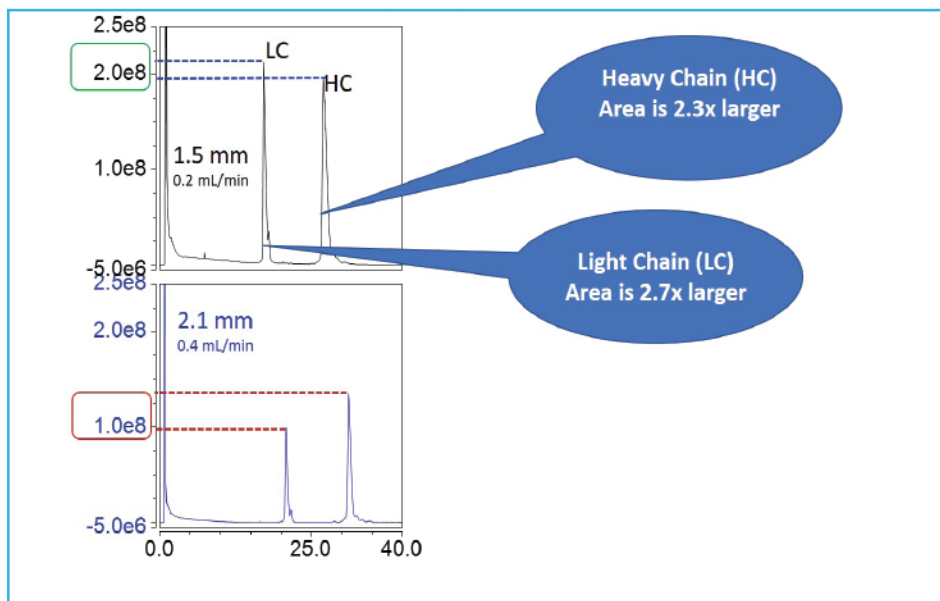




Demonstration of Increased Sensitivity with Reduced Trastuzumab Using a 1.5 mm ID Column

286-BIO



PEAK IDENTITIES

LC = Light Chain
 HC = Heavy Chain

TEST CONDITIONS:

Column: HALO 1000 Å Diphenyl, 2.7 μm, 1.5 x 150 mm
 Part Number: 9212X-702

Column: HALO 1000 Å Diphenyl, 2.7 μm, 2.1 x 150 mm
 Mobile Phase A: Water/0.1% DFA

B: 50% Acetonitrile/50% n-propanol/0.1%
 DFA

Gradient:	Time (min)	%B
	0.0	27
	40.0	36
	40.1	27
	45.0	27

Flow Rate: 0.2 mL/min for 1.5 mm ID
 0.4 mL/min for 2.1 mm ID

Back Pressure: 252 bar (1.5 mm)
 272 bar (2.1 mm)

Temperature: 60 °C

Injection Volume: 3 μL of 1.0 mg/mL reduced and
 alkylated trastuzumab

Sample Solvent: Water/0.1% TFA

LC System: Shimadzu Nexera X2

MS System: ThermoFisher Q Exactive

MS CONDITIONS:

Spray Voltage (kV): 3.8

Capillary temperature: 320 °C

Sheath gas: 35

Aux gas: 10

RF lens: 50

A separation of reduced and alkylated Trastuzumab is performed on a HALO 1000 Å Diphenyl column. Switching to a 1.5 mm ID column from the 2.1 mm ID provides a significant increase in sensitivity for the reduced and alkylated Trastuzumab along with a reduced flow rate. This increase in sensitivity can be achieved by using a 1.5 mm ID column in conjunction with optimized tubing post-column which provides a cheaper route for increased sensitivity without the investment into a specialized HPLC system.

