



**Thermo Scientific**

# **Dionex CarboPac MA1**

## **Column Product Manual**

**P/N: 065546-01**

**November 2013**

# **Product Manual**

**for**

## **Dionex CarboPac MA1 Guard Column**

4 x 50 mm, P/N 044066

## **Dionex CarboPac MA1 Analytical Column**

4 x 250 mm, P/N 044067

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## 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 Thermo Scientific Dionex CarboPac MA1 (4 mm) is optimized for weakly ionizable compounds such as sugar alcohols. The resin technology used in the Dionex CarboPac MA1 column is an efficient tool for the analysis of mono- and disaccharide alditols since they are oxy anions at pH values above 12.5. Because of its higher capacity, the macroporous Dionex CarboPac MA1 resolves many carbohydrates that are poorly retained on pellicular anion-exchange columns, and successfully separates alditols such as glycerol, arabitol, sorbitol, dulcitol and mannitol found in food products, physiological fluids, tissues and reduced glycoconjugate saccharides.

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, large linear polysaccharides, mono and disaccharide alditols. Each class of carbohydrates has different separation characteristics requiring different column chemistries.

## **Resin Characteristics:**

Particle Size:	7.5 µm
Pore Size:	microporous (< 10 Å)
Cross-linking:	15%
Ion exchange capacity:	1450 µeq per 4 × 250 mm column
Functional Group:	alkyl quaternary ammonium group

## **Latex Characteristics:    No Latex**

Latex Diameter:	N/A
Latex Cross-linking:	N/A

## **Typical Operating Parameters:**

pH range:	0–14
Temperature Limit:	4–60 °C
Pressure Limit:	2000 psi
Organic Solvent Limit:	0%
Typical eluents:	High purity water (18.2 megohm-cm), sodium hydroxide

## 2. System Requirements and Installation

### 2.1 System Requirements

#### 2.1.1 System Requirements for 4 mm Operation

The carbohydrate separations using the Dionex CarboPac MA1 columns are optimized for use with Thermo Scientific 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 Dionex CarboPac column and may contaminate the working electrode. Running a Dionex CarboPac column on a metal system voids the column warranty.

#### 2.1.2 Installation of Disposable Electrode into an ED50 Cell, pH-Ag/AgCl Reference Electrode or PdH Reference Electrode

The 2 mil-thick (0.002 in.) 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-5000 Ion Chromatography System Manual Doc. No. 065342 and User's Compendium for Electrochemical Detection Doc. No. 065340.

#### 2.1.3 System Void Volume

When using Dionex CarboPac MA1 columns, it is particularly important to minimize system void volume. The system void volume for 2 mm columns should be scaled down to at least 1/4 of the system volume in a standard system designed for 4 mm columns (4 mm system). For best performance, all of the tubing installed between the injection valve and detector should be 0.005" (P/N 044221) i.d. PEEK tubing for 2mm columns and 0.010" i.d. PEEK tubing (P/N 042260) for 4mm 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 4 mm System Injection Loop, 10 - 50 $\mu$ L

For most applications on a 4 mm analytical system, a 10 – 50  $\mu$ 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, however injecting more than 50  $\mu$ L of sample onto the 4 mm analytical column may result in decreased peak efficiency.

### 2.3 The Dionex CarboPac MA1 Guard Column

A Dionex CarboPac MA1 Guard Column is normally used with the Dionex CarboPac MA1 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 MA1 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex CarboPac MA1 Analytical Column.

## 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  $\mu\text{m}$ . 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 P/N UN 1824) for preparation.

### 3.3 Sodium Acetate

Thermo Scientific highly recommends the use of Dionex Sodium Acetate Reagent (P/N 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 MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily.
NEVER	run above 60°C and/or 2000 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  $\mu\text{m}$  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! After exposure to air NaOH pellets are coated with a layer of carbonate that can interfere with sample elution and detection.*

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 80.02g 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 with degassed, dionized 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)
0.3	24.0	15.7
0.48	38.4	25.1
0.612	49.0	32.0
1.0	80.0	52.3

**NOTE**

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

### 5.3 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](http://www.thermoscientific.com).

### 5.4 Verification of System Cleanliness

Prepare a new set of eluents as described in [Sections 5.1-5.2](#) 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.

