



CarboPac PA200 Columns *For Dual EGC ICS-6000*

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Product Manual

For CP-PA 200 Dual EGC ICS 6000

CarboPac PA200 Guard Column

CarboPac PA 200, 0.4 x 50 mm - Item # 302864

CarboPac PA 200, 1 x 50 mm - Item # 302862

CarboPac PA200 Analytical Column

CarboPac PA 200, 0.4 x 250 mm - Item # 302863

CarboPac PA 200, 1 x 250 mm - Item # 302861

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Revision 04, September 2020, Updated references to Dual EG Degasser.

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

1.1 CarboPac PA200

The CarboPac PA200 column is the latest addition to the CarboPac family of columns for carbohydrate separations. This column has been specially developed to provide high resolution separations of charged and neutral oligosaccharides and is the recommended column for these applications. The CarboPac PA200 columns are packed with a hydrophobic, polymeric, pellicular anion exchange resin stable over the range of pH 0–14. This unique pH-stability of the packing material allows the use of eluent compositions that are conducive to anodic oxidation of carbohydrates at gold electrodes.

Resin Characteristics:

Particle Size:	5.5 μm
Pore	Size:microporous (< 10 Å)
Cross-linking:	55%
Ion exchange capacity (1mm):	4.0 μeq per column
Ion exchange capacity (0.4mm):	0.64 μeq per column

Latex Characteristics:

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

Typical Operating Parameters:

pH range:	0–14
Temperature Limit:	4–60 °C
Pressure Limit:	4500 psi
Organic Solvent Limit:	100% compatible
Typical eluents:	potassium hydroxide, potassium methane sulfonate

2. Installation



NOTE

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

The proper configuration of an Ion Chromatography System (ICS) is dependent on column format. Although they can typically use the same system, the use of a 1-mm or 0.4-mm column requires different set up to ensure maximum performance. Under the Dual EG mode, a high-pressure isocratic pump is required for applications for both 1-mm and 0.4-mm column formats. For high pressure applications, the use of high-pressure fittings is recommended.

2.1 The Dionex High Pressure Ion Chromatography Systems

A minimum of a Dionex High Pressure Ion Chromatography System (HPIC) is recommended due to the higher backpressures generated at typical operational flow rates. Systems should have the capability to operate up to at least 5000 psi. Standard IC systems, with an upper limit of 3000 psi, are insufficient for proper column operation.

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 also contaminate the working electrode. Running a Dionex CarboPac column on a metal system voids the column warranty.



WARNING

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



NOTE

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

2.2 System Configuration

Configure the system with the Dionex AS-AP auto sampler on the left, the Dionex DC module in the middle and the Dionex EG module on the right, with the Dionex DP or SP module on top of the Dionex EG module. The system requires only deionized water as the feed eluent. Nitrogen or helium should be delivered to the water eluent bottle at about 5–6 psi.

To generate eluents of potassium methanesulfonate with excess potassium hydroxide for the application, a Dionex EGC 400 MSA is paired with a Dionex EGC 400 KOH for 1-mm column, or a Dionex EGC-MSA (Capillary) is paired with a Dionex EGC-KOH (Capillary) for 0.4-mm column. The system flow diagram for an analytical system is shown below in Figure 1, the flow diagram for a capillary system is shown in Figure 2.

Figure 1 Plumbing Schematic for Electrolytic Generation of Potassium Methanesulfonate Eluents Using a Dionex EGC 400 KOH and Dionex EGC 400 MSA Cartridge in an RFIC-EG System

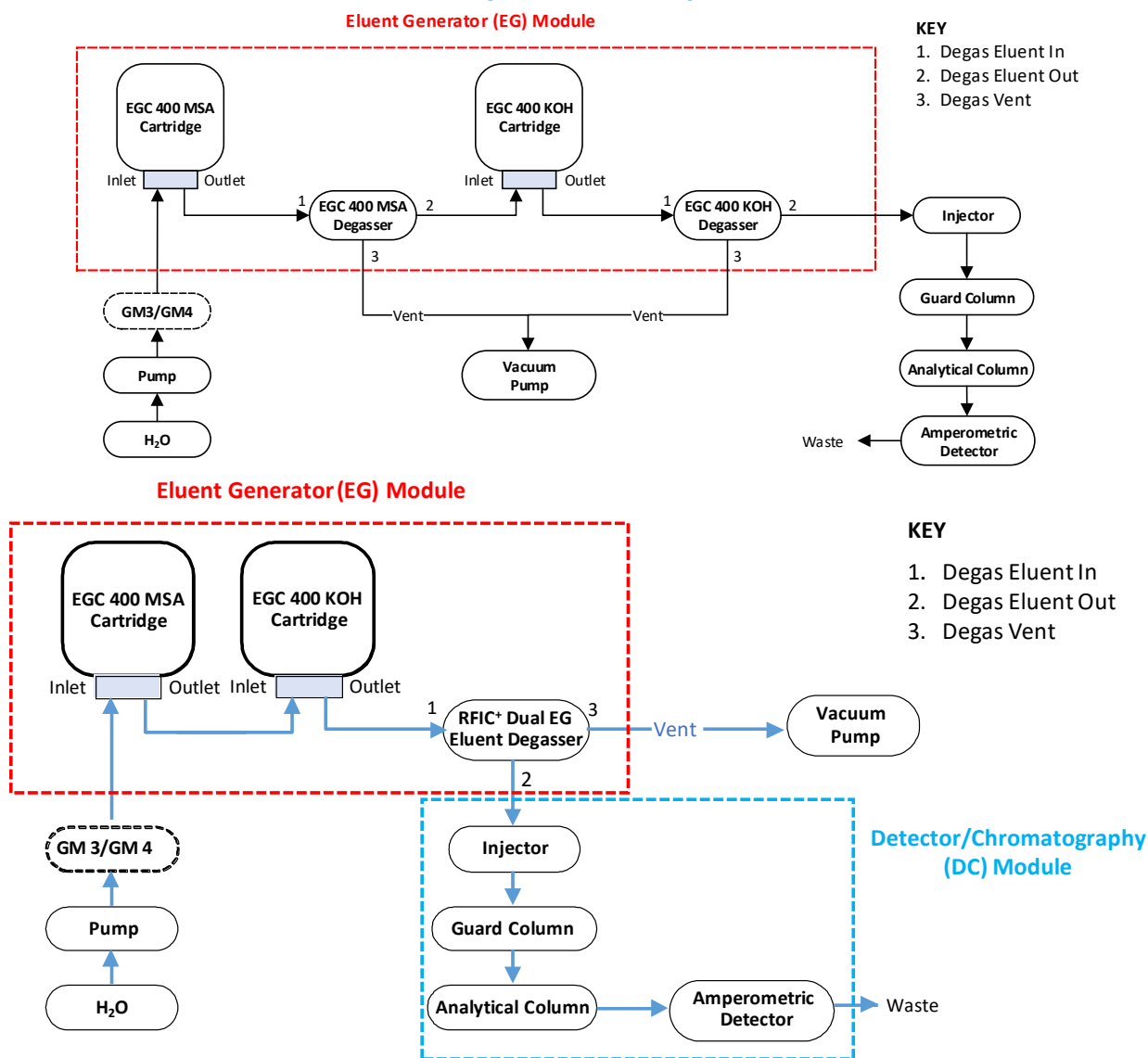
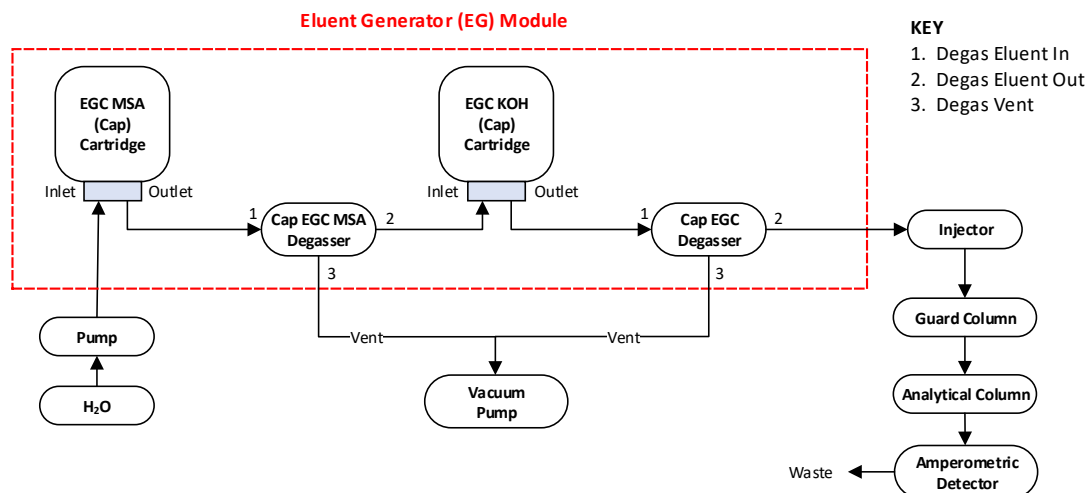


