

GIVING ION MOBILITY TO THE MASSES: WHAT COLLISIONAL-CROSS SECTION VALUES CAN DO FOR GLYCAN ANALYSIS OF BIOTHERAPUTIC MONOCLONAL ANTIBODIES

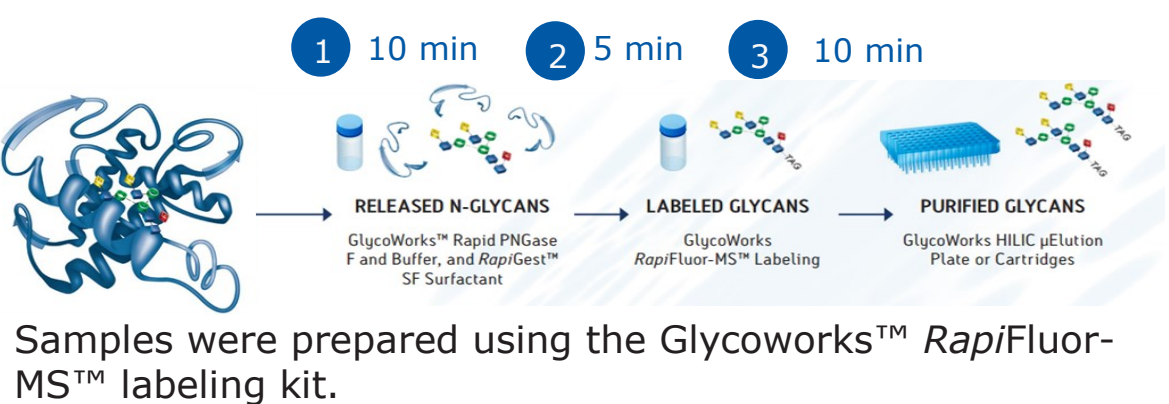
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INTRODUCTION

Glycan analysis is a key area of research in many scientific disciplines, including the health sciences and the biopharmaceutical industry. The path to structural characterization requires careful navigation in data analysis and is complicated by the multiple isomeric possibilities for a single composition. Glycan characterization frequently employs multiple analytical techniques and often relies on mass spectrometry (MS) and tandem MS, the latter requiring significant skill in spectral interpretation and is a laborious process and may require additional confirmatory experiments (e.g. exoglycosidase digestions). Here, we present the use of ion mobility (IMS)-MS data to improve glycan identification when performing LC retention time- (normalized in glucose units) based scientific library searches.¹

- Key Words**
- IMS: Ion mobility spectroscopy
 - RFMS: *RapiFluor*-MS™
 - GU: Glucose unit
 - CCS: Collision cross section
 - rCC: reduced CCS
 - HDMS: High Definition MS

