Optimization of Chromatography for Intact Mass Analysis of Monoclonal Antibodies Using bioZen™ WidePore C4 Columns

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Overview

The analysis of intact monoclonal antibodies (mAbs) by reversed phase HPLC is a common technique for assessing protein variation, such as clipping and heterogeneity due to post-translational modifications. When coupled with High Resolution Mass Spectrometers (HRMS) such as a Time of Flight (TOF) instrument, primary sequence can be confirmed and with sufficient spectral quality, minor isoforms and glycoforms can also be identified.

Of critical importance for intact mass applications is obtaining optimal peak shape to ensure that the HRMS can acquire spectral data. Further, peak broadening and peak tailing might lead to isoforms being excluded from the spectrum. Working with bioZen 2.6 µm WidePore C4 helps to resolve peak shape and peak broadening due to the high consistency of high performance core-shell particles found in these columns.

Although ion pair such as trifluoracetic acid (TFA) is volatile, it is ion suppressing and as such, if sample is limited or if sensitivity is prioritized over chromatography, analysis is limited to formic acid. Consequently, obtaining good quality chromatography can be challenging.

To improve peak shape, gradients must be optimized appropriately. Often a gradient slope as exceeding 4% B/column volume is implemented to ensure gaussian peak. This is contrary to typical intact reversed phase impurity analyses applications, where selectivity is often prioritized over peak shape. Gradient slope can be optimized by either shortening of the gradient program length, as in **Figure 1**, or directly changing starting and ending points for %B, as in **Figure 2**, both of which result in minimal peak widths.

Additionally, temperature can be modulated to improve peak shapes, namely, increases in temperature typically yield superior peak shapes. Provided the gradient program is run no more than 10 minutes, high temperature should not lead to significant on-column degradation **Figure 3** shows incremental improvements in peak shape as well as overall peak recoveries. Temperature in the context of the steep gradient slope is less impactful for selectivity but again, can lead to incremental improvements in peak shapes and heights and should be explored appropriately.

Finally, in running a somewhat "ballistic" gradient, i.e. gradient slopes exceeding 3% organic per column volume, lower flow-rates often produce better overall peak shapes. Again, this directly impacts %B per column volume. **Figure 4** shows these improvements as one decreases flow-rate, resulting in changes to gradient slope.

In conclusion, temperature and gradient slope are two critical method parameters to investigate for intact mass applications for mAbs when using bioZen™ 2.6 µm WidePore C4. In addition, gradient slope can be optimized by changes in gradient program (i.e. gradient start/end, program length) as well as flow-rate.

LC Conditions

Column: bioZen 2.6 µm WidePore C4
Dimensions: 100 x 2.1 mm
Part No.: 00D-4786-AN
Mobile Phase: A: 0.1% TFA in Water
B: 0.1 % TFA in Acetonitrile
Gradient **Time (min)** **% B**
See Figure 1 and 2

Flow Rate: See Figure 4
Injection Volume: 2 µL
Column Temperature: See Figure 3
Detection: UV-Vis @ 280 nm

Figure 1: Chromatogram stack showing difference in peak when varying gradient program. Black trace is run with gradient program of 15-70% B, blue trace is 10-90%. Flow-rate and gradient length is 0.5 mL/min and 4 minutes, respectively.

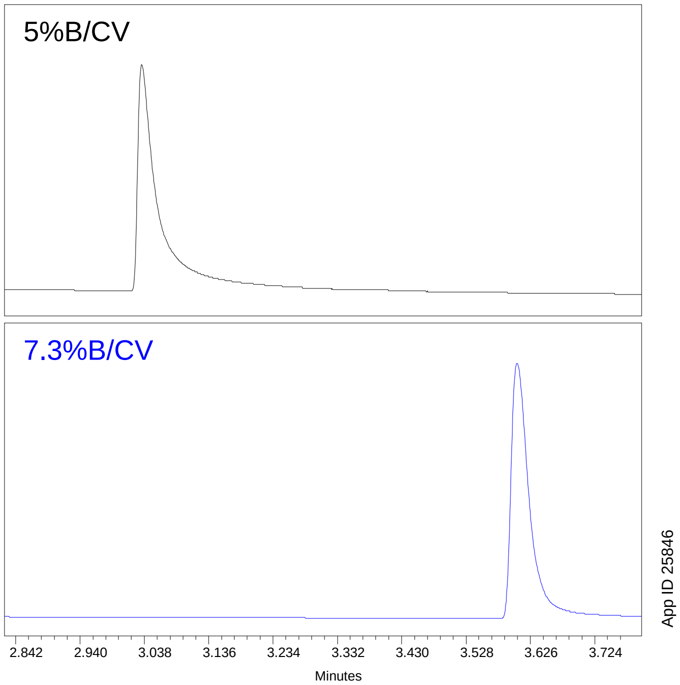


Figure 2: Chromatogram overlay showing the effect of gradient slope, with gradient program length (4 minutes) remaining constant. A change of 7.3% B per column volume yields superior peak shape.

