



CarboPac PA200 Columns

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Product Manual

for

Dionex CarboPac PA200 Analytical Columns

3 × 250 mm, (Item #: 062896)

Dionex CarboPac PA200 Guard Columns

3 × 50 mm, (Item #: 062895)

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Revision History:

Revision 02, November, 2018, Added Column Care Section. Transcribed content into the latest template.

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.

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1. Introduction

The Thermo Scientific™ Dionex™ 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 Thermo Scientific Dionex, simple to reproduce, and made possible by the pH stability of the Dionex CarboPac columns. The Dionex CarboPac PA200 (3mm) 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 Dionex CarboPac PA 100 for the separation of neutral and anionic oligosaccharide mixtures. If desalting of collected oligosaccharides is desired, the Dionex CarboPac PA200 column is the column of choice because lower salt concentrations are required for elution compared with the Dionex CarboPac PA100.

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.

Resin Characteristics:

Particle Size:	.5 µm
Pore Size:	microporous (< 10 Å)
Cross-linking:	55%
Ion exchange capacity:	35µeq per 3x250 mm column

Latex Characteristics:

Functional Group:	quaternary ammonium functionalized latex
Latex Diameter:	43 nm
Latex Cross-linking:	6%

Typical Operating Parameters:

pH range:	0–14
Temperature Limit:	4–60 °C
Pressure Limit:	3500 psi
Organic Solvent Limit:	100% compatible for cleaning only
Typical eluents:	High purity water (18.2 megohm-cm), sodium hydroxide, sodium acetate

2. System Requirements and Installation

2.1 System Requirements

2.1.1 System Requirements for column Operation

The carbohydrate separations using the Dionex CarboPac PA200 columns are optimized for use with Dionex Ion Chromatography systems equipped with electrochemical detection. It is highly recommended to ensure that the systems used for carbohydrate analysis are metal-free. Metal ions from a metal system will contaminate the CarboPac column and may contaminate the working electrode. Running a CarboPac column on a metal system voids the column warranty.

2.1.2 Installation of Disposable Electrode into an ED Cell, pH-Ag/AgCl Reference Electrode or PdH Reference Electrode

The 0.002 in. thick Teflon gaskets included in each package of disposable electrodes must be used, otherwise the disposable electrode product warranty is void. In addition, the quadruple waveform must be used for carbohydrate analysis otherwise the product warranty is void. Always wear gloves when handling electrodes. Never touch the electrode surface. To install a disposable working electrode and reference electrode (pH-Ag/AgCl or PdH) refer to Product Manual for Disposable Electrodes Doc. No. 065040, ICS-6000 Ion Chromatography System Manual Doc. No. 22181 and User's Compendium for Electrochemical Detection Doc. No. 065340.

2.1.3 System Void Volume

When using Dionex CarboPac PA200 columns, for best performance, all of the tubing installed between the injection valve and detector should be 0.005" (Item # 044221) i.d. PEEK tubing for 3 mm columns. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

2.2 The Injection Loop

2.2.1 The 3 mm System Injection Loop, 10 - 50 μ L

For most applications on a 3 mm analytical system, a 10 – 50 μ L injection loop is sufficient. Generally, you should not inject more than 40 nanomoles of any analyte onto the 4 mm analytical column. Injecting larger amounts of an analyte can result in overloading the column which can affect the detection linearity. For low concentrations, larger injection loops can be used to increase sensitivity.

2.3 The Dionex CarboPac PA200 Guard Column

A Dionex CarboPac PA200 Guard Column is normally used with the Dionex CarboPac PA 200 analytical column. Retention times will increase by approximately 20% when a guard column is placed in-line before the analytical column under isocratic conditions. A guard column is utilized to prevent sample contaminants from eluting onto the analytical column. It is easier to clean or replace a guard column than an analytical column. Replacing the Dionex CarboPac PA200 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex CarboPac PA200 analytical column.

2.4 Installing the Dionex CR-ATC Trap Column for Use with Dionex EGC

For Dionex CarboPac PA200 applications using the Dionex EGC KOH cartridge, a Dionex CR-ATC Continuously Regenerated Trap Column (Item# 060477) should be installed at the Dionex EGC eluent outlet to remove trace level anionic contaminants from the carrier deionized water. See the Dionex CR-TC Product Manual (Document No. 031910) for instructions.

2.5 Eluent Storage

Dionex CarboPac PA200 columns are designed to be used with hydroxide eluent systems. Storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents).



NOTE

For assistance, contact Technical Support for Thermo Scientific Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

3. 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. Thermo Scientific cannot guarantee proper column performance when alternate suppliers of chemicals or lower purity water are utilized.

3.1 Deionized Water

The deionized water used to prepare eluents should be Type I reagent grade water with a specific resistance of 18.2 megaohm-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 Sodium Hydroxide

Use 50% w/w sodium hydroxide (Certified Grade, Fischer Scientific Item # UN 1824) for preparation.

3.3 Sodium Acetate

Thermo Scientific highly recommends the use of Dionex Sodium Acetate Reagent (Item # 059326) for carbohydrate analysis. Thermo Scientific cannot guarantee proper detection performance when different grades or alternate suppliers of sodium acetate are utilized.

4. Before You Start

4.1 The Most Important Rules

ALWAYS	use 50% NaOH solution rather than NaOH pellets to make eluents
ALWAYS	use dedicated glassware and disposable glass or plastic ware for volume adjustments.
ALWAYS	keep your NaOH eluent blanketed with helium or nitrogen. Prepare new NaOH eluent if left unblanketed for more than 30 minutes.
ALWAYS	use EGC-KOH generated eluent when possible to avoid any eluent preparation issues
ALWAYS	pull at least 40 mL of new eluent through the lines when changing eluent or adding fresh eluent. This will ensure that your fresh eluent is primed through the lines up to the pump heads.
ALWAYS	verify the equilibration time necessary prior to injection to avoid baseline issues or artifacts or to avoid unnecessary increase in total method time.
NEVER	go to the next step of the installation if the previous step has failed.
NEVER	start an installation with any of the check list items below missing
NEVER	use 'communal' filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials.
NEVER	use methanol or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily.
NEVER	run above 60°C or 5000 psi.

4.2 Initial Check List

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

- ☐ Laboratory water unit delivering 18.2 megaohm-cm water at the installation site.
- ☐ Vacuum pump available for use with the vacuum filtration units.
- ☐ Sterile packed Nalgene Filtration units (pore size: 0.2 μm , filtered material: Nylon), 1 L funnel size
- ☐ Inert gas cylinder (helium or nitrogen) with a regulator valve (ca. 0–200 psi at the low-pressure side) and the appropriate size adaptors plus tubing.
- ☐ Sterile-packed 10 mL and 25 mL disposable pipets and suitable pipeting bulbs or pumps.
- ☐ Disposable, plastic (PE) large-size (at least 20 mL) syringe for priming the pump.
- ☐ Plastic eluent bottles.

5. Preparation of Eluents and Standards

**NOTE**

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

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

5.1 Eluent E1: 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. Note: Degassing by vacuum filtration through a 0.2 µm filter is a good alternative to degassing in a sonicator. Cap each bottle after the degassing step and minimize the length of time the bottle is opened to the atmosphere.

5.2 Eluent E2: 1 M Sodium Hydroxide

**NOTE**

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

Always store degassed NaOH eluents in plastic eluent bottles blanketed with helium or nitrogen to avoid carbon dioxide contamination from the air. Carbonate in the eluent can significantly reduce retention times for carbohydrates.

5.2.1 Sodium Hydroxide Eluent Concentration

Gravimetric Method

When formulating eluents from 50% sodium hydroxide, Thermo Scientific recommends weighing out the required amount of 50% sodium hydroxide. Use the assayed concentration value from the sodium hydroxide bottle.

Example: To make 1 L of 1 M NaOH use 80.02 g of 50% sodium hydroxide:

$$\text{For 1 M: } \frac{1 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 80.02 \text{ g diluted to 1 L}$$

Volumetric Method

Although it is more difficult to make precise carbonate-free eluents for gradient analysis volumetrically, you may choose to use the following formula to determine the correct volume of 50% sodium hydroxide to be diluted.

$$g = dvr$$

Where g = weight of sodium hydroxide required (g)
 d = density of concentrated solution (g/mL)
 v = volume of the 50% sodium hydroxide required (mL)
 r = % purity of the concentrated solution

Example: To make 1 L of 1M NaOH use 52.3 mL of 50% sodium hydroxide:

$$\text{For 1 M: } \frac{1 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53^* \text{ g/mL}} = 52.3 \text{ mL diluted to 1 L}$$

* This density applies to 50% NaOH. If the concentration of the NaOH solution is significantly different from 50%, the gravimetric method should be used instead.

