CarboPac PA20 Capillary Columns

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thermo scientific

For Research Use Only. Not for use in diagnostic procedures.

Product Manual

for

CarboPac PA20 Capillary
CarboPac PA20 0.4 x 150 mm (Item # 072072)

CarboPac PA20 Capillary Guard
CarboPac PA20 Guard 0.4 x 35 mm (Item # 072073)

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Revision History:

Revision 03, December 2018, Rebranded for Thermo Fisher Scientific. Added column care section.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument, but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

1.1 Capillary CarboPac PA20

The Capillary CarboPac PA20 0.4 x 150 mm column has been developed for use with the capillary format of the Dionex ICS-6000 system. It gives fast, efficient separations of monosaccharides with good spacing of monosaccharides. The Capillary CarboPac PA20 works best in combination with the eluent generator (EG) and the continuously regenerated anion trap column (CR-ATC). The instructions for assembling the capillary system with capillary CarboPac PA20 column set, EG and CR-ATC can be found in the ICS-6000 System Manual.

The Capillary CarboPac PA20 columns are packed with a hydrophobic, polymeric, pellicular anion exchange resin stable over the range of pH 0-14. This unique pH-stability of the packing material allows the use of eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes.

Resin Characteristics:
Particle Size: 6.0 µm
Pore Size: microporous (<10 Å)
Cross-linking: 55%
Ion exchange capacity: 1.16 µeq per 0.4 x 150 mm column

Latex Characteristics:
Functional Group: difunctional quaternary ammonium ion
Latex Diameter: 130 nm
Latex Cross-linking: 5.2%

Typical Operating Parameters:
pH range: 0-14
Temperature Limit: 4-60°C
Pressure Limit: 3500 psi
Organic Solvent Limit: 100% compatible
Typical eluents: High purity water (18 megohm-cm), potassium hydroxide from EG

1.2 CarboPac PA20 Anion Exchange Column Set

Part Number	Product Description
072072	Capillary CarboPac PA20 Analytical Column, 0.4x 150 mm
072073	Capillary CarboPac PA20 Guard Column, 0.4 x 35-mm
047078	BorateTrap, 4-mm

1.3 Capillary CarboPac PA20 Guard Column

A guard column is usually placed before the analytical column to prevent sample contaminants from eluting onto the analytical column. The addition of the guard to the analytical column also increases the column capacity by about 20%, which translates into an increase of about 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 just an analytical column, the gradient schedules should be increased by about 20%, to ensure similar resolution between the eluting peaks.

1.4 Borate Trap

Borate can affect peak symmetry of some monosaccharides, even when present in a low part-per-billion concentration range. Borate is one of the first ions to break through a water deionization system. Its presence in the water that is used to make up eluents for carbohydrate analysis can cause a significant loss of peak efficiency, especially for mannose and reduced monosaccharides. The BorateTrap (Item # 047078) is used immediately before the injection valve and serves to remove borate from the eluent just before chromatography.

Column: CarboPac™ PA20
Eluent: 10 mM NaOH, 10 ng/mL borate
Flow Rate: 0.008 mL/min
Detector: Pulsed amperometry, gold electrode

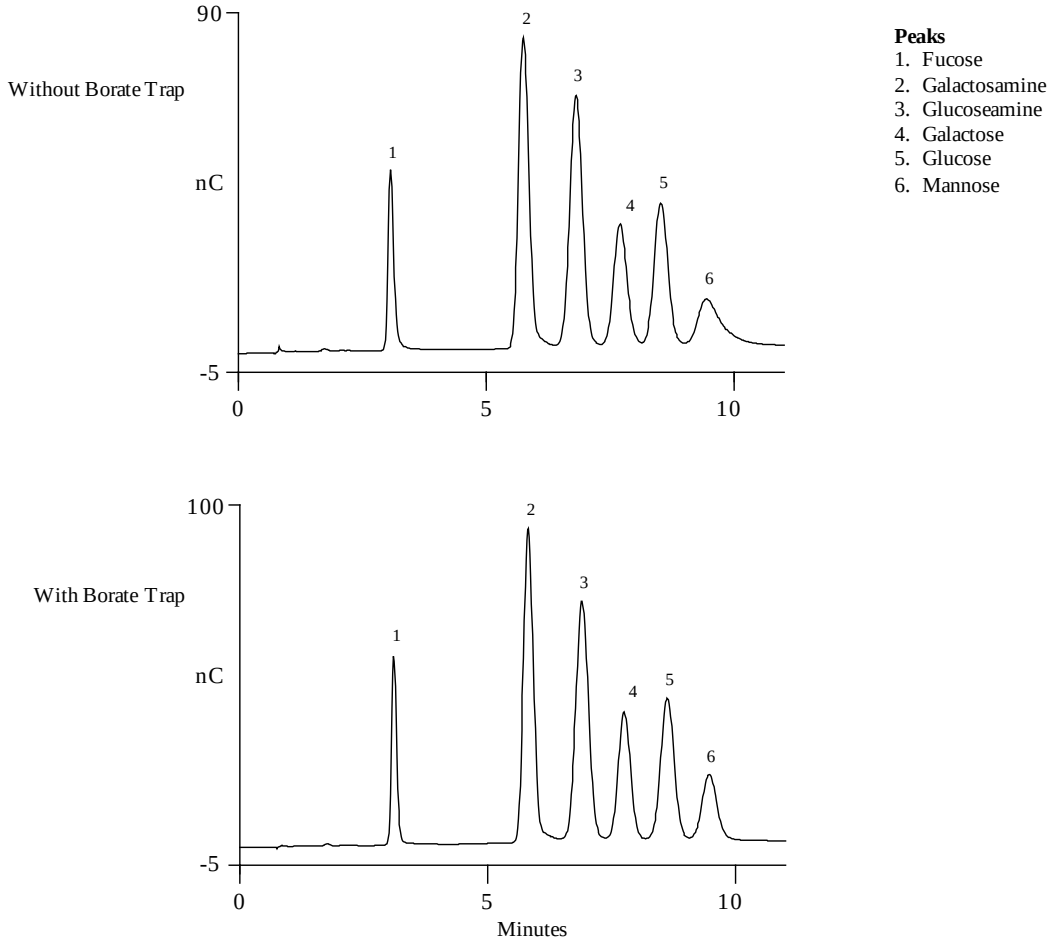


Figure 1
Effect of Borate and the BorateTrap™ on Monosaccharide Peak Symmetry

2. System Requirements

The Dionex Capillary Carbohydrate systems should be configured to comply with the following key requirements:

1. Water for preparing the eluent is kept under helium or nitrogen at all times
2. Use of KOH eluents from a compatible eluent generator
3. Accurate and precise flow rates at 0.001 to 0.1 mL/min
4. pH/Ag/AgCl or PdH reference electrode
5. Programmable pulsed amperometric waveforms with frequencies of 1 Hz or higher
6. Minimized contribution to the background signal by contaminants from the system and reagents
7. Constant temperature control of the guard column, separation column and detection cell.
8. Use of CR ATC and Degasser modules as a part of the DIONEX RFIC system

2.1 Capillary Carbohydrate System (Without Columns)

2.1.1 System Components and Description

The following system components are recommended for carbohydrate analysis.

1. AS autosampler with sample tray cooling
2. DP module (ICS-6000) configured for capillary flow rate range
3. EG module (ICS-6000) with EGC, and CR ATC
4. DC module (ICS-6000) configured with IC-Cube.
5. ICS-6000 IC Cube consisting of Suppressor Bypass, Eluent Degasser, injector with internal sample loop (0.4 µL) injector, column oven
6. Capillary electrochemical cell with a gold electrode, PdH or Ag/AgCl reference electrode
7. EO1 Eluent organizers

2.2 System Requirements

The carbohydrate separations with the Capillary CarboPac PA20 columns are optimized for use with ICS-6000 Capillary HPIC™ System. The key issue is that the pump should be configured for capillary flow rate range. All of the surfaces in contact with eluent and samples are metal-free.

Tubing anywhere between the injection valve and detector should be < 0.003 in I.D. PEEK tubing. Minimize the length of all liquid lines, but especially that of the tubing between the column and the detector cell. The use of larger diameter and/or longer tubing will decrease separation efficiency.

Each of the possible configurations offers multiple sampling option; however, a consistently reproducible quantitation and an absence of disturbing artifacts are achieved best using the "full loop" mode and in conjunction with a 0.4 µL internal loop of the injector valve. Good reproducibility of retention times requires the use of temperature control.

