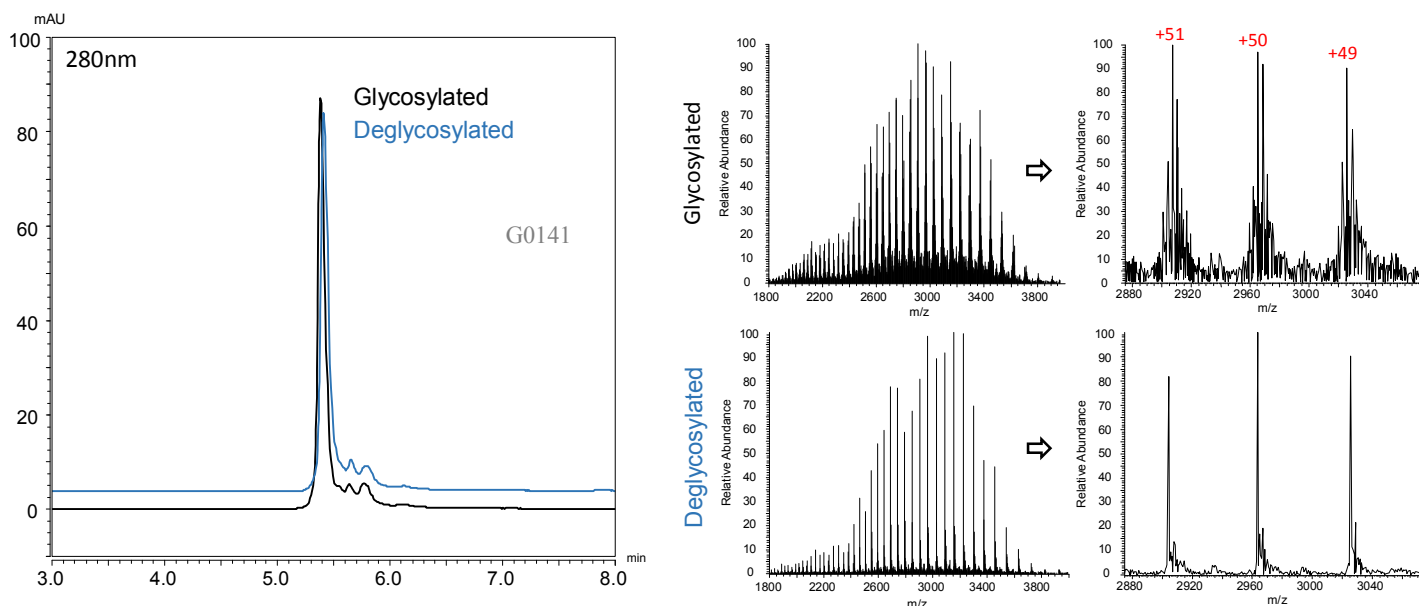


Application Note: 151-PR

## LC-MS Analysis of Trastuzumab Using HALO 1000Å C4



### LC Test Conditions:

Column: HALO 1000Å C4, 2.7  $\mu\text{m}$ , 2.1 x 150 mm  
Part Number: 92712-714  
Mobile Phase A: 10 mM difluoroacetic acid (DFA) in water  
Mobile Phase B: 10 mM difluoroacetic acid in 10/90 water/acetonitrile  
Gradient: 32-42% B in 10 min  
Flow Rate: 0.35 mL/min.  
Pressure: 184 bar  
Temperature: 80 °C  
Detection: 280 nm  
Injection Volume: 1  $\mu\text{L}$  of 2 mg/mL trastuzumab (glycosylated/deglycosylated)  
Sample Solvent: 0.1% DFA in 70/30 water/acetonitrile  
LC System: Shimadzu Nexera

