Heat and High pH Forced Degradation of Antibodies on a Biozen™ dSEC-2 Column

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Overview

The shelf life and storage considerations of various monoclonal antibodies (mAbs) heavily depend on their inherent biophysical properties. Size exclusion chromatography (SEC) provides an opportunity to elucidate the behaviors of proteinatious material with respect to time. Temperature and alkalinity can affect the longevity of an antibody in solution. In this application note, we demonstrate the aggregation propensity of multiple antibodies in response to their environmental conditions using a Biozen dSEC-2 column.

To test high temperature conditions for mAb degradation, we heated two antibodies, Herceptin and Cetuximab, at 60˚C while taking aliquots every 24 hours across 168 hours. High temperature stress is one of the most relevant conditions for providing information about potential long-term degradation at the intended storage condition. High temperature was found to accelerate the formation of aggregates (**Figures 1** and **2**) which were successfully separated from the monomer using the Biozen dSEC-2 column.

To evaluate pH, the storage solution was adjusted to pH 9 from the initial storage pH of 6-7. Infliximab and Cetuximab were each incubated under alkaline conditions at 37°C for 72 hours with aliquots sampled at 24-hour intervals. It was observed that the combination of physiological temperature as well as high pH leads to the degradation of the antibodies through cleavage of their disulfide bonds at the hinge and domain interfaces (**Figures 3** and **4**).

This application note demonstrates the applicability of the Biozen dSEC-2 column in understanding the biophysical integrity monoclonal antibodies and related proteins. Stability studies have been shown to generate impurities that feature a degree of denaturization thus exposing some hydrophobic regions of the folded protein's interior. The hydrophilic nature of the Biozen dSEC-2 stationary phase shows favorable aggregate profiles for forced degradation work under high salt conditions which is a known challenge when working with more hydrophobic hybrid particles for these studies.

LC Conditions

Column: Biozen 1.8 µm dSEC-2
Dimension: 300 x 4.6 mm
Part No.: 00H-4787-E0
Mobile Phase: 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2
Flow Rate: 0.35 mL/min
Injection Volume: 2 µL (Herceptin)
10 µL (Cetuximab, Infliximab)
Temperature: 25 °C
Detector: UV @ 280 nm
System: Waters® ACQUITY® UPLC H-Class
Samples:
1. Herceptin (10 mg/mL)
2. Cetuximab (2 mg/mL)
3. Infliximab (2 mg/mL)

Figure 1. Herceptin Day 0, 3, and 7 Forced Degradation by Heat Analysis on a Biozen™ dSEC-2 Column.

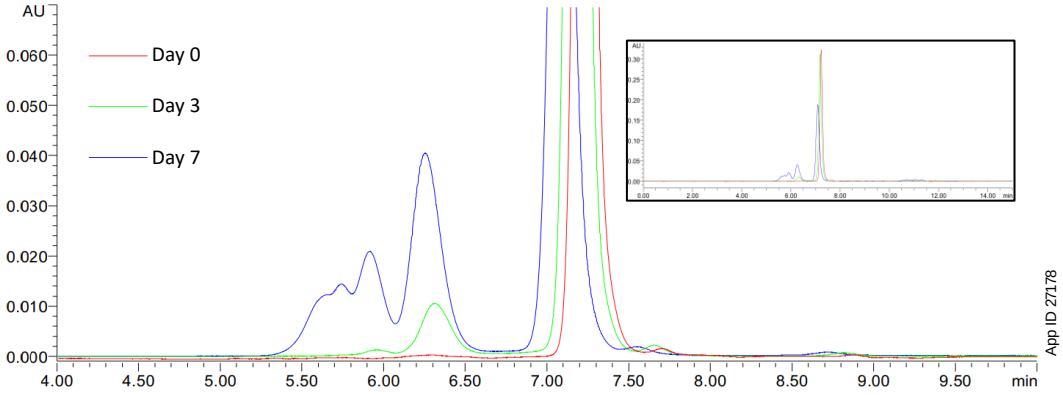


Figure 2. Cetuximab Day 0, 3, and 7 Forced Degradation by Heat Analysis on a Biozen dSEC-2 Column.

