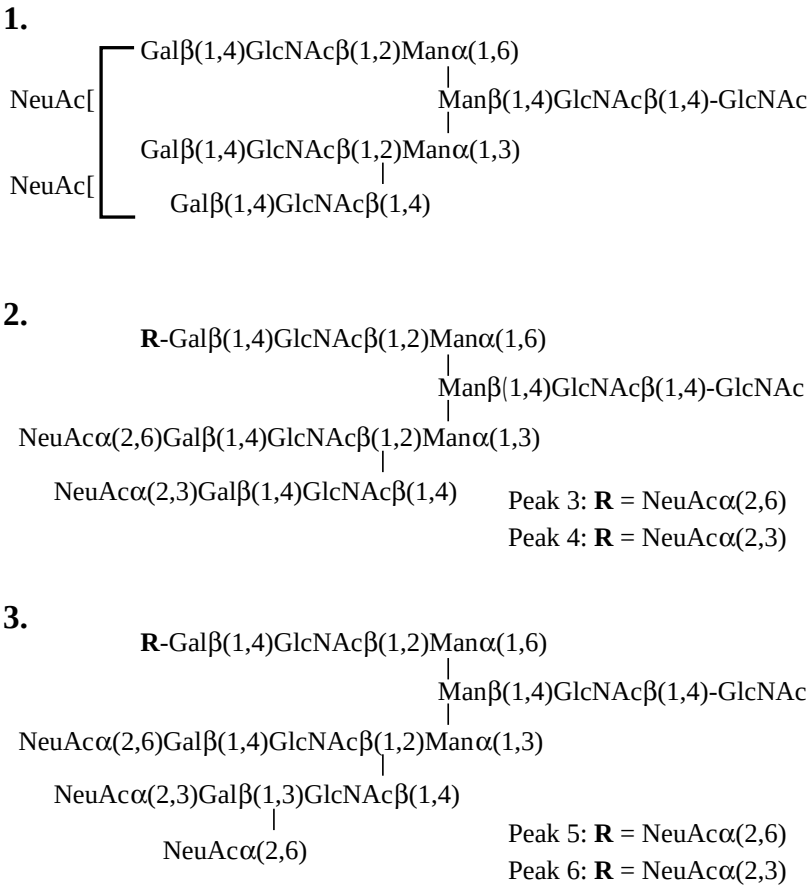


Figure 27
Major Carbohydrate Structures of Bovine Fetuin

Sample Volume:	10 µL
Analytical Column:	CarboPac PA100 (4 x 250 mm) and Guard
Eluent A:	100 mM NaOH
Eluent B:	500 mM NaOAc in 100 mM NaOH
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

Gradient Program

Time (min)	%A	%B	Comments
Initial	99	1	Initial conditions
0.0	99	1	Isocratic at 5 mM NaOAc
0.2	99	1	Start NaOAc gradient
10.0	90	10	Change slope of NaOAc gradient
50.0	55	45	End NaOAc gradient. Start column wash
50.1	0	100	Column wash
55.0	0	100	End column wash. Back to initial conditions
55.1	99	1	Start re-equilibration under initial conditons
70.0	99	1	End re-equilibration

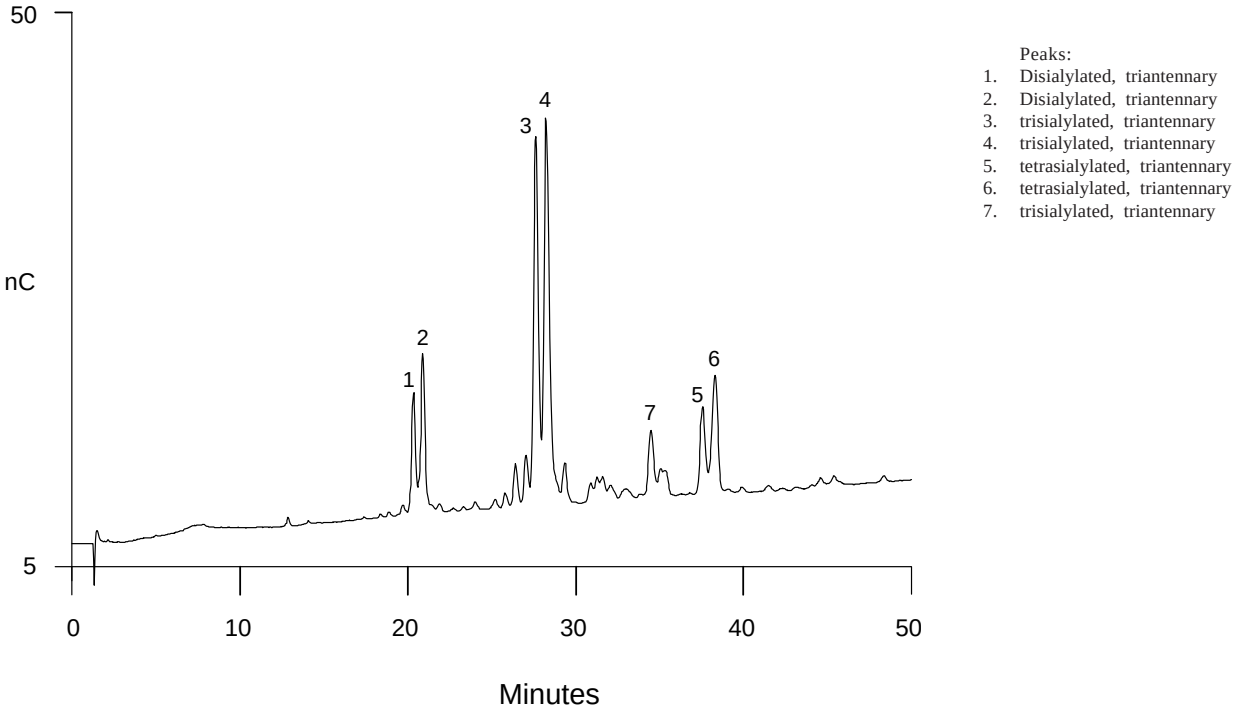


Figure 28
Separation of Fetuin *N*-linked Oligosaccharide Alditols

4.6.3 Oligosaccharide Profiles of Pure and Adulterated Orange Juice

Fruit juice adulteration is an economic and regulatory problem and a matter of worldwide concern. Fruit juices are targets for adulteration because of their high cost relative to sweeteners and other ingredients. The most common forms of adulteration are dilution and blending of inexpensive and synthetically produced juices into expensive ones, and addition of pulp wash. A common adulterant used to mask the effects of dilution and addition of other adulterants is beet medium invert sugar (BMIS). BMIS is partially inverted sucrose with a glucose:fructose:sucrose ratio of 1:1:2 which closely matches the ratio found in orange juice, and it is difficult to detect.

The basis for this method is that invert syrups contain traces of oligosaccharides not generally found in natural juices, so the selectivity of anion exchange chromatography for oligosaccharides, and the sensitivity and specificity of PAD is uniquely suited to this analysis. The major peaks at the start of the chromatogram are glucose, fructose, and sucrose which are natural components of fruit juices. The oligosaccharide 'fingerprint' region between 15 and 35 minutes shows significant differences between the pure and adulterated juice.

