Simplify carbohydrate analysis

Faster, easier, and more reliable carbohydrate analysis with Thermo Scientific Dionex CarboPac 1 mm format ion chromatography columns and Dual Eluent Generation

Carbohydrate analysis doesn't have to be complex

Carbohydrate analysis is an essential requirement for many food and beverage laboratories; it forms the basis of tests needed for food labeling and profiling for authenticity. It is also becoming increasingly important in biopharmaceutical laboratories; fast and effective glycoprotein testing is essential for the immunity screening required for growing vaccine, cell and gene therapy markets.

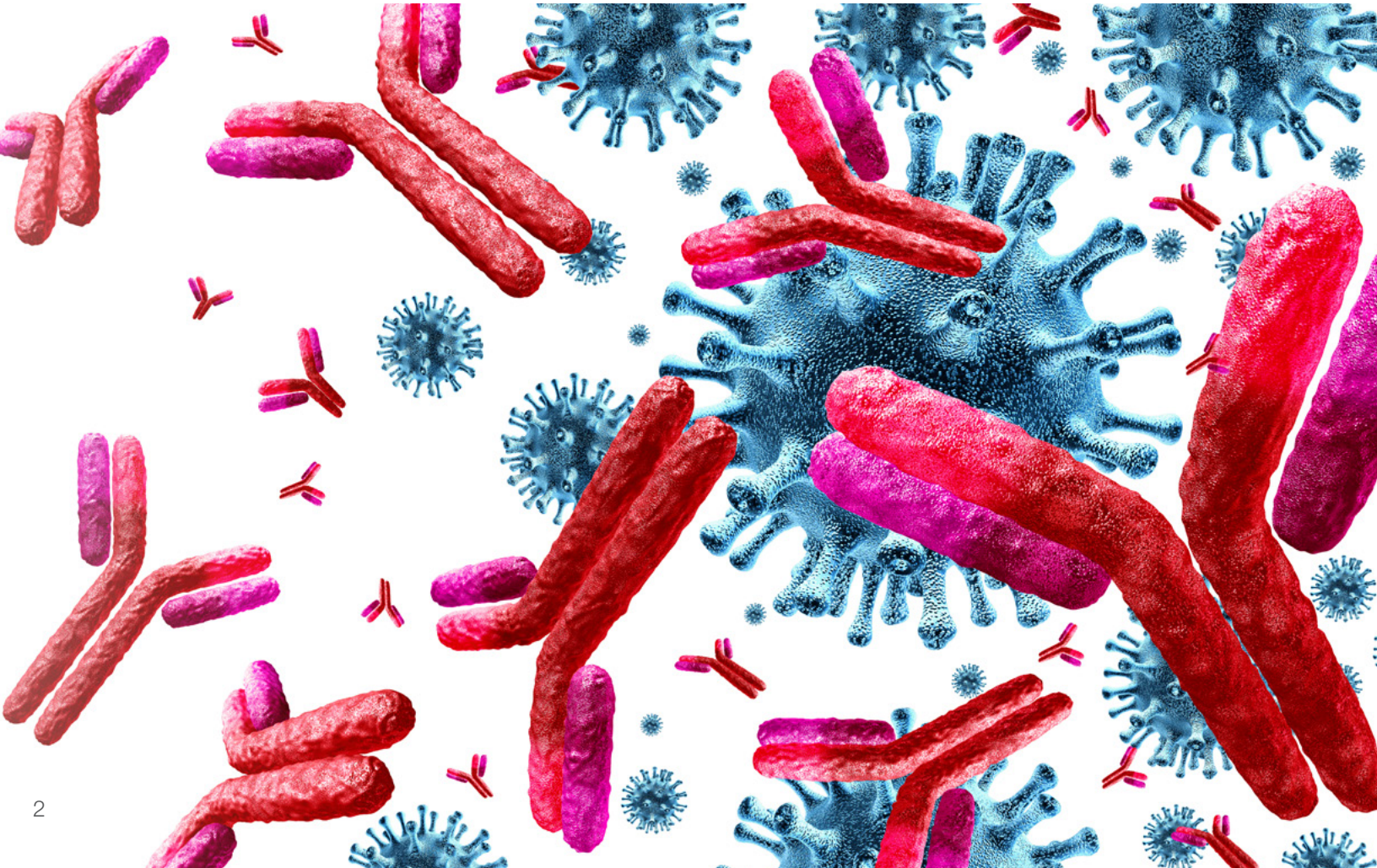
Traditionally, carbohydrate analysis has relied on complex and highly manual techniques, including:

