User Manual

Dionex CarboPac PA20-Fast-4µm

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thermo scientific

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Product Manual for

Dionex CarboPac PA20-Fast-4µm Guard Columns

4 × 30 mm (Item # 302748)
2 × 30 mm (Item # 302750)

Dionex CarboPac PA20-Fast-4µm Analytical Columns

4 × 100 mm (Item # 302747)
2 × 100 mm (Item # 302749)

Revision History:

Revision 01, June, 2017, Original Publication.

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 Dionex CarboPac PA20-Fast-4µm column

The Thermo Scientific™ Dionex™ CarboPac™ PA20-Fast-4µm (2mm and 4mm) columns are the latest additions to the CarboPac family of columns for carbohydrate separations. The new column was developed to provide fast, efficient separations for glycoprotein mono-saccharides with good spacing of mono-saccharides.

CarboPac™ PA20-Fast-4µm columns are packed with a hydrophobic, polymeric, microporous anion exchange resin stable over entire range of pH 0-14. The unique pH-stability of this packing allows eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes. Note that the use of small resin particles results in higher backpressures compared to the 6 µm resin particles in earlier Dionex CarboPac products. Therefore the Dionex CarboPac PA20-Fast-4µm will likely require the use of High Pressure IC (HPIC™) systems such as the Dionex ICS-5000$^+$ or Dionex Integrion™ HPIC system, or will have to be operated at lower flow rates.

1.1.1 Resin Characteristics:

Particle Size:	4 µm
Pore Size:	microporous
Cross-linking:	55%
Ion exchange capacity:	60 µeq per 4 x 100 column
	18 µeq per 4 x 30 column
	15 µeq per 2 x 100 column
	4.5 µeq per 2 x 30 column

1.1.2 Latex Characteristics:

Functional Group:	Quaternary ammonium ion
Latex Diameter:	130 nm
Latex Cross-linking:	5.2 %

1.1.3 Typical Operating Parameters:

pH range:	0-14
Temperature Limit:	4-60°C
Pressure Limit:	5000 psi

2. Installation

NOTE

Read the instrument manuals. This manual assumes that you are using Thermo Scientific Dionex instrumentation and are familiar with the installation and operation of the Thermo Scientific Dionex Ion Chromatograph (IC). If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.

The proper configuration of an Ion Chromatography System (ICS) is dependent on column format. Although they can typically use the same system, the use of a 2 mm or 4 mm column requires different set up to ensure optimized performance. The selected format and analysis type will affect the type of pump recommended. A gradient pump is designed to blend and pump isocratic, linear, or gradient mixtures of up to four mobile phase components at precisely controlled proportions and flow rates. An isocratic pump is for applications not requiring gradient or proportioned eluent capabilities. For high pressure applications, the use of high pressure fittings is recommended.

2.1 The Dionex High Pressure Ion Chromatography Systems

A minimum of a Dionex High Pressure Ion Chromatography (HPIC) system is recommended when running Dionex CarboPac PA20-Fast-4µm columns due to the higher backpressures generated at typical operational flow rates with 4 µm resins. Systems should have the capability to operate up to at least 5000 psi. Standard IC systems, with an upper limit of 3000 psi, will usually be inadequate for optimized column operation.

With this column Thermo Scientific recommends the use of metal-free system components. Metal ions will contaminate the Dionex CarboPac column and may also contaminate the working electrode. Running a Dionex CarboPac column on a metal system will void the column warranty.

WARNING

Care should always be taken not to exceed the maximum operating pressure of the system component. ICS systems with lower backpressure capabilities are not recommended as reduced flow rates may result in loss of performance.

NOTE

Contact your local representative for information on how to customize your system to your application needs.

2.2 System Requirements

Dionex CarboPac Columns are designed to run on Dionex Ion Chromatographs equipped with electrochemical detectors. We recommend the use of ferrules and fittings rated with a pressure of >5000 psi. The use of precut tubing, complete with high pressure fittings and ferrules, is also recommended, and Thermo Scientific Dionex IC PEEK Viper™ Fittings will help ensure easier and more secure installation.

2.2.1 Installation of Disposable Electrode into a Dionex ED50 Cell, pH-Ag/AgCl Reference Electrode or PdH Reference Electrode

The 2 mil (0.002") thick Teflon gaskets included in each package of disposable electrodes are usually required; otherwise, the disposable electrode product warranty may be voided. Other approved gaskets include 15 mil (0.015") (Item # 057364) and 62 mil (0.062") thickness (Item # 075499). When using the 62 mil gasket an alternate spacer block must also be used (Item # 085325). A gasket is always required because it forms the flow-through channel. In addition, the quadruple waveform must be used for carbohydrate analysis otherwise the disposable electrodes will fail quickly, and their product warranties will be 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-5000 Ion Chromatography System Manual Doc. No. 065342 and User's Compendium for Electrochemical Detection Doc. No. 065340.

2.2.2 System Void Volume

When using Dionex CarboPac PA20-Fast-4µm columns, it is important to minimize system void volume. The system void volume for 2 mm columns should be scaled down to 1/4 of the system volume in a standard system designed for 4 mm columns (4 mm system). For best 2mm performance, all of the tubing installed between the injection valve and detector should be 0.005" I.D. PEEK tubing (Item # 044221), for optimal performance with 4mm columns use 0.010" I.D. PEEK tubing (Item # 042690). Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

2.3 The Injection Loop

2.3.1 The 4 mm System Injection Loop, 10 - 25 µL

For most applications on a 4 mm analytical system, equipped with the 0.002" gaskets, a 10 – 25 µL injection loop will generally be sufficient. Avoid injecting more than 25 µM (~4.5 ppm) of any saccharide onto the 4 mm analytical column when using a 10 µL injection loop. Injecting larger amounts of an analyte can overload the column and detector, which can affect detection linearity. For low concentrations, larger injection loops can be used to increase sensitivity, however injection loops larger than 10 µL tend to have an adverse effect on peak shape.

2.3.2 The 2 mm System Injection Loop, 2 - 10 µL

For 2 mm column applications, a 2 – 10 µL injection loop is typically recommended. One should not inject more than 25 µM of any saccharides onto the 2 mm analytical column when using a 2.5 µL loop. Injecting larger amounts can result in overloading the column or detector which can affect detection linearity. For low concentrations, larger injection loops can be used to increase sensitivity. Install an injection loop less than or equal to one-fourth of the volume used with 4 mm analytical columns.