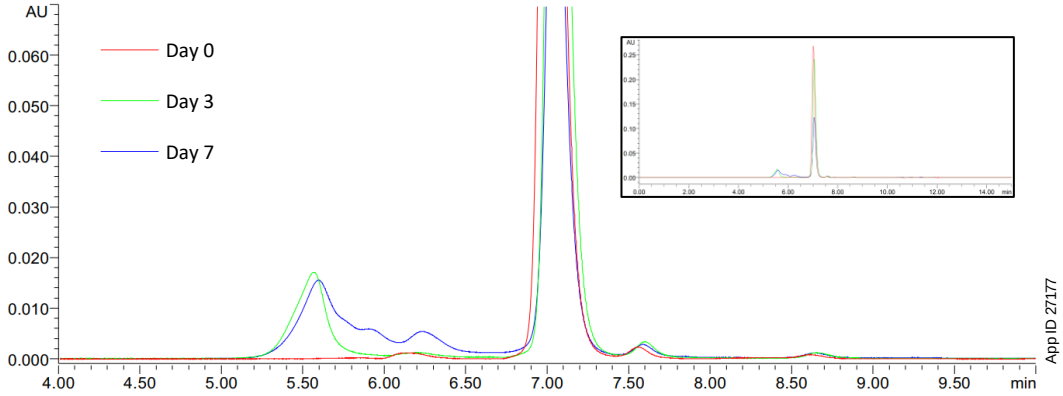


Figure 3. Cetuximab Day 0 and 3 Forced Degradation by High pH Analysis on a Biozen™ dSEC-2 Column.

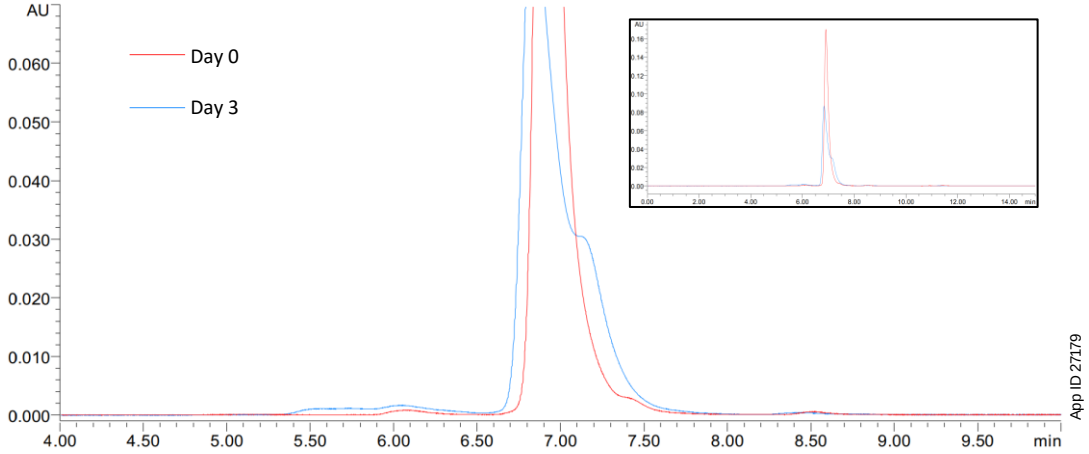
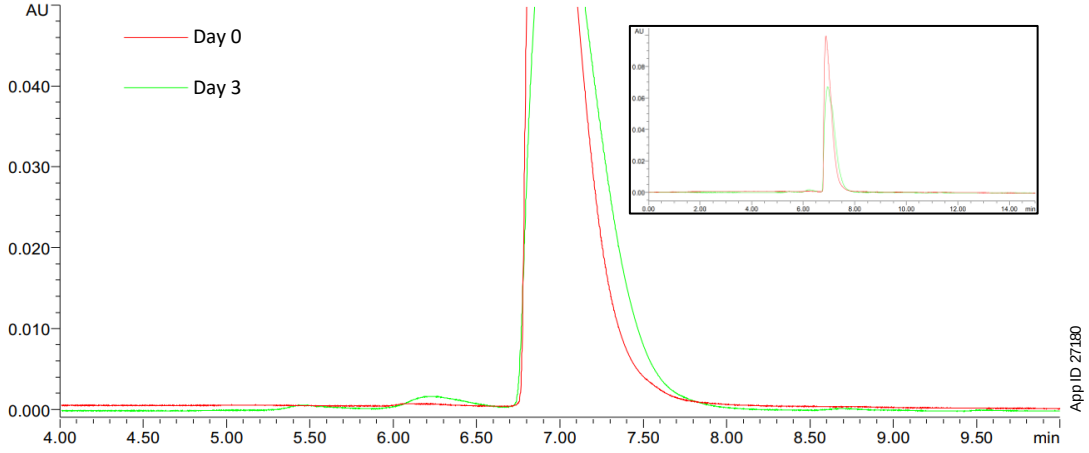


Figure 4. Infliximab Day 0 and 3 Forced Degradation by High pH Analysis on a Biozen dSEC-2 Column.



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