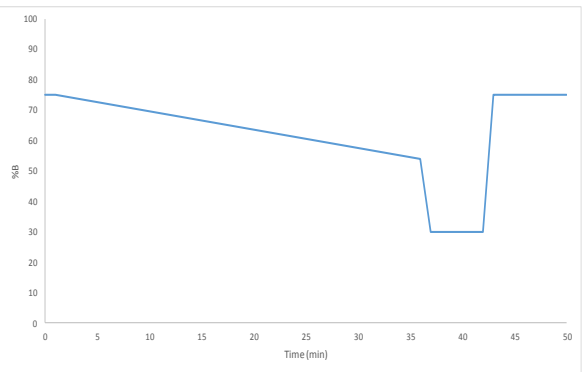
SAMPLE PREPARATION



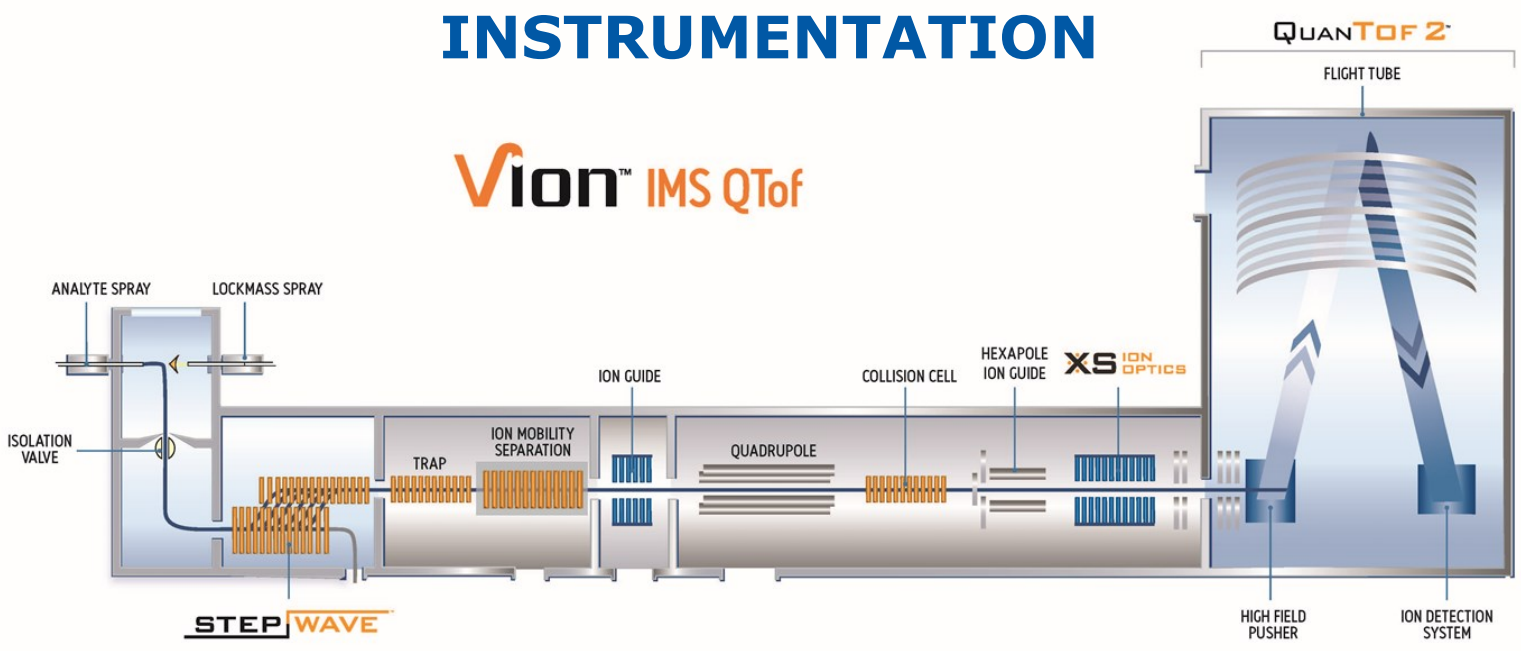
INSTRUMENT CONDITIONS

| | | | |
|----------------------------|--|--|--|
| LC Conditions | | | |
| LC System: | | | |
| LC Column: | | | |
| | | | |
| Column Temp.: | | | |
| Sample Temp.: | | | |
| Fluorescence Detection: | | | |
| | | | |
| Gradient Conditions | | | |
| Mobile Phase A: | | | |
| Mobile Phase B: | | | |

