



Dionex CarboPac PA20 Columns

031884 Revision 06 • November 2018

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Product Manual

for

Dionex CarboPac PA20 Guard Columns

(3 x 30 mm, Item #: 060144)

Dionex CarboPac PA20 Analytical Columns

(3 x 150 mm, Item #: 060142)

Dionex CarboPac PA20 Fast Sialic Analytical Column

(3 x 30 mm, Item #: 076381)

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Revision History:

Revision 06, November 2018, Added Column Care Section. Content transcribed into the latest template.

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 PA20

The Thermo Scientific™ Dionex™ CarboPac™ family of columns is designed to address the analytical requirements of a wide range of carbohydrate chemists. Underivatized carbohydrates are separated at high pH, in an approach that is unique to Thermo Scientific, simple to reproduce, and made possible by the pH stability of the Dionex CarboPac columns. The Dionex CarboPac PA20 (3 mm) analytical columns are recommended for monosaccharide composition analysis. It had been the best column to combine with the Thermo Scientific Dionex AminoTrap™ for glycoprotein monosaccharide composition analysis, before the introduction of the Dionex CarboPac PA20 column.

The Dionex AminoTrap column removes the problematic amino acids out of the chromatogram, thereby eliminating a possibility of their co-elution with carbohydrates. The Dionex CarboPac PA20 also works well in combination with the Dionex AminoTrap and EGC eluent generator or cartridge for monosaccharide analysis. For detailed information on Dionex CarboPac PA20, AminoTrap column and EGC, refer to the product manual Document No. 031884, 065362 (CPPA20), 031197 (AminoTrap) and 065018 (EGC).

Resin Characteristics:

| | |
|------------------------|----------------------|
| Particle Size: | 6.0 µm |
| Pore Size: | Microporous (< 10 Å) |
| Cross-linking: | 55% |
| Ion exchange capacity: | 65 µeq per column |

Latex Characteristics:

| | |
|----------------------|-----------------------------|
| Functional Group: | difunctional quaternary ion |
| Latex Diameter: | 130 nm |
| Latex Cross-linking: | 5.2% |

Typical Operating Parameters:

| | |
|------------------------|---|
| pH range: | 0–14 |
| Temperature Limit: | 4–60 °C |
| Pressure Limit: | 3500 psi |
| Organic Solvent Limit: | 100% compatible |
| Typical eluents: | High purity water (18.2 megohm-cm), potassium hydroxide, sodium hydroxide, sodium acetate |

1.2 CarboPac PA20 Anion Exchange Columns

| Part Number | Product Description |
|-------------|---|
| 060142 | CarboPac PA20 Analytical Column, 3-mm |
| 060144 | CarboPac PA20 Guard Column, 3-mm |
| 076381 | CarboPac PA20 Fast Sialic Analytical Column, 3-mm |

1.3 CarboPac PA20 Guard Column

A guard column is usually placed before the analytical column to prevent sample contaminants from eluting onto the analytical column. The addition of the guard to the analytical also increases the column capacity by about 20%, which translates into an increase of about 20% in the retention times for isocratic runs. If a guard is added to a system running a gradient method that was initially developed for just an analytical column, the gradient schedules should be increased by about 20%, to ensure similar resolution between the eluting peaks.

1.4 CarboPac PA20 Fast Sialic Analytical Column

The CarboPac PA20 Fast Sialic Analytical column has been tested specifically for sialic acid applications. A guard column or Amino Trap column is not necessary for these applications.

2. System Requirements and Installation

2.1 System Requirements

2.1.1 System Requirements for 3mm Operation

The carbohydrate separations using the Dionex CarboPac PA20 columns are optimized for use with Dionex Ion Chromatography systems equipped with electro chemical detection. It is highly recommended to ensure that the systems used for carbohydrate analysis are metal-free.

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

The 2 mil-thick Teflon gaskets included in each package of disposable electrodes must be used (Note: 1 mil-thick Teflon gaskets for use with capillary flow rates have to be ordered separately, PN 072117); otherwise, the disposable electrode product warranty is void. In addition, the quadruple waveform must be used for carbohydrate analysis otherwise the product warranty is void. Always wear gloves when handling electrodes. Never touch the electrode surface. To install a disposable working electrode and reference electrode (pH-Ag/AgCl or PdH) refer to Product Manual for Disposable Electrodes Doc. No. 065040, ICS-6000 Ion Chromatography System Manual Doc. No. 22181 and User's Compendium for Electrochemical Detection Doc. No. 065340.

2.1.3 System Void Volume

When using CPPA20 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 0.005" I.d. PEEK tubing for 3mm columns. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.

2.2 The Injection Loop

2.2.1 The 3 mm System Injection Loop, 10 - 50 μ L

For most applications on a 3 mm analytical system, a 10 – 50 μ L injection loop is sufficient. Generally, you should not inject more than 40 nanomoles of any analyte onto the 3 mm analytical column. Injecting larger amounts of an analyte can result in overloading the column which can affect the detection linearity. For low concentrations, larger injection loops can be used to increase sensitivity.

2.3 The Dionex CarboPac PA20 Guard Column

A Dionex IonPac CarboPac PA20 Guard Column is normally used with the CarboPac PA20 Analytical/Capillary Column. Retention times will increase by approximately 20% when a guard column is placed in-line before the analytical/capillary column under isocratic conditions. A guard column is utilized to prevent sample contaminants from eluting onto the analytical/capillary column. It is easier to clean or replace a guard column than an analytical/capillary column. Replacing the Dionex CarboPac PA20 Guard Column at the first sign of peak efficiency loss or decreased retention time will prolong the life of the Dionex CarboPac PA20 Analytical/Capillary Column.

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

For Dionex IonPac CarboPac PA20 applications using the Dionex EGC KOH cartridge, a Dionex CR-ATC Continuously Regenerated Trap Column (Item# 060477 or 072078) should be installed at the Dionex EGC eluent outlet to remove trace level anionic contaminants from the carrier deionized water. See the Dionex CR-TC Product Manual (Document No. 031910) for instructions.

2.5 Eluent Storage

Dionex CarboPac PA20 columns are 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 solvents).



NOTE

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

3. Purity Requirements for Chemicals

Obtaining reliable, reproducible and accurate results requires eluents that are free from impurities and prepared only from the chemicals recommended below. Thermo Scientific cannot guarantee proper column performance when alternate suppliers of chemicals or lower purity water are utilized.

3.1 Deionized Water

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

3.2 Sodium Hydroxide

Use 50% w/w sodium hydroxide (Certified Grade, Fischer Scientific Item # UN 1824) for preparation.

3.3 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 are utilized.

4. Before You Start

4.1 The Most Important Rules

| | |
|--------|--|
| ALWAYS | use 50% NaOH solution rather than NaOH pellets to make eluents |
| ALWAYS | use dedicated glassware and disposable glass or plastic ware for volume adjustments. |
| ALWAYS | keep your NaOH eluent blanketed with helium or nitrogen. Prepare new NaOH eluent if left unblanketed for more than 30 minutes. |
| ALWAYS | use EGC-KOH generated eluent when possible to avoid any eluent preparation issues |
| ALWAYS | pull at least 40 mL of new eluent through the lines when changing eluent or adding fresh eluent. This will ensure that your fresh eluent is primed through the lines up to the pump heads. |
| ALWAYS | verify the equilibration time necessary prior to injection to avoid baseline issues or artifacts or to avoid unnecessary increase in total method time. |
| NEVER | go to the next step of the installation if the previous step has failed. |
| NEVER | start an installation with any of the check list items below missing |
| NEVER | use ‘communal’ filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials. |
| NEVER | use MeOH or other organic solvents as rinse fluid in the autosampler. Use only water, replaced daily. |
| NEVER | run above 60°C or 5000 psi. |

4.2 Initial Check List

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

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

5. Preparation of Eluents and Standards



NOTE

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

For the monosaccharide compositional analysis, it is not necessary to prepare Eluent 4.