Sample Volume:	25 µL of 2 mg/mL
Analytical Column:	CarboPac PA100 (4 x 250 mm)
Eluent A:	NaOAc gradient in 100 mM NaOH
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold

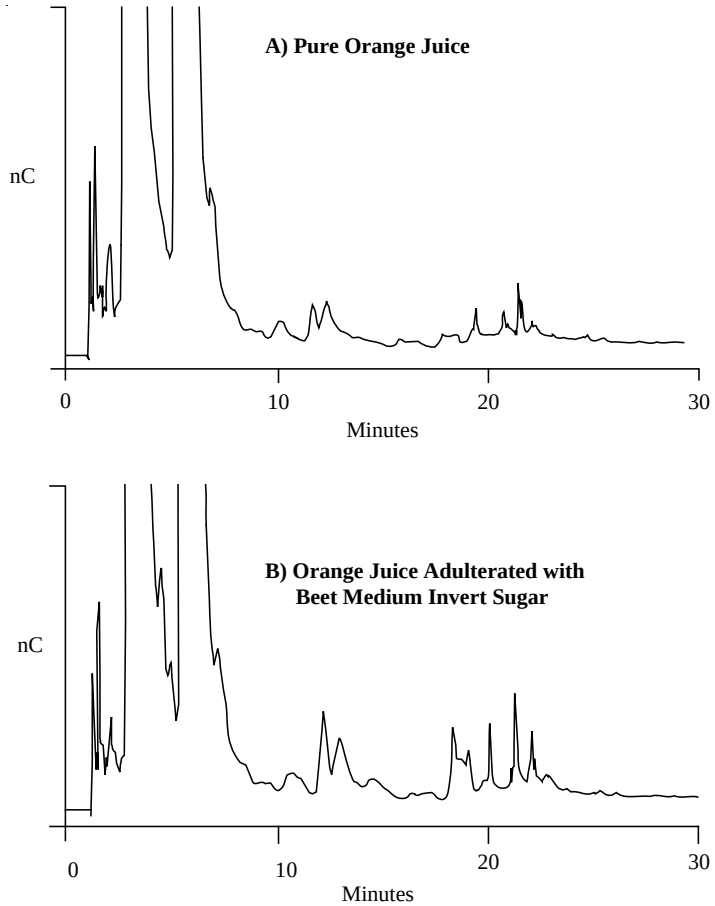


Figure 29
Oligosaccharide Profiles of Pure and Adulterated Orange Juice

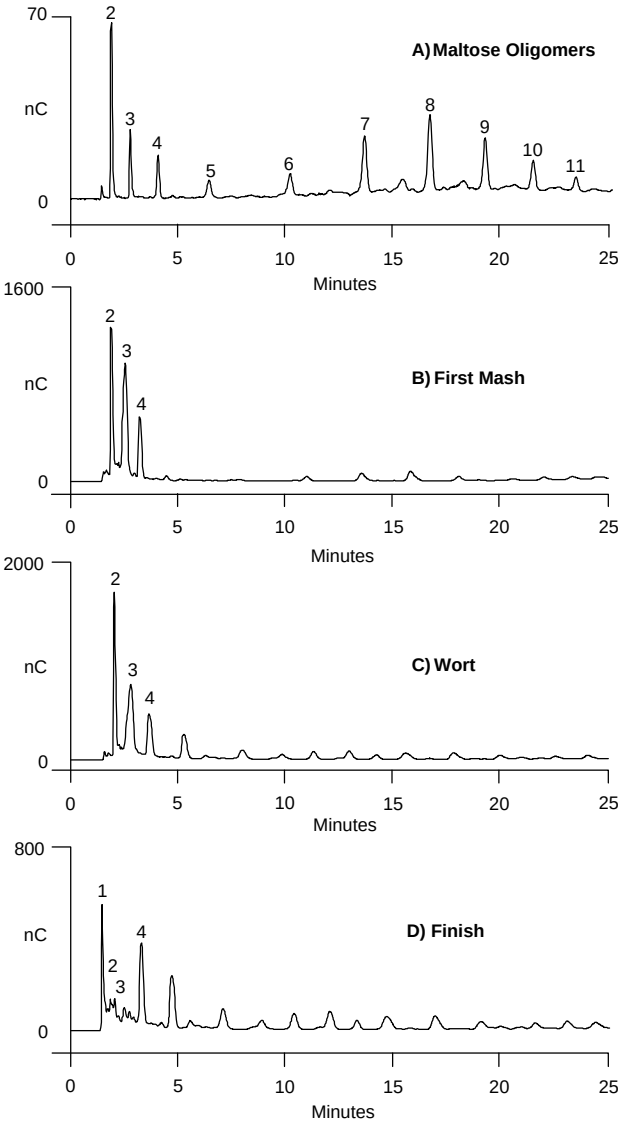
4.6.4 Oligosaccharide Profiling During Beer Production

Determining the levels of fermentable and non-fermentable sugars at every stage of beer production is important because fermentable sugars determine the final alcohol content, and non-fermentable sugars contribute to the flavor and 'body' of the final product. Sugars, sugar alcohols, alcohols, and glycols can be rapidly determined with high resolution at all phases of beer, wine or cider production.

A separation of maltose oligomers up to DP10 (degree of polymerization) with baseline resolution is shown in Panel A. Sugar and oligosaccharide profiles at various stages of the brewing processare shown in Panels B, C and D. All samples were diluted 1:10.

General Operating Conditions

Sample Volume:	10 µL
Analytical Column:	CarboPac PA100 (4 x 250 mm) and guard
Eluent A:	100 mM NaOH
Eluent B:	500 mM NaOAc in 100 mM NaOH
Flow rate:	1.0 mL/min
Detection:	Integrated Amperometry, quadruple waveform
Electrode:	Gold



Peaks:
1. Ethanol
2. Glucose
3. Maltose
4. Maltotriose
5. Maltotetraose
6. Maltopentaose
7. Maltohexaose
8. Maltoheptaose
9. Maltooctaose
10. Maltononaose
11. Maltodecaose

Peaks:
1. Ethanol
2. Glucose
3. Maltose
4. Maltotriose

Peaks:
1. Ethanol
2. Glucose
3. Maltose
4. Maltotriose

Peaks:
1. Ethanol
2. Glucose
3. Maltose
4. Maltotriose

Figure 30
Carbohydrate Profiles at Various Stages of Beer Production

SECTION 5 – TROUBLESHOOTING

5.1 Typical Problems Encountered

Table 14
Typical Problems

Symptom	Possible Causes
The pressure on the column is increasing over time	High system backpressure may be due to the system (e.g. pinched tubing or an obstructed fitting) or the column (e.g. fouled bed support or compressed resin bed)
Resolution between peaks has decreased. Peak efficiency has decreased	The column may be overloaded. Do not inject more than 10 nmol of any one analyte onto the analytical column. Overloading may be due to high salt concentration in the sample which is not detected by PAD but which may overload the column and/or foul the gold working electrode. The eluent may be accumulating carbonate. Prepare fresh eluents. Pump 200 mM NaOH through the system for 10-15 minutes to clean the column. If eluent concentrations are being made by proportioning, try making the eluent up in a single bottle to determine whether the proportioning valve is functioning correctly. If changes have been made to the system, check for excess lengths of tubing, larger than normal tubing diameter, and leaks.
Variations in Peak retention time	The column may not be adequately equilibrated or washed. A consistent and adequate method is important. Column washing may not be necessary between every run, especially with methods that use gradients that exceed 200 mM NaOH (600 mM for CarboPac MA1). If column washing is used every run, ensure that the equilibration time remains constant by making it part of a method. The eluent composition may not be correct. NaOH solutions can deteriorate when exposed to air. Prepare fresh eluents.
Spurious Peaks	There may be a contaminant in the eluent. Prepare fresh eluents. There may be a problem with the detector working or reference electrodes.

5.2 High System Backpressure

5.2.1 Finding the Source of High System Back Pressure

If you notice a significant increase in the system backpressure, it is possible that the inlet frit is plugged.

NOTE

Before replacing the inlet bed support assembly of the column, ensure the column is the cause of the excessive system backpressure.

Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the backpressure at your usual flow rate. It should not exceed 50 psi (0.34 MPa). Continue adding components back into the system, one-at-a-time (injection valve, columns, detector) while watching the system backpressure. Under the conditions of the standard chromatograms, the system backpressure should increase to approximately 1,150 psi with the addition of the CarboPac MA1 and CarboPac PA1 analytical columns. The backpressure should increase to approximately 1,600 psi with the addition of the CarboPac PA10 analytical column. If both the analytical and guard columns are in place, the system backpressure should increase to about 1,500 psi for the CarboPac MA1 and CarboPac PA1 and about 2,000 psi for the CarboPac PA10.

If you find that the increased backpressure is due to system components other than the column, please refer to the appropriate hardware Installation Instructions and Troubleshooting Guide for help.

5.2.2 Replacing the Column Bed Support Assemblies

If the column is determined to be the cause of the high backpressure, the inlet bed support assembly may need to be replaced. To change the inlet bed support assembly, refer to the following instructions using one to the two spare bed support assemblies included in the column box (P/N 042955 for the 4-mm bed support assembly or P/N 044689 for the 2-mm version).

NOTE
Replace the inlet bed support ONLY if the high backpressure persists

1. Disconnect the column from the system.

2. Support the column, inlet end up, in a clamp. Using two open end wrenches (5/16 and 9/16 inch), carefully unscrew the inlet (top) column fitting.

3. Remove the end fitting and tap it against a hard, flat surface to remove the bed support and seal assembly. If it does not come out, apply pressurized air to the inlet of the end fitting to remove the bed support and seal assembly. Discard the old bed support assembly.

