Comparison of UHPLC Particles for Intact Mass Subunit Analysis of Monoclonal Antibodies

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Overview

With many regulatory characterization requirements in biotherapeutic development, one of the essential requirements 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. The mAb can be further simplified by reducing to yield heavy chain and light chain. These subunits can then be analyzed by high resolution MS detection such as a time of flight (TOF) instrument to provide more insight on sample heterogeneity and glycoform identity that otherwise would not be determined at the intact level.

Because peak shapes are prioritized for intact mass LC methods, a relatively ballistic gradient (i.e. gradient slopes exceeding 5% organic per column volume) must be implemented to ensure that good peak shapes are maintained. The resulting Gaussian peak shape should facilitate the spectral acquisition of the MS. Although less of a concern at the intact level, when analyzing subunits, the separation may be adversely affected when running steep gradient slopes. Namely, a loss of resolution between the subunits may result in poorer spectral quality, because of partial coelution.

As such, the use of high efficiency UHPLC particles, i.e. sub-2 µm fully porous or more recently, superficially porous particles, have been more common for intact mass subunit MS analysis. Although both sub-2 µm fully porous particles and superficially porous particles can obtain acceptable resolution between mAb heavy chain and light chain, peak shapes may be less desirable using the sub-2 µm particle. This may impact both the spectrum quality, as well as the characterization of different variants. Utilizing a bioZen™ 2.6 µm WidePore C4 column compared to a sub-2 µm C4 allows for separation advantages for subunit analysis.

Figure 1 shows the separation obtained using a sub-2 µm fully porous particle for reduced NIST mAb. Separation of light chain and heavy chain is acceptable, though there is some peak tailing observed with the heavy chain fragment, even with the high gradient slope for the chromatographic method (7.9 % B/column volume).

Conversely, in **Figure 2**, we can observe the separation of reduced NIST mAb giving better peak shapes for both heavy and light chain. Further, a later eluting impurity, presumed deamidated variant because of the similar spectra obtained relative to the main peak, is separated.

In summary, the use of steep gradient slopes limits the separation when analyzing mAb subunits by intact mass. High efficiency particles could be adequate for this separation but the use of bioZen 2.6 µm WidePore C4 core-shell superficially porous particles may be optimal in instances where efficiency is required for detection and separation of low level impurities.

LC-MS Conditions

Column:	bioZen 2.6 µm WidePore C4, 400 Å
	Fully porous 1.7 µm C4, 300 Å
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 minutes
Flow-rate:	0.3 mL/min
Temperature:	80 °C
Detection:	QTOF
Injection:	2 µL
Sample:	NIST mAb, 1 mg/mL

Figure 1. Separation of NIST mAb Heavy and Light Chain on sub-2 µm, Fully Porous C4, 300Å