#### 5.4.1 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 200mM 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.

### 5.4.2 Verification of Column Cleanliness

Install the Dionex CarboPac MA1 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 MA1 is shipped in 612mM NaOH. Any column that is stored long-term should be stored in the same solution. To prepare the column for standard analysis, the Dionex CarboPac MA1 must be washed for at least one hour (two hours preferred) with 612 mM NaOH at appropriate flow rate. 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.

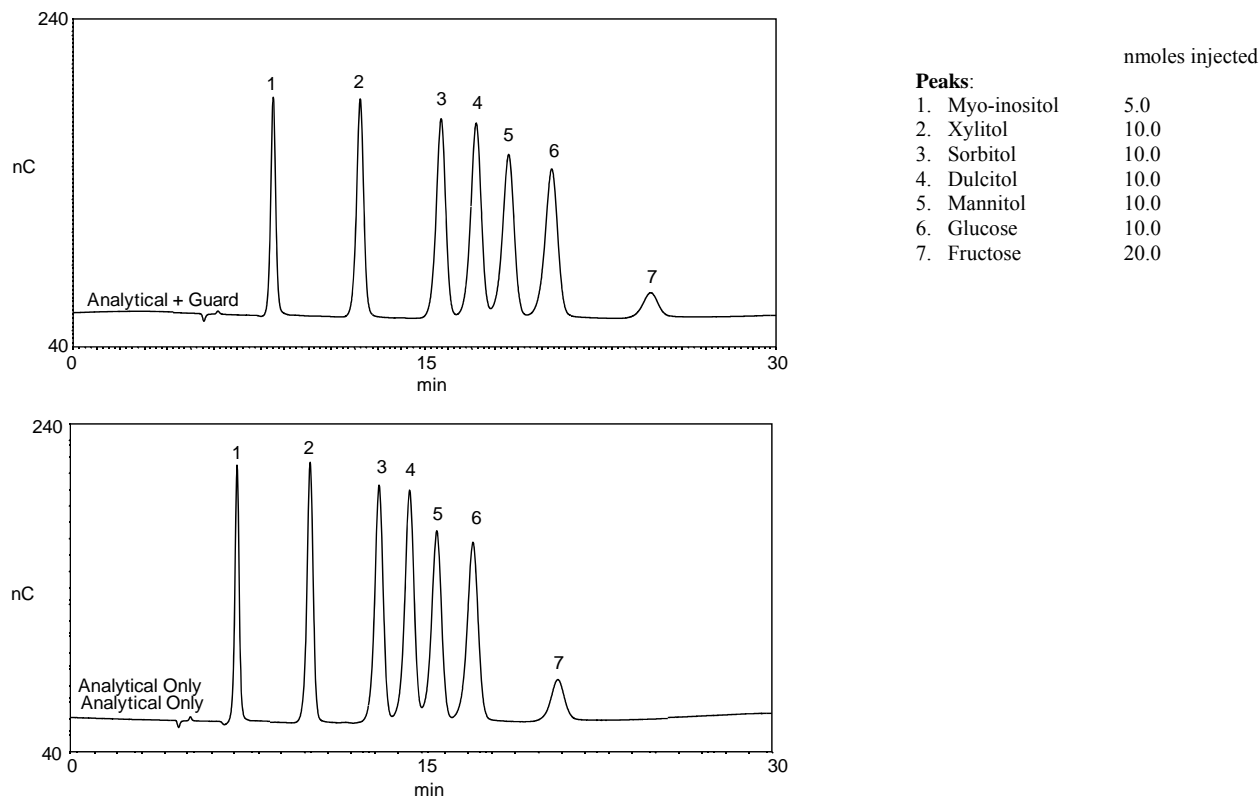


### 5.4.3 Production Test Chromatograms

Isocratic elution of the diluted Dionex CarboPac MA1 QAR standard on the Dionex CarboPac MA1 Analytical Column has been optimized utilizing a hydroxide eluent and can be used to test the performance of the Dionex CarboPac MA1 Column. The Dionex CarboPac MA1 Analytical Column should always be used with the