Figure 2 Plumbing Schematic for Electrolytic Generation of Potassium Methanesulfonate Eluents Using a Dionex EGC-MSA (Capillary) and Dionex EGC-KOH (Capillary) Cartridge in an RFIC-EG System



2.3 System Requirements

Dionex CarboPac Columns are designed to run on Dionex Ion Chromatographs equipped with electrochemical detectors. We recommend the use of ferrules and fittings rated with a pressure of >5000 psi. The use of precut tubing, complete with high pressure fitting and ferrules, is recommended for easier and more secure installation.

For 1-mm as well as for 0.4mm Dionex CarboPac PA200 Columns, 0.0025" i.d. PEEK (Item # 071870) tubing is required for all the connections after the EGC 400 MSA cartridge. Make sure that extra care is taken to minimize dead volume.

2.3.1 Installation of Disposable Electrode into a Dionex ICS-5000 ED Cell, pH-Ag/AgCl Reference Electrode or PdH Reference Electrode

The 1 mil (0.001") thick Teflon gaskets (Item # 072161) are required; otherwise, the disposable electrode 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.

In addition, the quadruple waveform must be used for carbohydrate analysis otherwise the product warranty is void. The standard, recommended carbohydrate waveform is shown in Table 1.

Table 1 Carbohydrate Quadruple Waveform

Time (sec)	Potential (V) vs. Ag/AgCl	Integration
0. 00	+0. 1	
0. 20	+0. 1	Begin
0. 40	+0. 1	End

0.41	-2.0
0.42	-2.0
0.43	+0.6
0.44	-0.1
0.50	-0.1



NOTE

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

2.3.2 System Void Volume

When using Dionex CarboPac PA200, 1 mm and 0.4 mm columns, it is particularly important to minimize system void volume. For best performance, all of the tubing installed between the injection valve and detector should be 0.0025" i.d. PEEK tubing (Item # 071870) for both 1mm and 0.4 mm. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

2.4 The Injection Volume

2.4.1 The 1 mm System Injection Volume, 0.4 μ L

For most applications on a 1 mm analytical system, a 0.4 μ L injection volume is sufficient. We recommend using the 4-port injection valve with an internal loop of 0.4 μ L (Item # 074699). 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 injection loops larger than 2 μ L (use 6 port valve and externally connect 2 μ L loop) may have an adversely affect peak shape.

2.4.2 The 0.4 mm System Injection Volume, 0.1 μ L

For most applications on a 0.4 mm analytical system, a 0.1 μ L injection volume is sufficient. We recommend using the 4-port injection valve with an internal loop of 0.1 μ L (Item # 0010-03-00041). 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. Injection loops larger than 0.4 μ L (Item # 074699) may have an adverse impact on peak shapes.

2.5 The Dionex CarboPac PA200 Guard Column

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

2.6 System Start-up



NOTE

Thermo Scientific recommends sanitizing the entire system (without installing the columns) with at least 2 hours of 100 mM KOH at 0.1 mL/min for 1 mm columns and at 0.02 mL/min for 0.4 mm columns using the KOH eluent generator cartridge.

In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA200 QAR eluent is 25-40nC.



NOTE

To ensure stable baseline and low background noise, it is crucial to have sufficient removal of the hydrogen and oxygen gas formed with the EGC generated eluents. For 1-mm system, connect the vent of the Dionex RFIC Dual EG Degasser to the Vacuum Port located at the back of the Dionex DP module. For 0.4-mm system, connect the vents of the Dionex RFIC Capillary EGC MSA Degasser and the Dionex RFIC Capillary Eluent Degasser to the Vacuum Port located at the back of the Dionex DP module. Make sure all connections and fittings for the vacuum degas are vacuum tight before using the system.



NOTE

Only turn on the EGC power when the system pressure reaches above 3000 psi. This step is very important to ensure best system performance.



NOTE

In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA200 QA and most applications is typically 25-45nC.

2.6.1 ICS-6000 Dual EG Start-Up Procedure for CarboPac PA200, 1 mm Column

For information regarding the ICS-6000 Dual EGC mode start-up procedure for 1-mm column, please refer to the ICS-6000 Dual EG Product Manual Doc. No. 065760 (see section 1.1.1).

