Improving Selectivity for Intact Mass Spectrometry of Biotherapeutic mAbs

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Overview

With many regulatory characterization requirements in biotherapeutic development, one essential requirement is intact liquid chromatography-mass spectrometry (LC-MS) by reversed phase chromatography. This technique rapidly provides data for monoclonal antibody (mAb) primary sequence confirmation, impurity identification, and heterogeneity information. High resolution mass spectrometry (HRMS) is the most common detection for intact MS analyses and is the focus of many methods in the literature.

An often-overlooked consideration of intact MS analysis is chromatographic media selection. Subtle changes in selectivity are likely to occur, even with columns of the similar particle morphology and stationary phase (e.g. – C4) mainly due to differences in pore size distribution and surface chemistry. To demonstrate these differences, we tested two columns with a core-shell particle and butyl stationary phase: bioZen™ 2.6 µm WidePore C4 and BIOshell™ 2.7 µm IgG C4.

For the comparison application, we evaluated a commercial biotherapeutic, rituximab, under LC-UV conditions using trifluoroacetic acid (TFA) as the mobile phase. The performance of the two columns is shown in **Figure 1**. As evident, there are minimal differences in the LC-UV trace, which shows both columns are suitable for this specific mAb.

However, when looking at intact LC-MS analysis of NIST mAb, separation profile differences are observed (**Figure 2**). In this case, the peak of interest is a light chain impurity of NIST mAb that elutes prior to the intact, full mAb. While both columns provide separation of the impurity, the baseline separation on the bioZen WidePore C4 column avoids any spectral overlap in the MS detector. Therefore, in the case of NIST mAb analysis, the stationary phase of the bioZen WidePore C4 provides the necessary separation relative to the BIOShell IgG C4. While this difference does not occur in all mAb assessments (e.g. – rituximab), it clearly demonstrates the differences in similar column options.

LC-UV Conditions (Figure 1)

Columns: bioZen 2.6 µm WidePore C4
BIOshell 2.7 µm IgG C4
Dimensions: 100 x 2.1 mm
Part No.: 00D-4786-AN (WidePore C4)
Mobile Phase A: 0.1 % TFA in Water
Mobile Phase B: 0.1 % TFA in Acetonitrile
Gradient Program: 30-40 % B in 5 min
Temperature: 60 °C
Flow Rate: 0.8 mL/min
Detection: UV @ 280 nm
Injection: 2 µL
Sample: Rituximab, 1 mg/mL

LC-MS Conditions (Figure 2)

Columns: bioZen 2.6 µm WidePore C4
BIOshell 2.7 µm IgG C4
Dimensions: 100 x 2.1 mm
Part No.: 00D-4786-AN (WidePore C4)
Mobile Phase A: 0.1 % Formic acid in Water
Mobile Phase B: 0.1 % Formic acid in Acetonitrile
Gradient Program: 5-90 % B in 6.5 min
Temperature: 0.3 mL/min
Flow Rate: 80 °C
Detection: QTOF
Injection: 2 µL
Sample: NIST mAb, 1 mg/mL

