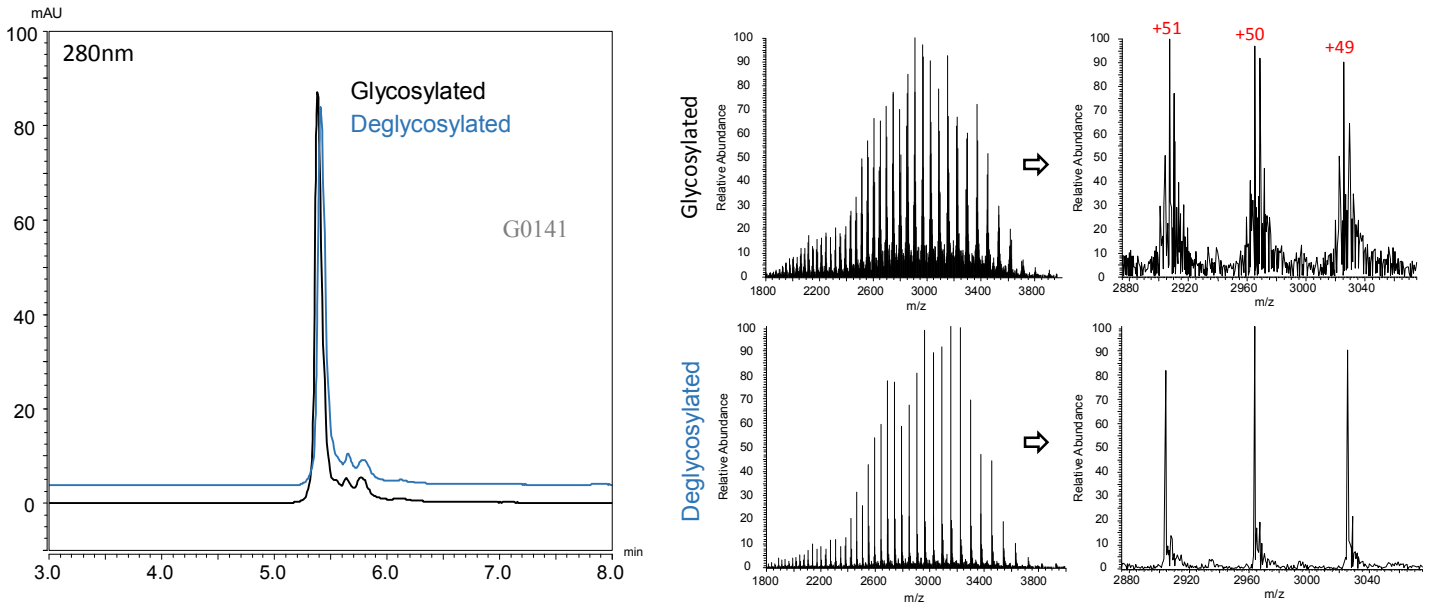
HALO | Fused-Core® Particle Technology

Application Note: 151-PR

LC-MS Analysis of Trastuzumab Using HALO 1000Å C4



LC Test Conditions:

Column: HALO 1000Å C4, 2.7 µm, 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 µL of 2 mg/mL trastuzumab (glycosylated/deglycosylated)
Sample Solvent: 0.1% DFA in 70/30 water/acetonitrile
LC System: Shimadzu Nexera

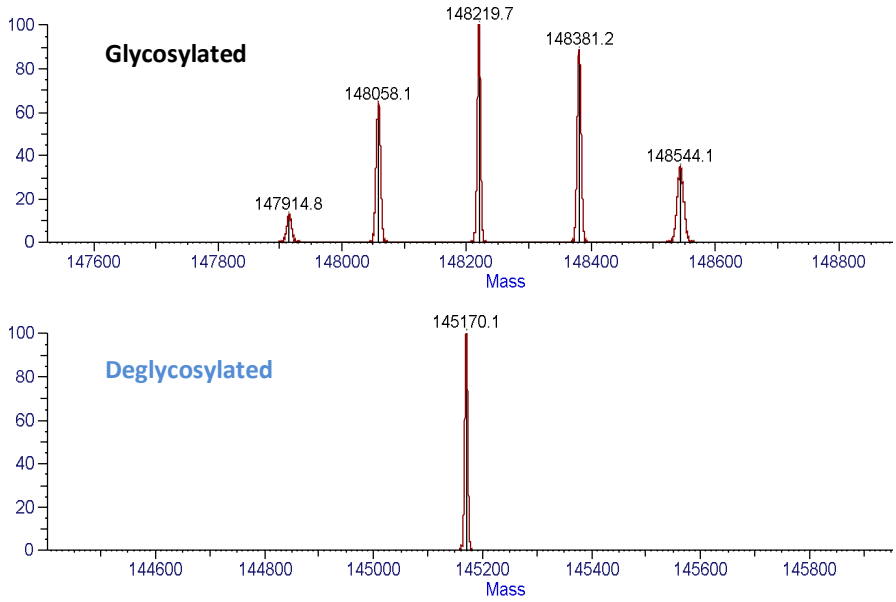
MS Test Conditions:

MS System: Thermo Fisher Orbitrap VelosPro ETD
Scan Time: 6 µscans/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



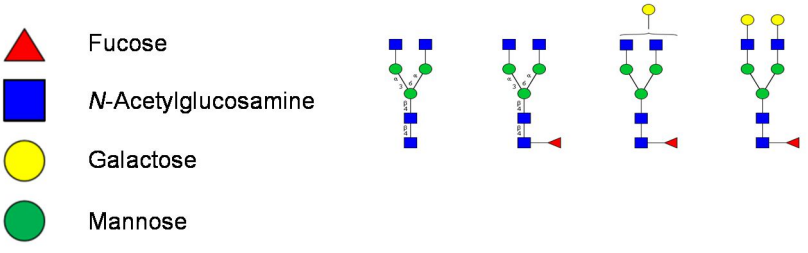
The structure of trastuzumab consists of two heavy chains and two light chains. Glycosylation occurs on the two heavy chains. One or more of the same or different carbohydrate moieties can be present on each heavy chain. Table 1 contains the combinations of sugars that correspond to the masses that were 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[1]	M[1]	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
[1]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

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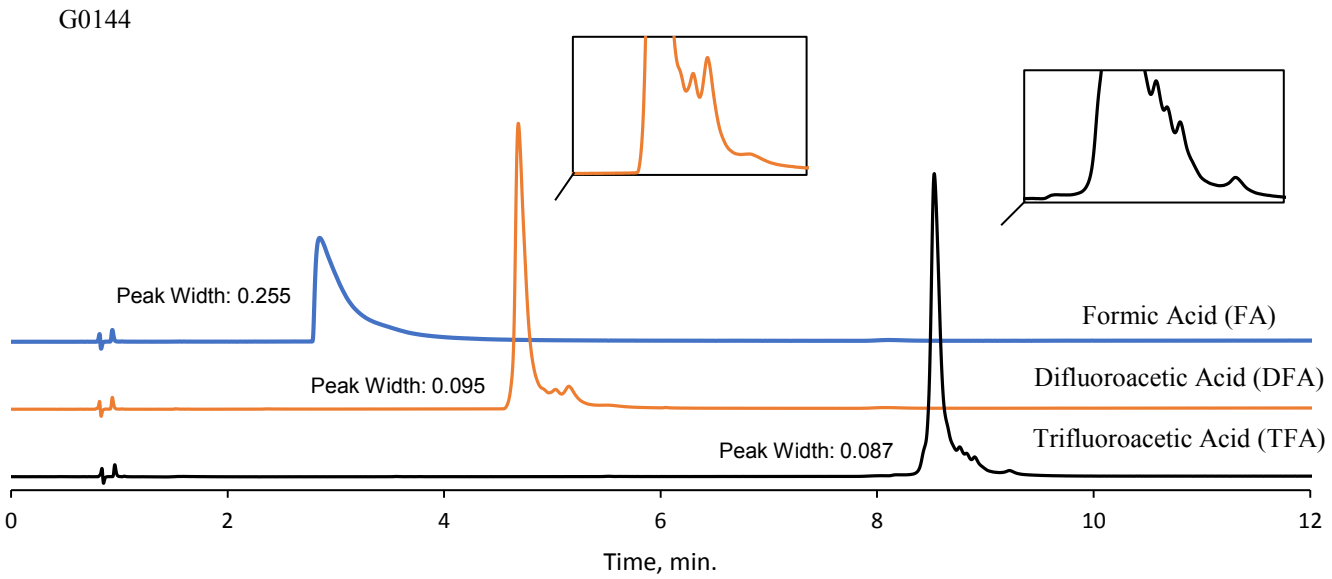
Application Note: 154-PR

Effect of Acid Modifiers on Intact mAb Peak Shape



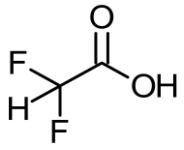
TEST CONDITIONS:

Column: HALO 1000Å C4, 2.7µm, 2.1 x 150 mm
Part Number: 92712-714
Mobile Phase:
A= Water, with 0.1% FA, DFA, or TFA as noted
B= 80/20 ACN/Water, with 0.1% FA, DFA, or TFA as noted
Gradient:

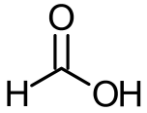
Time (min.)	%B
0.00	35.0
12.00	47.5

Flow Rate: 0.4 mL/min
Initial Pressure: 218 bar
Temperature: 80 °C
Injection Volume: 2 µL
Sample Solvent: 30/70 ACN/Water
Detection: UV 280 nm, PDA
Data Rate: 12.5 Hz
Response Time: 0.05 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

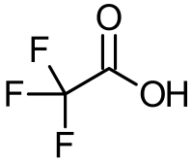
STRUCTURES:



Difluoroacetic Acid (DFA)



Formic Acid (FA)



Trifluoroacetic Acid (TFA)

Trastuzumab (~148 kDa) is a monoclonal antibody (mAb) used to treat breast cancer. TFA and DFA can be used as mobile phase additives instead of formic acid to provide much narrower and more symmetrical peaks, and to allow adjustments to retention and resolution among minor variants.

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