2.3.3 High Concentration Samples

For high concentration samples, a 0.4 µL internal injection valve (Item # 072050), a special 62 mil ED gasket (Item # 075499), and a compatible ED block, (Item # 085325), may be necessary to minimize the dilutions otherwise required. When using the 2 mm format for high concentration samples, a 15 mil gasket (Item # 057364) is recommended.

2.4 The Dionex CarboPac PA20-Fast-4µm Guard Column

A Dionex CarboPac PA20-Fast-4µm Guard Column is normally used with the Dionex CarboPac PA20-Fast-4µm Analytical Column. Retention times will increase by ~ 20% when a guard column is placed in-line before the analytical column, when using isocratic elution. A guard column helps prevent sample contaminants from fouling the analytical column. It is cheaper and easier to clean or replace a guard column than an analytical column. Replacing the Dionex CarboPac PA20-Fast-4µm Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex CarboPac PA20-Fast-4µm Analytical Column.

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

For Dionex CarboPac PA20-Fast-4µm applications using the Dionex EGC 500 KOH cartridge, a Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column (Item # 075550) or Dionex CR-ATC 600 Continuously Regenerated Anion Trap Column (Item # 088662) 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. 079684) for instructions.

2.6 System Start-up

2.6.1 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 10 mM KOH at 1.0 mL/min for 4 mm columns or 0.25 mL/min for 2 mm columns,

C. a length of narrow-bore tubing is installed between the injector and detector cell to generate ~1000 psi backpressure,

D. The column(s) are not yet installed.

Confirm that the pH is 11.8 ±1 pH unit. With the pH within this range, turn on the cell using the carbohydrate standard quad waveform (See Table 3, Section 6.3, Disposable Electrode Manual, document number 065040) and begin background signal monitoring from the Chromeleon™ control panel. Monitor the trace for at least 30 minutes. Confirm that the background is between 10 and 40 nC. If the background is above 50 nC or the pH is out of range, see the "Troubleshooting" section at the end of this manual.

NOTE

Thermo Scientific recommends sanitizing the entire system with 2M NaOH prior to initial start-up and after idle periods.

2.6.2 Verification of Column Cleanliness

Install the Dionex CarboPac PA20-Fast-4µm 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 PA20-Fast-4µm is shipped in 10 mM KOH. Any column that is stored long-term should be stored in the same solution. To prepare the column for standard analysis, the Dionex CarboPac PA20-Fast-4µm must be washed for at least 30 minutes (an hour preferred) with 100 mM KOH at the appropriate (column I.D. dependent) flow rate. Equilibrate the column set under the test chromatogram conditions (See QAR) and perform two blank injections (DI water) to ensure that DI water injection has no peaks.

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 the expected chromatographic performance, you are ready to proceed to running your application.

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

3. Operation

3.1 Dionex CarboPac PA20-Fast-4µm column Operational Parameters

- pH range: 0 - 14
- Temperature limit: 60°C
- Pressure limit: 5000 psi
- Organic Solvent Limit: 50% Acetonitrile, methanol, or acetone if required for cleaning
- Typical Eluent: Potassium hydroxide from the Eluent Generator (8-100 mM)
  Thermo Scientific does not recommend using eluent at less than 8 mM as this results in significantly lower detection response.
- Standard Flow Rate: 2 mm: 0.25 mL/min
  4 mm: 1.0 mL/min
- Maximum Flow Rate: 2 mm: 0.25 mL/min
  4 mm: 1.0 mL/min

3.1.1 The Best Operational Guidelines

3.1.1.1 ALWAYS…

- Use dedicated glassware and plasticware for sample handling.
- Use high purity water (≥18.2 MΩ-cm resistivity).
- Keep your eluents blanketed with ~ 3psi helium or nitrogen. Use new filtered water if left unblanketed for more than 30 minutes.

3.1.1.2 NEVER…

- Proceed to a next installation step 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 materials (e.g., cellulose derivatives).
- Use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily, or use sampler wash bottles blanketed with ~3 psi Nitrogen or helium.
- Run above 60°C or 5000 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.

- Laboratory water unit delivering ≥18.2 megohm-cm water at the installation site.
- Vacuum system for eluent vacuum filtration.
- 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.
- Plastic eluent bottles with gas-tight cap-fittings.

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

3.2.1 Deionized Water

Deionized water used to feed the Eluent Generator should be Type I reagent grade water with a specific resistance ≥18.2 megohm-cm. The water should be free from ionized impurities, organics, microorganisms, and particulate matter larger than 0.2 µm. UV treatment as a part of the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of UV lamps, ion exchange and adsorbent cartridges. All filters used for water purification must be free from electrochemically active components, including 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 significant gradient artifacts.

3.2.2 Potassium Hydroxide

Use a Dionex EGC 500 KOH Eluent Generator Cartridge installed with a Dionex CR-ATC 500 in the Dionex ICS-5000$^+$ EG module. A CR-ATC 600 should be used with Dionex Integrion HPIC systems. Manually-prepared KOH eluents will absorb CO_2 during and after preparation altering elution selectivity over time.

3.3 Preparation of Eluents and Standards

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 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.

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. NOTE: Select a vacuum level (e.g., ≤5mm Hg) that does not deform the plastic eluent container. Degas the reservoir for 5-10 minutes while the container is placed in a sonicator. Alternately, degas the aqueous eluent in a glass bottle at 27"Hg before transferring to the plastic bottle. 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 for the majority of cases. Online eluent generation and degassing is supported through the use of Thermo Scientific Dionex HPIC 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 outlet tubing. In the third step, select an appropriate KOH concentration (usually 10-14 mM) in the EG panel and verify that the eluent is exiting from the Dionex CR-ATC 500 outlet tubing, then turn on the Dionex CR-ATC 500 in the Chromeleon eluent generator panel.

3.4 Sample Preparation

The Dionex 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 strongly anionic compounds for the Dionex CarboPac columns (e.g., chloride, carbonate, phosphate, etc.).

IMPORTANT **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 Integrated Amperometry detection, eliminate electrochemically-active components (e.g., TRIS buffer, alcohols, and other hydroxylated compounds) from eluents and samples. Small amounts of organic solvents in the sample may not harm the column, although the organics may interfere with the chromatography or detection of the analytes of interest.