Sodium Hydroxide Eluents

Dilute the amount of 50% (w/w) NaOH Reagent specified in [Table 1](#). Prepare CarboPac Eluents with degassed, deionized water (18.2 megohm-cm) to a final volume of 1,000 mL using a volumetric flask. Avoid the introduction of carbon dioxide from the air into the aliquot of 50% (w/w) NaOH bottle or the deionized water being used to make the eluent. Do not shake the 50% (w/w) NaOH bottle or pipette the required aliquot from the top of the solution where sodium carbonate may have formed.

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

Eluent Concentration (M)	NaOH (50%) (g)	NaOH (50%) (mL)
0.15	12.0	7.8
0.5	40.0	26.2
0.8	64.0	41.8
1.0	80.0	52.3

5.3 Eluent E3: 0.1M Sodium Hydroxide / 0.1M Sodium Acetate

To maintain baseline stability, it is important to 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 eluents as follows:

Eluent A: x mM NaOH
 Eluent B: x mM NaOH, y mM NaOAc

To make one (1) liter of 0.1M sodium hydroxide/ 0.1M sodium acetate, dispense approximately 800 mL of DI water into a 1 L volumetric flask. Vacuum degas for approximately 5 minutes. Add a stir bar and begin stirring. Weigh out 8.2 g anhydrous, crystalline sodium acetate (Thermo Scientific Dionex Sodium Acetate Reagent, Item # 059326). Add the solid acetate steadily to the briskly stirring water to avoid the formation of clumps which are slow to dissolve. Once the salt has dissolved, remove the stir bar with a magnetic retriever. Add DI water to the flask to bring the volume to the 1 L mark.

Vacuum filter the solution through a 0.2 µm Nylon filter. This may take a while as the filter may clog with insoluble material from the sodium acetate. Using a plastic tip volumetric pipet, measure 5.2 mL of 50% (w/w) sodium hydroxide solution. Dispense the sodium hydroxide solution into the acetate solution about 1 inch under the surface of the acetate solution. The eluent should be kept blanketed under helium or nitrogen at 34 to 55 kPa (5–8 psi) at all times, and last about 1 week.

5.4 Eluent E4: 0.1M Sodium Hydroxide / 0.5 M Sodium Acetate

To maintain baseline stability, it is important to 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 eluents as follows:

Eluent A: x mM NaOH
 Eluent B: x mM NaOH, y mM NaOAc

To make one (1) liter of 0.1M sodium hydroxide/ 0.5M sodium acetate, dispense approximately 800 mL of DI water into a 1 L volumetric flask. Vacuum degas for approximately 5 minutes. Add a stir bar and begin stirring. Weigh out 41.0 g anhydrous, crystalline sodium acetate (Thermo Scientific Dionex Sodium Acetate Reagent, Item # 059326). Add the solid acetate steadily to the briskly stirring water to avoid the formation of clumps which are slow to dissolve. Once the salt has dissolved, remove the stir bar with a magnetic retriever. Add DI water to the flask to bring the volume to the 1 L mark.

Vacuumfilter the solution through a 0.2 µm Nylon filter. This may take a while as the filter may clog with insoluble material from the sodium acetate. Using a plastic tip volumetric pipet, measure 5.2 mL of 50% (w/w) sodium hydroxide solution. Dispense the sodium hydroxide solution into the acetate solution about 1 inch under the surface of the acetate solution. The eluent should be kept blanketed under helium or nitrogen at 34 to 55 kPa (5–8 psi) at all times, and last about 1 week.



NOTE

Thermo Scientific recommends the use of dedicated glassware, pipets and filtration apparatus for exclusive use in the preparation of carbohydrate eluents.

5.5 Sample Preparation

The Dionex CarboPac columns are strong anion exchangers. Thus, the normal caveats applicable to ion exchange chromatography apply to these columns. 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 Dionex CarboPac columns (e.g. chloride, carbonate, phosphate, etc.). It is best to avoid extremes of sample pH (especially extremely acidic 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.

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

When using pulsed electrochemical detection (PED) 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 Dionex CarboPac columns are extremely rugged, it is often worthwhile to analyze an aliquot of the sample directly, without any pre-column cleanup.

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 Web site at www.thermoscientific.com.

5.6 Verification of System Cleanliness

Prepare a new set of eluents as described in [Sections 5.1 through 5.4](#) and fill the eluent bottles. Set the eluent composition to 100% for each eluent line and draw out at least 40 mL of eluent from each eluent line.

5.6.1 System Background Check

This procedure is performed using the conditions of the test chromatogram. Make sure that

- A. the cell is not yet on,
- B. the pump is pumping 100 mM NaOH, 50 mM NaOAc or 200 mM KOH at 0.5 mL/min,
- C. a length of yellow tubing is installed between the injector and detector cell to generate ~1000 psi backpressure,
- D. the columns are not yet installed.

Confirm that the pH is between 12.8 and 13.4. With the pH within this range, turn on the cell using the quadruple waveform (See Table 3, Section 6.3 Disposable Electrode Manual, document number 065040) and begin monitoring the background signal from the control panel for at least 30 minutes. Confirm that the background is < 50 nC. If the background > 50 nC or the pH is out of range, see the “Troubleshooting” section at the end of this manual.

5.6.2 Verification of Column Cleanliness

Install the Dionex CarboPac PA200 column set only after the initial system test determines a background level within the specified range. A premature installation on a contaminated system will cause delays during the column equilibration.

The Dionex CarboPac PA200 is shipped in 100 mM NaOH + 100 mM NaOAc. Any column that is stored long-term should be stored in the same solution. Equilibrate the column set by performing two blank injections (DI water) under the test chromatogram conditions, including the column regeneration and re-equilibration steps.

Once the columns are equilibrated, inject a system suitability standard such as the column's QAR standard, to establish the performance of the column at start-up. This chromatogram can then be referred to when troubleshooting your system. Once you obtain your expected chromatography, you are ready to proceed to running your application.

Dionex recommends that the system suitability standard be run whenever you reinstall a column after long-term storage.

5.7 Carbohydrate Sialylated N-linked Alditol Standard

The Dionex OligoStandard, Sialylated N-Linked Alditols, Item # 043164 contains 25 nmol oligosaccharides purified from bovine fetuin. Dilute the standard prior to use, by adding a known volume of DI water (for example 1 mL). An injection of 25 μ L will then correspond to an injection of 625 pmol of the standard. Dionex recommends running this standard every time a new column is installed and subsequently anytime it becomes necessary to troubleshoot your system.

6. Applications

The CarboPac PA200 columns have been designed for isocratic or gradient separation using sodium hydroxide eluents up to a concentration of 1 M with sodium acetate gradients. 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 eluent 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.

6.1 Glycoprotein Characterization and Quality Control

Most secreted and cell surface proteins are glycosylated. They typically have multiple glycosylation sites, each site containing several structures. Therefore, a protein can exist in many glycoforms. Glycans present on the protein can have a profound effect on the protein structure, and on biological function. Therefore, the study of glycans is an important part of protein characterization. Biopharmaceuticals, including glycoproteins require thorough characterization and analysis to meet European, US and Japanese regulatory standards for New Drug Approval as defined by the International Conference for Harmonization (ICH) process. Even at clinical trial stage the national requirements for conduct of clinical studies necessitate clear identification, quality and purity of the investigational drug (FDA guidance documents for IND, MCA guidelines for CTX).

