Poster Reprint

ASMS 2025
Poster number MP 018

What information can a single quadrupole mass spectrometer provide for biopharmaceutical characterization of monoclonal antibodies?

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Introduction

Glycosylation is an important parameter to monitor in the biopharmaceutical industry because glycans play a role in the structural stability, safety, and efficacy of a therapeutic drug [1]. A common quality metric that is monitored repeatedly is to ensure that the relative abundances of protein glycosylation fall within accepted tolerances. If relative abundances do not pass predefined standards established by the quality control (QC) team, additional investigation into the manufacturing process for the therapeutic drug is required. Since mass spectrometry provides sensitive and robust methods for the characterization of monoclonal antibodies (mAbs), it is ideal to use mass spectrometry for glycan characterization. In this study, the Agilent InfinityLab Pro iQ Plus liquid chromatography/mass spectrometer (LC/MS) with improved mass range and superior mass spectral peak shape was used to detect glycosylation at the intact and subunit levels (HC, LC). The relative abundances of glycosylation after deconvolution illustrate that key information about protein glycosylation [2] can be measured on this instrument platform.

Experimental

50 µg of trastuzumab was reduced in the presence of 35 mM DTT (final concentration) for 30 minutes at 37°C. To quench the reduction reaction formic acid was added to a final v/v % of 1%. Intact and reduced trastuzumab were diluted using 0.1% formic acid in water to a final concentration of 250 ng/µL. LC/MS data was collected on an Agilent 1290 Infinity II Bio LC system coupled to an Agilent InfinityLab Pro iQ Plus single quadrupole LC/MS system. Deconvolution was processed using Agilent OpenLab CDS software, version 2.8.

Figure 1. Agilent 1290 Infinity II Bio LC system and Agilent Pro iQ Plus single quadrupole mass spectrometer.