Figure 1. Comparison of BIOshell™ IgG C4 with bioZen™ WidePore C4

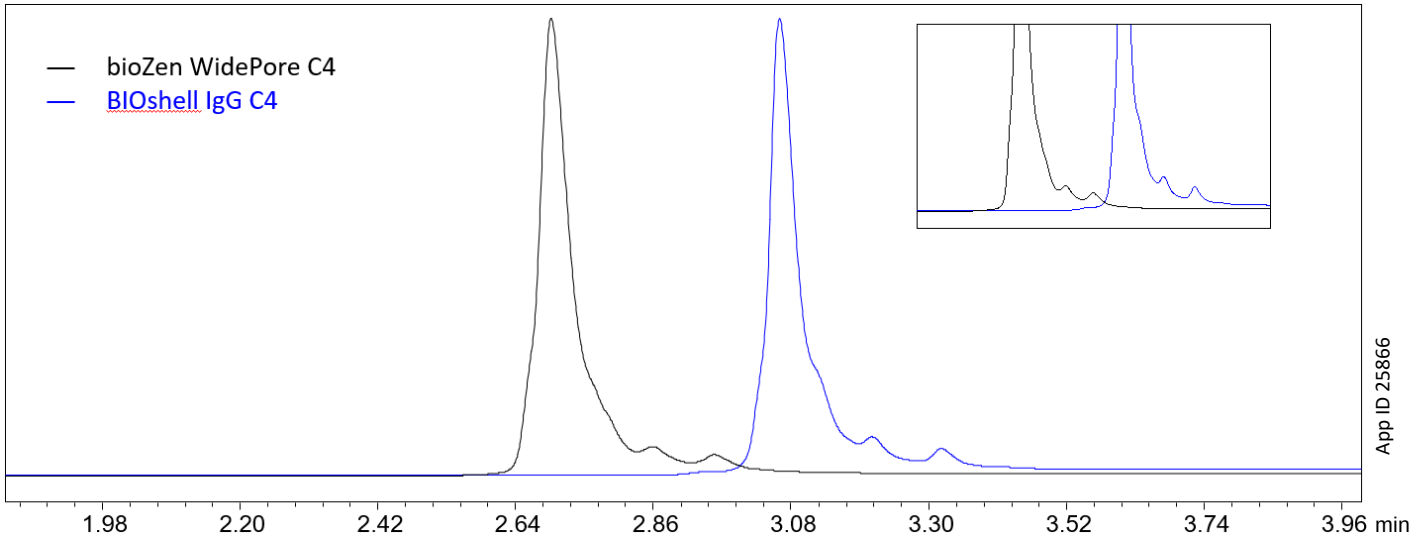
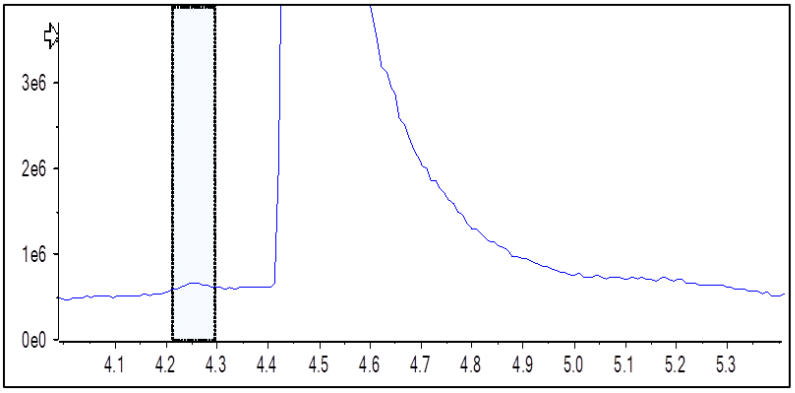
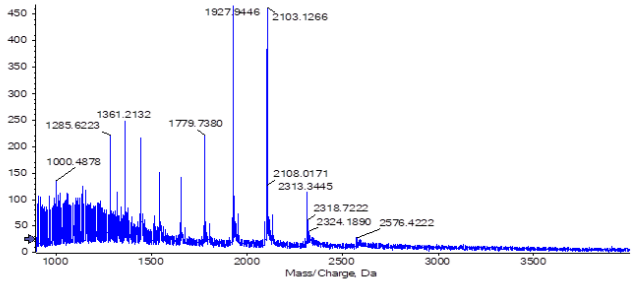


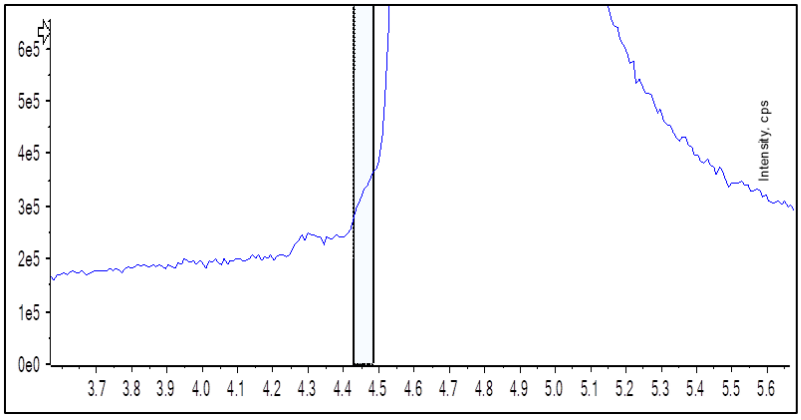
Figure 2. Intact LC-MS analysis of NIST mAb

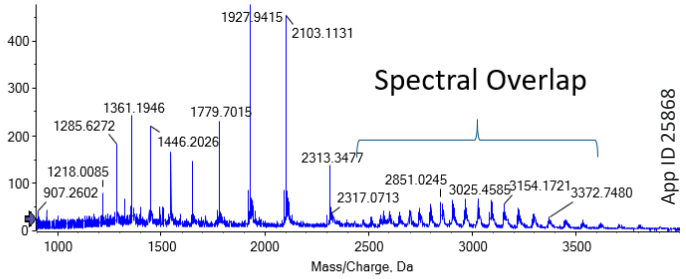
bioZen WidePore C4





BIOShell IgG C4





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