Dionex CarboPac PA300-4µm

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Thermo SCIENTIFIC

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Product Manual for
Dionex CarboPac PA300-4µm Column
2 × 250 mm (Item # 303346)

Dionex CarboPac PA300-4µm Guard Column
2 × 50 mm (Item # 303347)

Revision History:

Revision 01, April 2020, Original Publication.

Revision 02, September 2020, Added section 6.6 (Comparison between Ag/AgCl and PdH reference electrodes) and updated example applications.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

 **SAFETY**	***Indicates a potentially hazardous situation which, if not avoided, could result in death or serious injury.***
 **WARNING**	***Indicates a potentially hazardous situation which, if not avoided, could result in damage to equipment.***
 **CAUTION**	***Indicates a potentially hazardous situation which, if not avoided, may result in minor or moderate injury. Also used to identify a situation or practice that may seriously damage the instrument but will not cause injury.***
 **NOTE**	***Indicates information of general interest.***
IMPORTANT	***Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.***
Tip	***Highlights helpful information that can make a task easier.***

Contents

1. Introduction

1.1 Dionex CarboPac PA300-4µm column

The Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm column is the latest addition to the CarboPac family of columns offered for carbohydrate separations. The new column was developed for the high-resolution separations of complex oligosaccharides from biologics and food samples. This column is packed with polymeric, state-of-the-art supermacroporous resin coated with anion exchange latex particles and is stable over the entire range of pH 0-14. The unique pH stability of the packing material allows eluent compositions that are conducive to anodic oxidation of carbohydrates at the surface of gold electrodes.

High-pH anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) has been demonstrated to be effective in the separation and detection of carbohydrates ranging from small monosaccharides to branched oligosaccharides, and large linear polysaccharides. HPAE is proven to be capable of separating monosaccharides, positional linkage and branch isomers of branched oligosaccharides, and homopolymer oligosaccharides that differ only in length. Each class of carbohydrates has different separation characteristics requiring different column chemistries to address the need of this complex world of glycans.

Note that Dionex™ CarboPac™ PA300-4µm column uses 4µm supermacroporous resin particles to provide high resolution and high-capacity column. The high resolution provides better peak identification and high capacity allows the injection of more concentrated samples without overloading the column. The use of 4µm resin particles compared to the 6µm commonly found in earlier Dionex CarboPac products, the Dionex CarboPac PA300-4µm will require the use of high-pressure IC (HPIC™) systems such as the Thermo Scientific™ Dionex™ ICS-5000$^+$ HPIC™ system or the Thermo Scientific™ Dionex™ ICS-6000 HPIC™ system.

1.1.1 Resin / Latex Characteristics:

Particle Size:	4 µm (Analytical Column*)
Particle Size:	8 µm (Guard Column**)
Particle Cross-linking:	55%
Functional Group:	Quaternary ammonium functional groups
Latex Diameter:	60 nm
Ion exchange capacity:	85 µeq per 2 × 250 mm column. 1.3 µeq per 2 x 50 mm column

*Analytical Column Resin Composition: Supermacroporous polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene.

** Guard Column Resin Composition: Microporous polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene.

1.1.2 Typical Operating Parameters:

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

2. System Requirements and 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. A gradient pump is designed to blend and pump isocratic, linear, or gradient mixtures of up to four mobile phase components at precisely controlled proportions and flow rates. An isocratic pump is for applications not requiring gradient or proportioned eluent capabilities. For high-pressure applications, the use of high-pressure Viper™ fittings is recommended. Please use the following Item #'s to order Kit with Viper Fittings (4/2mm represent the ID of the separation column).

088804 – Dionex IC PEEK Viper Fittings Kit for 4 mm Dionex ICS-6000 system with Electrochemical Detector (ED)

302966 – Dionex IC PEEK Viper Fittings Kit for 2 mm Dionex ICS-6000 system with Electrochemical Detector (ED)

(To order the Item # ask your local sales representative for a quote)

2.1 The Dionex High-Pressure Ion Chromatography Systems

A Dionex High-Pressure Ion Chromatography System (HPIC) is recommended when running Dionex CarboPac PA300-4µm columns due to the higher backpressures generated at typical operational flow rates with 4µm resins. Systems should have the capability to operate up to at least 5000 psi. Standard IC systems, with an upper limit of 3000 psi, will be inadequate for optimized column operation.

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 will result in reduced throughput.

NOTE

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

2.2 System Requirements for Column Operation

Dionex CarboPac Columns are designed to run on Dionex Ion Chromatographs equipped with electrochemical detectors. We strongly recommend the use of Thermo Scientific Viper™ tubing. The Viper™ tubing will help ensure an easier and more secure installation. It is highly recommended to ensure that the system 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.

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

The 2 mil (0.002") thick Teflon gaskets included in each package of disposable electrodes are usually required; otherwise, the disposable electrode product warranty may be voided. A gasket is always required because it forms the flow-through channel. In addition, the quadruple waveform must be used for carbohydrate analysis otherwise the disposable electrodes will fail early, and their product warranties will be void. Always wear gloves when handling electrodes. Never touch the electrode surface. To install a disposable working electrode and reference electrode (pH-Ag/AgCl or PdH) refer to the Product Manual for Disposable Electrodes Doc. No. 065040, ICS-6000 Ion Chromatography System Manual Doc. No. 22181-97002 and User's Compendium for Electrochemical Detection Doc. No. 065340.

2.2.2 System Void Volume

When using a Dionex CarboPac PA300-4µm column, it is important to minimize system void volume. For the best 2 mm column performance, we strongly recommend the use of Thermo Scientific Viper™ fittings installed between the injection valve and detector. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

2.3 The Injection Loop

2.3.1 The 2 mm System Injection Loop, 2.5 µL to 10 µL

For 2 mm column applications, a 2.5 µL injection loop typically recommended. Injecting a larger amount can result in overloading the column or detector, which can affect detection linearity. For low concentrations, larger injection loops can be used to increase sensitivity.

2.4 Installing the Dionex BorateTrap Inline Trap Column

Borate is a known contaminant in laboratory water supplies. Borate contamination of chromatography eluents may be a result of degrading deionized water systems or as leachate from borosilicate glassware. If borate is present in the eluent, it binds both to the anion-exchange column and to the carbohydrate analytes. The carbohydrate-borate complex is less efficiently eluted from the anion-exchanger than is the carbohydrate, resulting in peak tailing, particularly where vicinal cis hydroxyl groups are present, such as for mannose and sugar alcohols.

For optimal performance during IC carbohydrate analysis using HPAE-PAD detection, remove borate contamination using the Thermo Scientific™ Dionex™ BorateTrap™ Inline Trap Column (Item # 047078). When placed between the eluent pump gradient mixture and the injection valve, the trap does not affect the efficiencies or retention times of the carbohydrate being analyzed. The Dionex BorateTrap column eliminates peak tailing for mannose, fructose, and sugar alcohols resulting from borate contamination.

2.5 The Dionex CarboPac PA300-4µm Guard Column

A Dionex CarboPac PA300-4µm Guard Column is normally used with the Dionex CarboPac PA300-4µm Analytical Column. Retention times will increase by ~5% when a guard column is placed in-line before the analytical column while using isocratic elution. A guard column helps prevent sample contaminants from fouling the analytical column. It is cheaper and easier to clean or replace a guard column than an analytical column. Replacing the Dionex CarboPac PA300-4µm Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex CarboPac PA300-4µm Analytical Column.

2.6 Procedure for Dionex EGC Installation and conditioning

For detailed information on how to install EGC KOH Cartridge, see Dionex Eluent Generator Cartridges Product Manual (Document # 065018).

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

For Dionex CarboPac PA300-4µm applications using the Dionex EGC 500 KOH cartridge, a Dionex CR-ATC 600 Continuously Regenerated Trap Column (Item # 088662) should be installed. See the Dionex CR-TC 600 Product Manual (Document No. 079684) for instructions.

2.8 Eluent Storage

The Dionex CarboPac PA300-4µm column is designed to be used with hydroxide eluent systems. Storage under a helium atmosphere ensures contamination-free operation and proper pump performance (nitrogen can be used if eluents do not contain organic solvents).

2.9 System Start-up

2.9.1 System Background Check

This procedure is performed using the conditions of the test chromatogram.

Make sure that…

A. the cell is not yet on,

B. the pump is pumping 10 mM NaOH at 0.25 mL/min

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

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

Confirm that the pH is 12.1 ±1 pH unit. With the pH within this range, turn on the cell using the carbohydrate standard quad waveform (See Table 3, Section 6.3, Disposable Electrode Manual, document number 065040) and begin background signal monitoring from the Chromeleon™ control panel. Monitor the trace for at least 30 minutes. Confirm that the background is between 10 and 35 nC. If the background is above 50 nC or the pH is out of range, see the "Troubleshooting" section at the end of this manual, and ensure that the vacuum degas system is operational.

NOTE

Thermo Scientific recommends sanitizing the entire system with at least 2 hours of 100mM KOH at 1.0mL/min using the KOH Eluent Generator cartridge. If the system does not use an eluent generator, 2M NaOH can be used prior to initial start-up and after long idle periods.

2.9.2 Verification of Column Cleanliness

Install the Dionex CarboPac PA300-4µm column set only after the initial system test determines a background level within the specified range. A premature installation on a contaminated system will cause delays during the column equilibration.

The Dionex CarboPac PA300-4µm is shipped in 10 mM NaOH. Any column that is stored long-term should be stored in the same solution. To prepare the column for standard analysis, the Dionex CarboPac PA300-4µm must be washed for at least 30 minutes (an hour preferred) with 200mM NaOH at 0.25 mL/min flow rate. Equilibrate the column set under the test chromatogram conditions (See Quality Assurance Report; QAR) and performing two blank injections (DI water) to ensure that DI water injection has no peaks.