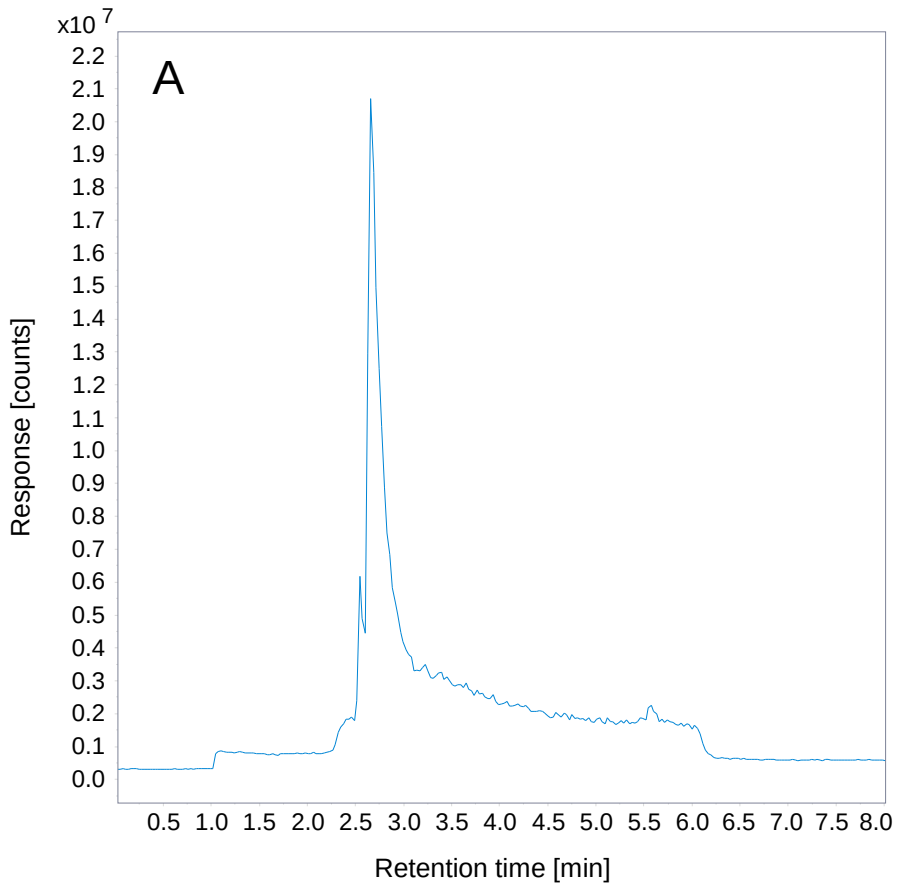
Experimental

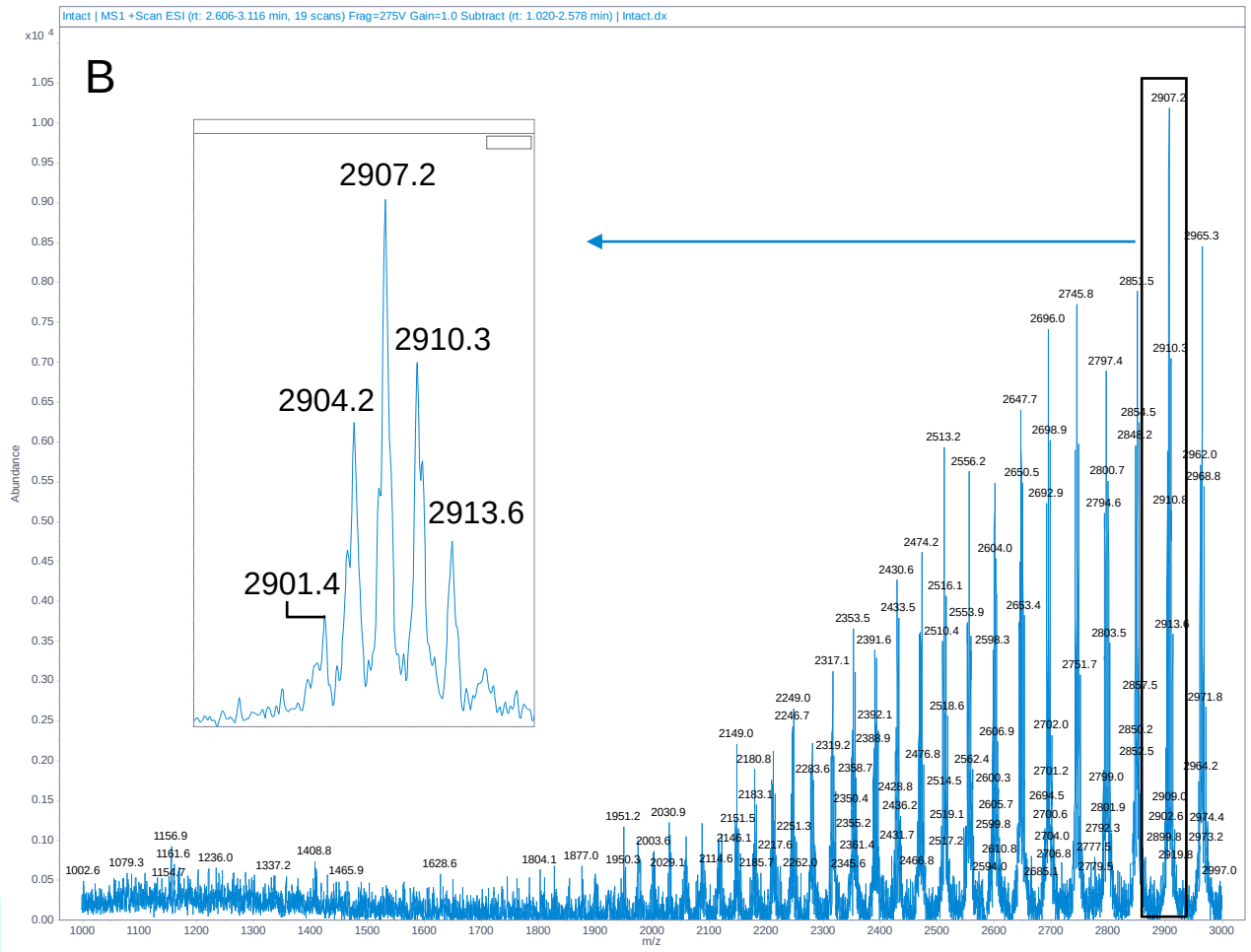
LC Conditions	
Solvent A	Water with 0.1% FA
Solvent B	ACN with 0.1% FA
Gradient (reduced)	0-0.1 min, 5-20% B 0.1-8 min, 20-40% B 8.1-9.1 min, 70% B 9.2-11 min, 5% B
Injection volume	2 µL
Flow rate	0.5 mL/min
Column temperature	80°C
MS Conditions	
Ion Source	Agilent Jet Stream ESI source
Polarity	Positive
Time Filter Window (min)	0.1
MS1 Scan Range	*m/z* 1000 to 3000 (intact) *m/z* 600 to 2400 (reduced)
Scan Time	1500 ms
Detector Gain Factor	1
Fragmentor	275 V (intact) 175 V (reduced)
Fragmentor Ramp?	Not checked
Data Storage	Profile
Gas Flow	12 L/min
Nebulizer	50 psi
Sheath Gas Flow	11 L/min
Capillary Voltage	4500 V
Nozzle Voltage	2000 V
Gas Temperature	350°C
Sheath Gas Temperature	360°C
Divert Valve	Enabled; LC flow to waste from 0 to 1 minutes
Postrun Diverter Position	To waste

