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Analytical Products

White Paper

Retention Behaviour of N-Acetyl-Hexosamine Isomers On A Supel™ Carbon LC Column

PGC LC-MS-based profiling of GlcNAc and GalNAc

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Abstract

While the use of porous graphitised carbon (PGC) columns for the separation of common N- and O-linked glycans ranging from 4-15 saccharide residues has been successful in many respects, it remains challenging to profile shorter glycans (structurally related mono- and disaccharides) in the same chromatographic run due to their relatively poor retention characteristics on conventional PGC LC columns.

In this work, we investigated the retention behaviour of two N-acetylhexosamine (HexNAc) epimers critically important in human glycobiology i.e., N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) in their non-reduced and reduced forms on a Supel™ Carbon LC column using a high-resolution Q-ToF mass spectrometer as the detector. We demonstrate that the Supel™ Carbon LC column was able to consistently retain and separate (fully or in part) both the non-reduced and reduced forms of the HexNAc isomers when analysed alone or in mixtures on a shallow LC gradient using conventional LC solvents. Particularly, the reduced forms of GlcNAc and GalNAc were well separated and exhibited excellent chromatographic behaviour including narrow peak width and symmetrical peak shape.

The chromatographic analyses of the non-reduced and reduced GalNAc and GlcNAc showed reproducible retention patterns as demonstrated by triplicate chromatographic runs of all samples documenting the robustness of the Supel™ Carbon LC column for the retention and separation of the HexNAc isomers. Both the reduced and non-reduced GlcNAc and GalNAc analytes consistently displayed differential retention patterns indicating that the subtle structural differences with respect to the spatial arrangement of the OH group on the fourth carbon atom on the pyranose ring impact the interaction of the HexNAc isomers with the PGC surface.

These observations document the interesting separation potential of the Supel™ Carbon LC column for the quantitative profiling of biologically important monosaccharides that have traditionally been challenging to separate and quantitatively analyse under conventional glycomics acquisition conditions. The advent of the Supel™ Carbon LC column, therefore, promises to expand our insights into the human glycome; the ability to effectively separate and quantify structurally similar monosaccharides.

Introduction

N-acetylglucosamine (GlcNAc) and N-acetylgalactosamine (GalNAc) are essential building blocks of complex carbohydrate structures (glycans) known to carry out wide-ranging biological functions in humans and all vertebrates. O-glycans are covalently attached to select threonine and serine residues in the protein backbone through an initiating GalNAc residue while for N-glycans an initiating GlcNAc residue is covalently bound to the protein backbone through select asparagine residues.[1] Since these glycan-forming monosaccharide residues have identical mass and elemental composition and only differ in their stereochemistry, mass spectrometry (MS) alone fails to accurately profile these HexNAc moieties and instead relies on chromatographic separation of these species ahead of the mass analysis. However, the chromatographic separation of GlcNAc and GalNAc on conventional

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liquid chromatography (LC) phases and with MS-compatible solvent systems has, to date, proven challenging due to the only subtle, structural differences between such monosaccharide isomers. Therefore, the field urgently requires new separation and detection methods for the rapid, robust, and accurate profiling of biologically relevant monosaccharides.

Porous graphitised carbon (PGC) is an important stationary phase in LC that retains non-polar analytes similarly to alkyl-bonded, reversed-phase chromatographic materials.[2] PGC has also been demonstrated to display a unique retention and selectivity characteristic of polar and charged compounds, such as complex carbohydrates (glycans). For this reason, PGC has been extensively used to separate glycan isomers in the emerging field of glycomics.[3]

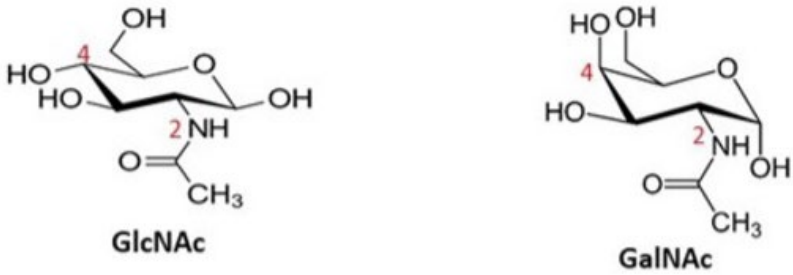
The aim of this work was to assess the abilities of a Supel™ Carbon LC column to retain and separate GalNAc and GlcNAc isomers in their reduced and non-reduced forms.

Experimental

GalNAc and GlcNAc monosaccharide standards as well as sodium borohydride were purchased from SigmaAldrich® (Castle Hill, NSW, Australia). In total, 2 mg of each monosaccharide standard was separately dissolved in 1 mL Milli-Q® water from which 10 µL aliquots (each having 20 µg monosaccharide) were made. 12 µL Milli-Q® water was added to all aliquots which were then dried in a SpeedVac™. Each aliquot was dissolved in 50 µL Milli-Q® water (concentration 0.4 µg HexNAc/µL) and PGC-LC-MS analyses were performed with and without reduction of the HexNAc moieties. The structures of non-reduced and reduced HexNAc moieties are displayed in **Figure 1**.

A

Non-reduced forms



B

Reduced forms

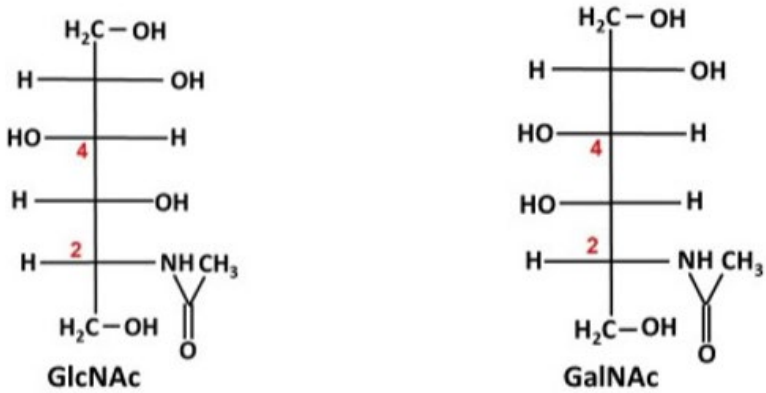


Figure 1. Overview of HexNAc (GlcNAc, left and GalNAc, right) isomers investigated with this study. The structure of each isomer is shown in their A) non-reduced and B) reduced (linear) form.

