Thermo Scientific

Dionex CarboPac SA10

Column Product Manual

P/N: 065384-03 July 2013

Thermo SCIENTIFIC

Product Manual

for

Dionex CarboPac SA10G Guard Columns

4 x 50 mm (P/N 074902)
2 x 50 mm (P/N 082323)

Dionex CarboPac SA10 Analytical Columns

4 x 250 mm (P/N 074641)
2 x 250 mm (P/N 082322)

For Research Use Only. Not for Use in Diagnostic Procedures.

Revision History:

Revision 03, July, 2013, Rebranded for Thermo Scientific. Added new 2 mm column sizes.

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 CarboPac SA10 column

The Thermo Scientific™ Dionex™ CarboPac™ SA10 (2 mm and 4mm) columns have been developed to provide fast, high-resolution separations for most mono- and disaccharides in biofuel and food & beverage research. The eight common biofuel sugars, fucose, sucrose, arabinose, galactose, glucose, xylose, mannose and fructose, can be separated on these columns in less than 10 minutes. The six common sugars in food & beverage industry, sucrose, glucose, fructose, lactose, cellobiose, and maltose, can be separated within 10 minutes on these columns.

The Dionex CarboPac SA10 columns are packed with a hydrophobic, polymeric, supermacroporous anion exchange resin stable over the range of pH 0-14. This unique pH-stability of the packing material allows the use of eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes.

1.1.1 Resin Characteristics:

Particle Size:	6 µm
Pore Size:	supermacroporous (2000 Å)
Cross-linking:	55%
Ion exchange capacity:	290 µeq per 4 x 250 mm column; 72.5 µeq per 2 x 250 column.

1.1.2 Latex Characteristics:

Functional Group:	quaternary ammonium ion
Latex Diameter:	55 nm
Latex Cross-linking:	4.5 %

1.1.3 Typical Operating Parameters:

pH range:	0-14
Temperature Limit:	4-60°C
Pressure Limit:	3500 psi
Organic Solvent Limit:	100% compatible
Typical eluents:	potassium hydroxide or sodium hydroxide

2. System Requirements

2.1 System Requirements and Installation

2.1.1 System Requirements for 2 mm and 4 mm Operation

The carbohydrate separations using the Dionex CarboPac SA10 columns are optimized for use with Dionex Ion Chromatography systems equipped with electro chemical detection. It is highly recommended to ensure that the systems used for carbohydrate analysis are metal-free. Metal ions from a metal system will contaminate the CarboPac column and may contaminate the working electrode. Running a CarboPac column on a metal system voids the column warranty.

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

The 2 mil thick Teflon gaskets included in each package of disposable electrodes must be used; otherwise, the disposable electrode product warranty is void. Other gaskets are available in 15 mil (P/N 057364) and 62 mil thickness (P/N 075499) which can also be used. When using the 62 mil gasket a special modified spacer block must also be used (P/N 075501). A gasket must always be used. 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 SA10 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 µL

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

2.2.2 The 2 mm System Injection Loop, 2 - 15 µL

For most applications on a 2 mm analytical system, a 2 – 15 µL injection loop is sufficient. Generally, you should not inject more than 10 nanomoles of any analyte onto a 2 mm analytical column. Injecting larger amount 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. Install an injection loop one-fourth or less (<15 µL) of the loop volume used with a 4 mm analytical system.

2.2.3 High Concentration Samples

For high concentration samples, a 0.4 µL internal injection valve (P/N 072050), special 62 mil thick ED gasket (P/N 075499), and a compatible ED block, (P/N 075501), may be needed to minimize requirement of dilution. When using the 2 mm format a 15 mil thick gasket (P/N 057364) is recommended.

2.3 The Dionex CarboPac SA10G Guard Column

A Dionex CarboPac SA10G Guard Column is normally used with the CarboPac SA10 Analytical Column. Retention times will increase by approximately 5% 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 SA10G Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex CarboPac SA10 Analytical Column.

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

For Dionex CarboPac SA10 applications using the Dionex EGC KOH cartridge, a Dionex CR-ATC 500 Continuously Regenerated Trap Column (P/N 075550) 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 500 Product Manual (Document No. 031910) for instructions.

2.5 System Start-up

2.5.1 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 KOH, 0.5 mL/min for 4 mm or 0.2 mL/min for 2 mm,
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 carbohydrate standard quad 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 baseline is < 30 nC. If the background > 30 nC or the pH is out of range, see the "Troubleshooting" section at the end of this manual.

NOTE

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

2.5.2 Verification of Column Cleanliness

Install the Dionex CarboPac SA10 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 SA10 is shipped in 1mM 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 SA10 must be washed for at least one hour (two hours preferred) with 100mM KOH 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.

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

3. Operation

3.1 CarboPac SA10 Column Operational Parameters

- pH range: pH = 0 - 14
- Temperature limit: 60°C
- Pressure limit: 3,500 psi
- Organic Solvent Limit: 100% Acetonitrile, methanol, acetone, if required for cleaning
- Typical Eluents: potassium hydroxide from the Eluent Generator
- Standard Flow Rate: 2 mm: 0.38 mL/min
  4 mm: 1.5 mL/min
- Maximum Flow Rate: 2 mm: 0.5 mL/min
  4 mm: 2.0 mL/min

3.1.1 The Most Important Rules

3.1.1.1 ALWAYS...

- use dedicated glassware and disposable glass or plastic ware for volume adjustments.
- use high purity water (18.2 MΩ-cm resistivity).
- keep your water blanketed with helium or nitrogen. Use new filtered water if left unblanketed for more than 30 minutes.

3.1.1.2 NEVER...

- go to the next step of the installation if the previous step has failed.
- start an installation with any of the check list items below missing.
- use 'communal' filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials.
- use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily. NEVER run above 60 °C or 3,500 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 pump available for use with the vacuum filtration units
- 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

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

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

3.2.2 Potassium Hydroxide

Use Dionex KOH Eluent Generator Cartridge installed with CR-ATC in the EG module.

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 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.

3.3.1 Deionized Water

Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir for 5-10 minutes while sonicating. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere. Vacuum filtration through 0.2 µm Nylon filters is a good alternative to vacuum degassing under sonication and is sufficient in the majority of cases. On-line degassing is supported through the use of the DP gradient pumping systems.