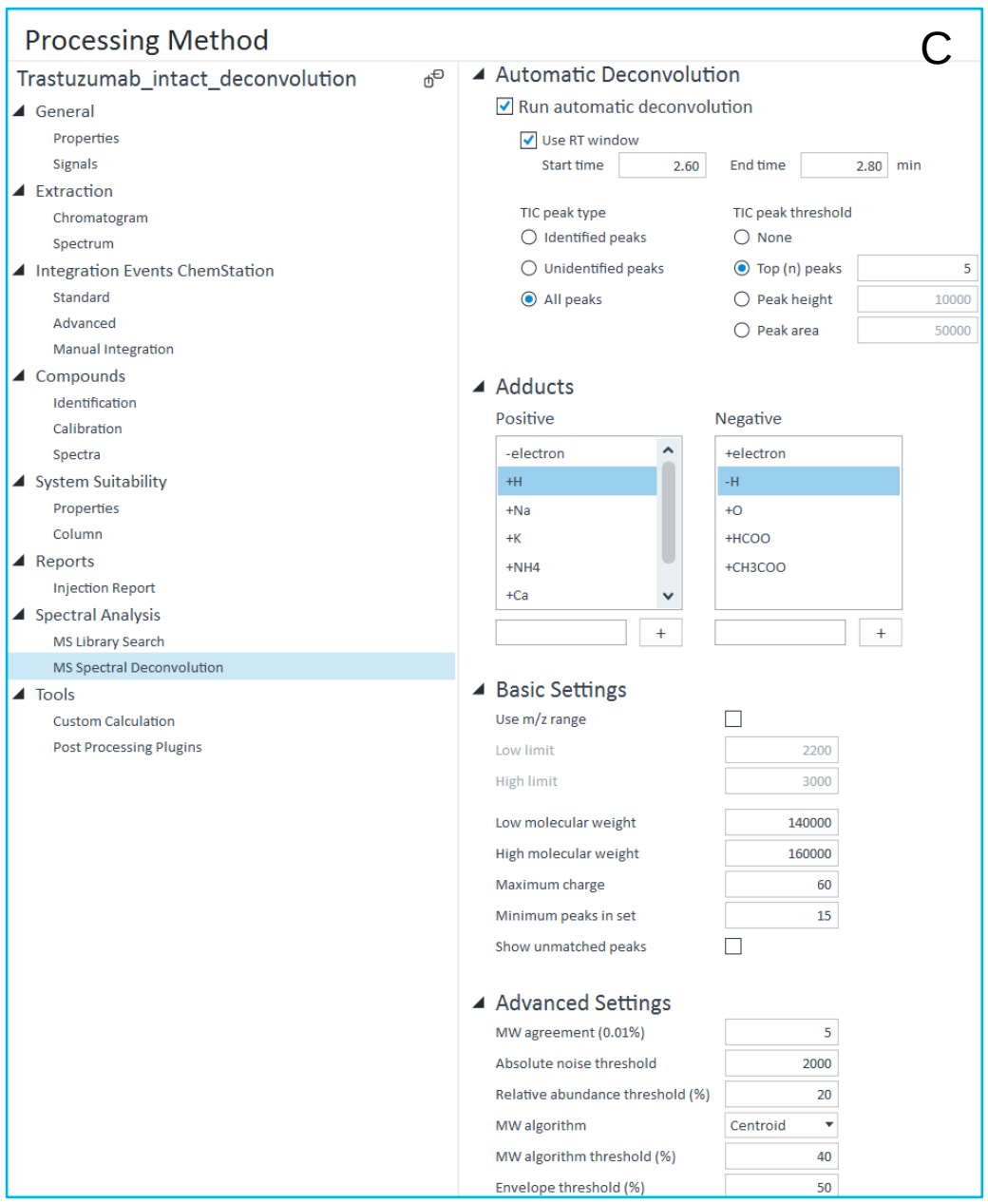
Table 1. LC/MS conditions for intact and reduced mAb analysis