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

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

3. Before You Start

3.1 Dionex CarboPac PA300-4µm column Operational Parameters

- pH range: 0 - 14
- Temperature limit: 4-60 °C
- Pressure limit: 5000 psi
- Organic Solvent Limit: 0-100%
- Typical Eluents: NaOH and NaOAc
- Standard Flow Rate: 2 mm: 0.25 mL/min
- Maximum Flow Rate: 2 mm: 0.30 mL/min
- Typical Back Pressure: 3000 psi, at 0.25 mL/min (analytical column)
- Typical Back Pressure: 200 psi, at 0.25 mL/min (guard column)

NOTE

Using hydroxide eluent concentration below 8 mM will produce a significantly lower detection response.
When using a high level of organic solvent, it is advised to add some base or acid to avoid any damage to the column performance

3.1.1 The Best Operational Guidelines

ALWAYS…	NEVER…
• Use a 50% (w/w) sodium hydroxide solution (ThermoFisher Scientific Item # 080389) as the source of sodium hydroxide. • Use dedicated plastic volumetric flasks and plastic serological pipettes for eluent preparation and volume adjustments. • Use plastic labware and eluent bottles for all eluents containing NaOH. NaOH dissolves borosilicate glass, releasing borate into the solution. • Use high purity water (>18.2 MΩ-cm resistivity) to prepare sodium hydroxide eluents. The water should be degassed, for best results, and prevent carbonate contamination. • Keep your NaOH eluent blanketed with helium or nitrogen. Prepare new NaOH eluent if left unblanketed for more than 30 minutes. • Use EGC-KOH generated eluent when possible to avoid any eluent preparation issues. • Purge at least 40 mL of new eluent through the lines when changing eluent or adding fresh eluent. This will ensure that your fresh eluent is primed through the lines up to the pump head. • Verify the equilibration time necessary before injection to avoid baseline issues or artifacts or to avoid an unnecessary increase in total method time.	• Proceed to the next installation step if the previous step has failed. • Start an installation with any of the checklist items below missing. • Use ‘communal’ filtration units or filters made of unknown or unsuitable materials (e.g., cellulose derivatives). • Use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily, or use sampler wash bottles blanketed with ~3 psi nitrogen or helium. • Run above 60 °C or 5000 psi.

3.1.2 Initial Check List

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

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

4. Purity Requirements for Chemicals

Obtaining reliable, reproducible, and accurate results require 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.

4.1.1 Deionized Water

Deionized water used to feed the Eluent Generator should be Type I reagent grade water with a specific resistance ≥18.2 MΩ-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. UV treatment as a part of the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of UV lamps, ion exchange, and adsorbent cartridges. All filters used for water purification must be free from electrochemically active components, including surfactants. Expanding their period of use beyond the recommended time may lead to bacterial contamination and as a result, a laborious cleanup may be required. The use of contaminated water for eluents can lead to high background signals and significant gradient artifacts.

4.1.2 Hydroxide

Use a Dionex EGC 500 KOH Eluent Generator Cartridge (Item # 075778) installed with CR-ATC 600 (Item # 088662) in the EG module. Manually prepared hydroxide eluents will absorb CO_2 during and after preparation altering elution selectivity over time. Use 50% w/w sodium hydroxide (Certified Grade, Thermo Fisher Scientific Item # 080389) for preparation.

4.1.3 Sodium Acetate

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

5. Preparation of Eluents and Standards

NOTE

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

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and electrochemically active impurities. Chemicals and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also helps to protect your columns and system components. Thermo Scientific cannot guarantee proper column performance when the quality of the chemicals, solvents, and water used to prepare eluents is substandard.

5.1 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. Degas the aqueous eluent in a glass bottle before transferring to the plastic bottle. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere. Vacuum filtration through 0.2 µm Nylon filters (Thermo Fisher Scientific Item # 164-0020) is a good alternative to vacuum degassing under sonication and is sufficient for most cases. On-line eluent and EG-produced eluent degassing is supported using the Thermo Scientific pumping systems.

5.2 0.2 M Sodium Hydroxide

NOTE

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

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

5.2.1 Sodium Hydroxide Eluent Concentration

Gravimetric Method

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

Example: To make 1 L of 0.2 M NaOH use 16.004 g of 50% sodium hydroxide:

For 0.2 M:

$$\frac{0.2 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 16.004 \text{ g diluted to 1 L}$$

Volumetric Method

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

g = dvr

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

Example: To make 1 L of 0.2 M NaOH use 10.46 mL of 50% sodium hydroxide:

For 0.2 M:

$$\frac{0.2 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% \times 1.53 \text{ g/mL}^*} = 10.46 \text{ mL diluted to 1 L}$$

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

Sodium Hydroxide Eluents

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

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

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

5.3 50 mM Sodium Hydroxide / 25 mM Sodium Acetate

Acetate has no buffering capacity at high pH, so to maintain baseline stability, it is important to keep the sodium hydroxide concentration constant during the sodium acetate gradient. This is achieved by making the eluents as follows:

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

To make one (1) liter of 50 mM sodium hydroxide/ 25 mM sodium acetate, dispense approximately 800 mL of vacuum-degassed water into a 1 L volumetric flask. Add a stir bar and begin stirring. Weigh out 2.05 g anhydrous, crystalline sodium acetate (Thermo Scientific Dionex Sodium Acetate Reagent, Item # 059326). Add the solid acetate steadily to the briskly stirring water to avoid the formation of clumps which are slow to dissolve. Once the salt has dissolved, remove the stir bar with a magnetic retriever. Add DI water to the flask to bring the volume to the 1 L mark.

Vacuum filter the solution through a 0.2 µm Nylon filter. This may take a while as the filter may clog with insoluble material from the sodium acetate. Using a plastic tip volumetric pipet, measure 4.0 g (approximately 2.6 mL) of 50% (w/w) sodium hydroxide solution. Dispense the sodium hydroxide solution into the acetate solution about 1 inch under the surface of the acetate solution. The eluent should be kept blanketed under helium or nitrogen at 34 to 55 kPa (5–8 psi) at all times, and it should last about 1 week.

5.4 0.1 M Sodium Hydroxide / 0.25 M Sodium Acetate

To make one (1) liter of 0.1M sodium hydroxide/ 0.25M sodium acetate, dispense approximately 800 mL of vacuum-degassed DI water into a 1 L volumetric flask. Vacuum degas for approximately 5 minutes. Add a stir bar and begin stirring. Weigh out 20.51 g anhydrous, crystalline sodium acetate (Thermo Scientific Dionex Sodium Acetate Reagent, Item # 059326). Add the solid acetate steadily to the briskly stirring water to avoid the formation of clumps which are slow to dissolve. Once the salt has dissolved, remove the stir bar with a magnetic retriever. Add DI water to the flask to bring the volume to the 1 L mark.

Vacuum filter the solution through a 0.2 µm Nylon filter. This may take a while as the filter may clog with insoluble material from the sodium acetate. Using a plastic tip volumetric pipet, measure 8.01 g (approximately 5.2 mL) of 50% (w/w) sodium hydroxide solution. Dispense the sodium hydroxide solution into the acetate solution about 1 inch under the surface of the acetate solution. The eluent should be always be kept a under helium or nitrogen at 34 to 55 kPa (5–8 psi), and last about 1 week.

NOTE

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

5.5 Sample Preparation

The Dionex CarboPac PA300-4µm column is an anion exchange column. Thus, the normal caveats applicable to ion exchange chromatography apply to this column. High salt concentrations in samples should be avoided where possible. Special care should be taken with samples containing high concentrations of strongly anionic compounds for the Dionex CarboPac PA300-4µm column (e.g. chloride, carbonate, phosphate, etc.). It is best to avoid extremes of sample pH (especially extremely acidic samples). The presence of anionic detergents (e.g., SDS) in samples should be avoided entirely. Nonionic or cationic detergents may be acceptable in low concentrations.

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.

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

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

Sample matrices in glycoprotein analysis can be simplified by performing a Western blot and selectively removing the carbohydrates from the PVDF membrane-bound proteins. Please ask for Dionex **Technical Note 30**, "Monosaccharide and Oligosaccharide Analysis of Glycoproteins Electrotransferred onto Polyvinylidene Fluoride (PVDF) membranes," or retrieve it from our Web site at www.thermoscientific.com.

6. Example Applications

The CarboPac PA300-4µm column is designed for isocratic and/or gradient separation of glycans using sodium hydroxide and sodium acetate eluent system. Analyte separation is highly dependent on hydroxide concentration in HPAEC. Some separations may require only an isocratic eluent method. 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.

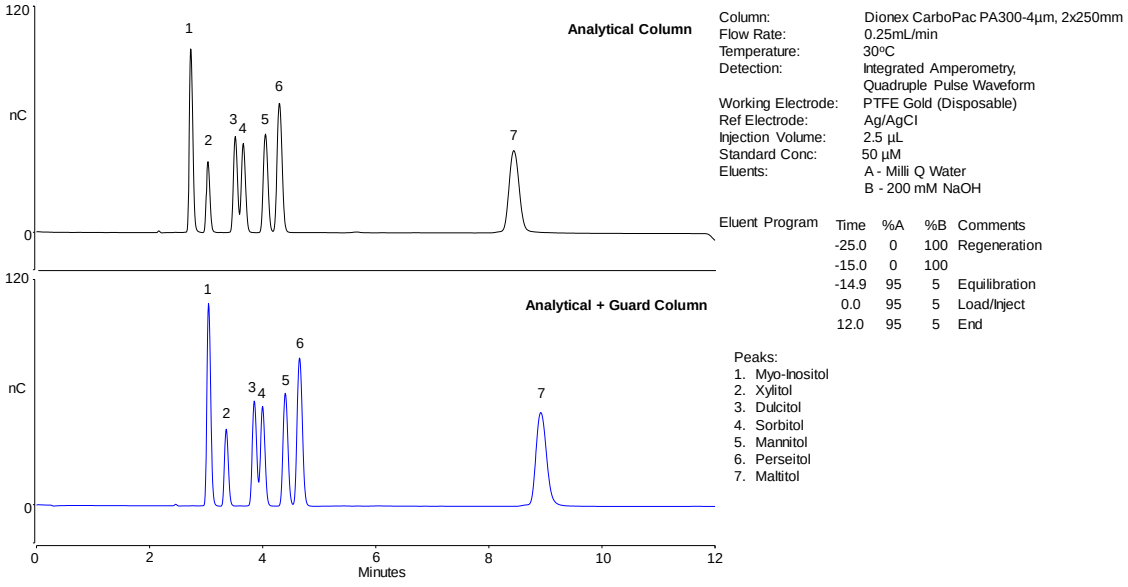
The following section provides an example of types of applications for which the Dionex CarboPac PA300-4µm column can be used. The chromatograms in this section were obtained using columns that reproduced the Quality Assurance Report on an optimized Ion Chromatograph. Different systems will differ slightly in performance due to slight differences in column set, system void volumes, liquid sweep-out times of different components and laboratory temperatures. Depending on your system, you may have to make small adjustments to your gradient conditions and/or operating temperature to achieve the required resolution for the analytes of interest.