3.3.2 Eluent: Potassium Hydroxide

The first step in the preparation of potassium hydroxide eluent is to degas an aliquot (typically 1000 mL) of the deionized water, as described above. In the second step, start the pump flow and verify that the water is exiting from the Eluent Generator exit tubing. In the third step, select an appropriate KOH concentration (usually 1.0 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 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 anions, which are strong eluents for the Dionex CarboPac columns (e.g. chloride, carbonate, phosphate, etc.). 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 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.

4. Example Applications

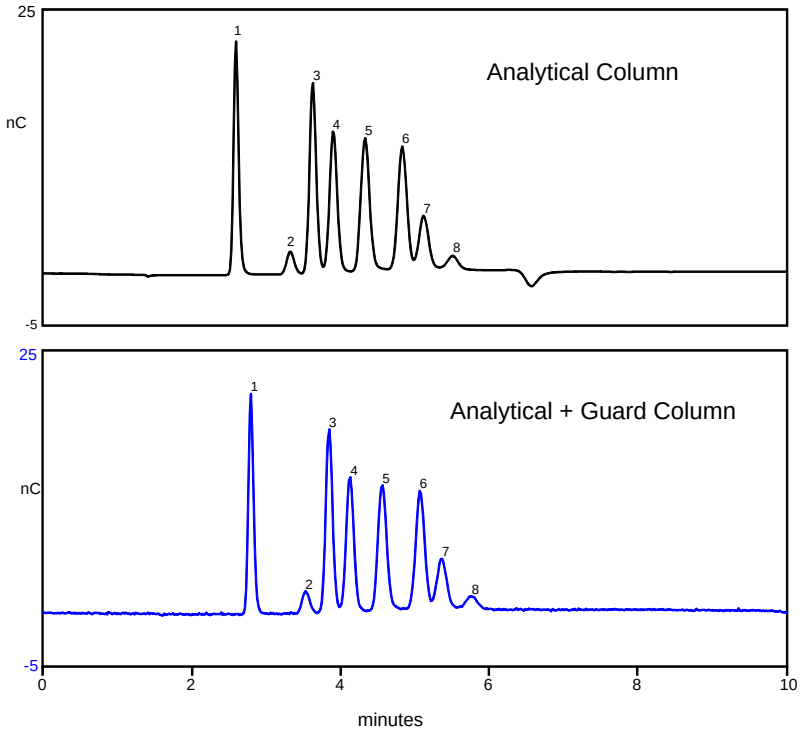
The following section provides an example of the types of applications for which the Dionex CarboPac SA10 is designed. The chromatograms in this section were obtained using columns that reproduced the Quality Assurance Report on an optimized Ion Chromatograph. Different systems will differ slightly in performance due to slight differences in column set, system voids volumes, liquid sweep-out times of different components and laboratory temperatures.

4.1 Production Test Chromatograms

Isocratic separation of mono and disaccharide standard (the 8 most common biofuel sugars of interest) the Dionex CarboPac SA10 analytical column has been optimized utilizing a hydroxide eluent and can be used to test the performance of the Dionex CarboPac SA10 Column. The Dionex CarboPac SA10 analytical column should always be used with the Dionex CarboPac SA10G guard column; the addition of the Guard column increases elution time by ~5% when compared to the Analytical Column by itself. To guarantee that all Dionex CarboPac SA10 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test at 45ºC.

The Dionex CarboPac SA10 column has been designed to provide fast speed separations for mono- and disaccharides. The eight biofuel sugars can be separated within 7 min on this column. Figures 1 and 2 show runs without and with a guard column for both the 4 mm and 2 mm formats.

Figure 1 Separation of mono- and disaccharides with and without guard column: 2 mm format



Column:	Dionex CarboPac SA10 2mm
Eluent :	1mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.38 mL/min
Inj. Volume:	2.5µL
Gasket:	2 mil
Temperature:	45C
Detection:	Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode:	PTFE Gold, disposable electrode

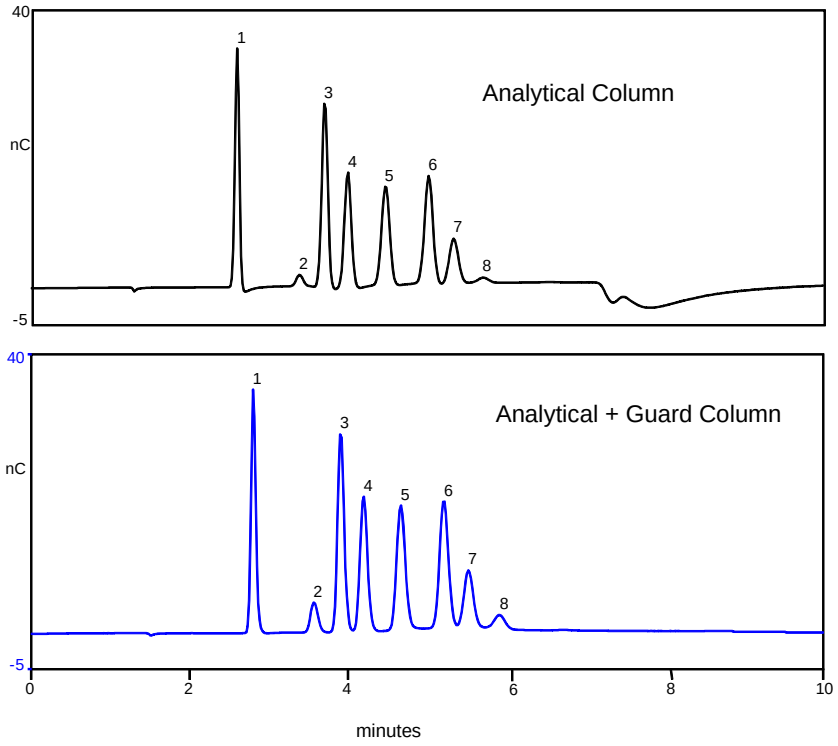
Reference Electrode: Silver /Silver Chloride

Standard: 10 mg/L

Peaks:

1. Fucose
2. Sucrose
3. Arabinose
4. Galactose
5. Glucose
6. Xylose
7. Mannose
8. Fructose

Figure 2 Separation of mono- and disaccharides with and without guard column, 4 mm format



Column:	Dionex CarboPac SA10 4mm
Eluent :	1mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	1.50mL/min
Inj. Volume:	10µL
Gasket:	2 mil
Temperature:	45C
Detection:	Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode:	PTFE Gold, disposable electrode

