Application brief | 004183

Dionex CarboPac PA300-4µm: A high-efficiency, high-resolution, all-purpose carbohydrate column

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Carbohydrates, sugars, glycans, monosaccharides, sugar alcohols, alditols

Introduction

Carbohydrate analysis is often challenging due to structural complexity and lack of chromophores. High-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD) overcomes these challenges, enabling high-pH separations and sensitive detection without the need for derivatization.

Choosing the right chromatographic column is crucial for effective carbohydrate analysis but can be challenging given the variety of available options. The Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm Column can resolve early eluting neutral carbohydrates while maintaining the resolution of later eluting charged glycans, making it an excellent all-purpose column for carbohydrate method development. Designed primarily for analyzing complex oligosaccharides from biologics and food samples, the Dionex CarboPac PA300-4µm Column excels in delivering high-efficiency and high-resolution separations of complex, heterogeneous samples. It is packed with state-of-the-art 4 µm supermacroporous resin coated with anion exchange latex particles, offering superior efficiency and resolution compared to 6 µm particles commonly found in typical ion chromatography columns.

In this experiment, various carbohydrate samples were analyzed using the Dionex CarboPac PA300-4µm Column and compared with the Thermo Scientific™ Dionex™ CarboPac™ PA200 Column, which is optimized for fast analysis of complex

carbohydrates such as oligosaccharides, linear polysaccharides, and galacto-oligosaccharides. This application brief highlights the versatility of the Dionex CarboPac PA300-4µm Column as a general-use, first-pass column for carbohydrate analysis, while presenting the highly efficient Dionex CarboPac PA200 Column as a superior choice for high-resolution fast analysis of oligo- and polysaccharide profiling.

Additionally, comparisons were made with a competitor column to demonstrate the versatility of the Dionex CarboPac PA300-4µm Column. For detection, PAD (with a disposable gold working electrode) and a palladium hydrogen (PdH) reference electrode were used for all tests.

Experimental

Equipment

Thermo Scientific™ Dionex™ ICS-6000 System, including:

- DP gradient pump module with degas option
- DC detector/chromatography module with HPAE-PAD
- Thermo Scientific™ Dionex™ AS-AP Autosampler

Software

Thermo Scientific™ Chromeleon 7.3.2 Chromatography Data System (CDS)

Consumables

- Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm Guard Column, 2 mm (P/N 303347)
- Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm Analytical Column, 2 mm (P/N 303346)
- Thermo Scientific™ Dionex™ CarboPac™ PA200 Guard Column, 3 mm (P/N 062895)
- Thermo Scientific™ Dionex™ CarboPac™ PA200 Analytical Column, 3 mm (P/N 062896)
- ThermoScientific™ Dionex™ BorateTrap™ Inline Trap Column, 4 mm (P/N 047078)
- Thermo Scientific™ Dionex™ ED Electrochemical Detector Gold on PTFE Disposable Electrode (P/N 066480)
- Thermo Scientific™ Dionex™ ED Electrochemical Detector Palladium Hydrogen Reference Electrode (P/N 072075)

Reagents

Deionized (DI) water, Type 1 reagent grade, 18 MΩ-cm resistivity or better; eluent water was degassed and blanketed with nitrogen gas

- Fisher Chemical™ sodium hydroxide, 50% w/w (SS254-500)
- Thermo Scientific™ sodium acetate, anhydrous (P/N 059326)

Instrument method

Systems	Dionex ICS-6000
Columns	Dionex CarboPac PA300-4µm, Analytical, 2 x 250 mm
	Dionex CarboPac PA300-4µm, Guard, 2 x 50 mm
Eluent	See below
Flow rate	See below
Column temperature	30 °C
Detection	PAD
Working electrode	Gold on PTFE disposable
Reference electrode	PdH
Waveform	Carbohydrate quad potential waveform for the ED

Separation conditions

Honey			
	PA300-4µm	PA200	Competitor
Flow rate*	0.28 mL/min	0.5 mL/min	0.7 mL/min
Eluent concentration	116 mM NaOH	30 mM NaOH	60 mM NaOH