Dionex CarboPac MA1 Guard Column; the addition of the Guard column increases elution time by ~20% when compared to the Analytical column by itself. To guarantee that all Dionex CarboPac MA1 Analytical columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test. An operating temperature of 30°C is used to ensure reproducible resolution and retention.

**Column:** Dionex CarboPac MA1 4 × 250 mm  
**Eluent:** 612 mM NaOH  
**Temperature:** 30°C  
**Flow Rate:** 0.4 mL/min  
**Inj. Volume:** 10 µL  
**Detection:** Integrated Amperometry, quadruple pulse waveform  
**Working Electrode:** PTFE Gold, disposable electrode  
**Reference Electrode:** Ag/AgCl  
**Diluted Standard:** (with DI Water)

**Figure 1** Dionex CarboPac MA1 Production Test Chromatograms (4 mm)



## 6. Applications

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

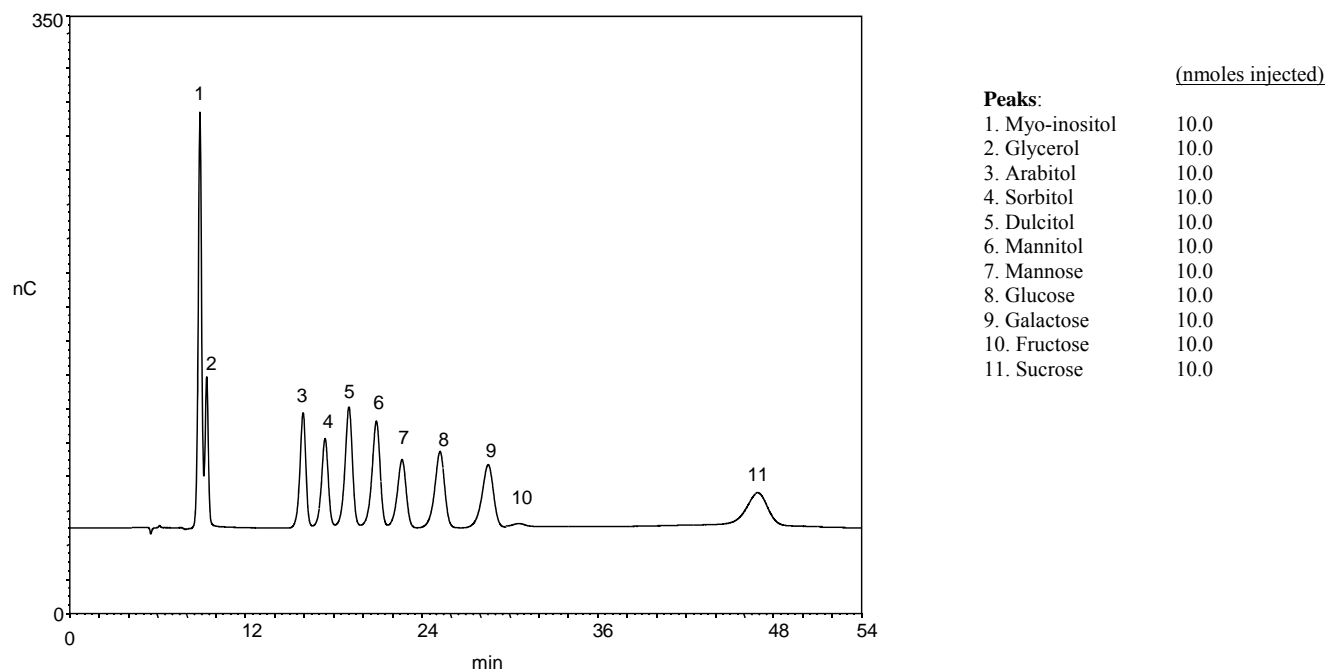
### 6.1 Separation of Sugar Alcohols and Monosaccharides Commonly Found in Food Products