Reductive beta-elimination was used to reduce the two HexNAc compounds to their corresponding alditol forms using an established protocol.[4] In short, the reductive beta-elimination was performed by adding 10 µL 1 M sodium borohydride solution (freshly made in 50 mM potassium hydroxide) to aliquots of the HexNAc compounds, which were then incubated at 50 °C overnight. After quenching the reaction with 2 µL glacial acetic acid, the reduced GlcNAc and GalNAc were desalted on custom-made ion exchange SPE columns. The desalted samples were dried in a SpeedVac™, dissolved in 150 µL methanol and dried again to remove any remaining borate from the reduction procedure. Finally, the reduced monosaccharides were dissolved in 50 µL Milli-Q® water for PGC LC-MS analysis using a high resolution 6538 Q-ToF mass spectrometer (Agilent) connected to an Agilent 1260 HPLC system and using a modified version of a previously used method (see **Table 1**). The corresponding non-reduced GalNAc and GlcNAc samples were analysed (both individually and in mixtures i.e. 1:1, 2:1 and 4:1 molar ratios of GlcNAc:GalNAc) directly on the same system after ion exchange SPE desalting as per above.

Table 1. Chromatographic conditions used in this study

LC Conditions	
Instrument:	Agilent 1260 HPLC
Column:	Supel™ Carbon LC, 150 x 1.0 mm I.D., 2.7 µm
Mobile phase:	A: 10 mM ammonium bicarbonate in water, pH 8 B: 10 mM ammonium bicarbonate in 70% acetonitrile
Gradient:	Time (min) A% B% 0 97 3 15 97 3 26 94 6 28 0.0 100 40 0.0 100 42 100 0.0 60 100 0.0
Flow rate:	20 µL/min
Pressure:	86 bar (at 97% solvent A)
Column temp.:	50 °C
Detector:	Q-ToF MS (high resolution, see below)
Injections:	1 µL (equivalent to 1.8 nmol for both reduced and non-reduced GlcNAc and GalNAc or dilution thereof of GalNAc for the mixtures)
Sample(s):	GalNAc, GlcNAc (reduced and non-reduced forms)
MS Conditions	MS1
Instrument:	Agilent 6538 UHD Accurate-Mass Q-ToF LC-MS system
Polarity:	ESI (-)
Spray voltage:	4,300 V
Capillary temp:	300 °C
Sheath gas:	20 p.s.i
Aux. gas:	3.5 L/min
m/z range:	150-2200 (a typical m/z range used in glycomics)

The retention behaviours of both reduced and non-reduced GalNAc and GlcNAc were investigated separately and in defined mixtures on a Supel™ Carbon LC column provided by Merck KGaA, Darmstadt, Germany. The reduced and non-reduced HexNAcs were monitored by performing extracted ion chromatograms (EICs) at m/z 220.0-220.4 and 222.0-222.4 corresponding to the expected molecular mass of the non-reduced and reduced HexNAc isomers, respectively.

Results & Discussion

The analysis of non-reduced and reduced GalNAc and GlcNAc samples on the Supel™ Carbon LC column are shown in **Figure 2-6**, **Figure 8-12**, and **Table 2**. The individual results are separately discussed below. While the chromatography, for unexplained reasons, was consistently poor for the non-reduced HexNAcs (poor peak capacity, broad peaks, weak MS signals) relative to the reduced counterparts, the generated data show that both non-reduced and reduced analytes are all reproducibly retained on the PGC-LC column as demonstrated by triplicate chromatographic runs of all samples (technical triplicates at the injection level).

The retention times of the non-reduced GlcNAc (18.5 min) and GalNAc (21.1 min) differed significantly on the Supel™ Carbon LC column, **Figure 2** and **Figure 3**. While the retention characteristics were highly reproducible, both the non-reduced GlcNAc and GalNAc analytes displayed very broad peaks (approximately 2 min peak width at half peak height) and relatively weak MS signals relative to their reduced counterparts, see below. The differential retention behaviour of non-reduced GlcNAc and GalNAc moieties suggests that the position of the -OH group linked to the fourth carbon atom in the pyranose ring (representing the structural difference between GalNAc and GlcNAc, **Figure 1A)** has a significant effect on the interaction with the PGC column.

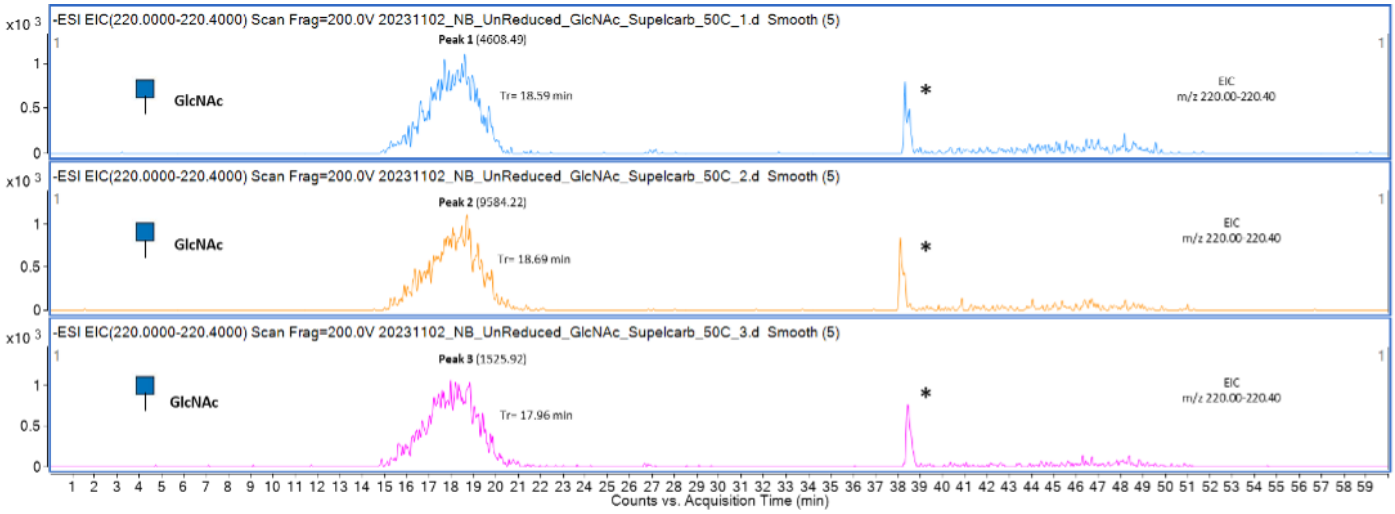


Figure 2. Retention behaviour of non-reduced GlcNAc (1.8 nmol/injection) on the Supel™ Carbon LC column as illustrated using extracted ion chromatograms (EICs) of the expected molecular mass of non-reduced GlcNAc (m/z 220.0-220.4). Blue squares: GlcNAc. Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] Numbered peaks across the chromatograms (peaks 1-48) are summarised in **Table 2**. *Unspecific analyte interference (not a monosaccharide). Tr denotes the retention time at the apex of the analyte elution peak, which, due to weak MS signals, varied considerably even though reproducible retention characteristics were observed over the three technical replicates.