6.1 Analysis of Sugar Alditols: A Quality Assurance Report (QAR)

Isocratic separation of mono- and di-saccharide alditol standards is used to test the performance of the Dionex CarboPac PA300-4µm column. While the CarboPac PA300-4µm analytical column should always be used with a CarboPac PA300-4µm Guard Column, the QAR chromatogram shipped with the analytical column does not employ a guard column. The addition of the Guard column will increase elution time by ~5% when compared to the Analytical Column by itself. To guarantee that all Dionex CarboPac PA300-4µm Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following quality assurance testing with sugar alditol standards at 30 ºC.

The Dionex CarboPac PA300-4µm column was developed to provide fast, high-resolution separations for of complex oligosaccharides from biologics and food samples. Using the Dionex CarboPac PA300-4µm column and 10 mM NaOH isocratically, the seven sugar alditols can be separated within 12 min as shown in Figure 1.

Figure 1 Dionex CarboPac PA300-4µm 2×250 mm: Without and With Guard Column



6.2 Characterization of Released Glycans Using CarboPac PA300-4µm

6.2.1 Bovine Fetuin N-Linked and O-Linked Glycan Alditol Profiling

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

The high resolution of the CarboPac PA300-4µm is demonstrated in the following example. CarboPac columns can separate mono, oligo, and polysaccharides based on fine structural differences in branching, linkage isomerism, anomericity and sialylation. In the fetuin oligosaccharide alditol standard shown in Figure 2, 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 α2-6 isomer is eluted before, and well resolved from, the α2-3 isomer.

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

Following enzymatic deglycosylation of N-linked glycans, the remaining O-linked glycans can be cleaved from the fetuin protein using standard β-elimination with subsequent sample cleanup. This produces two distinct populations of glycans from the glycoprotein: N-linked alditols (Figure 2) and O-linked alditols (Figure 3).

Figure 2 N-Linked Glycan Alditol Profile of Bovine Fetuin on Dionex CarboPac PA300-4µm

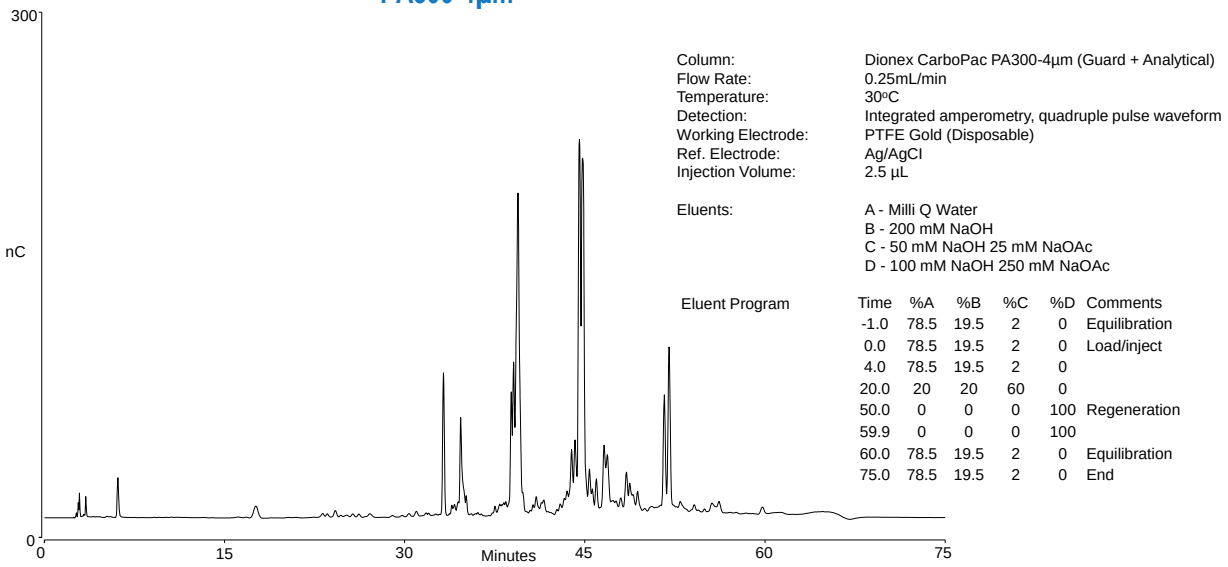
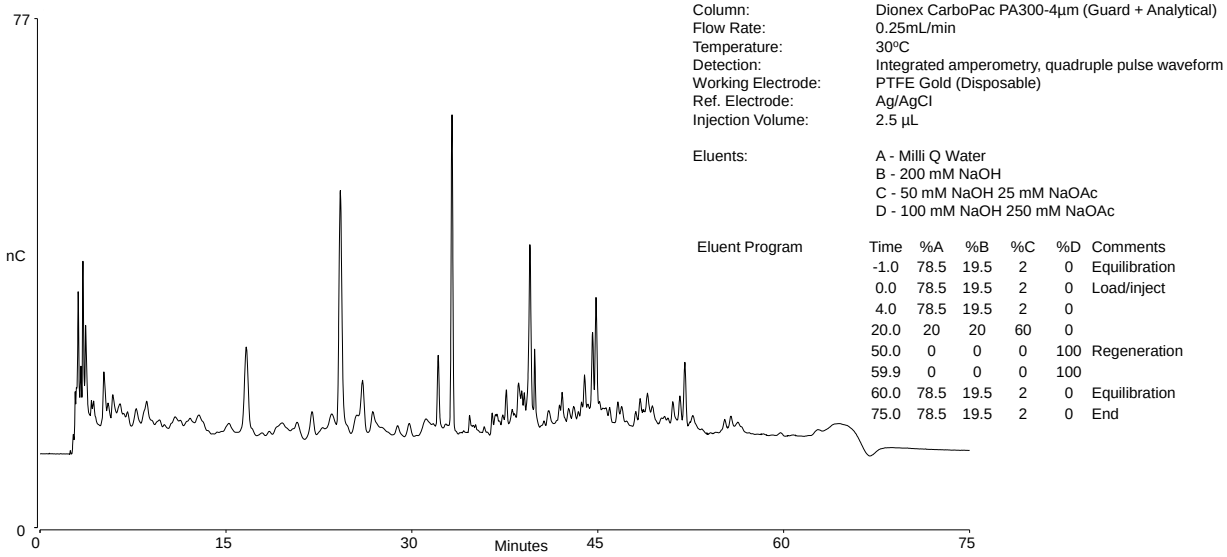


Figure 3 O-Linked Glycan Alditol Profile of Bovine Fetuin on Dionex CarboPac PA300-4µm



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30°C
Detection:	Integrated amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	Ag/AgCl
Injection Volume:	2.5 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

6.2.2 Porcine Gastric Type III Mucin O-Linked Glycan Alditol

Glycosylation in mammals represents a very diverse set of pre- and post-translational modifications. One of the most diverse and complex sets of glycans is found on mammalian mucin proteins. Mucins are heavily glycosylated proteins that are often involved in cellular recognition and signaling, and they often form chemical barriers on the extracellular surface of cells. Regulation of this family of proteins is thought to be tightly controlled, as the overexpression of many mucin proteins is strongly associated with many types of cancers.

The characterization of mucin proteins has been incredibly challenging largely due to the nature of mucin glycosylation. Mucins have tandem repeat sequences of amino acids with high concentrations of serine and threonine residues, and these often become saturated with O-linked glycans. O-linked glycosylation is non-template driven, and the glycans themselves are often complex in structure and composition, creating a high-density, heterogenous cluster of glycans on regions of the mucin protein. This complexity often inhibits analysis of the glycoproteins or glycopeptides, creating a need for additional technologies to characterize the sample.

Figure 4 shows a characteristic chromatogram of porcine gastric Mucin type III O-glycan on a CarboPac PA300-4µm column. Note the diversity of glycans present in the sample. O-linked glycans were released by β-elimination with subsequent sample cleanup.

Figure 5 demonstrates a chromatographic comparison of Mucin type III O-glycan traces using the CarboPac PA300-4µm compared to the CarboPac PA200 column. CarboPac PA300-4µm column exceeds the CarboPac PA200 performance in resolving the early eluting neutral O-glycans while maintaining the resolution of later eluted charged glycans.