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

5.1 Eluent E1: Deionized Water

Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir for 5–10 minutes while sonicating. Note: Degassing by vacuum filtration through a 0.2 µm filter is a good alternative to degassing in a sonicator. Cap each bottle after the degassing step and minimize the length of time the bottle is opened to the atmosphere.

5.2 Eluent E2, E3: 0.018 M / 1 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

Weight Method

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

Example: To make 1 L of 1 M NaOH use 80.02g of 50% sodium hydroxide:

$$\text{For 1 M: } \frac{1 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\%} = 80.02 \text{ g diluted to 1 L}$$

Volume Method

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

$$g = dvr$$

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

Example: To make 1 L of 1M NaOH use 52.3 mL of 50% sodium hydroxide:

$$\text{For 1 M: } \frac{1 \text{ mole/L} \times 40.01 \text{ g/mole}}{50\% * 1.53^* \text{ g/mL}} = 52.3 \text{ mL diluted to 1 L}$$

* This density applies to 50% NaOH. If the concentration of the NaOH solution is significantly different from 50% the upper (weight method) calculation should be used instead.

Sodium Hydroxide Eluents

Dilute the amount of 50% (w/w) NaOH Reagent specified in [Table 1](#) “Dilution of 50% (w/w) NaOH to 50% * 1.53* g/m Make Standard CPPA20 Eluents” with degassed, dionized water (18.2 megohm-cm) to a final volume of 1,000 mL using a volumetric flask. Avoid the introduction of carbon dioxide from the air into the aliquot of 50% (w/w) NaOH bottle or the deionized water being used to make the eluent. Do not shake the 50% (w/w) NaOH bottle or pipette the required aliquot from the top of the solution where sodium carbonate may have formed.

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

| Eluent Concentration (M) | NaOH (g) | NaOH (mL) |
|--------------------------|----------|-----------|
| 0.018 | 1.44 | 0.941 |
| 0.1 | 8.0 | 5.2 |
| 0.5 | 40.0 | 26.2 |
| 0.8 | 64.0 | 41.8 |
| 1.0 | 80.0 | 52.3 |

5.3 Eluent E4: 100 mM Sodium Hydroxide/ 1 M Sodium Acetate

To maintain baseline stability, it is important to keep the sodium hydroxide concentration constant during the sodium acetate gradient, because acetate has no buffering capacity at high pH. 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 0.1 M sodium hydroxide/ 1.0 M sodium acetate, dispense approximately 800 mL of DI water into a 1 L volumetric flask. Vacuum degas for approximately 5 minutes. Add a stir bar and begin stirring. Weigh out 82.0 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 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 kept blanketed under helium or nitrogen at 34 to 55 kPa (5–8 psi) at all times, and lasts about 1 week.



NOTE

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

5.4 Sample Preparation

The Dionex CarboPac columns are strong anion exchangers. Thus, the normal caveats applicable to ion exchange chromatography apply to these columns. High salt concentrations in the samples should be avoided wherever possible. Special care should be taken with samples containing high concentrations of anions, which are strong eluents for the Dionex CarboPac columns (e.g. chloride, carbonate, phosphate, etc.). It is best to avoid extremes of sample pH (especially extremely acidic samples). The presence of anionic detergents (e.g., SDS) in samples should be avoided entirely. Nonionic or cationic detergents may be acceptable in low concentrations.

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

When using pulsed electrochemical detection (PED) for detection, beware of high concentrations of electrochemically-active components (e.g., TRIS buffer, alcohols, and other hydroxylated compounds). Small amounts of organic solvents in the sample will not harm the column, although the organics may interfere with the chromatography or detection of the analytes of interest. If necessary, samples may be treated with reversed phase or ion exchange cartridges (such as the Dionex OnGuard cartridges) before analysis. However, because the Dionex CarboPac columns are extremely rugged, it is often worthwhile to analyze an aliquot of the sample directly, without any pre-column cleanup.

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

5.5 Verification of System Cleanliness

Prepare a new set of eluents as described in [Sections 5.1](#) and [5.2](#) and fill the eluent bottles. Set the eluent composition to 100% for each eluent line and draw out at least 40 mL of eluent from each eluent line.

5.5.1 System Background Check

This procedure is performed using the conditions of the test chromatogram. Make sure that

- A. the cell is not yet on,
- B. the pump is pumping 100 mM NaOH, 50 mM NaOAc or 200 mM KOH at 0.5 mL/min,
- C. a length of yellow tubing is installed between the injector and detector cell to generate ~1000 psi backpressure,
- D. the columns are not yet installed.

Confirm that the pH is between 12.8 and 13.4. With the pH within this range, turn on the cell using the quadruple waveform (See Table 3, Section 6.3 Disposable Electrode Manual, document number 065040) and begin monitoring the background signal from the control panel for at least 30 minutes. Confirm that the baseline is < 30 nC. If the background > 30 nC or the pH is out of range, see the “Troubleshooting” section at the end of this manual.

5.5.2 Verification of Column Cleanliness

Install the Dionex CarboPac PA20 column set only after the initial system test determines a background level within the specified range. A premature installation on a contaminated system will cause delays during the column equilibration.

The Dionex CarboPac PA20 is shipped in 18mM 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 PA20 must be washed for at least one hour (two hours preferred) with 200mM KOH or NaOH at appropriate flow rate. Equilibrate the column set by performing two blank injections (DI water) under the test chromatogram conditions, including the column regeneration and re-equilibration steps.

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

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

6. Applications

The CarboPac columns have been designed for isocratic or gradient separation utilizing sodium hydroxide eluents up to a concentration of 1M. Analyte separation is highly dependent on hydroxide concentration in HPAEC. Many 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 sodium hydroxide concentration increases. If a stronger eluent is needed to elute the analytes, a sodium acetate gradient can be used.

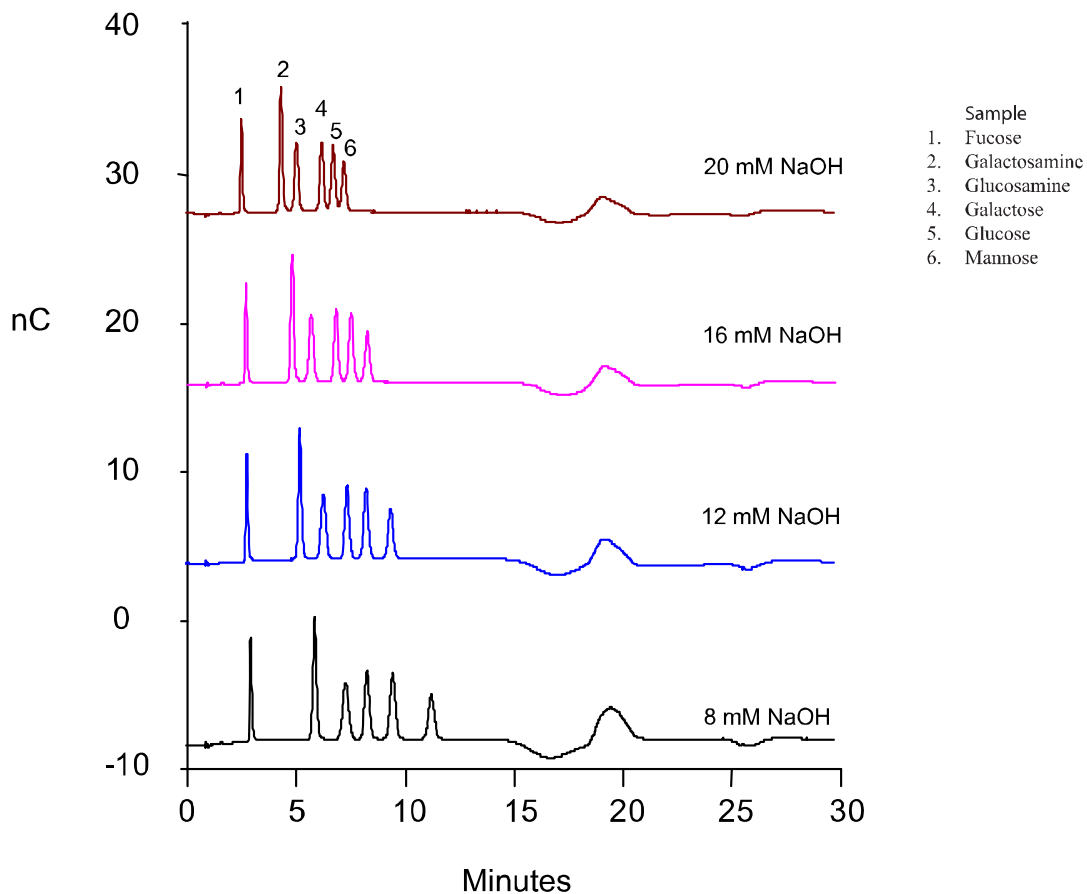
Depending upon your system, you may have to make small adjustments to your gradient conditions or operating temperature to achieve resolution of all analytes.