Despite reproducible retention across the triplicate chromatographic runs, the performance of the chromatography and MS was significantly weaker than the performance observed for the reduced HexNAc species, see below. To illustrate the compromised MS behaviour of the non-reduced HexNAcs, the average peak area (area under the curve) of the non-reduced GlcNAc and GalNAc were 5,239 and 9,044 at 1.8 nmol analyte mass, respectively, which was significantly lower (three orders of magnitude) compared to the corresponding peak areas of the matching, reduced species applied in identical amount (1,078,238 and 1,843,204, respectively). This difference can be attributed to a weaker ionisation potential of the non-reduced HexNAcs relative to the reduced species and the signal splitting across multiple analyte species for the non-reduced HexNAcs. Given the fact that non-reduced saccharides are known to exhibit multiple structural conformers due to mutarotation, giving rise to both the ring-opened form and the ring-closed alpha and beta anomers, then a broader elution pattern of the non-reduced HexNAcs (relative to the reduced counterparts) was expected; however, this may not be sufficient to explain the very poor peak capacity (minute-long elution) for non-reduced GlcNAc and GalNAc moieties on the Supel™ Carbon PGC LC column as observed in this study, which therefore remains partially unexplained.

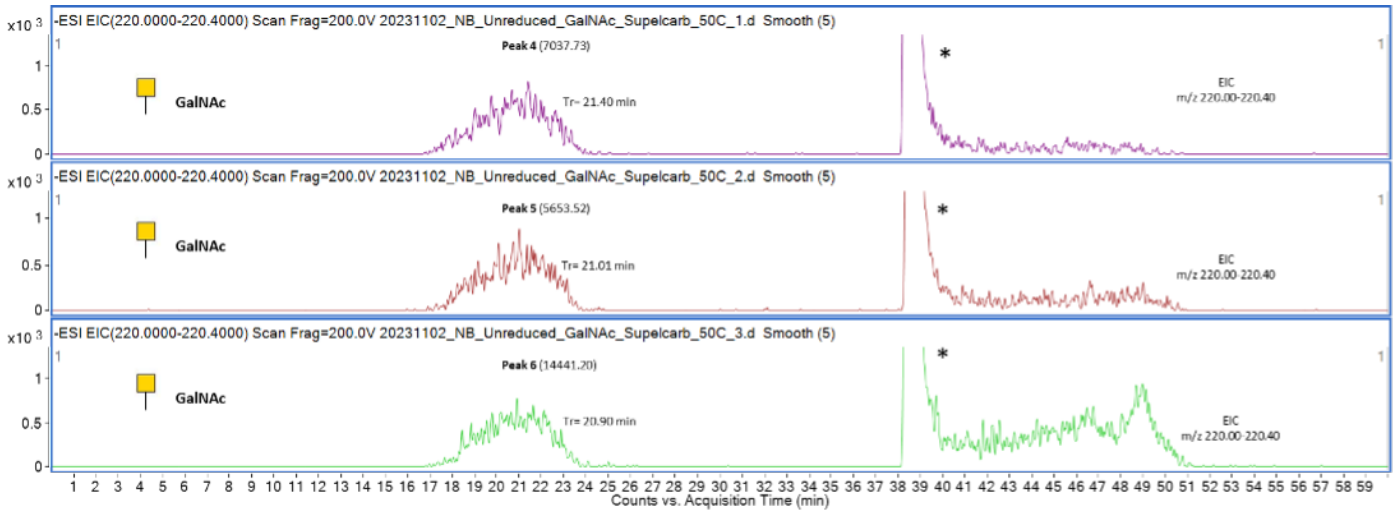


Figure 3. Retention behaviour of non-reduced GalNAc (1.8 nmol/injection) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of non-reduced GalNAc (m/z 220.0-220.4). Yellow squares: GalNAc. Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] All numbered peaks across all chromatograms (peaks 1-48) are summarised in **Table 2**. *Unspecific analyte interference (not a monosaccharide). Tr denotes the retention time at the apex of the analyte elution peak, which, due to weak MS signals, varied considerably despite that reproducible retention was observed over the three technical replicates.

Given their broad chromatographic elution pattern, the analysis of mixtures of non-reduced GlcNAc and GalNAc isomers studied in different molar ratios (4:1, 2:1 and 1:1, GlcNAc:GalNAc) resulted in partially overlapping peaks despite their ~3 min elution difference as measured based on the apex of their peaks, **Figure 4**-**6**. For the mixture with 4:1 molar ratio, the relative peak intensity of the two peaks was found to be approximately 5:1 GlcNAc:GalNAc, demonstrating, therefore, a quantitative profile matching largely the expected 4:1 ratio. In contrast, when the non-reduced HexNAc were studied individually (**Figure 2**-**3**), the non-reduced GalNAc was, for still unknown reasons, found to ionize better than the non-reduced GlcNAc counterpart. Encouragingly, the elution times of the non-reduced GlcNAc (18.4 min) and GalNAc (21.7 min) remained largely unchanged when analysed individually and in mixtures.