4. Place the new bed support assembly into the end fitting. Removal of the bed support may permit a small amount of resin to extrude from the column. Remove this with a flat surface such as a razor blade. Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

5. Screw the end fitting back into the column. Tighten it finger-tight, then an additional ¼ turn (25 in.lb.). Tighten further only if leaks are observed.

6. Reconnect the column to the system and resume operation.

Table 15
CarboPac Ordering Information

	CarboPac MA1 4 mm (P/N)	CarboPac PA1 2 mm (P/N)	CarboPac PA1 4 mm (P/N)	CarboPac PA10 2 mm (P/N)	CarboPac PA10 4 mm (P/N)	CarboPac PA100 2 mm (P/N)	CarboPac PA100 4 mm (P/N)
Analytical Column	044066	057178	035391	057180	046110	057182	043055
Guard Column	044067	057179	043096	057181	046115	057183	043054
Bed Support Assembly	042955	044689	042955	044689	042955	044689	042955
End Fitting	052809	043278	052809	043278	052809	043278	052809

CAUTION
If the end of the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column, but instead damage the column tube or break the end fitting.

NOTE
Replace the outlet support ONLY if the high pressure persists after replacement of the inlet fitting.

5.2.3 Column Cleaning to Remove Contamination

The CarboPac columns may be readily cleaned by rinsing with several column volumes of strong eluent. For the CarboPac MA1, rinse with approximately 10 column volumes of 0.2 -1.0 M NaOH. For the CarboPac PA1, CarboPac PA10 and CarboPac PA100, rinse with approximately 10 column volumes of 0.2 -1.0 M NaOH and/or strong sodium acetate (e.g.1 M of each). More stubborn contamination may require thorough cleaning of the column. Depending upon your column, use the appropriate procedure below.

NOTE

Only perform the procedures if washing with strong eluent does not restore the column to its working condition.

CarboPac MA1

a. Disconnect the amperometry cell lines from the system.

b. Wash the column extensively (50 column volumes) with water. At 0.4 mL/min, 50 column volumes will require about 3.5 hours.

c. Clean the column with 50 mL of a 1.0 M solution of acetic acid.

d. Wash the column with 50 mL of water.

e. Clean the column with 50 mL of a 1.0 M solution of sodium acetate.

f. Wash the column with 50 mL of water.

g. Clean the column with 50 mL of sodium hydroxide.

h. Equilibrate the column under the desired initial conditions. Test the column with standards (test chromatogram) to ensure column performance has been restored.

CarboPac PA1, CarboPac PA10, CarboPac PA100

a. Disconnect the amperometry cell lines from the system.

b. Clean the column with 60 mL of 1.0 M HCl or methanesulfonic acid.

c. Reconnect the cell and clean the column for 1.5 hours with 200 mM NaOH.

d. Equilibrate the column under the desired initial conditions. Test the column with standards (test chromatogram) to ensure column performance has been restored.

5.3 Poor Peak Resolution

Poor peak resolution may be due to any one or combination of the following problems.

5.3.1 Excessive System Void Volume

Ensure your chromatographic system does not have excessive lengths of tubing to interconnect the pump, injector, column(s) and/or detector. Also, check for and eliminate leaks. When 2-mm columns are used, replace all the 0.010" or larger ID tubing with red 0.005" ID tubing (P/N 052310).

5.3.2 Column Contamination

See Section 5.2.3.

5.3.3 Column Headspace

Operation of the column at flow rates which generate excessive backpressures, or backflushing the column, may introduce voids or channels in the packed bed. These will result in a loss of efficiency. Remove the column inlet fitting (see Section 5.2.2 “Replacing the Column Bed Assemblies”). If the packing material is not flush with the column tube, the resin bed has collapsed and the column will have to be replaced.

5.3.4 Column Overloading

Injecting more than 10 nmol of each analyte could cause poor peak resolution. Injecting less sample may produce a more acceptable chromatogram. The cause of the overloading may be due to conditions other than simple sample concentration. High salt concentrations may cause a sample to overload the column, even though only a very small amount of the analyte of interest (e.g. an oligosaccharide) is present in the sample. The capacity of a 2-mm ID column is 25% of a 4-mm ID column.

5.3.5 Incorrect Choice of Elution Conditions

In gradient analyses, the gradient may be too steep. In high pH anion exchange applications, the NaOH concentration may be too low for good adsorption of the analytes. NaOH concentrations that are too high can also cause problems. Na^+ can form bridges between vicinal oxyanions in solution and interfere with chromatography.

5.4 Variations in Peak Retention Time

Retention time variations may be due to one or more of the following problems.

5.4.1 Incorrect Eluent Concentration

Check for mistakes when preparing eluents. In addition, some eluents such as dilute NaOH solutions can deteriorate, either due to exposure to air or via microbial contamination. When in doubt, prepare fresh eluents. If helium blanketing of the eluents is omitted, atmospheric gases dissolve into the eluents. Dissolved oxygen can alter the baseline offset of the detector, and carbon dioxide, when dissolved in basic eluents, becomes carbonate, a divalent anion. This anion will reduce both retention and resolution when present in HPAE-PAD eluents.

5.4.2 Change in Flow Rate

If you are using pressurized eluents, check that the source of the pressurized gas is not empty or blocked. This will cause eluent starvation (cavitation) of the pump. Check the calibration of the pump to ensure that it has not changed.

5.4.3 Column Contamination

See Section 5.2.3.

5.5 Spurious Peaks

Spurious peaks may arise from one or more of the following problems.

5.5.1 Eluent Contamination

This is a serious problem in adsorption chromatography. For example, contaminants in water may adsorb to the column at initial conditions but be eluted during an analytical gradient. In this case, the longer you wait between runs, the larger the ghost peaks will appear. Always use high quality reagents and water. Filter solutions made from solid reagents. Water from HPLC-grade water systems may cause extraneous peaks and baseline drift, often due to microbial contamination in the water system. The outlet tubing is often the source of this contamination. Be extremely careful with water quality. Work at high sensitivity may benefit from recrystallized salts (e.g. sodium acetate).

5.5.2 Detector Problems

Pulsed amperometry is a very sensitive, electrochemical detection method. The working and reference electrodes should be carefully maintained. In particular, reference electrode problems can produce unusual baseline drifting and even apparent peaks. A defective reference electrode can cause a shift in potentials, thus affecting peak detection as well as baseline stability. However, a contaminated eluent could also result in a shift in potential, so remake the eluent as a first step. If this does not help, it may be necessary to replace the reference electrode.

The ED50 and ED gold electrodes should be flush with the surrounding plastic block. Its color should be slightly dull gold. Brown or black deposits on the electrode suggest severe fouling. See Document No. 031154, “Polishing Amperometry Cell Gold Working Electrodes,” on the Dionex Reference Library CD-ROM for the cleaning procedure.

The disposable gold electrode is the latest innovation in electrochemical detection, providing a new level of reproducibility and ease of use. They can be replaced more often without electrode reconditioning by polishing or other methods since they are inexpensive. (see Document No. 065040 "Product Manual for Disposable Electrodes")

SECTION 6 - INSTALLING A REFERENCE ELECTRODE IN ED50 AMPEROMETRY CELL

Users of ED (ICS-3000) please disregard this section.

Since April 2002, the amperometry cell for Dionex ED40, ED50, and ED50A detectors has been redesigned to facilitate installation of the reference electrode. Two new parts are included with the cell.

- A CHEMRAZ® O-ring (P/N 048410) (included in a bag labeled CHEMRAZ)
- An O-ring retainer (P/N 057192) (shipped in the reference electrode cavity)

IMPORTANT

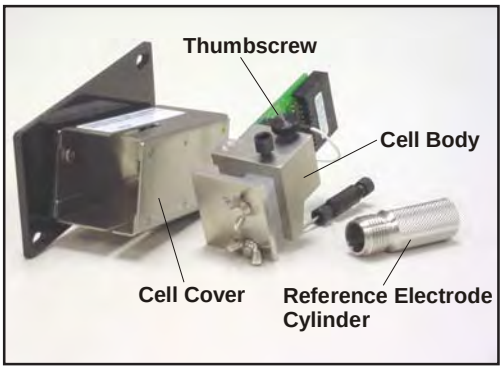
These instructions replace the reference electrode installation instructions in your detector operator's manual.