2.6.2 ICS-6000 Dual EG Start-Up Procedure for CarboPac PA200, 0.4 mm Column

For information regarding the ICS-6000 Dual EGC mode start-up procedure for 0.4-mm column, please refer to the ICS-6000 Dual EG Product Manual Doc. No. 065760 (see section 1.2.1).

2.6.3 Verification of Column Cleanliness

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

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

3. Operation

3.1 Dionex CarboPac PA200 column Operational Parameters

- pH 0-14
- Temperature limit: 4-60 °C
- Pressure limit: 4500 psi
- Organic Solvent Limit: 100% compatible
- Typical Eluents: potassium hydroxide, potassium methanesulfonate
- Standard Flow Rate: 1 mm: 0.063 mL/min
0.4 mm: 0.010 mL/min

3.1.1 The Best Operational Guidelines

3.1.1.1 ALWAYS...

- Use dedicated glass- and plastic- ware for sample handling.
- Use high purity water (≥ 18.2 M Ω -cm resistivity).
- Keep your water eluent bottle blanketed with ~ 5 psi helium or nitrogen. Use new filtered water if left un-blanketed for more than 30 minutes.

3.1.1.2 NEVER...

- Proceed to a next installation step if the previous step has failed.
- Start an installation with any of the check list items below missing.
- Use ‘communal’ filtration units or filters made of unknown or unsuitable (e.g., cellulose derivatives) materials.
- Use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily, or use sampler wash bottles blanketed with ~3 psi Nitrogen or helium.
- Run above 60 °C or 5000 psi.

3.1.2 Initial Check List

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

- Laboratory water unit delivering ≥ 18.2 megohm-cm water at the installation site.
- Vacuum system for eluent vacuum filtration
- Inert gas cylinder (helium or nitrogen) with a regulator valve (for example, a 0-200 psi gauge on the low-pressure side) and the appropriate size adaptors plus tubing
- Plastic eluent bottles with gas-tight cap-fittings.

3.2 Purity Requirements and Preparation of Eluents and Standards

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. The Dual EG platform offers the high-purity eluents with the ease of feeding only deionized water to the eluent bottle. To ensure high-quality performance, it is critical to use high-purity water and follow the proper preparation recommended below.

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 ≥ 18.2 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.

Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a modest vacuum on the filled reservoir with a vacuum pump. Select a vacuum (e.g., ≤ 5 mm Hg) that does not deform the plastic eluent container. 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 (Fisher Cat. # 0974046) is a good alternative to vacuum degassing under sonication and is sufficient for the majority of cases. On-line eluent and EG-produced eluents degassing is supported through the use of Thermo Scientific pumping systems.

3.2.2 Carbohydrate Sialylated N-linked Alditol Standard

The Dionex OligoStandard, Sialylated N-Linked Alditols, Item # 043164 contains 25 nmol oligosaccharides purified from bovine fetuin. Dilute the standard prior to use, by adding a known volume of DI water (for example 0.5 mL). Dionex recommends running this standard every time a new column is installed and subsequently anytime it becomes necessary to troubleshoot your system, with an injection of 0.4 μL for the 1-mm column and an injection of 0.1 μL for the 0.4-mm.

3.3 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.).

IMPORTANT **The presence of anionic detergents (e.g. SDS) in samples should be avoided entirely. Nonionic or cationic detergents may be acceptable in low concentrations.**

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

3.4 Method Development

For users who are familiar with the manually-prepared sodium acetate/sodium hydroxide eluent system, the general guideline to convert an existing method using the sodium acetate/sodium hydroxide eluents to a method using the EGC-generated potassium methanesulfonate/potassium hydroxide eluents is to follow an estimated conversion of concentration:

$$C_{\text{KMSA}} \approx (1/3 \text{ to } 1/2) * C_{\text{NaOAc}}$$

In practice, the selectivity is governed by a combination of potassium methanesulfonate and potassium hydroxide concentrations. Therefore, it's recommended to start from the above basic estimation, and make necessary adjustments to the concentrations of both potassium methanesulfonate and potassium hydroxide. To achieve good peak resolution and sensitivity, it's also recommended to use higher concentration of hydroxide when using potassium methanesulfonate/potassium hydroxide eluent, as compared to using sodium acetate/sodium hydroxide eluent. As the eluting strength of methanesulfonate ion is stronger than that of acetate ion, an increase in pH as a result of the increase in hydroxide concentration favors the ionization and the retention of the analytes.

3.5 System Re-start



NOTE

The systems under Dual EG mode are designed to be operated non-stop for months at a time. If the system has been shut down for more than 1-2 days, the system should be re-started using the following procedure (applied to both analytical and capillary systems):

3.5.1 System Re-Start Procedure for Dionex CarboPac PA200, 1 mm Column

For information regarding the ICS-6000 Dual EGC mode Re-Start Procedure for 1-mm column, please refer to the ICS-6000 Dual EG Product Manual Doc. No. 065760 (see section 1.1.3).

3.5.2 System Re-Start Procedure for Dionex CarboPac PA200 0.4-mm Column

For information regarding the ICS-6000 Dual EGC mode Re-Start Procedure for 0.4-mm column, please refer to the ICS-6000 Dual EG Product Manual Doc. No. 065760 (see section 1.2.3).

4. Applications

The CarboPac PA200 columns have been designed exclusively for isocratic or gradient separation of oligosaccharides using electrolytically generated potassium methanesulfonate and potassium hydroxide gradients with a combined concentration up to 200 mM. Certain separations require only an isocratic separation. However, some groups of analytes will require a step or gradient elution. Retention of carbohydrates can be varied with eluent concentration, in some cases changing the elution order as the eluent concentration increases.

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

4.1 Fetuin Oligosaccharide Alditol Profiling

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

The Dionex OligoStandard, Sialylated N-Linked Alditols, Item # 043164 contains 25 nmol oligosaccharides purified from bovine fetuin. Dilute the standard prior to use, by adding a known volume of DI water. Dionex recommends running this standard every time a new column is installed and subsequently anytime it becomes necessary to troubleshoot your system.