6.1 Runtime Optimization

The CarboPac PA20 has been designed to give good resolution between the components in the Monostandard Mix of Six monosaccharides under a variety of sodium hydroxide conditions. These conditions can be optimized depending upon the goal of the separation. The higher the sodium hydroxide concentration, the faster the peaks will be eluted but the less the resolution between the peaks. This phenomenon is illustrated in the chromatograms below. It is important to remember that despite the fact that the peaks are separated isocratically, at low hydroxide concentration it is still necessary to regenerate the column in order to remove carbonate buildup which will shorten the peak retention times over time.

Figure 1 Runtime Optimization

Column: CarboPac PA20 Analytical Column
 Gradient: 20 mM NaOH isocratic with a step to 200 mM NaOH at 10 min to regenerate the column.
 Flow Rate: 0.5 mL/min
 Temperature: Ambient
 Detection: Pulsed electrochemical detection, Au electrode
 Waveform: Quadruple potential

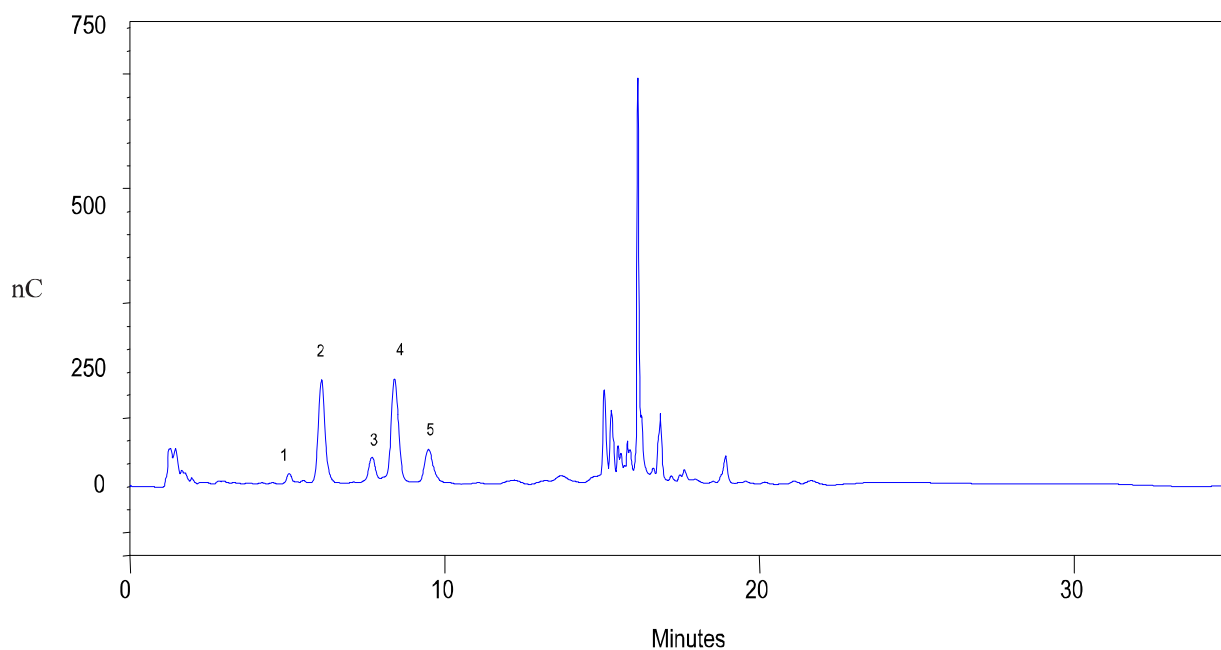


6.2 Common Monosaccharides from Mammalian Glycoproteins

Many mammalian proteins have carbohydrates attached to them. In many cases, the presence of the carbohydrate controls the biological activity of the protein or the rate at which it is cleared from the system. For example, certain glycosylated forms of tissue plasminogen activator (tPA) have more enzymatic activity than others. Erythropoietin shows complex effects if the protein is deglycosylated or the glycosylation is altered. Failure of secretion from the body, decreased stability and decreased biological activity occurs if multiple glycosylation sites are eliminated. Desialylation and/or less branched oligosaccharides give increased activity in vitro, but decreased activity in vivo. Thus, protein glycosylation is important to many scientists, including those making recombinant protein for therapeutic use.

The following conditions should be used for guidance. These conditions may be modified, as necessary, to suit your particular application needs.

Figure 2 Analysis of Monosaccharides from Hydrolyzed Rabbit Serum



Sample Prep: 280 μ L of 50% rabbit serum was hydrolyzed in 4M TFA for 4 h at 100°C. The sample was then speed vacuumed overnight to dryness and reconstituted in 560 μ L of water and then filtered through a 0.45 μ m nylon filter.

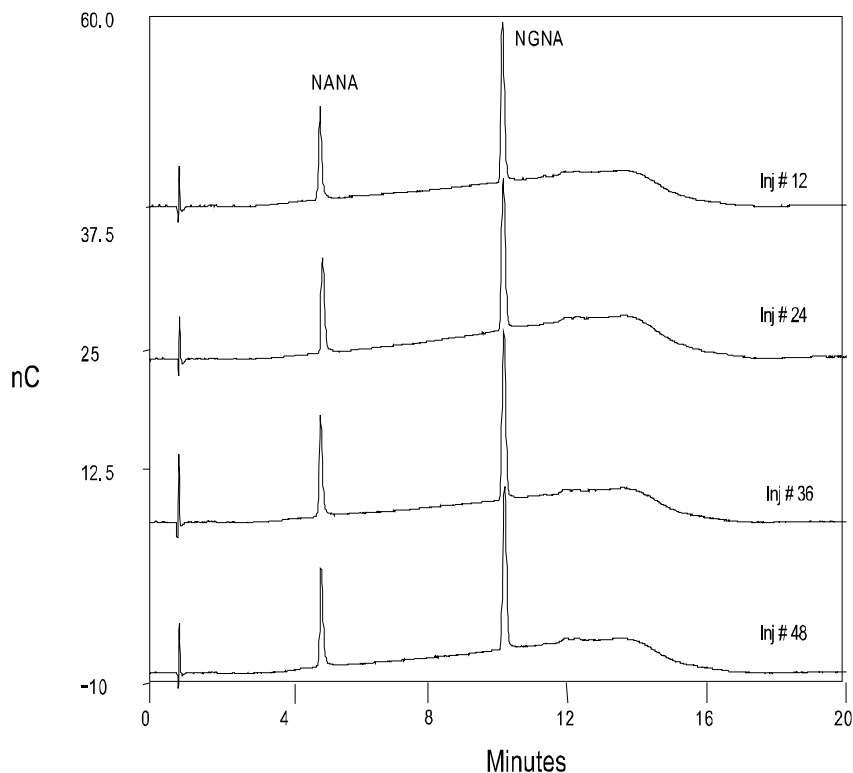
6.3 Sialic Acids

Sialic acids are a family of N- and O-substituted neuraminic acids that play an important role in physiology. They occupy terminal positions on many glycoproteins and serve as “markers” for protein removal from blood circulation. The amino group of neuraminic acid is linked to either an N-acetyl or N-glycolyl group which yields N-acetylneuraminic acid (Neu5Ac or NANA) or N-glycolylneuraminic acid (Neu5Gc or NGNA), respectively. Sialic acids are charged at neutral pH; at high pH many of the hydroxyl modifications are hydrolyzed and therefore cannot be analyzed using standard HPAE-PAD techniques. These substituted sialic acids can be separated at neutral pH and detected using PAD with the addition of post-column base. See Technical Note 41, “Analysis of Sialic Acids Using High-Performance Anion-Exchange Chromatography,” for details on eluent preparation, protein hydrolysis and digestion conditions and the use of 3-deoxy-D-glycero-D-galacto-2-nonulosonic acid (KDN) as an internal standard for optimal performance.