Figure 4 O-Linked Glycan Profile of Porcine Gastric Mucin Type III on Dionex CarboPac PA300-4µm

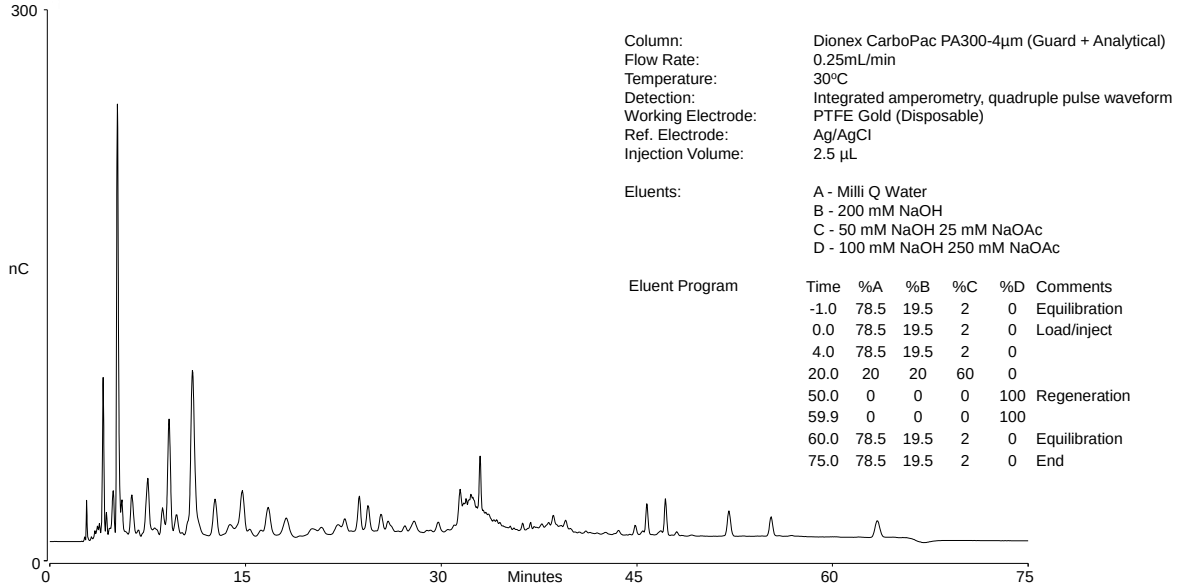
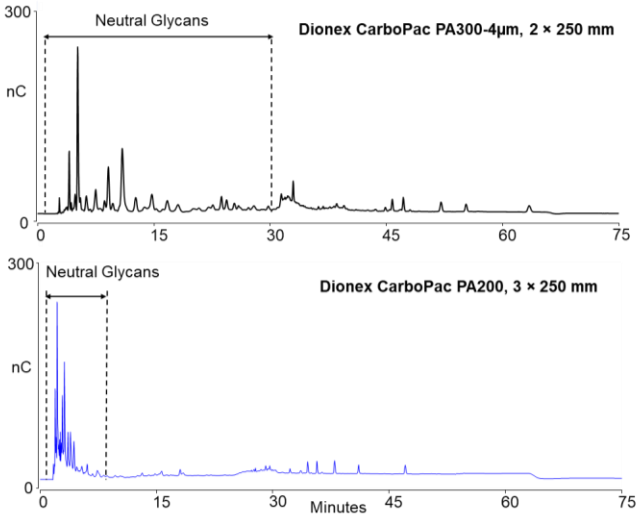


Figure 5 Dionex CarboPac PA300-4µm vs. CarboPac PA200 Separation of O-glycans Released from Porcine Mucin Type III.



Column:	Dionex CarboPac PA300-4µm, 2 × 250 mm (Guard + Analytical) Dionex CarboPac PA200, 3 × 250 mm (Guard + Analytical)
Flow Rate:	PA300: 0.25mL/min PA200: 0.50 mL/min
Temperature:	30°C
Detection:	Integrated amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	Ag/AgCl
Injection Volume:	2.5 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

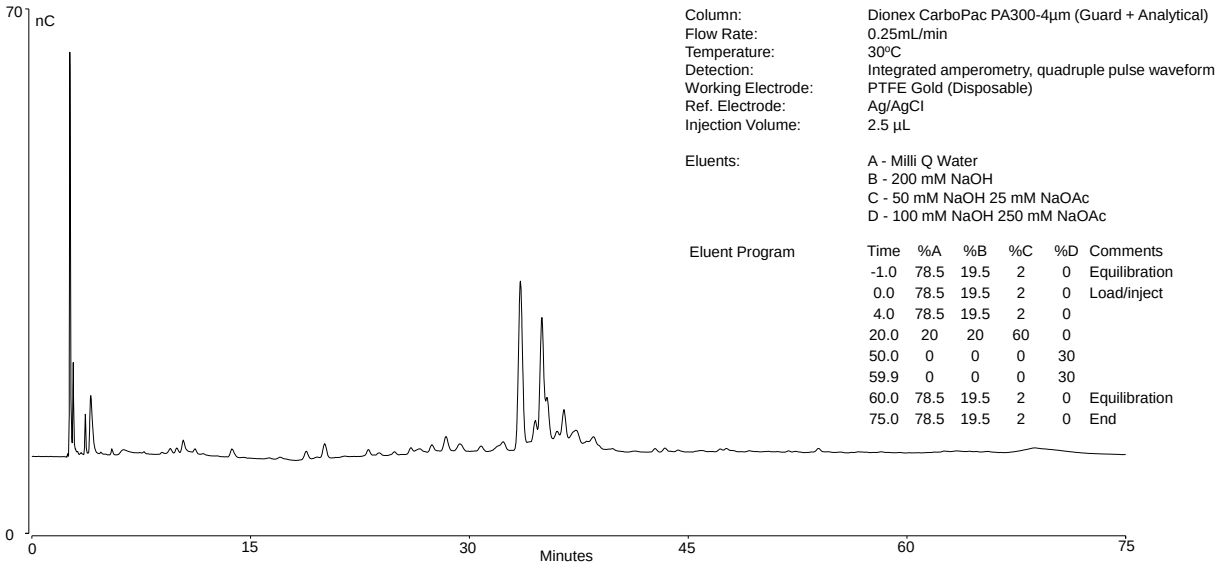
Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

6.2.3 N-Linked Glycan Profile of Trastuzumab (Herceptin).

Antibodies have become a cornerstone of numerous medical and scientific procedures, and they are therefore produced routinely for both medical and research applications. In the medical world, they are used as diagnostics, to detect cancers or infection by certain bacteria or viruses; as vaccines, to boost the body's immune response; and as therapeutics, to target foreign bacteria, viruses or cancerous cells.
Figure 6 below shows a chromatographic separation of N-glycans released from Trastuzumab, a groundbreaking monoclonal antibody used to treat HER2 positive breast, stomach, and esophageal cancers, using CarboPac PA300-4µm column.

Figure 6 N-Linked Glycan Profile of Trastuzumab (Herceptin) on Dionex CarboPac PA300-4µm

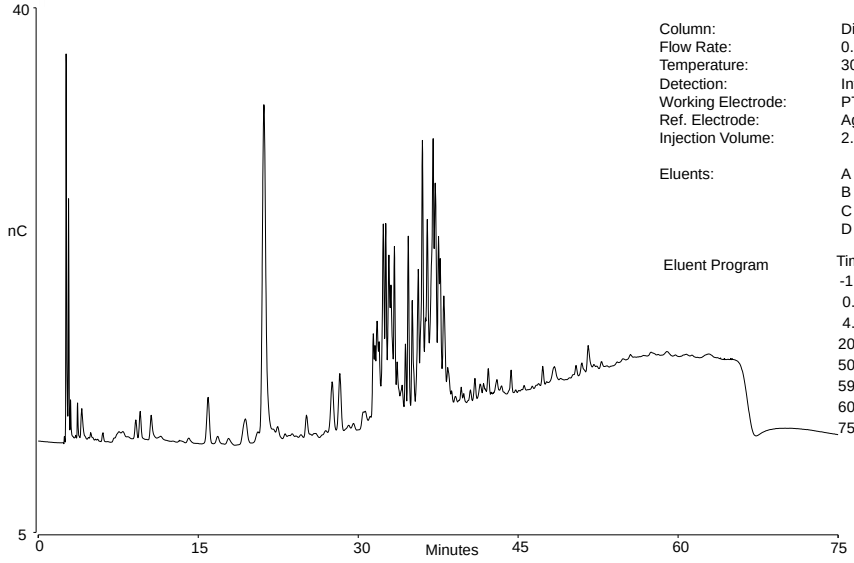


6.2.4 N-linked Glycan Profile of Bovine Milk Proteins

Glycosylation of milk proteins is associated with many functions, such as passive protection from pathogen binding to the intestinal cells, maintaining correct protein folding, protecting proteins from digestion, and facilitating the release of encrypted bioactive peptides. Structural characterization of milk glycans is critical in understanding the biological properties that are associated with the presence of glycans, and ultimately in determining milk's nutritional quality. N-linked glycans released from milk proteins include oligomannose, complex, and hybrid type, with an abundance of sialylation.

Figure 7 demonstrates N-glycan profile using CarboPac PA300-4µm column. N-glycans were released from 5 mg of bovine milk protein by PNGase F treatment, followed by the sample purification with a HyperSep™ Hypercarb™ Filter Plate (Item # 60110-504).