- **Complex derivatization** – with the addition of detectable groups, such as chromophores or fluorophores.
- **Time-consuming and manual eluent preparation** – with chemicals that are prone to quality and variability issues, which can cause carbonate formation and lowered pH.
- **The use of eluents with high pH** – leading to corrosion of metal-based chromatographic flow paths.
- **Complex and manual eluent preparation** – meaning poor method transfer, even between analysts in the same team.

These traditional techniques can cause serious problems in analysis workflows, leading to poor retention and peak resolution. In turn, this can result in poorly reproducible results and high chemical waste streams. With the creation of high pH eluents, long maintenance protocols are needed to flush and clean columns, which lead to equipment downtime or risk of corrosion.

But there is another way

The Thermo Scientific™ Dionex™ ICS-6000 high-pressure ion chromatography (HPIC) system simplifies carbohydrate analysis with its Thermo Scientific™ Dionex™ CarboPac™ 1 mm format IC columns and automated Dual Eluent Generation Cartridge (EGC).



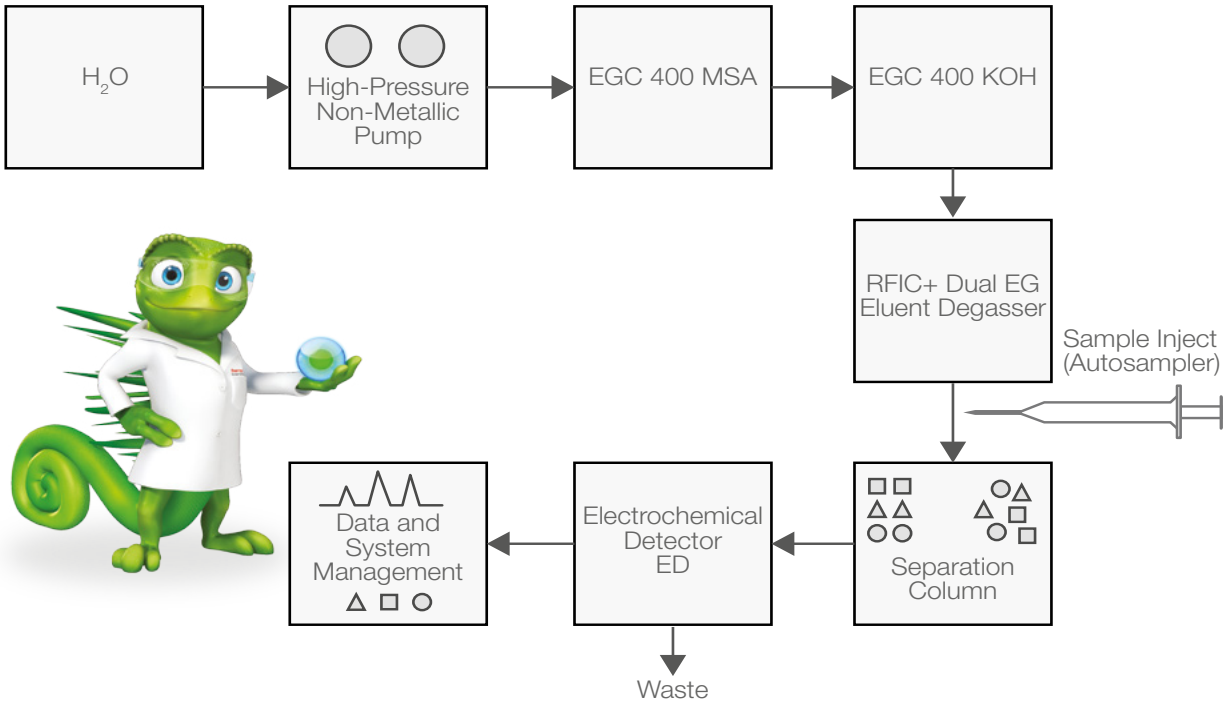
Automated eluent production: The key to simplified carbohydrate analysis

Most issues related to traditional carbohydrate analysis lie within the eluent production stage. Since complex oligo- and polysaccharides are weak acids, they require an eluent with an extremely basic pH to better ionize the carbohydrates and sugar alcohols.

