

Thermo Scientific™ Dionex™ CarboPac™ PA20 1 mm columns for ICS-6000 systems in the Dual EGC mode

Improved reproducibility and accuracy for the analysis of simple sugars

Keywords: Monosaccharides, disaccharides, carbohydrate, sialic acids, high performance, anion exchange chromatography with pulsed amperometric detection, potassium hydroxide, methanesulfonic acid, dual eluent generator cartridge

Benefits

- Predictable, high-resolution, separation of monosaccharides, disaccharides and some oligosaccharides using electrolytically generated potassium methanesulfonate and potassium hydroxide
- Rugged all-purpose column to determine monosaccharides and disaccharides in a variety of samples
- Column chemistry has USP designation L69 and is used in the USP's Heparin Sodium monograph and General Chapter <210> Monosaccharide Analysis

The [Thermo Scientific™ Dionex™ CarboPac™ PA20 1 mm column](#) is a specialized anion-exchange column designed to be used with high performance anion exchange chromatography with pulsed amperometric detection



(HPAE-PAD) to deliver high resolution separations of simple sugars. Electrochemical detection is used to measure the current resulting from oxidation of the carbohydrate at the surface of a working electrode. During oxidation reactions, electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the electrochemical cell. Detection is sensitive and highly selective for electroactive species, because many potentially interfering species cannot be oxidized or reduced and are not detected. Pulsed amperometry, which uses a repeating sequence of potentials, is a reproducible and sensitive detection method for carbohydrates. The Dionex CarboPac PA20 1 mm column is recommended for [Dual Eluent Generation Cartridge \(Dual EGC\)](#) mode applications using a [Thermo Scientific™ Dionex™ ICS-6000 HPIC™ System](#).

Predictable, high-resolution, separation of carbohydrates

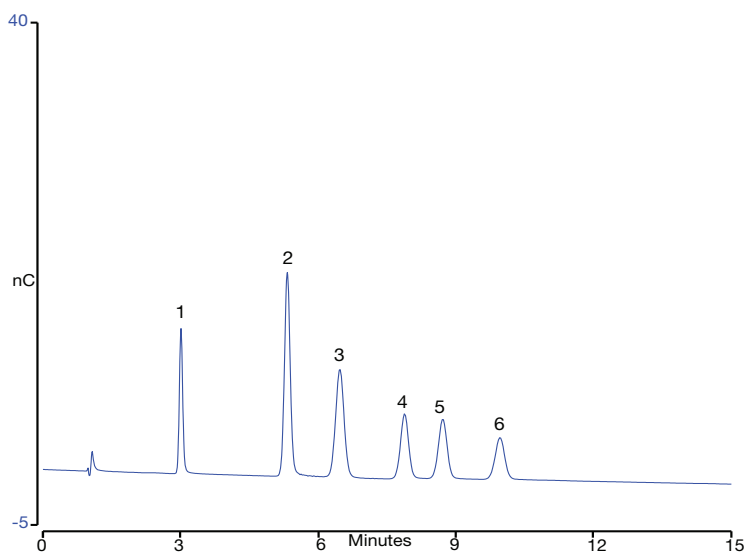
The Dionex CarboPac PA20 1 mm columns are designed primarily for use with the Dionex ICS-6000 HPIC system in Dual EGC mode. The chromatogram below shows a separation of six monosaccharides using a Dionex CarboPac PA20 1 mm column with a guard column on the Dionex ICS-6000 HPIC system in dual EGC mode. The figure shows that the column provides excellent resolution between the six underivatized monosaccharides commonly found in mammalian glycoproteins when using the dual EGC mode to electrolytically generate potassium hydroxide to separate the monosaccharides and potassium

methanesulfonate and potassium hydroxide to clean the column when analyzing samples containing amino acids, peptides, and other strongly anionic species.