Intact trastuzumab TIC, raw mass spectrum, and deconvoluted spectrum

Intact trastuzumab can be measured on the Agilent InfinityLab Pro iQ Plus single quadrupole mass spectrometer due to its extended mass range up to *m/z* 3000. The relative abundances shown in the raw MS spectrum (inset of Figure 2B) match what is observed in the deconvoluted mass spectrum (Figure 2D).







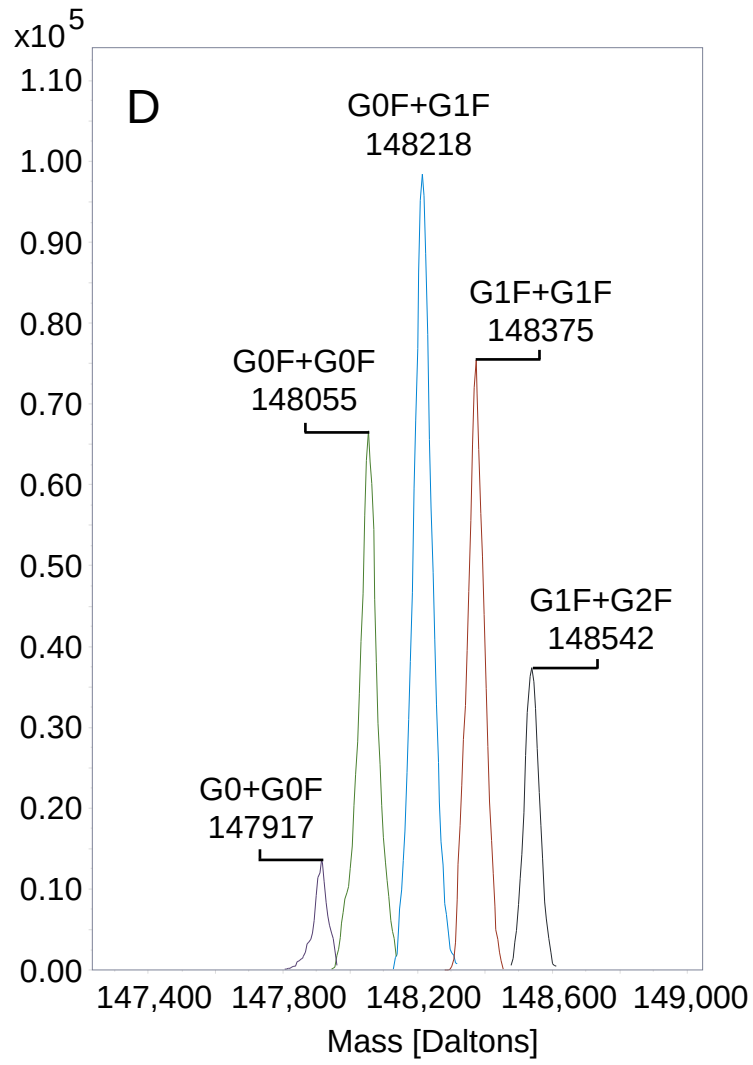


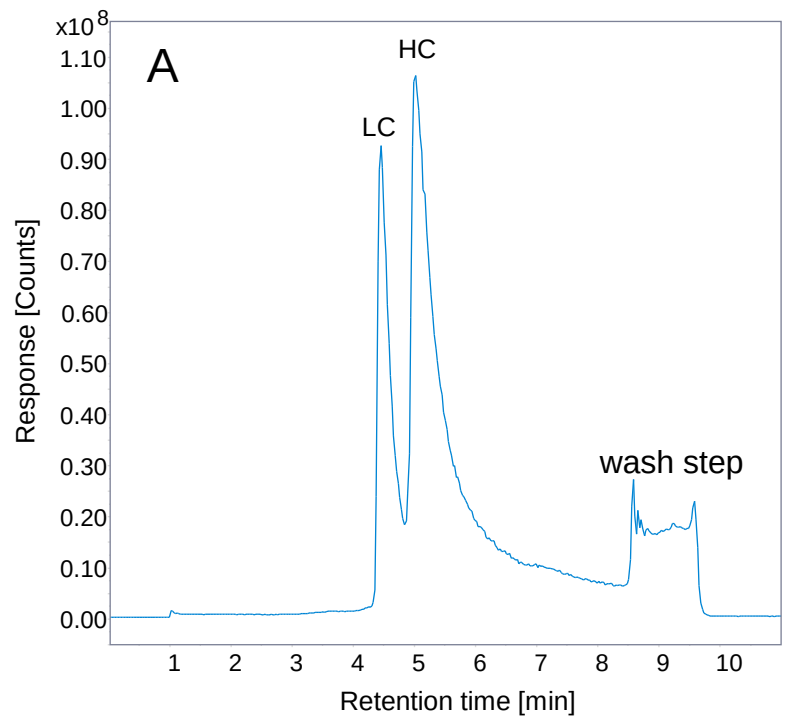