4. Example Applications

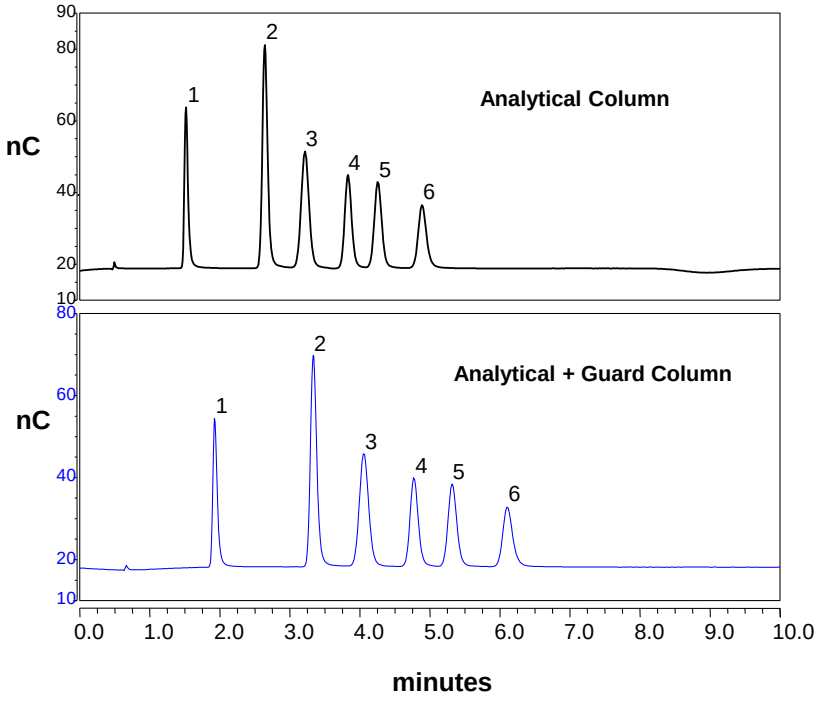
The following section provides an example of the types of applications for which the Dionex CarboPac PA20-Fast-4µm is designed. The chromatograms in this section were obtained using columns that reproduced the Quality Assurance Report on an optimized Ion Chromatograph. Different systems will differ slightly in performance due to slight differences in column set, system void volumes, liquid sweep-out times of different components, and laboratory temperatures.

4.1 Production Test Chromatograms

Isocratic separation of mono-saccharide standards (the 6 most common sugars of interest).
These sugars are used to test the performance of the Dionex CarboPac PA20-Fast-4µm column. While the Dionex CarboPac PA20-Fast-4µm analytical column should always be used with a Dionex CarboPac PA20-Fast-4µm Guard Column, the example chromatogram developed on all shipped columns does not employ a guard column. Addition of the Guard column will increase elution time by ~20% when compared to the Analytical column by itself. To guarantee that all Dionex CarboPac PA20-Fast-4µm Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test at 30ºC.

The Dionex CarboPac PA20-Fast-4µm column has been designed to provide fast separations of common mono-saccharides. Using 10mM KOH, the six sugars can be separated within 7 min on the 100 mm long columns for both the 2mm ID and 4mm ID column formats (Figures 1 & 2).

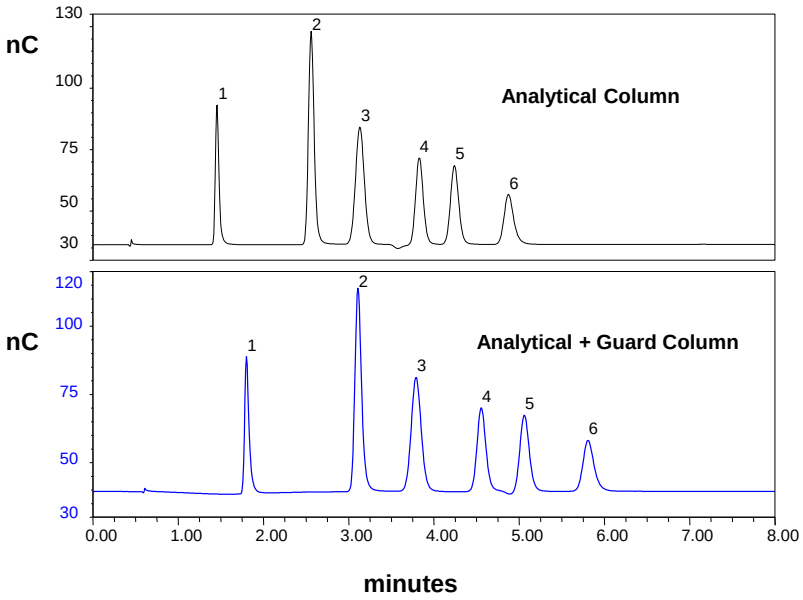
Figure 1 Production Test Chromatogram for Dionex CarboPac PA20-Fast-4µm 2 x 100 mm Column



Column: CarboPac PA20-Fast-4µm 2mm
Eluent: 10mM KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30ºC
Flow Rate: 0.25mL/min
Inj Volume: 2.5µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard Peaks:	Name	Concentration (pmol)
1,	Fucose	25
2,	Galactosamine	25
3,	Glucosamine	25
4,	Galactose	25
5,	Glucose	25
6,	Mannose	25

Figure 2 Production Test Chromatogram for Dionex CarboPac PA20-Fast-4µm 4 x 100 mm Column



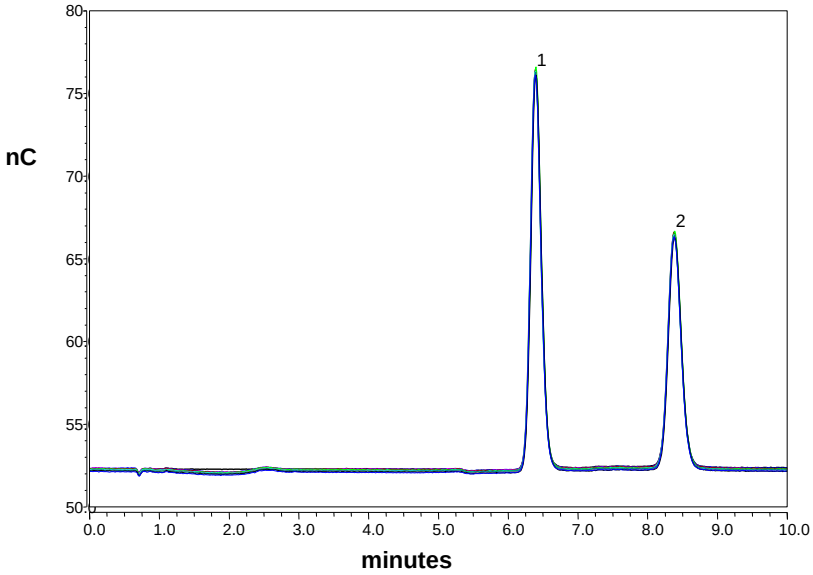
Column: CarboPac PA20-Fast-4µm 4mm
Eluent: 10mM KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30°C
Flow Rate: 1.0mL/min
Inj Volume: 10µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard Peaks: Name		Concentration (pmol)
1,	Fucose	100
2,	Galactosamine	100
3,	Glucosamine	100
4,	Galactose	100
5,	Glucose	100
6,	Mannose	100

4.2 Analysis of Peach Iced Tea

Figure 3 shows a sample of commercially available peach flavored iced tea diluted 1:1000, filtered through a 0.45 µm membrane, and injected onto a Dionex CarboPac PA20-Fast-4µm column.