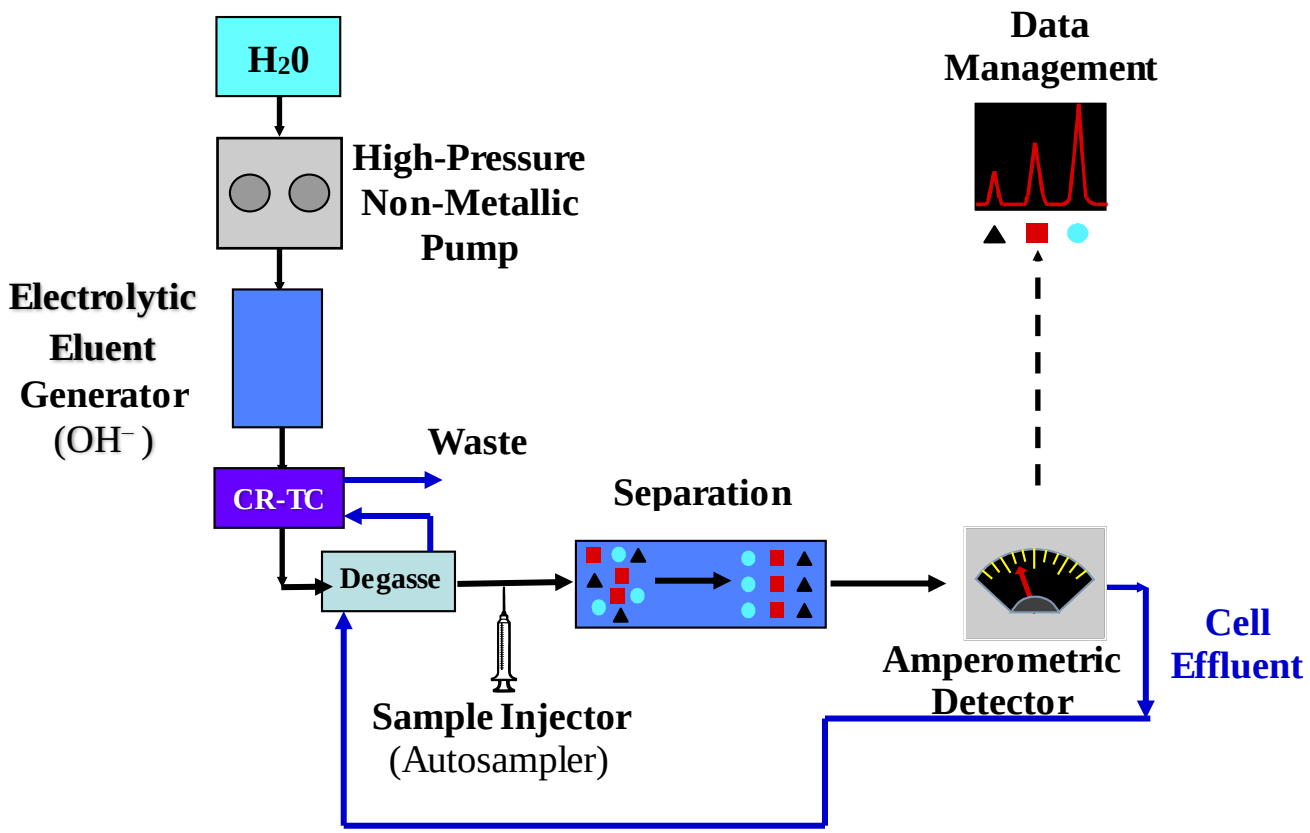


Figure 2
Block Diagram of a Capillary Reagent-Free™ Ion Chromatography System with Electrochemical Detection

2.3 Gold Working Electrodes

Carbohydrates separated by high pH anion exchange chromatography are detected by pulsed electrochemical detection and the signal is reported in Coulombs (C). Electrochemical detection is used to measure the current or charge resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. During oxidation reactions electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the amperometry cell. Detection is sensitive and highly selective for electroactive species, since many potentially interfering species cannot be oxidized or reduced, and are not detected. When a single potential is applied to the working electrode, the detection method is DC amperometry. Pulsed amperometry and integrated amperometry employ a repeating sequence of potentials. Pulsed electrochemical detection at a gold working electrode is a reproducible and sensitive method for the detection of all carbohydrates of molecular weight up to ten-thousand.

Although carbohydrates can be oxidized at a gold working electrode, some products of the oxidation reaction poison the surface of the electrode, inhibiting further analyte oxidation. By repeatedly pulsing between optimized high positive and negative potentials, a stable and active electrode surface can be maintained. However, the gold working electrode is very slowly consumed during this process and will eventually need to be replaced. Occasionally the electrode may be 'poisoned' by other contaminants, resulting in a significantly reduced response. When this occurs, the active surface of conventional (non disposable) electrodes can be renewed. However, this can be a tedious and time-consuming process.

The Dionex disposable gold electrodes (Item # 060139 for 6, Item # 060216 for 4 packages of 6) make electrode reconditioning by polishing and other methods unnecessary. They are less expensive and can thus be replaced more often than the conventional electrodes. The more frequent replacement of working electrodes renders electrochemical detection more predictable and reproducible. The disposable electrodes also make easier any troubleshooting of electrochemical detection problems. The gold hydroxide (AuOH) catalyzed mode of oxidation of carbohydrates differs from the gold oxide catalyzed oxidation of amino acids at higher potentials. Although both gold electrodes can be mounted in the same ED50 detection cell, and thus in principle it is feasible to convert a gold electrode from one mode of detection to another, in practice this may require an extensive period of time and is thus not recommended. The Au electrodes for carbohydrate analysis have been tested for and are guaranteed to work for carbohydrate analyses.

WARNING

Dionex Technical Note 21
You MUST USE the quadruple waveform (Waveform A) with disposable electrodes. Waveform B and Waveform C CANNOT BE USED with Disposable Electrodes. Waveforms B and C will strip the gold surface of the disposable electrode within 24 hours.

2.4 Electrochemical Capillary Cell and Electrodes

Part Number	Product Description
061749	Conventional gold working electrode
045972	Cell gasket for conventional gold working electrode
066480	Disposable gold electrodes on PTFE (6-packs)
060139	Disposable gold electrodes on Polyester (6-pack)
072161	Cell gasket for use with PN060139 at capillary flow rates
072044	Capillary electrochemical detection cell
074221	PEEK inlet capillary ID 0.0025" for PN 072044
061879	pH-Ag/AgCl reference electrode
072162	Gasket for use with pH/Ag/AgCl reference electrode at capillary flow rates
072075	Pd/H reference electrode
072214	Gasket for the PdH reference electrode

2.5 System Start-up

Configure the system with the AS autosampler on the left, the DC module in the middle and the pump on the right. EG Module should be placed on top of the pump. Nitrogen or helium should be delivered to the eluent organizer with about 5-6 psi at each bottle. Make all fluidic and electrical connections, but do not install the column yet. Instead install some backpressure tubing, such as a length of 0.0025" I.D tubing between the injector and detector cell inlet. Minimize the number of unions and the length of all the liquid lines. Tubing between the injection valve and the detector, on either side of the column, should be 0.0025" ID PEEK tubing. The use of larger tubing will decrease separation efficiency. Verify that the modules are communicating.

2.5.1 System Rinse

1. DO NOT install the Capillary CarboPac PA20 column before confirming that the background < 30nC. If experiencing a higher background rinse the system with 2 M NaOH.
2. Prepare a solution of 2M NaOH to rinse each bottle, by diluting 104 mL of 50% sodium hydroxide to 1 L with deionized water.
3. Place the 2 N NaOH in a pre-rinsed bottle and place the eluent line in it. Withdraw at least 40 mL of sodium hydroxide from the line into waste, using a priming flow rate.
4. Close the solvent draw-off valve and leave the pump running at 0.05 ml/min for 15 minutes.
5. Make sure that all surfaces come into contact with the sodium hydroxide; rotate the injector valve.
6. Repeat the process with 18 megohm-cm water.

3. Operation

3.1 CarboPac PA20 Column Operational Parameters

pH range: pH = 0 - 14
Temperature limit: 60°C
Pressure limit: 3,500 psi
Organic Solvent Limit: 100% Acetonitrile, methanol, acetone, if required for cleaning
Typical Eluents: High purity water (18 megohm-cm), potassium hydroxide from the Eluent Generator

3.1.1 The Most Important Rules

ALWAYS…

- use dedicated glassware and disposable glass or plastic ware for volume adjustments.
- keep your water blanketed with helium or nitrogen. Use new filtered water if left unblanketed for more than 30 minutes.
- use 0.4 µL loop size; larger loops will cause loss of resolution.

NEVER…

- go to the next step of the installation if the previous step has failed.
- start an installation with any of the check list items below missing.
- use 'communal' filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials.
- use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily. NEVER run above 60 °C or 3,200 psi.

3.1.2 Initial Check List

The following items MUST be available in your lab. The absence of any of these may compromise your analysis.

1. Laboratory water unit delivering 18.2 megohm-cm water at the installation site.
2. Vacuum pump available for use with the vacuum filtration units.
3. Inert gas cylinder (helium or nitrogen) with a regulator valve (for example, a 0-200 psi gauge on the low pressure side) and the appropriate size adaptors plus tubing.
4. Mixture of 6 carbohydrates standard.
5. Sterile-packed 10 mL and 25 mL disposable pipets and suitable pipeting bulbs or pumps.
6. Disposable, plastic (PE) large-size (at least 20 mL) syringe for priming the pump.
7. Plastic eluent bottles.

3.2 Purity Requirements for Chemicals

Obtaining reliable, reproducible and accurate results requires eluents that are free from impurities and prepared only from the chemicals recommended below. DIONEX cannot guarantee proper column performance when alternate suppliers of chemicals or lower purity water are utilized.

3.2.1 Deionized Water

The deionized water used to feed the Eluent Generator should be Type I reagent grade water with a specific resistance of 18 megohm-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. The availability of UV treatment as a part of the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free from electrochemically active surfactants. Expanding their period of use beyond the recommended time may lead to bacterial contamination and as a result, a laborious cleanup may be required. Use of contaminated water for eluents can lead to high background signals and gradient artifacts.

3.2.2 Potassium Hydroxide

Use Dionex Capillary Format of KOH Eluent Generator Cartridge installed with CR ATC in the EG5000 module.