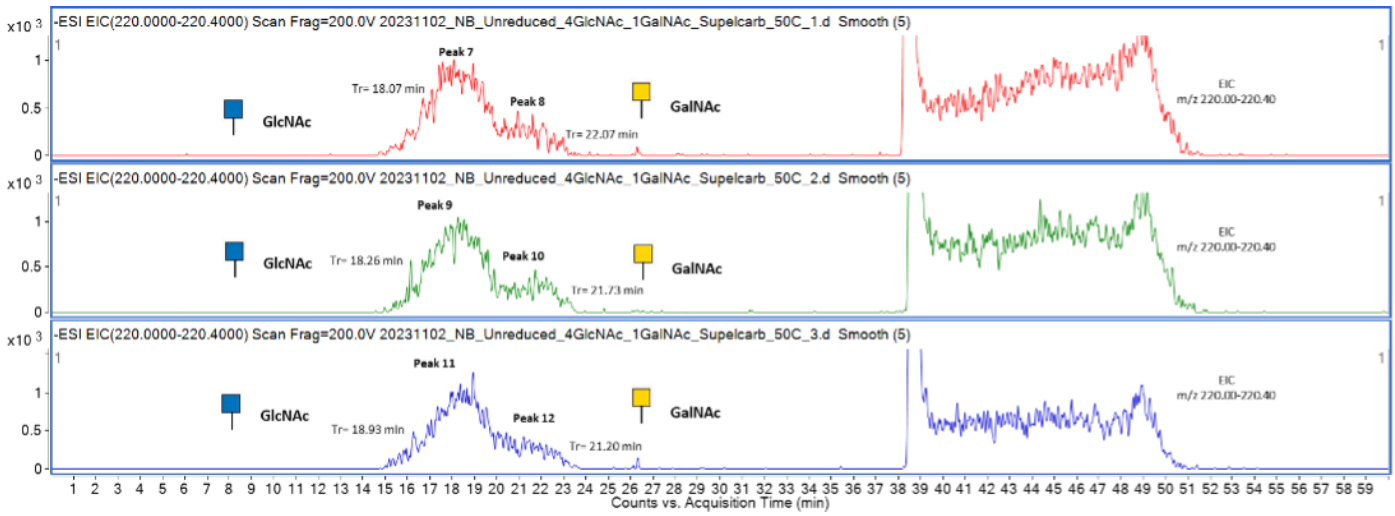


Figure 4. Retention behaviour of non-reduced GlcNAc and GalNAc applied in 4:1 ratio (1.8 nmol non-reduced GlcNAc and 0.45 nmol non-reduced GalNAc) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of non-reduced HexNAc analytes (m/z 220.0-220.4). Yellow squares: GalNAc, Blue squares: GlcNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] Numbered peaks across all chromatograms (peaks 1-48) are summarised in **Table 2**. *Unspecific analyte interference (not a monosaccharide). Tr denotes the retention time at the apex of the analyte elution peaks, which, due to weak MS signals, varied considerably even though reproducible retention was observed over three technical replicates.

The mixture containing a 2:1 GlcNAc/GalNAc molar ratio resulted in similar chromatograms again with partially overlapping peaks resulting from the broad peak shape with an average retention time of 18.3 min for GlcNAc and 21.8 min for GalNAc, **Figure 5**. Like the 4:1 mixture (**Figure 4**), the 2:1 mixture showed a largely quantitative profile of non-reduced GlcNAc and GalNAc (~2.5:1) matching approximately the expected quantitative ratio of the analysed mixture.

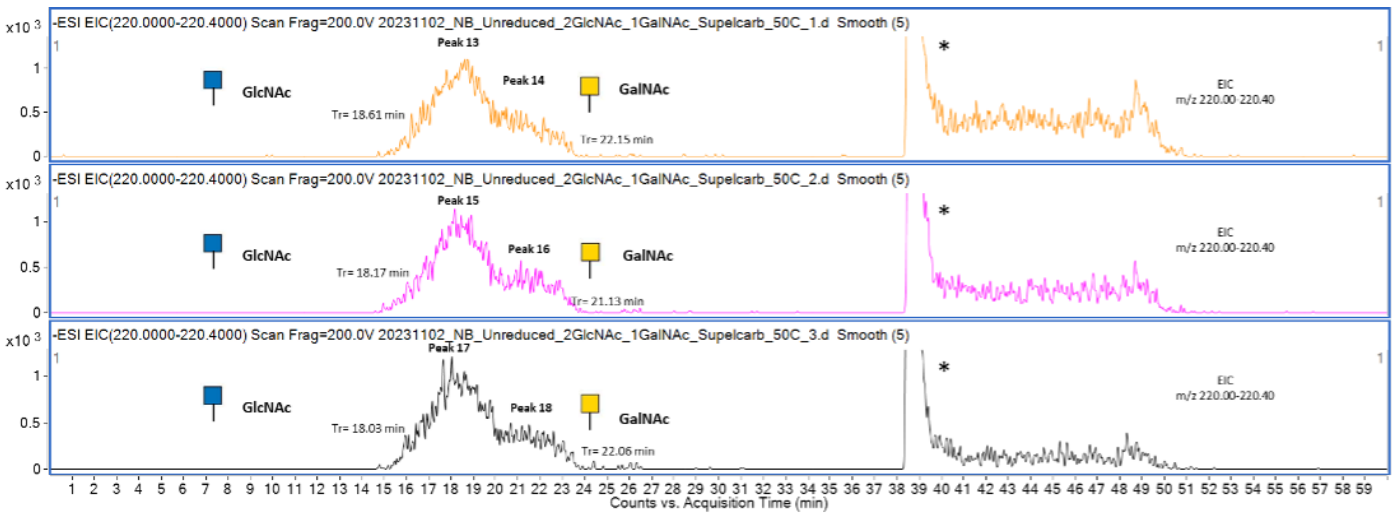


Figure 5. Retention behaviour of non-reduced GlcNAc and GalNAc applied in 2:1 ratio (1.8 nmol non-reduced GlcNAc and 0.9 nmol non-reduced GalNAc) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of the non-reduced HexNAc moieties (m/z 220.0-220.4). Yellow squares: GalNAc, Blue squares: GlcNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] Numbered peaks in all chromatograms (peaks 1-48) are summarised in **Table 2**. *Unspecific analyte interference (not a monosaccharide). Tr denotes the retention time at the apex of the analyte elution peaks, which, due to weak MS signals, varied considerably despite that reproducible retention was observed over the three technical replicates.