The following conditions should be used as guidance. These may be modified, as necessary, to suit your particular application needs.

Figure 3 Sialic Acid Analysis using the CarboPac PA20

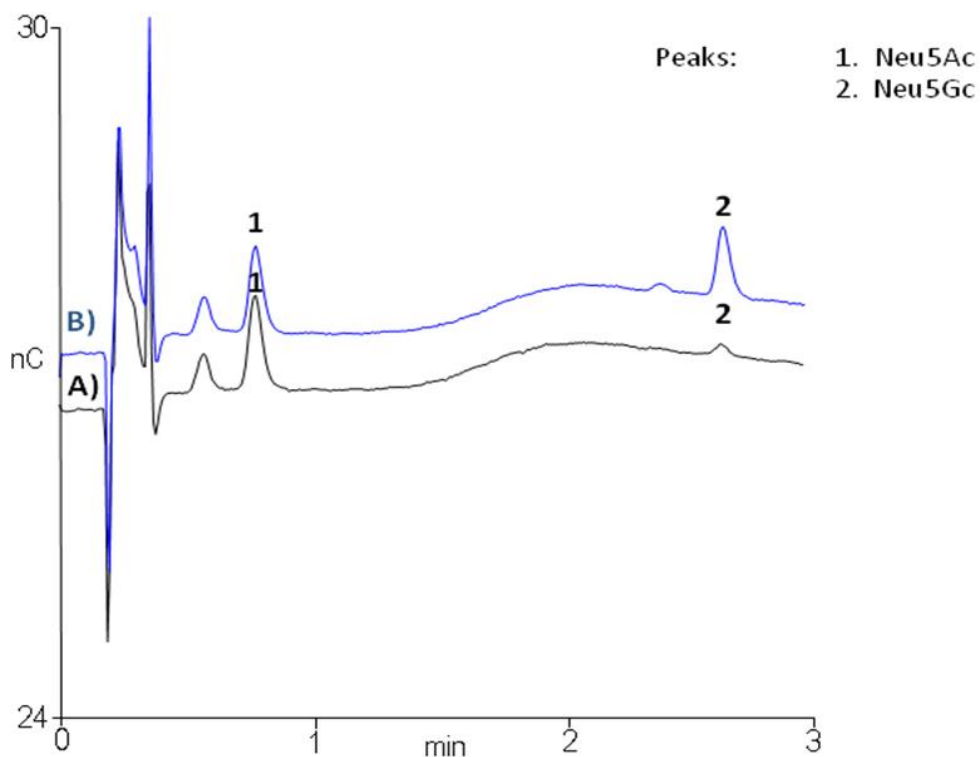
| | |
|------------|--|
| Column: | CarboPac PA20 and Guard |
| Gradient: | 20 - 200 mM NaOAc in 100 mM NaOH over 10 min |
| Flow Rate: | 0.5 mL/min |
| Detection: | Pulsed electrochemical detection, Au electrode |
| Waveform: | Quadruple potential |
| Sample: | NANA, NGNA |



6.4 Rapid Sialic Acid Analysis

For rapid sialic acid determination in glycoprotein hydrolyzates, the CarboPac PA20 Fast Sialic column is recommended. This column requires no guard column. The conditions below were developed for acetic acid protein hydrolyzates and are presented for general guidance. They may be modified to meet the needs of other sialic acid determination applications.

Figure 4 Sialic Acid Analysis using the CarboPac PA20 Fast Sialic Column



6.5 Carbohydrates in Foods

Isocratic sodium or potassium hydroxide eluents with concentrations on the range of 18-100 mM are typically used for the separation of monosaccharides, although for monosaccharides that are important in dietary fiber analysis, the eluent may be weaker. For all of these isocratic runs, it is important to regenerate the column using a step gradient to about 300 mM at the end of the run, to remove more strongly retained sample matrix components and ensure stable run times.

Occasionally, it may be necessary to use an extremely weak eluent or even water. Under these conditions, post column addition of 300 mM hydroxide is necessary for detection, although if the sensitivity is adequate, the added complexity of post-column hydroxide addition may not be worth the effort. For example, coffee sugars can be separated and detected with 2 mM NaOH with sufficient sensitivity to obviate the need for post-column base addition.

The following conditions should be used as guidance. These may be modified, as necessary, to suit your particular application needs.

Figure 5 Separation of Coffee Sugars Using the CarboPac PA20

| | |
|------------|--|
| Column: | CarboPac PA20 |
| Eluent: | 2 mM NaOH, isocratic |
| Flow Rate: | 0.5 mL/min |
| Detection: | Pulsed electrochemical detection, Au electrode |
| Waveform: | Quadruple potential |