3.3 Preparation of Eluents and Standards

Always sanitize the entire analyzer with 2M NaOH prior to initial start-up (see Section 2.5.2) and after idle periods.

NOTE

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and electrochemically active 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 your ion exchange columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents is substandard.

3.3.1 Deionized Water

Vacuum degas the 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. Vacuum filtration through 0.2 µm Nylon filters is a good alternative to vacuum degassing under sonication and is sufficient in the majority of cases. On-line degassing is available in the DP gradient pumping systems.

3.3.2 Eluent: Potassium Hydroxide

The first step in the preparation of potassium hydroxide eluent is to degas an aliquot (typically 1000 mL) of the deionized water, as described above. In the second step, start the pump flow and verify that the water is exiting from the Eluent Generator exit tubing. In the third step, select an appropriate KOH concentration (usually 10 mM) in the EG panel and verify that the eluent is exiting from the CR ATC outlet tubing, then turn on the CR ATC in the Chromeleon eluent generator panel.

3.4 Standard Mixture of Six Monosaccharides

The Dionex MonoStandard, Mix of Six, Item # 043162 contains 100 nmol each of L-fucose, D-galactosamine, D-glucosamine, D-galactose, D-glucose and D-mannose. Dilute the standard by adding 1.0 mL DI water to the vial containing the monosaccharide standard. The concentration of each of the monosaccharides will then be 100 µM. Take a 100 µL aliquot of the diluted standard and add 900 µL of water to make a 10 µM solution. Inject 0.4 µL volumes to compare with the column test chromatogram, when installing a new column or troubleshooting a separation.

3.5 Sample Preparation

The CarboPac columns are strong anion exchangers. Thus, the sample matrix precautions applicable to ion exchange chromatography apply to these columns. High salt concentrations in the samples should be avoided where 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.). Avoid extremes of sample pH (especially extremely acid samples). The presence of anionic detergents (e.g. SDS) in samples should be avoided entirely. Nonionic or cationic detergents may be acceptable in low concentrations.

When using pulsed amperometric 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.

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. Please ask for DIONEX Technical Note 30, "Monosaccharide and Oligosaccharide Analysis of Glycoproteins Electrotransferred onto Polyvinylidene Fluoride (PVDF) Membranes," or retrieve it from our website at www.dionex.com.

3.6 Introduction to the Detection Methods

The carbohydrate oxidation at gold electrodes is made possible by a rapid sequence of potentials (waveform) adjusted between the working electrode (gold) and the reference electrode (Ag/AgCl). Resulting currents are measured by integration during a short time interval of the detection waveform. The standard, recommended carbohydrate waveforms are shown in Table 1.

Table 1
Carbohydrate Quadruple Waveform

Time (s)	Ag/AgCl reference potential (mV)	PdH potential for 10 mM KOH (mV)	Integration
0	100	950	
0.2	100	950	Start
0.4	100	950	End
0.41	-2000	-1150	
0.42	-2000	-1150	
0.43	600	1450	
0.44	-100	750	
0.5	-100	750	

NOTE

Do not polish a new gold electrode prior to use.
NEVER POLISH the disposable gold electrodes.

Refer to "Section 5 – Troubleshooting Guide" of this manual for an overview of reconditioning techniques for conventional gold working electrodes.

The reference electrode for the ED is either a combination pH-Ag/AgCl electrode or a PdH electrode. For carbohydrate analysis, the former electrode is used in the Ag mode. Always verify the correct selection of reference electrode is made in the program file and on the Chromeleon ED panel prior to turning the cell voltage on.

Always have available at least one unused "known good" pH-Ag/AgCl reference electrode. If stored in saturated KCl, a reference electrode can be kept for years with its reference potential virtually unchanged. In contrast, the pH-Ag/AgCl reference electrodes mounted inside the electrochemical cell and exposed to flowing potassium or sodium hydroxide have only a limited lifetime of approximately 3 to 6 months. As a result of prolonged exposure to alkaline solutions, the ~~0.1 M~~ KCl solution inside the reference electrode gradually becomes alkaline and the silver chloride layer on the Ag wire immersed into that solution either dissolves or converts to a mixture of silver oxide and silver hydroxide. As that happens, the reference potential shifts and becomes increasingly unstable. Shifting reference potential is experienced by the user either as an unusually high background or as a decrease in signal response. A combination of both effects is also possible.

The PdH electrode does not undergo any change during a prolonged exposure to alkaline conditions. However, its potential can change slightly during a longer storage outside of the detection cell.

Never leave pH-Ag/AgCl electrode reference electrode inside a disconnected electrochemical cell.

CAUTION

A pH-Ag/AgCl electrode reference electrode can be irreversibly damaged by drying out. This happens most frequently by leaving the reference electrode inside a disconnected electrochemical cell. Always remove the pH-Ag/AgCl electrode reference cell from the electrochemical cell, when the system is not in proper use (i.e. cell inlet and outlet are not plugged or connected to a flowing eluent). After removal from the electrochemical cell, keep the reference electrode immersed in 3M KCl solution (224 g KCl/L) at all times.

3.7 Column Start-Up Requirements

Follow the instructions in section 2.5 when starting up a new system. Before installing the column, filter a fresh aliquot of ultrapure water and transfer it into an eluent container blanketed by inert gas. Pump 40 mL of water into the waste at priming flow rate. Stop the flow, and complete the tubing connections from the pump to the eluent generator, CR ATC, IC-Cube, detector cell and to waste. Follow the instructions in the ICS-6000 manual in making the connections. Close the priming valve, start the pump, select 10 mM KOH concentration in the eluent generator panel. Do not install the capillary column yet. Perform the following background check.

3.7.1 Verification of System Cleanliness

This section is performed using the conditions of the chromatogram in Figure 3.

Make sure that:

1. The cell is not yet on.
2. The pump is pumping at 0.008 mL/min and the EG is set at 12 mM KOH.
3. A length of 0.0025" ID tubing is installed between the injector and detector cell to generate ~1000psi backpressure.
4. The column is not yet installed.

Confirm that the pH reading on the Chromeleon EC Detector or Home panel is between 11.3 and 12.00 (only with pH-Ag/AgCl reference electrode). With the pH within this range, specify a reference electrode, turn on the cell using the appropriate quadruple waveform in Table 1 (Section 3.6) and begin monitoring the background signal from the control panel for at least 30 minutes. Confirm that the baseline is <30nC. If the background >30 nC or the pH is out of range, see the "Troubleshooting" section at the end of this manual.

3.7.2 Installation of the Capillary CarboPac PA20 Column

Install the CarboPac PA20 column set only after the initial system test (3.7.1) determines a background level within the specified range. A premature installation on a contaminated system will cause delays during column equilibration. The following procedure should be followed after any long term column storage (>1 week).

- Remove the 0.0025" tubing from the injector to the detector and install the Capillary CarboPac PA20 column set, but do not connect it to the cell; instead send the effluent from the column straight to waste at first. ***The Capillary CarboPac PA20 columns are shipped in 10 mM KOH***.
- To prepare the column for optimum performance, the CarboPac PA20 must be washed for 2 hours at 0.010 mL/min using 200 mM KOH. ***Have the column outlet connected to the inlet tubing of the Suppressor Bypass module in the IC Cube.***
- Following the wash, equilibrate the column at 10 mM KOH for 15 – 25 minutes.
- Next, switch your injection valve to LOAD (the loop now contains eluent equivalent to the initial conditions) and then connect the column to the cell.
- Reconnect the detection cell outlet to the inlet tubing of the Suppressor Bypass.
- When using 10 mM KOH, column washing with 100 mM KOH for 5-10 minutes between analyses is recommended to remove carbonate. Without such step, carbonate may accumulate on the column causing a gradual reduction of retention times. For example, a typical elution program for the six monosaccharide standard at a flow rate of 0.008 ml/min consists of 12 min at 10 mM KOH, 5 min at 100 mM KOH and 10 min of re equilibration in 10 mM KOH. A high accumulation of carbonate indicated by significantly shorter retention times can be corrected by a 2-hour rinse with 200 mM KOH at 0.010 mL/min.

4. Example Applications

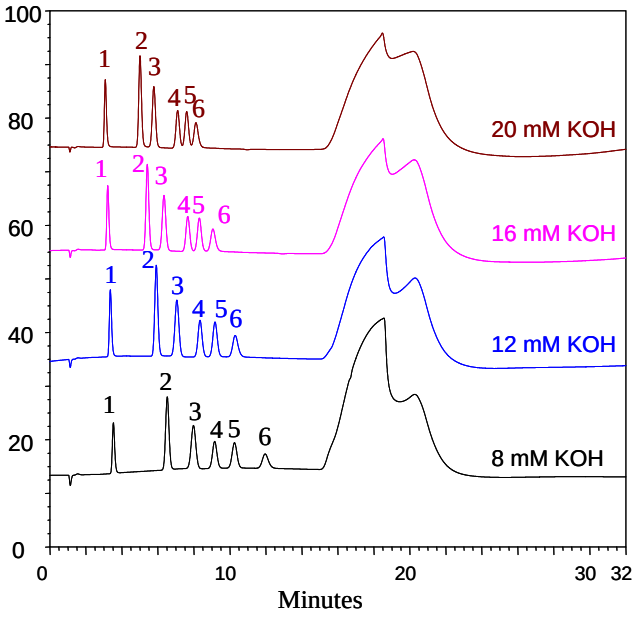
The CarboPac columns have been designed for isocratic or gradient separation utilizing potassium or sodium hydroxide eluents up to a concentration of 1M. Analyte separation is highly dependent on hydroxide concentration in HPAEC. Many separations require only an isocratic separation. However, some groups 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.