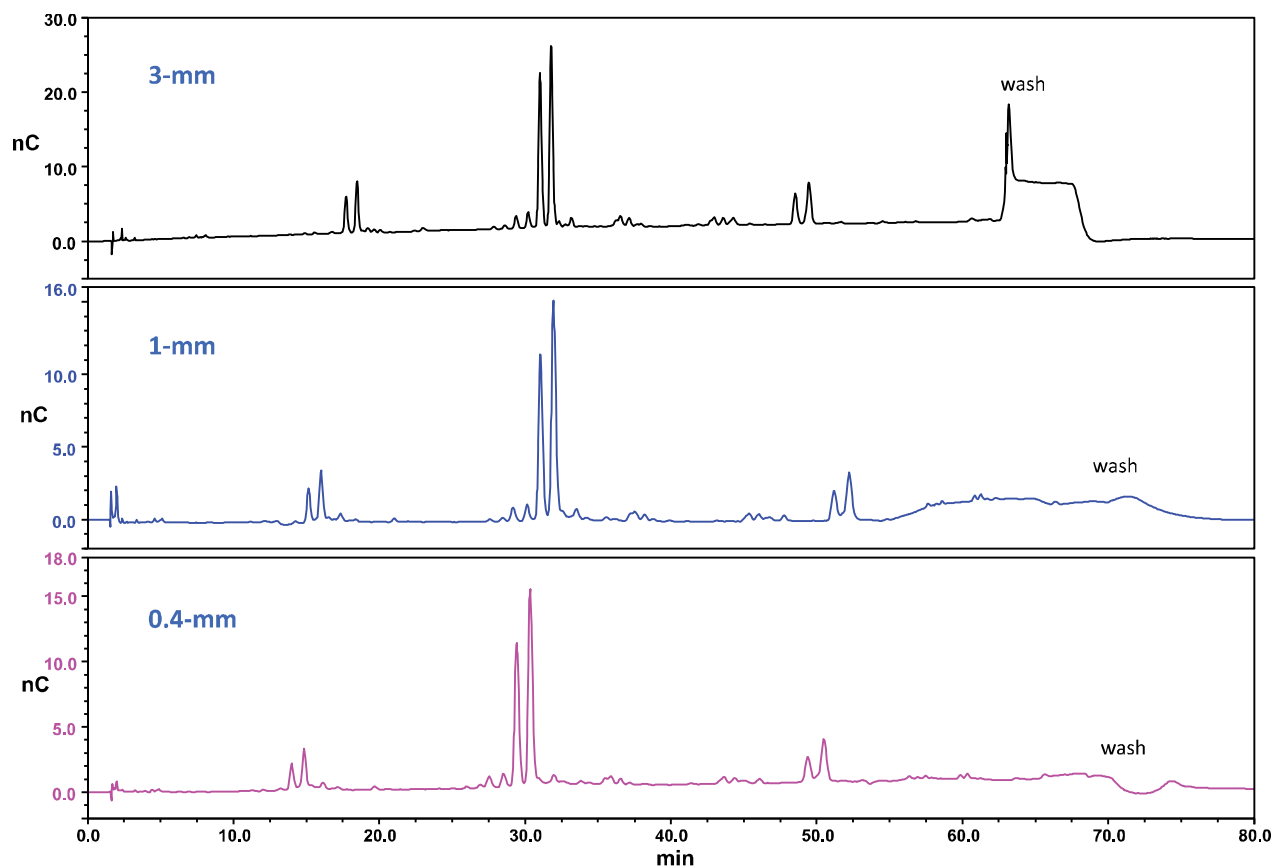
The CarboPac PA200, 3 mm column (Document No. 031992) is NOT compatible with the ICS-6000 Dual EG mode but it is recommended to be used with manually-prepared sodium acetate/sodium hydroxide eluents. The illustration of the application on the CarboPac PA200, 3 mm column is for comparison only, to demonstrate the comparable performance of the CarboPac PA200, 1 mm and 0.4 mm columns with the ease of use.

Columns:	CarboPac PA200, 3-mm (guard + separator) CarboPac PA200 1-mm (guard + separator) CarboPac PA200 0.4-mm (guard + separator)
Gradient:	CarboPac PA200, 3-mm: 0-60 min: 20-150 mM NaOAc in 100 mM NaOH 60-65 min: 500 mM NaOAc in 100 mM NaOH 65-80 min 20 mM NaOAc in 100 mM NaOH CarboPac PA200, 1-mm: 0-50 min: 15-64 mM KMSA in 136 mM KOH 50-60 min: 80 mM KMSA in 90 mM KOH 60-65 min: 100 mM KMSA in 100 mM KOH 65-80 min: 15 mM KMSA in 136 mM KOH CarboPac PA200, 0.4-mm:

0-50 min: 15-64 mM KMSA in 136 mM KOH
 50-60 min: 80 mM KMSA in 90 mM KOH
 60-65 min: 100 mM KMSA in 100 mM KOH
 65-80 min: 15 mM KMSA in 136 mM KOH

Flow Rate: CarboPac PA200, 3-mm: 0.5 mL/min
 CarboPac PA200, 1-mm: 0.063 mL/min
 CarboPac PA200, 0.4-mm: 0.010 mL/min
 Detection: CarboPac PA200, 3-mm: PAD, Au on PTFE, 2 mil gasket, Ag/AgCl ref.
 CarboPac PA200, 1-mm: PAD, Au on PTFE, 1 mil gasket, Ag/AgCl ref.
 CarboPac PA200, 0.4-mm: PAD, Au on PTFE, 1 mil gasket, Ag/AgCl ref.
 Samples: 50 μ mol/L fetuin oligosaccharide alditol standard

Figure 3 Fetuin Oligosaccharide Profiles: CarboPac PA200, 3-mm vs. 1-mm vs. 0.4mm