Figure 7 N-Linked Glycan Profile of Bovine Milk Protein on Dionex CarboPac PA300-4µm



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30ºC
Detection:	Integrated amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	Ag/AgCl
Injection Volume:	2.5 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

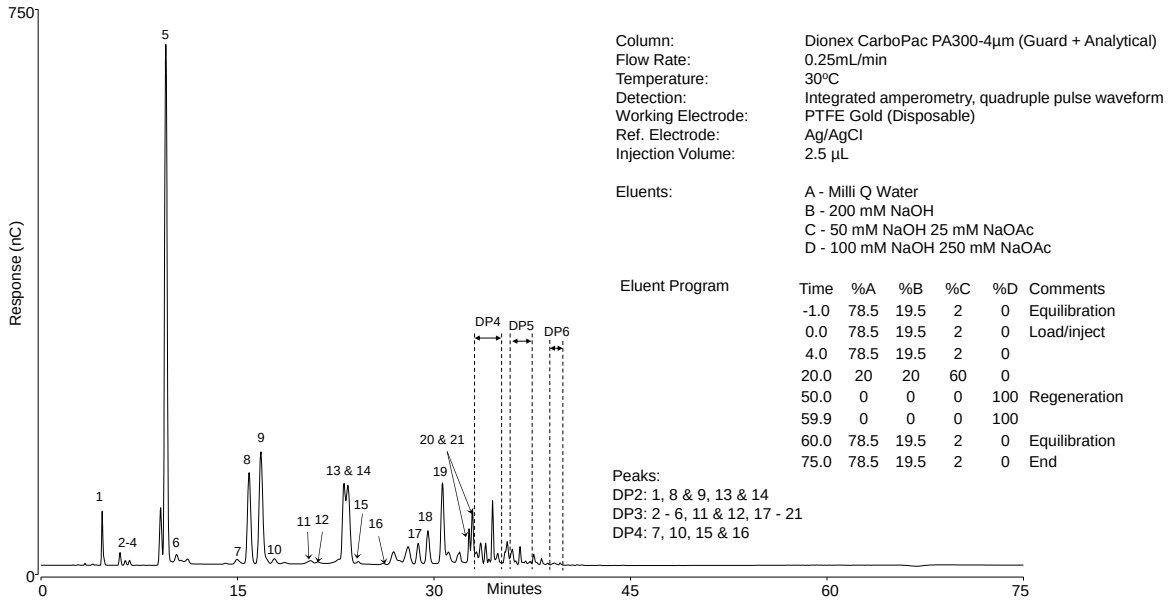
6.3 Analysis of Free Complex Carbohydrates in Food

6.3.1 Galacto-oligosaccharides (GOS) Syrup

Galacto-oligosaccharides (GOS) are a group of non-digestible carbohydrates that are increasingly being used as functional food ingredients. The GOS fractions comprise galactose oligosaccharides with a terminal glucose, varying in type of glycosidic linkages between monosaccharide units and in the degree of polymerization (DP). The differences in GOS structures can affect their roles on gut microbiota.

Figure 8 demonstrates the separation of oligosaccharides in Bimuno® GOS syrup with a CarboPac PA300-4µm column. Samples were prepared by dissolving 20 mg of Bimuno® GOS syrup in 10 mL of water to make a stock solution, diluting the stock to make a solution with the final concentration of 400 mg/L, and filtering through a 0.2 µm filter. The mixture of GOS is separated mainly based on their size (DP). The early eluting peaks (RT earlier than 33 minutes) are mainly DP2 and DP3 oligosaccharides, with a presence of low-abundant DP4 molecules. Peaks eluting between 33 and 35 minutes are mainly DP4. Peaks present between 35 and 37 minutes are mainly DP5. Peaks eluting near 39 minutes are mainly DP6. The size of the oligosaccharides was identified by the HPAE-MS platform.

Figure 8 Oligosaccharides Profile in GOS Syrup on Dionex CarboPac PA300-4µm



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30°C
Detection:	Integrated amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	Ag/AgCl
Injection Volume:	2.5 µL

Eluents:
A - Milli Q Water
B - 200 mM NaOH
C - 50 mM NaOH 25 mM NaOAc
D - 100 mM NaOH 250 mM NaOAc

Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

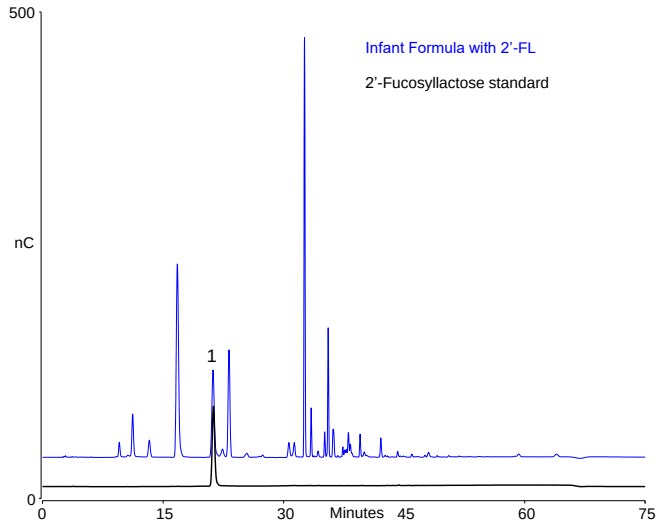
Peaks:
DP2: 1, 8 & 9, 13 & 14
DP3: 2 - 6, 11 & 12, 17 - 21
DP4: 7, 10, 15 & 16

6.3.2 Free Oligosaccharides in Infant Formula with 2'-FL HMO

Human milk oligosaccharides (HMOs) are a family of free glycans that contains diverse structures and are highly abundant in human milk. Numerous publications have demonstrated the importance of HMOs not only in enhancing the development of the intestinal microbiota of newborns, but also bolstering the immune system in breastfed infants. Therefore, HMOs are a vital component of infant nutrition. The most abundant HMO in most mothers' breast milk is 2′-fucosyllactose (2'-FL). Preclinical data suggests that the addition of HMOs to infant formula is safe, resulting in the addition of 2′-FL HMO to some commercial infant formulas.

Figure 9 shows the oligosaccharide profile in a commercial infant formula product with 2'-FL. The sample containing 0.1 mL of infant formula product was diluted and passed through a HyperSep™ Hypercarb™ Filter Plate (Item # 60110-504) for cleanup prior to analysis. The peak eluted at 21.275 min is 2'-FL, and the identity was confirmed with a 2'-FL standard purchased from Biosynth Carbosynth®.

Figure 9 Oligosaccharides Profile in Infant Formula with 2'-FL on Dionex CarboPac PA300-4µm



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30°C
Detection:	Integrated amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	Ag/AgCl
Injection Volume:	2.5 µL

Eluents:
A - Milli Q Water
B - 200 mM NaOH
C - 50 mM NaOH 25 mM NaOAc
D - 100 mM NaOH 250 mM NaOAc

Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

Peak:
1: 2'-Fucosyllactose
RT: 21.275 min

6.4 Structural Characterization of Complex Carbohydrates Using CarboPac PA300-4µm Column in the IC-MS platform

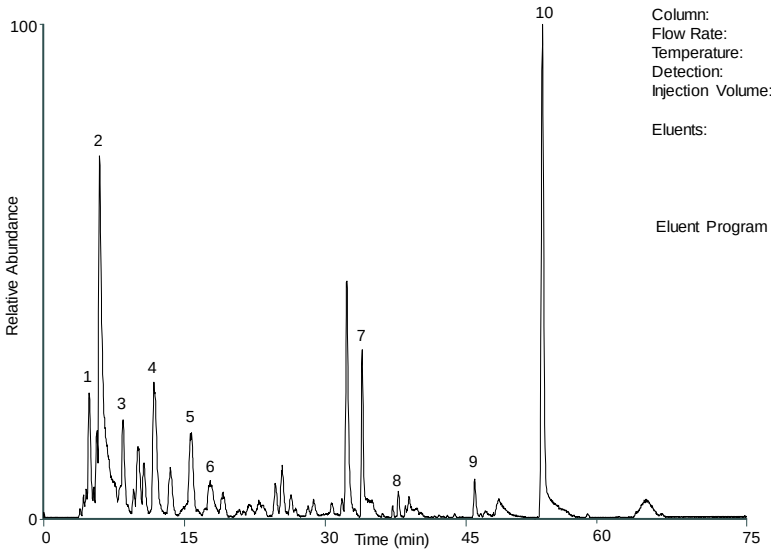
6.4.1 Porcine Gastric Type III Mucin O-Linked Glycan Alditol by IC-MS

High resolution separations, especially to separate populations of chemically similar but structurally heterogenous glycans, becomes important for subsequent mass spectrometric characterization. When the CarboPac PA300-4µm column is used in an IC-MS workflow, it enables the identification of glycan structures.
Figure 10 demonstrates the base peak chromatogram of porcine gastric mucin type III O-glycans injected into an Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ Mass Spectrometer. A selection of peaks are numbered and identified below in Table 2. Peak compositional identities were confirmed with previously published data (Jin, C., Kenny, D. T., Skoog, E. C., Padra, M., Adamczyk, B., Vitizeva, V., Thorell, A., Linden S. K., & Karlsson, N. G. (2017). Structural diversity of human gastric mucin glycans. *Molecular & Cellular Proteomics, 16*(5), 743-758).

In this application example, we demonstrate that IC-MS platform supports simultaneous separation and detection of neutral and charged (sialylated and sulfated) glycans without the need for derivatization. High resolution MS data and tandem MS/MS spectrum with diagnostic fragments provide a highly reliable structural annotation of heterogenous glycans. The unique IC-MS workflow presented here provides confirmatory, orthogonal information for glycan analysis.

Figure 11 show a characteristic chromatogram and the ability for the CarboPac PA300-4µm to resolve compositional isomers.

Figure 10 Base Peak Chromatogram of O-Linked Glycans of Porcine Gastric Mucin Protein on Dionex CarboPac PA300-4µm



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30ºC
Detection:	Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ MS
Injection Volume:	20 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

Eluent Program

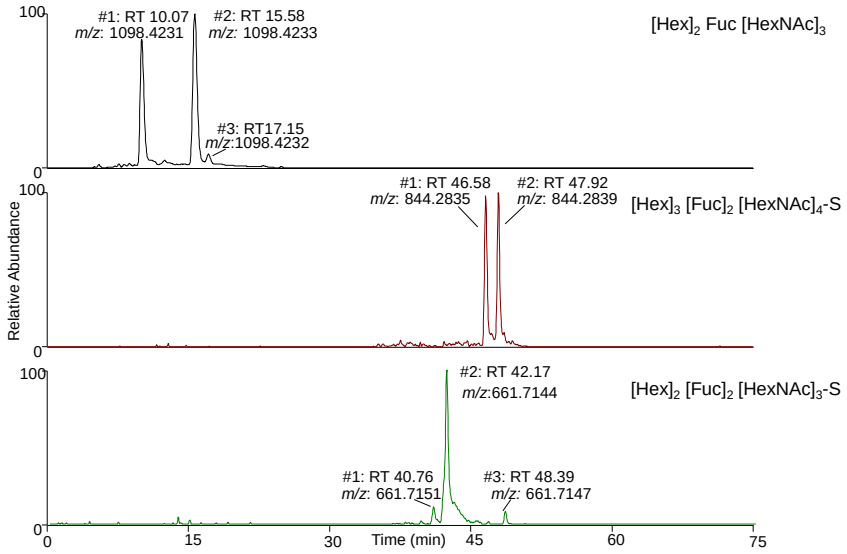
Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

Table 2 The Compositional Identities of Selected Peaks in Figure 10.