Depending upon your system, you may have to make small adjustments to your gradient conditions or operating temperature to achieve resolution of all analytes.

4.1 Runtime Optimization

The CarboPac PA20 has been designed to give good resolution between the components in the Monostandard Mix of Six monosaccharides under a variety of potassium hydroxide conditions. These conditions can be optimized depending upon the goal of the separation. The higher the potassium hydroxide concentration, the faster the peaks will be eluted but the less the resolution between the peaks. This phenomenon is illustrated in the chromatograms below. It is important to remember that despite the fact that the peaks are separated isocratically, at low hydroxide concentration it is still necessary to regenerate the column in order to remove carbonate buildup which will shorten the peak retention times over time.

Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	8, 12, 16,or 20 mM KOH (12 min) / 100 mM KOH (5 min) / 10 mM KOH (15 min) (EG)
Flow Rate:	8 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Reference Electrode:	PdH
Electrode:	Au
Gasket Thickness:	1 mil
Sample:	Standard (10 µM)



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

Figure 3
Runtime Optimization: Six Monosaccharides

Table 2
Analytical performance: separation of monosaccharides using the capillary CarboPac PA20 column

	LOD (µM)	Linear Range (µM)	Correlation Coefficienct
Fuc	0.024	0.024 – 50	0.9958
GalN	0.018	0.018 – 25	0.9998
GlcN	0.029	0.029 – 25	0.9949
Gal	0.054	0.054 – 25	0.9964
Gla	0.056	0.056 – 50	0.9986
Man	0.068	0.068 – 50	0.9956

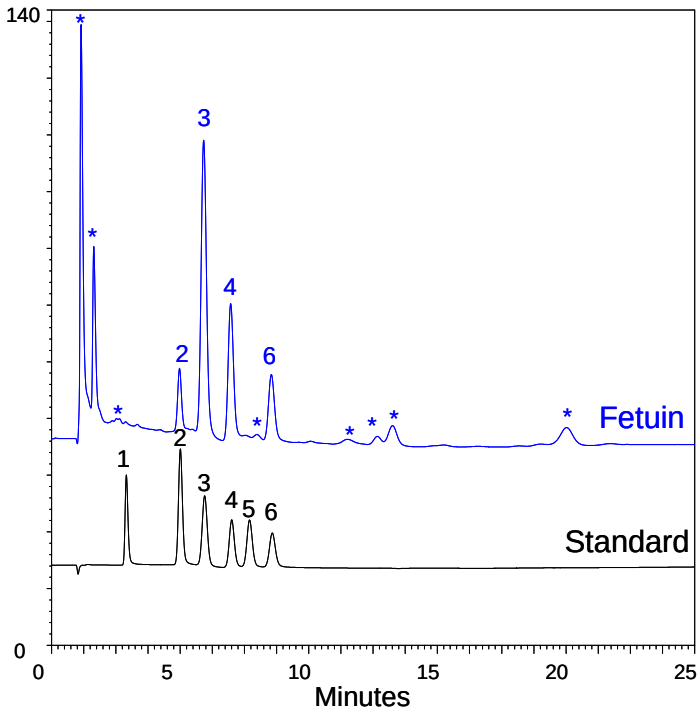
NOTE ***LOD: Limit of Detection as 3x of noise, Injection Volume: 0.4 µL.***

4.2 Common Monosaccharides from Mammalian Glycoproteins

Many mammalian proteins have carbohydrates attached to them. In many cases, the presence of the carbohydrate controls the biological activity of the protein or the rate at which it is cleared from the system. For example, certain glycosylated forms of tissue plasminogen activator (tPA) have more enzymatic activity than others. Erythropoeitin shows complex effects if the protein is deglycosylated or the glycosylation is altered. Failure of secretion from the body, decreased stability and decreased biological activity occurs if multiple glycosylation sites are eliminated. Desialylation and/or less branched oligosaccharides give increased activity in vitro, but decreased activity in vivo. Thus protein glycosylation is important to many scientists, including those making recombinant protein for therapeutic use.

The following conditions should be used for guidance. These conditions may be modified, as necessary, to suit your particular application needs.

Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	12 mM KOH (25 min)/ 100 mM KOH (15 min) / 12 mM KOH (20 min) (EG)
Flow Rate:	9 µL/min
Injection Volume:	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Electrode:	Disposable Au
Reference:	PdH
Gasket Thickness:	1 mil
Sample:	Fetuin hydrolysate



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

Figure 4
Monosaccharides in Fetuin Hydrolysate

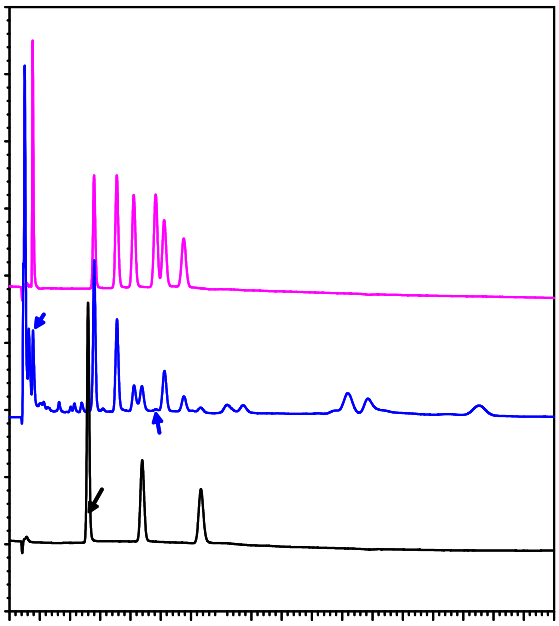
4.3 Carbohydrates in Foods

Sodium or potassium hydroxide eluents with concentrations in the range of 10-100 mM are typically used for the separation of monosaccharides, although for monosaccharides that are important in dietary fiber analysis, the eluent may be weaker. For all of these isocratic runs, it is important to regenerate the column using a step gradient to about 180 mM at the end of the run, to remove more strongly retained sample matrix components and ensure stable run times.

Occasionally, it may be necessary to use an extremely weak eluent or even water. For example, coffee sugars can be separated and detected with 3 mM KOH with sufficient sensitivity.

The following conditions should be used as guidance. These may be modified, as necessary, to suit your particular application needs.

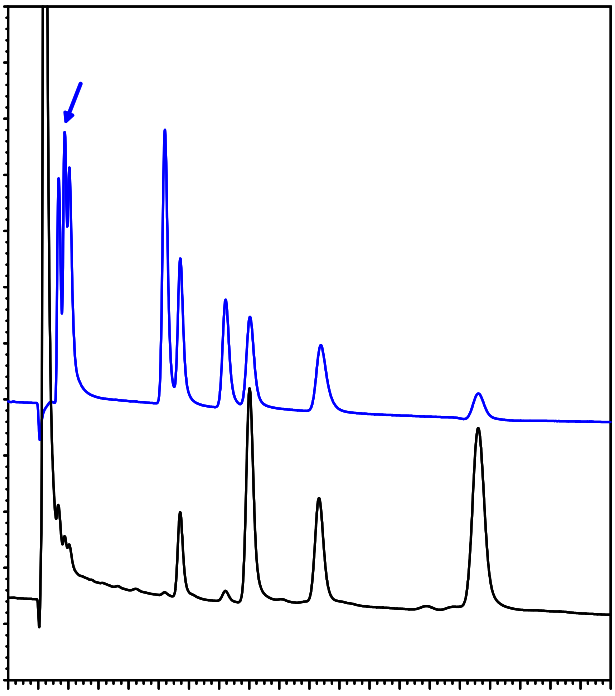
Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	3 mM KOH (45 min) / 100 mM KOH (5 min) / 3 mM KOH (20 min) (EG)
Flow Rate:	9 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Reference Electrode:	PdH
Electrode:	Au
Gasket Thickness:	1 mil
Sample:	Standards 1 and 2 (10 µM) / Coffee (1:1000 dilution)



Peaks:
1. Mannitol
2. Galactosamine
3. Arabinose
4. Galactose
5. Glucose
6. Sucrose
7. Xylose
8. Mannose
9. Fructose
10. Ribose
*. Unknown

Figure 5
Separation of Coffee Sugars

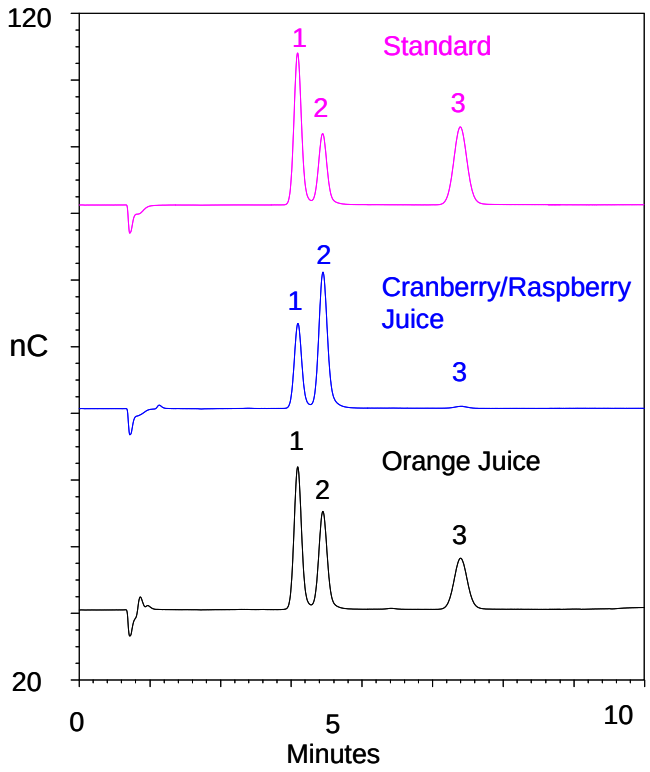
Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	10 mM KOH (20 min) / 100 mM (5 min) / 10 mM KOH (20 min) (EG)
Flow Rate:	9 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Reference Electrode:	PdH
Electrode:	Au
Gasket Thickness:	1 mil
Sample:	Wine sample (1:500 dilution) / Standard (10 µM)