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

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

<b>Column:</b>	Dionex CarboPac MA1 Analytical and Guard Column (4mm)
<b>Eluent:</b>	480 mM NaOH
<b>Temperature:</b>	30°C
<b>Inj. Volume:</b>	10µL
<b>Flow Rate:</b>	0.4 mL/min
<b>Detection:</b>	Integrated Amperometry, quadruple pulse waveform
<b>Working Electrode:</b>	PTFE Gold, disposable electrode
<b>Reference Electrode:</b>	Ag/AgCl

**Figure 2** Separation of Common Food Alditols

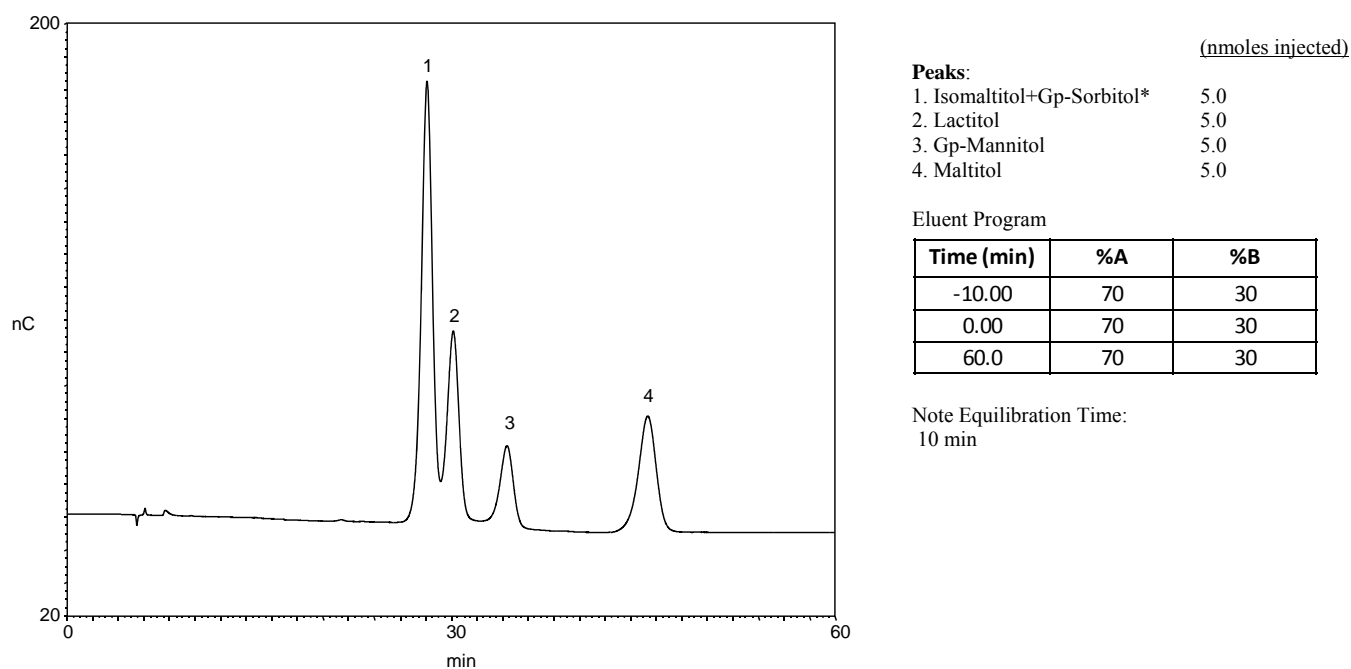


## 6.2 Analysis of Sweeteners

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

**Column:** Dionex CarboPac MA1 4 × 250 mm Analytical and Guard Column  
**Eluent:** 300 mM NaOH  
**Temperature:** 30°C  
**Inj. Volume:** 10 µL  
**Flow Rate:** 0.4 mL/min  
**Detection:** Integrated Amperometry, quadruple pulse waveform  
**Working Electrode:** PTFE Gold, disposable electrode  
**Reference Electrode:** Ag/AgCl

**Figure 3 Separation of Disaccharide Alcohols in Sweeteners**



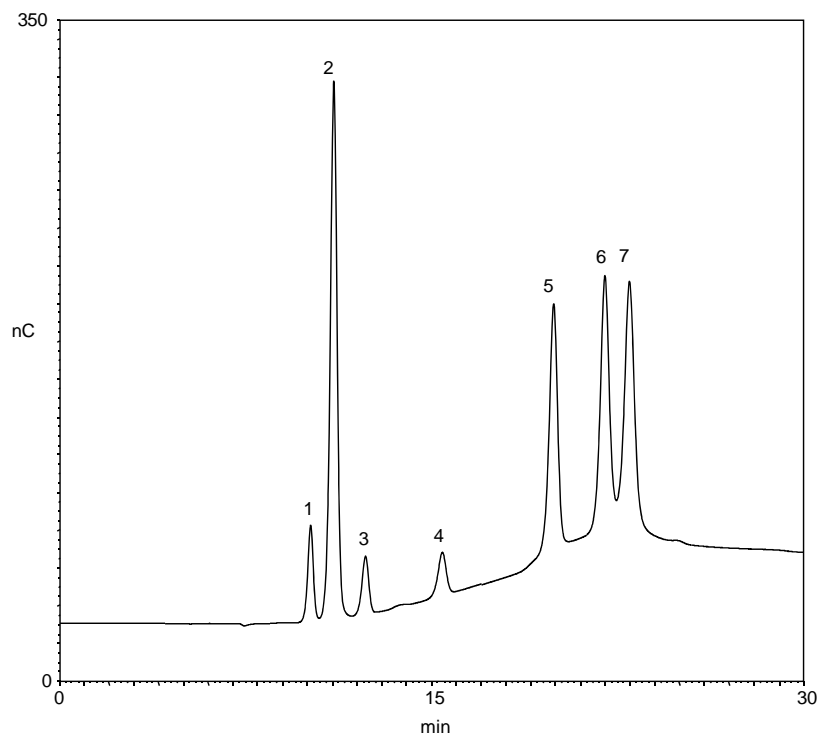
\* Please Note: Gp-Mannitol standard came from a product called isomalt, which in addition to Gp-mannitol has Gp-sorbitol

### 6.3 Alditols found in Physiological Fluids

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

**Column:** Dionex CarboPac MA1 Analytical and Guard Column (4mm)  
**Eluent:** A – DI Water  
 B – 1.0 M NaOH  
**Temperature:** 30°C  
**Flow Rate:** 0.4 mL/min  
**Inj. Volume:** 10 µL  
**Detection:** Integrated Amperometry, quadruple pulse waveform  
**Working Electrode:** PTFE Gold, disposable electrode  
**Reference Electrode:** Ag/AgCl

**Figure 4 Separation of Alditols Found in Physiological Fluids**



Peaks:	(nmoles injected)
1. Glycerol	10
2. Myo-inositol	10
3. Scyllo-inositol	0.5
4. Erythritol	100
5. Arabitol	10
6. Sorbitol	10
7. Dulcitol	10