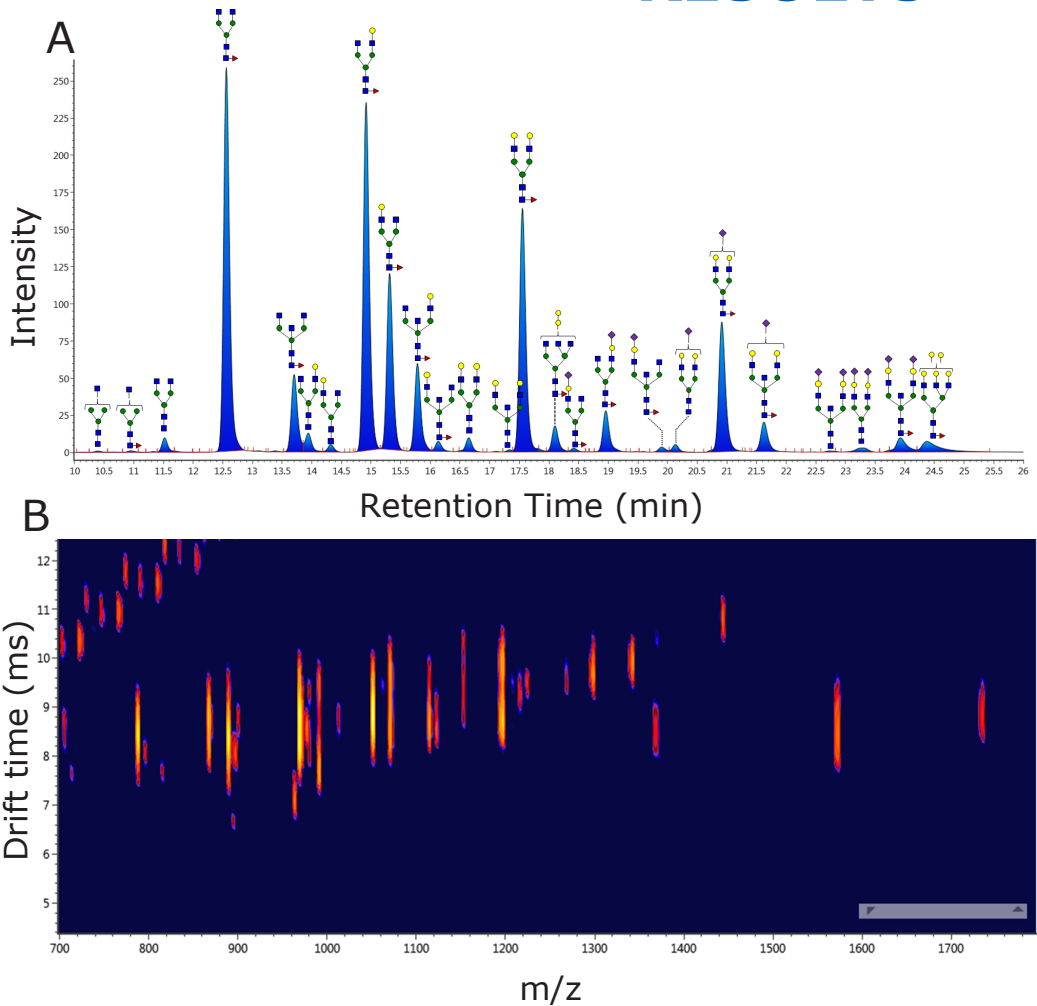
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|----------------------|--|--|--|
| MS Conditions | | | |
| MS System: | | | |
| Analyzer Mode: | | | |
| | | | |
| Capillary Voltage: | | | |
| Cone Voltage: | | | |
| <i>m/z</i> Range | | | |
| Scan Rate: | | | |
| Acquisition: | | | |
| Instrument Control: | | | |
| Informatics: | | | |
| | | | |
| Scientific Library: | | | |