Peaks:
1. Unknown
2. Sorbitol
3. Mannitol
4. Trehalose
5. Galactosamine
6. Arabinose
7. Galactose
8. Glucose
9. Unknown
10. Fructose
11-12. Unknown
13. Proline

Figure 6
Wine Sample and Standard

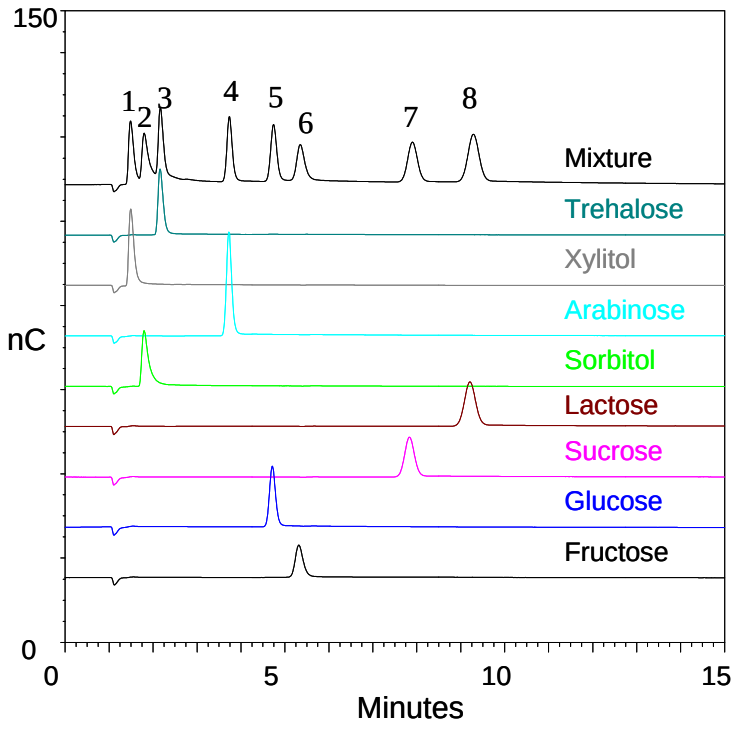
Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	50 mM KOH (EG)
Flow Rate:	10 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Reference Electrode:	PdH
Electrode:	Au
Gasket Thickness:	1 mil
Sample:	Juice samples (1:5000 dilution) / Standard (20 µM)



Peaks:
1. Glucose
2. Fructose
3. Sucrose

Figure 7
Juice Samples and Standard

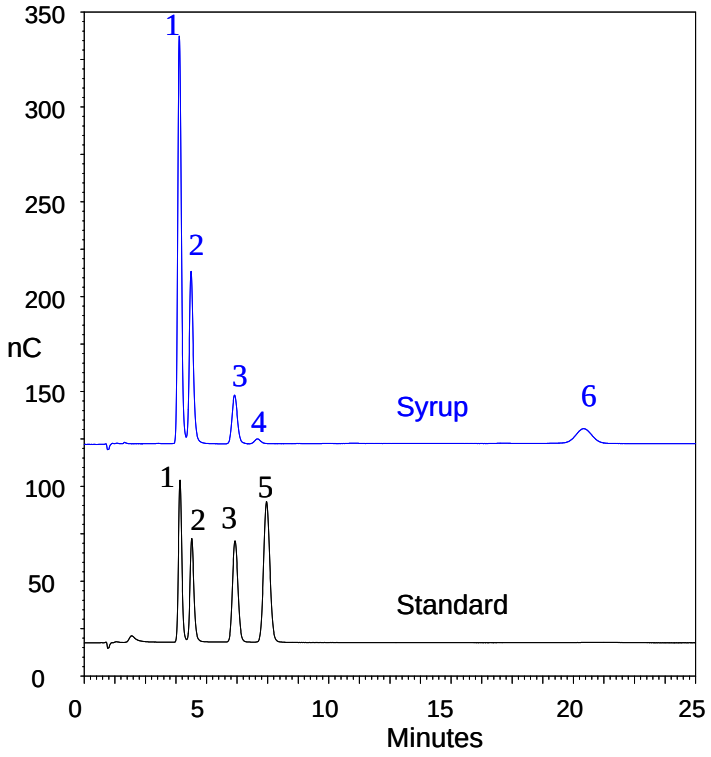
Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	52 mM KOH (EG)
Flow Rate:	8 µL/min
Injection Volume:	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Reference Electrode:	PdH
Electrode:	Au
Gasket Thickness:	1 mil
Sample:	Standards (10 µM)



Peaks:
1. Xylitol
2. Sorbitol
3. Trehalose
4. Arabinose
5. Glucose
6. Fructose
7. Sucrose
8. Lactose

Figure 8
Sugar Alcohols, Mono- and Disaccharides which are Usually Analyzed in Dietary Fiber

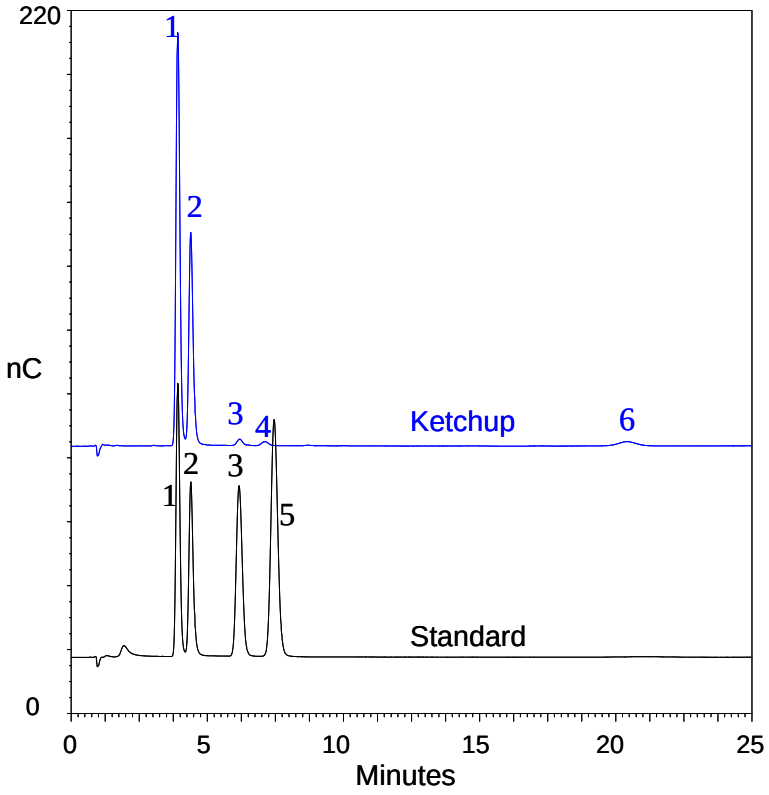
Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	50 mM KOH (EG)
Flow Rate:	9 µL/min
Injection Volume:	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Electrode:	Disposable Au
Reference:	PdH
Gasket Thickness:	1 mil
Sample:	Sample (10,000 dilution) / Standard (50 µM)



Peaks:
1. Glucose
2. Fructose
3. Sucrose
4. Unknown
5. Lactose
6. Unknown

Figure 9
Chocolate Syrup

Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	50 mM KOH (EG)
Flow Rate:	9 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Electrode:	Disposable Au
Reference:	PdH
Gasket Thickness:	1 mil
Sample:	Sample (10,000 dilution) / Standard (50 µM)



Peaks:
1. Glucose
2. Fructose
3. Sucrose
4. Unknown
5. Lactose
6. Unknown

Figure 10
Ketchup

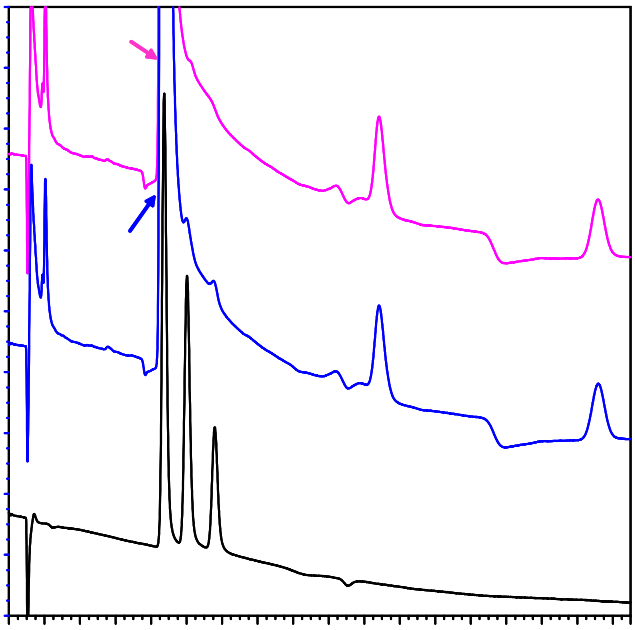
4.4 Carbohydrates in Bio- and Pharmaceutical Products and Processes

The United States Food and Drug Administration (FDA) and the regulatory agencies in other countries require that pharmaceutical products be tested for composition to verify their identity, strength, quality and purity. Recently, attention has been given to inactive ingredients as well as active ingredients. Many of the ingredients are non-chromophoric and cannot be visualized by absorbance detection. However, carbohydrates, glycols, sugar alcohols and sulfur-containing compounds can be oxidized and therefore detected by amperometric detection.