Inulin			
	PA300-4µm	PA200	Competitor
Flow rate*	0.25 mL/min	0.5 mL/min	0.8 mL/min
Eluent	A: 200 mM NaOH B: 200 mM NaOH/ 500 mM sodium acetate	A: 100 mM NaOH B: 100 mM NaOH/ 500 mM sodium acetate	A: 100 mM NaOH B: 100 mM NaOH/ 500 mM sodium acetate
Eluent condition	0–5 minutes: A: 60% B: 40% 5–60 minutes: A: 60–0% B: 40–100% 60–62 minutes: A: 0% B: 100% 62–62.1 minutes: A: 0–60% B: 100–40% 62.1–77 minutes: A: 60% B: 40%	0–0.1 minutes: A: 80% B: 20% 0.1–60 minutes: A: 80–10% B: 20–90% 60–62 minutes: A: 10% B: 90% 62–62.1 minutes: A: 10–80% B: 90–20% 62.1–77 minutes: A: 80% B: 20%	0–0.1 minutes: A: 100% B: 0% 0.1–12 minutes: A: 100–64% B: 0–36% 12–60 minutes: A: 64–10% B: 36–90% 60–60.1 minutes: A: 10–100% B: 90–0% 60.1–75 minutes: A: 100% B: 0%

Alditol			
	PA300-4µm	PA200	Competitor
Flow rate*	0.25 mL/min	0.5 mL/min	0.7 mL/min
Eluent	A: 10 mM NaOH B: 200 mM NaOH	A: DI water B: 50 mM NaOH C: 100 mM NaOH/ 500 mM sodium acetate	A: DI water B: 50 mM NaOH C: 100 mM NaOH/ 500 mM sodium acetate
Eluent concentration	0–12 minutes: A: 100% B: 0% 12–12.1 minutes: A: 100–0% B: 0–100% 12.1–22 minutes: A: 0% B: 100% 22–22.1 minutes: A: 0–100% B: 100–0% 22.1–37 minutes: A: 100% B: 0%	0–10 minutes: A: 98% B: 2% C: 0% 10–10.1 minutes: A: 98–90% B: 2–0% C: 0–10% 10.1–15 minutes: A: 90% B: 0% C: 10% 15 – 15.1 minutes: A: 90–98% B: 0–2% C: 10–0% 15.1 – 25 minutes: A: 98% B: 2% C: 0%	0–10 minutes: A: 98% B: 2% C: 0% 10–10.1 minutes: A: 98–90% B: 2–0% C: 0–10% 10.1–15 minutes: A: 90% B: 0% C: 10% 15–15.1 minutes: A: 90–98% B: 0–2% C: 10–0% 15.1–25 minutes: A: 98% B: 2% C: 0%

A Dionex ICS-6000 system with proportioning valve and manually prepared eluents is required.

For the analyses of carbohydrates, detailed technical information can be found in Technical Notes 71 and 70671.[1,2] These technical notes can be downloaded from Thermo Scientific™ AppsLab Library of Analytical Applications.

*It should also be noted that the 2 mm Dionex CarboPac PA300-4µm allows a lower flow rate than the larger bore column, leading to less eluent usage with similar separation.

Carbohydrates in honey

Fructose and glucose are major components of sugars in honey. Profiling minor honey sugars may be useful for the determination of floral origin and as a parameter of honey's quality.

Figure 1 shows the separation of typical sugars found in a honey sample using the Dionex CarboPac PA300-4µm, the Dionex CarboPac PA200, and a competitor column. Though the exact separation differs slightly, all three columns demonstrate similar performance.

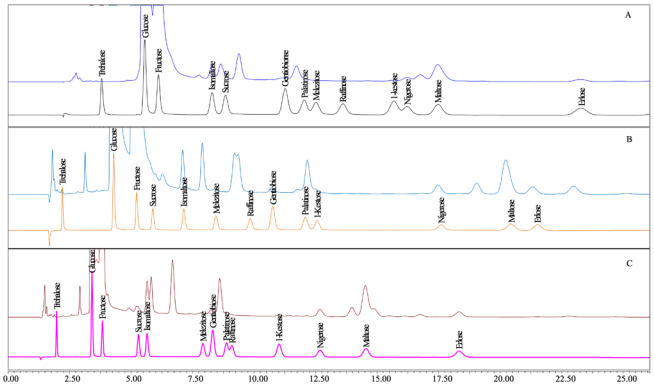

Figure 1: Analysis of simple carbohydrates in honey, compared with standards. A: CarboPac PA300-4µm. B: CarboPac PA200. C: Competitor column.