Peak	RT (min)	Observed *m/z*	Theoretical *m/z*	Mass accuracy (ppm)	Composition	Charge state
1	4.85	733.2896	733. 2884	1.64	Hex Fuc $[HexNAc]_2$	1
2	6.08	530.2080	530.2090	1.89	Hex Fuc HexNAc	1
3	8.46	733.2892	733. 2884	1.09	Hex Fuc $[HexNAc]_2$	1
4	11.97	1041.4006	1041.3992	1.34	$[Hex]_2$ $[Fuc]_2$ $[HexNAc]_2$	1
5	15.58	1098.4233	1098.4206	2.46	$[Hex]_2$ Fuc $[HexNAc]_3$	1
6	17.66	1203.4506	1203.4520	1.16	$[Hex]_3$ $[Fuc]_2$ $[HexNAc]_2$	1
7	34.01	821.3063	821.3045	2.19	NeuAc Hex Fuc HexNAc	1
8	38.19	852.2811	852.2848	4.34	N-glycan, hybrid, sulfated	2
9	45.92	1121.3568	1121.3559	0.80	$[Hex]_2$ $[Fuc]_2$ $[HexNAc]_2$-S	1
10	53.19	813.2474	813.2452	2.70	Hex Fuc $[HexNAc]_2$-S	1

Hex: Hexose; Fuc: Fucose; HexNAc: N-Acetylhexosamine; NeuAc: N-Acetylneuraminic acid; S: Sulfated

Figure 11 Dionex CarboPac PA300-4µm Column Provides Isomer Resolution of Both Neutral and Sulfated O-glycans from Porcine Gastric Mucin Type III Sample

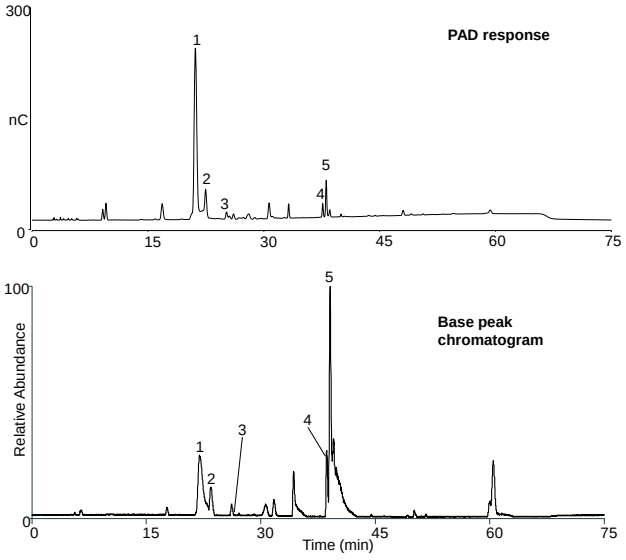


6.4.2 Analysis of Bovine Milk Free Oligosaccharides Using IC-MS

Oligosaccharides are bioactive molecules that have a variety of health benefits to consumers. One of the known health benefits is the use of oligosaccharides as prebiotics, which play an important role in establishing the intestinal microbiota by selectively simulating the growth of beneficial bacteria. Bovine milk is a promising candidate source for novel functional carbohydrates. It contains oligosaccharides that have structures similar to those found in human milk, and there is a growing interest in examining the structures of those milk oligosaccharides to understand their potential biological benefits.

Figure 12 shows the analysis of oligosaccharides extracted from 0.1 mL of a commercial, lactose-free bovine milk sample after sample cleanup with a HyperSep™ Hypercarb™ Filter Plate. The purified oligosaccharide sample was analyzed using HPAE-PAD and IC-MS individually via two independent injections. The chromatograph clearly shows the ability of the CarboPac PA300-4µm column to separate charged, sialylated glycan structures from neutral, non-sialylated structures. The peak identities are confirmed with tandem mass spectrometry analysis and analytical standards purchased from Biosynth Carbosynth®. The chromatograms obtained from two different detectors are similar, yet with some variations in peak responses. Response factors provided by the two different detection methods for each glycan species is dependent on the glycan structures.

Figure 12 Analysis of Free Oligosaccharides from Commercial, Lactose-Free Bovine Milk with a Dionex CarboPac PA300-4µm Column



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30°C
PAD Detection:	Integrated Amperometry, Quadruple Pulse Waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	Ag/AgCl
Mass spectrometer:	Q Exactive™ HF-X Hybrid Quadrupole-Orbitrap™ MS
Injection Volume:	10 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

Eluent Program

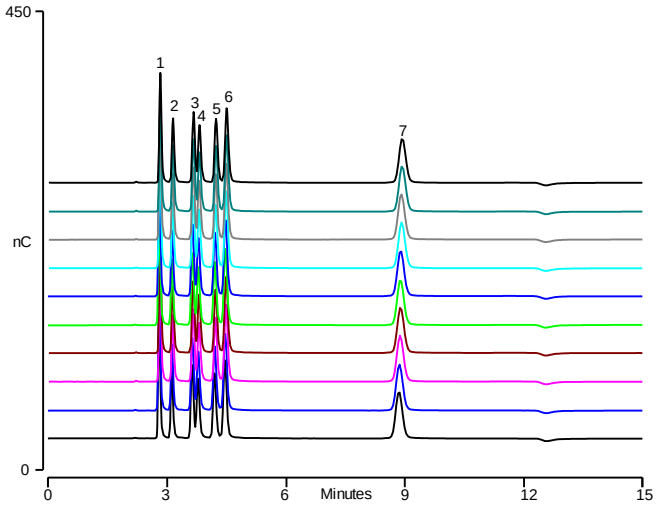
Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

Peaks:
1: $[Hex]_3$
2: $[Hex]_2$ HexNAc
3: Lacto-N-neotetraose
4: 6'-sialyllactose
5: 3'-sialyllactose

6.5 Robustness and ruggedness of Dionex CarboPac PA300-4µm

The Dionex CarboPac PA300-4µm column provides consistent chromatography performance and highly reproducible separation. To demonstrate the ruggedness of the Dionex CarboPac PA300-4µm column, a mixture of seven sugar alditols was injected three times every 10th run for a total of 100 back-to-back runs. Every 10th run is displayed here in Figure 13, showing high reproducibility for the Dionex CarboPac PA300-4µm column.

Figure 13. Stability Test on a Dionex CarboPac PA300-4µm Column



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Eluent:	10mM KOH
Flow Rate:	0.25mL/min
Temperature:	30°C
Detection:	Integrated Amperometry, Quadruple Pulse Waveform
Working Electrode:	PTFE Gold (Disposable)
Ref Electrode:	Ag/AgCl
Injection Volume:	2.5 µL
Standard Conc:	50 µM

Peaks:
1. Myo-Inositol
2. Xylitol
3. Dulcitol
4. Sorbitol
5. Mannitol
6. Perseitol
7. Maltitol

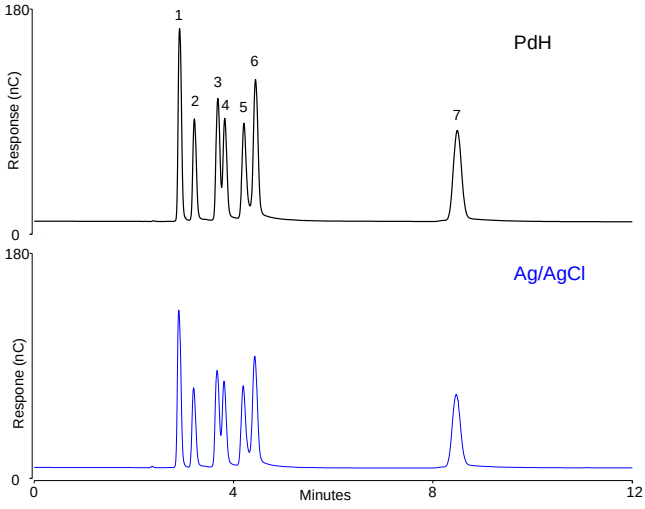
6.6 Comparison between Ag/AgCl and PdH reference electrodes

The Electrochemical Detector (ED) cell is a miniature flow-through amperometric detection cell that includes three different electrodes: a titanium cell body (the counter electrode), a working electrode, and a reference electrode. For carbohydrate applications, pulsed amperometric detection is typically performed with a series of potentials at a gold working electrode where the analytes are detected.

A reference electrode is required to assure application of the proper working electrode potential. Both silver/silver chloride (Ag/AgCl) electrodes and palladium hydrogen (PdH) reference electrodes have been commonly used in carbohydrate applications. In this section, the performance of a PdH reference electrode was demonstrated and compared to a Ag/AgCl reference electrode for two carbohydrate applications: a mixture of sugar alditols (shown in figure 14) and N-glycans released from bovine fetuin glycoprotein (shown in figure 15). In both applications, the signal response was similar, but slightly higher, with the PdH reference electrode.

Please refer to Thermo Scientific Electrochemical Detection User's Compendium (http://tools.thermofisher.com/content/sfs/manuals/Man-065340-Electrochemical-Detection-Man065340-EN.pdf) for detailed instructions on the installation of a PdH reference electrode.