**Gradient Program**

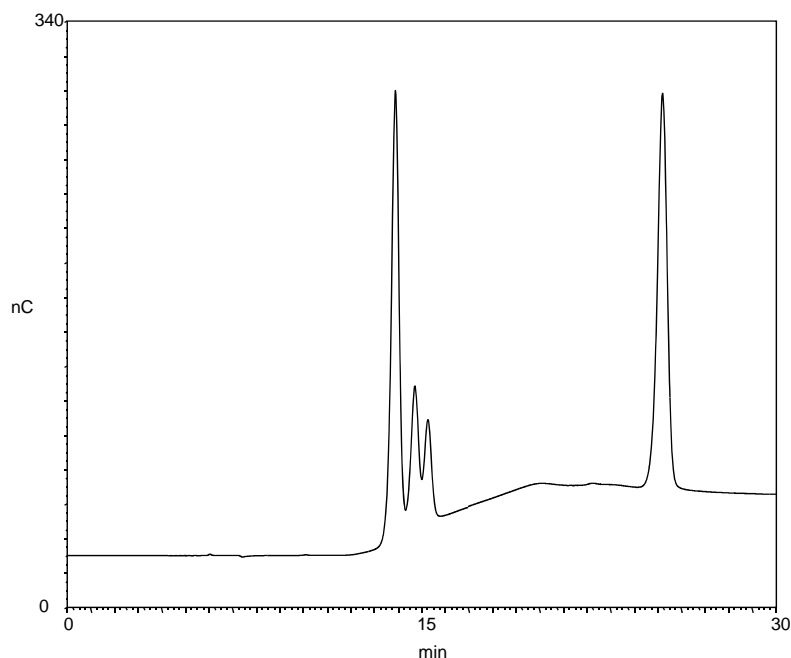
Time (min)	%A	%B
-20.00	92	8
0.0	92	8
4.0	92	8
15.1	30	70
25.2	30	70
26.3	92	8
35.3	92	8

## 6.4 Separation of Monosaccharide Alditols Released by $\beta$ -elimination from Glycoproteins

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

**Column:** Dionex CarboPac MA1 Analytical and Guard Column (4mm)  
**Eluent:** A – DI Water  
 B – 1.0 M NaOH  
**Temperature:** 30°C  
**Flow Rate:** 0.4 mL/min  
**Inj. Volume:** 10  $\mu$ L  
**Detection:** Integrated Amperometry, quadruple pulse waveform  
**Working Electrode:** PTFE Gold, disposable electrode  
**Reference Electrode:** Ag/AgCl

**Figure 5** Separation of Monosaccharide Alditols Released by Direct  $\beta$ -elimination from Glycoproteins



Peaks:	(nmoles injected)
1. Fucitol	10.0
2. GalNAcol	2.0
3. GlcNAcol	2.0
4. Mannitol	10.0

