

HPAE-PAD

HPAE-PAD analysis of galactosyloligosaccharide-containing samples using dual eluent generation cartridge mode

Authors

Manali Aggrawal, Sachin Patil, and Jeffrey Rohrer

Thermo Fisher Scientific, Sunnyvale, CA

Keywords

Dionex ICS-6000, Dionex CarboPac PA200 column (1 mm i.d.), Dionex EGC 400 MSA, Dionex EGC 400 KOH, prebiotics, Dual EGC mode, GOS

Goal

To demonstrate equivalency between HPAE-PAD chromatography of galactosyloligosaccharides (GOS) obtained using Dual EGC mode and manually prepared eluents

Introduction

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of beneficial bacteria in the colon.¹ GOS are considered a prebiotic and are found in commercially available infant and adult food products. GOS are prepared from lactose and comprise a chain of galactose units, which arise through consecutive transgalactosylation reactions, with a terminal glucose unit. The degree of polymerization (DP) of GOS can vary quite markedly, depending mainly on the type of enzyme used and the degree of lactose conversion.²

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) is one of the most widely used methods for profiling oligosaccharides and quantification of known oligosaccharides. HPAE-PAD uses direct detection. In other words, no sample derivatization is required.^{3,4} Thermo Scientific Application Note 1151 (no longer available) described an HPAE-PAD profiling method for GOS in prebiotic dietary supplements using manually prepared sodium hydroxide/sodium acetate (NaOH/NaOAc) eluent gradients with a Thermo Scientific™ Dionex™ CarboPac™ PA200 3 mm column set on a Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system.

After publication, we learned that the commercial dried GOS sample also contained maltodextrins, and peaks labeled as GOS were, in fact, maltodextrins. Maltodextrins are frequently used as an additive in the spray drying process to enhance yield.⁴ We obtained a maltodextrin-free liquid GOS sample (GOS syrup) and repeated the work. The new work was documented in Application Note 71993.⁵ The same dried GOS sample was used to produce the original Application Note 72714 (no longer available), which analyzed the sample using an HPAE-PAD system operating in Dual Eluent Generation Cartridge (Dual EGC) mode and also labeled maltodextrin peaks as GOS.

This application note describes the analysis of the GOS syrup sample with a Dual EGC mode HPAE-PAD system and replaces the original AN72714. The GOS chromatography obtained with the Dual EGC mode is compared with that obtained using manually prepared eluent in AN71993.⁵ GOS analysis using Dual EGC mode offers several advantages. The Dual EGC mode (Figure 1) uses two eluent generator cartridges in series, one containing methanesulfonic acid (MSA) and the other containing potassium hydroxide (KOH), to electrolytically generate KOH and KMSA. The electrolytically generated eluents can replace sodium hydroxide/sodium acetate eluents used for oligosaccharide separations. This operating mode is applicable to analytical (1.0 mm) column format. Dual EGC mode offers improved reproducibility and eluent gradient capabilities using an isocratic pump, eliminates manual preparation of eluents, maximizes instrument uptime, and minimizes pump maintenance. It also eliminates the potential risk of system contamination that can result from the use of lower quality sodium acetate.

Experimental

Equipment and consumables

- Thermo Scientific™ Dionex™ ICS-6000 HPLC™ System including:
 - Dionex ICS-6000 DP Isocratic Pump and/or a single isocratic pump module with degas
 - Dionex ICS-6000 EG Eluent Generator module with Dionex RFIC+ Dual EG eluent degasser (P/N 22181-60951)
 - Dionex ICS-6000 DC Detector/Chromatography module
 - 4-port Valve Rebuild Kit (P/N 074699), which includes a 0.4 µL injection loop
 - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler with Sample Syringe, 250 µL (P/N 074306) and Buffer line, 1.2 mL (P/N 074998)
- Thermo Scientific™ Dionex™ EGC 400 KOH Eluent Generator Cartridge (P/N 302766)
- Thermo Scientific™ Dionex™ EGC 400 MSA Eluent Generator Cartridge (P/N 302767)
- Thermo Scientific™ Dionex™ ED Electrochemical Detector (P/N 072042)
- Thermo Scientific™ Dionex™ ICS-6000 ED Electrochemical Cell (P/N 072044)
- pH, Ag/AgCl Reference Electrode (P/N 061879)
- Gold on PTFE Disposable Electrode for 1 mm applications (P/N 303397)