The most common forms of eluent creation involve combining sodium or potassium hydroxide and acetate; however, the quality of these chemicals can be highly variable, and this affects the resulting quality of the eluent. Since hydroxide readily absorbs carbon dioxide, carbonates are easily formed, lowering the pH and causing issues with reproducibility.

Used as part of the Dionex ICS-6000 HPIC system with the CarboPac 1 mm IC columns, Dual EG cartridges automatically combine purified potassium hydroxide with methanesulfonate to create accurate eluents with exact concentrations. The analyst simply installs the cartridges within the Dionex ICS-6000 HPIC system and adds 18.2 MΩ-cm deionized water. The system software electrolytically controls the exact eluent concentrations, even adjusting the concentration gradient during analysis, if needed.

Dionex ICS-6000 HPIC system — complete with system software and touchscreen controls.



The Dionex ICS-6000 system configuration under the Dual EGC mode.

Reproducibility and reliability assured

The Dual EG cartridge is designed specifically for the CarboPac PA1 and PA20 1 mm format IC columns.

CarboPac PA1 columns

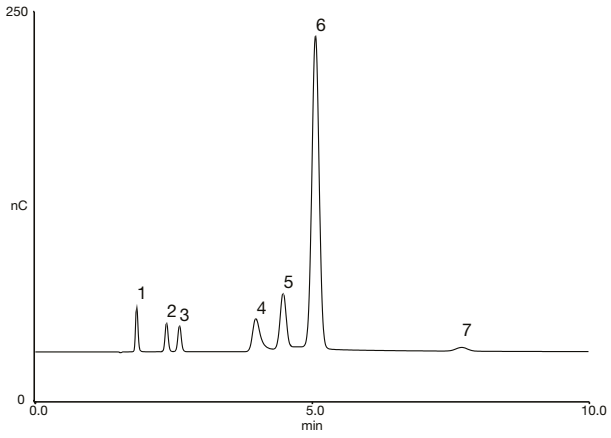
The CarboPac PA1 column is a rugged, all-purpose 1 mm diameter column designed to measure mono-, di- and oligosaccharides in a variety of complex matrices, especially in food and beverage analysis.

CarboPac PA20 columns

The CarboPac PA20 column is a highly sensitive 1 mm diameter column designed to measure mono-, di- and oligosaccharides, including glycoproteins, in biopharmaceutical workflows and advanced food and beverage analysis.

In both CarboPac columns, the 1 mm diameter ensures a low flow rate, giving similar chromatography profiles to different formats of CarboPac columns, but with the added benefit of a cleaner background from using cleaner eluent. This enables even trace levels of complex carbohydrates to be detected without derivatization and increases the overall sensitivity of the analysis.

When these highly effective pellicular resin columns are combined with the automated and electrolytically controlled Dual EGC, the eluent concentration is precisely defined. This translates into reliable and reproducible results.



Column:	Dionex CarboPac PA1 1mm analytical/guard column
Eluent:	150 mM KOH/0 mM MSA
Eluent source:	Dionex EGC 400 KOH cartridge
Flow rate:	0.063 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:	Ag/AgCl
Sample:	Agave syrup standard

Peaks:	mg/L
1. Inositol	2.5
2. Sorbitol	2.5
3. Mannitol	2.5
4. HMF*	10.0
5. Glucose	10.0
6. Fructose	100.0
7. Sucrose	2.5

*Hydroxymethylfurfural

Gradient:	0-6 min: 3 mM MSA/102 mM KOH
	6-12 min: 14 mM MSA/108 mM KOH

Separation of sugars in an agave syrup standard using a CarboPac PA1 1 mm column with the Dionex ICS-6000 system in Dual EGC mode.

