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

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INTRODUCTION

Human Milk Oligosaccharides (HMOs) are composed of 5 different monosaccharides: D-glucose, D-galactose, L-fucose, N-acetylneuraminate, and N-acetylgalcosamine. Approximately 200 unique structures have been identified, with the diversity among individual mothers being considerable, varying from as few as 23 and up to 130 different oligosaccharides detected. 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-tride (Galβ1-4GlcNAcβ1-3Galβ1-4Glc) 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
- Glucose
- Galactose
- N-Acetylgalcosamine
- Fucose
- Neu5Ac (N-Acetylneuraminic acid)

Fucosyllectoses
- Galβ1-4Glc
- Galβ1-4GlcNAcβ1-3Galβ1-4Glc

Sialyllectoses
- Neu5Acβ1-2/3Galβ1-4Glc
- Neu5Acβ1-2/3Galβ1-4Glc

Complex HMOs
- Galβ1-4GlcNAcβ1-3Galβ1-4Glc (Neu5Ac2-3/6Galβ1-4Glc)
- Galβ1-4GlcNAcβ1-3Galβ1-4Glc (Neu5Ac2-3/6Galβ1-4Glc)

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 or 1 M) and NaOAc (10 mM), and a CarboPac PA1 column at 22 °C.

CONCLUSIONS

Several of the mono-, di- and oligosaccharides investigated exhibited maximum retention at discrete concentrations of NaOH. The observed retention properties of the investigated saccharides provide information for designing gradient elution strategies. Separation of the HMO building blocks glucose, galactose and N-acetylgalcosamine was indicated as optimizable with gradient methods in the concentration range of 5 to 15 mM NaOH, while disaccharide building blocks could be separated using a NaOAc concentration gradient.

REFERENCES

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