Reference Electrode: Silver /Silver Chloride

Standard: 10 mg/L each

Peaks:

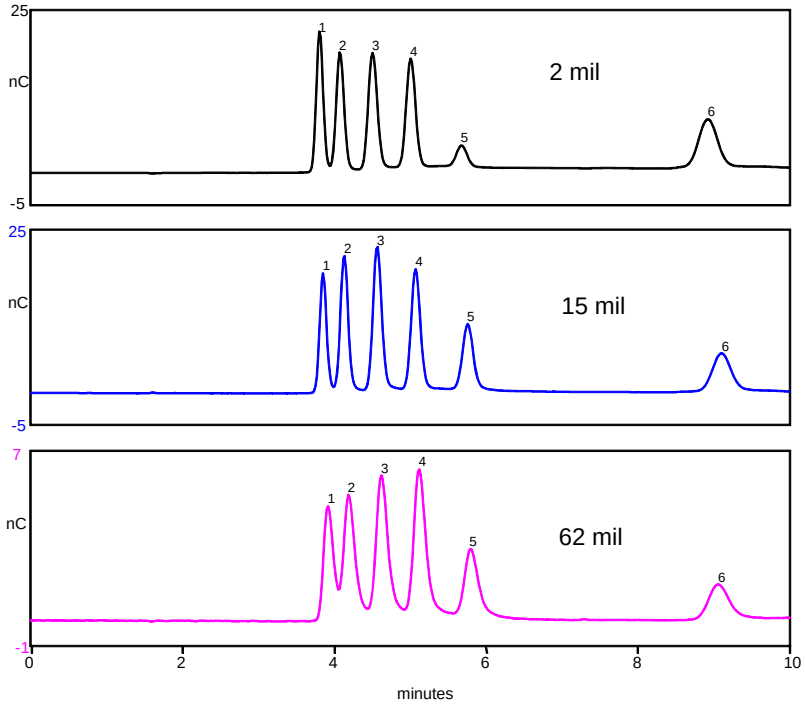
1. Fucose
2. Sucrose
3. Arabinose
4. Galactose
5. Glucose
6. Xylose
7. Mannose
8. Fructose

4.2 Comparison of Gasket Thickness Using Standards and Corn Stover Sample

Figure 3 and figure 4 demonstrates that gasket thickness may not be as important if the sample concentration is not high. The Dionex CarboPac SA10 (4 mm) chromatography looks good (Figure 4) when using 2 mil, 15 mil or 62 mil gasket and sugar samples with relatively lower concentrations. However, Dionex CarboPac SA10 (2 mm) chromatography is affected negatively (Figure 3) by using 62 mil gasket (excessive peak tailing and loss of resolution). The optimum gasket thickness for the 2 mm SA10 is either 2 mil or 15 mil when using a low concentration sample or standard.

Biomass samples often contain high concentration (over 100 g/L) sugar contents, which usually require a dilution factor of 1000 to avoid saturating the column or the detector. By reducing the detector sensitivity with 62 mil thick ED gasket, corn stover hydrolysate sample with ~150 g/L total sugar concentration can be analyzed quantitatively on the column after 50× dilution (Figures 5 & 6). One can use a standard injection valve with PEEK injection loops and various gasket sizes depending on customer preference. Figures 5 and 6 show 50:1 dilution of a corn stover sample using a 2.5 µL injection loop for 2 mm formats and 10 µL for 4 mm formats using three different gasket thickness. As you can see when using high concentration samples, 62 mil gasket is optimum for the Dionex CarboPac SA10 (4 mm) and 15 mil gasket is optimum for the Dionex CarboPac SA10 (2 mm).

Figure 3 Comparison of Various Thickness Gaskets with Low Concentration Standards: 2 mm Format



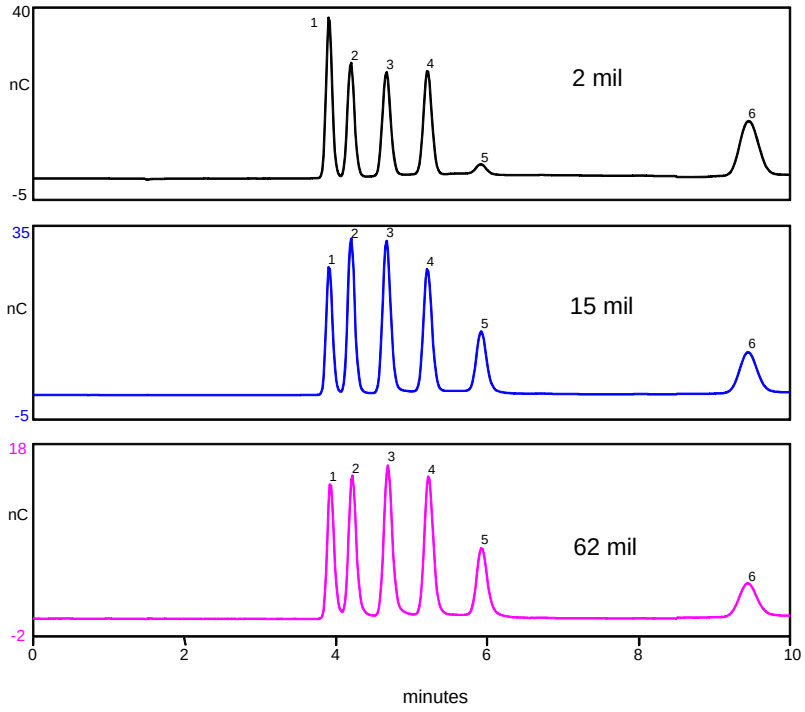
Column:	Dionex CarboPac SA10 Guard+ Analytical 2mm
Eluent :	1mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.38 mL/min
Inj. Volume:	2.5µL
Temperature:	45C
Detection:	Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode:	PTFE Gold, disposable electrode

Reference Electrode: Silver /Silver Chloride

Standard: 15 mg/L each
Peaks:

1. Arabinose
2. Galactose
3. Glucose
4. Xylose
5. Fructose
6. Cellobiose

Figure 4 Comparison of Various Thickness Gaskets with Low Concentration Standards: 4 mm Format