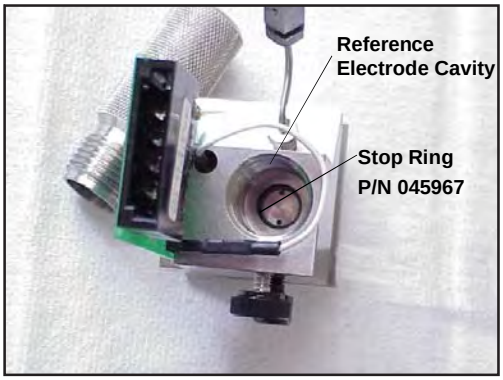
Equipment

To complete this installation procedure, you will need:

- A pair of tweezers
- Deionized water

Installation Procedure





Step 1

Remove the cell from the box. Loosen the cell cover thumbscrew and remove the cover. Unscrew the reference electrode cylinder and remove it from the cell body. NOTE: The photo below shows the mounting plate for an AS50 autosampler compartment. Mounting hardware varies, depending on where the cell is installed.

Step 2

Remove the O-ring retainer from the reference electrode cavity. Verify that the stop ring is at the bottom of the reference electrode cavity. If you used the cell previously, rinse and dry the cavity to remove any particulate matter such as salt crystals, etc. Make sure that the inlet and outlet are open to avoid any hydraulic pressure buildup when inserting the reference electrode.

Step 3

Remove the reference electrode from its box. Remove the electrode from the storage bottle by partially unscrewing the bottle cap and pulling the electrode out of the opening in the cap. Rinse the electrode thoroughly in deionized water to remove any precipitated salt.

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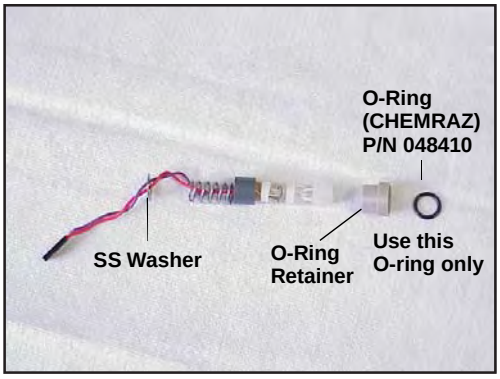
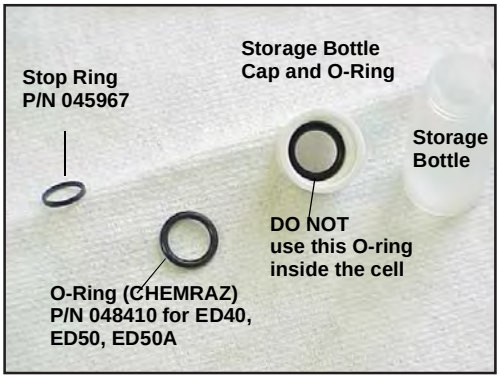


Step 4

Verify that you have all of the necessary parts.

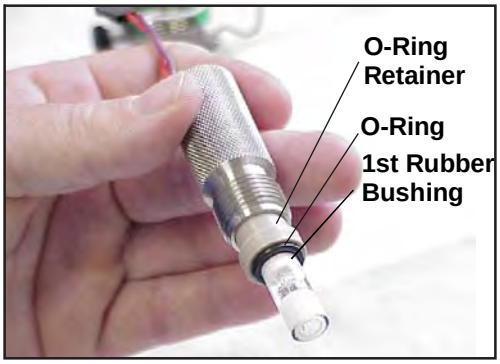


NOTE

The O-ring inside the storage bottle cap and the CHEMRAZ O-ring are made from different materials. To prevent leaks, use only the CHEMRAZ O-ring.

Step 5

Pull the J2 connector through the opening in the electrode cylinder.



Step 6

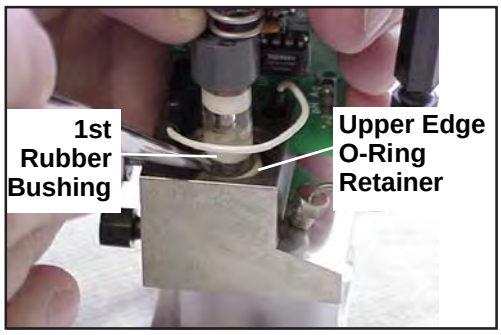
Slide the PEEK O-ring retainer and CHEMRAZ O-ring above the first rubber bushing.

IMPORTANT

While installing the electrode, maintain all parts in a vertical orientation, with the bottom of the electrode pointing down. This avoids bubble formation and helps ensure correct installation of the electrode.

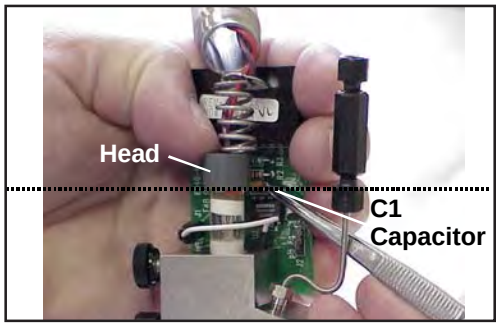
Step 7

Carefully insert the reference electrode into the reference electrode cavity until it touches the stop ring. The first rubber bushing will be visible above the cell body.



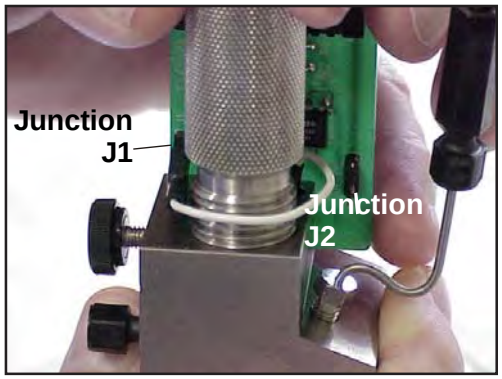
Step 8

Using tweezers, slide the O-ring retainer and O-ring all the way down until there is about 2 to 3 mm between the upper edge of the O-ring retainer and the bushing.



Step 9

When the reference electrode is in the correct position, the lower edge of the head is roughly at the same height as the C1 capacitor on the electronics card.



Step 10

While still keeping all parts in a vertical orientation, screw the electrode cylinder into the cell body and finger tighten.

Step 11

Connect the J2 connector to junction J2 on the electronics card and verify that the white working electrode lead wire is connected to junction J1.

Step 12

Slide the cell cover back over the cell body, making sure that the cable connector on the end of the electronics card lines up with the opening in the cell cover. Tighten the thumbscrew.

SECTION 7 – DIONEX LITERATURE

The following literature is available on the DIONEX Reference Library CD-ROM, or contact your local DIONEX representative.

Technical Note 20	"Analysis of Carbohydrates by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection"
Technical Note 21	"Optimal settings for Pulsed Amperometric Detection of Carbohydrates using DIONEX Pulsed Electrochemical and Amperometric Detectors"
Technical Note 30	"Monosaccharide and Oligosaccharide Anlaysis of Glycoproteins Electrotransferred onto Polyvinylidene Fluoride (PVDF) Membranes"
Technical Note 36	"Analysis of Exoglycosidase Digestions of N-Linked Oligosaccharides using HPAE-PAD"
Technical Note 40	"Glycoprotein Monosaccharide Analysis using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) and Eluent Generation"
Technical Note 41	"Analysis of Sialic Acids Using high Performance Anion Exchange Chromatography"
Technical Note 42	"Glycoprotein Oligosaccharide Analysis using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection"
Technical Note 53	"Determination of Glycoprotein Monosaccharide Composition by HPAE-PAD using On-line Electrolytically Generated Eluents
Technical Note 71	"Eluent Preparation for High-Performance-Anion-Exchange Chromotography with Pulsed Amperometric Detection
Application Note 46	"Ion Chromatography – A Versatile Technique for the Analysis of Beer"
Application Note 65	"Analysis of Inositol Phosphates"
Application Note 66	"Determination of Neomycin B and Impurities using HPAE-PAD"
Application Note 67	"Determination of Plant-Derived Neutral Oligo - and Polysaccharides"
Application Note 82	"Analysis of Fruit Juice Adulterated with Medium Invert Sugar from Beets"
Application Note 83	"Size Exclusion Chromatography of Polysaccharides with Pulsed Amperometric Detection"
Application Note 87	"Determination of Sugar Alcohols in Confections and Fruit Juices by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection"
Application Note 92	"The Determination of Sugars in Molasses by High Performance Anion Exchange with Pulsed Amperometric Detection"
Application Note 105	"Glycosylation Analysis of Human Serum Transferrin Glycoforms using Pellicular Anion Exchange Chromatography"
Application Note 117	"Quantification of Carbohydrates and Glycols in Pharmaceuticals"
Application Note 122	"The Determination of Carbohydrates, Alcohols and Glycols in Fermentation Broths"
Application Note 141	"Improved Long-Term Stability of N-Acetylneuramaic Acid and N-Glycolyl-Neutaminic Acid Peak Area Responses using Waveform A, a Quadruple Potential Waveform"
Application Note 147	"Determination of Polydextrose in Foods by AOAC Method 2000"
Application Note 150	"Determination of Plant-Derived Neutral Oligo - and Polysaccharides using the CarboPac PA 200"
Application Note 151	"Determination of Sucralose in Reduced-Carbohydrate Colas using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection"
Application Note 155	"Determination of Trans-Galactooligosaccharides in Foods by AOAC Method 2001.02"
Application Note 159	"Determination of Sucralose using HPAE-PAD"
Application Note 186	"Analysis of Paromomycin by HPAE-IPAD"