Another area of great interest to the pharmaceutical chemist is the optimization of fermentation broths. If the correct nutrients and amino acids for a given culture are determined, and monitored, so that the correct concentrations are always maintained, then the culture has the best chance of producing the optimum yield. The CarboPac PA20 can be used on-line to monitor fermentation broths and cell cultures. Alternatively, if there is more interest in monitoring the amino acid content, then the AminoPac PA10 (Item# 055406) should be considered for direct detection, on-line.

The following conditions should be used as guidance. These may be modified, as necessary, to suit your particular application needs.

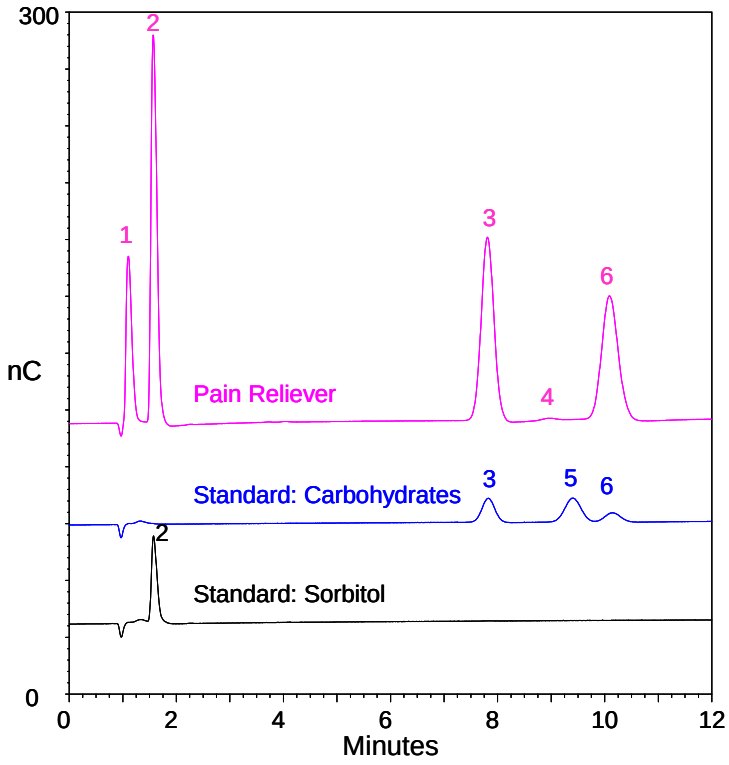
Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	7 mM KOH (35 min) / 100 mM KOH (5 min) / 7 mM KOH (20 min) (EG)
Flow Rate:	9 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Electrode:	Disposable Au
Reference:	PdH
Gasket Thickness:	1 mil
Sample:	Standard (10 µM) / Spiked Sample (10,000 dilution spiked with 1 µM Suc and Fru) / Sample (10,000 dilution)



Peaks:
1. Glucose
2. Sucrose
3. Fructose
4-6. Unknown

Figure 11
Cell Culture Medium: Yeast Extract-Peptone-Dextrose (YPD)

Column:	CarboPac PA 20 (0.40 x 150 mm)
Temperature:	30 °C
Eluent:	10 mM KOH (12 min) / 100 mM KOH (5 min) / 10 mM KOH (20 min) (EG)
Flow Rate:	8 µL/min
Injection Volume :	0.40 µL
Detection Method:	PAD (Carbohydrate Quadruple Waveform)
Electrode:	Disposable Au
Reference:	PdH
Gasket Thickness:	1 mil
Sample:	Sample (10,000 dilution) / Standard (Glc, Suc and Fru: 10 µM) / Standard (Sorbitol: 10 µM)



Peaks:
1. Unknown
2. Sorbitol
3. Glucose
4. Unknown
5. Sucrose
6. Fructose

Figure 12
Pediatric Pain Reliever and Standards

5. Troubleshooting

Problems such as sample contamination, imprecision during sample transfer, problems during peptide or protein hydrolysis, may be related to specific experimental protocols.

Make sure to follow the rules from Section 3.1.1, "The Most Important Rules," and to recheck all of the items from Section 3.1.2, "Initial Check List."

5.1 High Background

While it may be possible to obtain reasonable performance even with elevated levels of detection background according to some requirements, high background frequently brings about an increased size of gradient artifacts and can be accompanied by a presence of ghost peaks. Detection sensitivity may also change suddenly when the detection background is too high.

A background of >30 nC with 10 mM potassium hydroxide at 0.008 mL/min and 30°C using the quadruple waveform shown in Table 1 indicates one of the following possibilities:

5.1.1 Incorrect detection parameters

Verify that "Ag" or "PdH" is specified in Chromeleon EC Detector panel. Check all values of waveform in the program against those in Table 1, "Carbohydrate Quadruple Waveforms," in Section 3.6. Make sure the correct waveform for the selected reference mode is being applied. If the pH reading in 10 mM KOH is above 13.2 replace the pH/Ag/AgCl reference electrode. The pH/Ag/AgCl reference electrode should be calibrated only once during the initial installation if the pH readout is to be used as an indication of reference potential shift.

5.1.2 Compromised working electrode surface

Briefly install a new working electrode and check the background as above, If the reading remains > 30 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. Otherwise continue with your work with the new electrode installed.

5.1.3 Column contamination

Remove the column set from the system first and replace it with a length of 0.0025" ID PEEK tubing, generating a pressure drop between 1000 and 2000 psi. If the background reading improves after the column is removed from the system, go to section 5.3.

5.1.4 Water contamination

Prepare eluents using a freshly filtered aliquot of water, investigate the source of contamination in the original aliqout of water.

5.1.5 System contamination

If the background remains high even with fresh water and without the column, carry out the 2 M sodium hydroxide rinse described in section 5.5.

5.2 Decreased Detection Sensitivity

Always confirm the loss of response by performing at least one injection of the 0.4 µL of 10 µM monosaccharide standard mix as illustrated in Figure 3 (8 mM KOH). This is to make sure that a decreased level of response is not being caused by system problems discussed in Section 5.4.2.

Any decrease in detection sensitivity means that the working electrode surface has been affected. The operator should install a new working electrode. Spare gold working electrodes should always be available in order to avoid unnecessary delays.

IMPORTANT
Never install a new electrode without an aggressive system cleanup (Section 5.6, "EDTA Cleanup")
Exceptions to this rule are described below.

Exception:
If pH/Ag/AgCl reference electrode is installed, check the pH reading in 10 mM KOH. The pH/Ag/AgCl reference electrode should be calibrated only once during the initial installation if the pH readout is to be used as an indication of reference potential shift. If the value is >13.2, install a new pH/Ag/AgCl electrode and then install a new gold working electrode. The system cleanup is not necessary. The decrease in sensitivity was caused by a gold-oxide-buildup on the electrode surface. This was because the reference potential was too high. The non-disposable gold working electrode can be reconditioned by the repair polishing described in Section 5.7.1, "Mechanical Polishing."

After installing a new working electrode (with or without the complete system cleanup), confirm the normal detection sensitivity. Carry out the monosaccharide injection test illustrated in Figure 2. Should the response be still too low (peak area of glucose < 0.7 nC min for 0.4 µL injections of 10 µM standard), immediately remove the new working electrode from the system.

5.3 Column Problems

The guard column protects the main column not only from contamination but also from excessive pressure fluctuations caused by the instrument or by operator errors. Have the guard column installed at all times, disconnect it only during some of the testing described in this section, or when priming the pump to prevent accidental over pressure.
The column set is causing the high background if the background reading decreases after the column is replaced by a section of PEEK tubing, as described in Section 5.1 item 3, "Column Contamination."

5.3.1 Column Set Causing High Background

If the column has been determined to be the cause of the high background, as described above, replace the column.

5.3.2 Peak Efficiency and Resolution are Decreasing

Always have a spare guard available.
Peak deformations may sometimes be caused by sample matrix.

1. Run a standard separation with the Guard column removed from the system. Install a new Guard column should the separation improve with the old Guard removed. It is quite common to replace the Guard column several times during the lifetime of an analytical column.
2. Verify that only the 0.0025" I.D. tubing is installed for all connections between injector and detector.
3. Check for proper installation of ferrules on all PEEK tubing starting with the injector outlet and all other connectors to the detection cell inlet. Check the quality of cuts at the end of each tubing.
4. Check temperature settings in your method and/or actual temperature in your column oven.
5. The column may be overloaded. Try to inject a smaller amount of your sample or dilute the sample more.
6. If all of the above does not lead to an improved separation, the resin bed of the main column has been damaged and the main column must be replaced.