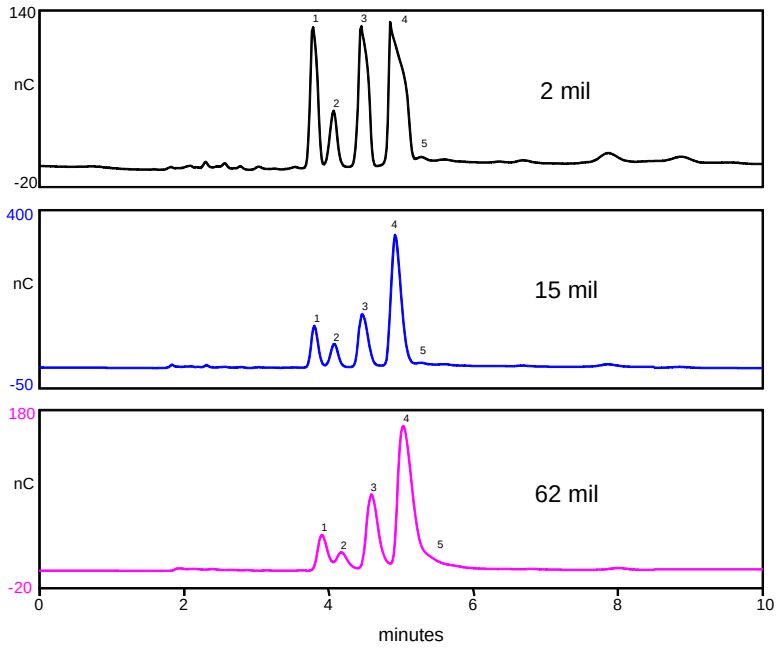


Column: Dionex CarboPac SA10 Guard+ Analytical 4 mm
Eluent : 1mM KOH
Eluent Source: Dionex EGC 500 KOH cartridge
Flow Rate: 1.50 mL/min
Inj. Volume: 10µL
Temperature: 45C
Detection: Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode: PTFE Gold, disposable electrode
Reference Electrode: Silver /Silver Chloride

Standard: 15 mg/L each
Peaks:

1. Arabinose
2. Galactose
3. Glucose
4. Xylose
5. Fructose
6. Cellobiose

Figure 5 Comparison of Various Gaskets with 50:1 Corn Stover Sample: 2 mm Format

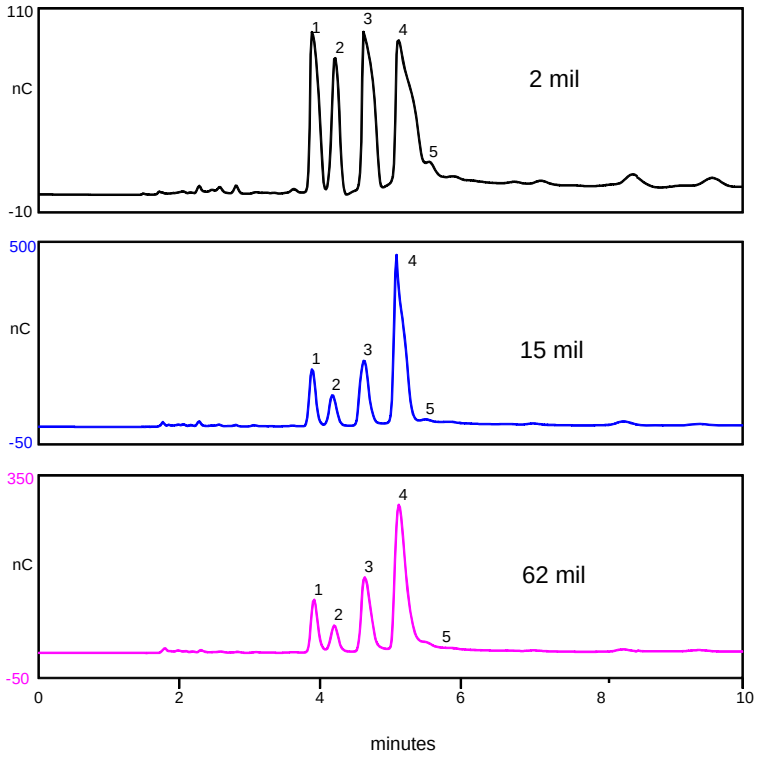


Column: Dionex CarboPac SA10 Guard+ Analytical 2 mm
Eluent : 1mM KOH
Eluent Source: Dionex EGC 500 KOH cartridge
Flow Rate: 0.38 mL/min
Inj. Volume: 2.5µL
Temperature: 45C
Detection: Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode: PTFE Gold, disposable electrode
Reference Electrode: Silver /Silver Chloride

Sample: Corn Stover (1:50 Dilution)
Peaks:

1. Arabinose
2. Galactose
3. Glucose
4. Xylose
5. Mannose

Figure 6 Comparison of Various Gaskets with 50:1 Corn Stover Sample: 4 mm Format



Column:	Dionex CarboPac SA10 Guard+ Analytical 4 mm
Eluent :	1mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	1.50 mL/min
Inj. Volume:	10µL
Temperature:	45C
Detection:	Integrated Amperometry , Carbohvdrate Standard Ouad Waveform
Working Electrode:	PTFE Gold, disposable electrode

Reference Electrode: Silver /Silver Chloride

Sample: Corn Stover (1:50 Dilution)