SECTION 8 – SELECTED BIBLIOGRAPHY

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SECTION 9 – APPENDIX

9.1 k′ Table for the CarboPac MA1 Analytical Column

Table 16

k′ Values of Selected Analytes

Eluent Concentration (M NaOH)

Analyte	**0.05**	**0.14**	**0.25**	**0.38**	**0.50**
Glycerol	1.13	0.99	0.89	0.80	0.72
myo-Inositol	1.32	1.08	0.86	0.69	0.56
scyllo-Inositol	1.63	1.30	1.02	0.81	0.64
GlcNol	1.81	1.40	1.09	0.89	0.75
Fucitol	1.94	1.63	1.40	1..18	1.05
Erythritol	2.02	1.71	1.44	1.25	1.13
GalNol	2.29	1.81	1.39	1.13	0.95
GalNAcol	2.35	1.81	1.38	1.12	0.95
GlcNAcol	2.61	1.96	1.48	1.18	0.95
Xylitol	3.09	2.48	1.95	1.59	1.35
Arabitol	4.69	3.62	2.76	2.24	1.92
Sorbitol	6.43	4.72	3.33	2.55	2.06
Dulcitol	6.52	5.04	3.73	2.87	2.26
Adonitol	7.09	5.31	3.83	2.96	2.43
Mannitol	8.98	6.38	4.37	3.28	2.63
Fucose	10.34	4.72	2.52	1.69	1.25
Isomaltose	12.22	8.15	4.89	3.30	2.43
Lactitol	14.97	9.61	5.49	3.57	2.43
gp-Mannitol	15.66	10.36	6.18	4.15	3.05
GalN	18.56	7.16	3.39	2.13	1.48
GlcN	20.88	7.71	3.61	2.24	1.55
Maltitol	31.21	17.25	8.80	5.44	3.67
Glucose	>32	15.70	7.19	4.31	2.91
Mannose	>32	13.55	6.15	3.72	2.53
Galactose	>32	17.82	8.25	4.99	3.43

9.2 PED-2 and PAD-2 Configurations

Table 17 “Hardware Configurations”, lists different detector configurations for the older Dionex detectors. The Basic Settings are in widespread use. The Basic settings may cause baseline instability due to detector sensitivity to dissolved oxygen in the eluent. The Advanced Settings should minimize baseline drift and sensitivity to dissolved oxygen. The body of the table contains the section numbers where the pulse conditions are described.

Table 17
Hardware Configurations

Detector	PED-2		PAD-2	
Cell	pH-Ag/AgCl Reference Electrode	Ag/AgCl	Standard	Solvent Compatible
Basic Settings	8.3.1	8.3.3	3.4.1	3.4.1
Advanced Settings	8.3.2	8.3.4	8.4.2	8.4.3

9.3 PED-2 Settings

9.3.1 PED-2 with the pH Half of the Combination pH-Ag/AgCl Reference Electrode: Basic Settings

If the cell response using these settings is unstable, or there is evidence of baseline drift, try the advanced settings in section 8.3.2, “PED-2 with the pH Half of the Combination pH Ag/AgCl Reference Electrode: Advanced Settings.”

Table 18
Basic PED-2 Settings

Time (sec)	Potential (V)	Integrate
t_1 0.00	+0.35	
0.28	+0.35	Begin
0.48	+0.35	End
t_2 0.49	+0.95	
0.61	+0.95	
t_3 0.62	-0.25	
0.69	-0.25	

DIP switch setting: Switch S4, only Position 2 on

These settings may not be the same as stored in Program 2 in the PED-2. You may need to edit this program before use. Refer to the PED-2 Operator’s Manual *(Document No. 034730)* on the Dionex Reference Library CD-ROM.

9.3.2 PED-2 with the pH Half of the Combination pH-Ag/AgCl Reference Electrode: Advanced Settings

The following settings are recommended for monosaccharide methods as an alternative to the Basic Settings in Section 9.3.1, "PED-2 with the pH half of the combination pH-Ag/AgCl reference electrode: Basic Settings". These settings should minimize baseline drift and sensitivity to dissolved oxygen. These settings also give a small improvement (from approximately 20 to 50%) in sensitivity compared to the basic settings. Response appears to be independent of NaOH concentration above15 mM. The settings are particularly useful when using broad pH gradients.

Table 19
PED-2 Basic Settings

[NaOH]	Time (s)	Potential (V)	Integrate
15-200mM	t_1 0.00	+0.40	
	0.20	+0.40	Begin
	0.40	+0.40	End
	t_2 0.41	+1.00	
	0.60	+1.00	
	t_3 0.61	+0.25	
	1.00	+0.25	

DIP Switch Setting: Switch S4, only Position 2 ON

The pH-Ag/AgCl electrode should not be used at NaOH concentrations above 200 mM.

9.3.3 PED-2 with Ag/AgCl Reference Electrode: Basic Settings

The following settings are in widespread use. These settings may cause baseline instability due to detector sensitivity to dissolved oxygen in the eluent.

If the detector response using these settings is unstable or there is evidence of baseline drift, try the Advanced Settings in Section 9.3.4.

These settings should also be used when using the Ag/AgCl half of the combination pH-Ag/AgCl electrode as the reference (Switch 4, only Position 3 ON). Note these settings are not the same as those stored in Program 1 in the PED-2. You will need to edit Program 1 before use. Please refer to the PED-2 *Operator's Manual* on the Dionex Reference Library CD-ROM.

Table 20
PED-2 with Ag/AgCl Basic Settings

Time (sec)	Potential (V)	Integrate
t_1 0.00	+0.05	
0.28	+0.05	Begin
0.48	+0.05	End
t_2 0.49	+0.60	
0.61	+0.60	
t_3 0.62	-0.60	
0.69	-0.60	

9.3.4 PED-2 with Ag/AgCl Reference Electrode: Advanced Settings

The following settings are recommended for monosaccharide methods as an alternative to the Basic Settings in Section 9.3.3, "PED-2 with Ag/AgCl reference electrode: Basic Settings". These settings should minimize baseline drift and sensitivity to dissolved oxygen. These settings also give a small improvement (from approximately 20 to 50%) in sensitivity compared with the basic settings. Response appears to be independent of NaOH concentration above15 mM. The settings are particularly useful when using broad pH gradients. These settings should also be used when using the Ag/AgCl half of the pH-Ag/AgCl electrode as the reference (Switch S4, only Position 3 ON).

Table 21
PED-2 with Ag/AgCl Advanced Settings

[NaOH]	Time (s)	Potential (V)	Integrate
15-900mM	t_1 0.00	+0.05	
	0.20	+0.05	Begin
	0.40	+0.05	End
	t_2 0.41	+0.75	
	0.60	+0.75	
	t_3 0.61	+0.15	
	1.00	+0.15	

9.4 PAD-2 Settings

9.4.1 PAD-2: Basic Settings

The following settings for the Standard and Solvent compatible cells are in widespread use. These settings may cause baseline instability due to detector sensitivity to dissolved oxygen in the eluent.