INSTRUMENTATION



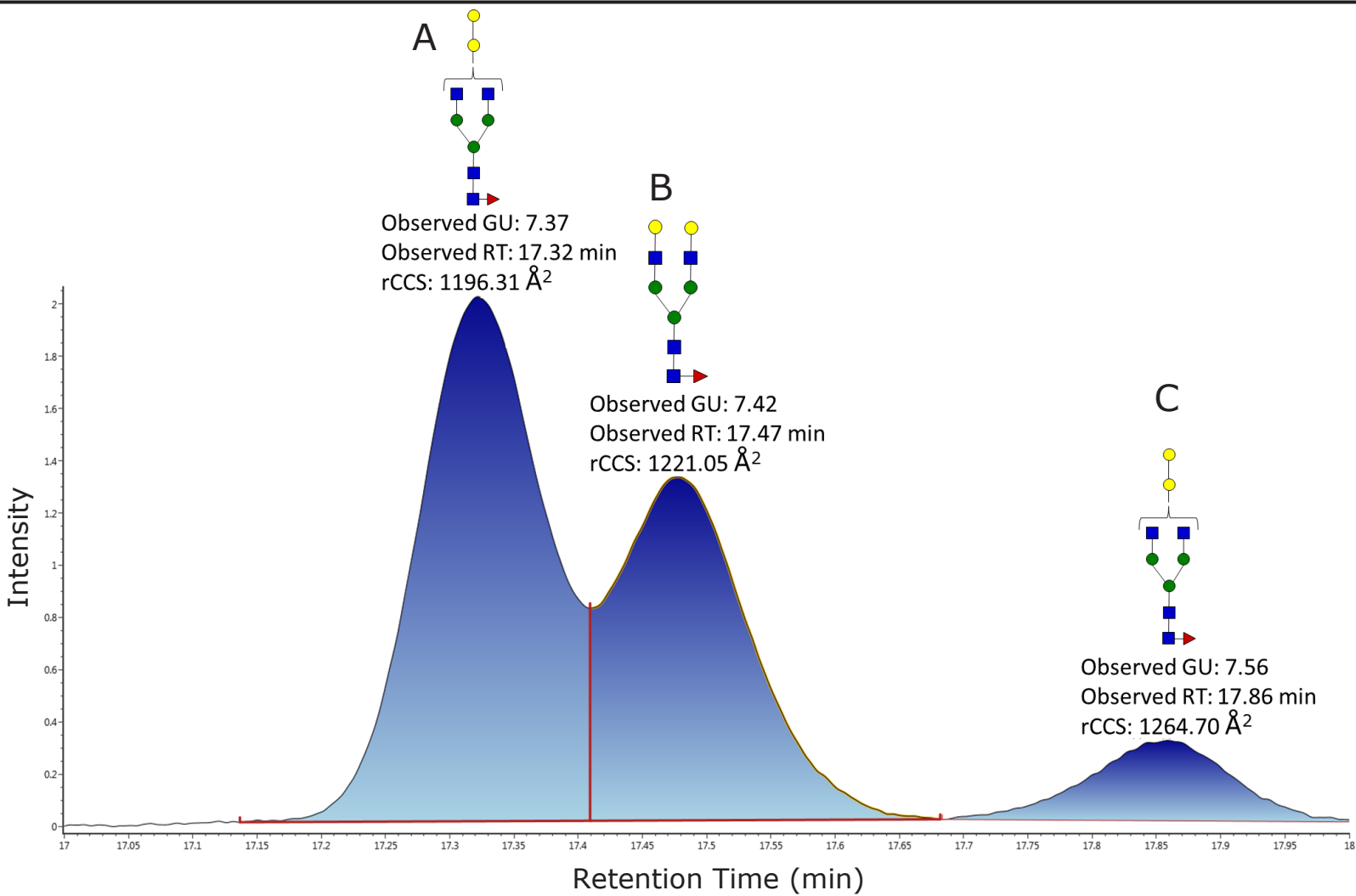