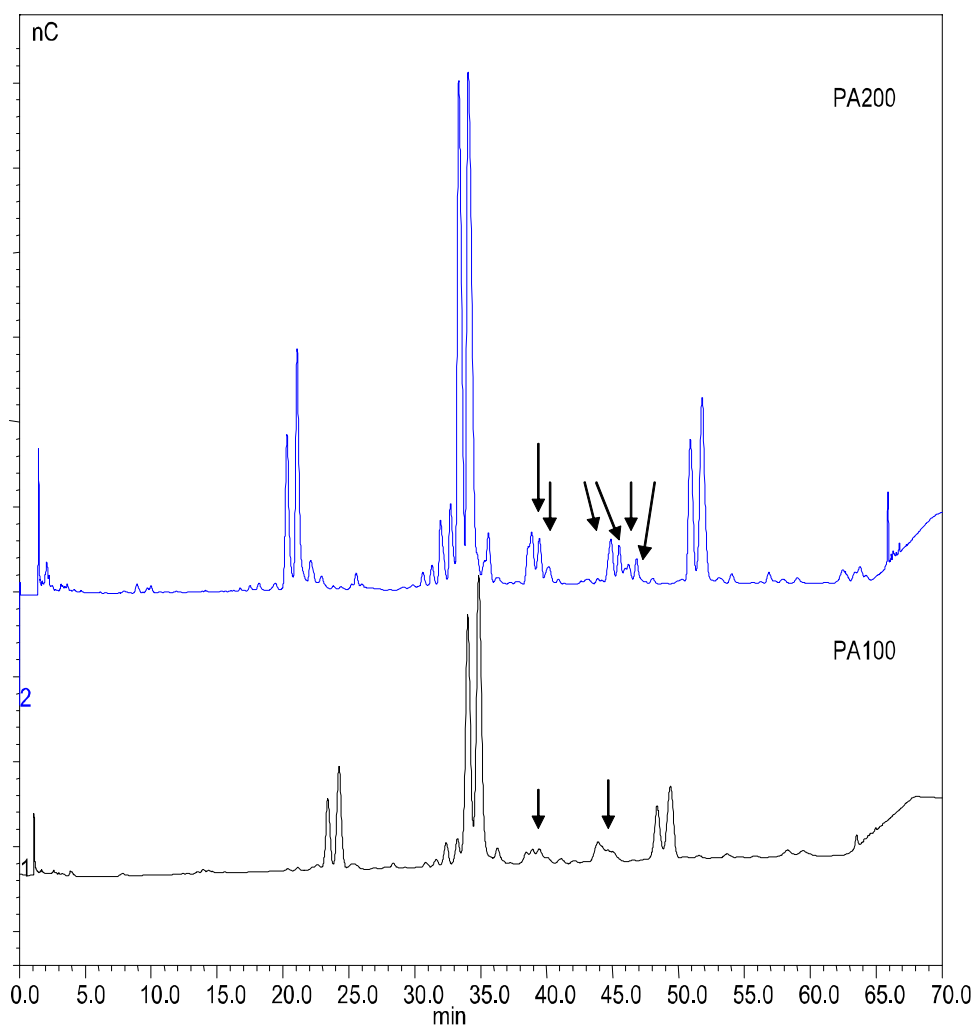
6.1.1 Fetuin Oligosaccharide Alditol Profiling

The high resolution of the CarboPac PA200 is exemplified in the following example. CarboPac columns separate mono-, oligo and polysaccharides on the basis of fine structural differences in branching, linkage isomerism, anomericity and sialylation. In the fetuin oligosaccharide alditol standard shown below, peaks are separated according to branching, sialylation and linkage isomerism. The disialylated biantennary peaks are eluted before the trisialylated triantennary peaks which are eluted before the tetrasialylated tetraantennary peaks. In addition, within each grouping, the α 2-6 isomer is eluted before, and well resolved from, the α 2-3 isomer.

The Dionex OligoStandard, Sialylated N-Linked Alditols, P/N 043064 contains 25 nmol oligosaccharides purified from bovine fetuin. Dilute the standard prior to use, by adding a known volume of DI water (for example 1 mL; a 25 μ L injection corresponds to 625 pmol of oligosaccharide). Dionex recommends running this standard every time a new column is installed and subsequently anytime it becomes necessary to troubleshoot your system.

Figure 1 Fetuin Oligosaccharide Profiles: CarboPac PA200 vs. CarboPac PA100

Columns:	CarboPac PA200 (3 x 250 mm) and CarboPac PA100 (4 x 250 mm)
Gradient:	PA 200: 20–150mM NaOAc in 100 mM NaOH over 1 h PA100: 20–200 mM NaOAc in 100 mM NaOH over 1 h
Flow Rate:	PA200: 0.5 mL/min PA100: 1mL/min
Detection:	Pulsed amperometry, QP waveform, gold electrode
Samples:	Fetuin oligosaccharide alditol std



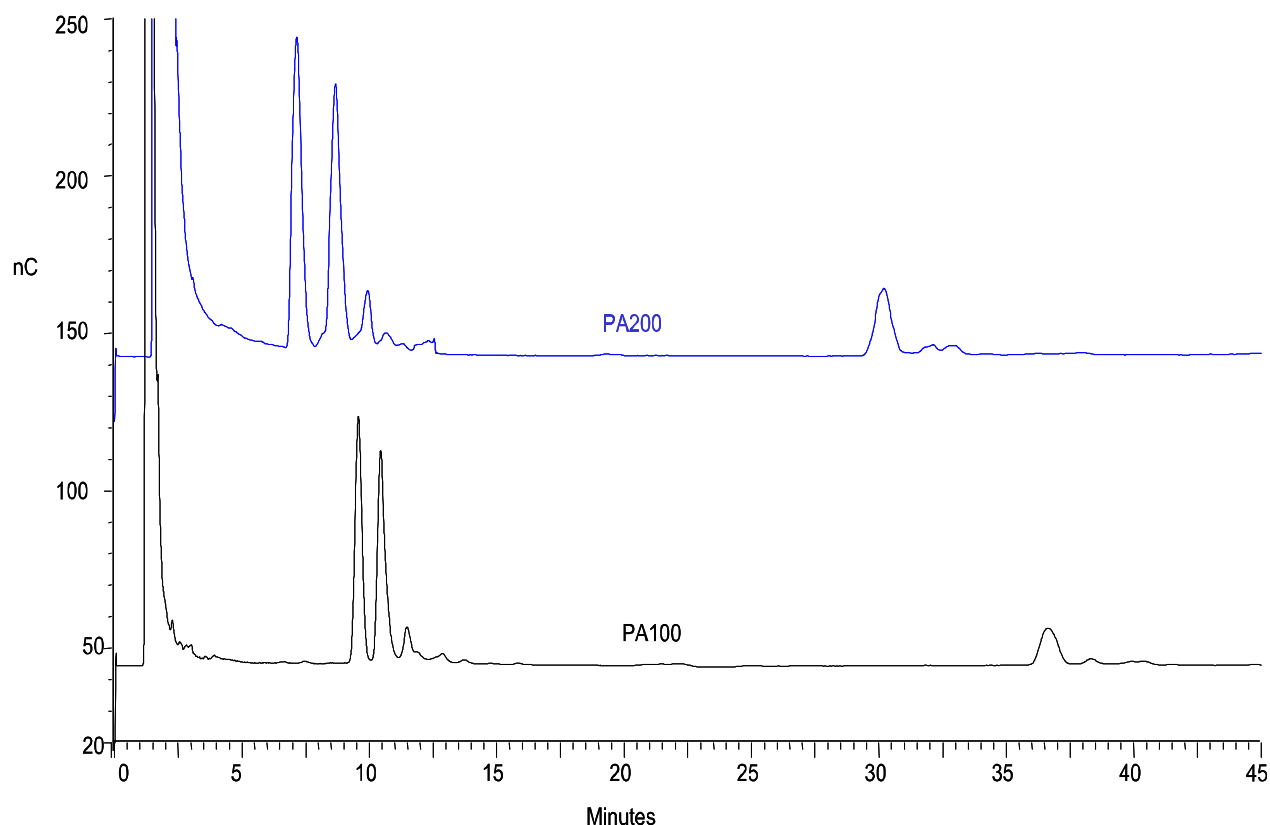
6.1.2 Antibodies Separations

Today, monoclonal antibodies comprise the cornerstone of numerous medical and scientific procedures and thus are produced routinely for both medical and research applications. In the medical world, they are used as diagnostics, to detect cancers or infection by certain bacteria or viruses; as vaccines, to boost the body's immune response; and as therapeutics, to target foreign bacteria, viruses or cancerous cells.

Monoclonal Antibody Profile

Figure 2 CarboPac PA200 vs. CarboPac PA100 Separation of Monoclonal Antibody N-linked Oligosaccharides