### Gradient Program

Time (min)	%A	%B
-10.00	90	10
0.00	90	10
4.70	90	10
15.80	30	70
30.00	30	70

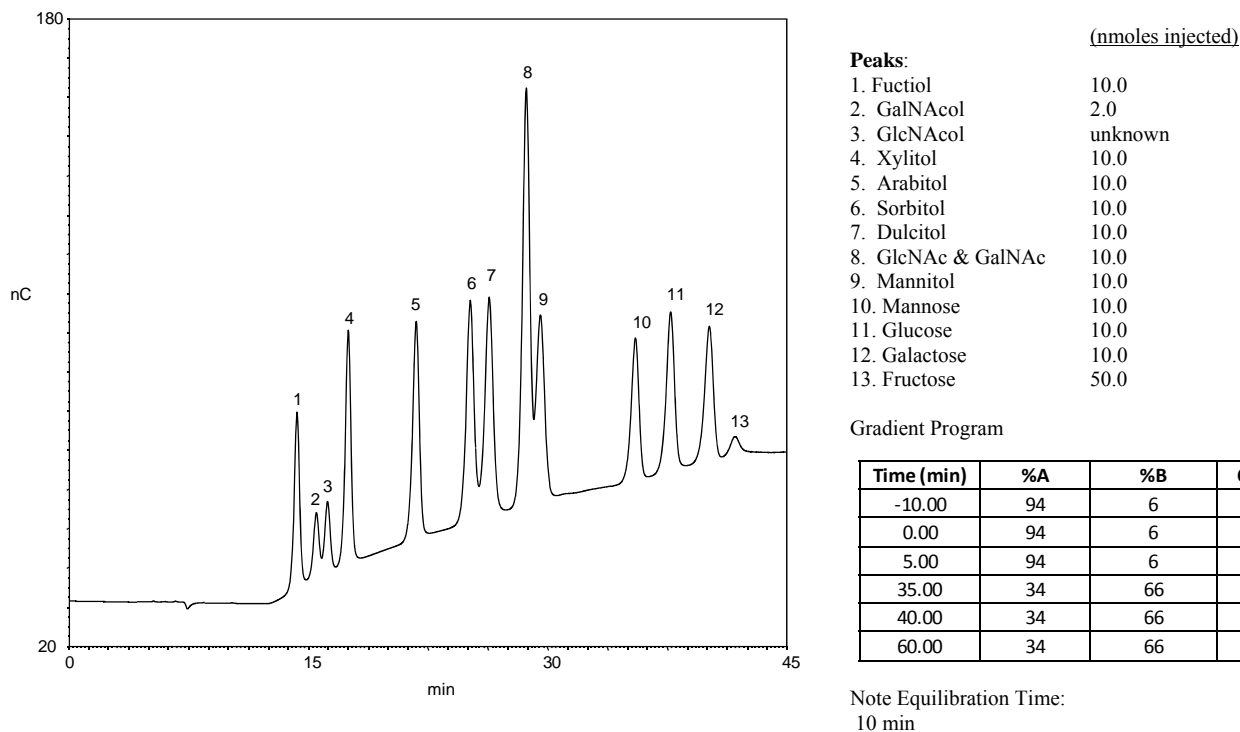
Note Equilibration Time:  
10 min

## 6.5 Separation of Monosaccharide Alditols Released by Hydrolysis of $\beta$ -elimination Glycoconjugates

This example chromatogram shows the separation of monosaccharides derived from glycoprotein oligosaccharides. Oligosaccharides are released by reductive  $\beta$ -elimination which converts only the terminal peptide-linked sugar to the alditol form. The terminally-reduced oligosaccharide is chemically or enzymatically digested into a mixture containing one alditol (Peaks 1, 2, 3, 4, 7, 8 and 10) and one or more non-reducing monosaccharides. Arabitol (Peak 5) serves as an internal standard. An initial isocratic step at 60 mM NaOH separates N-acetyl glucosaminitol (GlcNAcol) and N-acetyl galactosaminitol (GalNAcol) from one another and from fucitol. The gradient that follows this isocratic separation accelerates the elution of mannitol as well as any oligosaccharide alditols that may have been released during this  $\beta$ -elimination reaction and positions the elution of several aldoses potentially present so they do not interfere with the other analytes.

**Column:** Dionex CarboPac MA1 Analytical and Guard Column (4mm)  
**Eluent:** A – DI Water  
 B – 1.0 M NaOH  
**Temperature:** 30°C  
**Flow Rate:** 0.4 mL/min  
**Inj. Volume:** 10  $\mu$ L  
**Detection:** Integrated Amperometry, quadruple pulse waveform  
**Working Electrode:** PTFE Gold, disposable electrode  
**Reference Electrode:** Ag/AgCl

**Figure 6** Separation of Alditols and Aldoses Released by Hydrolysis of  $\beta$ -eliminated Oligosaccharides with Arabitol as Internal Standard



## 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 compare 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 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 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 0.4 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 > 30 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. Otherwise continue with your work with the new electrode installed.
- 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 MA1 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 described in a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac MA1 QAR eluent (612mM NaOH) is 50-70nC. 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, “Column Cleanup”.