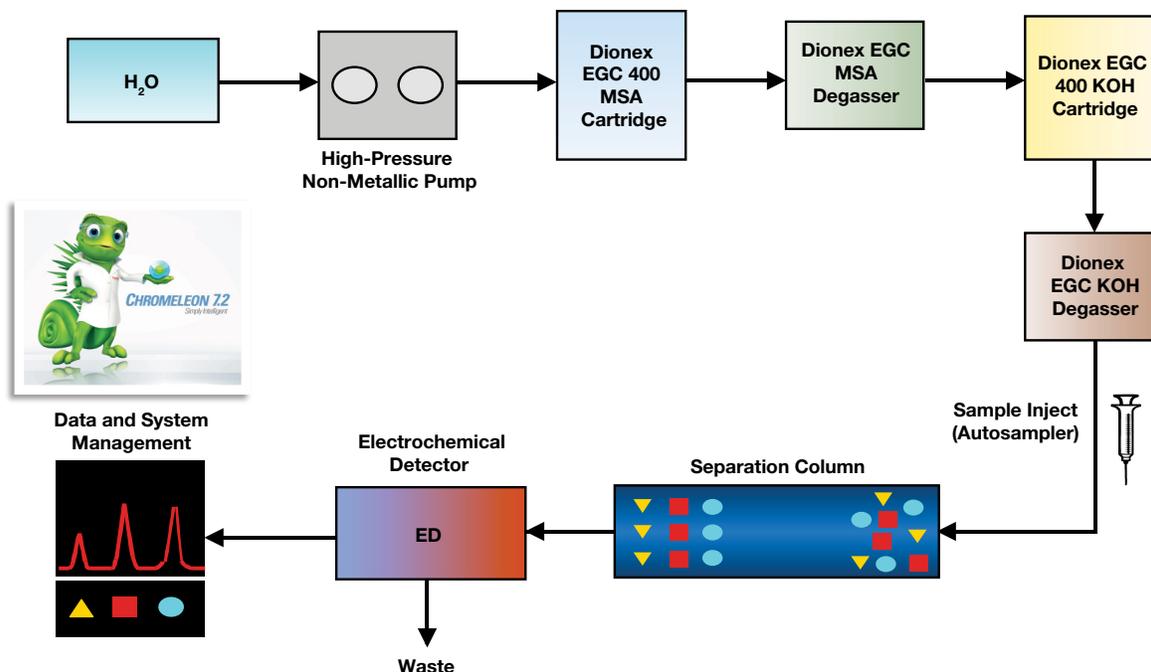


Figure 1. Dual EGC mode schematic

- Thermo Scientific™ Dionex™ IC PEEK Viper™ Fittings Kit for Dionex ICS-6000 HPIC system running Dual EG application (1 mm) (P/N 303377)
- Dionex AS-AP Autosampler Vials 10 mL (P/N 074228)
- Thermo Scientific™ Nalgene™ Syringe Filters, PES, 0.2 µm (Fisher Scientific P/N 09-740-61A)
- AirTite™ All-Plastic Norm-Ject™ Syringes, 5 mL, Sterile (Fisher Scientific P/N 14-817-28)
- Thermo Scientific™ Nalgene™ 1000 mL, 0.2 µm Nylon Filter Units (P/N 09-740-46)
- Amicon™ Ultra-15 Centrifugal Filter Unit with Ultracel™-3 membrane (P/N UFC900396)

Software

- Thermo Scientific™ Chromeleon™ Chromatography Data System software version 7.2 SR 7

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Bimuno™ GOS syrup (www.bimuno.com) containing 52% w/w galactooligosaccharide, kindly provided by the company.

Preparation of solutions and reagents

Dual EGC eluent

To generate eluents comprising potassium methanesulfonate and potassium hydroxide, a Dionex EGC 400 MSA cartridge is paired with a Dionex EGC 400 KOH cartridge. The Chromeleon CDS software version 7.2 SR 7 provides the feature of Dual EGC Control, which will track the amounts of MSA and KOH used and calculate the remaining cartridge lifetimes. Dual EGC mode guarantees high purity eluents, eliminates system contamination related to sodium acetate quality, and improves method precision and productivity.