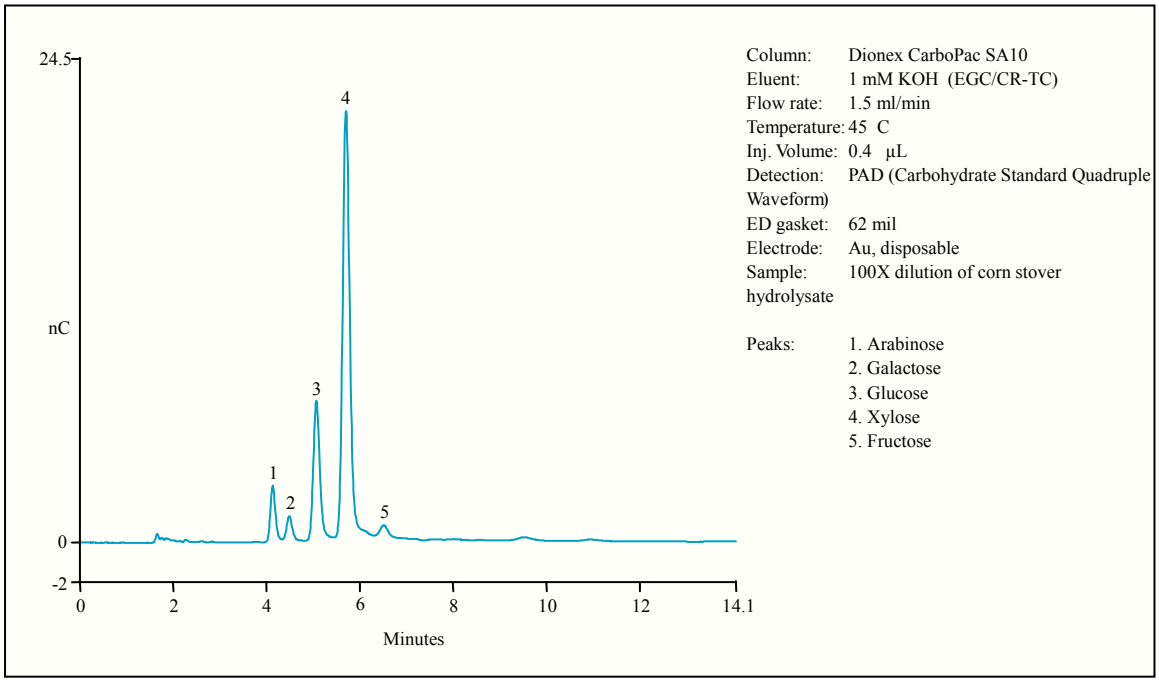
Peaks:

1. Arabinose
2. Galactose
3. Glucose
4. Xylose
5. Mannose

4.3 High concentration sample loading and analysis of Corn Stover Hydrolysate mono and disaccharides

Pulsed amperometric detection (PAD) is well known for high sensitivity. Biomass samples often contain high concentration (over 100 g/L) sugar contents, which usually require a dilution factor of 1000 to avoid saturating the column or the detector. By reducing the injection volume to 0.4 µL with an internal injection valve, and reducing the detector sensitivity with 62 mil thick ED gasket, corn stover hydrolysate sample with ~150 g/L total sugar concentration can be analyzed quantitatively on the column after 100× dilution.
The customer is not limited to this approach for high concentration samples. One can use a standard injection valve with PEEK injection loops and various gasket sizes depending on customer preference. Figures 5 and 6 show 50:1 dilution of a corn stover sample using a 2.5 µL injection loop for 2 mm formats and 10 µL for 4 mm formats. Shown in figures 9 and 10 are corn stover samples at two different dilutions with a 15 mil gasket for 2mm and with a 62 mil gasket for the 4mm CarboPac SA10. Included in these two figures is also an injection of the QAR standard used for testing these columns.

Figure 7 Analysis of corn stover hydrolysate: 4 mm Format



NOTE

Alternate approaches for reducing required dilutions is to use a 0.4µl injection valve with a 62 mil gasket, P/N 075499, and modified spacer block, P/N 075501.

Figure 8 Linearity on the CarboPac SA10 Column

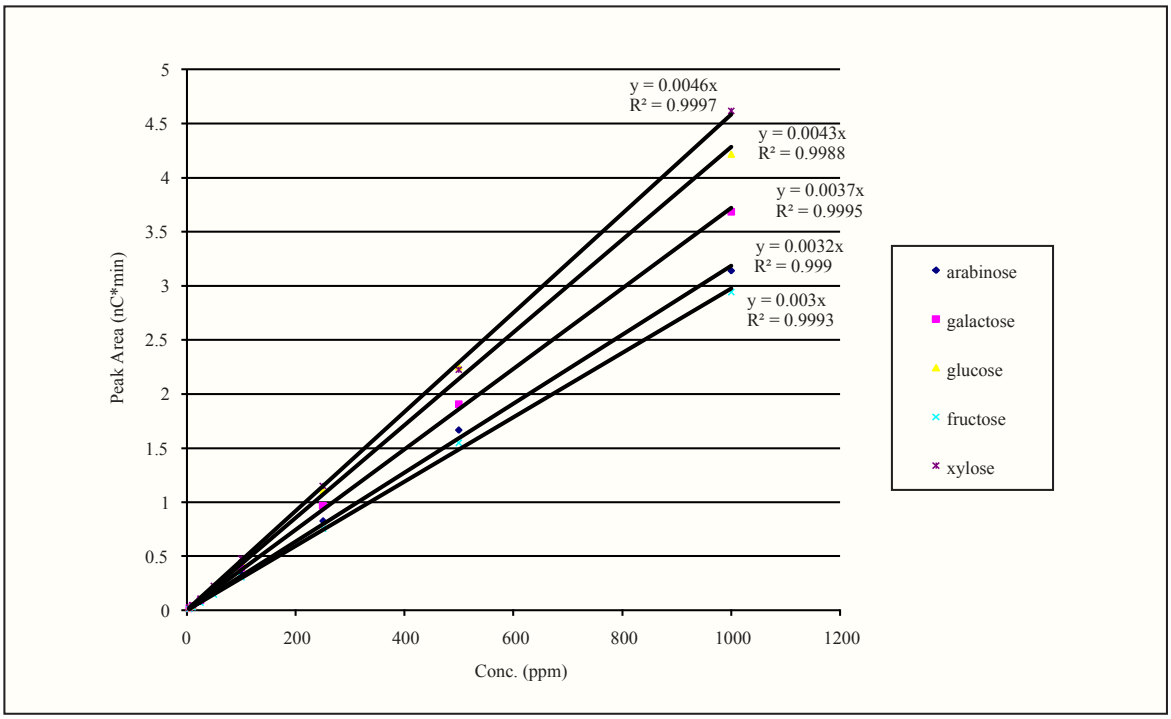
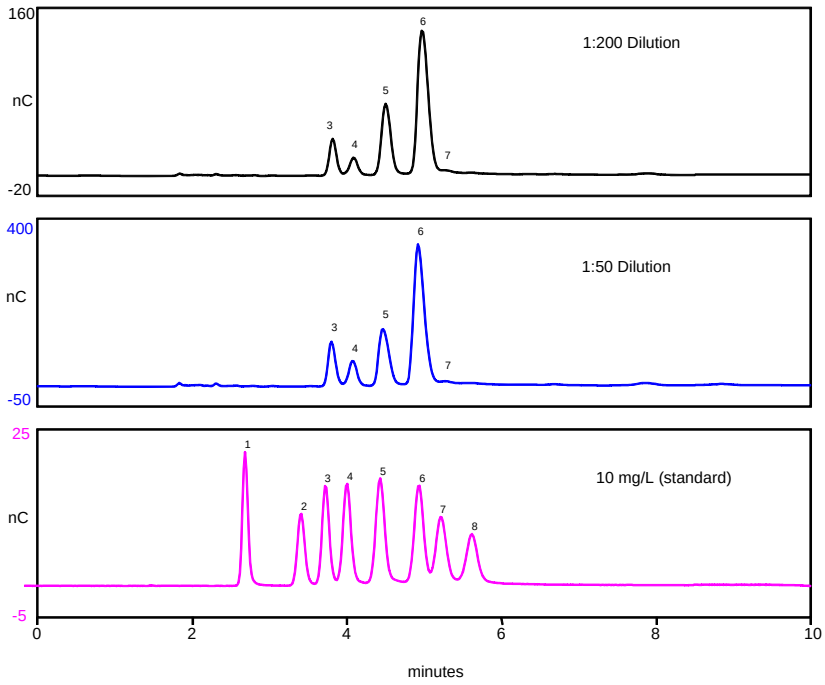


