Loading Capacity of Biozen™ dSEC-2 Column Under SEC-HRMS Conditions

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Overview

Monoclonal antibodies (mAbs), mAb-conjugates, and other protein therapeutics (collectively termed biologics) are increasingly prominent in modern medicine. Aggregation, fragmentation, misfolding, and unfavorable glycosylation profiles of biologics, are among major concerns for the likelihood of decreased product efficacy and safety. Analytical methods for assessment of biologics under non-denaturing ("native") conditions can yield critical insights into the quality of biologics. The combination of size exclusion chromatography (SEC), high resolution mass spectrometry (HRMS), and bioinformatic technologies have been developed to improve biologic quality assessment. However, few integrated platforms adopting the employment of advanced SEC, HRMS, and analytical software have been developed and applied.

Although challenging, it is desirable to operate under higher concentrations of Ammonium Acetate for better chromatography, improved preservation of native mAb folding, and changes in sensitivity. Increases in conductivity while using higher concentrations of Ammonium Acetate can result in excessive electrospray currents, ultimately decreasing sensitivity. To address these concerns, the insertion of resistor tubing between the electro spray ionization (ESI) probe and the high voltage ground enables the use of higher concentrations of Ammonium Acetate in the mobile phase by reducing the ESI amperage to an acceptable level. This expands the experimental space considerably, as the higher ionic strength mobile phase is no longer a limitation and increases sensitivity.

In this application note, the use of resistor tubing and 200 mM Ammonium Acetate in the mobile phase was implemented to measure loading capacity of a Biozen 1.8 µm dSEC-2 column.

We tested the ability of increased sensitivity to yield accurate results with low sample loads, a common necessity. Some low abundance variants were below the limit of detection in the lower sample load injections. However, when the injection quantity exceeded the limit of detection for lower abundance variants, minor secondary exclusions began to appear.

LC Conditions

Column: Biozen 1.8 µm dSEC-2
Dimensions: 150 x 2.1 mm
Part No.: 00F-4787-AN
Mobile Phase: 100 mM Ammonium Acetate (no resistor tubing)
200 mM Ammonium Acetate (with resistor tubing)
Flow Rate: 90 µL/min
Injection Volume: Varying, see **Table 1**
Temperature: 30 °C
Instrument: Vanquish™ UHPLC
Detector: Q Exactive™ Plus
Detection: HRMS
Sample: Trastuzumab (10 mg/mL)

HRMS Conditions

Scan Type: Full ESI-MS (high range)
Scan Range: 2,500 to 8,000 m/z
In-source CID: 50.0 eV
Resolution: 17,500
Polarity: Positive
Microscans: 10
Lock Masses: Off
Automatic Gain Control: 3e6
Max Inject Time: 200 ms
Spray Voltage (kV): 3.5
Capillary Temperature: 340 °C

Figure 1. Resistor Tubing From the ESI Probe to Ground.