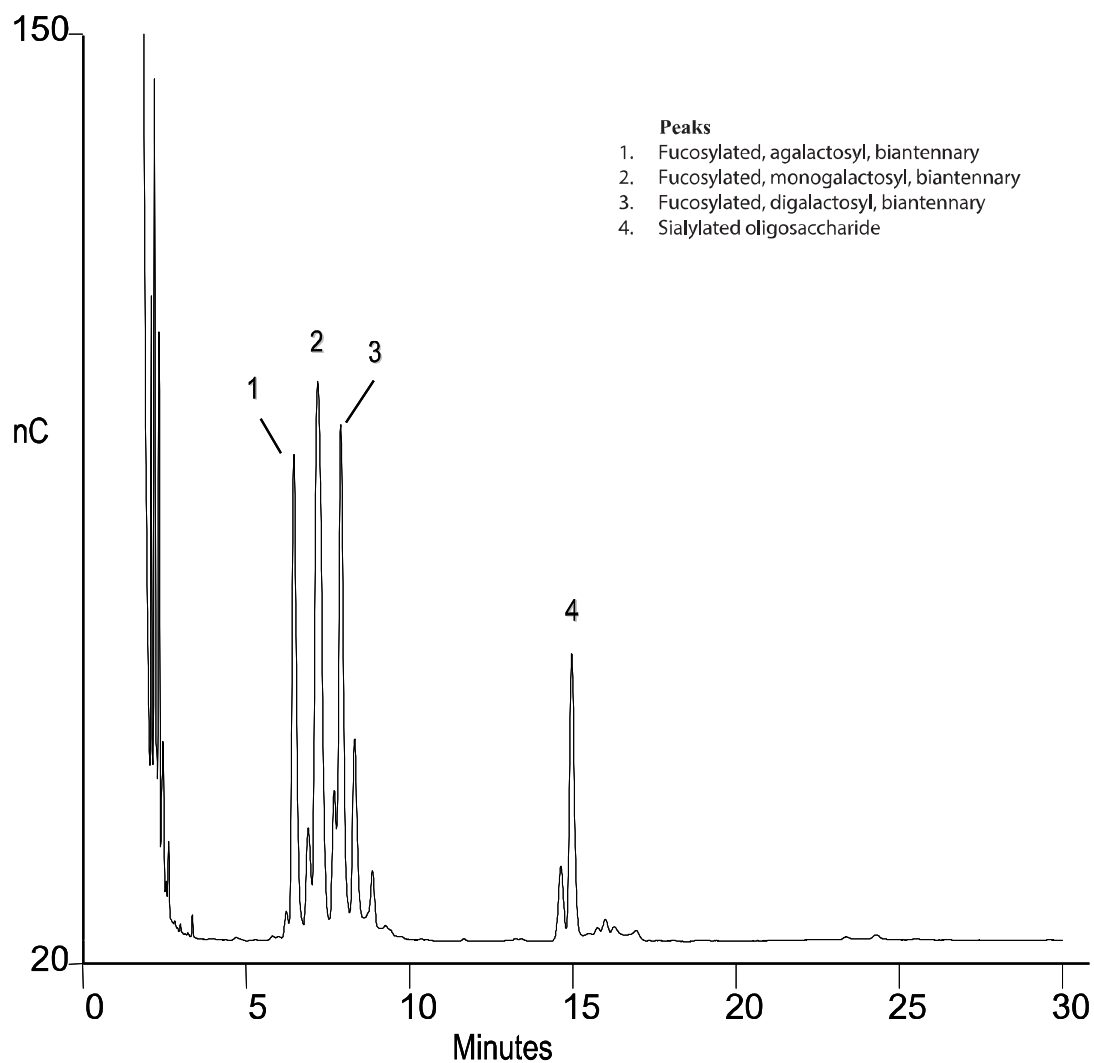
Columns:	CarboPac PA200 (3 x 250 mm) and CarboPac PA100 (4 x 250 mm)
Gradient:	10 mM or 20 mM – 150 mM NaOAc in 100 mM NaOH over 60 min
Flow Rate:	
PA200:	0.5 mL/min
PA100:	1 mL/min
Detection:	Pulsed amperometry, QP waveform, gold electrode
Sample:	48 h PNGase F digest of monoclonal antibody (100 µg)



Polyclonal Antibody Profile

Figure 3 Polyclonal Antibody Profile

Column: CarboPac PA200 and guard
Eluent: Sodium acetate gradient in 100 mM sodium hydroxide
Flow Rate: 0.5 mL/min
Detection: Pulsed amperometry, gold electrode
Sample: PNGase-F digest of hIgG



6.2 Profiling of Complex Plant Carbohydrates

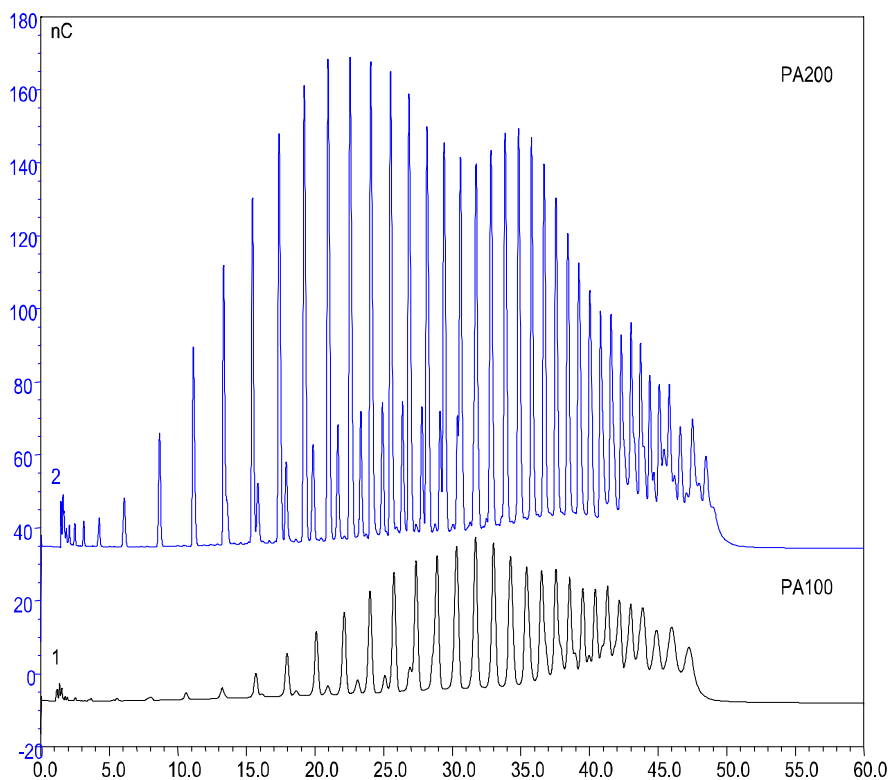
6.2.1 Profiling of Inulins

Inulins and fructooligosaccharides (FOS) are increasingly being used as functional food ingredients. Chain length distribution profiles of commercial products such as those derived from inulin can be determined by using HPAE-PAD with gradient elution. By adjusting the initial gradient profile, smaller oligofructose chains can be distinguished from the inulin chains and separations up to and exceeding DP80 are possible.

Quantification of individual inulin oligomers requires a knowledge of the PAD response factors. These have been determined for the Fn and GFn oligomers from DP 2 to 8 and from DP 11 to 17, by isolation of 5–20 mg quantities of the pure oligomers using preparative scale RP-18 HPLC (see reference 9, section 8.6.2). Unfortunately, pure fractions in the range DP 6–10 could not be obtained due to coelution problems. For DP >17, the response factor appears to change very slowly with increasing DP, and relative response factors can be obtained by interpolation. Semi-preparative scale (9- and 22- mm i.d.) CarboPac PA1 and PA100 columns are available and could be used for isolation of mg quantities of all the inulin oligomers. A method that both identifies and quantifies FOS products, directly, is detailed in reference 8 (section 8.6.2).

Figure 4 Inulin Profiles: CarboPac PA200 vs. CarboPac PA100

Columns:	CarboPac PA200 (3 x 250 mm) and CarboPac PA100 (4 x 250 mm)
Gradient:	120–320 mM NaOAc in 100 mM NaOH over 40 min
Flow Rate:	PA200: 0.5 mL/min PA100: 1 mL/min
Detection:	Pulsed amperometry, QP waveform, gold electrode
Samples:	Inulin from chicory (Sigma)