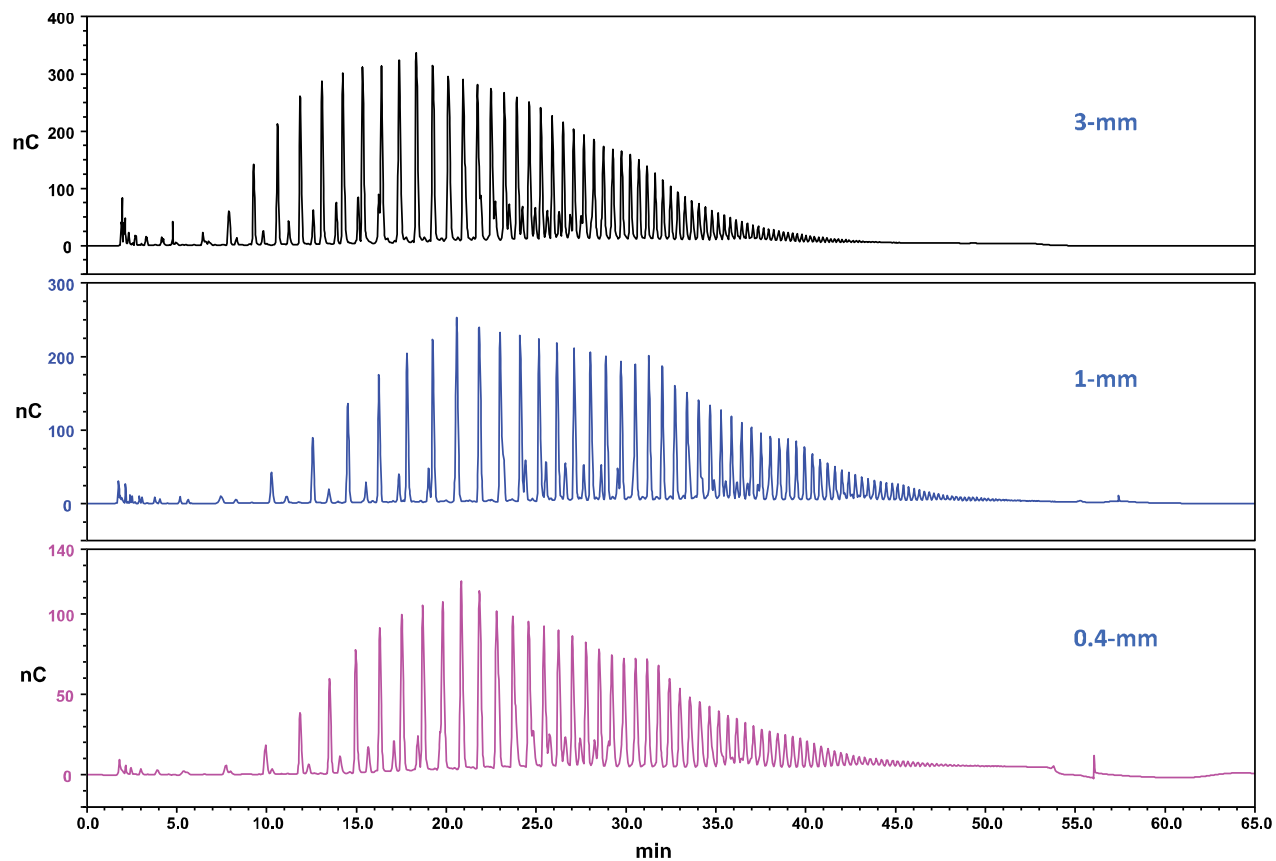
4.2 Profiling of Inulin

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

The CarboPac PA200, 3 mm column (Document No. 031992) is NOT compatible with the ICS-6000 Dual EG mode but it is recommended to be used with manually-prepared sodium acetate/sodium hydroxide eluents. The illustration of the application on the CarboPac PA200 3-mm column is for comparison only, to demonstrate the comparable performance of the CarboPac PA200, 1 mm and 0.4 mm columns with the ease of use.

Columns:	CarboPac PA200, 3-mm (guard + separator) CarboPac PA200, 1-mm (guard + separator) CarboPac PA200, 0.4-mm (guard + separator)
Gradient:	CarboPac PA200, 3-mm: 0-45 min: 100-430 mM NaOAc in 100 mM NaOH 45-50 min: 430 mM NaOAc in 100 mM NaOH 50-65 min 100 mM NaOAc in 100 mM NaOH CarboPac PA200, 1-mm: 0-45 min: 40 mM KMSA/60 mM KOH to 156 mM KMSA/22 mM KOH 45-50 min: 156 mM KMSA/22 mM KOH 50-65 min: 40 mM KMSA/ 60 mM KOH CarboPac PA200, 0.4-mm 0-45 min: 40 mM KMSA/70 mM KOH to 190 mM KMSA/10 mM KOH 45-50 min: 190 mM KMSA/10 mM KOH 50-65 min: 40 mM KMSA/70 mM KOH
Flow Rate:	CarboPac PA200, 3-mm: 0.5 mL/min CarboPac PA200 1-mm: 0.063 mL/min CarboPac PA200 0.4-mm: 0.010 mL/min
Detection:	CarboPac PA200, 3-mm: PAD, Au on PTFE, 2 mil gasket, Ag/AgCl ref. CarboPac PA200, 1-mm: PAD, Au on PTFE, 1 mil gasket, Ag/AgCl ref. CarboPac PA200, 0.4-mm: PAD, Au on PTFE, 1 mil gasket, Ag/AgCl ref.
Samples:	5 mg/mL Inulin from chicory (Sigma)