Figure 9 Comparison of Various Dilutions of Corn Stover Sample Using 15 mil Gasket: 2 mm Format



Column:	Dionex CarboPac SA10 Guard+ Analytical 2 mm
Eluent :	1mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	0.38 mL/min
Inj. Volume:	2.5µL
Temperature:	45C
Detection:	Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode:	PTFE Gold, disposable electrode

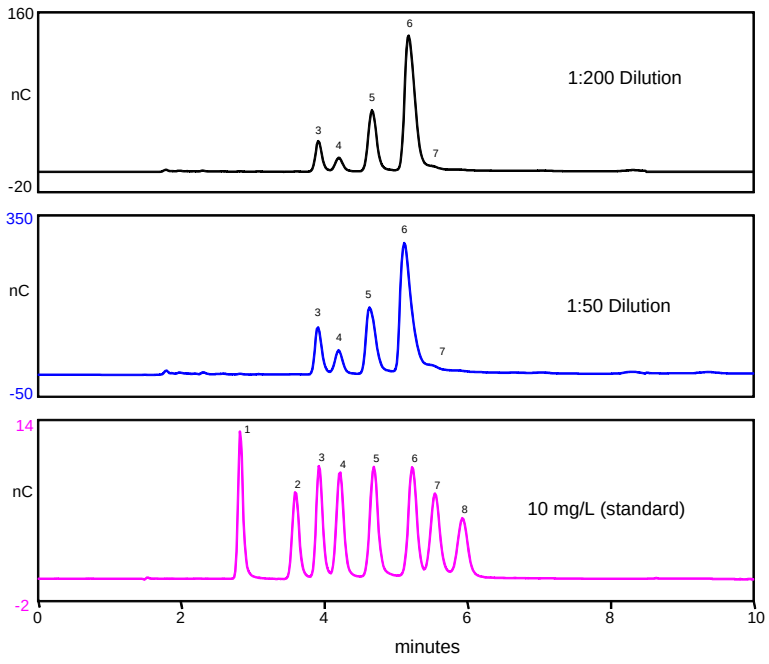
Reference Electrode: Silver /Silver Chloride

Sample: Corn Stover (1:200 and 1:50 Dilution)

Peaks:

1. Fucose
2. Sucrose
3. Arabinose
4. Galactose
5. Glucose
6. Xylose
7. Mannose
8. Fructose

Figure 10 Comparison of Various Dilutions of Corn Stover Sample Using 62 mil Gasket: 4 mm Format



Column:	Dionex CarboPac SA10 Guard+ Analytical 4 mm
Eluent :	1mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge
Flow Rate:	1.5 mL/min
Inj. Volume:	10 µL
Temperature:	45C
Detection:	Integrated Amperometry , Carbohydrate Standard Quad Waveform
Working Electrode:	PTFE Gold, disposable electrode

Reference Electrode: Silver /Silver Chloride

Sample: Corn Stover (1:200 and 1:50 Dilution)

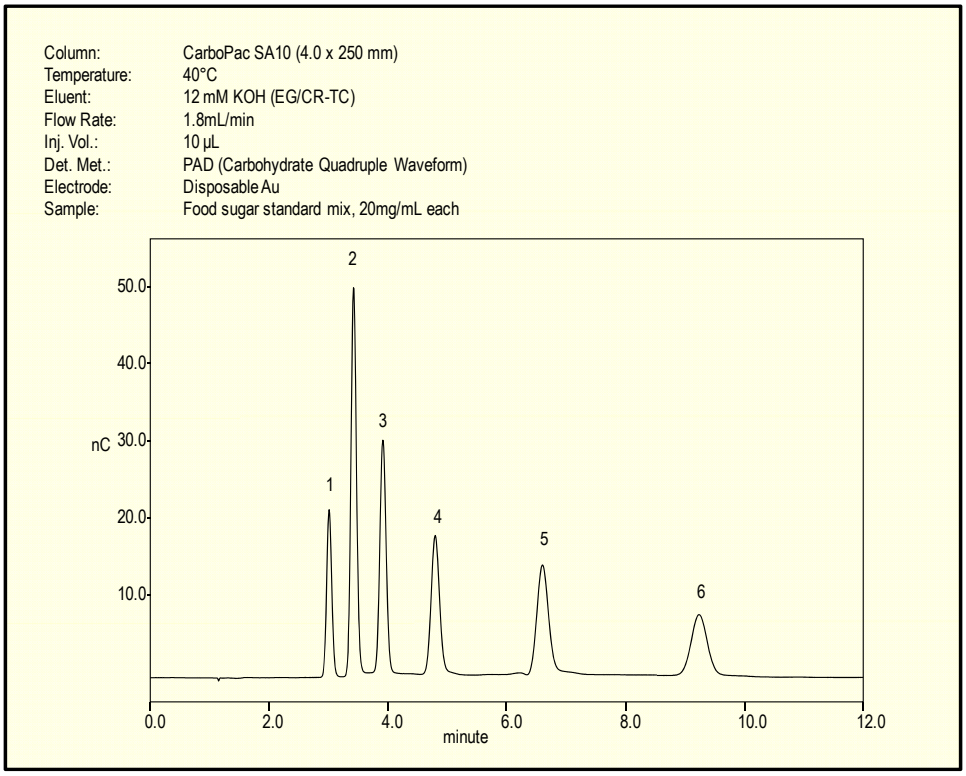
Peaks:

1. Fucose
2. Sucrose
3. Arabinose
4. Galactose
5. Glucose
6. Xylose
7. Mannose
8. Fructose

4.4 Food and Beverage

The Dionex CarboPac SA10 column has been designed to provide fast separations primarily for mono- and disaccharides biofuel samples, however one can also use this column for the sugars found in food and beverages. The six food sugars shown below in Figure 11 can be separated within 10 min on this column.