Figure 3 Analysis of Peach Iced Tea on the Dionex CarboPac PA20-Fast-4µm 4 x 100 mm Column



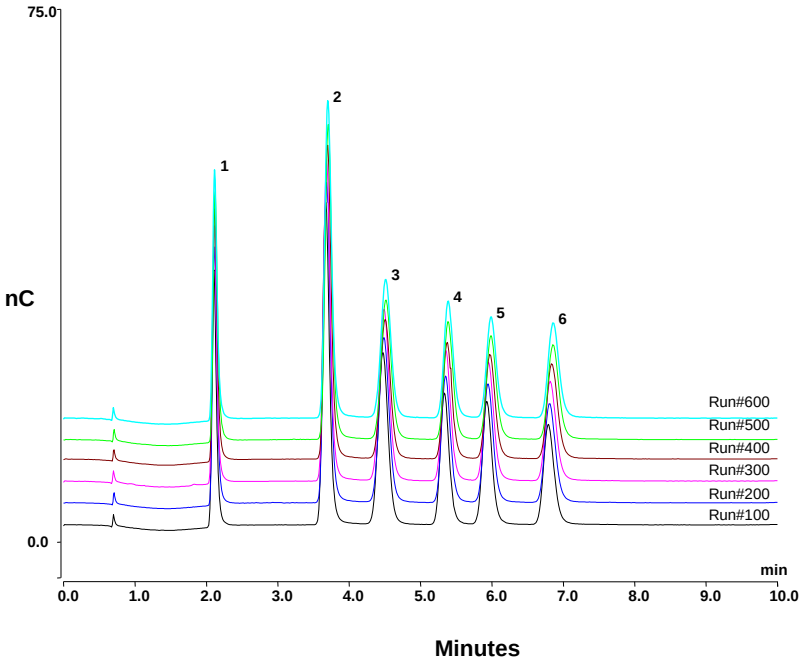
Column: CarboPac PA20-Fast-4µm 4mm
Eluent: 10mM KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30°C
Flow Rate: 0.89mL/min
Inj Volume: 2.5µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard Peaks: Name	
1,	Glucose
2,	Fructose

4.3 Ruggedness and Reproducibility

The Dionex CarboPac PA20-Fast-4µm column provides consistent chromatography performance and highly reproducible separations. To demonstrate the ruggedness of the Dionex CarboPac PA20-Fast-4µm columns, they were subjected to five days of continuous operation with injections being made every twelve minutes for a total of 600 injections. Figures 4 & 5 below show overlays of every 100th injection, demonstrating very good reproducibility for both formats.

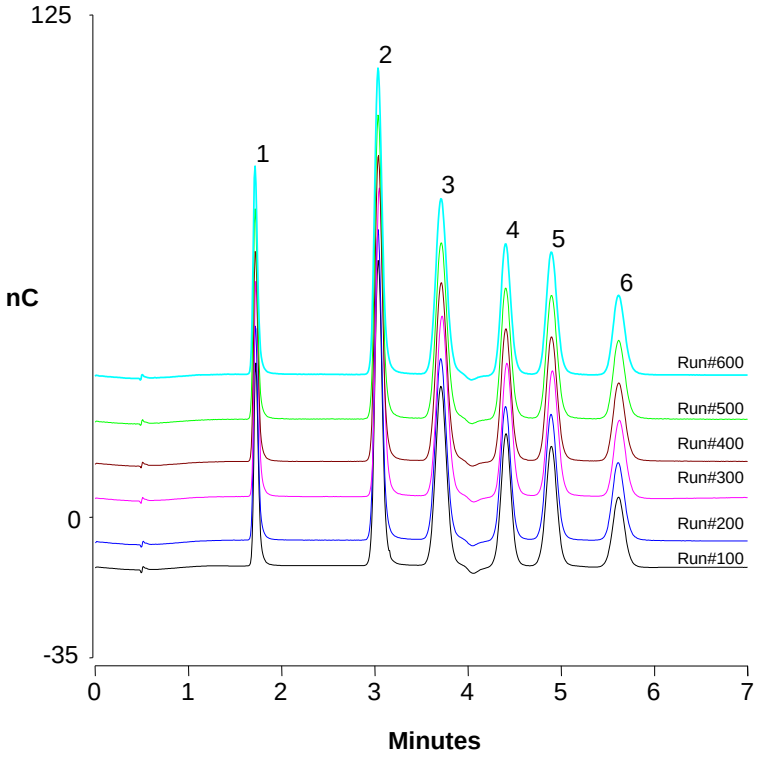
Figure 4 Overlay of Every 100th Injection of a Mixed Monosaccharide Standard on the Dionex CarboPac PA20-Fast-4µm Column (2 mm)



Column: CarboPac PA20-Fast-4µm
2x100mm + 2x30mm
Eluent: 10mM KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30°C
Flow Rate: 0.25mL/min
Inj Volume: 2.5µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard Peaks: Name		Concentration (pmol)
1,	Fucose	25
2,	Galactosamine	25
3,	Glucosamine	25
4,	Galactose	25
5,	Glucose	25
6,	Mannose	25

Figure 5 Overlay of Every 100th Injection of a Mixed Monosaccharide Standard on the Dionex CarboPac PA20-Fast-4µm Column (4 mm)