Figure 14. Comparison between a PdH and a Ag/AgCl Reference Electrode for Analysis of Sugar Alditols on Dionex CarboPac PA300-4µm



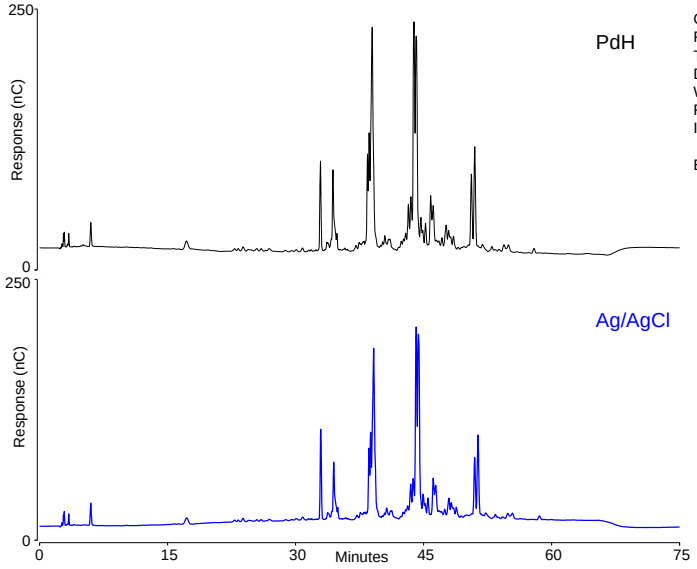
Column:	Dionex CarboPac PA300-4µm, 2x250mm
Flow Rate:	0.25mL/min
Temperature:	30 °C
Detection:	Integrated Amperometry, Quadruple Pulse Waveform
Working Electrode:	PTFE Gold (Disposable)
Ref Electrode:	PdH or Ag/AgCl
Injection Volume:	2.5 µL
Standard Amount:	50 µM
Eluents:	A - Milli Q Water B - 200 mM NaOH

Eluent Program

Time	%A	%B	Comments
-25.0	0	100	Regeneration
-15.0	0	100	
-14.9	95	5	Equilibration
0.0	95	5	Load/Inject
12.0	95	5	End

Peaks:
1. Myo-Inositol
2. Xylitol
3. Dulcitol
4. Sorbitol
5. Mannitol
6. Perseitol
7. Maltitol

Figure 15. Comparison between a PdH and a Ag/AgCl Reference Electrode for Analysis of N-linked Glycan Alditol Profile of Bovine Fetuin on Dionex CarboPac PA300-4µm



Column:	Dionex CarboPac PA300-4µm (Guard + Analytical)
Flow Rate:	0.25mL/min
Temperature:	30 °C
Detection:	Integrated amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold (Disposable)
Ref. Electrode:	PdH or Ag/AgCl
Injection Volume:	2.5 µL
Eluents:	A - Milli Q Water B - 200 mM NaOH C - 50 mM NaOH 25 mM NaOAc D - 100 mM NaOH 250 mM NaOAc

Eluent Program

Time	%A	%B	%C	%D	Comments
-1.0	78.5	19.5	2	0	Equilibration
0.0	78.5	19.5	2	0	Load/inject
4.0	78.5	19.5	2	0	
20.0	20	20	60	0	
50.0	0	0	0	100	Regeneration
59.9	0	0	0	100	
60.0	78.5	19.5	2	0	Equilibration
75.0	78.5	19.5	2	0	End

7. Troubleshooting Guide

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using Dionex CarboPac columns. For more information on problems that originate with the Ion Chromatograph (IC), refer to the Troubleshooting Guide in the appropriate operator's manual. Remember that some of the problems may be related to parts of your experimental protocol (sample contamination, imprecision during sample transfer, 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.

NOTE

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

7.1 High Back Pressure

7.1.1 Finding the Source of High System Pressure

Column pressure, (after subtracting the system pressure) for the Dionex CarboPac PA300-4µm Analytical Column should be close to the pressure listed in the QAR when using the test chromatogram conditions. If a Dionex CarboPac guard and analytical column are both installed, column pressure will increase by 5-10 % over the pressure listed in the QAR for the column. If the total system pressure is much higher than expected, it is advisable to determine the cause of the high system pressure.

A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. If necessary, measure the pump flow rate by collecting the DI H_2O eluent for a specified time at operating pressure, and measure the collected amount using an analytical balance. This data (weight/time) will give actual flow rate.

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

C. To determine which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. 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 pressure with the eluent generator connected should be <400 psi. Continue adding system components (backpressure coil (if present), injection valve, column(s), and detector) one by one, while monitoring the system pressure. The pressure should increase by the sum of the measured pressures of the individual guard and analytical columns (see product QAR) when the CarboPac Guard and Analytical columns are connected.

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

7.1.2 Replacing Column Bed Support Assemblies for 2 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 analytical column) into the end fitting. Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Drop the bed support assembly into the end fitting, making sure that the bed support assembly is centered at the bottom of the end fitting. Tap the end fitting gently on a hard surface to reorient the bed support assembly as necessary in order to properly situate the bed support assembly in the end fitting.

CAUTION

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting.

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

7.1.3 Filter Eluent

Eluents containing particulate material or bacteria may clog the column inlet bed support. Filter eluents through a 0.2 µm Nylon or PES (PolyEtherSulfone) filter (We recommend the Thermo Scientific, Nalgene Sterile Disposable Filter Units with Nylon Membrane, Item # 164-0020). DO NOT use a cellulosic filter (e.g., cellulose acetate or regenerated cellulose) as these will introduce cellulosic polymers into your eluent, and thus many Integrated Amperometric peaks will appear, even with blank “injections”.

7.1.4 Filter Samples

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

7.2 High Background

While it may be possible to obtain reasonable performance even with elevated levels of detection background according to some requirements, high background frequently brings about increased gradient artifacts and can be accompanied by a presence of ghost peaks. Detection sensitivity may also change suddenly when the detection background is too high. A background >35 nC with 10 mM hydroxide at 0.25 mL/min and 30°C using the quadruple waveform indicates one of the following possibilities:

A. Incorrect detection parameters.
Verify that Ag/AgCl is specified as a reference electrode. Check all of the waveform values in the program against those in the Disposable Electrode Manual. If the pH reading with 10 mM NaOH is above 13.2 replace the reference electrode.

B. Compromised working electrode surface.
Briefly install a new working electrode and check the background as above. If the reading remains > 35 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. If the detector background signal is in the 10-35 nC range, continue with your work with the new electrode installed.

C. Column contamination: Remove the column set from the system first and replace it with a length of yellow PEEK tubing, generating a pressure drop between 1000 and 2000 psi. If the background reading improves after the column is removed from the system, go to Appendix A, “CarboPac PA300-4µm Column Care”.

D. Water contamination: Prepare eluents using a fresh ultra-pure water from another source. If the background is reduced, investigate the source of contamination in the original source of water.

E. System Contamination: If the background remains high even with fresh water and without the column, carry out a 2 M sodium hydroxide rinse. In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA300-4µm QAR eluent is 10-35nC. If the background is much higher, determine the cause of high background. Consider the possibility that the eluent filter might be cellulosic, as that will introduce very high signal and noise.

7.2.1 Preparation of Eluents

A. Make sure that the eluents are made correctly.

B. Make sure that the eluents are made from chemicals with the recommended purity.

C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 18.2 MΩ-cm or greater

7.2.2 Dionex CR-ATC Column

A. When using eluent generator (Dionex EGC 500 KOH) to generate eluent, install a Dionex CR-ATC 500 Anion Trap Column.

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

7.2.3 A Contaminated Guard or Analytical Column

A. Remove the columns from the system.

B. Install a back-pressure coil that generates approximately 2000 psi and continue to pump eluent. If the background decreases, the column(s) is (are) the cause of the high background.

C. To eliminate downtime, clean or replace the analytical column at the first sign of column performance degradation. Clean the column as instructed in, "Appendix A, Dionex CarboPac PA300-4µm Column Care".

7.3 Poor Resolution

One of the unique features of Dionex CarboPac columns is the fast equilibration time in gradient applications from the ending eluent (high ionic strength) to the beginning eluent (low ionic strength). The actual equilibration time depends on the ratio of the strongest eluent concentration to the weakest eluent concentration and application flow rate. Typically, equilibration times range from 10 to 15 minutes at 0.25 mL/min for 2 mm ID columns.

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

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

A. Keep the eluent concentrations constant and adjust the gradient time. This is the simplest way to compensate for total system differences if resolution is the problem.

B. Change the initial and/or final eluent concentration and adjust the gradient time. This approach requires more time to develop and more experience with methods development work. Its advantage is that it allows a method to be tailored for a particular application, where selectivity, resolution, and total run time are optimized. Be aware that poor peak resolution can be due to any or all of the following factors.

7.3.1 Loss of Column Efficiency

A. Check to see if headspace has developed in the guard or analytical column. This is usually due to improper use of the column such as exposing it to high pressures. Remove the column's inlet end fitting (see Section 7.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body to the top, the column must be replaced.

B. Extra-column effects can result in sample band dispersion or band broadening. Make sure you are using viper fittings for all connections, including the sample loop (if necessary, PEEK tubing with an ID of no greater than 0.005" can be used if junctions are fitted and tightened appropriately). For PEEK tubing, cut the tubing lengths as short as possible. Check for leaks.

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

D. Bypassing the pre-column heater unit if the extra volume introduced by this unit is the cause for peak broadening or loss of efficiency.

7.3.2 Shortened Retention Times

NOTE

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

A. Check the flow rate. See if the eluent flow rate is equivalent to the flow rate specified by the analytical protocol. Collect the eluent for a specified time after the column and measure the eluent flow rate gravimetrically using an analytical balance.