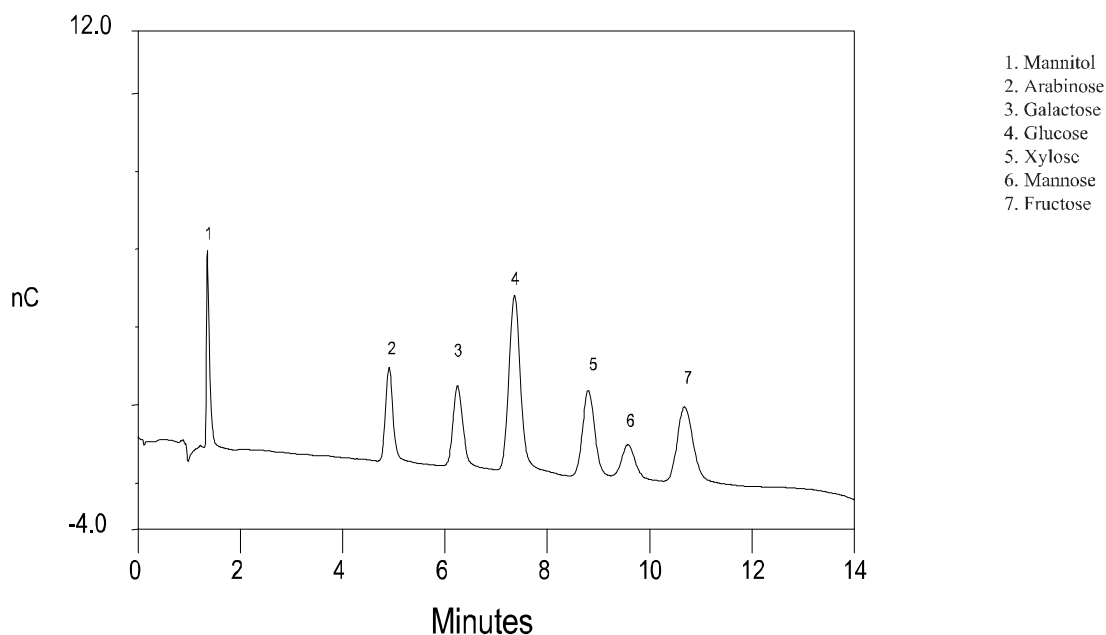


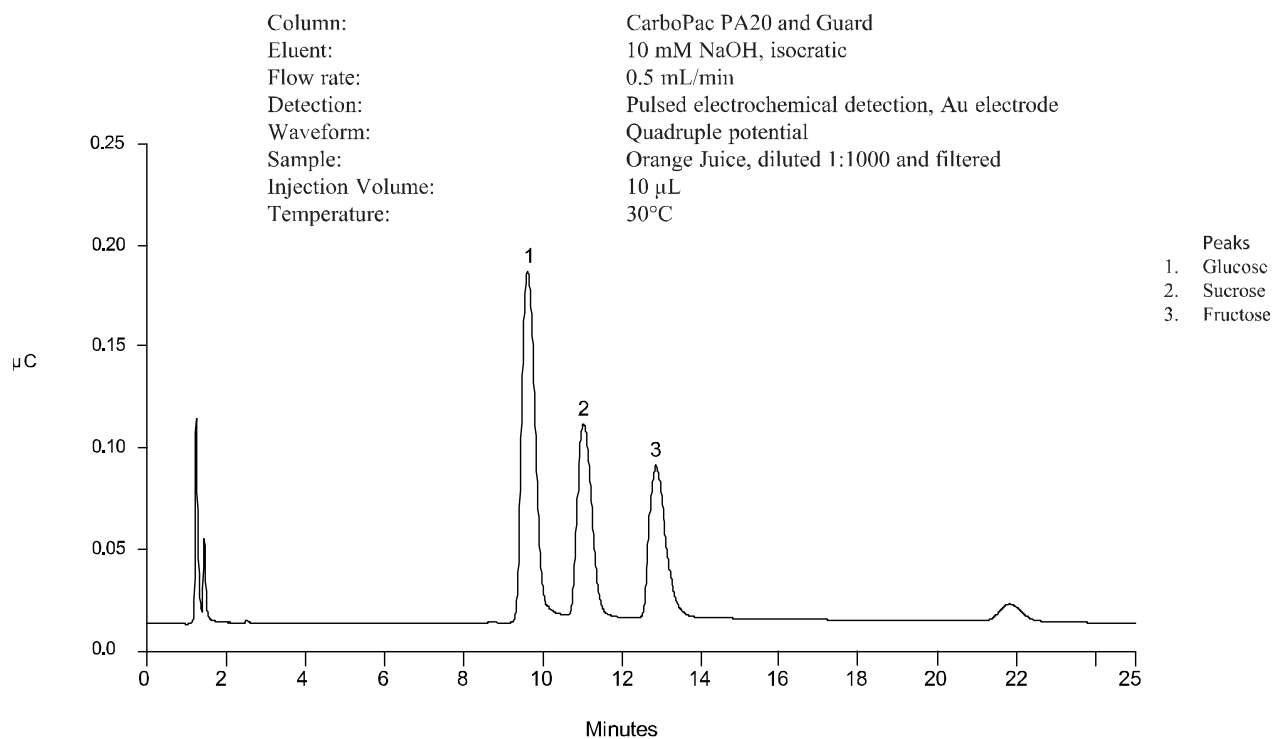
Figure 6 Analysis of Orange Juice using the CarboPac PA20