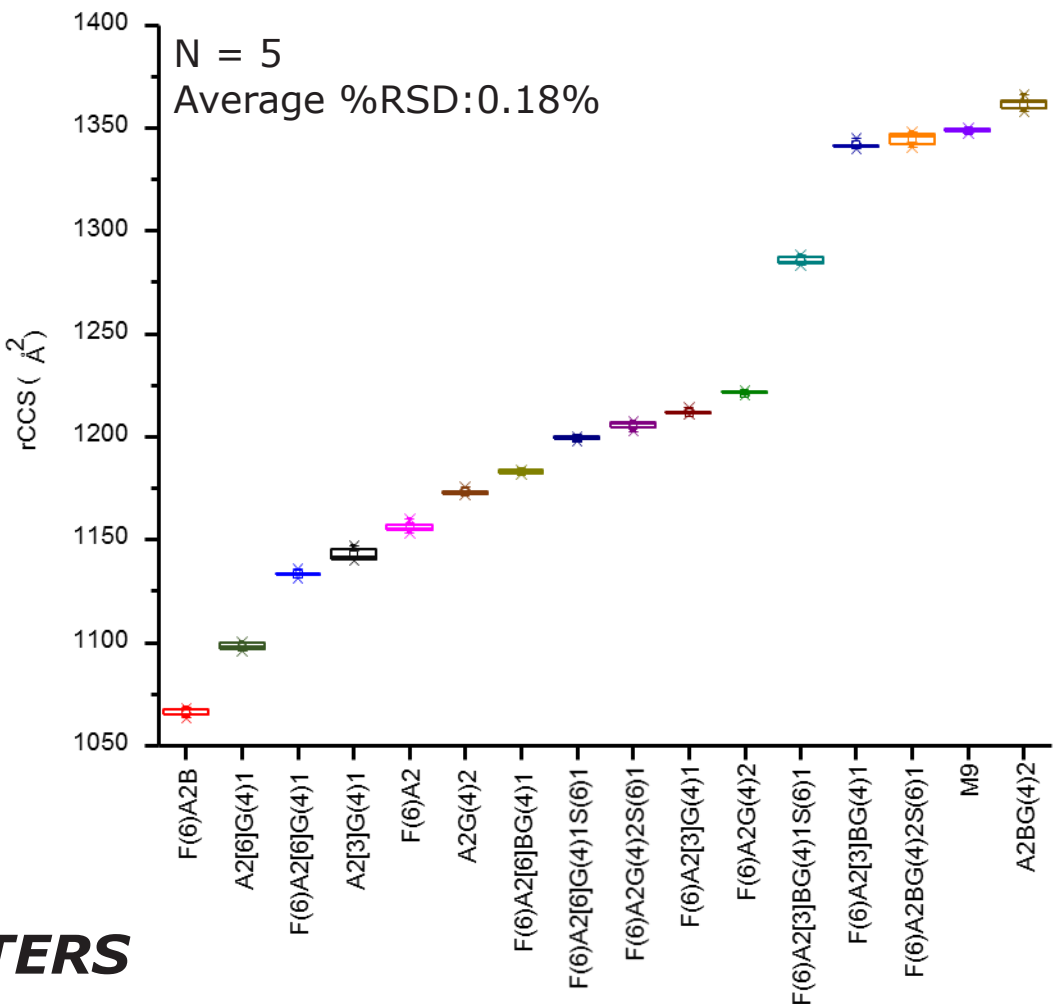
RESULTS