Food applications

Determination of common sugars in orange juice

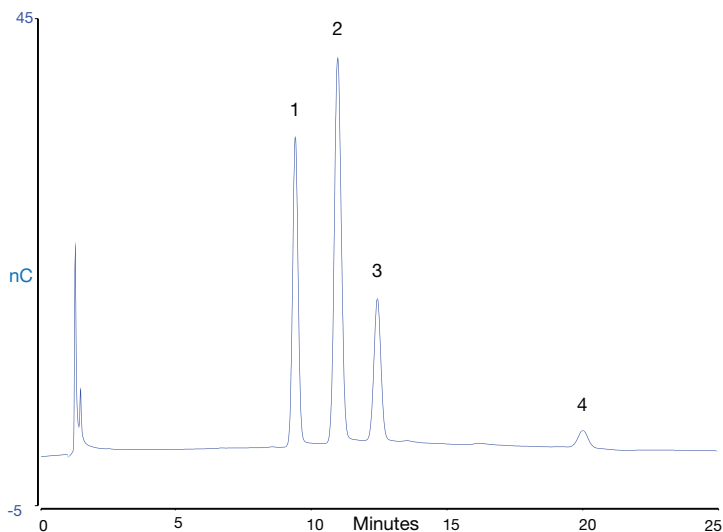
The Dionex CarboPac PA20 1 mm column does an excellent job for the determination of common sugars in orange juice samples (Figure 2). Note that due to the high sensitivity of PAD, the orange juice sample was diluted a thousand-fold with deionized water to avoid overloading the column and/or the detector. This separation also features a regeneration step to ensure retention time reproducibility when analyzing food samples.



Column: See Chromatogram
 Eluent source: Dionex Dual EGC -400 KOH / MSA cartridge
 Program profile: Regen: 100 mM KMSA and 100mM KOH for 5 min
 200mM KOH for 15 min
 EQ: 10 mM KOH for 25 min prior to injection
 Inject: 10 mM KOH for 15 min
 Flow rate: 0.056 mL/min
 Inj. volume: 0.4µL
 Gasket: 1 mil
 Temperature: 30°C
 Detection: Integrated amperometry, quadruple pulse waveform
 Working electrode: PTFE gold, disposable electrode
 Reference electrode: Silver /silver chloride

Peaks:	pmoles (Injected)
1. Fucose	4.0
2. Galactosamine	4.0
3. Glucosamine	4.0
4. Galactose	4.0
5. Glucose	4.0
6. Mannose	4.0

Figure 1. Separation of six monosaccharides using the Dionex CarboPac PA20 1 mm column with the Dionex ICS-6000 HPIC system in Dual EGC mode.



Column: Dionex CarboPac PA20 1mm Analytical + Guard Column
 Eluent source: Dionex Dual EGC-400 KOH / MSA cartridge
 Program profile: Regen:100 mM KMSA and 100 mM KOH for 5 min
 200 mM KOH for 15 min
 EQ: 10 mM KOH for 20 min prior to injection
 Inject: 10 mM KOH for 25 min
 Flow rate: 0.056 mL/min
 Inj. volume: 0.4 µL
 Gasket: 1 mil
 Temperature: 30 °C
 Detection: Integrated amperometry, quadruple pulse waveform
 Working electrode: PTFE Gold, disposable electrode
 Reference electrode: Silver/silver chloride
 Sample: Orange juice (Dilution 1:1000)