Figure 7 Separation of Monosaccharides Important in Dietary Fiber Analysis

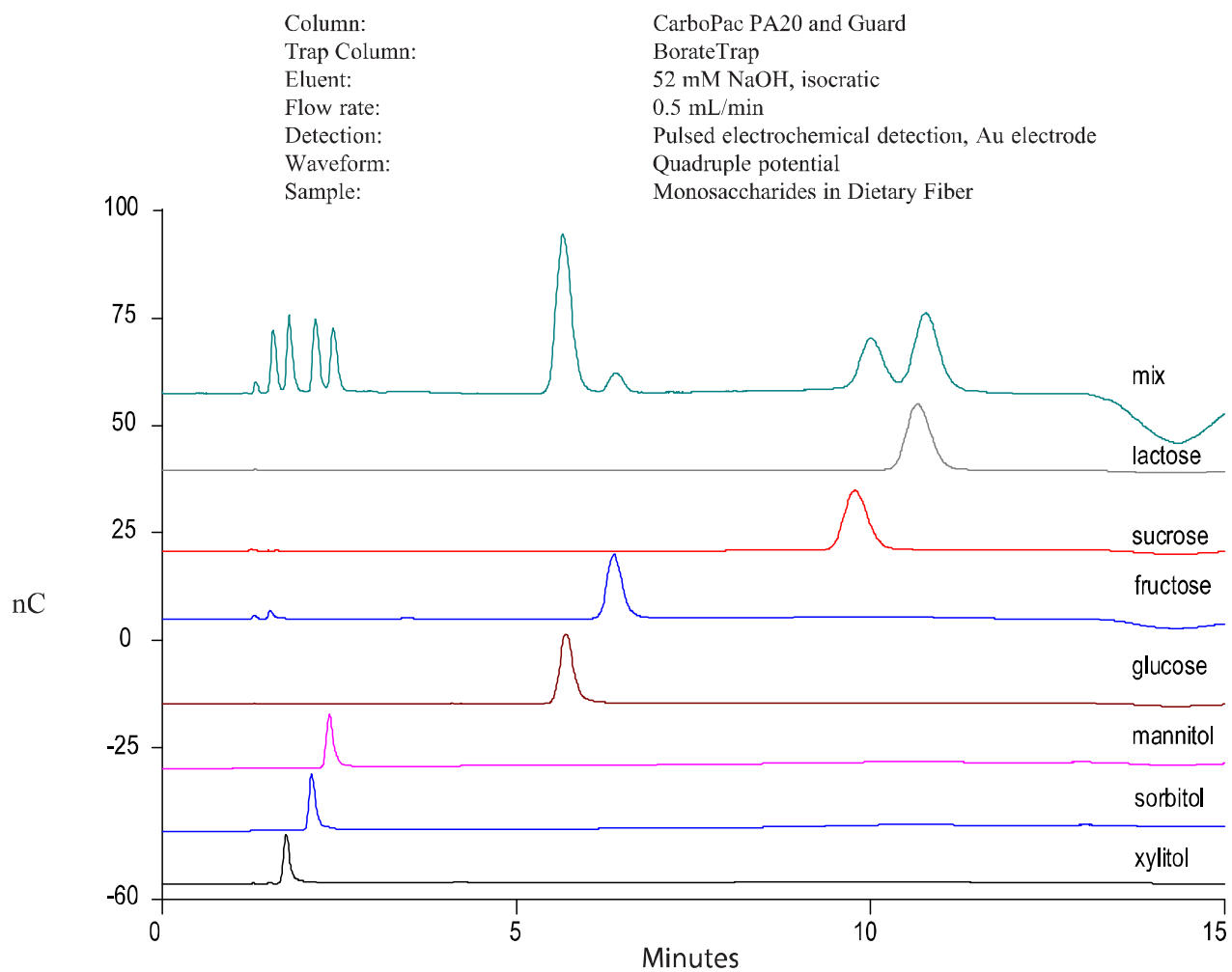


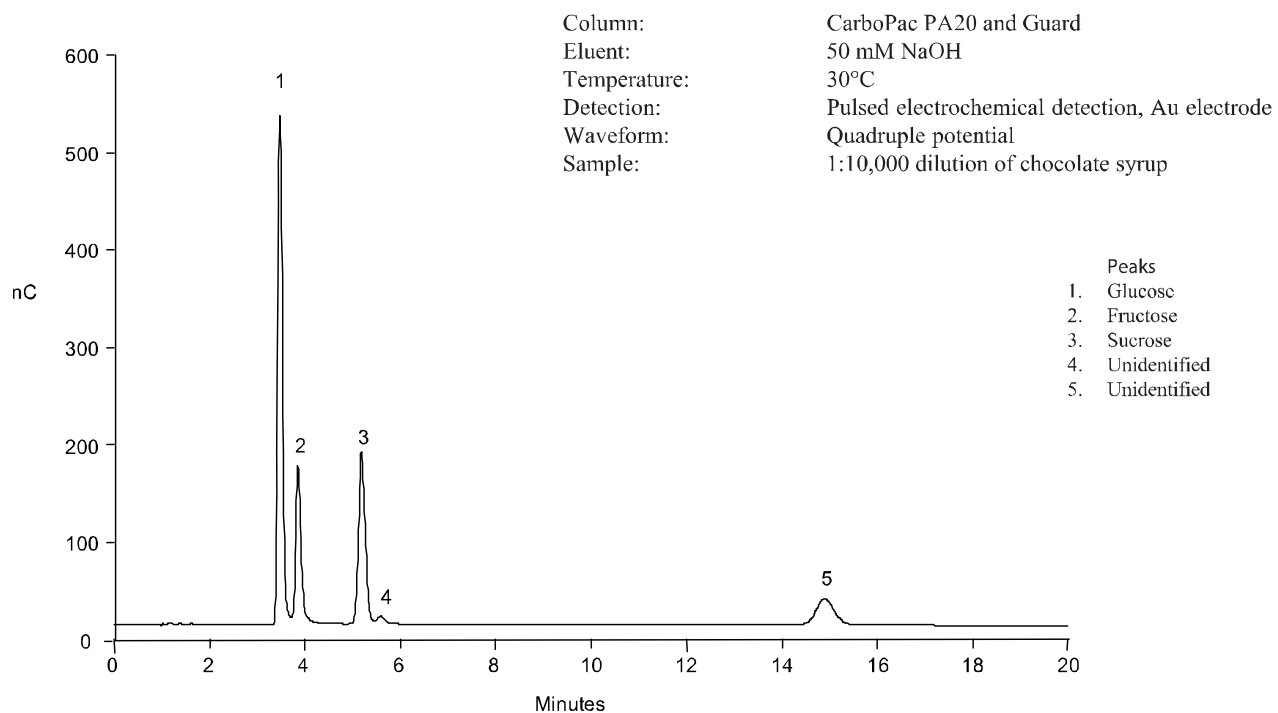
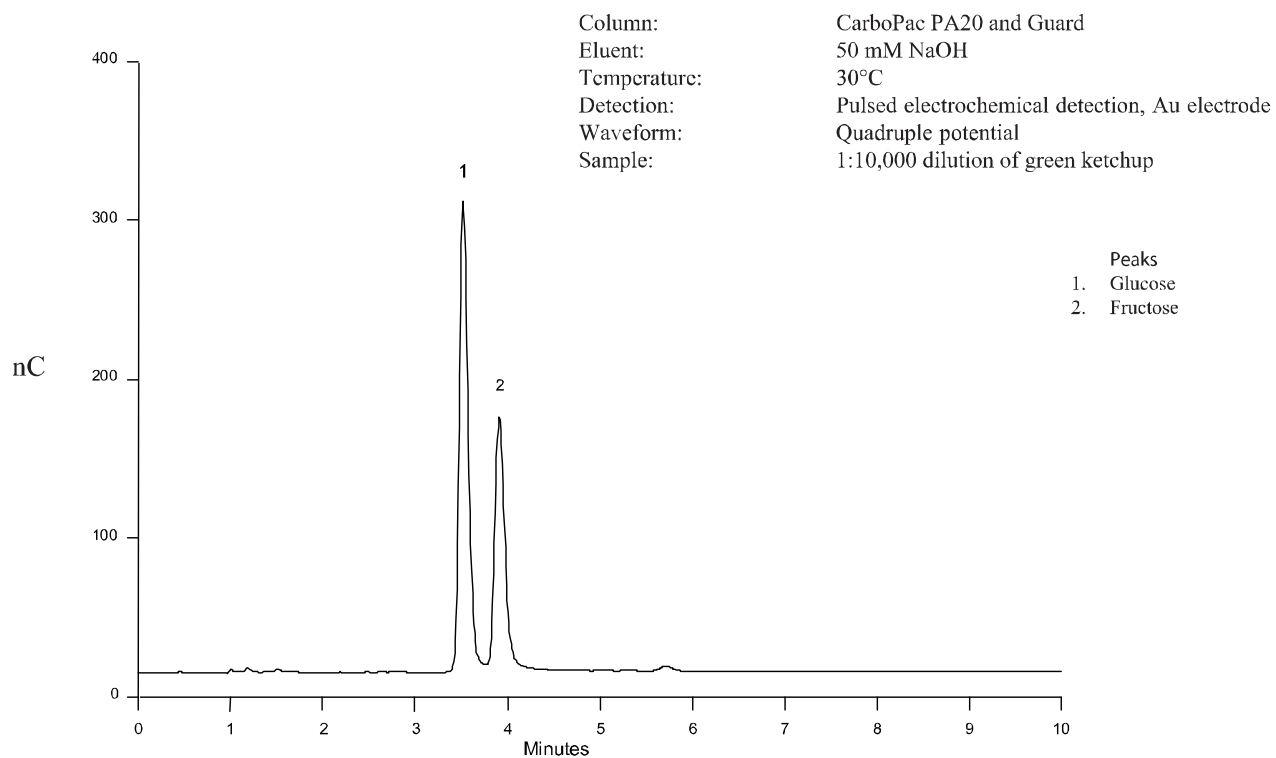
Figure 8 Analysis of Chocolate Syrup using the CarboPac PA20

Figure 9 Analysis of Green Ketchup using the CarboPac PA20



6.6 Carbohydrates in Bio- and Pharmaceutical Products and Processes

The United States Food and Drug Administration (FDA) and the regulatory agencies in other countries require that pharmaceutical products be tested for composition to verify their identity, strength, quality and purity. Recently, attention has been given to inactive ingredients as well as active ingredients. Many of the ingredients are non-chromophoric and cannot be visualized by absorbance detection. However, carbohydrates, glycols, sugar alcohols and sulfur-containing compounds can be oxidized and therefore detected by amperometric detection.

Another area of great interest to the pharmaceutical chemist is the optimization of fermentation broths. If the correct nutrients and amino acids for a given culture are determined, and monitored, so that the correct concentrations are always maintained, then the culture has the best chance of producing the optimum yield. The CarboPac PA20 can be used on-line to monitor fermentation broths and cell cultures. Alternatively, if there is more interest in monitoring the amino acid content, then the AminoPac PA 10 (Item# 055406) should be considered for direct detection, on-line.

The following conditions should be used as guidance. These may be modified, as necessary, to suit your particular application needs.

Figure 10 Analysis of Yeast Extract-Peptide-Dextrose (YPD) Broth Supernatant

| | |
|--------------|---|
| Column: | CarboPac PA20 and Guard |
| Eluent: | 10 mM NaOH followed by 200 mM NaOH for 10 min to regenerate the column. |
| Temperature: | 30°C |
| Detection: | Pulsed electrochemical detection, Au electrode |
| Waveform: | Quadruple potential |
| Sample: | 1:1,000 dilution of YPD broth supernatant |