Table 22
PAD-2 Basic Settings

	Time- setting	Time (msec)	Potential (E)	Potential (V)
t_1	5	480	E1	+0.05
t_2	2	120	E2	+0.60
t_3	1	60	E3	-0.60

*DIP switch setting: Range 2

If the cell response using these settings is unstable or there is evidence of baseline drift, try the advanced settings in Section 9.4.2, "PAD-2 with the Standard Cell: Advanced Settings" or Section 9.4.3, "PAD-2 with the Solvent Compatible Cell: Advanced Settings".

9.4.2 PAD-2 with the Standard Cell: Advanced Settings

The following settings are recommended for monosaccharide methods as an alternative to the Basic Settings in Section 9.4.1, "PAD-2: Basic Settings". These settings should minimize baseline drift and sensitivity to dissolved oxygen. These settings also give a small improvement (from approximately 20 to 50%) in sensitivity compared with the basic settings. Response appears to be independent of NaOH concentration above 15 mM. The settings are particularly useful when using broad pH gradients.

Table 23
PAD-2 Advanced Settings

[NaOH] (mM)		Time- setting	Time (msec)	Potential (E)	Potential (V)
15-50	t_1	4	420	E1	+0.05
	t_2	3	180	E2	+0.80
	t_3	6	360	E3	-0.15
50-500	t_1	4	420	E1	+0.05
	t_2	3	180	E2	+0.75
	t_3	6	360	E3	-0.20
>500	t_1	4	420	E1	+0.00
	t_2	3	180	E2	+0.70
	t_3	6	360	E3	-0.25

*DIP switch setting: Range 2

When using the standard PAD-2 cell with a broad sodium hydroxide gradient, use a combination of potential settings that cover the range required by the hydroxide concentrations is use. For example, for a 20 to 600 mM sodium hydroxide gradient, use the highest value for E2 (+0.80) and the lowest value for E3 (-0.25). It has been found experimentally that the noise and cell response are the least sensitive to changes in pH when E1 is set at 0.0V when this pulse sequence is used.

9.4.3 PAD-2 with the Solvent Compatible Cell: Advanced Settings

The following settings are recommended for monosaccharide methods as an alternative to the Basic Settings in Section 9.4.1, "PAD-2: Basic Settings". These settings should minimize baseline drift and sensitivity to dissolved oxygen. These settings also give a small improvement (from approximately 20 to 50%) in sensitivity compared with the basic settings. Response appears to be independent of NaOH concentration above15 mM. The settings are particularly useful when using broad pH gradients.

Table 24
PAD-2 with Solvent Compatible Cell Advanced Settings

[NaOH] (mM)		Time- setting	Time (msec)	Potential (E)	Potential (V)
15-900	t_1	4	420	E1	+0.05
	t_2	3	180	E2	+0.75
	t_3	6	360	E3	-0.15

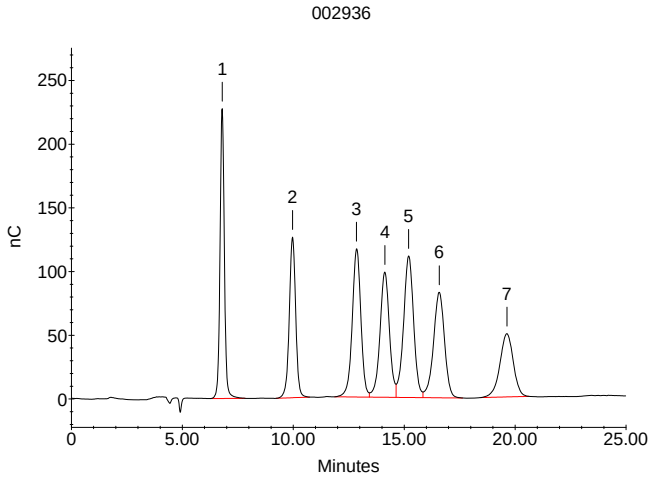
*DIP switch setting: Range 2

APPENDIX A - QUALITY ASSURANCE REPORTS

A.1 CarboPac MA1 (4 x 250 mm)

Product No. 044066

Serial No. : 002936 **Pressure (PSI) : 1290** **Date : 6/14/2005 9:56:21 AM**



Eluent: 612 mM NaOH
Flow Rate: 0.4 mL/min

ED40 Operating Parameters:
ED40 Gold Electrode with Ag/AgCl reference electrode.
Waveform:
Select preloaded waveform
Carbohydrates (Quad. Potential)

#	Time (sec)	Potential (V)	Integration
1	0.00	0.10	
2	0.20	0.10	Begin
3	0.40	0.10	End
4	0.41	-2.00	
5	0.42	-2.00	
6	0.43	0.60	
7	0.44	-0.10	
8	0.50	-0.10	

Injection Volume: 10 µL
Storage Solution: Eluent, 612 mM NaOH

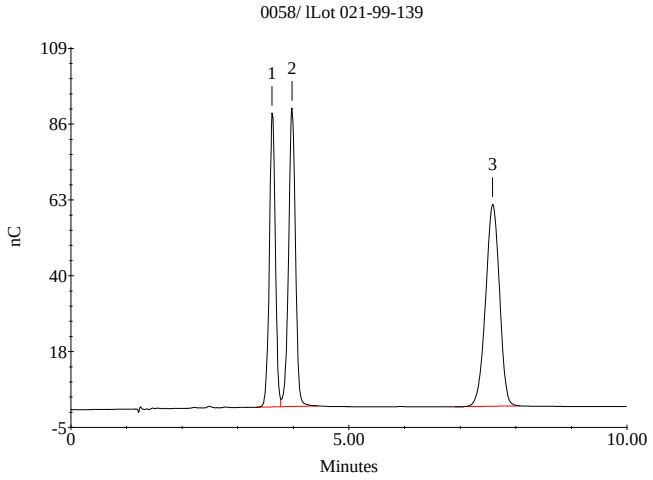
Peak Information : Found Components

Peak No.	Retention Time	Name	(nmoles)	Efficiency	Asymmetry (10%)	Resolution
1	6.80	Myo-Inositol	50.0	5457	1.0	7.01
2	9.97	Xylitol	100.0	5488	1.0	4.64
3	12.85	Sorbitol	100.0	5269	1.0	1.75
4	14.13	Dulcitol	100.0	5465	n/a	1.32
5	15.20	Mannitol	100.0	5120	0.9	1.55
6	16.58	Glucose	100.0	5036	0.9	3.00
7	19.63	Fructose	200.0	5054	0.9	n/a

A.2 CarboPac PA1 (2 x 250 mm)

Product No. 057178

Serial No. : 0058/ lLot 021-99-139 **Pressure (PSI) : 1233**



The monosaccharide lot test analysis is available upon request

Eluent: 200 mM NaOH
Flow Rate: 0.25 mL/min
Diluted Standard (with DI water):
55 nmol/mL Glucose
83 nmol/mL Fructose
146 nmol/mL Sucrose
Injection Volume: 2.5 µL
ED40 Operating Parameters:
ED40 Gold Electrode
with Ag/AgCl reference electrode.
Waveform:
Select preloaded waveform
Carbohydrates (Quad. Potential)

Time	Potential	Integration
0.00	+0.10	
0.20	+0.10	Begin
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	+.60	
0.44	-.10	
0.50	-.10	

Storage Solution: 200 mM NaOH

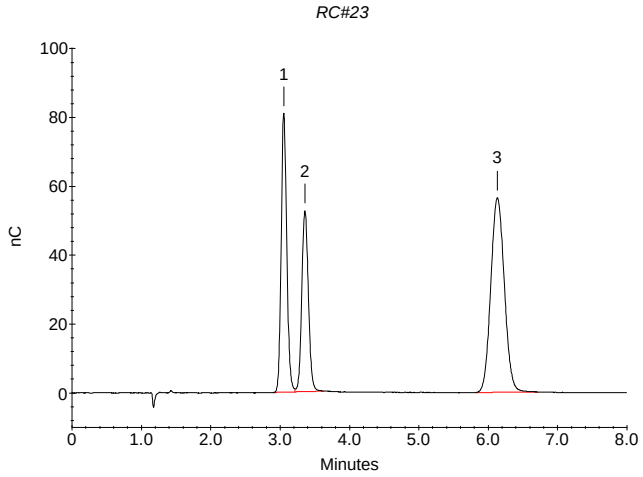
Peak Information : Found Components

Peak No.	Retention Time	Name	(nmol)	Theoretical Plates (USP)	Asymmetry (10%)	Resolution
1	3.62	Glucose	0.14	4822	1.1	1.64
2	3.98	Fructose	0.20	4994	0.9	10.22
3	7.58	Sucrose	0.37	4225	1.0	n/a