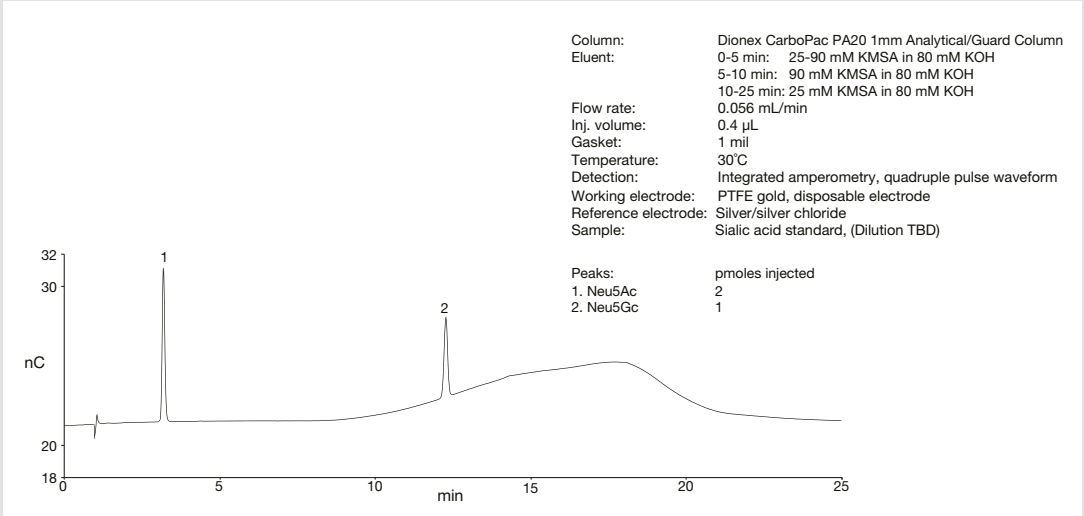
Reduce waste and maintenance tasks

Manual eluent preparation often means increased waste due to inconsistencies, and long and laborious cleaning sparging.

With the narrow 1 mm diameter, lower flow rates are naturally achieved with the CarboPac columns. This means less eluent is needed to deliver superior results and waste streams are immediately reduced. Since the Dual EGC relies on automated, electrolytic generation, chemicals are carefully controlled, manual handling is avoided and human error is removed; the operator simply adds the EG cartridges and water.

Some workflows, such as those used in food testing and biopharmaceutical analysis, can leave residuals that are hard to remove and require frequent cleaning with high concentrations of hydroxide. Traditional metal chromatographic systems cannot withstand these concentrations and are susceptible to corrosion. Column contamination needs to be removed with cleaning cycles and this means downtime.

Since concentrations of up to 200 mM hydroxide can be electrolytically generated with Dual EG, and the Dionex ICS-6000 system can withstand these elevated levels, the columns can be cleaned *in situ* without having to do offline cleaning, saving time. This means reduced downtime and fewer maintenance cycles, helping to increase productivity and decrease the total cost for each analysis task.



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

Reliable results for agave syrup analysis

Agave syrup has gained popularity in recent years due to its low glycemic index when compared to sugar or honey. As imports increase, there is a higher risk of adulteration with substances such as high-fructose corn syrup, so oligosaccharide profiling has become more important.

Three different agave syrups were tested with the Dionex ICS-6000 system with CarboPac 1 mm columns and Dual EGC in the following chromatographic conditions.

Chromatographic conditions

Parameter	Value
Columns	Dionex CarboPac PA1 Guard, 1 × 50 mm (P/N 303273) Dionex CarboPac PA1 Separation, 1 × 250 mm (P/N 303272)
Eluent	Gradient (Table 1)
Flow rate	0.063 mL/min
Column temperature	30 °C
Injection volume	0.4 µL
Autosampler temperature	5 °C
Reference electrode	Ag/AgCl
Working electrode	Disposable electrode gold, with a 1 mil (25 µm) gasket
Detection	Pulsed Amperometric Detector (Electrochemical Detection)
Detection compartment temperature	30 °C
Detection waveform	Gold, Carbohydrates, 4-Potential (Table 2)
System backpressure	-3300 psi (100 psi - 0.8894 MPa)
Run time	70 min