5.4 System Problems

5.4.1 High Detection Background Caused by the System

1. Verify the problem is neither the detector (see Section 5.1 item 1, "Incorrect detection parameter" and item 2, "Compromised working electrode surface") nor the column (see Section 5.1 item 3, "Column Contamination") related.
2. With injector, column and detector cell installed (cell voltage off) carry out the 2M NaOH wash as described in Section 5.5, "Sodium Hydroxide Cleanup."
3. Prepare a new aliquot of filtered water and transfer it into a rinsed out, inert gas blanketed eluent bottle.
4. Rinse eluent lines with the freshly filtered water (at least 40 mL by priming speed

5.4.2 No Peaks, Poor Peak Area Reproducibility or Too Small Peak Areas

1. Check the position and filling levels of sample vials in the autosampler.
2. Check injector needle-height setting.
3. Check injection port transfer volume calibration
4. Check values of injection parameters (AS injection volume, sequence injection volume, syringe speed).
5. Service the injection valve (and diverter valve if using sequential injection for two channels). Check for leaks, Tefzel fragments, or sediments inside the valve.

5.4.3 Large Baseline Dip in the Chromatogram

A large baseline dip appearing between 17 and 19 minutes when the guard column is installed is usually caused by oxygen in the sample injected. The 'oxygen dip' is normal and can be reduced in magnitude with higher KOH concentration in the eluent.

5.4.4 Incorrect or Variable Retention Times

1. Check the operational parameters of your CR ATC.
2. Prime the pump if necessary.
3. Measure the flow rate by weighing out the eluent collected during exactly sixty minutes of flow. Recalibrate the pump if necessary.
4. The re-equilibration period at the end of the program with a 100-200 mM KOH rinse is too short.
5. Samples containing high salt content (>50 mM) will decrease the retention times.

5.4.5 Unidentified Peaks Appear Alongside the Expected Analyte Peaks

During the 100-200 mM KOH rinse, a number of small peaks may appear. These peaks are usually due to trace contaminants in the water supply. The contaminants accumulate on the column during the isocratic section of the chromatogram and are released, frequently as irregular baseline deformations or sharp spikes, with the increasing eluent strength. Some trace contaminants can co-elute with monosaccharides, compromising accuracy of quantitation at lower concentrations.

If extraneous peaks are observed even after the water supply is excluded as a possible cause, clean the autosampler lines and sample loop. The autosampler should be cleaned using the following protocol:

Disconnect the column and detector cell from the autosampler.

Set the pump to 100% deionized water.

Place the following solutions in the autosampler and inject in sequence:

1. 1 M NaOH
2. Deionized water
3. IPA
4. Deionized water
5. 1 M HCl
6. Deionized water

5.5 Sodium Hydroxide Cleanup

The sodium hydroxide (2 M) rinse used to decrease column or system-related elevated background is essentially identical to the rinse performed during an installation of a new system, Section 2.5.1, "System Rinse." Following the rinse, check the background again while pumping the 10 mM potassium hydroxide and repeat the rinse at least once if necessary. Leave the old gold working electrode in place during the first and second checking of the detection background. Use a new or reconditioned electrode only if the background remains high even after the second rinse. Should the new electrode also produce a reading of > 30 nC, remove it from the system within 30 minutes, rinse it with water and reinstall the old electrode. In case the repeated rinse does not lower the background, perform the EDTA cleanup described in Section 5.6, "EDTA Cleanup." Then try the background with the old electrode first and if necessary only briefly with the new electrode again. In case the new electrode delivers < 30 nC, leave it in the system, and if non-disposable electrodes are used, recondition the old electrode using the chemical cleanup described in Section 5.7.3.

5.6 EDTA Cleanup

1. Install new Pump Seals
2. Wash System with 6.5 mM (2.4 g/L) Na_2EDTA (must be disodium EDTA) MW 372 g/mol
   a. Remove the column.
   b. Remove gold electrode from the cell, close the cell again using an empty holder block over a gasket.
   c. Restore liquid connection between injector valve and detection cell (column has been removed)
   d. Empty the contents of eluent container, rinse it with 1 L of 18 megohm water and discard the water.
   e. Filter 1 L of 18 megohm water through 0.2 µm Nylon filter.

Do not use any other filter material than Nylon for eluent filtration.

NOTE

   f. Transfer filtered water into eluent container. Pump at least 30 mL of water into pump waste at priming flow rate.
   g. Stop the pump. Close the priming valve. Pump at least 50 mL of water from eluent container through the system into detector waste at 1 mL/min.
   h. Toward the end of the water rinse, turn the injection valve at least 3 times.
   i. Prepare 1L of 6.5 mM Na_2EDTA and filter it through a 0.2 µm Nylon filter. Discard water from eluent container and replace it by the filtered aliquot of 6.5 mM EDTA.
   j. Pump at least 30 mL of EDTA into pump waste at priming flow rate.
   k. Stop the pump. Close the priming valve. Pump (1 mL/min) at least 100 mL of EDTA from eluent container through the system into detector waste.
   l. Toward the end of the EDTA rinse, turn the injection valve at least 3 times.
   m. Carry out steps d to h again. Rinse the system (column remains out of the system) with the initial eluent composition (10 mM KOH).
   n. Re-install the capillary column set and using the rate of 0.010 mL pump for at least 10 minutes from the column to waste.
   o. Open the working electrode side of the cell, remove the gasket and rinse the sealing surface with 18 megohm water.
   p. Reassemble cell with a new disposable electrode and a new 1 mil PTFE gasket
   q. Reconnect the detection cell to the capillary column and perform the separation illustrated in Figure 3 (8 mM KOH). Repeat cleanup and install a new column if the peak area for glucose is < 0.7 nC min.

5.7 Reconditioning of Gold Electrodes

IMPORTANT
The following procedures apply only to non-disposable gold working electrodes.
Do not recondition disposable electrodes.

5.7.1 Mechanical Polishing

1. Polish with coarse polishing compound (Item# 36319) for 10 minutes with as much strength as you can sustain.
2. Apply several mL of water to a fresh polishing pad (Item# 36121) and 'polish' for one minute. This step removes the coarse polishing powder particles imbedded in the gold material.
3. Polish with fine polishing compound (Item# 36318) for at least 10 minutes. Use a fresh piece of polishing cloth.
4. Apply several mL of water to another piece of fresh polishing cloth and 'polish' for I minute. This step removes the fine polishing powder particles imbedded in the gold material.
5. Reassemble the ED cell and apply an appropriate Table 1 waveform under initial conditions. If necessary, wait for at least 2 hours for the response to stabilize. In many cases, it is useful to wait overnight. Repeat the entire polishing procedure until the background drops below 30 nC, or 10 µM glucose response increases above 0.7 nC min under the conditions shown in Figure 3 (8 mM KOH)

5.7.2 Sanding of Receded Gold Working Electrodes

IMPORTANT
This entire procedure should be used only for seriously damaged or receded non-disposable gold working electrodes. Do not sand disposable gold electrodes.

1. Sanding off of the gold electrodes is always done with a subsequent coarse and fine polishing as described above.
2. The only reason to sand off an electrode is to make the gold electrode flush with the KEL-F surface.
3. Use a fresh 600-grit sand paper. Make sure that the KEL-F surface remains planar. If the surface is not planar, the ED cell will leak. The cell gasket will not have the required uniform seal around the entire flow path inside the assembled cell.
4. Sand for less than 1 minute (continuous sanding only to bring the KEL-F to the same level as gold), rinse off the powder residue with deionized water. Polish the rinsed electrode on a clean polishing pad (Item # 36121) with deionized water to remove last traces of the powder residue. Rinse the water again.

5.7.3 Chemical Reconditioning of Gold Working Electrodes

The chemical method of reconditioning removes chemical contamination from the non-disposable working electrode surface and restores the electrode performance. Disposable electrodes should simply be replaced. If the electrode has been passivated by excessive gold oxide formation (see, for example, section 5.2 "Exception", high reference potential), the chemical cleaning will not restore the electrode performance.

Wear gloves and safety glasses whenever handling chromic acid solutions.

SAFETY

A. Preparation of Chromic Acid
Dissolve/suspend 1 g of sodium chromate in 1 mL of water in a 100 mL glass beaker, slowly add 10 mL of concentrated sulfuric acid with constant stirring. Store the solution in a suitable glass vessel.

Chromic acid is corrosive and carcinogenic.
Follow all usual precautions and proper disposal procedures.

WARNING

B. Reconditioning of Electrodes

Before, during and after the reconditioning, avoid any skin contact with the gold electrodes.

NOTE

Put the working electrode on a clean filter resting on a horizontal surface. Using a fresh glass transfer pipet, apply one or two droplets of chromic acid to the electrode surface. The chromic acid should form a hemisphere (approximately 2-3 mm in diameter) covering the entire gold surface and surrounding polymeric material. Leave the reagent in place for 10 minutes. Rinse the chromic acid off with DI water, then rinse the entire electrode with water again and dry it with a clean airflow.

5.8 Failed pH/Ag/AgCl Reference Electrode

The first indication that a pH/Ag/AgCl reference electrode has failed is a pH readout outside of the expected range of pH 12-13. A reference electrode can be irreversibly damaged by drying out. This happens most frequently by leaving the reference electrode inside a disconnected ED. Always remove the reference electrode when the system is not in proper use (i.e. cell inlet and outlet are not plugged or connected to a flowing eluent). After removal from the ED cell, keep the pH/Ag/AgCl reference electrode immersed in 3 M KCl solution (224 g KCl/L) at all times. The PdH electrode is stored dry.