Figure 4 Inulin Profiles: CarboPac PA200, 3-mm vs. 1-mm vs. 0.4-mm



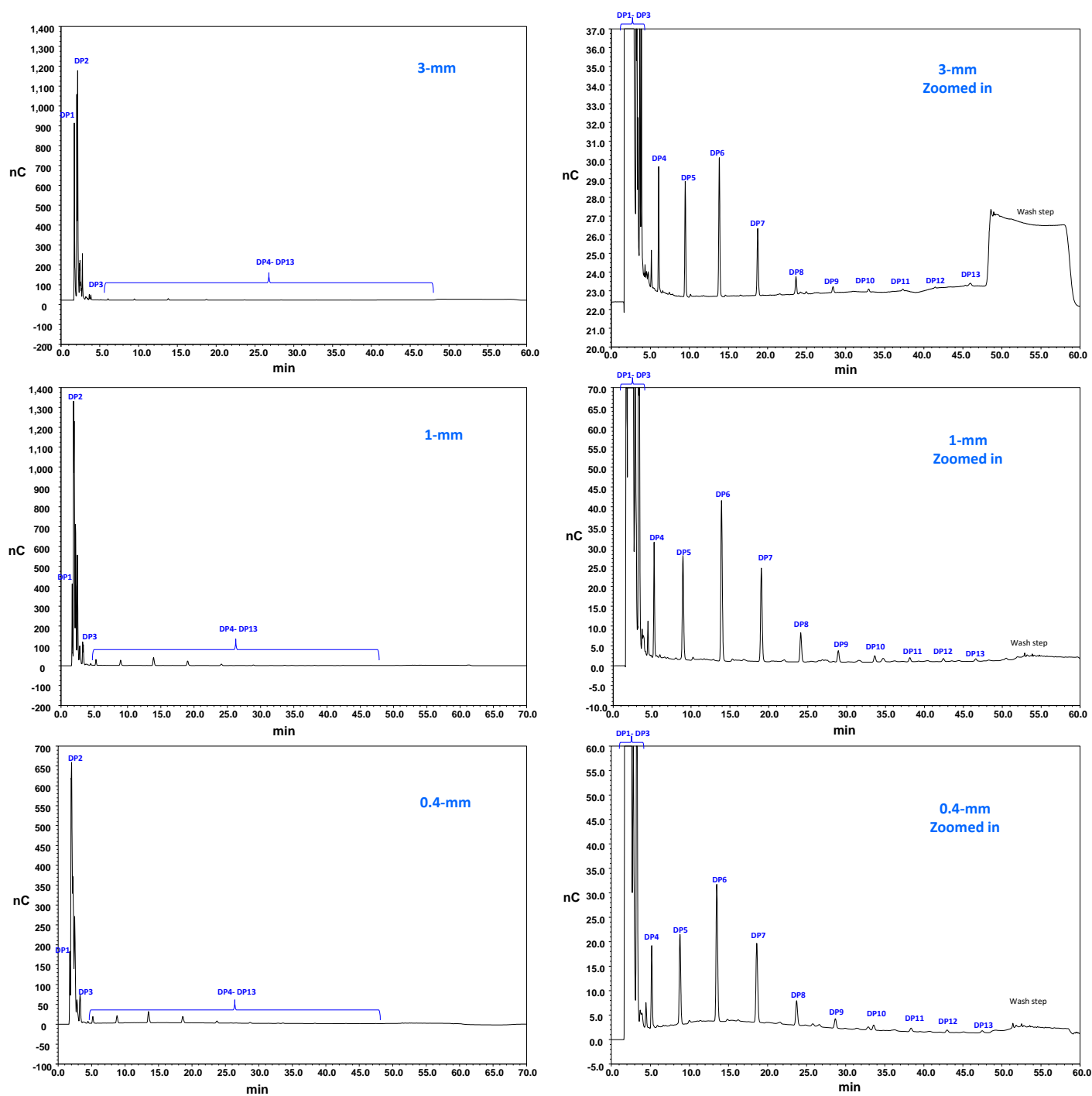
4.3 Profiling of Maltodextrin

Maltodextrin is a polysaccharide that is a common ingredient in packaged foods. It's a non-sweet nutritive polymer of glucose, in which the glucoses are joined by α -1,4 linkages with occasional branches of α -1,6 linked glucose. Maltodextrin is typically composed of a mixture of chains that vary from three to 17 glucose units long.

The CarboPac PA200, 3 mm column (Document No. 031992) is NOT compatible with the ICS-6000 Dual EG mode but it is recommended to be used with manually-prepared sodium acetate/sodium hydroxide eluents. The illustration of the application on the CarboPac PA200, 3 mm column is for comparison only, to demonstrate the comparable performance of the CarboPac PA200, 1 mm and 0.4 mm columns with the ease of use.

Columns:	CarboPac PA200, 3-mm (guard + separator) CarboPac PA200, 1-mm (guard + separator) CarboPac PA200, 0.4-mm (guard + separator)
Gradient:	CarboPac PA200, 3-mm: -5 min: 50 mM NaOAc in 100 mM NaOH 0-45 min: 50-150 mM NaOAc in 100 mM NaOH 45-55 min 500 mM NaOAc in 100 mM NaOH 55-60 min: 50 mM NaOAc in 100 mM NaOH CarboPac PA200, 1-mm: 0-45 min: 20-70 mM KMSA in 70 mM KOH 45-55 min: 100 mM KMSA in 70 mM KOH 55-70 min: 20 mM KMSA in 70 mM KOH CarboPac PA200, 0.4-mm 0-45 min: 18-65 mM KMSA in 70 mM KOH 45-55 min: 100 mM KMSA in 70 mM KOH 55-70 min: 18 mM KMSA in 70 mM KOH
Flow Rate:	CarboPac PA200, 3-mm: 0.5 mL/min CarboPac PA200 1-mm: 0.063 mL/min CarboPac PA200 0.4-mm: 0.010 mL/min
Detection:	CarboPac PA200, 3-mm: PAD, Au on PTFE, 2 mil gasket, Ag/AgCl ref. CarboPac PA200, 1-mm: PAD, Au on PTFE, 1 mil gasket, Ag/AgCl ref. CarboPac PA200, 0.4-mm: PAD, Au on PTFE, 1 mil gasket, Ag/AgCl ref.
Sample:	Bimuno probiotic food supplement powder

Figure 5 Maltodextrin Profiles: CarboPac PA200 3-mm vs. 1-mm vs. 0.4-mm



5. Troubleshooting Guide

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



NOTE

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

5.1 High Back Pressure

5.1.1 Finding the Source of High System Pressure

Column pressure, (after subtracting the system pressure) for the Dionex CarboPac PA200 Analytical Column should be close to the pressure listed in the QAR when using the test chromatogram conditions. If a Dionex CarboPac guard and analytical column are both installed, column pressure will increase by approximately 20% over the pressure listed in the QAR for the column. If the total system pressure is much higher than expected, it is advisable to determine the cause of the high system pressure.

- A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. If necessary, measure the pump flow rate by collecting the DI H₂O eluent for a specified time at operating pressure, and measure the collected volume using an analytical balance. This data (weight/time) will give actual flow rate.
- B. Determine which part of the system is causing the high pressure. High pressure could be due to plugged or constricted tubing, an injection valve with a clogged port, a column bed support clogged with particulates, or a clogged detector cell.

To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the Dionex EGC 400 MSA cartridge for 1-mm system (the Dionex EGC (Cap) MSA cartridge for 0.4-mm system), 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). The total pressure with the Dionex EGC 400 MSA cartridge, Dionex RFIC EGC 400 MSA degasser, Dionex EGC 400 KOH cartridge and Dionex RFIC EGC 400 KOH degasser for 1-mm system (the Dionex EGC (Cap) MSA cartridge, Dionex RFIC capillary EGC MSA degasser, Dionex EGC-KOH (Cap) cartridge, and Dionex RFIC EGC capillary eluent degasser for 0.4-mm system) connected should be <1000 psi. Continue adding system components (backpressure coil (if present), injection valve, column(s), and detector) one by one, while monitoring the system pressure. The pressure should increase by the sum of the measured pressures of the individual guard and analytical columns (see product QAR) when the CarboPac Guard and Analytical columns are connected.

- C. A Dionex High-Pressure In-Line Filter positioned between the Pump and Eluent Generator (or injection valve if and EGC is not installed) should be installed to prevent particulates from blocking the system

5.1.2 Replacing Column Bed Support Assemblies for 1 mm column

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

- A. Disconnect the column from the system.
- B. Carefully unscrew the inlet (top) column fitting. Use two open-end wrenches.
- C. Remove the bed support. Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you do not scratch the walls of the end fitting. Discard the old bed support assembly.
- D. Place a new bed support assembly (provided with each 1-mm 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.



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 (3.5 lbf-in). Tighten further only if leaks are observed. It is important you do not overtighten the end fitting or you will strip the threads on the column!
- F. Reconnect the column to the system and resume operation.