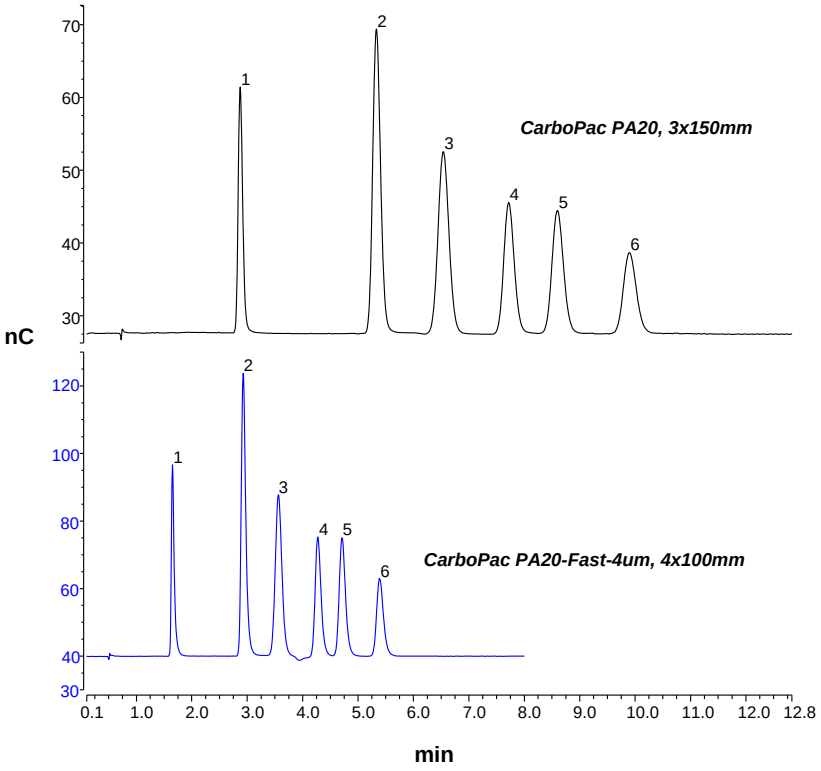
Column: CarboPac PA20-Fast-4µm 4mm
Eluent: 10mM KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30°C
Flow Rate: 0.89mL/min
Inj Volume: 10µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard Peaks:	Name	Concentration (pmol)
1,	Fucose	100
2,	Galactosamine	100
3,	Glucosamine	100
4,	Galactose	100
5,	Glucose	100
6,	Mannose	100

4.4 Dionex CarboPac PA20-Fast-4µm Column vs. Dionex CarboPac PA20 Column

The Dionex CarboPac PA20-Fast-4µm column packed with 4 µm resin particles provides faster separations and more efficient peaks when compared to a standard Dionex CarboPac PA20 column packed with 6.5 µm resin particles. Figure 6 below shows the same amount of monosaccharide mixed standard injected on both a Dionex CarboPac PA20-Fast-4µm 4 x 100 mm column and a standard Dionex CarboPac PA20 3 x 150 mm column. Notice the run time is reduced by approximately 50% without sacrificing performance.

Figure 6 Comparison of Dionex CarboPac PA20-Fast-4µm 4 x 100 mm and Dionex CarboPac PA20 3 x 150 mm Columns



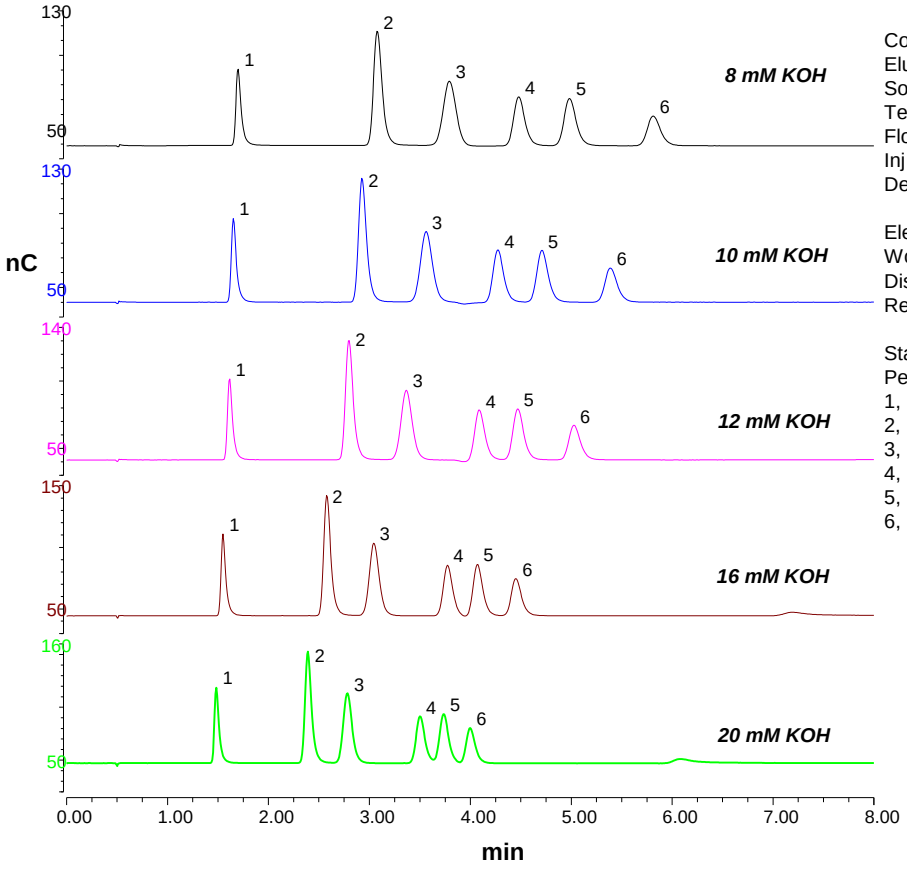
Eluent: 10mM KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30°C
Flow Rate: 0.50 mL/min for 3x150mm column
0.89 mL/min for 4x100mm column
Inj Volume: 10µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard
Peaks: Name
1, Fucose
2, Galactosamine
3, Glucosamine
4, Galactose
5, Glucose
6, Mannose

4.5 Effect of Hydroxide Concentration on Runtime

The Dionex CarboPac PA20-Fast-4µm column has been designed to provide good resolution between monosaccharides over a variety of hydroxide conditions. These conditions can be optimized depending on the goal of the separation. Figure 7 below shows the effect of hydroxide concentration on the separation of a mixed monosaccharide standard. Higher hydroxide concentrations will elute peaks faster albeit with less resolution between them.