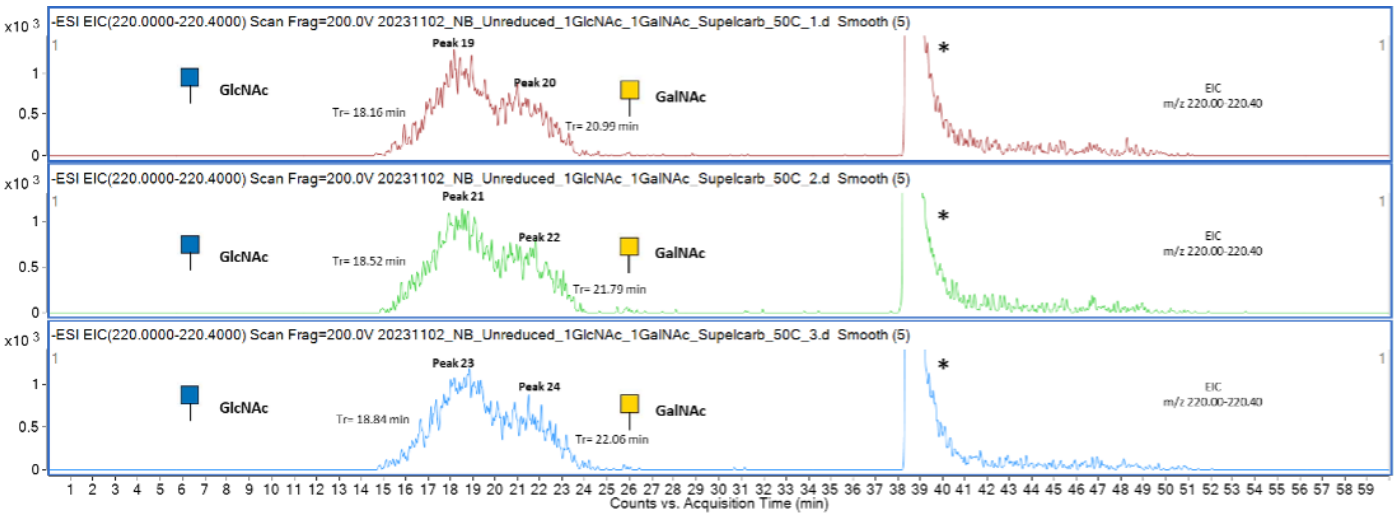


Figure 6. Retention behaviour of non-reduced GlcNAc and GalNAc applied in 1:1 ratio (1.8 nmol non-reduced GlcNAc and 1.8 nmol non-reduced GalNAc) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of the non-reduced HexNAc moieties (m/z 220.0-220.4). Yellow squares: GalNAc, Blue squares: GlcNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] Numbered peaks across all chromatograms (peaks 1-48) are summarised in **Table 2**. *Unspecific analyte interference (not a monosaccharide). Tr denotes the retention time at the apex of the analyte elution peaks, which, due to weak MS signals, varied considerably despite that reproducible retention was observed over the three technical replicates.

Overlapping chromatographic peaks were also observed when the two non-reduced HexNAcs were applied in 1:1 molar ratio on the Supel™ Carbon PGC LC column, **Figure 6**. The broad elution profile, which was reproducible and similar to the 4:1 and 2:1 mixtures, suggests that the non-reduced HexNAc isomers take multiple conformers and that these exhibit different interactions with the PGC LC column resulting in wider peaks. Again, the analysis was largely quantitative showing ~1.5:1 ratio of the non-reduced GlcNAc and GalNAc signal in reasonable agreement with the expected 1:1 ratio of this sample.

Reproducible and chromatographically much better results were obtained with the reduced GlcNAc and GalNAc analytes. As expected, the molecular mass increased by two protons for both reduced HexNAc moieties (m/z 222.0) relative to their non-reduced counterparts (m/z 220.0), **Figure 7**.

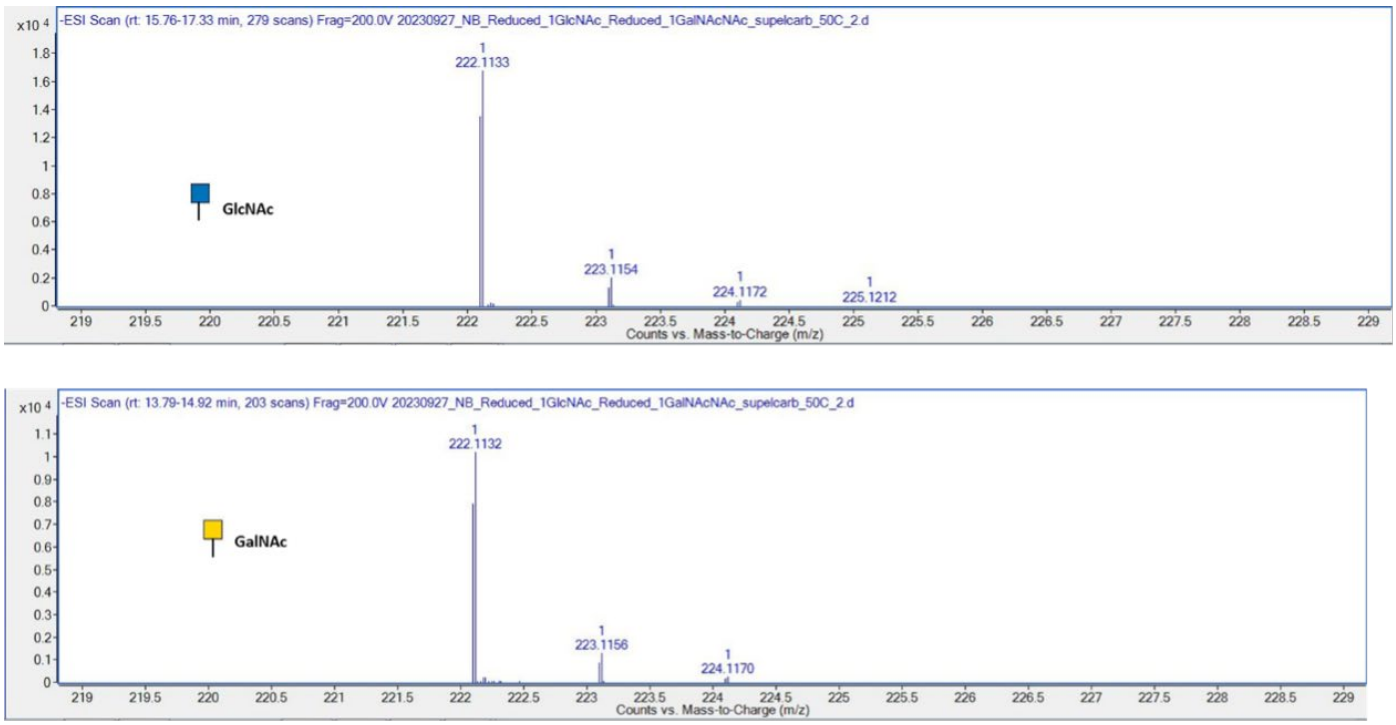


Figure 7. Mass spectra of the reduced GlcNAc (top) and reduced GalNAc (bottom) showing the expected isotopic distribution for the reduced HexNAc species. Blue squares: GlcNAc; Yellow squares: GalNAc. Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] See also **Table 2** for overview of all structures.