B. Check to see if the eluent compositions and concentrations are correct. An eluent that is too concentrated will cause the peaks to elute earlier. If eluent concentration is too concentrated, prepare fresh eluent.

NOTE

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

C. Column contamination can lead to a loss of column capacity. Highly retained contaminants will tend to occupy anion exchange sites limiting the number of sites available for retention of the analytes. Refer to "Appendix A, Dionex CarboPac PA300-4µm Column Care," for recommended column cleanup procedures.

NOTE

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

D. Diluting the eluent will improve peak resolution but will also increase the retention times. If a 10% dilution of the eluent is not sufficient to obtain the desired peak resolution, or if the resulting increase in retention times is unacceptable, try cleaning the column (see Appendix A, Dionex CarboPac PA300-4µm Column Care).

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

NOTE

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

7.3.3 Loss of Resolution for early eluting peaks

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

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

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

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

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

7.3.4 Spurious Peaks

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

NOTE

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

B. The injection valve may need maintenance. When an injection valve is actuated, the possibility of creating a baseline disturbance exists. This baseline upset may appear as a peak of varying size and shape. This will occur when the injection valve needs to be cleaned or 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.

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

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

A. Check the position and filling levels of sample vials in the autosampler.

B. Check injector needle-height setting.

C. Check the injection valve orientation. If the valve is installed upside down, or if it is plumbed 180° out of the correct orientation, switching from load to inject will bypass the loop. If this is confirmed, re-plumb the valve in the correct orientation.

D. Check each line of the schedule for proper injector parameters. Use full-loop sampling and appropriate loop size.

E. Check the total transfer volume (TLV) and recalibrate this volume. If the newly calibrated volume has changed dramatically from the previously recorded value, service the autosampler transfer line.

F. Service the injection valve (check for leaks, Tefzel fragments, or sediments inside the valve).

G. Remove the borate trap from the system and perform a quality assurance test of the column (Appendix B) without borate trap in-line. If the old borate trap was the cause of loss of peaks, replace the contaminated borate trap with a new one and/or clean the contaminated borate trap using the following protocol:

- Set up a stand-alone pump with following wash solutions:
  a) 1M Methanesulfonic acid (MSA)
  b) 2M NaOH
- Connect the inlet of the contaminated borate trap to the pump outlet, and direct the outlet of the borate trap to a waste container
- Wash the borate trap at 1mL/min with 1M MSA for at least 2 hours, and then with 2M NaOH for 2 hours
- Test the cleaned borate trap under the quality assurance report for CarboPac PA300-4µm conditions (Appendix B) and ensure QAR data is acceptable.

7.3.6 Large Baseline Dip in the Chromatogram

A large baseline dip appearing into the chromatogram is usually caused by oxygen in the sample injected. This ‘oxygen dip’ is normal and can be reduced in magnitude with higher hydroxide concentrations in the eluent. If the sample vial is emptied causing the sampler to “inject” air, this dip will dramatically increase.

7.3.7 Unidentified Peaks Appear with Expected Analyte Peaks

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

Some trace contaminants can co-elute with glycans, compromising accuracy of quantitation at lower concentrations. If extraneous peaks are observed even after the water supply is excluded as a possible cause, clean the autosampler lines and sample loop. The autosampler should be cleaned using the following protocol:

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

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

7.3.8 Decreased Detection Sensitivity

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

Any decrease in detection sensitivity means that the working electrode surface has been affected. The operator should install a replacement (disposable) working electrode. Spare reference electrodes and disposable gold working electrodes should always be available in order to avoid unnecessary delays.

Exceptions:

Check the pH reading. If the value is out of range or >13.2, install a new reference electrode and then install a new gold disposable working electrode. The system cleanup is not necessary. The decrease in sensitivity can be caused by a loss of surface area on the disposable electrode, or by deposition of gold-oxide on the conventional electrode surface because the reference potential was too high. The conventional gold working electrode can be reconditioned by polishing.

Peak heights will also increase with increasing eluent concentrations, especially between 1 and 10 mM. 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 be significantly higher at 12mM. You can expect a peak area (and height) decrease whenever reducing your eluent strength below 8 mM.

After installing a new working electrode (disposable or conventional, with or without the complete system cleanup), confirm the expected detection sensitivity. Should the response be too low, immediately remove the new working electrode from the system to minimize its contamination.

7.3.9 Excessive Gradient Drift

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

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

B. Set column temperature to 30 °C and wash the guard and analytical column with 1M NaOH for at least four hours (preferably overnight). For high concentrations of NaOH, we recommend bypassing the ED cell and diverting the column effluent to waste.

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

Refer to the Product Manual for Disposable Electrodes (Doc. No. 065040), Dionex ICS-6000 Ion Chromatography System Manual (Doc. No. 22181-97002) or User's Compendium for Electrochemical Detection (Doc. No. 065340) for any help necessary with electrochemical detection, working and reference electrodes.

Appendix A: Dionex CarboPac PA300-4µm 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 PA300-4µm column is 5,000 psi (34.47 MPa).

A.2 Column Start-Up

The Dionex CarboPac columns are shipped using sodium hydroxide (see QAR) as the storage solution. Use manually prepared sodium hydroxide eluent employed in the Quality Assurance Report (QAR). Install the column in the chromatography module and direct the column effluent to waste for 60 minutes, and then connect to the ED cell. It is recommended to clean the column for 1 to 2 hours with 200 mM NaOH at 0.25 mL/min for 2 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 7.3 Poor Resolution and Section 7.3.1 Loss of Column Efficiency.

IMPORTANT ***When making any tubing connections (column installation, replacing tubing etc.), it is recommended to make these connections with the pump turned off.***

A.3 Column Storage

Store the CarboPac PA300-4µm column in 10 mM hydroxide eluent. Flush the column for a minimum of 20 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

A.4 Dionex CarboPac PA300-4µm Column Cleanup

The Dionex CarboPac PA300-4µm can be readily cleaned by rinsing the column with ~ 60 column volumes of 200 mM NaOH. More stubborn contamination problems may necessitate a thorough column cleaning. Use the following steps to thoroughly clean the Dionex CarboPac PA300-4µm; use 0.25 mL/min for 2mm Column formats to avoid 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, but ensure the flow direction is as shown on the column label. Do not direct flow backward through the column(s).

CAUTION

When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Otherwise, contaminants that have accumulated on the guard column elute onto the analytical column causing irreversible damage. If in doubt, clean each column separately.

B. Wash the Dionex CarboPac PA300-4µm column with deionized water (18.2 MΩ-cm) for about 30 minutes and then clean with 1 M MSA for one to two hours at 0.25mL/min.

C. Wash the column with deionized water for about 30 minutes.

D. Then clean the Dionex CarboPac PA300-4µm with 200 mM NaOH for at least two hours.

E. Reconnect column to the cell and equilibrate the column with the desired initial conditions; test the column performance using the QAR standard and eluent.

Appendix B: Quality Assurance Report

Device Monitoring Enabled
and Viper Fitting Ready

Dionex CarboPac™ PA300-4µm
Analytical (2 x 250 mm)
Product No. 303346

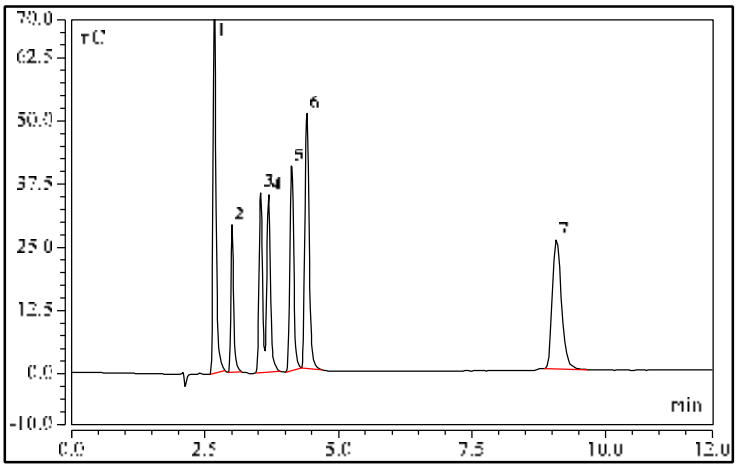
Date: 3/17/2020
Serial No. : 200401001
Lot No. : 01610061B

Eluent Flow Rate: 0.25 mL/min
Temperature: 30 °C
Detection: Electrochemical Detection, quadruple waveform
Injection Volume: 2.5 µL
Storage Solution: Eluent

Eluent Composition
%A: 200 mM NaOH
%B: 10 mM NaOH

Eluent Profile

Time	%A	%B	Comment
-25.00	100	0	Regeneration
-15.00	100	0	
-14.99	0	100	Equilibration
0.00	0	100	Inject
12.00	0	100	End



ED40 Operating Parameter

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

[1] **Reference Electrode M** Ag/AgCl

No.	Peak Name	Ret.Time (min)	Asymmetry (AIA)	Resolution (EP)	Efficiency (EP)	Amount Injected pmol
1	Myo-Inositol	2.7	1.6	3.49	12848	125
2	Xylitol	3.0	1.4	5.04	15304	125
3	Sorbitol	3.5	n.a.	1.28	15144	125
4	Dulcitol	3.7	n.a.	3.40	15023	125
5	Mannitol	4.1	1.5	2.14	15937	125
6	Perseitol	4.4	1.3	20.66	15160	125
7	Maltitol	9.1	1.5	n.a.	13743	125

QA Results:

Analyte	Parameter	Specification	Results
Maltitol	Efficiency	>=8100	Passed
Maltitol	Retention Time	8.4-10.1	Passed
Maltitol	Asymmetry	1.0-1.8	Passed
	Pressure	<=3850	2752

Production Reference:
Datasource: QAR7
Directory: CarboPac\CP_PA300
Sequence: CP_PA300-4µm_2x250mm
Sample No.: 1

7.2.10.23925

Chromeleon™ Thermo Fisher Scientific