Figure 2. (A) Total ion chromatogram for 500 ng of intact trastuzumab. (B) Raw MS spectrum measured on the Agilent InfinityLab Pro iQ Plus LC/MS system. The inset shows MS spectral peak shapes. (C) Processing parameters used to deconvolute intact trastuzumab data. (D) Deconvoluted mass spectrum illustrated in OpenLab CDS software.

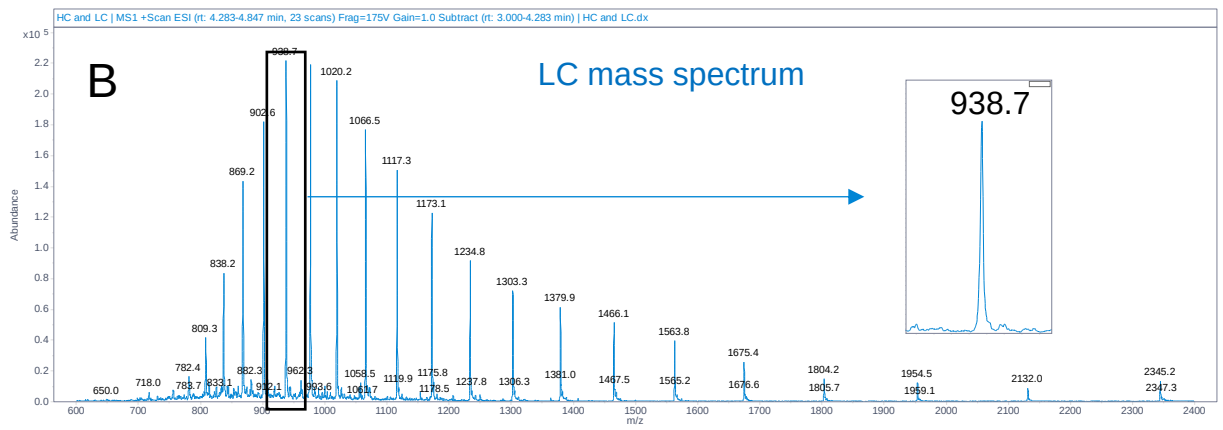
Results and Discussion

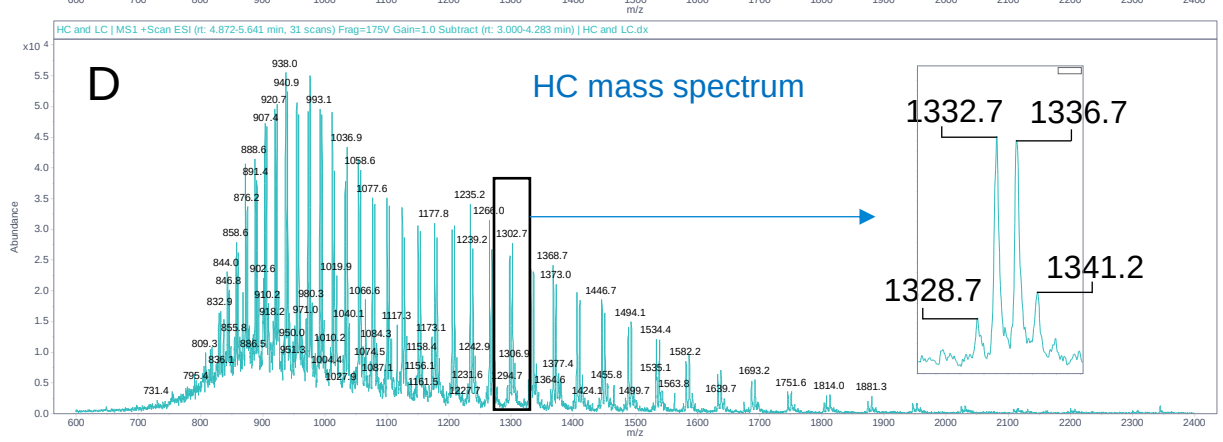
Reduced trastuzumab TIC, raw mass spectrum, and deconvoluted spectrum