The HexNAc reduction significantly shortened the retention of the reduced GlcNAc (16.2 min, relative to 18.4 min for the non-reduced counterpart) and led to markedly sharper peaks, **Figure 8**. Similar changes were observed for the reduced GalNAc (14.1 min), which also eluted well ahead of the non-reduced counterpart (21.1 min) and again with dramatically improved chromatographic peak shapes, **Figure 9**. Both the reduced GlcNAc and GalNAc displayed a high peak capacity (approx. 20-30 s peak width at half peak height) relative to the minute-long elution of the non-reduced species. The shorter retention time and narrow peak shape of the reduced HexNAc isomers can be attributed to an altered electrostatic charge distribution of the single alditol forms created upon saccharide reduction.

The transfer of two protons from the reducing agent (sodium borohydride) is known to result in the reduction of the aldehyde groups on the HexNAc moieties and leads to the formation of sugar alcohols exhibiting a single alditol conformation. This process is accompanied by a ring opening of the pyranose moiety and the formation of linear carbohydrates (**Figure 1B**). These structural changes influence the physicochemical properties of the HexNAc molecules, which seemingly also manifest in their chromatographic behaviour which are observed as reduced retention times and narrow elution characteristics on the Supel™ Carbon PGC LC column (**Figure 8-9**).

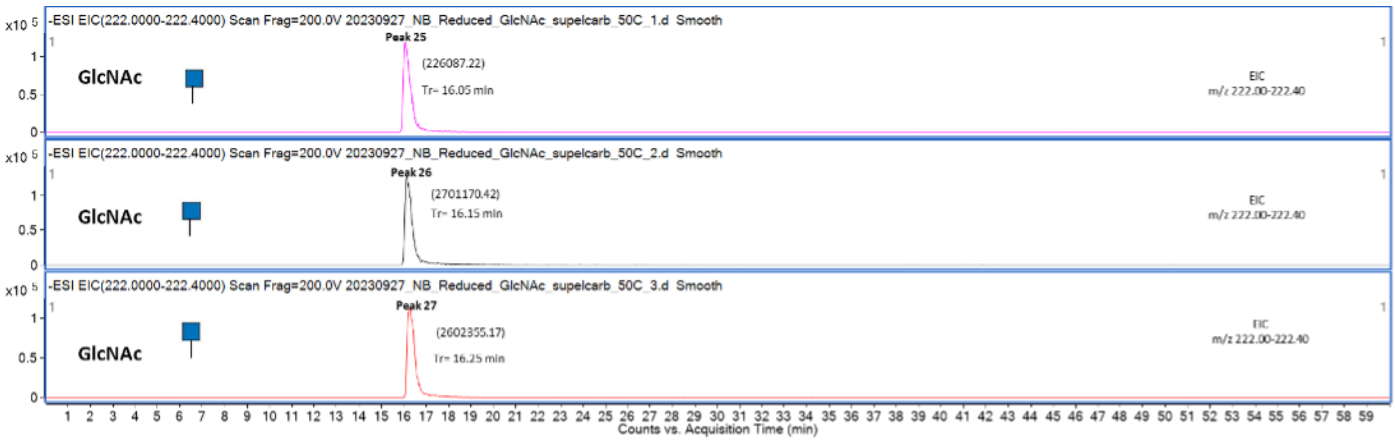


Figure 8. Retention behaviour of reduced GlcNAc (1.8 nmol/injection) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of the reduced HexNAc moieties (m/z 222.0-222.4). Blue squares: GlcNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] See also **Table 2** for overview of all structures and LC retention times of all peaks across the chromatograms (peak 1-48). Tr denotes the retention time at the apex of the analyte elution peaks.

Contrary to the analyses of the non-reduced HexNAcs, which showed a greater retention of non-reduced GalNAc over non-reduced GlcNAc, the reduced GlcNAc (~16.15 min, **Figure 8**) exhibited a greater retention than the reduced GalNAc (14.05 min, **Figure 9**). The retention time difference between the two reduced HexNAc isomers was about 1.85 min indicating that the spatial location of the -OH group on the third carbon atom in the linear (reduced) molecule has considerable effect on the interaction of the two isomers with the Supel™ Carbon LC column and that these interactions differ from the non-reduced counterparts. Importantly, highly reproducible and relatively symmetrical peak patterns were consistently observed for both the reduced GlcNAc and GalNAc.

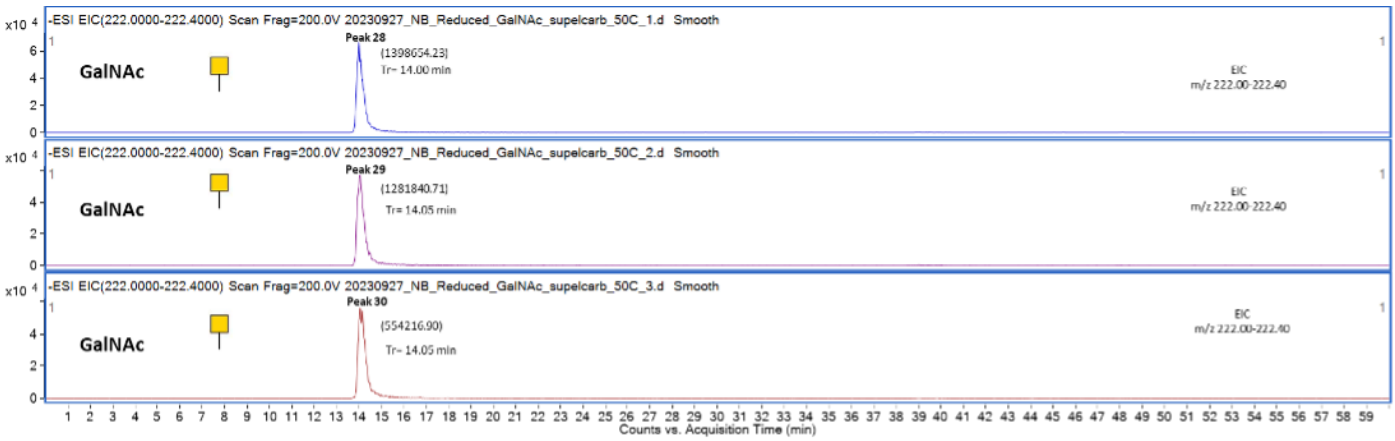


Figure 9. Retention behaviour of reduced GalNAc (1.8 nmol/injection) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of the reduced HexNAc moieties (m/z 222.0-222.4). Yellow squares: GalNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] See also **Table 2** for overview of all structures and LC retention times of all peaks (peak 1-48). Tr denotes the retention time at the apex of the analyte elution peaks.