Profiling of inulin

Inulins are naturally occurring polyfructans produced by plants with 11 to 60 degrees of polymerization (DP). Figure 2 shows the profile of chicory inulin on a Dionex CarboPac PA300-4µm Column compared to a Dionex CarboPac PA200 Column and a competitor column. Although peak assignment and identification is difficult without a commercial standard, similar numbers of peaks were observed by all tested columns with the Dionex CarboPac PA200 Column showing a slightly better resolution.

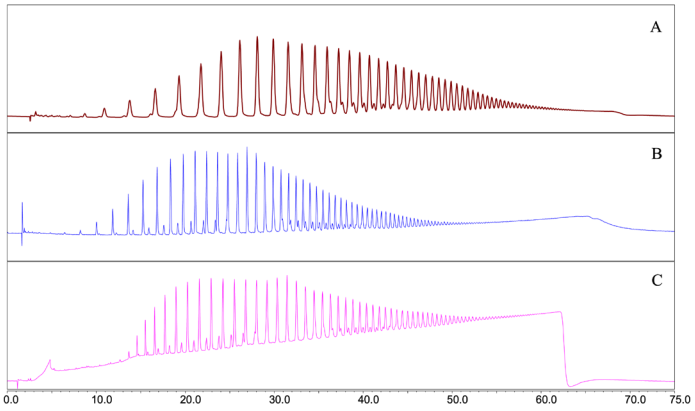

Figure 2: Analysis of Inulin. A: CarboPac PA300-4µm. B: CarboPac PA200. C: Competitor column.

Alditols

Figure 3 shows the separation of seven alditols. Only the Dionex CarboPac PA300-4µm Column was able to provide the resolution, demonstrating the versatility of this column.

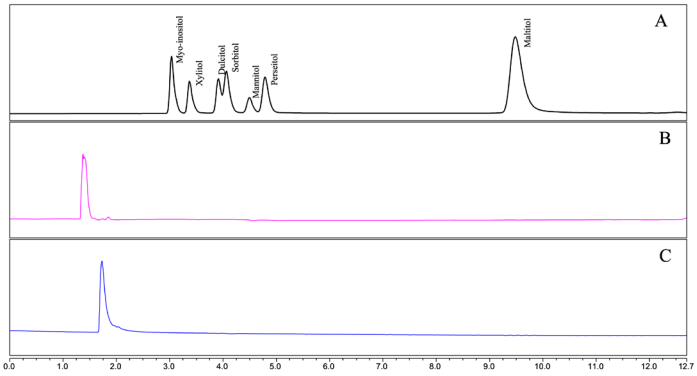

Figure 3: Analysis of alditols. A: CarboPac PA300-4µm. B: CarboPac PA200. C: Competitor column.

Conclusion

The Dionex CarboPac PA300-4µm Column excels in the efficient separation of complex, heterogeneous samples, offering superior chromatographic resolution for both neutral and charged glycans of various sizes. Its capability to separate a wide range of carbohydrates with differing sizes and charge states makes it an ideal choice for initiating method development. Moreover, the microbore column's smaller internal diameter reduces eluent consumption, promoting a more environmentally friendly approach.

This column is complementary to the Dionex CarboPac PA200 Column for high-efficiency and high-resolution separations of oligosaccharide and polysaccharide.

Reference

1. Rohrer, J. Technical Note 71: Eluent preparation for high-performance anion exchange chromatography with pulsed amperometric detection, Thermo Scientific AppsLab Library. (2017). http://tools.thermofisher.com/content/sfs/brochures/TN-71-Eluent-Preparation-for-High-Performance-Anion-Exchange-Chromatogarphy-with%20APD-TN-70669.pdf (accessed).

2. Rohrer, J. Technical Note 70671: Carbohydrate analysis by high-permormance anion exchange chromatography with HPAE-PAD, Thermo Scientific AppsLab Library. https://appslab.thermofisher.com/App/4930/tn70671-carbohydrate-analysis-by-highperformance-anionexchange-chromatography-with-pulsed-amperometric-detection-hpaepad (accessed).

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