Figure 7 Effect of Hydroxide Concentration on the Dionex CarboPac PA20-Fast-4µm 4 x 100 mm Column



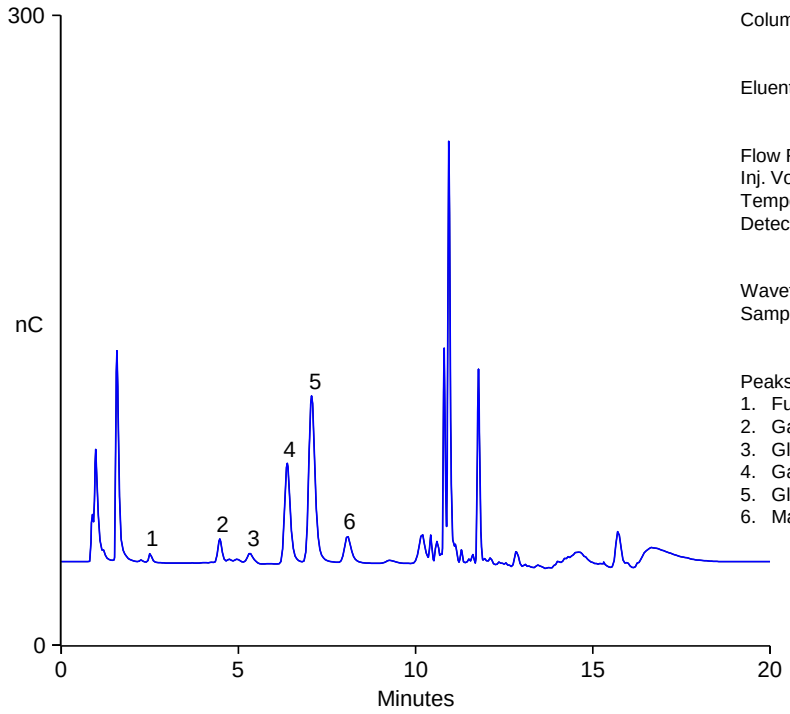
Column: CPPA20-Fast-4um, 4x100mm
Eluent: KOH
Source: Dionex EGC KOH Cartridge
Temperature: 30°C
Flow Rate: 0.89 mL/min
Inj Volume: 10µL
Detection: Integrated Amperometry
quadruple-pulse waveform
Electrodes
Working: Au (Carbohydrate-Disposable)
Reference: Ag/AgCl

Standard
Peaks: Name
1, Fucose
2, Galactosamine
3, Glucosamine
4, Galactose
5, Glucose
6, Mannose

4.6 Determination of Carbohydrates in Algae Biomass

Algae provide an attractive feedstock for production of sustainable transportation fuels. The sugars in algal biomass are typically broken down chemically or biochemically followed by fermentation. To maximize the biofuel yield, it is critical to quantify the released carbohydrates during biofuel production. As shown in Figure 8 below, the Dionex CarboPac PA20-Fast-4µm column provides a fast, robust, accurate, and quantitative analytical method for carbohydrate determination in algal biomass samples.

Figure 8 Carbohydrate Determination in Algal Biomass Samples Using the Dionex CarboPac PA20-Fast-4µm 2 x 100 mm Column



Columns:	Dionex CarboPac PA20-Fast-4µm Analytical and Guard columns (2 mm)
Eluent:	10 mM KOH from 0 to 8 minutes 100 mM KOH from 8 to 14 minutes 10 mM KOH from 14 to 20 minutes
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL
Temperature:	30˚C
Detection:	Pulsed amperometric detection with disposable gold working electrode
Waveform:	Carbohydrates, Quad Potential
Sample:	Algae biomass sample hydrolyzed with TFA and diluted 1:100

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

5. 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, 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.

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.

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Column pressure (after subtracting the system pressure) for the Dionex CarboPac PA20-Fast-4µm Analytical Column should be close to the pressure listed in the QAR when using the test chromatogram conditions. If a Dionex CarboPac guard and analytical column are both installed, column pressure will increase by approximately 20% over the pressure listed in the QAR for the column. 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. If necessary, measure the pump flow rate by collecting the DI H_2O eluent for a specified time at operating pressure, and measure the collected volume using an analytical balance. This data (weight/time) will give actual flow rate.

B. Determine which part of the system is causing the high pressure. High pressure could be due to plugged or constricted tubing, an injection valve with a clogged port or worn rotor, a column bed support clogged with particulates, or a clogged detector cell.

C. 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. Monitor 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). The pressure with the eluent generator connected should be < 400 psi. Continue adding system components (backpressure coil (if present), injection valve, column(s), 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.

D. Measure the system back pressure by attaching a short piece of new 0.010" tubing in place of the column.

E. A High-Pressure In-Line Filter (Item # 074505) positioned between the pump and injection valve should be installed to prevent particulates from blocking the system.

5.1.2 Replacing Column Bed Support Assemblies for 2 mm and 4 mm columns

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 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.

5.1.3 Filter Eluent

Eluents containing particulate material or bacteria may clog the column inlet bed support. Filter eluents through a 0.2 µm Nylon or PES (PolyEtherSulfone) filter. DO NOT use a cellulosic filter (e.g., cellulose acetate or regenerated cellulose) as these will introduce cellulosic polymers into your eluent, and thus many Integrated Amperometric peaks will appear, even with blank "injections".

5.1.4 Filter Samples

Samples containing particulate material may clog the column inlet bed support. Filter samples through a 0.2 µm Nylon or PES (PolyEtherSulfone) filter prior to injection.

Please note the comments in section 5.1.3 above.

5.2 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 >35 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 of the waveform values in the 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 > 35 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. If the detector background signal is in the 10-35 nC range, 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, "Dionex CarboPac PA20-Fast-4µm 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 a 2 M sodium hydroxide rinse. In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA20-Fast-4µm using QAR eluent is 10 – 35 nC. If the background is much higher, determine the cause of high background. Consider the possibility that the eluent filter might be cellulosic, as that will introduce very high signal and noise.

5.2.1 Preparation of Eluents

A. The Dionex CarboPac PA20-Fast-4µm column is not designed for routine use of manually prepared eluents. We recommend the use of a Dionex EGC 500 KOH cartridge and Dionex CR-ATC 500 (or equivalent) for best results.

B. Make sure that the deionized water used has a specific resistance of 18.2 megohm-cm or greater.

5.2.2 Dionex CR-ATC Column

A. When using a Dionex ICS-5000$^+$ EG eluent generator with a Dionex EGC 500 KOH cartridge to generate eluent, install a Dionex CR-ATC 500 Continuously Regenerated Anion Trap Column. When using a Dionex Integrion HPIC system with a Dionex EGC 500 KOH cartridge to generate eluent, install a Dionex CR-ATC 600 Continuously Regenerated Anion Trap Column.

B. If the background is elevated due to contamination of the Dionex CR-ATC 500, please refer to Sections 5.3 and 6 in the Dionex CR-TC Product Manual (Document No. 079684) for corrective action.

5.2.3 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, Dionex CarboPac PA20-Fast-4µm Column Care".