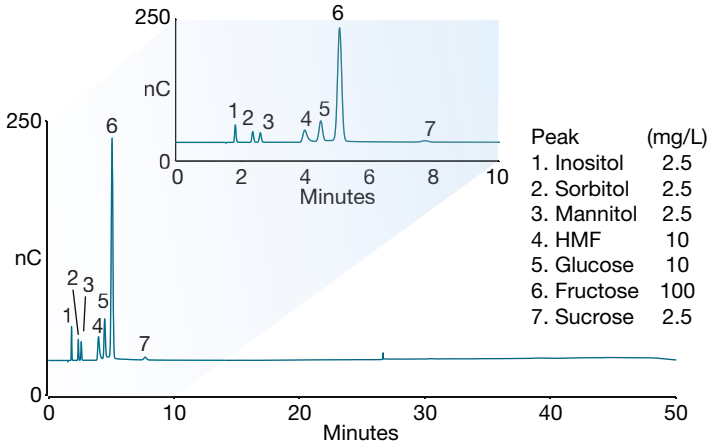
Eluent gradient

Time (min)	KMSA (mM)	KOH (mM)
-20	3	102
0	3	102
6	3	102
12	14	108
30	70	130
40	100	100
40.1	3	102
50	3	102

Carbohydrates, 4-potential waveform

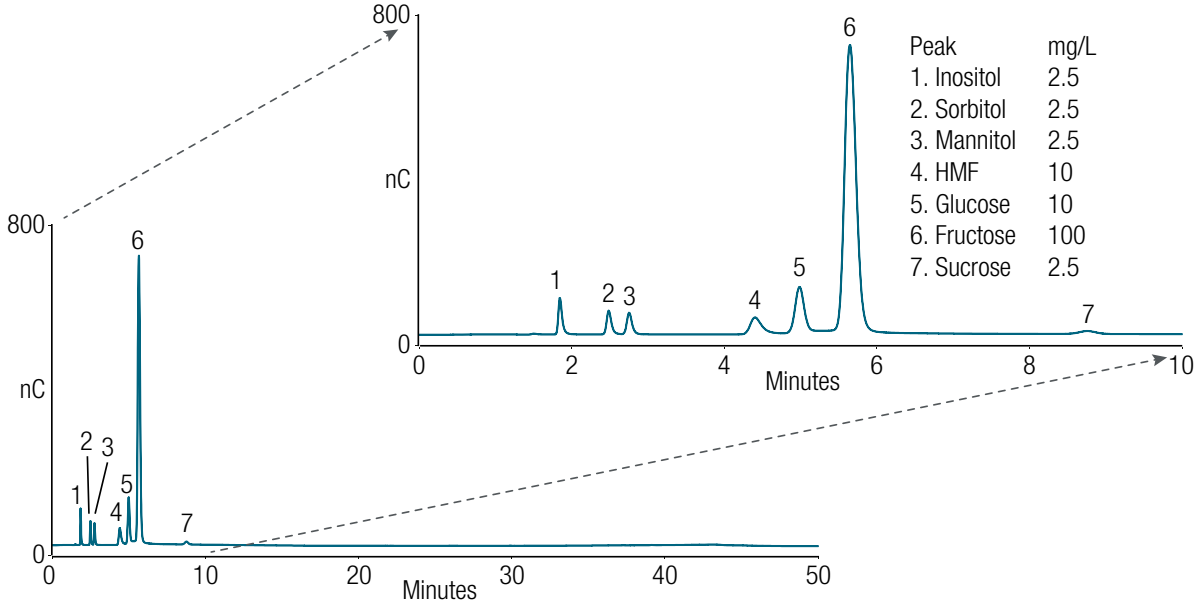
Time (s)	Potential (V) vs Ag/AgCl	Integration
0	0.1	Off
0.2	0.1	On
0.4	0.1	Off
0.41	-2	Off
0.42	-2	Off
0.43	0.6	Off
0.44	-0.1	Off
0.5	-0.1	Off

The system showed excellent resolution of the seven main sugars in agave syrup in line with the governmentally-approved characterization from the producing country, Mexico, and in line with the Norma Oficial Mexicana (NOM) approved methods.



Separation of seven agave sugar standards using the CarboPac PA1 1 mm column and Dual EGC.