Peaks
1. Glucose
2. Sucrose
3. Fructose
4. Unknown

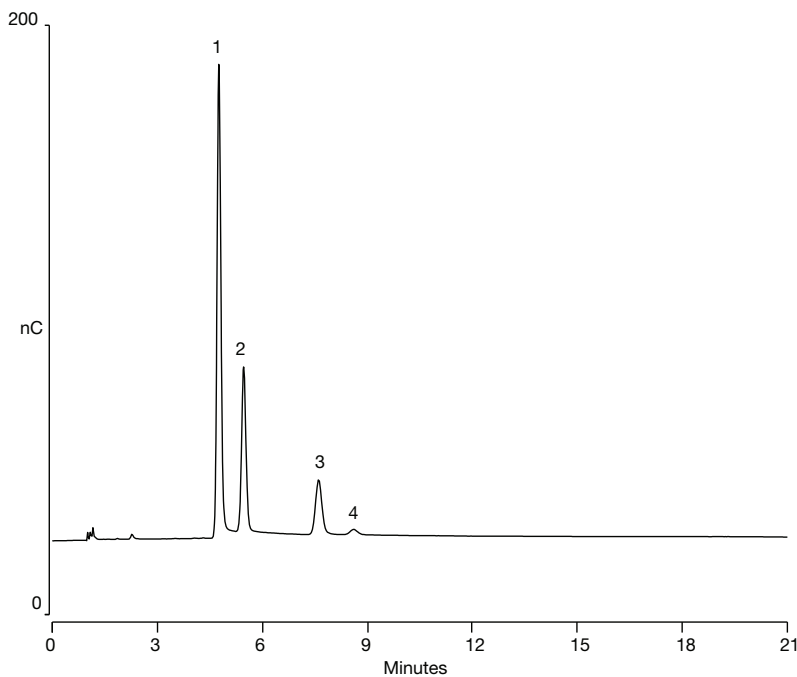
Figure 2. Separation of carbohydrates in orange juice using the Dionex CarboPac PA20 1 mm column with the Dionex ICS-6000 HPIC system in Dual EGC mode.

Determination of sugars in chocolate syrup

Chocolate has been traditionally used as a flavoring ingredient in various food products such as cakes, ice creams, and milk shakes. Chocolate syrup also known as chocolate-flavored condiments, is a concentrated solution of chocolate primarily used as a topping or dessert sauce in various desserts. Many of these chocolate products are analyzed for their sugar content to precisely control the quality of the final product. Figure 3 shows the separation of glucose, fructose, and sucrose from chocolate syrup in less than 10 min using electrolytically generated potassium methanesulfonate and potassium hydroxide. Like the previous examples, the carbohydrates are separated with potassium hydroxide and the column is cleaned with a combination of potassium hydroxide and potassium methanesulfonate.

Sialic acid analysis

Sialic acids are a family of N- and O-substituted neuraminic acids that play an important role in physiology. Sialic acid can serve a critical role in mediating the effectiveness of recombinant therapeutic glycoproteins, and sialylation must be controlled and monitored during the manufacturing process. The presence or absence of sialic acid on the non-reducing terminal of glycans can affect the pharmacokinetics of the protein, as well as, its potential immunogenicity. Sialic acids 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 an N-glycolyl group. These yield N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc), respectively. Figure 4 shows the separation of Neu5Ac and Neu5Gc in a standard. Using electrolytically generated potassium methanesulfonate and potassium hydroxide gradients, Neu5Ac and Neu5Gc are well resolved in 12 min.



Column: Dionex CarboPac PA20 Analytical + Guard Column
Eluent source: Dionex Dual EGC-400 KOH / MSA cartridge
Program profile: Regen: 100 mM KMSA and 100mM KOH for 5 min
200mM KOH for 15 min
EQ: 50 mM KOH for 20 min prior to injection
Inject: 50 mM KOH for 15 min
Flow rate: 0.056 mL/min
Inj. volume: 0.4µL
Gasket: 1 mil
Temperature: 30°C
Detection: Integrated amperometry, quadruple pulse waveform
Working electrode: PTFE gold, disposable electrode
Reference electrode: Silver/silver chloride
Sample: Chocolate syrup, (Dilution 1:10,000)

Peaks
1. Glucose
2. Sucrose
3. Fructose
4. Unknown

Figure 3. Separation of carbohydrates in chocolate syrup using the Dionex CarboPac PA20 1 mm column with the Dionex ICS-6000 HPIC system in Dual EGC mode.

Column: Dionex CarboPac PA20 1mm Analytical/Guard Column
 Eluent: 0-5 min: 25-90 mM KMSA in 80 mM KOH
 5-10 min: 90 mM KMSA in 80 mM KOH
 10-25 min: 25 mM KMSA in 80 mM KOH
 Flow rate: 0.056 mL/min
 Inj. volume: 0.4 µL
 Gasket: 1 mil
 Temperature: 30°C
 Detection: Integrated amperometry, quadruple pulse waveform
 Working electrode: PTFE gold, disposable electrode
 Reference electrode: Silver/silver chloride
 Sample: Sialic acid standard, (Dilution TBD)