5.3 Poor Resolution

One of the unique features of Dionex CarboPac columns is the fast equilibration time in gradient applications from the ending eluent (high ionic strength) to the beginning 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 1.0 mL/min for 4 mm ID columns.

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

Due to different system configurations, the delivered gradient profile may not exactly 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 experience with methods development work. Its advantage is that it allows a method to be tailored for a particular application, where selectivity, resolution, and total run time are optimized. Be aware poor peak resolution can be due to any or all of the following factors.

5.3.1 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 5.1.2, "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. If it does, replace the inlet bed support.

B. Extra-column effects can result in sample band dispersion, or band broadening. Make sure you are using PEEK tubing with an ID of no greater than 0.010" for 4 mm systems or 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.

C. If tubing is not connected properly from the inlet and outlet of the column, it can cause low efficiency. When installing Dionex CarboPac columns, it is recommended to turn off the pump while connecting the column inlet and the column outlet to the detector. This will help avoid any slippage of the ferrule when attempting to secure the fitting under elevated pressure conditions.

5.3.2 Shortened Retention Times

NOTE

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

A. Check the flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Collect the eluent for a specified time after the column, and measure the eluent flow rate 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 earlier. If eluent concentration is too concentrated, prepare fresh eluent.

NOTE

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

C. Column contamination can lead to a loss of column capacity. Highly retained contaminants will tend to occupy anion exchange sites limiting the number of sites available for retention of the analytes. Refer to "Appendix A, Dionex CarboPac PA20-Fast-4µm 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, but will also increase the 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, try cleaning the column (see Appendix A, Dionex CarboPac PA20-Fast-4µm 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. Capacity close to the original capacity should be restored by this treatment, since the contaminants should be cleared 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.

5.3.3 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. Check the flow rate of the pump, as pump flow rate will affect the eluent concentration in an RFIC-EG system. Ensure the Eluent Generator is set to the correct eluent concentration.

B. Column overloading may be an issue. Reduce the amount of sample injected onto the column by either diluting the sample or injecting a smaller volume.

C. Sluggish operation of the injection valve may also cause this, due to partially plugged port faces. Refer to the valve manual for instructions.

D. Improperly swept volumes anywhere in the system prior to the guard and analytical/capillary columns may reduce resolution of early peaks. Swap components, one at a time, in the system prior to the analytical/capillary column and test for early eluting peak resolution after every component change.

5.3.4 Spurious Peaks

A. The column(s) 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 will 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 may appear as a peak of varying size and shape. This will occur when the injection valve needs to be cleaned or serviced (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 system 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.

C. Another potential cause of spurious peaks or dips is amperometric reference electrode offset. Since the reference electrode is continually exposed to hydroxide, the Cl^- in the Ag/AgCl electrode will eventually be exchanged for hydroxide (OH^-). This will result in a voltage offset, and result in delivery of waveform potentials that differ from the programmed values by the offset potential. This may result in unexpected peaks and dips in the chromatographic carbohydrate retention window. If this is suspected, perform the reference electrode calibration in Chromeleon. If the offset is $\geq$ 25 mV, replace the ED cell reference electrode.

5.3.5 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 the injection valve orientation. If the valve is installed upside down, or if it is plumbed 180° out of the correct orientation, switching from load to inject will actually bypass the loop. If this is confirmed, re-plumb the valve in the correct orientation.
D. Check the Chromeleon instrument method and sequence for proper injector parameters. Use full-loop sampling and appropriate loop size.
E. Service the injection valve (check for leaks or sediments inside the valve).

5.3.6 Large Baseline Dip in the Chromatogram

A large baseline dip appearing 15-25 minutes into the chromatogram is usually caused by oxygen in the sample injected. This 'oxygen dip' is normal and can be reduced in magnitude with higher NaOH concentration in the eluent.

5.3.7 Unidentified Peaks Appear with Expected Analyte Peaks

During an acetate or hydroxide gradient, a number of small peaks may appear. These peaks are usually due to trace contaminants in the water supply used to prepare eluents. The contaminants accumulate on the column during the isocratic, or low eluent strength section of the chromatogram, and are eluted as irregular baseline deformations or sharp spikes with the increasing eluent strength.

Some trace contaminants can co-elute with glycans, 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 µL full loop injections:
    1. 1 M NaOH
    2. Deionized water
    3. IPA
    4. Deionized water
    5. 1 M HCl
    6. Deionized water

Sometimes multiple cycles of each solution may be required. Also, if you suspect a dirty sample loop or injection valve, rinse them with above protocol.

5.3.8 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 4.1. 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 replacement (disposable) working electrode. Spare reference electrodes gold working electrodes should always be available in order to avoid unnecessary delays.

Exceptions:

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 disposable working electrode. The system cleanup is not necessary. The decrease in sensitivity can be caused by a loss of surface area on the disposable electrode, or by deposition of gold-oxide on the conventional electrode surface because the reference potential was too high. A conventional gold working electrode can be reconditioned by polishing.

Peak heights will also increase with increasing eluent concentrations, especially between 1 and 10 mM KOH. This is due to improvement of the kinetics in the electrode detection related to ionic strength and pH effects. If you run the same standard at 1 mM KOH and 12 mM KOH, peak heights will be significantly higher at 12 mM KOH. You can expect a peak area (and height) decrease whenever reducing your eluent strength below 10 mM KOH.

After installing a new working electrode (disposable or conventional, with or without the complete system cleanup), confirm the expected detection sensitivity. Test sensitivity using the Dionex MonoStandard Mix of Six (Item # 043162). Should the response be too low, immediately remove the new working electrode from the system to minimize its contamination.

5.3.9 Excessive Gradient Drift

The magnitude of the gradient baseline drift can be minimized by high eluent strength column wash steps during the times when the system is not in use for sample or standard analysis. This will keep the column conditioned, free from buildup of carbonate and other contaminants, and ready for analysis.

A. Make sure the gradient drift is not caused by the eluents and/or detector cell (working or reference electrodes).

B. Set 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 necessary repeat the wash with 100 mM NaOH / 950 mM sodium acetate at 40°C.

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

Refer to the Product Manual for Disposable Electrodes (Doc. No. 065040), Dionex ICS-5000 Ion Chromatography System Manual (Doc. No. 065342) or User's Compendium for Electrochemical Detection (Doc. No. 065340) for any help necessary with electrochemical detection, working and reference electrodes.

Appendix A – Column Care

A.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 PA20-Fast-4µm column is 5,000 psi (34.47 MPa).