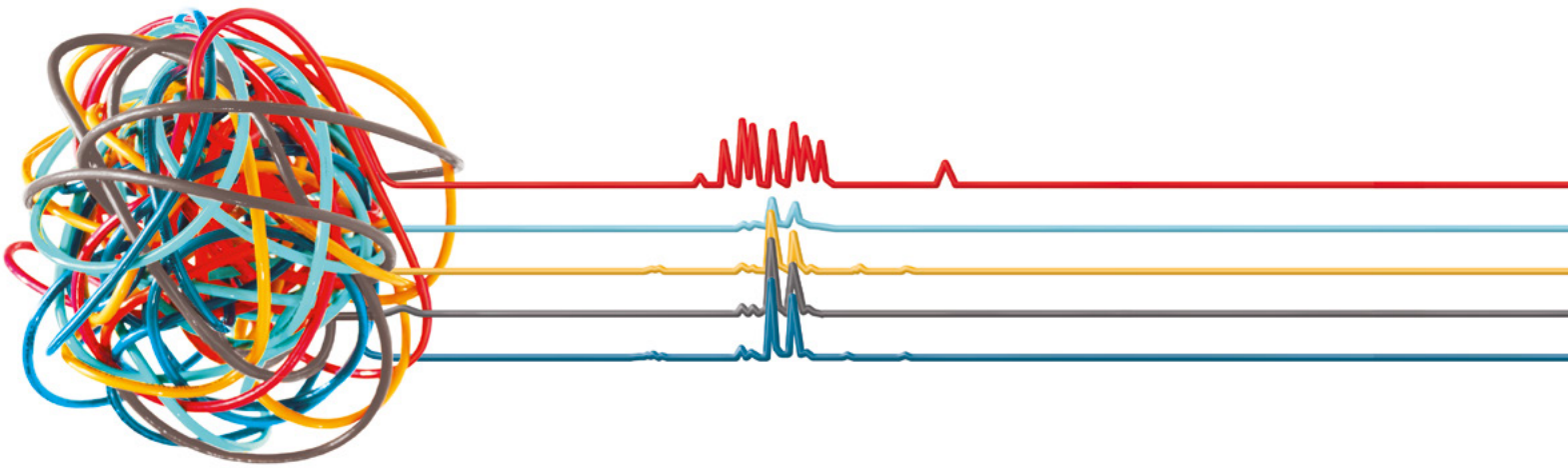
The Dual EGC method also produced identical results when compared with manually prepared eluent, with the added benefit of reducing waste and saving time in eluent production.



Manually prepared eluent

A CarboPac column with Dual EGC for every type of carbohydrate analysis

Column	Part number - format capacity (µeq/column)	Recommendations	Official method/target applications	Application notes
Dionex CarboPac PA1 columns for Dual EGC mode on the Dionex ICS-6000 HPIC system	303272 - 1 × 250 mm (6.25 µeg) 303273 - 1 × 50 mm (1.25 µeq)	Columns for predictable, high resolution, isocratic and gradient separation of monosaccharides, and disaccharides using the Dual EGC mode	Predictable, high resolution, separation of monosaccharide and disaccharides using electrolytically generated potassium methanesulfonate, and potassium hydroxide	AN73896: Carbohydrate analysis of agave syrup using HPAE-PAD in Dual EGC mode
Dionex CarboPac PA20 columns for Dual EGC mode on the Dionex ICS-6000 HPIC system	303369 - 1 × 250 mm (7.2 µeq) 303370 - 1 × 50 mm (1.4 µeq)	Columns for predictable, high resolution, isocratic and gradient separation of monosaccharides, disaccharides, and sialic acids using the Dual EGC mode	Predictable, high resolution, separation of monosaccharide, disaccharides and sialic acids using electrolytically generated potassium methanesulfonate, and potassium hydroxide	AN73986: Determination of Trans-Galactooligosaccharides in Foods using HPAE-PAD in Dual EGC mode
Dionex CarboPac PA200 columns for Dual EGC mode on the Dionex ICS-6000 HPIC system	302861 - 1 × 250 mm (4 µeq) 302862 - 1 × 50 mm (0.8 µeq)	Columns for predictable high resolution, isocratic and gradient separation of oligosaccharides using the Dual EGC mode, Thermo Scientific™ Dionex™ RFIC systems to electrolytically generate KOH/KMSA eluents by using a methanesulfonic acid (MSA) EGC cartridge, and a potassium hydroxide (KOH) EGC cartridge in series with each other	Predictable, and accurate high resolution separations of oligosacharides, linear polysacharides, and galactooligosaccharides	AN72714: HPAE-PAD analysis of galactosyl oligosaccharide-containing samples using Dual EGC cartridges



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