Analysis of mixtures containing defined amounts of reduced GlcNAc and GalNAc revealed that the retention times of the reduced species was identical to those observed during the individual analysis of reduced GlcNAc (14.4 min) and reduced GalNAc (16.2 min), **Figure 10-12**. Contrary to the largely quantitative data obtained with the mixtures of non-reduced GlcNAc and GalNAc, we found that the reduced GlcNAc analyte was favoured (over-represented) when in a mixture with reduced GalNAc (approximately 12:1) as compared to the expected 4:1 molar ratio for the studied sample, **Figure 10**. A better ionisation was also observed when analysing reduced GlcNAc alone relative to reduced GalNAc, but the molecular basis for this bias remains otherwise unexplained at this stage.

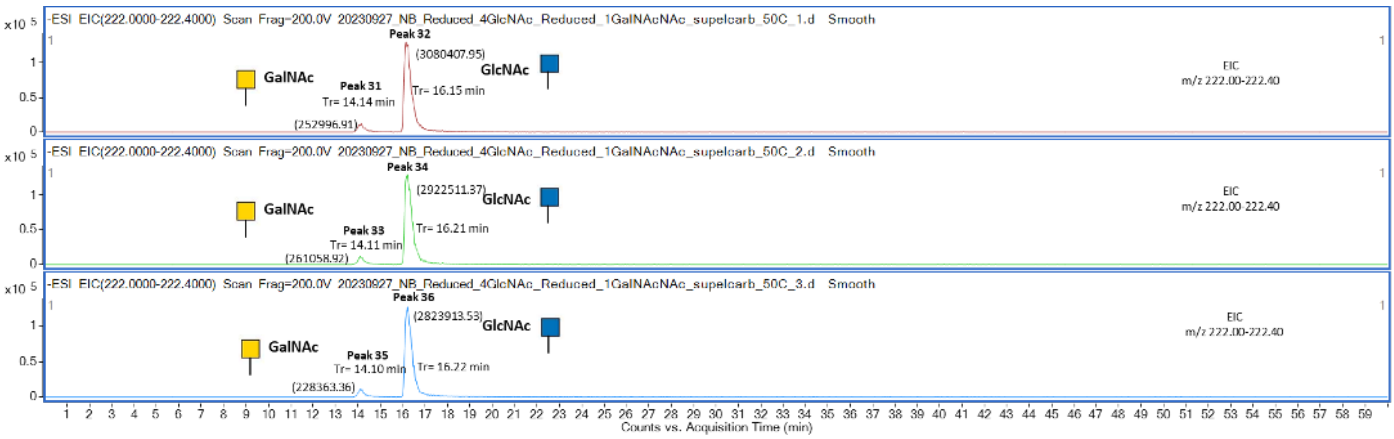


Figure 10. Retention behaviour of reduced GlcNAc and GalNAc applied in 4:1 ratio (1.8 nmol reduced GlcNAc and 0.45 nmol reduced GalNAc) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of the reduced HexNAc moieties (m/z 222.0-222.4). Blue squares: GlcNAc, yellow squares: GalNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] See also **Table 2** for overview of all structures and LC retention times of all peaks across the chromatograms (peak 1-48). Tr denotes the retention time at the apex of the analyte elution peaks.

Consistently, the 2:1 mixture containing 1.8 nmol GlcNAc and 0.9 nmol GalNAc showed a 16.3 min average retention time for reduced GlcNAc and 14.1 min average retention time for reduced GalNAc, **Figure 11**. These profiles were also consistent with the quantitative bias towards reduced GlcNAc over reduced GalNAc (in this sample ~5.5:1 whereas a 2:1 ratio was expected).

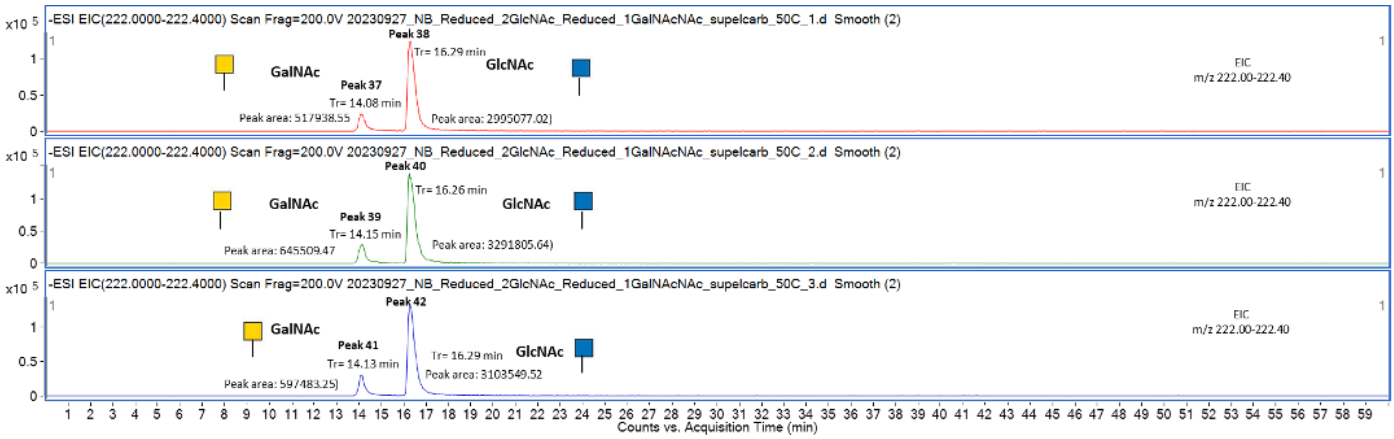


Figure 11. Retention behaviour of reduced GlcNAc and GalNAc mixed in a 2:1 ratio (1.8 nmol reduced GlcNAc and 0.9 nmol reduced GalNAc) on the Supel™ Carbon PGC LC column as illustrated using EICs of the expected molecular mass of the reduced HexNAc moieties (m/z 222.0-222.4). Blue squares: GlcNAc, yellow squares: GalNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans.[5] See also **Table 2** for overview of all structures and LC retention times of all peaks across the chromatograms (peak 1-48). Tr denotes the retention time at the apex of the analyte elution peaks.