The Dual EGC mode of operation is available on the Dionex ICS-6000 HPIC system to support the analysis of complex carbohydrates using electrolytically generated eluents. The Dionex EGC 400 cartridges are designed to enable operation at flow rates between 20 µL/min and 200 µL/min, the flow rate range necessary for use with 1 mm diameter columns. The Dionex EGC 400 MSA cartridge is connected to a Dionex EGC 400 KOH cartridge to generate KOH/KMSA up to 200 mM (combined potassium concentration) at flow rates of 20–63 µL/min, and up to 63 mM at flow rates up to 200 µL/min.

Conditions	
IC system	Dionex ICS-6000 HPIC system
Columns	Dionex CarboPac PA200 guard column (1 × 50 mm, P/N 302862) Dionex CarboPac PA200 analytical column (1 × 250 mm, P/N 302861)
Eluent source	Dionex EGC 400 MSA eluent generator cartridge in series with Dionex EGC 400 KOH eluent generator cartridge
Eluents	Potassium methanesulfonate /potassium hydroxide (KMSA/KOH)
Gradient	5 to 75 mM KMSA in 50 mM KOH from 0 to 35 min, 75 mM KMSA in 50 mM KOH from 35 to 45 min, 5 mM KMSA/50 mM KOH from 45 to 75 min
Flow rate	0.063 mL/min
Injection volume	0.4 µL (full loop)
Temperature	30 °C (column and detector compartments)
Detection	Pulsed amperometric, Gold on PTFE disposable working electrode, Ag/AgCl reference, 1 mil gasket
Run time	75 min

Carbohydrate 4-potential waveform for the ED				
Time (s)	Potential (V)	Gain region	Ramp	Integration
0.00	+0.1	Off	On	Off
0.20	+0.1	On	On	On
0.40	+0.1	Off	On	Off
0.41	-2.0	Off	On	Off
0.42	-2.0	Off	On	Off
0.43	+0.6	Off	On	Off
0.44	-0.1	Off	On	Off
0.50	-0.1	Off	On	Off

Standard solutions

Bimuno GOS standard

Weigh 1 g of Bimuno GOS syrup into a 125 mL polypropylene bottle. Add DI water to 100 g to make a 10,000 mg/L stock standard. Store the stock standard at 4 °C. Using this stock standard, freshly prepare working standards (100–5,000 mg/L) before analysis to avoid degradation.

System preparation and configuration

To achieve the best chromatography, we recommend a clean-up of the entire system (without installing the columns) for at least 2 h with 100 mM KOH at 0.1 mL/min. To ensure a stable baseline and low background noise, it is crucial to have sufficient removal of the hydrogen and oxygen gases formed with the production of the EGC-generated eluents. For a 1-mm system, connect the vents of the Dionex RFIC EGC 400 MSA Degasser and the Dionex RFIC EGC 400 KOH Degasser to the Vacuum Port located at the back of the Dionex DP pump module. Ensure all connections and fittings for the vacuum degas are vacuum-tight before using the system. Keep the eluent water blanketed under 34–55 kPa (5–8 psi) of nitrogen at all times to reduce carbonate and microorganism contamination. Only turn on the EGC power when the system pressure is above 2,600 psi. This step is important to ensure best system performance. In a properly working system, the electrochemical detection (ED) background for the Dionex CarboPac PA200 column Quality Assurance Report chromatography and most applications is typically 25–45 nC. Follow the flow diagram (Figure 1) to plumb the consumables and modules of the Dionex ICS-6000 HPIC system for Dual EGC mode. Note: Plumbing must be with narrow bore tubing [Dionex IC PEEK Viper Fitting Kit for Dionex ICS-6000 HPIC system running Dual EG application (1 mm)]. Deionized water is pumped first into the Dionex EGC 400 MSA cartridge, and then the MSA is passed into the Dionex EGC 400 KOH cartridge to titrate the potassium hydroxide to potassium methanesulfonate. By balancing the concentration of the two cartridges, pure KMSA can be generated. By generating an excess of KOH compared to MSA, a basic solution of KMSA plus KOH can be generated (Basic Eluent mode). By generating an excess of MSA compared to KOH, an acidic solution of KMSA plus MSA can be generated (Acidic Eluent mode). The system can be switched between the two modes as needed. For this application, we are only using the Basic Eluent mode.