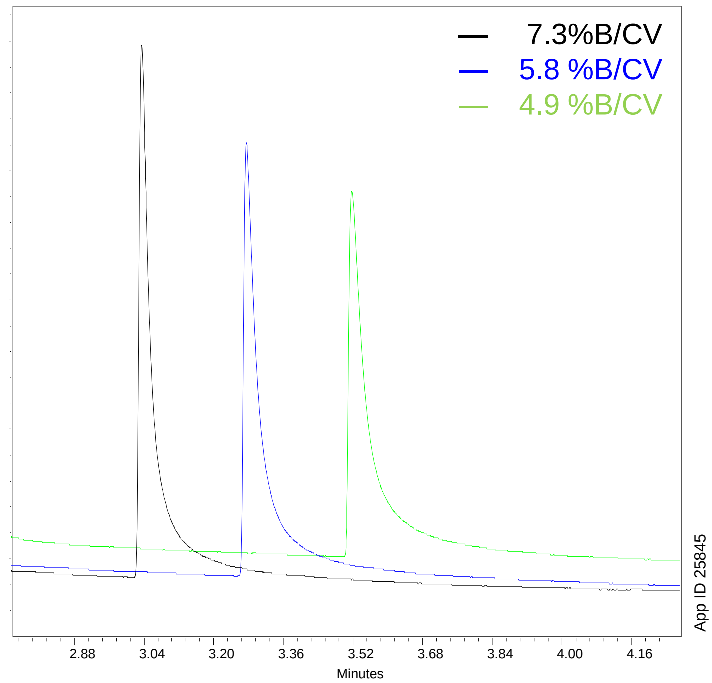


Figure 3: Overlay showing the effect of temperature on peak shape. Peak shapes and recoveries are nominally improved with increasing temperature, though little change to selectivity nor retention time.

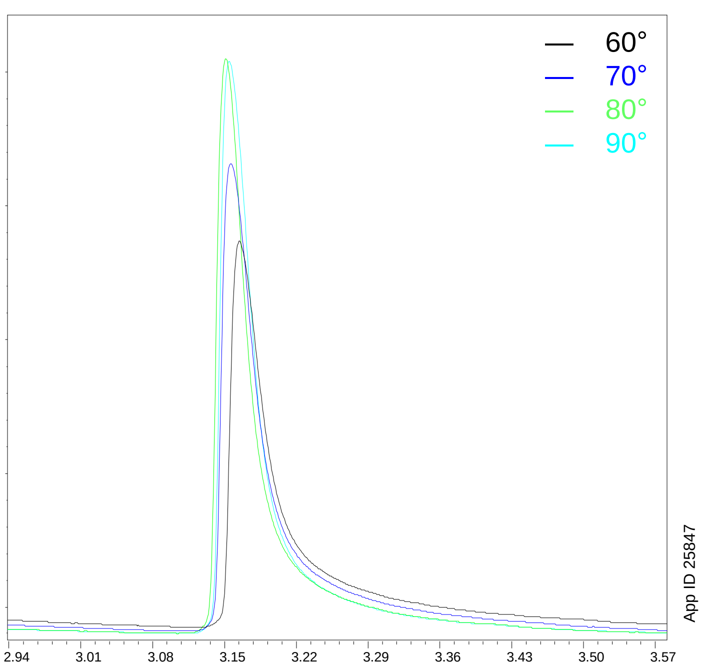
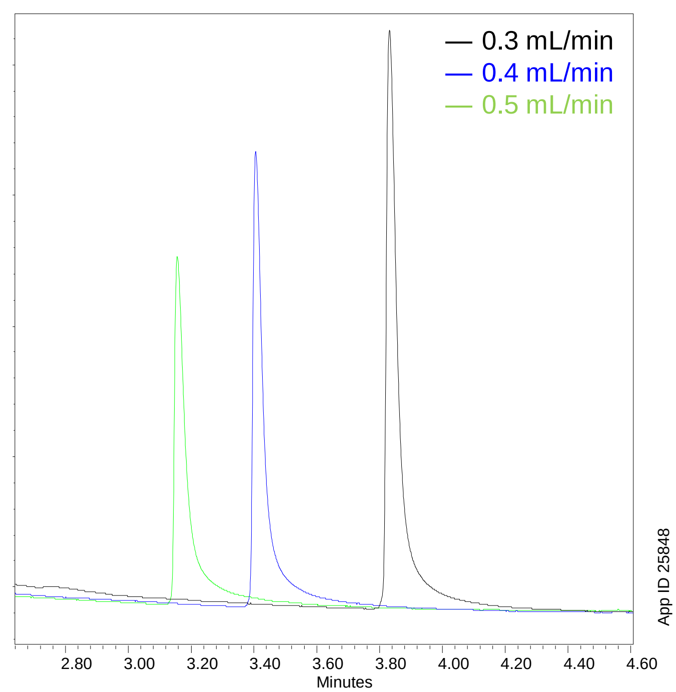


Figure 4: Overlay showing changes in flow-rate while maintaining gradient length (4 minutes) 0.3 mL/min giving optimal peak shapes and heights. This is a direct result of the impact lower flow-rate has on gradient slope, with 0.3 mL/min giving the highest (7%B/CV) when compared to other linear velocities.



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