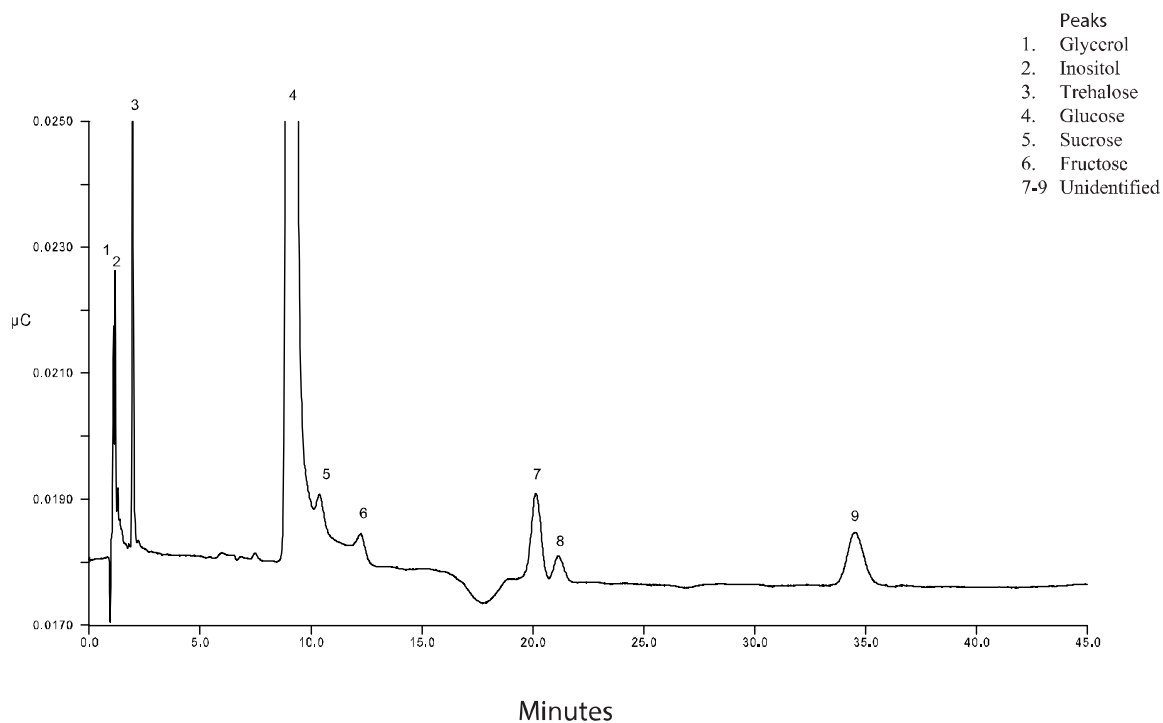


Figure 11 Analysis of a Pediatric Oral Decongestant using the CarboPac PA20

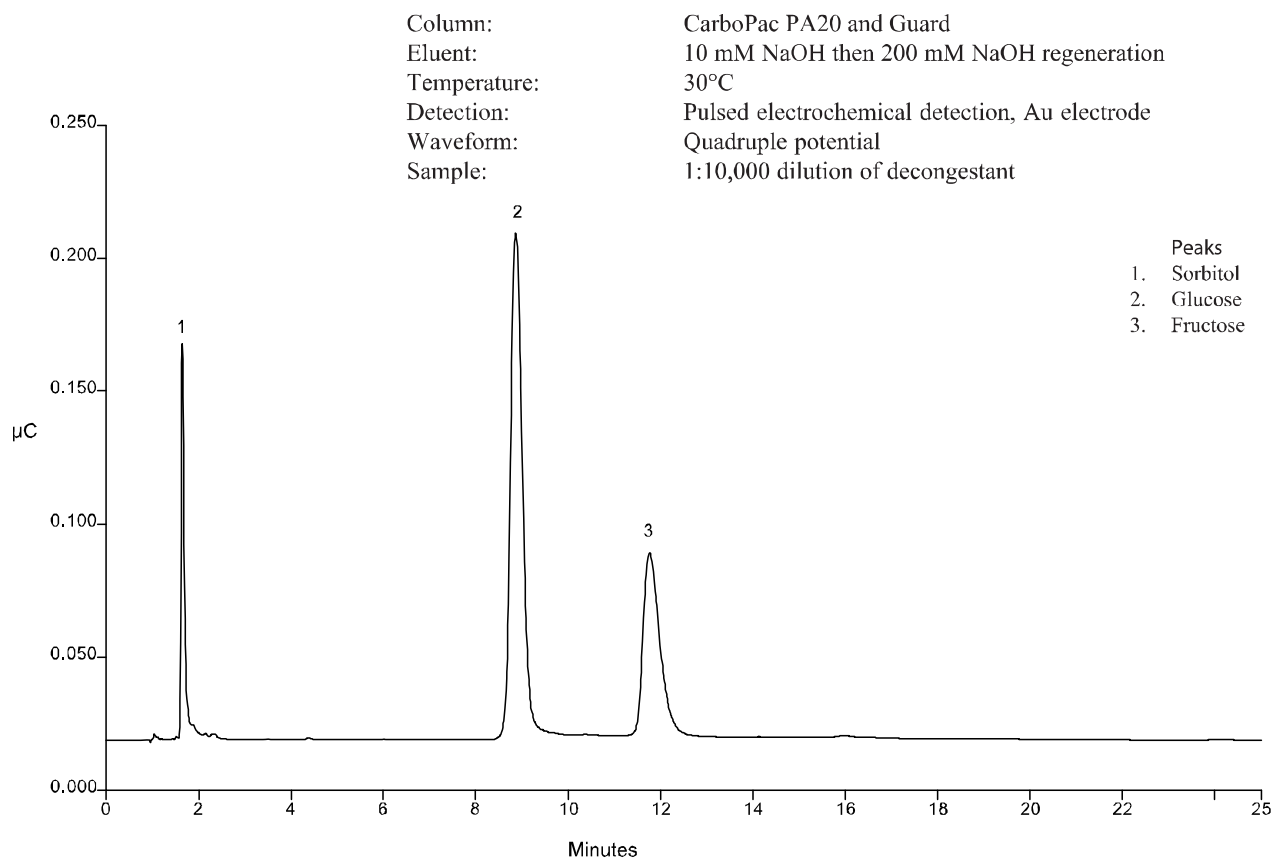
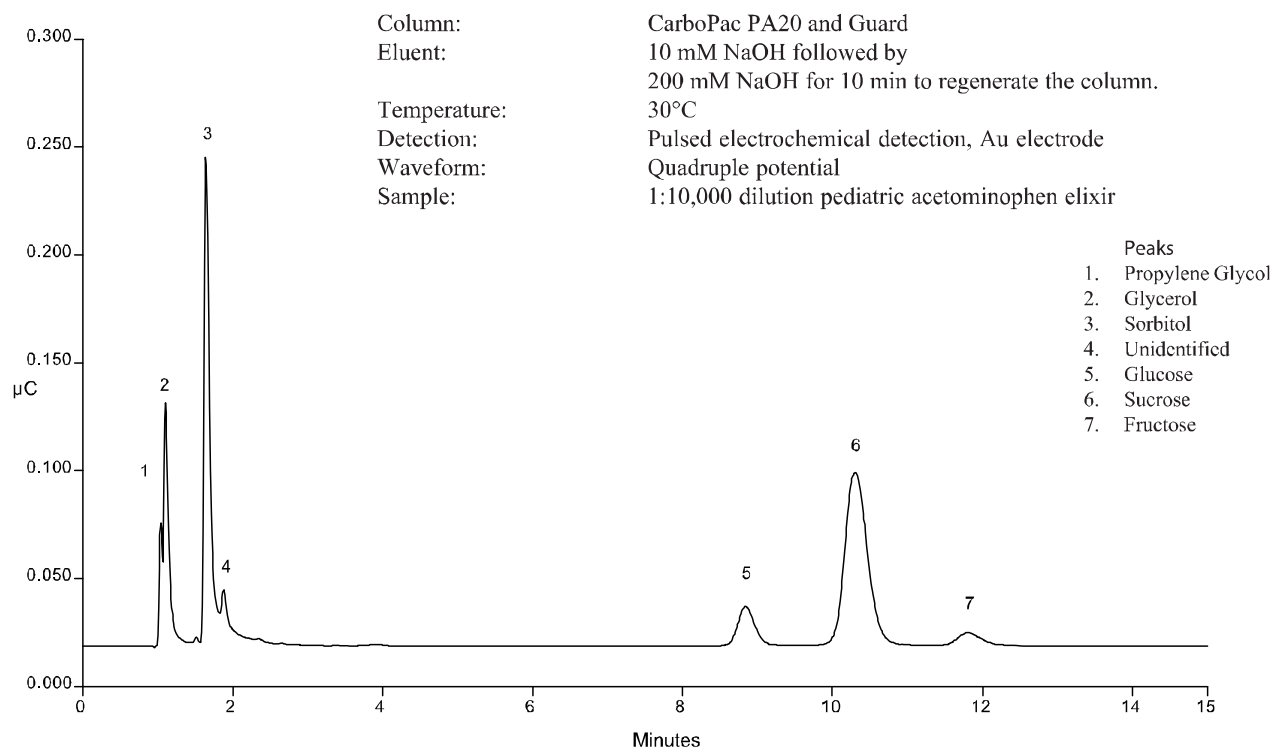


Figure 12 Analysis of a Pediatric Acetaminophen Elixir



7. Troubleshooting Guide

Remember that some of the problems may be related to parts of your experimental protocol (sample contamination, imprecision during sample transfer, problems during peptide or protein hydrolysis, etc.). The following text should help you to locate and eliminate problems traceable to the carbohydrate hardware and chemistries. It also provides a selection of cleanup and reconditioning procedures that have been found effective by many users.

