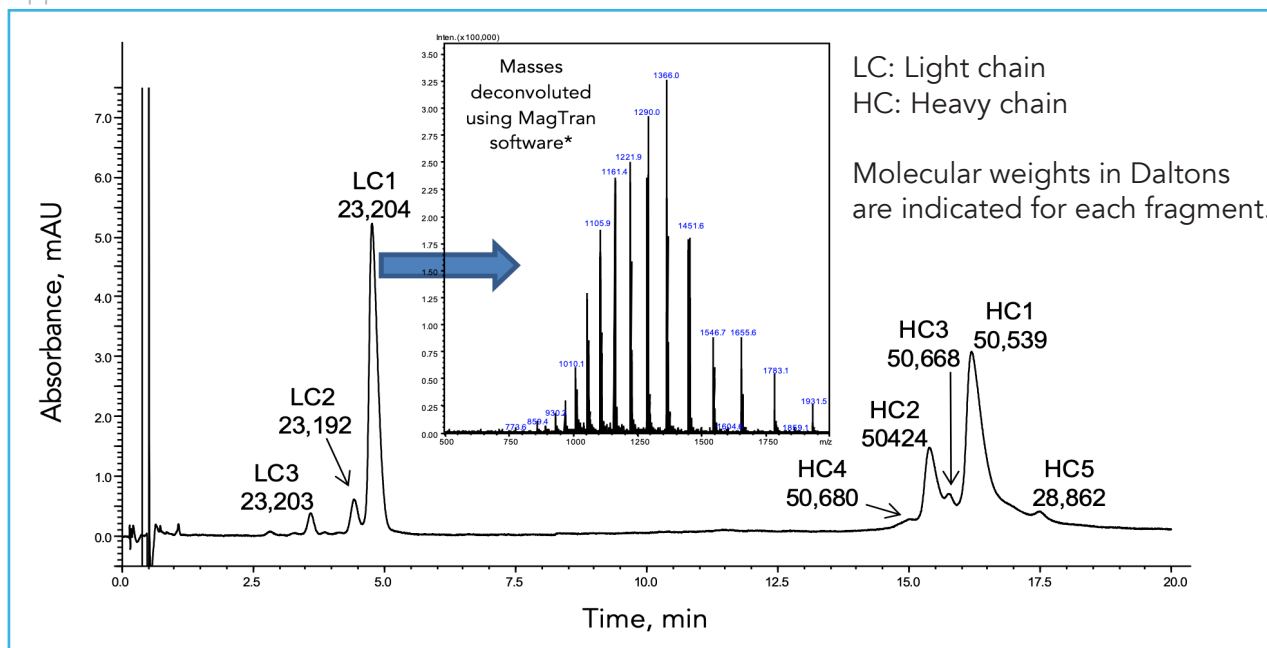




LC-MS Analysis of Reduced IgG1 Monoclonal Antibody Fragments Using HALO 400 Å C4

Application Note 125-PR



TEST CONDITIONS:

Column: HALO 400 Å C4, 3.4 μm,
2.1 x 100 mm

Part Number: 93412-614

Mobile Phase:

A: 0.5% formic acid with 20 mM ammonium formate

B: 45% acetonitrile/45% isopropanol/0.5% formic acid/9.5% water with 20 mM ammonium formate

Gradient: 29–32% B in 20 min

Flow Rate: 0.4 mL/min

Pressure: 20 bar

Temperature: 80 °C

Detection: 280 nm and MS using 2 pps scan rate from 500 to 2000 m/z

Injection Volume: 2 μL of 2 μg/μL reduced and alkylated IgG1

Sample Solvent: 0.25% formic acid in water

MS Parameters: Positive ion mode, ESI at +4.5 kV, 400°C heat block, 225°C capillary

LC-MS System: Shimadzu Nexera and LCMS-2020 (single quadrupole MS)

HALO 400 Å C4 has the low pH and high temperature stability that is required to analyze reduced and alkylated IgG1 using MS compatible mobile phase. The use of 80 °C enables improved peak shape while the high resolution MS allow complete analysis of the IgG1 fragments that are present.

Adapted from J. Chromatogr. A 1315 (2013) 118-126.

*Z. Zhang, A.G. Marshall, J. Am. Soc. Mass Spectrom. 9 (1998) 225.