A.3 CarboPac PA1 (4 x 250 mm)

Product No. 035391

Serial No. : RC#23 **Pressure (PSI) : 820**



Eluent: 200 mM NaOH
Flow Rate: 1.0 mL/min
Diluted Standard (with DI water):
55 nmol/mL Glucose
83 nmol/mL Fructose
. 146 nmol/mL Sucrose
Injection Volume: 10 µL
ED40 Operating Parameters:
ED40 Gold Electrode
with Ag/AgCl reference electrode.
Waveform:
Select preloaded waveform
Carbohydrates (Quad. Potential)

#	Time (sec)	Potential (V)	Integration
1	0.00	0.10	
2	0.10	0.10	Begin
3	0.20	0.10	End
4	0.21	-2.00	
5	0.22	-2.00	
6	0.23	0.60	
7	0.24	-0.10	
8	0.27	-0.10	

The monosaccharide lot test analysis is available upon request

Storage Solution: 200 mM NaOH

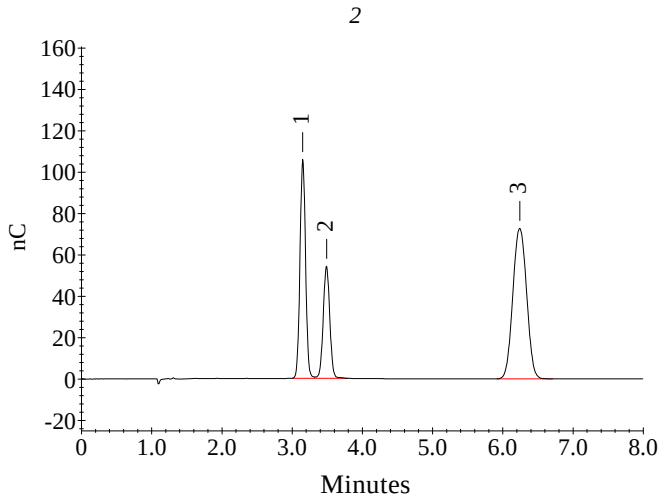
Peak Information : Found Components

Peak No.	Retention Time	Name	(nmoles)	Efficiency	Asymmetry (10%)	Resolution
1	3.05	Glucose	0.55	6367	1.2	1.87
2	3.36	Fructose	0.83	5828	1.1	10.24
3	6.13	Sucrose	1.46	4460	1.1	n/a

A.4 CarboPac PA1 (9 x 250 mm)

Product No. 039686

Serial No. : 2 **Pressure (PSI) : 1410**



Eluent: 200 mM NaOH
Flow Rate: 5.0 mL/min
Diluted Standard (with DI water):
55 nmol/mL Glucose
83 nmol/mL Fructose
146 nmol/mL Sucrose
Injection Volume: 25 µL
ED40 Operating Parameters:
ED40 Gold Electrode
with Ag/AgCl reference electrode.
Waveform:
Select preloaded waveform
Carbohydrates (Quad. Potential)

Time (sec)	Potential (V)	Integration
0.00	0.10	
0.20	0.10	Begin
0.40	0.10	End
0.41	-2.00	
0.42	-2.00	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	

Storage Solution: 200 mM NaOH

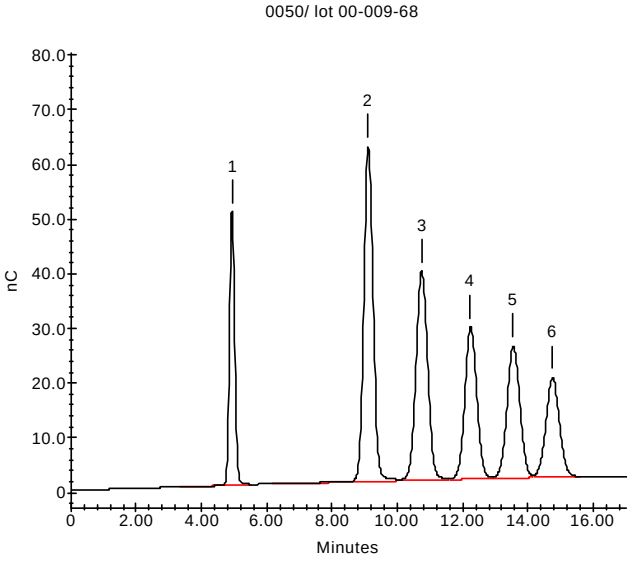
Peak Information : Found Components

Peak No.	Retention Time	Name		Efficiency	Asymmetry (10%)	Resolution
1	3.15	Glucose	1.38	6542	1.0	2.02
2	3.49	Fructose	2.08	5755	0.9	9.73
3	6.24	Sucrose	3.65	4263	1.0	n/a

A.5 CarboPac PA10 (2 x 250 mm)

Product No. 057180

Serial No. : 0050/ lot 00-009-68 **Pressure (PSI) : 1978**



Eluent A: 200 mM NaOH
Eluent B: 18mM NaOH
Flow Rate: 0.25 mL/min
Diluted Standard (with DI water): 50 nmol/mL
Injection Volume: 2.5 µL
Gradient:

Time	%EA	%EB	Comment
0.00	100	0	Regenerate
15.00	100	0	
15.10	0	100	Re-equilibrate
30.00	0	100	
30.10	0	100	Inject
31.00	0	100	

ED40 Operating Parameters:
ED40 Gold Electrode
(Use the pre-loaded waveform With Ag/AgCl reference electrode)

Time (sec)	Potential (V)	Integration
0.00	0.05	
0.20	0.05	Begin
0.40	0.05	End
0.41	0.75	
0.60	0.75	
0.61	-0.15	
1.00	-0.15	

Storage Solution: 18 mM NaOH

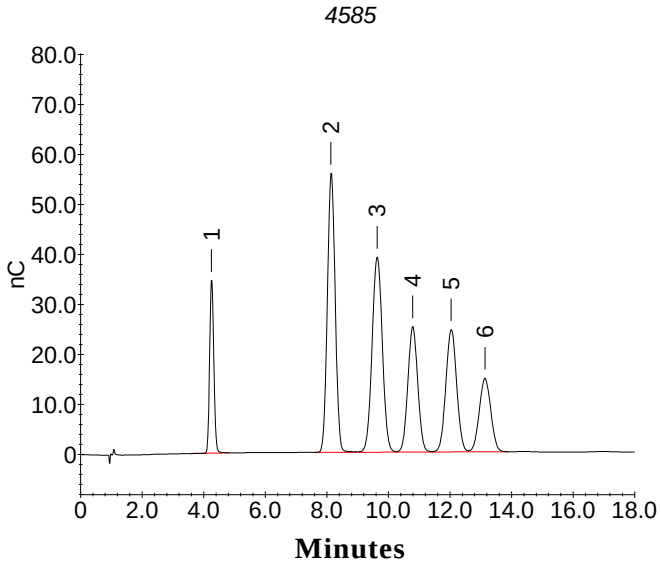
Peak Information : Found Components

Peak No.	Retention Time	Name	(nmoles)	Efficiency	Asymmetry (10%)	Resolution
1	4.93	Fucose	0.125	3371	1.1	9.52
2	9.10	Galactosamine	0.125	4575	1.2	2.69
3	10.73	Glucosamine	0.125	3989	1.1	2.21
4	12.23	Galactose	0.125	5182	1.1	1.86
5	13.55	Glucose	0.125	5417	1.1	1.54
6	14.75	Mannose	0.125	5159	1.1	5.01

A.6 CarboPac PA10 (4 x 250 mm)

Product No. 046110

Serial No. : 4585 **Pressure (PSI) : 2030**



Eluent A: 200 mM NaOH
Eluent B: 18mM NaOH
Flow Rate: 1.0 mL/min.
Diluted Standard (with DI water): 50 nmol/mL
Injection Volume: 10 µL
Gradient:

Time	%EA	%EB	Comment
0.00	100	0	Regenerate
15.00	100	0	
15.10	0	100	Re-equilibrate
30.00	0	100	
30.10	0	100	Inject
31.00	0	100	

ED40 Operating Parameters:
ED40 Gold Electrode
(Use the pre-loaded waveform With Ag/AgCl reference electrode)

