Analysis of human milk oligosaccharides using high-performance anion-exchange chromatography with pulsed amperometric detection

Lie, Aleksander; Pedersen, Lars Haastrup

Publication date:
2016

Document Version
Publisher's PDF, also known as Version of record

Analysis of human milk oligosaccharides using high-performance anion-exchange chromatography with pulsed amperometric detection

Aleksander Lie, Lars H. Pedersen
Department of Chemistry and Bioscience,
Aalborg University, Denmark
E-mail: alie@bio.aau.dk

AALBORG UNIVERSITY
DENMARK

Introduction

Human Milk Oligosaccharides (HMOs) are composed of 5 different monosaccharides: D-glucose, D-galactose, *N*-acetylglucosamine, L-fucose, and *N*-acetylneuraminic acid. Approximately 200 unique structures have been identified, ranging in degree of polymerization from 3 to 22. The diversity found among individual mothers is considerable, ranging from as few as 23 and up to 130 different oligosaccharides. HMOs are known as beneficial for infant health and development, and have received increasing attention in recent years [1].

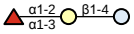
Syntheses of this unique family of lactose-based molecules necessitates analysis methods that can provide separation and quantification of the common structural constituents mentioned, as well as the disaccharide lactose (Galβ1-4Glc) and oligosaccharides such as lacto-*N*-triose II (GlcNAcβ1-3Galβ1-4Glc), lacto-*N*-tetraose (Galβ1-3GlcNAcβ1-3Galβ1-4Glc), and lacto-*N*-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc), among others (see below). In the present work, isocratic analyses of various saccharides were performed to serve as a basis for the development of more complex chromatographic methods.

Human milk oligosaccharide structures

Monosaccharide building blocks

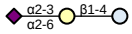
○	Glc	Glucose
○	Gal	Galactose
□	GlcNAc	*N*-Acetylglucosamine
△	Fuc	Fucose
◇	Neu5Ac	*N*-Acetylneuraminic acid

Fucosyllactoses



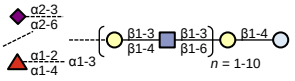
Fucα1-2/3Galβ1-4Glc

Sialyllactoses



Neu5Acα2-3/6Galβ1-4Glc

Complex HMOs



Neu5Acα2-3/6 / Fucα1-2/3/4(Galβ1-3/4GlcNAcβ1-3/6)$_n$ Galβ1-4Glc

High-performance anion-exchange chromatography

High-performance anion-exchange chromatography (HPAE) with pulsed amperometric detection (PAD) is a method highly suited for analysis of carbohydrates. HPAE with alkaline eluents results in retention of neutral carbohydrates depending on the number of charged groups in the molecule, pH and concentration of competing anions. The PAD provides sensitivity for carbohydrates in the pmol-range, although the detection response is dependent on eluent pH [2].

Samples prepared from pure standards were eluted using water and aqueous solutions of NaOH (100 mM) and NaOAc (10 mM), through a CarboPac PA1 column at 22 °C.

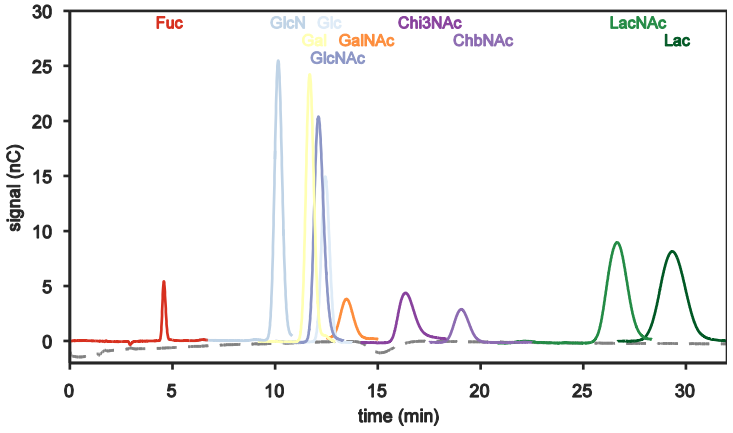


Figure 1: Chromatogram overlay from HPAE analyses of mono-and oligosaccharides.
Isocratic elutions of standards at 25 mM NaOH and 1 mM NaOAc after 10 min equilibration. Injection of pure water subtracted (- - -). See fig. 2 for full names of compounds.