Peaks: pmoles injected
 1. Neu5Ac 2
 2. Neu5Gc 1

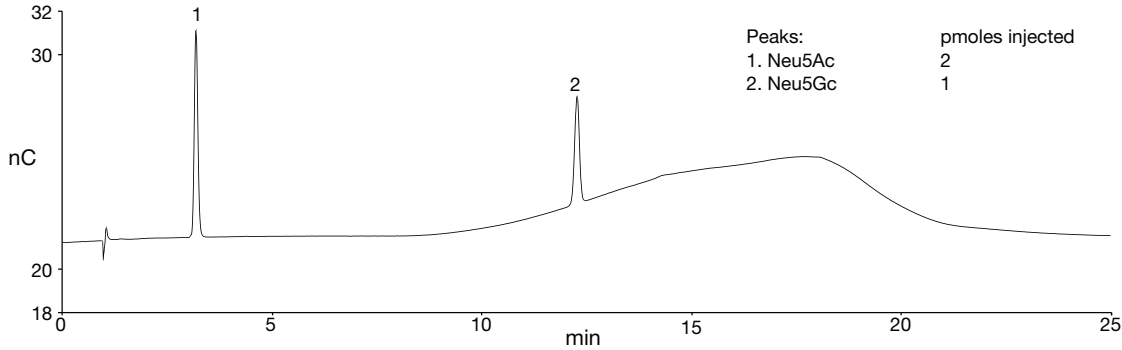


Figure 4. Separation of a sialic acid standard using the Dionex CarboPac PA20 1 mm column with the Dionex ICS-6000 HPIC system in Dual EGC mode.

Exceptional performance

The Dionex CarboPac PA20 1 mm column is a specialized anion-exchange column designed to be used with PAD to deliver high resolution separations of mono-, di-saccharides, both neutral and charged, and sialic acids. The resin consists of 5.5 µm diameter nonporous beads covered with a fine latex of functionalized

Thermo Scientific™ Dionex™ MicroBead™ resin. This pellicular resin structure permits excellent mass transfer, resulting in high resolution chromatography and rapid re-equilibration. The resulting polymer has a pH tolerance range from 0-14 and is 100% compatible with common organic solvents used in HPLC.

Specifications

Resin compositions	5.5 µm Diameter Substrate (Ethylvinylbenzene 55% Crosslinked with Divinylbenzene) Agglomerated with 240 nm MicroBead Difunctional Quaternary Ammonium Ion (5% Crosslinked)
Anion exchange capacity	7.2 µeq/column
pH range	0-14
Temperature range	4 °C – 60 °C
Organic solvent limit	100% compatible with common HPLC solvents (methanol, acetonitrile) for the columns
Typical eluents	Potassium hydroxide, Potassium methanesulfonate
Recommended flow rate	0.056 mL/min
Maximum pressure	5000 (34500 kPa)

Ordering information

Description	Part Number
Thermo Scientific™ Dionex™ CarboPac PA20 1 mm Analytical Column (1 x 150 mm)	303369
Thermo Scientific™ Dionex™ CarboPac PA20 1 mm Guard Column (1 x 30 mm)	303370
Thermo Scientific™ Dionex™ EGC 400 KOH Eluent Generator Cartridge	302766
Thermo Scientific™ Dionex™ EGC 400 MSA Eluent Generator Cartridge	302767
Thermo Scientific™ Dionex™ Gold on PTFE Disposable Electrode with 1 mil gasket kit for 1 mm and capillary applications (6 pack)	303397
Thermo Scientific™ Dionex™ Ag/AgCl pH Reference Electrode	061879
Thermo Scientific™ Dionex™ Palladium Hydrogen Reference Electrode	072075
Thermo Scientific™ Dionex™ O-ring for PdH Reference Electrode for analytical format (included in 072075)	030839
Thermo Scientific™ Dionex™ Gasket for PdH Reference Electrode for capillary format (included in 072075)	072214

Find out more at thermofisher.com/complexcarbs

For Research Use Only. Not for use in diagnostic procedures. © 2021 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representatives for details. **PS73869-EN 0221S**