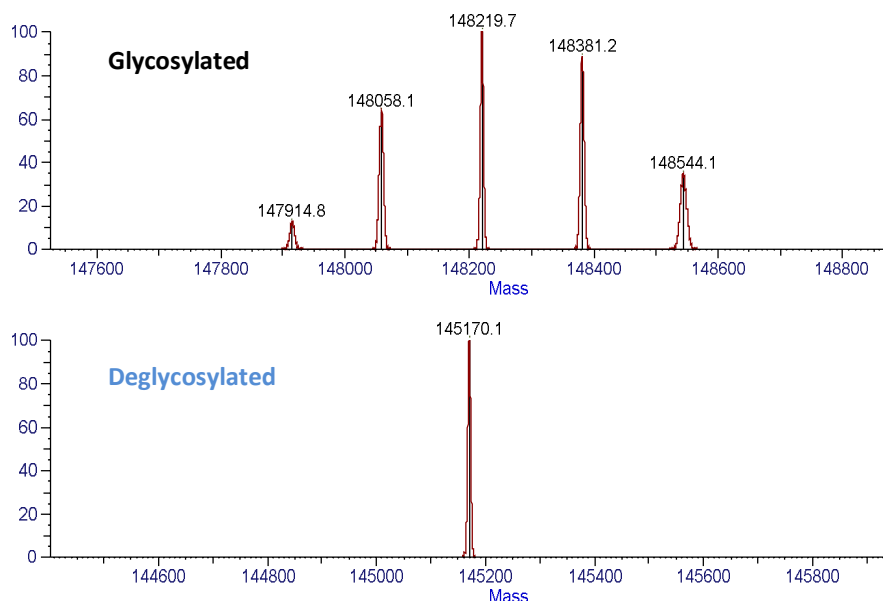
### MS Test Conditions:

MS System: Thermo Fisher Orbitrap VelosPro ETD  
Scan Time: 6  $\mu\text{s}$  scans/250 ms max inject time  
Scan range: 1800 to 4000 m/z  
MS parameters: Positive ion mode, ESI at +4.0 kV, 225°C capillary

LC-MS analysis using a HALO 1000Å C4 Protein column has been used to analyze two samples of the monoclonal antibody, trastuzumab: glycosylated and enzymatically deglycosylated. Minor variant structures are observed in both the glycosylated and deglycosylated monoclonal IgG (small peaks after main peak), indicating that the polypeptides are structure variants.

The glycosylation profile of therapeutic mAbs is an important characteristic, which must be monitored throughout the manufacturing process. Determination of the mass of the deglycosylated IgG confirms the identity and integrity of the protein.

## Deconvoluted Spectra and Peak Information



The structure of trastuzumab consists of two heavy chains and two light chains. Glycosylation occurs on the two heavy chains. One or more of the same or different carbohydrate moieties can be present on each heavy chain. Table 1 contains the combinations of sugars that correspond to the masses that were observed upon deconvolution of the mass spectrum on the previous page. The last column is the mass of the deglycosylated trastuzumab, which results from enzymatic cleavage of the glycans by PNGase F.

**Table 1:**

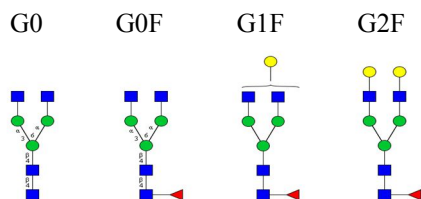
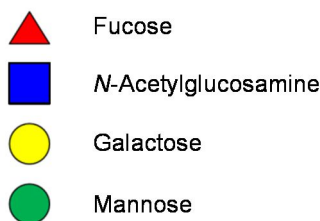
GLYCANS:	G0/G0F		G0F/G0F		G1F/G0F		G1F/G1F, G2F/G0F		G1F/G2F		Deglycosylated Trastuzumab	
	T <sup>1</sup>	M <sup>1</sup>	T	M	T	M	T	M	T	M	T	M
Trastuzumab	147911	147915	148057	148058	148219	148220	148381	148381	148543	148544	145167	145170
ΔMass (glyc) Trastuzumab	2744	2745	2890	2888	3052	3050	3214	3211	3376	3374	—	3

T = Theoretical mass

M = Measured mass

<sup>1</sup>All masses reported in Daltons

Glycan Descriptions:



### Deconvolution Parameters:

Minimum Adjacent Charges 3 - 6  
 Noise Rejection 95% Confidence  
 m/z Range 1800 - 4000  
 Mass Tolerance 20 ppm  
 Charge State Range 40 - 120  
 Choice of Peak Model Intact Protein