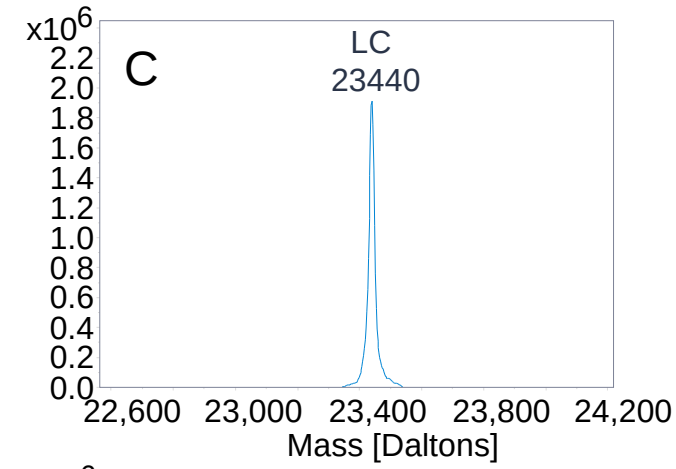
To add an additional dimension to glycosylation profiling, an intact mAb can be reduced into subunits to reduce complexity.

- For trastuzumab the site of glycosylation follows a consensus sequence (i.e., glycosylation occurs at asparagine residues that follow an NXS/T motif)
- Light chain does not have an NXS/T site in its amino acid sequence, therefore a single peak is observed in the raw MS spectrum
- The inset of Figure 3D shows four peaks in the raw MS spectrum that are consistent with the G0, G0F, G1F, and G2F glycans.
- Measured relative abundances at the intact and reduced levels allows the QA/QC team to determine if there is significant deviation from established tolerances









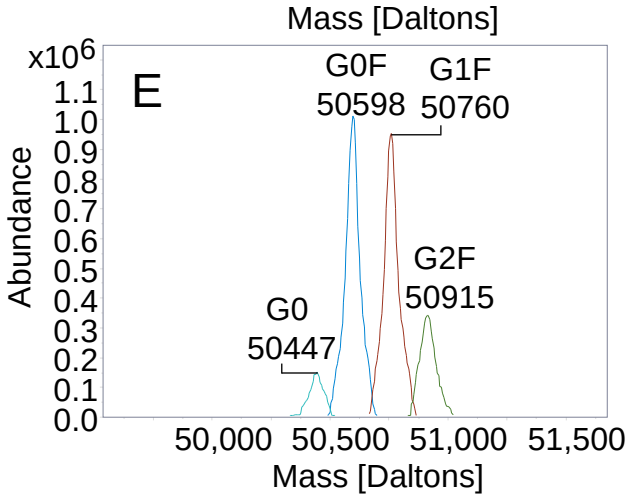


Figure 3. (A) TIC for 500 ng of reduced trastuzumab. (B+D) Raw MS spectrum for the trastuzumab light chain and heavy chain, respectively. The insets show the raw spectral quality on the instrument. (C+E) Deconvoluted mass spectrum for trastuzumab light chain and heavy chain, respectively.

Conclusions

The Agilent InfinityLab Pro iQ Plus single quadrupole LC/MS system enables glycosylation profiling at the intact and reduced levels with extended mass range and superior MS spectral peak shapes

- LC/MS conditions were optimized to detect five glycosylated forms of intact trastuzumab (~148 kDa), four glycosylated forms for the trastuzumab heavy chain (~50 kDa), and the trastuzumab light chain (~23 kDa)
- Deconvoluted mass spectra measured at the intact levels show relative abundances that were consistent with results measured on Agilent high-resolution accurate mass spectrometry systems

References

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DE-006362

Published in USA, May 15,2025

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