## 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 1mL/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 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.

### 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 "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 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, "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  $\mu$ 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 5.6.3](#). 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 necessary 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-5000 Ion Chromatography System Manual Doc. No. 065342 and User's Compendium for Electrochemical Detection Doc. No. 065340 for any help necessary with electrochemical detection, working and reference electrodes.

# Appendix A – Dionex CarboPac MA1 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 MA1 column is 2,000 psi (13.79 MPa).

## A.2 Column Start-Up

The Dionex 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 1.3 Poor Resolution and Section 1.3.1 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), 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.

## A.4 Column Cleanup

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

- 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.*

- B. Wash the Dionex CarboPac MA1 extensively (50-column volumes) with water. At 0.4 mL/min, 50-column volumes will require approximately 3.5 hours.
- C. Clean the column with 50 mL of a 1.0 M solution of acetic acid.
- D. Wash the column with 50 mL of water.
- E. Clean the column with 50 mL of a 1.0 M solution of sodium acetate.
- F. Wash the column with 50 mL of water.
- G. Clean the Dionex CarboPac MA1 with 50 mL of 1.0 M NaOH.
- H. Equilibrate the Dionex CarboPac MA1 to the desired initial conditions. Test the column with standards to ensure that good column performance has been restored.

# Appendix B – Quality Assurance Reports (QAR)

**Dionex CarboPac™ MA1**  
**Analytical (4 x 250 mm)**  
**Product No. 044066**

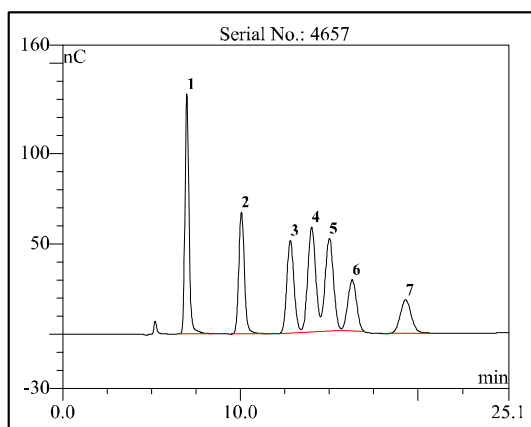
**Date:** 12-Sep-13 10:16  
**Serial No. :** 004657  
**Lot No. :** 011-28-046A

**Eluent:** 612 mM NaOH  
**Eluent Flow Rate:** 0.4 mL/min  
**Temperature:** Ambient Temperature  
**Detection:** Electrochemical Detection  
**Injection Volume:** 10 µL  
**Storage Solution:** Eluent

## ED40 Operating Parameter

Time	Potential <sup>1</sup> 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

<sup>1</sup> Reference Electrode Mode: Ag/AgCl



No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Amount Injected (nmoles)
1	Myo-Inositol	7.0	1.2	6.32	4723	5.0
2	Xylitol	10.1	1.2	4.24	5026	10.0
3	Sorbitol	12.8	1.1	1.53	4776	10.0
4	Dulcitol	14.0	n.a.	1.16	4602	10.0
5	Mannitol	15.0	n.a.	1.45	4700	10.0
6	Glucose	16.3	1.0	2.94	5004	10.0
7	Fructose	19.3	1.1	n.a.	4721	20.0

## QA Results:

Analyte	Parameter	Specification	Results
Glucose	Efficiency	>=4500	Passed
Glucose	Retention Time	15.9-18.1	Passed
Glucose	Asymmetry	0.9-1.7	Passed
	Pressure	<=1430	1107

## Production Reference:

Datasource: Resin  
 Directory: RTA\RTA\_6  
 Sequence: 1487868\_CP\_MA1\_4X250MM\_SS  
 Sample No.: 80

6.80 SR12 Build 3578 (207169) (Demo-Installation)

Chromleon™ Thermo Fisher Scientific

066947-05 (QAR)