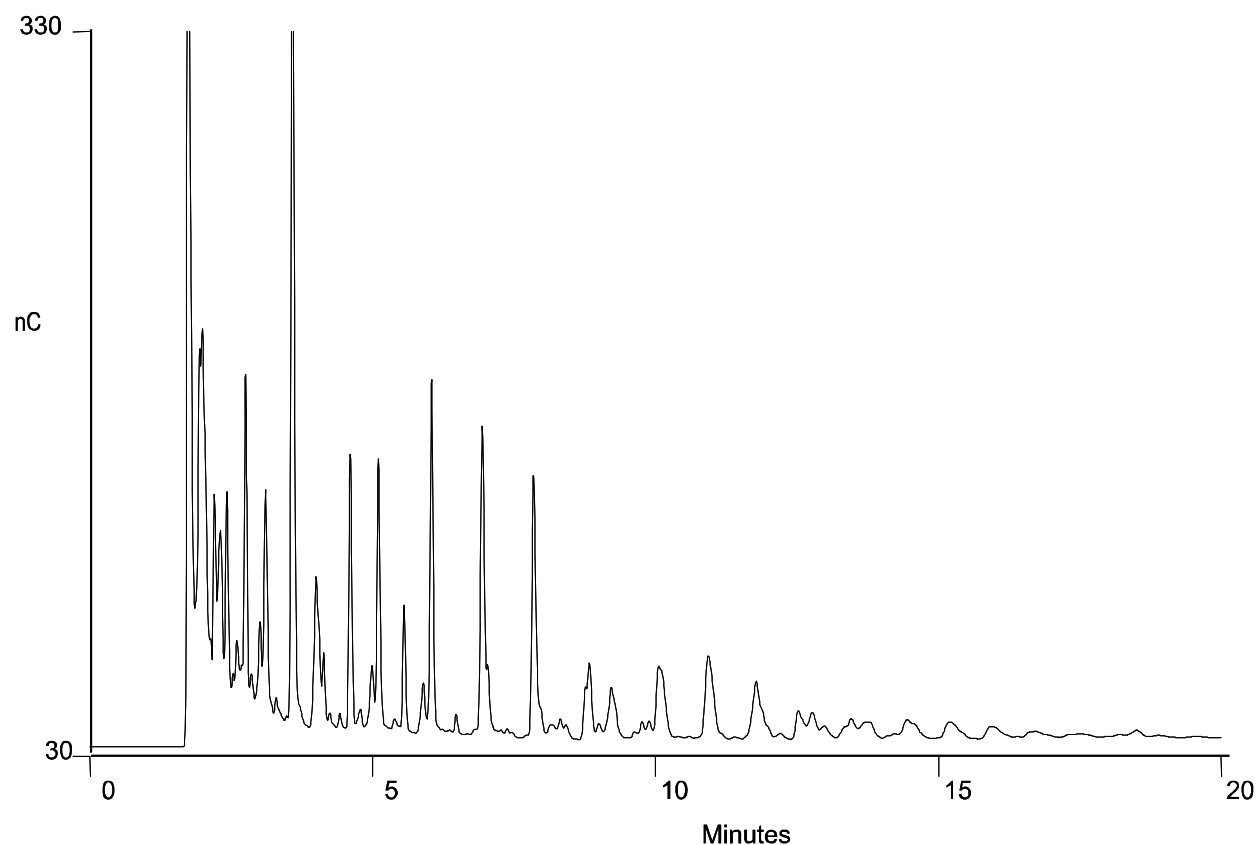


6.2.2 Amylopectins

HPAE-PAD with gradient elution has been used for structural studies on starch-derived materials such as amylopectins since the chain length distribution is an important parameter for characterizing the molecular structure. These distributions can be used as fingerprints for the amylopectin source.

Figure 5 Amylopectins Separated on the CarboPac™ PA200 Column

Column:	CarboPac PA200 and guard
Eluent:	Sodium acetate gradient in 100 mM Sodium hydroxide 70 to 300 mM in 30 min
Flow Rate:	0.5 mL/min
Temperature:	30 °C
Injection Vol:	5 µL from 10 µL loop
Detection:	Pulsed amperometry, gold electrode
Sample:	Red Hook Amber Ale 1:50 dilution

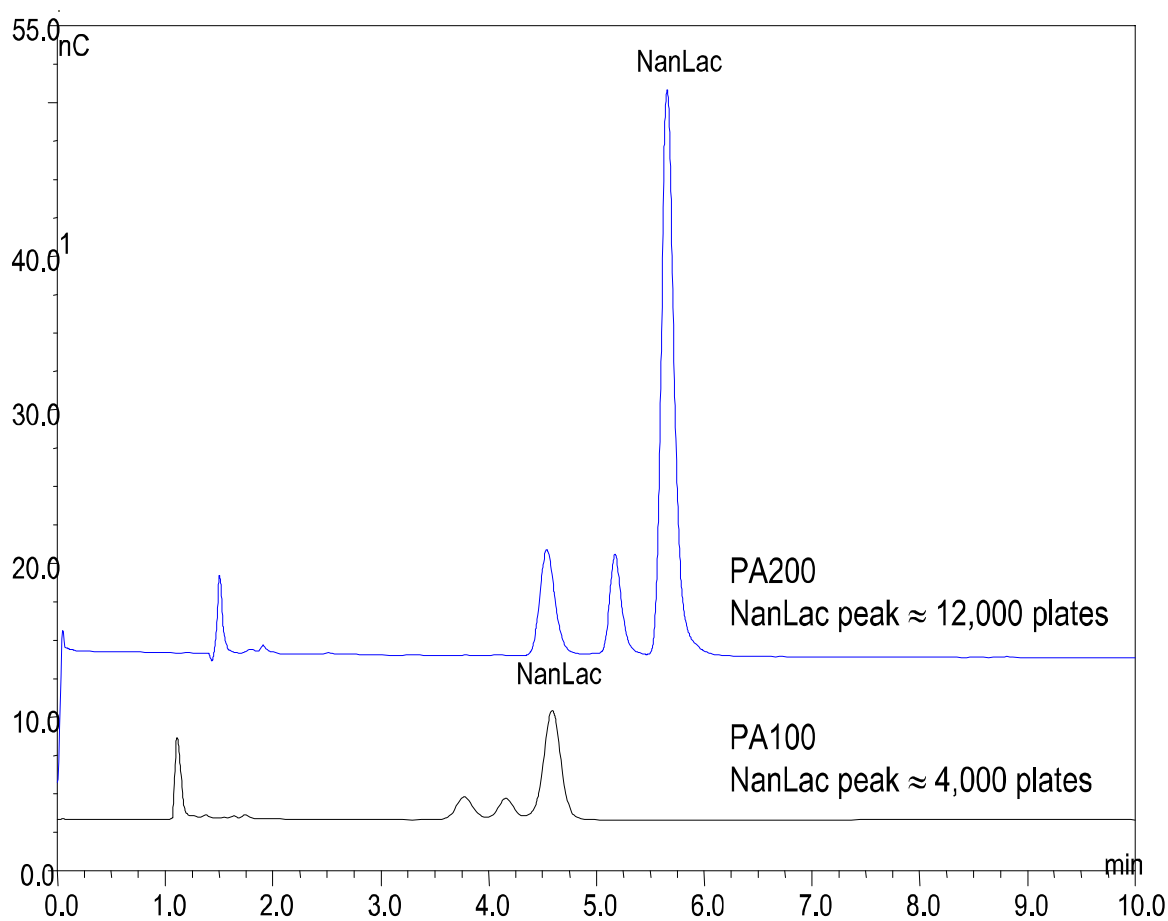


6.3 Sialyl Lactose (N-Acetylneuraminy-Lactose)

The following chromatography is used as the quality assurance test for the CarboPac PA200 column. Every CarboPac PA200 column is tested with respect to sialyl lactose peak efficiency. Chromatography of sialyl lactose on the CarboPac PA200 column exhibits greater than a twofold increase in peak efficiencies compared to chromatography of sialyl lactose on the CarboPac PA100 column.

Figure 6 Chromatography of NanLac on the CarboPac PA200 exhibits > 2x the peak efficiencies compared to the CarboPac PA100

Columns:	CarboPac PA200 (3 x 250 mm) or CarboPac PA100 (4 x 250 mm)
Gradient:	
	PA200: 100 mM NaOH, 50 mM NaOAc isocratic
	PA100: 100 mM NaOH, 100 mM NaOAc isocratic
Flow Rate:	
	PA200: 0.5 mL/min
	PA100: 1 mL/min
Detection:	Pulsed amperometry, QP waveform, gold electrode
Sample:	NanLac Std