Column wash and equilibration between injections for EGC method

For this method, perform a wash step after every injection to maintain column performance. The wash step consists of 10 min of 75 mM KMSA in 50 mM KOH. This wash will ensure stable retention times and assist in maintaining a clean electrode. For good retention time reproducibility, the column must be equilibrated to the starting gradient conditions prior to each injection, and the re-equilibration period should be tightly controlled. In the separation shown here, the column set was re-equilibrated at initial conditions for 15 min prior to the next injection.

Results and discussion

HPAE-PAD profiling of Bimuno GOS syrup

Figures 2A and 2B show the HPAE-PAD profiles of the Bimuno GOS syrup sample obtained using Dual EGC mode and manually prepared eluents, respectively. In Figure 2A, oligosaccharides were separated on a Dionex CarboPac PA200 1 mm column set using a Dionex EGC 400 MSA cartridge connected in series to a Dionex EGC 400 KOH cartridge. A solution of Bimuno GOS syrup was prepared, and an aliquot (0.4 µL) of the solution was injected onto the column and eluted at 0.063 mL/min with a linear gradient of KMSA (5–75 mM in 35 min) in KOH (50 mM KOH). For manually prepared eluent-based separation (Figure 2B), oligosaccharides were separated on a Dionex CarboPac PA200 3 mm column set using a gradient of 20 to 100 mM sodium acetate in 50 mM sodium hydroxide. The injection volume and flow rate were 10 µL and 0.5 mL/min, respectively. Refer to AN71993 for more information on the manually prepared eluent separation of GOS.⁵

Analysis of Bimuno GOS syrup by Dual EGC mode HPAE-PAD showed a complex pattern of peaks, with decrease in intensity with increasing retention time. This result is similar to that obtained using manually prepared eluent. Coulier et al. observed a similar complex pattern for the HPAE-PAD profile of Vivinal™ GOS.⁶ Instead of a single peak, clusters of peaks were observed in the GOS profile at regular intervals indicating increasing molecular weight. The retention time of a homologous series of carbohydrates increases as the degree of polymerization increases, and thus each successive cluster of peaks represents GOS that have one more galactose than the previous peak cluster. Individual peaks within a cluster represent structural isomers of the same DP.⁷