5.1.3 Filter Samples

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

5.2 High Background

While it may be possible to obtain reasonable performance even with elevated levels of detection background according to some requirements, high background frequently brings about an increased size of gradient artifacts and can be accompanied by a presence of ghost peaks. Detection sensitivity may also change suddenly when the detection background is too high. A background >50 nC with 8 mM KMSA/150 mM KOH at 0.0.063 mL/min for the 1-mm system and 0.010 mL/min for 0.4-mm system 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.
 - B. Compromised working electrode surface.
Briefly install a new working electrode and check the background as above. If the reading remains > 50 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. Otherwise continue with your work with the new electrode installed.
 - C. Insufficient degassing vacuum.
Verify that the vacuum applied to both the Dionex RFIC EGC 400 MSA degasser (Item # 22181-60210) and the Dionex RFIC EGC 400 KOH degasser (Item # 22181-60201) are above 27 inch Hg for 1-mm system (the Dionex RFIC Capillary EGC MSA Degasser (Item # 22181-60211) and the Dionex RFIC Capillary Eluent Degasser (Item # 22181-60202) for 0.4-mm system).
- In the case that the vacuum doesn't work properly, after the vacuum is restored, install the restriction tubing in place of the column. With EGC power OFF, turn on the pump to wash the system of the residual gas for 2 h. After that, install the column and turn on the pump with EGC power OFF, keep the pump running for 15 min before turning EGC power back on.
- D. Column contamination: Remove the column set from the system first and replace it with a length of 0.001" i.d. PEEK tubing, generating a pressure drop over 2000 psi. If the background reading improves after the column is removed from the system, go to [Appendix A, "CarboPac PA200 Column Care" in Product Manual of CarboPac PA200 Columns For Dual EGC ICS-6000 \(Document No. 065734\)](#).
 - E. 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.
 - F. System Contamination: If the background remains high even with fresh water and without the column, carry out the 2 M sodium hydroxide rinse. In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA200 QAR eluent is 25-40nC. If the background is much higher, determine the cause of high background.
 - G. Minor leak at the ED cell. Due to the low flow rate, the minor leak at the ED cell might not set off the leak alarm. The minor leak can cause increased background and noise. Verify that there is no leak at the ED cell. If leaking around the 1mil gasket is observed, remove the gasket, carefully dry with lint free cloth the wet ED surface and replace with new 1mil gasket.
 - H. Always confirm the loss of response by performing at least one injection of the system suitability standard mix as described in Section 4.1 in Product Manual of CarboPac PA200 Columns for Dual EGC ICS-6000 (Document No. 065734). This is to make sure that a decreased level of response is not being caused by system problems.

5.2.1 Preparation of Eluents

- A. The Dionex CarboPac PA200 1 mm and 0.4 mm columns are not compatible with manually prepared eluents. The use of a Dionex EGC 400 MSA cartridge and a Dionex EGC 400 KOH cartridge is required.
- B. Make sure that the deionized water used has a specific resistance of 18.2 megohm-cm or greater.

5.2.2 A Contaminated Guard or Analytical Column

- A. Remove the columns from the system.
- B. Install a back-pressure coil that generates approximately 2000 psi and continue to pump eluent. If the background decreases, the column(s) is (are) the cause of the high background.
- C. To eliminate downtime, clean or replace the analytical column at the first sign of column performance degradation. Clean the column as instructed in, [“Appendix A, Dionex CarboPac PA200 Column Care”](#) in [Product Manual of CarboPac PA200 Columns For Dual EGC ICS-6000 \(Document No. 065734\)](#).

5.3 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 15 to 20 minutes for both 1-mm and 0.4-mm columns.

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.

5.3.1 Loss of Column Efficiency

- A. Check to see if headspace has developed in the guard or analytical column. This is usually due to improper use of the column such as exposing it to high pressures. Remove the column's inlet end fitting (see Section 5.1.2, "Replacing Column Bed Support Assemblies" in [Product Manual of CarboPac PA200 Columns For Dual EGC ICS-6000 \(Document No. 065734\)](#)). 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 0.0025" for both 1 mm and 0.4 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. 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 Dionex CarboPac columns, it is recommended to turn off the pump while connecting the column inlet and the column outlet to the detector. This will avoid any slippage of the ferrule under high pressure conditions which can cause low peak efficiencies.

5.3.2 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.
- 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, Dionex CarboPac PA200 Column Care](#)" in [Product Manual of CarboPac PA200 Columns For Dual EGC ICS-6000 \(Document No. 065734\)](#), for recommended column cleanup procedures.



NOTE

Possible sources of column contamination are impurities in injected samples 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. After cleaning the column, follow the system re-start procedure (see Section 3.5 System Re-start) BEFORE reinstall the column 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. 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.

5.3.3 Loss of Resolution for early eluting peaks

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

- A. Improper eluent concentration may be the problem if retention time is less than expected. Check the flow rate of the pump, as pump flow rate will affect the eluent concentration in an RFIC-EG system. Ensure the Eluent Generator is set to the correct eluent concentration.
- B. Column overloading may be 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.

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.

5.3.4 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” in [Product Manual of CarboPac PA200 Columns For Dual EGC ICS-6000 \(Document No. 065734\)](#).

**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 re-torqued (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.

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

- A. Check the position and filling levels of sample vials in the auto sampler.
- B. Check the transfer line volume.
- C. Check injector needle-height setting.
- D. Check if there is any bubble trapped in the sample syringe of the auto sampler.
- E. Check each line of the schedule for proper injector parameters. Revert to full loop and column appropriate sample loop size.
- F. Service the injection valve (check for leaks, Tefzel fragments, or sediments inside the valve).

5.3.6 Large Baseline Dip in the Chromatogram

A large baseline dip appearing later in the chromatogram 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.

5.3.7 Unidentified Peaks Appear with Expected Analyte Peaks

During a potassium methanesulfonate/ potassium 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.

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

- A. Disconnect the column and detector cell from the auto sampler.
- B. Set the pump to 100% deionized water.
- C. Place the following solutions in the auto sampler and inject in sequence. Use 0.4 μL and 0.1 μL full loop injections for 1 mm and 0.4 mm systems respectively:
 1. 1 M NaOH
 2. Deionized water
 3. IPA
 4. Deionized water
 5. 1 M HCl
 6. Deionized water

5.3.8 Decreased Detection Sensitivity

Always confirm the loss of response by performing at least one injection of the system suitability standard mix as described in Section 4.1 in [Product Manual of CarboPac PA200 Columns For Dual EGC ICS-6000 \(Document No. 065734\)](#). 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.

Exceptions:

Check the pH reading. If the value is out of range or >13.2 , install a new reference electrode and then install a new gold 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.

Peak heights will increase with increasing eluent concentrations. This is due to improvement of the kinetics in the electrode detection related to ionic strength and pH effects. If you run the same standard at 1mM and at 12 mM, peak heights will increase at 12mM. Hence you will see a decrease in peak heights whenever going to lower eluent concentrations as well.

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.