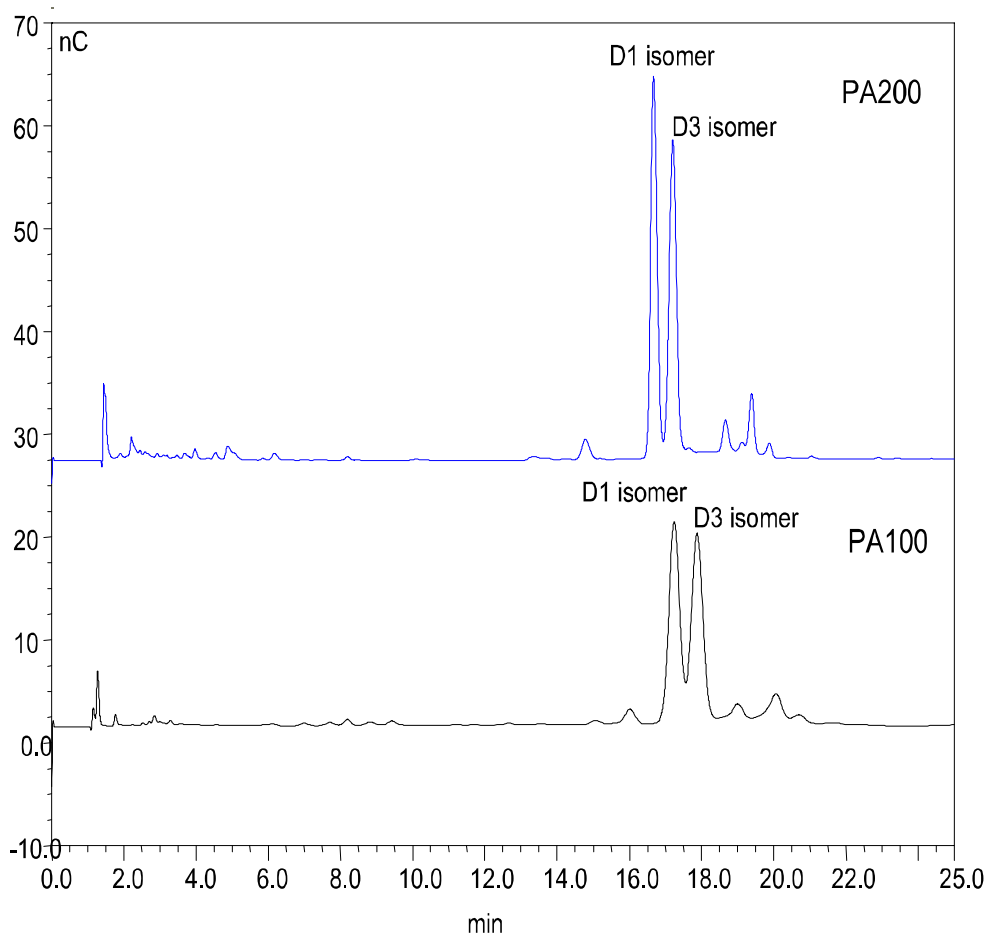


6.4 Separation of Mannose 7 D1, D3 Isomers

High mannose isomers that frequently would not be distinguishable by other techniques are well separated on both the CarboPac PA200 and PA100 columns. The separation of these isomers is better on the CarboPac PA200 column.

Figure 7 CarboPac PA200 vs. CarboPac PA100 Separation of Mannose 7 D1, D3 Isomers

Columns:	CarboPac PA200 (3 x 250 mm) or CarboPac PA100 (4 x 250 mm)
Gradient:	0–200 mM NaOAc in 100 mM NaOH over 110 minutes
Flow Rate:	PA200: 0.5 mL/min PA100: 1 mL/min
Detection:	Pulsed amperometry, QP waveform, gold electrode
Sample:	Mannose 7 isomers (Dextra Labs):



6.5 Separation of Neutral N-Linked Oligosaccharides

N-linked glycoproteins may exhibit micro-heterogeneity with respect to the N-linked oligosaccharides that may be present. Neutral N-linked oligosaccharides released from glycoproteins could be of the complex or high mannose variety. A comparison of chromatography of neutral and high mannose oligosaccharides on the CarboPac PA 200 column compared to the CarboPac PA 100 column shows that the separation window is wider for the CarboPac PA 200 column. Early eluting oligosaccharides on the CarboPac PA 100 column tend to elute earlier on the CarboPac PA 200 column.

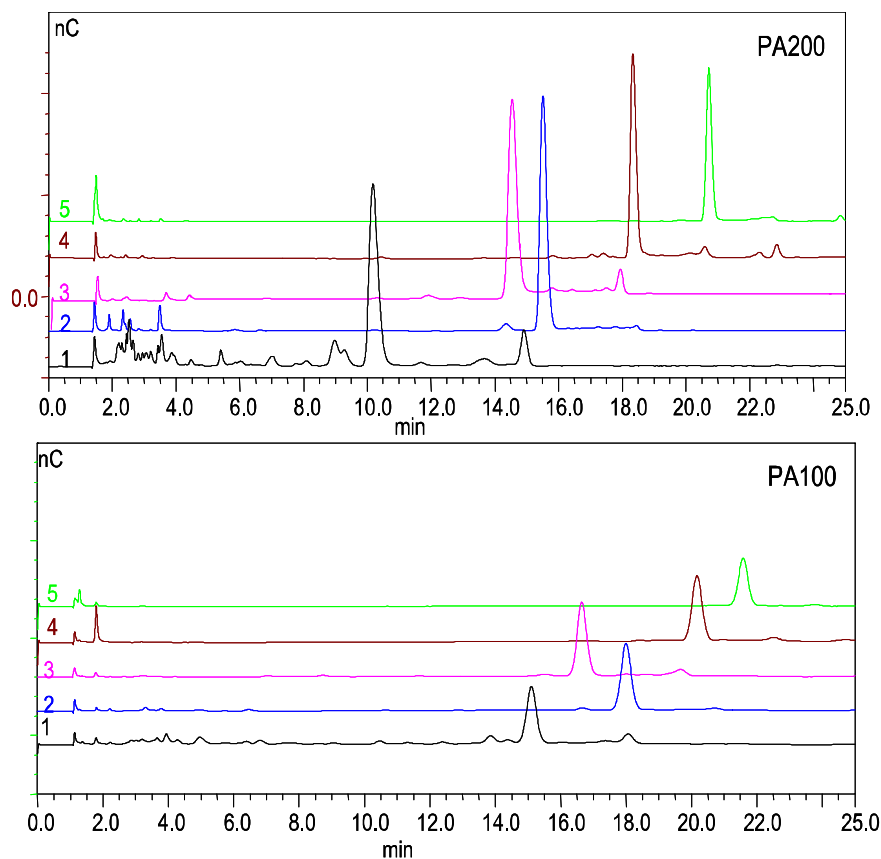
6.5.1 Complex Neutral Structures

Figure 8 CarboPac PA200 vs. CarboPac PA100 Separation of Neutral N-linked Oligosaccharides (complex neutral structures)

Columns: CarboPac PA200 (3 x 250 mm) or CarboPac PA100 (4 x 250 mm)
 Gradient: 0–200 mM NaOAc in 100 mM NaOH over 110 minutes
 Flow Rate: PA200: 0.5 mL/min
 PA100: 1 mL/min
 Detection: Pulsed amperometry, QP waveform, gold electrode

Samples:

1. Asialo agalacto biantennary standard
 2. Asialo galacto biantennary standard
 3. Asialo galacto fuco biantennary standard
 4. Asialo triantennary standard
 5. Asialo tetraantennary standard
- (All standards are from Dextra Labs)



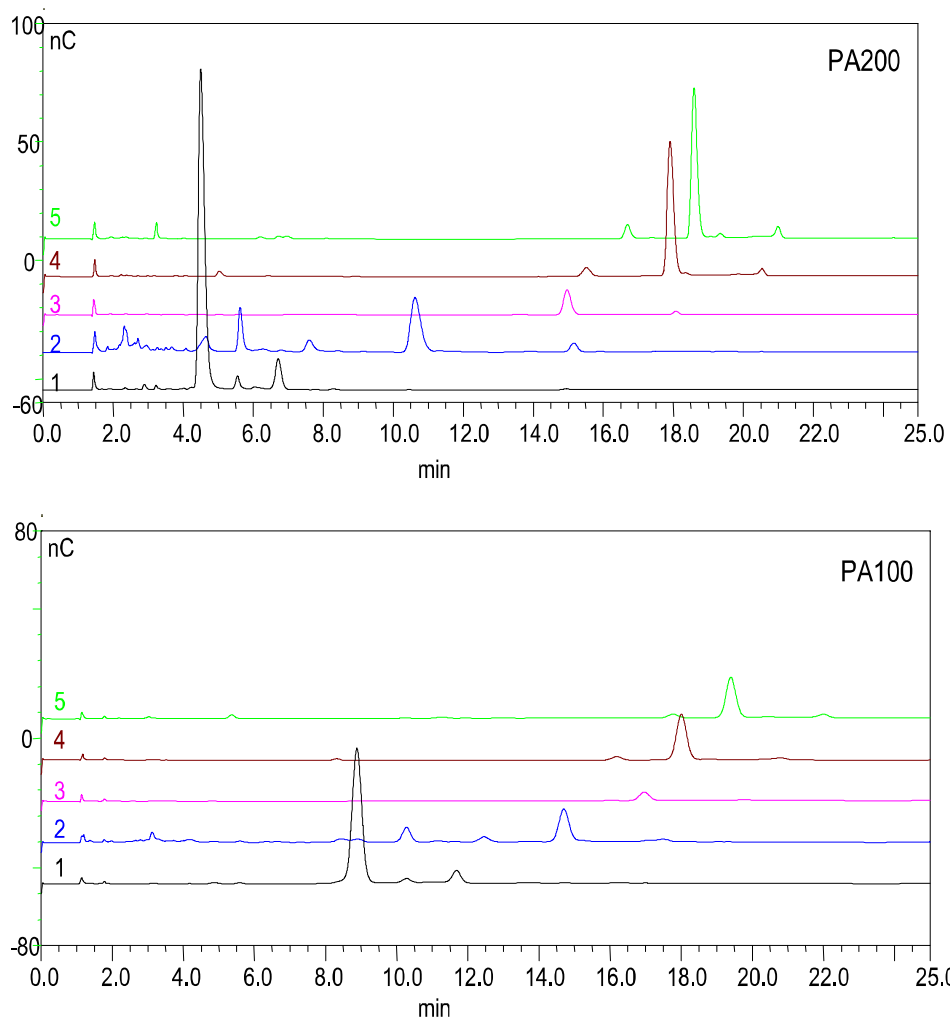
6.5.2 High Mannose Structures

Figure 9 CarboPac PA200 vs. CarboPac PA100 Separation of Neutral N-linked Oligosaccharides (high mannose structures)