Isocratic elutions of mono- and oligosaccharides

Various mono- and oligosaccharides were analysed under isocratic conditions with different eluent concentrations of NaOH in the range 5-75 mM, while the concentration of NaOAc was maintained at 1 mM. The resulting retention times and separation were considered.

The investigated saccharides exhibited different retention properties as an effect of NaOH-concentration, and several were shown to be maximally retained at different concentrations (see fig. 2). Most of the monosaccharides co-eluted or eluted in close proximity at the upper range of the conditions studied, with even some changes in the elution order observed. However, variations in retention facilitating optimisation of separation were observed at lower NaOH-concentrations.

As building blocks for HMOs, the separation of glucose, galactose and *N*-acetyl-glucosamine were of particular interest. These saccharides all exhibited the lowest retention times at 5 mM NaOH, while the highest retention times were seen at 17, 20 and 14 mM, respectively. For glucose and galactose, the largest difference in retention times was at 10 mM, though all these conditions gave estimated resolutions more than sufficient for baseline separation ($R_s \geq 1.5$). For glucose and *N*-acetylglucosamine the largest difference in retention times was at 7 mM NaOH, and up to 17 mM the resolution indicated baseline separation, while above this NaOH-concentration the resolution decreased

These results indicated that gradient elution with increasing concentration of NaOH in the range 5-15 mM over the time range of the retention times of Glc, Gal and GlcNAc could be used to optimise separation and analysis time for these monosaccharides.

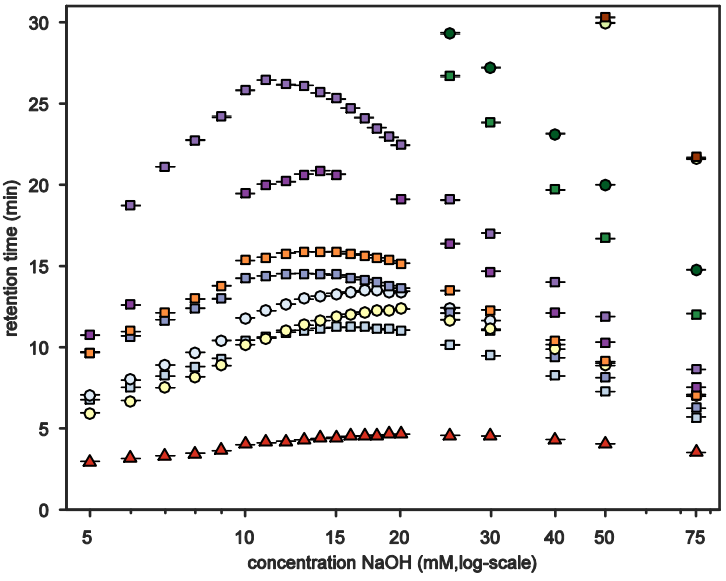


Figure 2: Effect of eluent concentration of NaOH on retention times of mono- and oligosaccharides.
△ fucose, ○ galactose, □ glucosamine, ○ glucose, □ *N*-acetylglucosamine, □ *N*-acetylgalactosamine, □ *N,N',N''*-triacetylchitotriose, □ *N,N'*-diacetylchitobiose, □ *N*-acetyllactosamine, ● lactose, ○ 6'-galactosyl-lactose, □ lacto-*N*-triose II.
Error bars indicate relative standard deviations calculated from fucose as internal standard.

Conclusion

Several of the mono-, di- and oligosaccharides investigated exhibited maximal retention at different concentrations of NaOH.

The different retention properties of the investigated saccharides provide information for developing gradient elution separations.

Separation of the HMO building blocks glucose, galactose and *N*-acetylglucosamine was indicated as optimisable with gradient methods in the concentration range of 5 to 15 mM NaOH.

References
1. Bode L, Jantscher-Krenn E. (2012) Adv. Nutr., 3: 383S
2. Lee YC. (1990) Anal. Biotechnol., 189: 151

This work was performed as part of the project 'OliGram: Design and gram scale enzymatic synthesis of Human milk oligosaccharides', funded by the Danish Strategic Research Council.