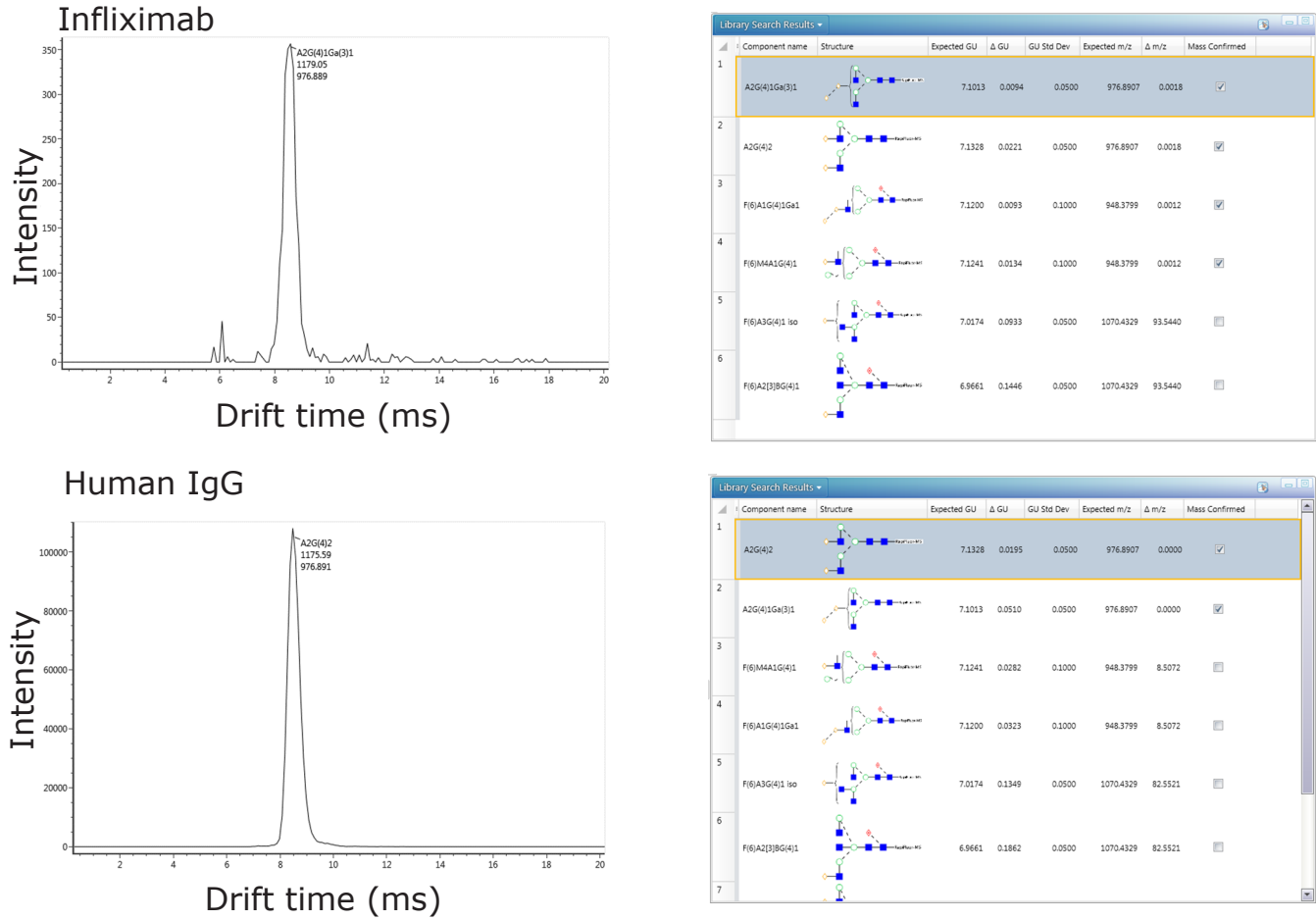
We identified 25 mass confirmed RFMS-labeled glycans from pooled human IgG using our Glucose Unit library, shown in Panel A. Panel B shows a plot of *m/z* vs drift time. The majority of the ions were doubly-charged and had drift times between 6.5-12.5 ms. From these drift times, Unifi automatically calculated collision cross sections (CCS) and reduced CCSs (rCCS) values, based on a calibration using a polyalanine ladder.

