Polysialylated N-Glycans Identified in Human Serum Through Combined Developments in Sample Preparation, Separations and ESI-MS

Scott R. Kronewitter, Ioan Marginean, Jonathan T. Cox, Rui Zhao, Clay D. Hagler, Anil K. Shukla, Timothy S. Carlson, Joshua N. Adkins, David G. Camp II, Ronald J. Moore, Karin D. Rodland, Richard D. Smith*

Biological Sciences Division, Pacific Northwest National Laboratory, P.O. Box 999, Richland, Washington, 99352

*Corresponding author: rds@pnnl.gov

The following supplementary text sections S1-2 are included below

S1: Two Stage Desalting
S2: Acid Gradient Chromatography
S3: Supplementary Table 1 Caption: Glycan Library
S4: Supplementary Table 2 Caption and Headers: Human Serum Glycans

References

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S1: Two Stage Desalting

Sample desalting was performed as the final step to consolidate peaks because multiply-charged glycans can have a mixture of protons and cation adducts that can further divide the signal and complicate analysis. Sodium-proton exchanges are common (especially on sialic acid) when sodium is present\(^1\) and ammoniated adducts are detected when ammonia compounds are used in sample preparation (most likely from ammonia borane reduction).\(^2\) Solid phase extraction was used as the initial desalting step to remove the bulk of the buffer/matrix salts, and small molecules. The glycans were absorbed onto the graphite stationary phase and desalted by washing with a relatively large amount of water (10 mL) considering the initial sample was 100 µL in volume. The final desalting was performed on-column in a preparatory step prior to the analytical gradient and subsequent SPIN-MS analysis. The end product after ionization was effectively sodium free and the glycans were detectable predominantly in a protonated form across many charge states. Figure S1 shows the 2+ charge state of \(\text{Hex}_3\text{HexNAc}_4\text{FucNeu5Ac}\) at \(m/z\) 1040.89 primarily in the fully protonated form.

\(^{1}\) Sodium-proton exchanges are common (especially on sialic acid) when sodium is present.

\(^{2}\) Ammoniated adducts are detected when ammonia compounds are used in sample preparation (most likely from ammonia borane reduction).
S2: Acid Gradient Chromatography

The reduction step added the smallest mass possible to the reducing end (a hydrogen atom) and allowed for the maximum separation of the native glycans by HPLC because the branched portion of the glycans generally dictates separation behavior rather than a bulky, reducing end chemical modification.

Graphitized carbon stationary phase material, considered one of the best performing materials for glycan separation with regards to resolution and robustness, was utilized in this study. An example of the separation of the high performing graphite columns was shown with the α, β ring anomer conformations of the unreduced 6’ isomer of sialyllactose. The EIC of sialyllactose is presented in Figure S3, m/z 634.22.

However, large acidic glycans were typically challenging to elute from graphite columns using standard AcN/water reversed phase gradients with 0.1% formic acid. While formic acid as the ion-pairing reagent generally provided better conventional electrospray sensitivity, trifluoroacetic acid (TFA), when added to the mobile phase, typically produced sharper chromatographic peaks that can offset its lower ionization efficiencies. A further benefit of TFA as the ion-pairing reagent was observed when eluting acetic glycans during solid phase extraction. To balance all of these benefits and disadvantages, an AcN/water hybrid reversed phase gradient was developed to include an acid gradient that started with 0.1% formic acid and ended with 0.2% TFA. This strategy provided optimal sensitivity and electrospray performance for early eluting glycans (neutral glycans) and effectively desorbed the later eluting acidic glycans as the TFA composition increased.
**Supplementary Table 1 Caption: Glycan Library**

This table represents the glycan library used to populate the targets in GlyQ-IQ. The key columns required are the empirical Formula, Monoisotopic Mass, glycan code and a unique target ID. The glycan code corresponds to the number of monosaccharides in the target (hexose – N-Acetylhexosamine – Fucose – Neu5Ac – Adduct). For example, if a sodiated mass is to be searched, the adduct integer needs to be increased to 1 and the empirical formula is updated by removing a hydrogen and adding a sodium. In this case, the target ID is a numerical representation of the glycan code where 2 digits are reserved for each monosaccharide and adduct.

**Supplementary Table 2 Caption: Human Serum Glycans**

GlyQ-IQ results from a search with the human serum retrosynthetic glycan library targets list. The “Compositions” page included GlyQ-IQ results where the most abundant isomer for each composition is reported. The “Isomers” page reports all the detailed isomer information corresponding to chromatographically separated glycan isomer peaks. Column Definitions are as follows:

- **Target ID**: A numerical glycan code where 2 digits correspond to each monosaccharide count in the glycan code.
- **Glycan Code**: A string representation of the glycan composition with the following order (Hexose – N-acetylhexosamine – Fucose – NeuAc – Adduct).
- **Empirical Formula**: Elemental formula of the glycan.
- **Charge State**: Detected charge state of the glycan
- **Mono Mass Theoretical**: Calculated mass of the monoisotopic mass of the glycan
- **m/z Theoretical**: Calculated mass to charge ratio of the monoisotopic mass of the glycan based on the charge state
- **Mono Mass Observed**: Observed monoisotopic mass of the glycan calculated from
- **m/z Observed**: Observed mass to charge ratio of the monoisotopic peak in the data
- **Elution Time Observed**: Centroided elution time of the glycan in the dataset
- **Scan Number**: Centroided MS scan of the glycan in the dataset. Signal averaging is centered around this scan
- **Abundance**: MS abundance from signal averaged scan
- **Insource Fragmentation Correlation Score**: Correlation between the target and the insource fragment or 1-monosachardide larger glycan.
- **Name**: Name of monosaccharide difference corresponding to the ions correlated.
- **Parent Area**: MS abundance of the correlated ion from the signal averaged scan
- **LC Peak LM_R2**: Coefficient of determination Gaussian peak model fit to the smoothed data
- **Global Charge State Min**: lowest charge state observed in the dataset
- **Global Charge State Max**: highest charge state observed in the dataset
Global Aggregate Abundance: summed abundance corresponding to the area under the fit elution profiles including all charge states detected.

Confidence: Number of GlyQ-IQ variates used in the annotation. “4-var” includes (Accurate Mass, Isotope Profile Fit, Elution Profile Fit and Glycan Family Relationships. “5-var” includes the 4-var metrics in addition to the insource fragmentation detection and validation. “Fragment” includes the 4-var metrics and the identification of the candidate target as a fragment using insource fragmentation correlation information.

Average Mono Mass: averaged monoisotopic mass calculated from one or more charge states detected.

PPM: parts-per-million mass accuracy calculated from the observed averaged monoisotopic mass and the calibrated theoretical mass.

Iso Fit Score: Fit score indicating the goodness of fit of the observed isotope profile and its model.

Charge State Correlation: Correlation between extracted ion chromatograms from multiple charge states when detected.