Columns: CarboPac PA200 (3 x 250 mm) or CarboPac PA100 (4 x 250 mm)
 Gradient: 0–200 mM NaOAc in 100 mM NaOH over 110 minutes
 Flow Rate: PA200: 0.5 mL/min
 PA100: 1 mL/min
 Detection: Pulsed amperometry, QP waveform, gold electrode

Samples:

1. mann 3 standard
 2. mann 5 standard
 3. mann 6 standard
 4. mann 7 standard
 5. mann 8 standard
- (All standards are from Dextra Labs)



7. Troubleshooting Guide

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Dionex CarboPac columns. For more information on problems that originate with the Ion Chromatograph (IC), refer to the Troubleshooting Guide in the appropriate operator's manual. Remember that some of the problems may be related to parts of your experimental protocol (sample contamination, imprecision during sample transfer, problems during peptide or protein hydrolysis, etc.). The following text should help you to locate and eliminate problems traceable to the carbohydrate hardware and chemistries. It also provides a selection of cleanup and reconditioning procedures that have been found effective by many users.

For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

7.1 High Back Pressure

7.1.1 Finding the Source of High System Pressure

Total system pressure for the Dionex CarboPac Guard plus the Dionex CarboPac Analytical Column when using the test chromatogram conditions should be close to the pressure listed in the QAR. If CarboPac guard and analytical column is being installed on the system, column pressure will increase by approximately 20% as compared to pressure listed in the QAR for each column type. If the total system pressure is much higher than expected, it is advisable to determine the cause of the high system pressure.

- A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the pump flow rate if necessary with an analytical balance.
- B. Determine which part of the system is causing the high pressure. High pressure could be due to a plugged tubing or tubing with collapsed or pinched walls, an injection valve with a clogged port, a column with particulates clogging the bed support, a clogged High-Pressure In-Line Filter, or the detector cell.

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 200 psi. (unless a backpressure coil has been installed between the pump outlet and the injection valve in which case, first disconnect the eluent line from the pump to the backpressure coil). Continue adding system components (backpressure coil (if present), injection valve, column(s), suppressor and detector) one by one, while monitoring the system pressure. The pressure should increase by the sum of the measured pressures of the individual guard and analytical columns (see product QAR) when the CarboPac Guard and Analytical columns are connected.

7.1.2 Replacing Column Bed Support Assemblies

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the Ship Kit.

- A. Disconnect the column from the system.
- B. Carefully unscrew the inlet (top) column fitting. Use two open-end wrenches.
- C. Remove the bed support. Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you do not scratch the walls of the end fitting. Discard the old bed support assembly.

- D. Place a new bed support assembly (provided with each analytical column) into the end fitting. 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. Drop the bed support assembly into the end fitting, making sure that the bed support assembly is centered at the bottom of the end fitting. Wrap the end fitting gently on a hard surface to reorient the bed support assembly as necessary in order to properly situate the bed support assembly in the end fitting.



CAUTION

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct 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 the end fitting.

- E. While holding the column in an inverted configuration, tighten the end fitting back onto the column. Tighten it finger-tight, then an additional 1/4 turn (25 in-lb). Tighten further only if leaks are observed.
- F. Reconnect the column to the system and resume operation.

7.1.3 Filter Eluent

Eluents containing particulate material or bacteria may clog the column inlet bed support. Filter eluents through a 0.45 µm Nylon or PTFE filter.

7.1.4 Filter Samples

Samples containing particulate material may clog the column inlet bed support. Filter samples through a 0.45 µm filter prior to injection.

7.2 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 >50 nC with 10 mM sodium hydroxide at 0.5 mL/min and 30°C using the quadruple waveform indicates one of the following possibilities:

- A. Incorrect detection parameters.
Verify that Ag/AgCl is specified as a reference electrode. Check all values of waveform in program against those in the Disposable Electrode Manual. If the pH reading with 10 mM NaOH or KOH is above 13.2 replace the reference electrode.
- B. Compromised working electrode surface.
Briefly install a new working electrode and check the background as above. If the reading remains > 50 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.
- C. Column contamination: Remove the column set from the system first and replace it with a length of yellow 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 [Appendix A, “CarboPac PA200 Column Care”](#).
- D. Water contamination: Prepare eluents using a fresh ultra pure water from another source. If the background is reduced, investigate the source of contamination in the original source of water.

- E. **System Contamination:** If the background remains high even with fresh water and without the column, carry out the 2 M sodium hydroxide rinse. In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA200 QAR eluent is 50-70 nC. If the background is much higher, determine the cause of high background.

7.2.1 Preparation of Eluents

- A. Make sure that the eluents are made correctly.
- B. Make sure that the eluents are made from chemicals with the recommended purity.
- C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 megohm-cm or greater.

7.2.2 CR-ATC Column

- A. When using eluent generator (EGC-KOH) to generate eluent, install a Dionex CR-ATC Anion Trap Column.
- B. If the background is elevated due to contamination of the Dionex CR-ATC, please refer to Sections 5.3 and 6, in the Dionex CR-ATC Product Manual (Document No. 031910) for corrective action.

7.2.3 A Contaminated Guard or Analytical Column

- A. Remove the columns from the system.
- B. Install a back-pressure coil that generates approximately 2000 psi and continue to pump eluent. If the background decreases, the column(s) is (are) the cause of the high background.
- C. To eliminate downtime, clean or replace the analytical column at the first sign of column performance degradation. Clean the column as instructed in, “[Appendix A, CarboPac PA200 Column Care](#)”.

7.3 Poor Resolution

One of the unique features of Dionex CarboPac columns is the fast equilibration time in gradient applications from the last eluent (high ionic strength) to the first eluent (low ionic strength). The actual equilibration time depends on the ratio of the strongest eluent concentration to the weakest eluent concentration and application flow rate. Typically, equilibration times range from 10 to 15 minutes at 1 mL/min.

If increased separation is needed for early eluting peaks, reduce the initial eluent concentration.

Due to different system configurations, the gradient profile may not match the gradient shown in example applications in the product column manual. Gradient conditions can be adjusted to improve resolution or to adjust retention times either by changing the gradient timing or by changing the initial and/or final eluent concentration.

- A. Keep the eluent concentrations constant and adjust the gradient time. This is the simplest way to compensate for total system differences if resolution is the problem.
- B. Change the initial and/or final eluent concentration and adjust the gradient time. This approach requires more time to develop and more knowledge in methods development. The advantage is that it allows a method to be tailored for a specific application, where selectivity, resolution, and total run time can be optimized. Be aware poor peak resolution can be due to any or all of the following factors.

7.3.1 Loss of Column Efficiency

- A. Check to see if headspace has developed in the guard or analytical column. This is usually due to improper use of the column such as exposing it to high pressures. Remove the column's inlet end fitting (see Section 7.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body all the way to the top, the column must be replaced.
- B. Extra-column effects can result in sample band dispersion, making the peaks' elution less efficient. Make sure you are using PEEK tubing with an ID of no greater than 0.010" for 4 mm systems or no greater than 0.005" for 2 mm systems to make all eluent liquid line connections between the injection valve and the detector cell inlet. Cut the tubing lengths as short as possible. Check for leaks. For capillary systems, only use precut tubing of the same type.
- C. If tubing is not connected properly from the inlet and outlet of the column, it can cause low efficiency. When installing CarboPac columns, it is recommended to turn off the pump while connecting the column inlet and the column outlet to the detector. This will avoid any slippage of the ferrule under high pressure conditions which can cause low peak efficiencies.

7.3.2 Shortened Retention Times



NOTE

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast.

- A. Check the flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using an analytical balance.
- B. Check to see if the eluent compositions and concentrations are correct. An eluent that is too concentrated will cause the peaks to elute faster. Prepare fresh eluent.



NOTE

If you are using a gradient pump to proportion the eluent, components from two or three different eluent reservoirs, the resulting eluent composition may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.

- C. Column contamination can lead to a loss of column capacity. Highly retained contaminant ions will occupy a portion of the anion exchange sites limiting the number of sites available for retention of analyte ions. Refer to "[Appendix A, CarboPac PA200 Column Care](#)", for recommended column cleanup procedures.



NOTE

Possible sources of column contamination are impurities in chemicals and in the deionized water used for eluents or components of the sample matrix. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of 18.2 megohm-cm.