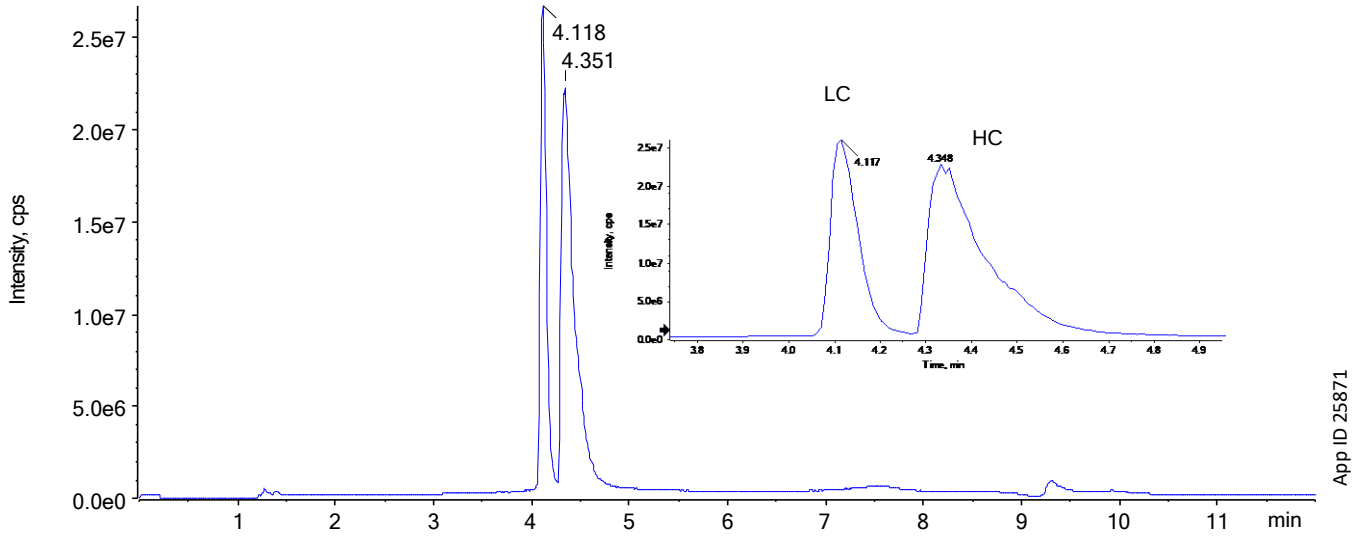
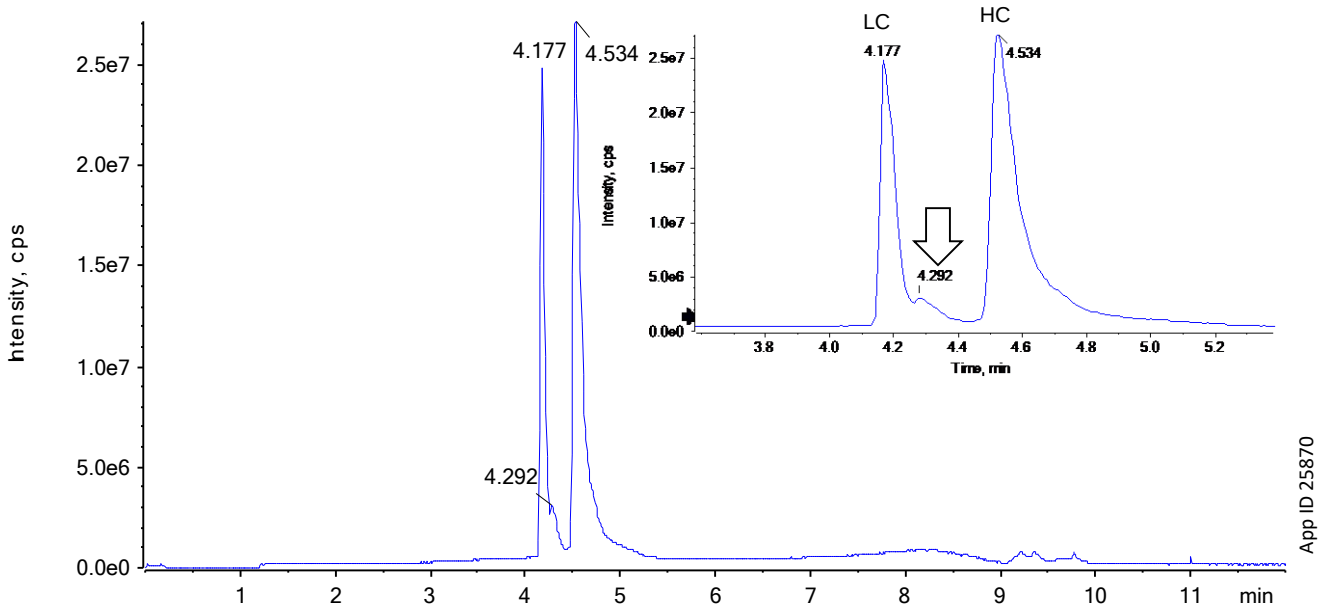


Figure 2. Separation of NIST mAb Heavy and Light Chain on bioZen™ WidePore C4, 400 Å



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