7.1 High Background

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

A background >30 nC with 10 mM sodium hydroxide at 0.5 mL/min and 30°C using the quadrupole waveform indicates one of the following possibilities:

- A. Incorrect detection parameters.
Verify that Ag/AgCl is specified as a reference electrode. Check all values of waveform in program against those in the Disposable Electrode Manual. If the pH reading with 10 mM NaOH or KOH is above 13.2 replace the reference electrode.
- B. Compromised working electrode surface.
Briefly install a new working electrode and check the background as above. If the reading remains >30 nC, remove the new electrode within 30 minutes and continue testing for column or system contamination. Otherwise continue with your work with the new electrode installed.
- C. Column contamination.
Remove the column set from the system first and replace it with a length of yellow PEEK tubing, generating a pressure drop between 1000 and 2000 psi. If the background reading improves after the column is removed from the system, go to [Section 7.3](#).
- D. Water contamination.
Prepare eluents using a fresh ultra-pure water from another source. If the background is reduced, investigate the source of contamination in the original source of water.
- E. System contamination.
If the background remains high even with fresh water and without the column, carry out the 2M sodium hydroxide rinse described in [Section 7.5](#).

7.2 Decreased Detection Sensitivity

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

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

Exception:

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

After installing a new working electrode (with or without the complete system cleanup), confirm the normal detection sensitivity. Carry out a test with a reference standard. Should the response be too low, immediately remove the new working electrode from the system.

7.3 Column Problems

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

The column set is causing the high background if the background reading decreases after the column is replaced by a section of PEEK tubing, as described in [Section 5.5.1](#).

7.3.1 Excessive Gradient Rise

The magnitude of the gradient rise can be minimized by running high eluent strengths during the times when the system is not in use for sample or standard analysis. This will keep the column conditioned, free from carbonate buildup, and ready for analysis.

- A. Make sure the gradient rise is not caused by the system and/or detector cell.
- B. Increase column temperature to 40°C and wash the guard and column with 200mM NaOH or KOH for at least four hours (preferably overnight). Run a blank gradient at 30°C and if necessary repeat the clean-up with 100mM NaOH, 950 mM sodium acetate wash at 40°C .

7.3.2 Peak Efficiency and Resolution are Decreasing

Always have a spare Guard available. Peak deformations may sometimes be caused by sample matrix.

- A. Run a standard separation with the Guard column removed from the system. Install a new Guard column should the separation improve with the old Guard removed. It is common to replace Guard columns several times during the lifetime of an analytical column.
- B. Verify that correct tubing is installed for all connections between injector and detector, see [Section 2.1.3](#).
- C. Check for proper installation of ferrules on all PEEK tubing starting with the injector outlet and all other connectors to the ED cell inlet.
- D. Check temperature settings in your method and/or actual temperature in your column oven.
- E. The column may be overloaded. Try to inject a smaller amount of your sample or dilute the sample more.
- F. If all of the above does not lead to an improved separation, the resin bed of the main column has been damaged and the main column must be replaced.

7.4 System Problems

7.4.1 High Detection Background Caused by the System

- A. Verify the problem is neither the detector nor column related.
- B. Prepare new eluents.
- C. Rinse all three eluent lines with the new eluents.

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

- A. Check the position and filling levels of sample vials in the autosampler.
- B. Check injector needle-height setting.
- C. Check each line of the schedule for proper injector parameters. Revert to full loop and column appropriate sample loop size.
- D. Service the injection valve (check for leaks, Tefzel fragments, or sediments inside the valve).

7.4.3 Large Baseline Dip in the Chromatogram

A large baseline dip appearing between 17 and 19 minutes when the guard column is installed is usually caused by oxygen in the sample injected. The 'oxygen dip' is normal and can be reduced in magnitude with higher NaOH concentration in the eluent.

7.4.4 Incorrect or Variable Retention Times

- A. Check your eluent preparation procedure for possible errors.
- B. Prime the pump if necessary. Set the eluent composition for 100% for each eluent and draw out at least 40 mL of eluent from each of the lines.
- C. Wash the column with 200mM KOH or NaOH for two hours or longer to eliminate the excessive carbonate contamination of the column when ran with 18mM KOH or NaOH for extended period of time.
- D. Verify if the equilibration time after the strong wash is optimum by increasing the equilibration time.
- E. Measure the flow rate by weighing out the eluent collected during exactly five minutes of flow. Recalibrate the pump if necessary.
- F. Samples containing high salt content (> 50 mM) will decrease the retention times.
- G. Check and/or service the pump's proportioning valve. With the pumping turned off, the flow through the pump outlet tubing (disconnected from the injector) should be zero in all eluent positions. Check this separately for each eluent line at 100% setting.

7.4.5 Unidentified Peaks Appear with Expected Analyte Peaks

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

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

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

7.5 Sodium Hydroxide Cleanup

The sodium hydroxide (200mM NaOH or KOH) rinse used to decrease column or system-related elevated background is essentially identical with the rinse performed during an installation of a new system. Following the rinse, check the background again while pumping the 10 mM sodium hydroxide and repeat the rinse at least once more if necessary. Leave the old gold working electrode in place during the first and second checking of the detection background. Use a new or reconditioned electrode only if the background remains high even after the second rinse. Should the new electrode also produce a reading of > 30 nC, remove it from the system within 30 minutes, rinse it with water and reinstall the old electrode.

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

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

8. Column Care

8.1 Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for Dionex CarboPac PA20 column is 3,500 psi (24.12MPa).

8.2 Column Start-Up

The Dionex CarboPac columns are shipped using 10mM sodium hydroxide (see QAR) as the storage solution. Use Dionex EGC-500 KOH cartridge to generate the eluent employed in the Quality Assurance Report (QAR). Install the column in the chromatography module and direct the column effluent to waste for 60 minutes, and then connect to the ED cell. It is recommended to clean the column for 1 to 2 hours with 200mM KOH at 0.008mL/min 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.

| | |
|------------------|--|
| IMPORTANT | <i>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.</i> |
|------------------|--|

8.3 Column Storage

For short-term storage (< 1 week), the QAR Eluent is acceptable, for long-term storage (> 1 week), employ the storage solution described on the QAR. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

8.4 Column Cleanup

The Dionex CarboPac PA20 can be readily cleaned by rinsing the column with ~ 60 column volumes of 200 mM KOH. More stubborn contamination problems may necessitate a thorough column cleaning. Use the following steps to thoroughly clean the column; use appropriate flow rate to avoid over-pressurization of the column:

| | |
|------------------|--|
| IMPORTANT | <i>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.</i> |
|------------------|--|

- A. Clean the Dionex CarboPac PA20 column with 1M NaOH for at least two hours.
- B. Reconnect column to the cell and equilibrate the column with the desired initial conditions; test the column performance using the QAR standard and eluent.