5.3.9 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. Set column temperature to 40 °C and wash the guard and column with 100 mM KMSA/100 mM KOH for at least four hours (preferably overnight). Run a blank gradient at 30 °C and if necessary repeat the cleanup with 100 mM KMSA/100 mM KOH wash at 40 °C.

5.4 Reconditioning or Replacement of the Gold (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 PA200 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 PA200 column is 4,500 psi (31.03 MPa).

A.2 Column Start-Up

The Dionex CarboPac columns are shipped using potassium methanesulfonate/potassium hydroxide/ (see QAR) as the storage solution. Use Dionex EGC-400 MSA/KOH cartridges to generate eluent shown in the Quality Assurance Report (QAR). Install the column in the chromatography module and direct the column effluent to waste for 60 minutes, and then connect to the ED cell. It is recommended to clean the column for 1 to 2 hours with 100mM KOH at 0.063 mL/min for 1 mm and at 0.010 μ L/min for 0.4 mm column to ensure good chromatography without baseline artifacts. Test the column performance under the conditions described in the QAR. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

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

IMPORTANT

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

A.3 Column Storage

For short-term storage (< 1 week), 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 Dionex CarboPac PA200 Column Cleanup

The Dionex CarboPac PA200 can be readily cleaned by an approximate 60-column-volume rinse with 100 mM KOH. More stubborn contamination problems may necessitate thoroughly cleaning the column. Use the following steps to thoroughly clean the Dionex CarboPac PA200 at a flow rate similar to application flow rate or half the application flow rate to avoid the over-pressurization of the column:

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



CAUTION

When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each column separately.

- B. Wash the Dionex CarboPac PA200 with deionized water (18.2 megohm-cm) for about 30 minutes and then clean with 200 mM MSA for one to two hours at appropriate flow rate.
- C. Wash the column with deionized water for about 30 minutes.
- D. Then clean the Dionex CarboPac PA200 with 100 mM KOH for at least one hour.
- E. Reconnect column to the cell and equilibrate the Dionex CarboPac PA200 to the desired initial conditions and test the performance using QAR standard and eluent.

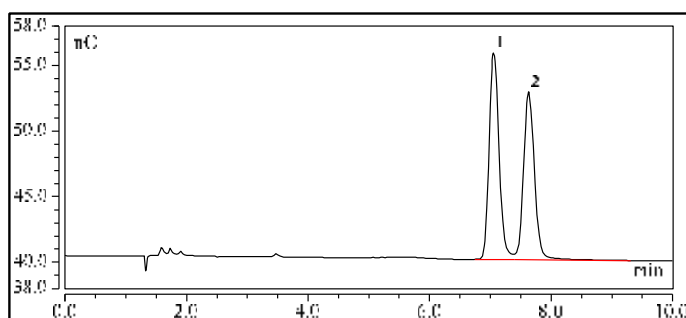
Appendix B – Quality Assurance Report (QAR)

Device Monitoring Enabled
and Viper Fitting Ready

Dionex CarboPac™ PA200
Analytical (1 x 250 mm)
Product No. 302861

Date: 04-Oct-17 16:33
Serial No. : 171005253
Lot No. : 20172203

Eluent: 150 mM KOH/ 8 mM MSA
Eluent Flow Rate: 0.063 mL/min
Temperature: 30 °C
Detection: Electrochemical Detection
Injection Volume: 0.4 µL
Storage Solution: Eluent



Wave Form		
Time	Potential ¹	Integration
0.00	0.10	
0.20	0.10	Begin
0.40	0.10	End
0.41	-2.00	
0.42	-2.00	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	

¹ Reference Electrode Mode: Ag/AgCl

No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Amount Injected (pmoles)
1	alpha-(2,6)-NAN-lactose	7.1	1.3	1.91	9530	6.1
2	alpha-(2,3)-NAN-lactose	7.6	1.3	n.a.	9393	8.5

QA Results:

Analyte	Parameter	Specification	Results
alpha-(2,3)-NAN-lactose	Efficiency	>=7650	Passed
alpha-(2,3)-NAN-lactose	Asymmetry	1.0-1.5	Passed
alpha-(2,3)-NAN-lactose	Retention Time	6.4-8.1	Passed
	Pressure	<=2750	1902

Production Reference:

Datasource: CMDev7
Directory: _QAR\Column\CP_PA200
Sequence: CP-PA200_1x250
Sample No.: 4

7.2.7.10369

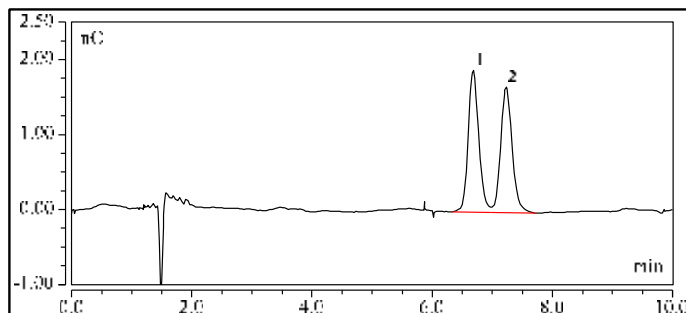
Chromleon™ Thermo Fisher Scientific

Appendix B – Quality Assurance Report (QAR)

Dionex CarboPac™ PA200
Capillary (0.4 x 250 mm)
Product No. 302863

Date: 20-Nov-17 10:38
Serial No. : 001000
Lot No. : 01701631

Eluent: 150 mM KOH/ 8 mM MSA
Eluent Flow Rate: 0.01 mL/min
Temperature: 30 °C
Detection: Electrochemical Detection
Injection Volume: 0.1 µL
Storage Solution: Eluent



<u>Wave Form</u>		
Time	Potential ¹	Integration
0.00	0.10	
0.20	0.10	Begin
0.40	0.10	End
0.41	-2.00	
0.42	-2.00	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	

¹ Reference Electrode Mode: Ag/AgCl

No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Amount Injected (pmoles)
1	alpha-(2,6)-NAN-lactose	6.7	1.2	1.66	7266	1.5
2	alpha-(2,3)-NAN-lactose	7.2	1.3	n.a.	7278	2.1

QA Results:

Analyte	Parameter	Specification	Results
alpha-(2,3)-NAN-lactose	Efficiency	>=5850	Passed
alpha-(2,3)-NAN-lactose	Asymmetry	1.0-1.5	Passed
alpha-(2,3)-NAN-lactose	Retention Time	6.4-8.1	Passed
	Pressure	<=2750	2143

Production Reference:

Datasource: CMDev7
 Directory: _QAR\Column\CP_PA200
 Sequence: CP-PA200_Cap_1x250
 Sample No.: 1

7.2.7.10369

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