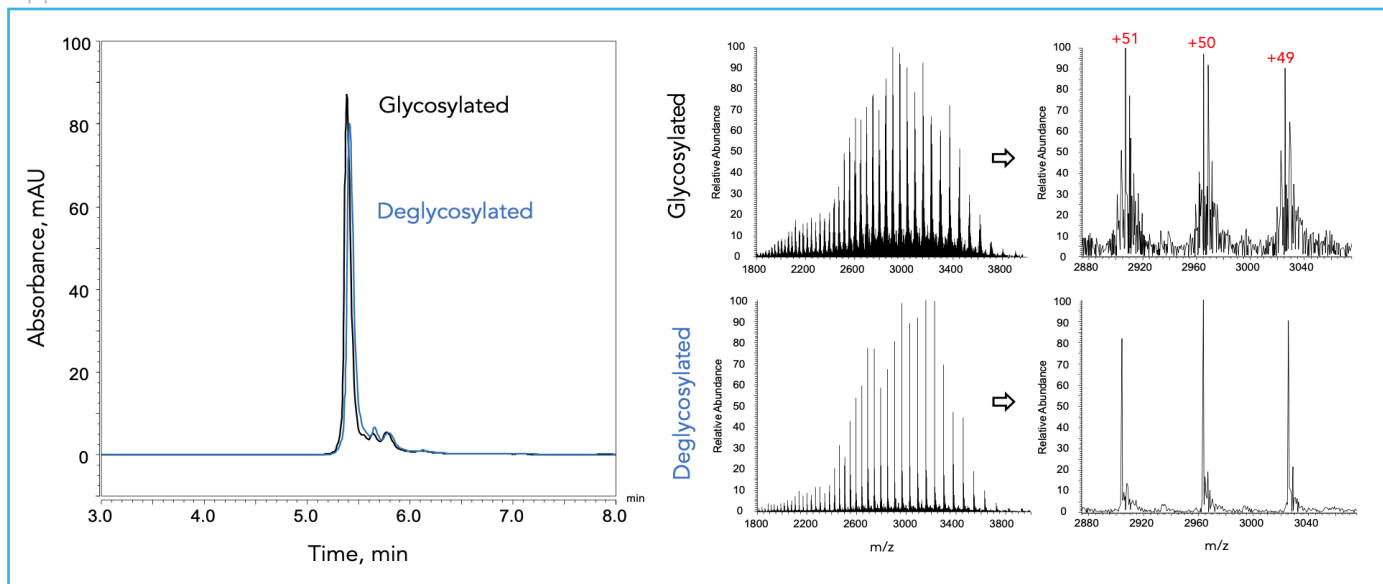




LC-MS Analysis of Trastuzumab Using HALO® 1000 Å C4

Application Note 151-PR



LC TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 μm ,
2.1 x 150 mm

Part Number: 92712-714

Mobile Phase:

A: 10 mM difluoroacetic acid (DFA) in water
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: 1.0 μL of 2 mg/mL trastuzumab
(glycosylated/deglycosylated)

Sample Solvent: 0.1% DFA in 70/30 water/acetonitrile

LC System: Shimadzu Nexera

LC-MS analysis using a HALO 1000 Å 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.

MS TEST CONDITIONS:

MS System: Thermo Fisher Orbitrap VelosPro ETD

Scan Time: 6 μs 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