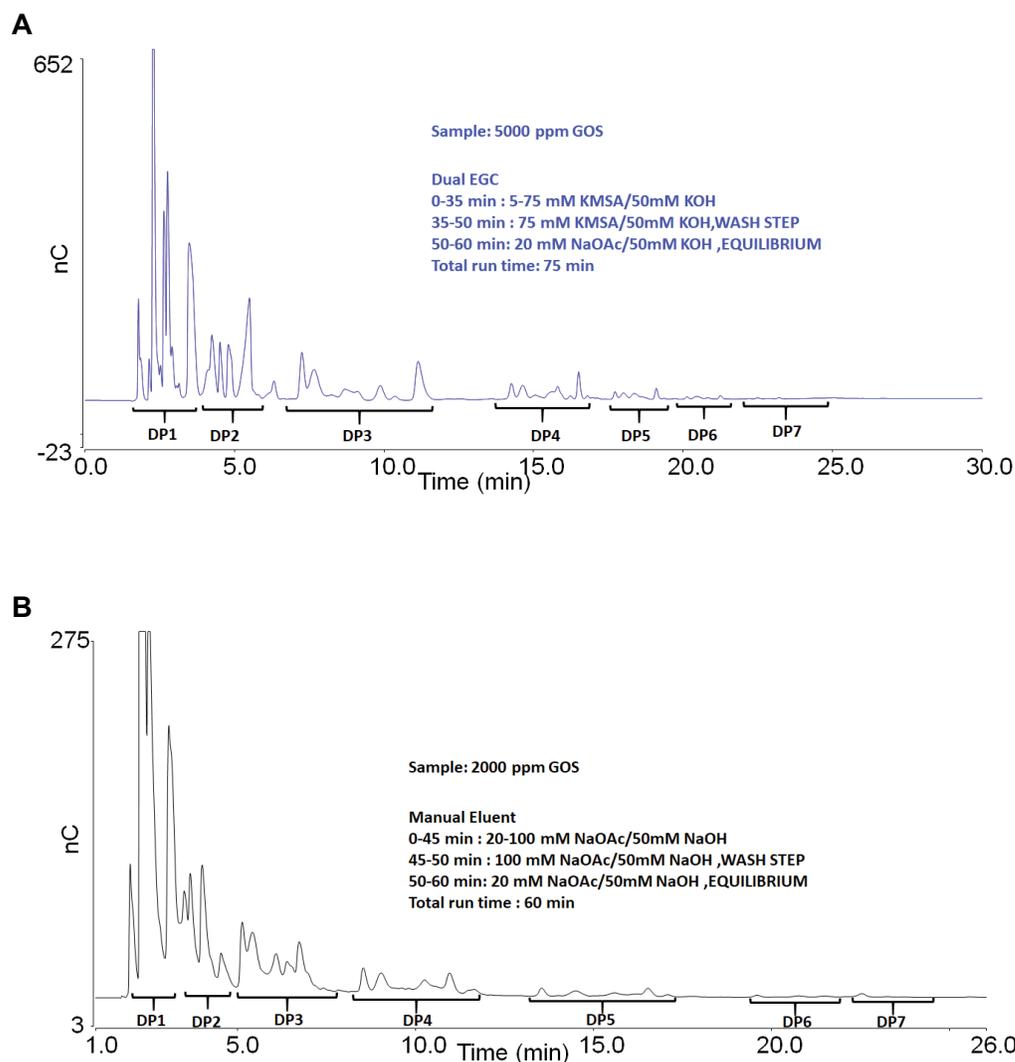


Figure 2. Bimuno GOS analysis using (A) Dual EGC mode (KOH/KMSA) on a 1 × 250 mm Dionex CarboPac PA200 column and (B) manually prepared eluents (NaOH/NaOAc) on a 3 × 250 mm Dionex CarboPac PA200 column

Conclusion

Our study demonstrated GOS separation using Dual EGC mode and a Dionex CarboPac PA200 1 mm column. The results reported here update an earlier application note that used a GOS sample containing maltodextrin and incorrectly reported maltodextrin peaks as GOS peaks. The GOS sample used here

does not contain maltodextrin. The resultant chromatography is comparable to that obtained using manually prepared eluents and to that reported in the literature. The results obtained here imply that electrolytically generated eluents can be used and will greatly simplify routine GOS analysis.

References

1. Macfarlane, G.; Steed, H.; Macfarlane, S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J. Appl. Microbiol.* **2008**, *104*, 305–344.
2. Cardelle-Cobas, A.; Villamiel, M.; Olano, A.; Corzo, N. Study of galactooligosaccharide formation from lactose using pectinex-Ultra SP-L. *J. Sci. Food Agr.* **2008**, *88*, 954–961.
3. Rohrer, J. S. High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) for the Determination of Oligosaccharides in Food and Agricultural Products. (2003) ACS Symposium Series 849: Oligosaccharides in Food and Agriculture (Ed. Eggleston, G. and Côté, G. L.), Oxford University Press, Chap. 2, 16–31.
4. Bhandari, B., Datta, N., Howes, T. Problems Associated with Spray Drying of Sugar-Rich Foods. *Dry. Technol.* **1997**, *15*(2), 671–684.
5. Thermo Scientific Application Note 71993 (AN71993). Profiling galactosyloligosaccharide-containing samples by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD). [Online] <https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-71993-ic-galactosyloligosaccharides-prebiotics-an-71993-en.pdf>
6. Coulier, L.; Timmermans, J.; Bas, R.; Dool, R.V.; Haaksman, I.; Klarenbeek, B.; Slaghek, T.; Dongen, W.V. In-depth characterization of prebiotic galactooligosaccharides by a combination of analytical techniques. *J. Agric. Food Chem.* **2009**, *57* (18), 8488–8495.
7. Patil, S.; Rohrer, J. An improved method for galactosyl oligosaccharide characterization. *J. Chromatogr. B.* **2021**, *1184*, 122967.

 Learn more at thermofisher.com/ic

General Laboratory Equipment – Not For Diagnostic Procedures. © 2022-2024 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. AirTite and Norm-Ject are trademarks of Products Co., Inc. Amicon and Ultracel are trademarks of Merck KGaA. Bimuno is a trademark of Clasado Ltd. Vivinal is a trademark of FrieslandCampina Ingredients. 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 manner 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 representative for details. **AN72714-EN 0224M**