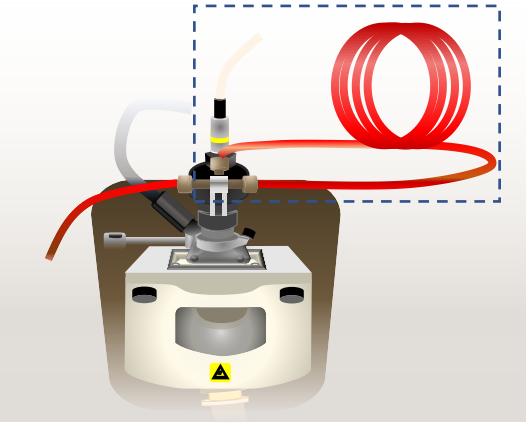
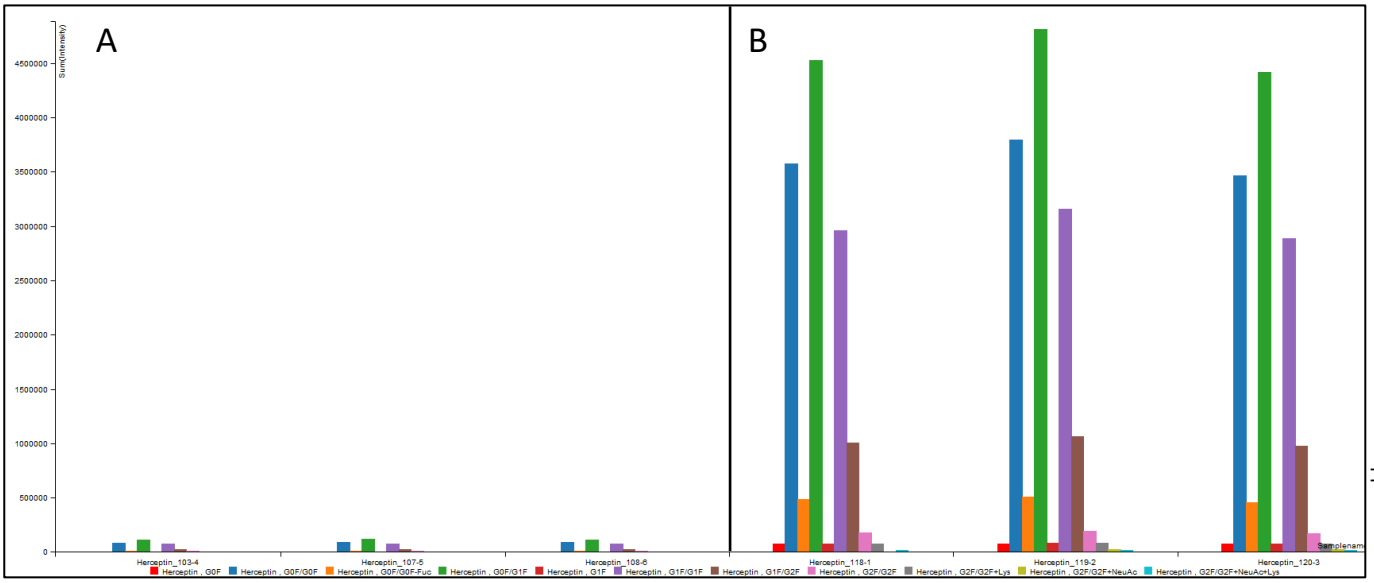


Figure 2. Trastuzumab Signal A) in the Absence of Resistor Tubing and B) in the Presence of Resistor Tubing.



100 mM Ammonium Acetate 200 mM Ammonium Acetate

Table 1. Identity and Relative Abundance of Trastuzumab Variants From the Main (Monomer-containing) SEC Chromatogram Peak.

Name ↑ / Sample name	250 ng	500 ng	1 µg	2.5 µg	5 µg	15 µg	25 µg
	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Herceptin , G0F			0.44	0.56	0.56	0.63	0.62
Herceptin , G0F/G0F	27.51	27.63	27.26	27.24	27.28	27.18	27.41
Herceptin , G0F/G0F-Fuc	3.13	3.65	3.59	3.57	3.67	3.70	3.80
Herceptin , G0F/G1F	36.49	34.87	34.95	34.85	34.62	34.54	34.71
Herceptin , G1F		0.21	0.48	0.63	0.59	0.63	0.64
Herceptin , G1F/G1F	23.47	22.93	23.07	22.98	22.79	22.85	22.70
Herceptin , G1F/G2F	7.95	7.99	7.77	7.75	7.87	7.82	7.76
Herceptin , G2F/G2F	1.44	1.48	1.44	1.48	1.42	1.44	1.40
Herceptin , G2F/G2F+Lys		0.63	0.72	0.62	0.68	0.65	0.64
Herceptin , G2F/G2F+NeuAc+Lys			0.19	0.23	0.20	0.23	0.21
Herceptin , G2F/G2F+NeuAc+Lys+Hex				0.11	0.10	0.09	0.10

Figure 3. Total Ion Chromatogram (TIC) Overlay of Trastuzumab Using "Resistor Tubing." Inset is a Deconvoluted Spectra (27+ Charge State) From the Main SEC Chromatographic Peak of Trastuzumab.