With a "known good" reference electrode it is possible to carry out one of the following checks of the reference electrode being used in the ED cell:

A. Immerse the "known good" reference electrode and the tested electrode into the same 0.1 M KCl solution. Using a voltmeter, measure the potential between the two electrodes. Use the contact number 1 of the blue reference electrode connector (ICS-3000 or ICS-6000) for the measurement. Discard and replace any tested electrode that differs by more than 30 mV from the "known good" Ag/AgCl reference.

B. Simply replace the electrode you wish to check with a "known good" reference electrode inside the ED50. Apply the voltage to the cell. Discontinue using the checked electrode if insertion of the "known good" electrode decreased the background from > 30 nC to <30 nC.

Immediately remove the "known good" electrode and store it properly. This referencing procedure will work as long as you do not leave your "known good" electrode inside the ED cell for more than a few minutes at a time and store it properly (immersed in 3 M KCl) in the intervening periods of time.

NOTE

6. Good Practices for Successful HPAE-PAD

6.1 Good Practices

1. Always use a guard column
2. Keep tubing lengths to a minimum and change tubing from the injector to the column, between columns, and to the detector at least once a year.
3. Change the autosampler needle every 6 months to a year.
4. Regularly test the autosampler to ensure that it is functioning properly (see TN40, "Glycoprotein Monosaccharide Analysis Using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)," for an example)
5. Develop all new methods with waveform A, the quadruple waveform (see TN21, "Optimal Settings for Pulsed Electrochemical Detection of Carbohydrates Using the Dionex Electrochemical Detector.").
6. Establish a system suitability standard, such as the mixture of 6 monosaccharides used by Dionex for the test chromatogram.
7. Change the reference electrode every 3 months and be sure to calibrate it at initial installation.
8. Disconnect the column from the cell when cleaning the column
9. Control the column and cell temperature at 30°C.
10. Calibrate the pump flow rate every three months and repair (pump seals, check valves) as necessary.

6.2 System Parameters to Monitor

When your system has been installed and optimized, note the values of the following parameters. These values can be checked periodically to ensure that the system is still functioning optimally.

1. System backpressure should not exceed 3300 psi.
2. System background under your running conditions. This value is typically 20–35 nC using the quadruple waveform.
3. Baseline noise for 1 minute intervals, should be < 60 pC peak-to-peak.
4. The pH reading of the pH/Ag/AgCl reference electrode should be between 11 and 12.5 for 10 mM KOH. If there is no reading, the reference electrode has failed.
5. Mannose asymmetry should be less than 1.2, typically. If it is greater than 1.2, then the probable source of the problem is the water source. This can be confirmed by installing a BorateTrap to see if the asymmetry improves
6. Reproducibility of peak areas under conditions of Figure 3 (8 mM KOH) should be <3% RSD (n=10).
7. Reproducibility of retention times under conditions of Figure 3 (8 mM KOH) should be < 0.2% (n=10).

6.3 Shutting Down an HPAE-PAD System

1. It is not necessary to shut down capillary systems for short periods of time. The flow can be maintained for weeks at a time without refilling the eluent bottles. The turning off cell voltage is recommended during the periods of inactivity, however. It will extend the useful life of the working electrode.
2. For long-term, fill the column with strong eluent, remove from the system and plug the ends.
3. Remove the reference electrode and put it in its container with a saturated KCl solution (see the detector manual for more information). PdH reference electrodes are stored dry.
4. Pump water through the system (each channel used). This can be done at 2 mL/min when no column is in-line. Flush the autosampler.

6.4 Restarting an HPAE-PAD System

1. Pump water at 0.1 mL/min (no column in-line) through each eluent channel, or withdraw at least 20 mL from each line using a priming flow rate.
2. Replace the water with the appropriate eluent and pump the eluent through each line at 0.1 mL/min to replace the water in the lines, or withdraw at least 20 mL from each line using a priming flow rate.
3. Install the column, wash with strong eluent at 0.01 mL/min for 30 minutes into waste
4. Reinstall the reference electrode.
5. Flush the autosampler, make sure the flush line is free of air bubbles.
6. Run the system with strong eluent and evaluate the background.

7. PAD Positive Compounds Versus Hydroxide Concentrations

Retention Times (min)

Analyte	10 mM	50 mM	100 mM	250 mM
Glycerol	1.14	1.15	1.17	1.15
Erythritol	1.24	1.24	1.25	1.22
Arabitol	1.51	1.48	1.46	1.38
Methanol	1.59	1.58	1.61	1.61
Galactitol	1.69	1.65	1.61	1.48
Ribitol	1.71	1.67	1.61	1.48
Sorbitol	1.74	1.67	1.62	1.47
Trehalose	2.23	2	1.87	1.62
Fucose	3.23	2.29	1.9	1.46
Maltitol	4.82	3.86	3.17	2.25
2-Deoxy-D-Glucose	5.31	3.43	2.59	1.8
Galactosamine	5.84	3.19	2.33	1.62
Rhamnose	6.03	3	2.19	1.57
Arabinose	7	3.86	2.73	1.83
Glucosamine	7.16	3.49	2.46	1.67
Galactose	8.95	4.83	3.34	2.03
Glucose	9.93	4.88	3.3	2.05
Mannose	11.4	4.86	3.18	1.97
Sucrose	11.45	7.91	5.59	3.25
Xylose	11.67	5.26	3.45	2.08
Fructose	13.29	5.78	3.64	2.2
Ribose	15.37	6.36	4.01	2.33
Oxygen Dip	17.9	18.18	N/A	18.1
Raffinose	22.04	13.84	9.06	4.45
Alpha-Lactose	22.5	9.42	5.55	2.78
Cellobiose	43.65	15.45	8.53	3.74
Melibiose	N/A	6.83	4.3	2.32
Turanose	N/A	14.77	7.95	3.57
Gentiobiose	N/A	14.79	8.56	3.69
Palatinose	N/A	15.65	8.66	3.85
Maltose	> 60	27.54	13.86	5.13
Maltotriose	> 60	> 60	50.89	12.93
Maltotetraose	> 60	> 60	> 60	34.65
Maltopentaose	> 60	> 60	> 60	> 60

8. HPAE-PAD Carbohydrate Resources

None of these resources specifically use the CarboPac PA20; however, the following Technical Notes, Application Notes and articles can be used to determine starting conditions for separations on the CarboPac PA20. The CarboPac PA20 is designed to have shorter run times and higher efficiencies.

8.1 Basic HPAE-PAD Resources

1. Dionex Technical Note 20, "Analysis of Carbohydrates by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)"
2. Dionex Technical Note 21, "Optimal Settings for Pulsed Electrochemical Detection of Carbohydrates Using the Dionex Electrochemical Detector"
3. Rocklin R.D., et al. (1998), *Anal. Chem.*, **70**, 1498-1505 (Quadruple waveform)

8.2 HPAE-PAD Monosaccharide Resources

1. Dionex Technical Notes 30, 40, 53
2. Dionex Application Notes 117, 122
3. Application Update 125
4. Weitzhandler, M., et al. (1996) *Anal. Biochem.*, **241**, 128-134
5. Weitzhandler M., et al (1996) *Anal. Biochem.*, **241**, 135-136
6. Hanko V. and Rohrer J., (2000), *Anal. Biochem.*, **283**, 192-199 (Fermentation Broths)
7. Rohrer J., (2000), *Anal. Biochem.*, **283**, 3-9 (sialic acids)
8. Rohrer J., et al. (1998), Glycobiology, **8**, 35-43 (sialic acids)

9. Column Care

9.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for Dionex Capillary CarboPac PA20 column is 5,000 psi (34.47 MPa).

9.2 Column Start-Up

The Dionex Capillary CarboPac columns are shipped using 10mM potassium hydroxide (see QAR) as the storage solution. Use Dionex EGC-500 KOH cartridge to generate the eluent employed in the Quality Assurance Report (QAR). Install the column in the chromatography module and direct the column effluent to waste for 60 minutes, and then connect to the ED cell. It is recommended to clean the column for 1 to 2 hours with 200mM KOH at 0.008mL/min to ensure good chromatography without baseline artifacts. Test the column performance under the conditions described in the QAR. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

IMPORTANT ***When making any tubing connections (column installation, replacing tubing etc), it is recommended to make these connections with the pump turned off. This will avoid any slippage of the ferrule under high pressure conditions.***

9.3 Column Storage

For short-term storage (< 1 week), the QAR Eluent is acceptable, for long-term storage (> 1 week), employ the storage solution (18mM NaOH) described on the QAR. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

9.4 Dionex Capillary CarboPac PA20 Column Cleanup

The Dionex Capillary CarboPac PA20 can be readily cleaned by rinsing the column with ~ 60 column volumes of 200 mM KOH. More stubborn contamination problems may necessitate a thorough column cleaning. Use the following steps to thoroughly clean the Dionex Capillary CarboPac PA20; use 0.008 mL/min to avoid over-pressurization of the column:

CAUTION ***When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Otherwise, contaminants that have accumulated on the guard column elute onto the analytical column causing irreversible damage. If in doubt, clean each column separately.***

A. Clean the Dionex Capillary CarboPac PA20 column with 1M KOH or NaOH for at least two hours.

B. Reconnect column to the cell and equilibrate the column with the desired initial conditions; test the column performance using the QAR standard and eluent.