In the 1:1 mixture, the reduced GlcNAc and GalNAc analytes also exhibited reproducible elution times of 16.3 min and 14.1 min, respectively, **Figure 12**. In this sample containing equimolar amounts of GlcNAc and GalNAc, we found the quantitative signal to be ~2.5:1 of GlcNAc:GalNAc confirming the quantitative bias towards GlcNAc when analysing mixtures of the reduced HexNAc species under the investigated conditions.

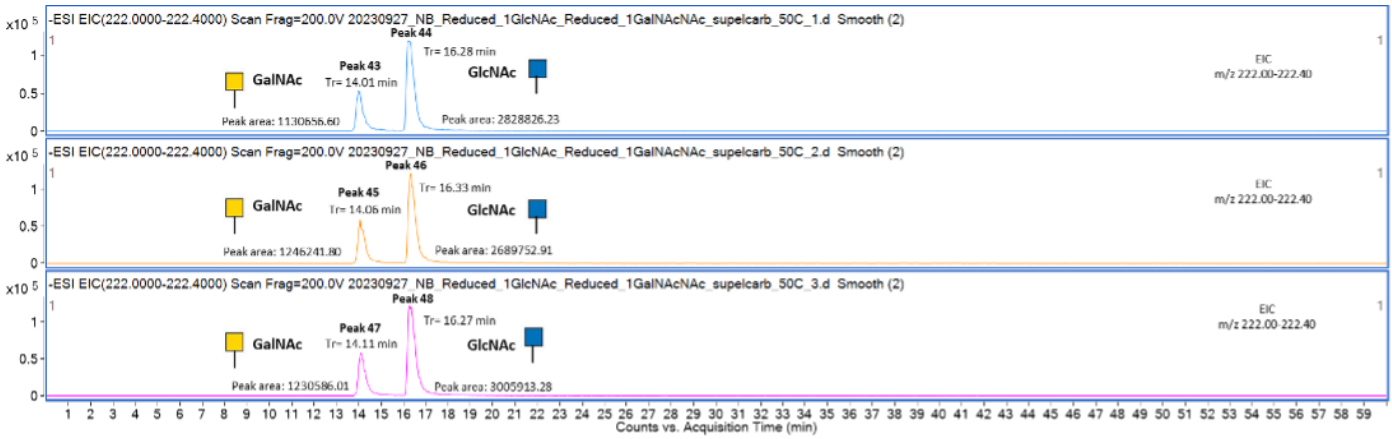


Figure 12. Retention behaviour of reduced GlcNAc and GalNAc applied in 1:1 ratio (1.8 nmol reduced GlcNAc and 1.8 nmol reduced GalNAc) on the Supel™ Carbon LC column as illustrated using EICs of the expected molecular mass of the reduced HexNAc moieties (m/z 222.0-222.4). Blue squares: GlcNAc, yellow squares: GalNAc; Cartoons are depicted according to the latest Symbol Nomenclature for Glycans[5]. See also **Table 2** for overview of all structures and LC retention times of all peaks across the chromatograms (peak 1-48). Tr denotes the retention time at the apex of the analyte elution peaks.

Table 2. Overview of retention times and m/z values of non-reduced and reduced GlcNAc and GalNAc

LC Peak	Glycan Structure	Retention Time (min)	m/z
1-3	Non-reduced GlcNAc	18.6, 18.7, 18.0	220.0
4-6	Non-reduced GalNAc	21.4, 21.0, 20.9	220.0
7, 8, 9, 10, 11, 12	Non-reduced GlcNAc/GalNAc 4:1	18.1, 22.1, 18.3, 21.7, 18.9, 21.2	220.0
13, 14, 15, 16, 17, 18	Non-reduced GlcNAc/GalNAc 2:1	18.6, 22.2, 18.2, 21.1, 18.0, 22.1	220.0
19, 20, 21, 22, 23, 24	Non-reduced GlcNAc/GalNAc 1:1	18.2, 21.0, 18.5, 21.8, 18.8, 22.1	220.0
25, 26, 27	Reduced GlcNAc (1.8 nmol)	16.1, 16.2, 16.3	222.0
28, 29, 30	Reduced GalNAc (1.8 nmol)	14.0, 14.1, 14.1	222.2
31, 32, 33, 34, 35, 36	Reduced GlcNAc/GalNAc 4:1	14.1, 16.2, 14.1, 16.2, 14.1, 16.2	222.2
37, 38, 39, 40, 41, 42	Reduced GlcNAc/GalNAc 2:1	14.8, 16.3, 14.2, 16.3, 14.1, 16.3	222.2
43, 44, 45, 46, 47, 48	Reduced GlcNAc/GalNAc 1:1	14.0, 16.3, 14.1, 16.3, 14.1, 16.3	222.2

Conclusion

We have here investigated the retention behaviour of two non-reduced and two reduced HexNAc isomers (i.e. GalNAc and GlcNAc) and studied the separation of these biologically relevant monosaccharides in various mixtures on a Supel™ Carbon LC column. We have demonstrated that the Supel™ Carbon LC column is able to consistently retain all four monosaccharide species in a highly reproducible manner. The data indicated that separation was effectively achieved for the GlcNAc and GalNAc in their reduced form (narrow peak width) under the tested conditions that are compatible with typical glycomics applications. While the non-reduced HexNAc species were reproducibly retained and exhibited different retention characteristics, their broad elution pattern and poor MS signal response resulting from their different conformers meant that these species were not well separated and profiled on the Supel™ Carbon LC column, which therefore requires further optimisation.

These observations document the interesting and rather unique separation potential of the Supel™ Carbon LC column for the profiling of biologically important monosaccharides which therefore promise to expand our analytical capabilities for studying a more complete population of glycans within the glycome and thus yield new quantitative insights into the extreme structural diversity and functional roles of the human glycome.

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Product List

Description	Cat. No.
Featured Products	
Supel™ Carbon LC, 150 x 1.0 mm I.D., 2.7 µm	**59978-U**
GalNAc	**A2795-10MG**
GlcNAc	**A8625-10MG**
Solvents and Reagents	
Acetonitrile, hypergrade for LCMS LiChrosolv®	**1.00029-2.5L**
Ammonium bicarbonate	**11213-1KG-R**
Methanol LiChrosolv®	**1.06007.4000**
Sodium borohydride	**213462-25G**

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