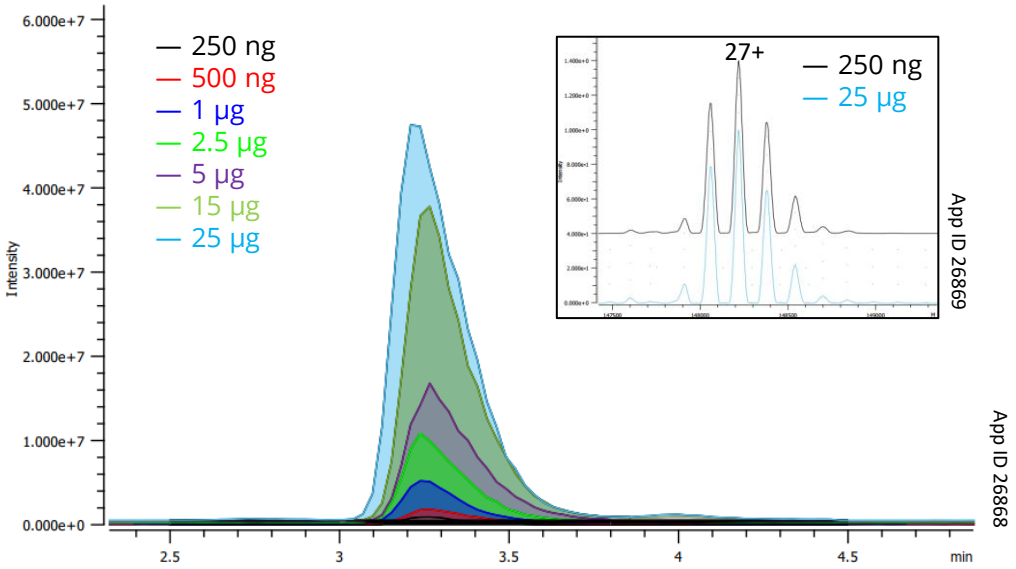
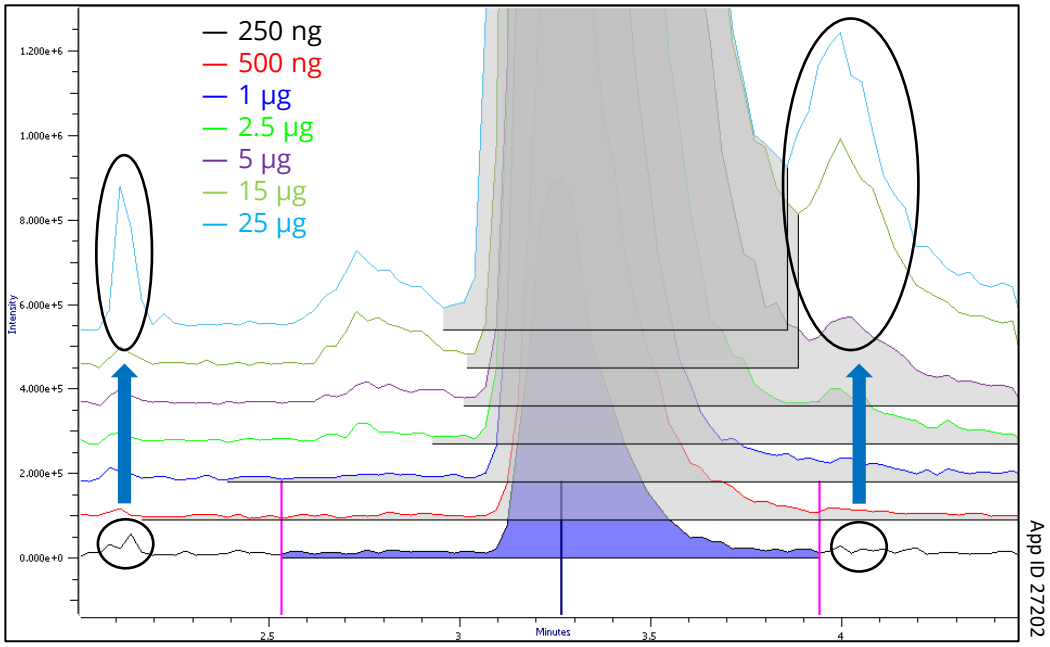


Figure 4. Total Ion Chromatogram (TIC) Overlay of Trastuzumab Using "Resistor Tubing." The Higher Loading Mass of 25 µg Shows Minor Secondary Exclusions Leading to a Slight Overload of the Column.



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