rCCS values were used due to their charge-independent nature and were calculated by $CCS = (rCCS * \mu^{1/2})/z$ where $\mu = (M_i * M_n) / (M_i + M_n)$, $M_i = m/z * z$, and $M_n =$ Drift gas mass

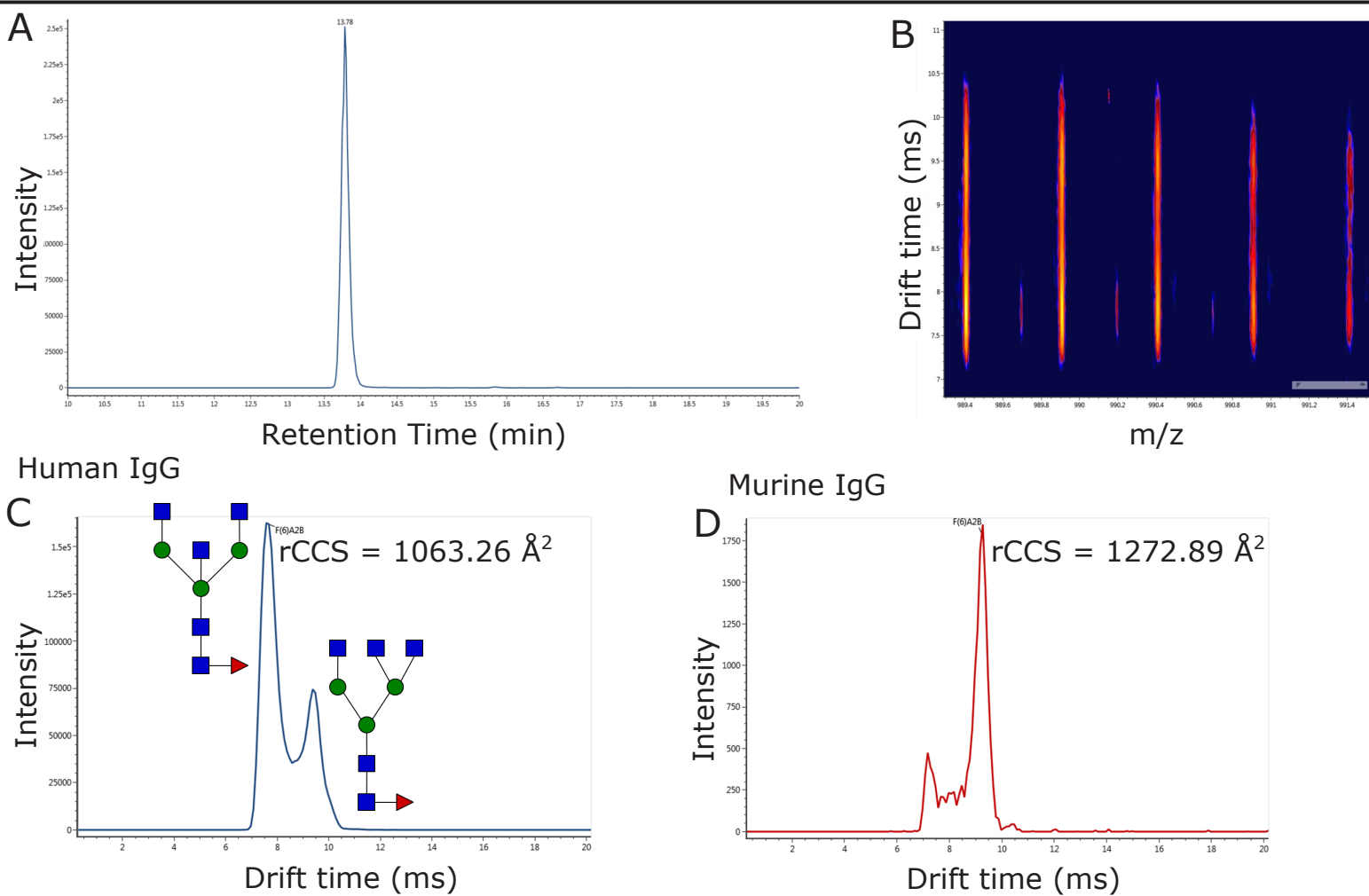
Reproducibility of Glycan Reduced Collision Cross Sections