Figure 11 Separation of food & beverage sugars: 4 mm Format



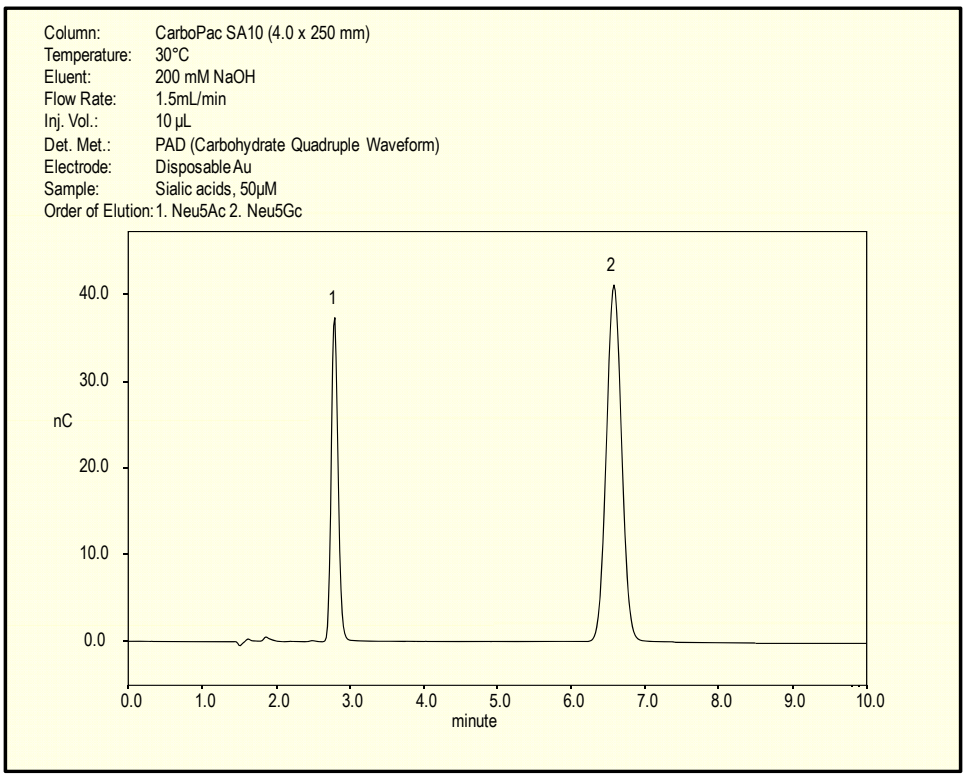
Peaks:

1. Sucrose
2. Glucose
3. Fructose
4. Lactose
5. Cellobiose
6. Maltose

4.5 Sialic acid

Another type of sample which can be analyzed with the Dionex CarboPac SA10 column includes sialic acids. However for these samples one must use manually prepared eluent to achieve the high concentration needed to elute such compounds.

Figure 12 Separation of Sialic Acids



5. Troubleshooting

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.

5.1 High Background

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

A background >30 nC with 1 mM potassium hydroxide at 1.5 mL/min (4mm)/ 0.38 mL/min (2mm) and 45°C using the carbohydrate standard quad 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 1 mM 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 Section 5.3.
D. Water contamination.
   Prepare eluents using 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.

5.2 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 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.

5.3 Column Problems

The guard column protects the main column not only from contamination but also from excessive pressure fluctuations caused by the instrument or by operator errors. Have the guard column installed at all times, disconnect it only during some of the testing described in this section, or when priming the pump to prevent accidental over pressure.

The column set is causing the high background if the background reading decreases after the column is replaced by a section of PEEK tubing, as described in Section 2.5.1. See Section 5.5 for the column clean up.

5.3.1 Peak Efficiency and Resolution are Decreasing

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

A. Run a standard separation without the guard column (analytical column only). If the separation improves, install a new guard column.. It is common to replace Guard columns several times during the lifetime of an analytical column.
B. Verify that correct tubing is installed for all connections between injector and detector, see Section 2.1.3.
C. Check for proper installation of ferrules on all PEEK tubing starting with the injector outlet and all other connectors to the ED cell inlet.
D. Check temperature settings in your method and/or actual temperature in your column oven.
E. The column may be overloaded. Try to inject a smaller amount of your sample or dilute the sample more.
F. If all of the above does not lead to an improved separation, the resin bed of the main column has been damaged and the main column must be replaced.

5.4 System Problems

5.4.1 High Detection Background Caused by the System

A. Verify the problem is neither the detector nor column related.
B. Replace DI water with fresh DI water.
C. Rinse all eluent lines with fresh DI water.

5.4.2 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).

5.4.3 Large Baseline Dip in the Chromatogram

A baseline dip appearing between 5 and 10 minutes is usually caused by oxygen in the sample injected. The ‘oxygen dip’ is normal and can be reduced in magnitude with higher KOH concentration in the eluent.

5.4.4 Incorrect or Variable Retention Times

A. Check your eluent preparation procedure for possible errors.
B. Prime the pump if necessary. Set the eluent composition for 100% for each eluent and draw out at least 40 mL of eluent from each of the lines.
C. Wash the column with 200mM KOH for two hours or longer to eliminate excessive carbonate contamination of the column.
D. Verify if the equilibration time after the strong wash is optimum by increasing the equilibration time.
E. Measure the flow rate by weighing out the eluent collected during exactly five minutes of flow. Recalibrate the pump if necessary.
F. Samples containing high salt content (> 50 mM) will decrease the retention times.
G. Check and/or service the pump's proportioning valve. With the pumping turned off, the flow through the pump outlet tubing (disconnected from the injector) should be zero in all eluent positions. Check this separately for each eluent line at 100% setting.