Time (sec)	Potential (V)	Integration
0.00	0.05	
0.20	0.05	Begin
0.40	0.05	End
0.41	0.75	
0.60	0.75	
0.61	-0.15	
1.00	-0.15	

Storage Solution: 18 mM NaOH

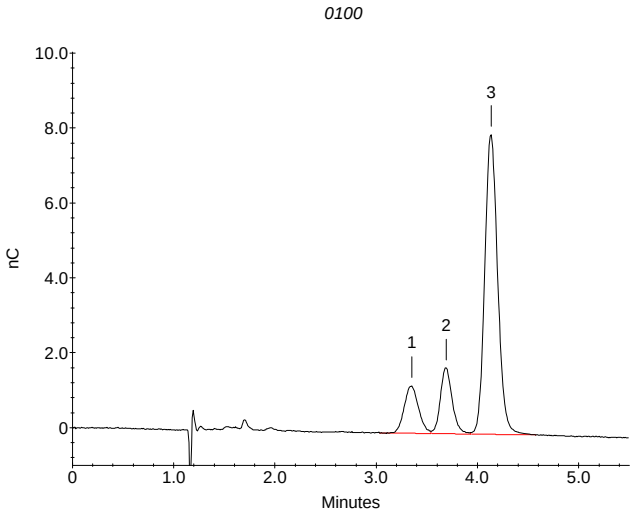
Peak Information : Found Components

Peak No.	Retention Time	Name	(nmoles)	Efficiency	Asymmetry (10%)	Resolution
1	4.25	L-Fucose	0.5	4447	1.3	10.57
2	8.13	D-Galactosamine	0.5	4565	1.1	2.65
3	9.63	D-Glucosamine	0.5	3499	1.1	1.85
4	10.78	D-Galactose	0.5	5259	1.1	2.01
5	12.03	D-Glucose	0.5	5459	1.0	1.62
6	13.13	D-Mannose	0.5	5504	1.0	n/a

A.7 CarboPac PA100 (2 x 250 mm)

Product No. 057182

Serial No. : 0100 **Pressure (PSI) : 2020**



Eluent: 100 mM NaOH + 100 mM CH_3COONa
Flow Rate: 0.25 mL/min
Standard: N-Acetylneuraminyl-lactose
Diluted Standard (with DI water) = 12.0 nmol/mL
Injection Loop: 2.5 µL
(0.118 nmol total per injection of unknown and two isomers)
Detection: ED40 gold electrode
Use preloaded waveform with Ag/AgCl reference electrode

Time (sec)	Potential (v)	Integration
0.0	+0.10	
0.20	+0.10	Begin
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.60	
0.44	-0.10	
0.50	-0.10	

Storage Solution: Eluent

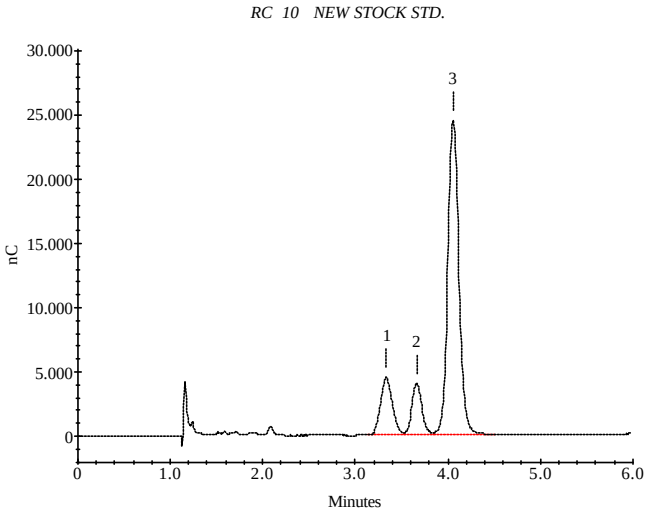
Peak Information : Found Components

Peak No.	Retention Time	Name	Theoretical Plates (USP)	Asymmetry (10%)	Resolution
1	3.35	Unknown	2525	1.1	1.38
2	3.69	Alpha(2,6)NAN-LAC	5024	1.1	1.90
3	4.13	Alpha(2,3)NAN-LAC	5021	1.1	n/a

A.8 CarboPac PA100 (4 x 250 mm)

Product No. 043055

Serial No. : RC 10 NEW STOCK STD. Pressure (PSI) : 2000



Eluent: 100 mM NaOH + 100 mM CH_3COONa
Flow Rate: 1.0 mL/min
Standard: N-Acetylneuraminyl-lactose
Diluted Standard (with DI water): 12.0 nmol/mL
Injection Loop: 10 µL
(0.47 nmol total per injection of unknown and two isomers)
Detection: ED40 gold electrode
Use preloaded waveform with Ag/AgCl reference electrode

Time (sec)	Potential (v)	Integration
0.0	+0.10	
0.20	+0.10	Begin
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.60	
0.44	-0.10	
0.50	-0.10	

Storage Solution: Eluent

Peak Information : Found Components

Peak No.	Retention Time	Name	Theoretical Plates (USP)	Asymmetry (10%)	Resolution
1	3.33	Unknown	3265	1.3	1.47
2	3.67	alpha (2,6) NAN-Lac	5856	1.2	1.73
3	4.06	alpha (2,3) NAN-Lac	5613	1.1	n/a

APPENDIX B - COLUMN CARE

B.1 CarboPac MA1

If the normal wash does not restore the column to normal performance, use the following procedure:

A. Disconnect the amperometry cell tubing from the system.

B. Wash the CarboPac MA1 extensively (50-column volumes) with water. At 0.4 mL/min, 50-column volumes will require approximately 3.5 hours.

C. Clean the column with 50 mL of a 1.0 M solution of acetic acid.

D. Wash the column with 50 mL of water.

E. Clean the column with 50 mL of a 1.0 M solution of sodium acetate.

F. Wash the column with 50 mL of water.

G. Clean the CarboPac MA1 with 50 mL of 1.0 M NaOH.

H. Equilibrate the CarboPac MA1 to the desired initial conditions. Test the column with standards to ensure that good column performance has been restored.

B.2 CarboPac PA 1

The CarboPac PA1 can be readily cleaned by an approximate 10-column-volume rinse with 0.2–1 M NaOH, and/or strong sodium acetate (e.g., 1 M). More stubborn contamination problems may necessitate thoroughly cleaning the column. Use the following steps to thoroughly clean the CarboPac PA1:

A. Disconnect the amperometry cell lines from the system.

B. Wash the CarboPac PA1 with 150 mL water and then clean with 60 mL 1 M HCl or 1 M methanesulfonic acid.

C. Wash the CarboPac PA1 with 150 mL water.

D. Reconnect the cell and clean the CarboPac PA1 with 50 mL 200 mM NaOH.

E. Equilibrate the CarboPac PA1 to the desired initial conditions and test it for performance with the standards provided (e.g. 16 mM NaOH, monostandards).

B.3 CarboPac PA10

The CarboPac PA10 can be readily cleaned by an approximate 10-column-volume rinse with 200–1,000 mM NaOH, and/or strong sodium acetate (e.g., 1 M each). More stubborn contamination problems may necessitate thoroughly cleaning the column. Use the following steps to thoroughly clean the CarboPac PA10:

A. Disconnect the amperometry cell lines from the system.

B. Wash the CarboPac PA10 with 150 mL 20 mM NaOH and then clean with 60 mL 1 M HCl or 1 M methanesulfonic acid.

C. Wash the CarboPac PA10 with 150 mL 20 mM NaOH.

D. Reconnect the cell and clean the CarboPac PA10 with 50 mL 200 mM NaOH.

E. Equilibrate the CarboPac PA10 to the desired initial conditions and test it for performance with the standards provided (e.g., 18 mM NaOH, monostandards).

B.4 CarboPac PA100

The CarboPac PA100 can be readily cleaned using a 10-column-volume elution with either strong NaOH, and/or strong sodium acetate (e.g., up to 1 M each). More stubborn contamination problems may necessitate "recycling" the column. Practically put, this requires the operator to:

A. Wash the column thoroughly with deionized water.

B. Wash the column for 1 hour or more with 1 M HCl solution after disconnecting the detector cell from the column outlet.

C. Wash the column(s) with 20-column volumes of deionized water.

D. Reconnect the cell and wash the column extensively with 200 mM NaOH.

E. The column(s) can now be equilibrated to the desired initial conditions and tested for performance with the standards provided or the benchmark performance test of your choice.