- D. Diluting the eluent will improve peak resolution, however will also increase the analytes' retention times. If a 10% dilution of the eluent is not sufficient to obtain the desired peak resolution, or if the resulting increase in retention times is unacceptable, clean the column (see [Appendix A, CarboPac PA200 Column Care](#)).

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes directing the column effluent to waste. Then connect the column to the electrochemical detector cell. No water wash is necessary. The column is equilibrated when consecutive injections of the standard result in reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should have been eluted from the column.



NOTE

For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

7.3.3 Loss of Resolution for early eluting peaks

If poor resolution or efficiency is observed for early eluting peaks compared to the later eluting peaks, check the following:

- A. Improper eluent concentration may be the problem if retention time is less than expected. If manually prepared eluent is used, remake the eluent as required for your application and ensure that the water and chemicals used are of the required purity. If Dionex eluent generator is used to generate the eluent; check the flow rate, as pump flow rate will affect the eluent concentration.
- B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical/capillary column by either diluting the sample or injecting a smaller volume onto the column.
- C. Sluggish operation of the injection valve may be the problem due to partially plugged port faces. Refer to the valve manual for instructions.
- D. Improperly swept out volumes anywhere in the system prior to the guard and analytical/capillary columns may be the problem. Swap components, one at a time, in the system prior to the analytical/capillary column and test for early eluting peak resolution after every system change.

7.3.4 Spurious Peaks

- A. The columns may be contaminated. If the samples contain an appreciable level of polyvalent ions and the column is used with a weak eluent system, the retention times for the analytes will then decrease and spurious, inefficient (broad) peaks may show up at unexpected times. Clean the column as indicated in “Column Care”.



NOTE

For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office.

- B. The injection valve may need maintenance. When an injection valve is actuated, the possibility of creating a baseline disturbance exists. This baseline upset can show up as a peak of varying size and shape. This will occur when the injection valve needs to be cleaned or retorqued (see injection valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.

7.3.5 No Peaks, Poor Peak Area Reproducibility or too Small Peak Areas

- A. Check the position and filling levels of sample vials in the autosampler.
- B. Check injector needle-height setting.
- C. Check each line of the schedule for proper injector parameters. Revert to full loop and column appropriate sample loop size.
- D. Service the injection valve (check for leaks, Tefzel fragments, or sediments inside the valve).

7.3.6 Large Baseline Dip in the Chromatogram

A large baseline dip appearing is usually caused by oxygen in the sample injected. The ‘oxygen dip’ is normal and can be reduced in magnitude with higher NaOH concentration in the eluent.

7.3.7 Unidentified Peaks Appear with Expected Analyte Peaks

During the acetate or hydroxide gradient, 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:

- A. Disconnect the column and detector cell from the autosampler.
- B. Set the pump to 100% deionized water.
- C. Place the following solutions in the autosampler and inject in sequence. Use 25 μ L full loop injections:
 1. 1 M NaOH
 2. Deionized water
 3. IPA
 4. Deionized water
 5. 1 M HCl
 6. Deionized water

7.3.8 Decreased Detection Sensitivity

Always confirm the loss of response by performing at least one injection of the system suitability standard mix as described in [Section 6.1](#). This is to make sure that a decreased level of response is not being caused by system problems.

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.

Exception:

Check the pH reading. If the value is out of range or >13.2 , install a new reference 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 because the reference potential was too high. The non-disposable gold working electrode can be reconditioned by polishing.

After installing a new working electrode (with or without the complete system cleanup), confirm the normal detection sensitivity. Carry out a test with a reference standard. Should the response be too low, immediately remove the new working electrode from the system.

7.3.9 Excessive Gradient Rise

The magnitude of the gradient rise can be minimized by running high eluent strengths during the times when the system is not in use for sample or standard analysis. This will keep the column conditioned, free from carbonate buildup, and ready for analysis.

- A. Make sure the gradient rise is not caused by the system and/or detector cell.
- B. Increase column temperature to 40°C and wash the guard and column with 1M NaOH or KOH for at least four hours (preferably overnight). Run a blank gradient at 30°C and if needed repeat the clean-up with 100 mM NaOH, 950 mM sodium acetate wash at 40°C .

7.4 Reconditioning or Replacement of the Gold (conventional or disposable) Electrodes or Replacement of the Reference Electrode

Refer to Product Manual for Disposable Electrodes Doc. No. 065040, Dionex ICS-6000 Ion Chromatography System Manual Doc. No. 22181 and User's Compendium for Electrochemical Detection Doc. No. 065340 for any help necessary with electrochemical detection, working and reference electrodes.

8. Column Care

8.1 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 CarboPac PA200 column is 5,000 psi (34.47 MPa).

8.2 Column Start-Up

The CarboPac columns are shipped using Sodium hydroxide (see QAR) as the storage solution. Prepare the eluent shown on 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. 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.

If chromatographic efficiency or resolution is poorer than the QAR, see Sections 7.3 Poor Resolution and Section 7.3.1 Loss of Column Efficiency.

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.

8.3 Column Storage

For short-term storage (< 1 week), use Eluent, for long-term storage (> 1 week), see 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.

8.4 CarboPac PA200 Column Cleanup

The CarboPac PA200 can be readily cleaned by an approximate 50-column-volume rinse with 0.5–1 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 PA200 at a flow rate similar to application flow rate or half the application flow rate to avoid the over-pressurization of the column:

- A. Disconnect column from the ED cell and direct the column effluent to waste. If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels.



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. Contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each column separately.

8 – Column Care

- B. Wash the CarboPac PA200 with deionized water (18.2 megohm-cm) for about 30 minutes and then clean with 1 M Methanesulfonic acid (MSA) for one to two hours at appropriate flow rate (1mL/min for 4mm and 0.25mL/min for 2mm column).
- C. Wash the column with deionized water for about 30 minutes.
- D. Then clean the CarboPac PA200 with 2 M NaOH for at least one hour.
- E. Reconnect column to the cell and equilibrate the CarboPac PA200 to the desired initial conditions and test the performance using QAR standard and eluent.

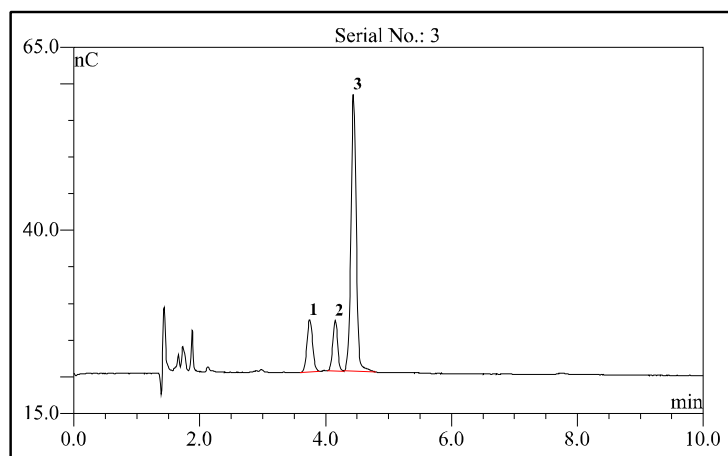
9. Quality Assurance Report

CarboPac™ PA200
Analytical (3 X 250 mm)
Product No. 062896

Date: 10-Mar-04 17:00
Serial No. : 000003
Lot No. : 014-01-086E

Flow Rate: 0.50 mL/min
Detection: ED50
Temperature: 30°C
Injection Volume: 10 µL
Diluted Standard: 12 nmol/mL in DI Water
 Each analyte is 0.12 nmoles
 per injection.
Storage Solution: Eluent

Eluent Composition
 %A: 100 mM NaOH + 50 mM Sodium acetate



Quadruple Waveform

Time	Potential	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	

Reference Electrode: Ag/AgCl
Electrode Mode: Ag

No.	Peak Name	Ret.Time (min)	Asymmetry (EP @ 10%)	Resolution (EP)	Efficiency (EP)
1	Unknown	3.74	1.11	2.68	8076
2	alpha-(2,6)-NAN-Lac	4.15	1.01	2.05	14365
3	alpha-(2,3)-NAN-Lac	4.44	1.02	n.a.	14721

QA Results:

Analyte	Parameter	Specification	Results
alpha-(2,3)-NAN-Lac	Efficiency	>=10800	PASS
alpha-(2,3)-NAN-Lac	Asymmetry	1.00-1.54	PASS
alpha-(2,3)-NAN-Lac	Retention Time	4.17-4.83	PASS
	Pressure	<=2640	1740

Production Reference:

Datasource: CON SQL local
 Sequence: CP PA200

Sample No.: 1

6.50 SP3 Build 980
 Chromeleon® Dionex Corp. 1996-2002