5.4.5 Unidentified Peaks Appear with Expected Analyte Peaks

Some trace contaminants can co-elute with mono and disaccharides, 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 KOH
    2. Deionized water
    3. IPA
    4. Deionized water
    5. 1 M HCl
    6. Deionized water

5.5 Potassium Hydroxide Cleanup

The potassium hydroxide (200mM KOH) rinse used to decrease column or system-related elevated background is essentially identical with the rinse performed during an installation of a new system. Following the rinse, check the background again while pumping the 1 mM potassium hydroxide and repeat the rinse at least once more if necessary. Leave the old gold working electrode in place during the first and second checking of the detection background. Use a new or reconditioned electrode only if the background remains high even after the second rinse. Should the new electrode also produce a reading of > 30 nC, remove it from the system within 30 minutes, rinse it with water and reinstall the old electrode.

5.6 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, 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 – Quality Assurance Reports

Dionex CarboPac™ SA 10
Analytical (2 x 250 mm)
Product No. 082322

Date:	03-Jun-13 16:29
Serial No. :	000005
Lot No. :	2006-09-126E

Eluent:	1 mM KOH
Eluent Source:	EGC-KOH
Eluent Flow Rate:	0.38 mL/min
Temperature:	45 °C
Detection:	Electrochemical Detection, quadruple waveform
Injection Volume:	2.5 µL
Storage Solution:	1.0 mM KOH

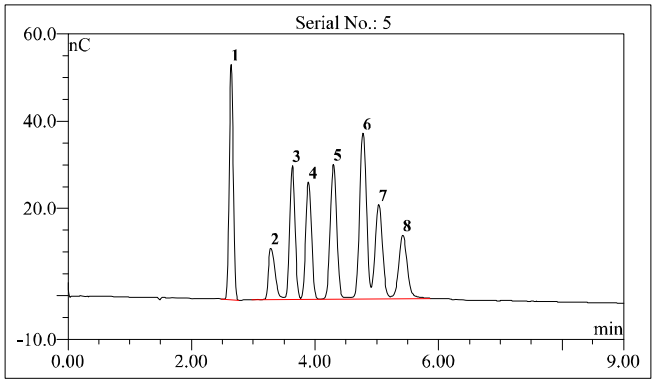
Eluent Profile

Time	Conc. (mM)	Comment
-0.10	1	
0.00	1	Inject
9.00	1	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 (EP)	Efficiency (EP)	Resolution (EP)	Peak Width (50%) min	Concentration (mg/L)
1	Fucose	2.64	1.07	7060	4.0	0.07	10.0
2	Sucrose	3.28	1.64	4555	2.0	0.11	10.0
3	Arabinose	3.63	1.04	8047	1.6	0.10	10.0
4	Galactose	3.90	1.01	7619	2.1	0.11	10.0
5	Glucose	4.30	1.04	7813	2.4	0.11	10.0
6	Xylose	4.78	n.a.	8327	1.2	0.12	10.0
7	Mannose	5.03	n.a.	8015	1.7	0.13	10.0
8	Fructose	5.43	1.07	8086	n.a.	0.14	10.0

QA Results:

Analyte	Parameter	Specification	Results
Glucose	Asymmetry	0.90-1.54	Passed
Arabinose	Efficiency	<=4950	Passed
Xylose	Retention Time	4.45-5.55	Passed
Xylose	Resolution	>=1.0	Passed
	Pressure	<=2200	1428

Production Reference:

Datasource:	QAR
Directory	CarboPac\Fast-Carb
Sequence:	SA-10_2X250MM
Sample No.:	3

6.80 SR11 Build 3161 (184582) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific

Dionex CarboPac™ SA 10
Analytical (4 x 250 mm)
Product No. 074641

Date: 20-May-13 10:46
Serial No. : 001417
Lot No. : 012-26-102

Eluent: 1 mM KOH
Eluent Source: EGC-KOH
Eluent Flow Rate: 1.5 mL/min
Temperature: 45 °C
Detection: Electrochemical Detection, quadruple waveform
Injection Volume: 10 µL
Storage Solution: 1.0 mM KOH

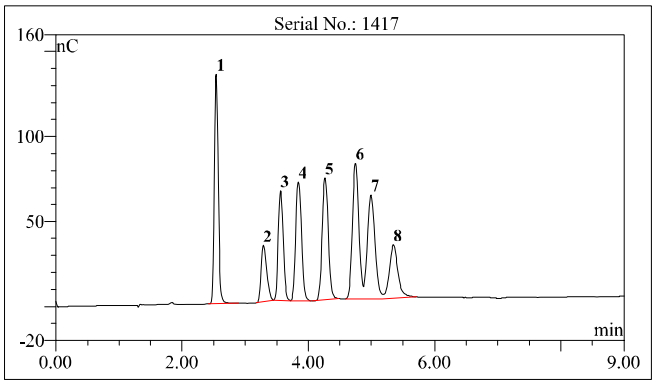
Eluent Profile

Time	Conc. (mM)	Comment
-0.10	1	
0.00	1	Inject
9.00	1	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 (EP)	Efficiency (EP)	Resolution (EP)	Peak Width (50%) min	Concentration (mg/L)
1	Fucose	2.54	1.11	7339	5.2	0.07	10.0
2	Sucrose	3.28	1.42	6073	1.7	0.10	10.0
3	Arabinose	3.56	1.16	8889	1.7	0.09	10.0
4	Galactose	3.84	1.12	7782	2.4	0.10	10.0
5	Glucose	4.27	1.08	8976	2.6	0.11	10.0
6	Xylose	4.75	n.a.	9760	1.2	0.11	10.0
7	Mannose	4.99	n.a.	8360	1.6	0.13	10.0
8	Fructose	5.35	n.a.	8637	n.a.	0.14	10.0

QA Results:

Analyte	Parameter	Specification	Results
Glucose	Asymmetry	0.90-1.54	Passed
Arabinose	Efficiency	<=4950	Passed
Xylose	Retention Time	4.45-5.55	Passed
Xylose	Resolution	>=1.0	Passed
	Pressure	<=2200	1806

Production Reference:
Datasource: QAR
Directory CarboPac\Fast-Carb
Sequence: SA-10_4X250MM
Sample No.: 1

6.80 SR11 Build 3161 (184582) (Demo-Installation)

Chromeleon™ Thermo Fisher Scientific

074899-04 (QAR)