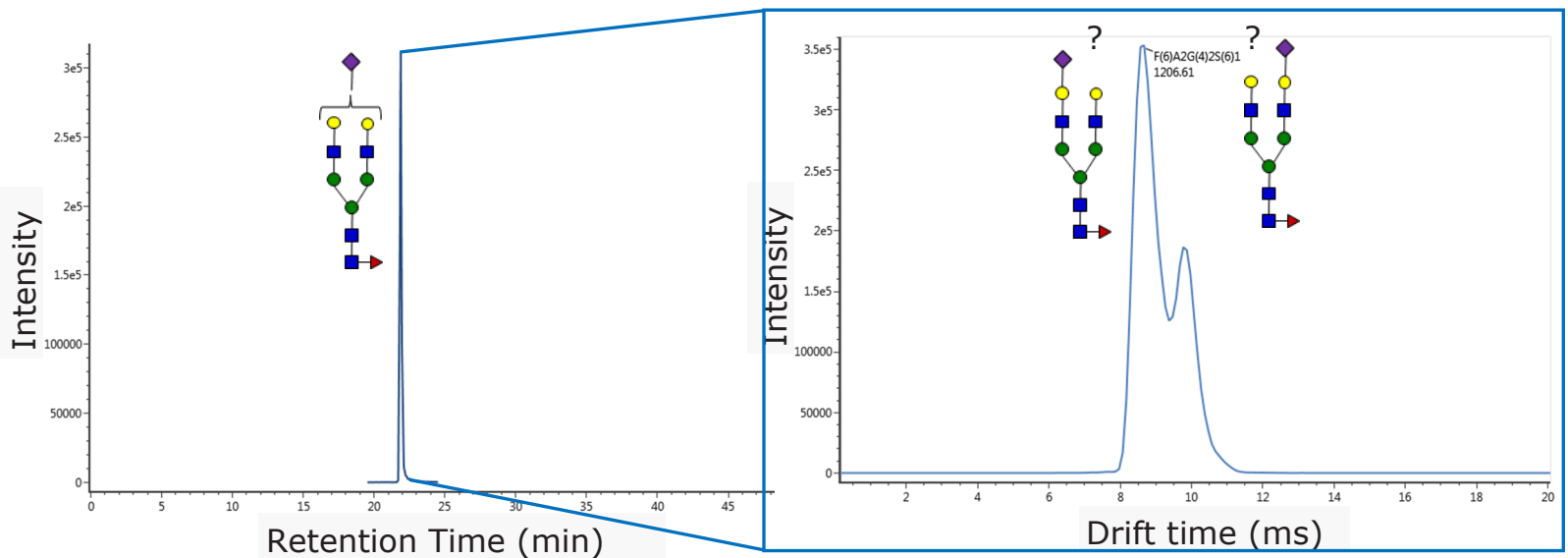
We investigated the use of rCCSs to assist our GU library searching. With a search tolerance of 0.2 GU units, we are sometimes challenged by isomeric species that have GU values that are within this tolerance, as shown in the FLR chromatogram above. However, Isomers A and B have rCCS values sufficiently different and we can correctly assign each peak without the need for additional experiments. To ensure that we did have the correct assignments, MS^E experiments were performed and Isomer A produced an abundant ion at *m/z* 528.19, indicating the presence of α-linked galactoses while Isomer B generated an intense ion at *m/z* 366.14, confirming both galactoses were attached to GlcNAcs.



This figure demonstrates the usefulness of rCCS values. In an infiximab sample, our library search indicated that the glycan eluting at 17.4 min was A2G(4)1Ga(3)1 based on GU values. Unfortunately for this low abundance glycan, tandem MS experiments were not able to conclusively confirm this assignment. However, the rCCS value (1179 Å²) was nearly identical to that of A2G(4)2 (1175 Å²) from human IgG where MS^E did confirm the assignment. Using rCCS values, we believe that the correct assignment for this glycan in infiximab is A2G(4)2.



An interesting trend was observed for bisecting glycans during HDMS analyses. Panel A of this figure shows the extracted ion chromatogram for the *m/z* value of the F(6) A2B glycan and only one chromatographic peak was present. However, IMS separation, shown in Panels B and C, reveals two distinct peaks, suggesting a possible isomeric species was present in human IgG. Interestingly, the IMS profile shifted the predominant peak to the lower mobility species in a different monoclonal antibody. These two species were observed to have significantly different rCCS values.



IMS also appears to be able to resolve the sialic acid positional isomers of F(6) A2G(4)2S1. However, at this time, there is still some ambiguity as to which isomer is associated with each IMS peak.

CONCLUSIONS

- rCCS values can improve the confidence of GU library search results, particularly for isomeric glycans that have GU values within the search tolerance.
- rCCS values can add an extra dimension of knowledge and may be useful in cases when tandem MS data is inconclusive.
- Possible IMS resolution of glycan isomers within a given chromatographic peak was observed.

Reference

Yu, Y. Q. N-linked Glycan Characterization and Profiling: Combining the Power of Accurate Mass, Reference Glucose Units, and UNIFI Software for Confident Glycan Assignments. 2014.