A.2 Column Start-Up

The Dionex CarboPac columns are shipped using potassium hydroxide (see QAR) as the storage solution. Use a 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 100 mM KOH at 0.8 mL/min for 4mm and at 0.20 mL/min for 2mm column 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.

If chromatographic efficiency or resolution is poorer than the QAR, see Sections 5.3 Poor Resolution and Section 5.3.1 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.

A.3 Column Storage

For short-term storage (< 1 week), the QAR eluent (10 mM KOH) is acceptable, for long-term storage (> 1 week), employ the storage solution 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.

A.4 Dionex CarboPac PA20-Fast-4µm Column Cleanup

The Dionex CarboPac PA20-Fast-4µm can be readily cleaned by rinsing the column with ~ 60 column volumes of 100 mM KOH or NaOH. More stubborn contamination problems may necessitate a thorough column cleaning. Use the following steps to thoroughly clean the Dionex CarboPac PA20-Fast-4µm; use 0.5 mL/min for 4mm ID columns and 0.12 mL/min for 2mm Column formats 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 CarboPac PA20-Fast-4µm column with 1M KOH or NaOH for at least one hour.

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.

Appendix B – Quality Assurance Reports

Device Monitoring Enabled
and Viper Fitting Ready

Dionex CarboPac™ PA20-Fast-4µm
Analytical (2 x 100 mm)
Product No. 302749

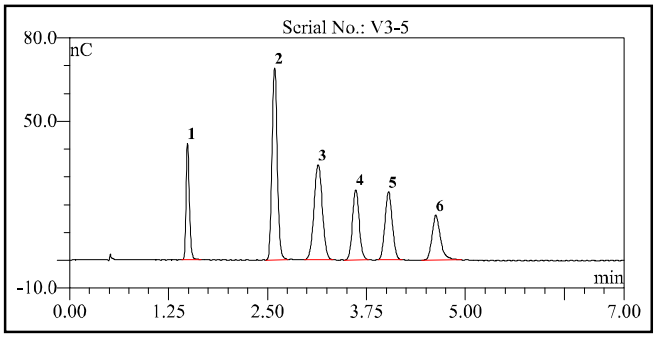
Date: 13-Jun-17 09:48
Serial No. : V3-5
Lot No. : 2016-06-097

Eluent Flow Rate:	0.25 mL/min
Temperature:	30 °C
Detection:	Electrochemical Detection
Injection Volume:	10 µL
Recommended Trap:	BorateTrap
Storage Solution:	Eluent

Eluent Composition
%A: 100 mM KOH
%B: 10 mM KOH

Eluent Profile

Time	%A	%B	Comment
-40.00	100	0	Regeneration
-30.05	100	0	
-30.00	0	100	Equilibration
0.00	0	100	Inject
12.00	0	100	End



ED40 Operating Parameter

Time	Potential [1]	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	

[1] **Reference Electrode Mode:** Ag/AgCl

No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Amount Injected (nmoles)
1	Fucose	1.49	1.27	11.16	6075	0.1
2	Galactosamine	2.59	1.09	3.50	7304	0.1
3	Glucosamine	3.14	1.00	2.70	4179	0.1
4	Galactose	3.61	1.09	2.54	8516	0.1
5	Glucose	4.03	1.11	3.35	8953	0.1
6	Mannose	4.62	1.40	n.a.	9787	0.1

QA Results:

Analyte	Parameter	Specification	Results
Galactosamine	Efficiency	>=5400	Passed
Galactosamine	Asymmetry	1.00-1.65	Passed
Mannose	Retention Time	4.25-5.35	Passed
Galactose	Resolution	>=1.62	Passed
	Pressure	<=4180	2430

Production Reference:
Datasource: QAR
Directory _RFID\CarboPac\CP_PA20-Fast_4µm
Sequence: CP_PA20_2x100-Fast-4µm
Sample No.: 1

6.80 SR15 Build 4656 (243203) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific

Device Monitoring Enabled
and Viper Fitting Ready

Dionex CarboPac™ PA20-Fast-4µm
Analytical (4 x 100 mm)
Product No. 302747

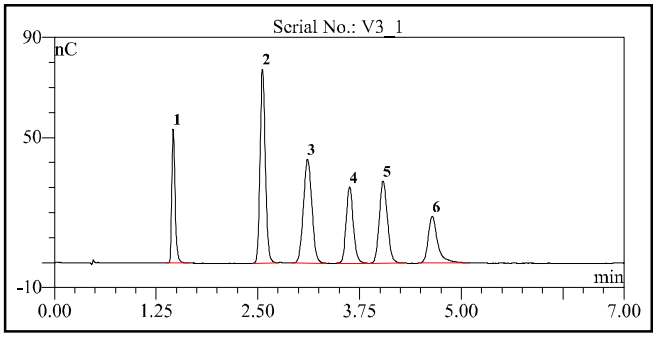
Date: 06-Jun-17 16:15
Serial No. : V3_1
Lot No. : 2016-06-097

Eluent Flow Rate:	1.0 mL/min
Temperature:	30 °C
Detection:	Electrochemical Detection
Injection Volume:	10 µL
Recommended Trap:	BorateTrap
Storage Solution:	Eluent

Eluent Composition
%A: 100 mM KOH
%B: 10 mM KOH

Eluent Profile

Time	%A	%B	Comment
-40.00	100	0	Regeneration
-30.05	100	0	
-30.00	0	100	Equilibration
0.00	0	100	Inject
12.00	0	100	End



ED40 Operating Parameter

Time	Potential [1]	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	

[1] **Reference Electrode Mode:** Ag/AgCl

No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Amount Injected (nmoles)
1	Fucose	1.46	1.49	11.66	6802	0.1
2	Galactosamine	2.56	1.25	3.58	7533	0.1
3	Glucosamine	3.11	1.09	2.96	4209	0.1
4	Galactose	3.63	1.20	2.44	8425	0.1
5	Glucose	4.04	1.19	3.24	7947	0.1
6	Mannose	4.64	1.67	n.a.	9299	0.1

QA Results:

Analyte	Parameter	Specification	Results
Galactosamine	Efficiency	>=5400	Passed
Galactosamine	Asymmetry	1.00-1.65	Passed
Mannose	Retention Time	4.25-5.35	Passed
Galactose	Resolution	>=1.62	Passed
	Pressure	<=4180	2958

Production Reference:
Datasource: QAR
Directory _RFID\CarboPac\CP_PA20-Fast_4µm
Sequence: CP_PA20_4X100-FAST-4µM
Sample No.: 1

6.80 SR15 Build 4656 (243203) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific