Qualitative Characterization and Quantitative Assessment of Monoclonal Antibodies Using Protein Metrics and nSMOL coupled with the Shimadzu LCMS-9030 QTOF

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1. Introduction

In this poster, we use the new LC-MS 9030 Q-ToF to qualitatively characterize NIST mAb reference standard as model of biotherapeutic monoclonal antibodies (Figure 1). Assessments of intact profile, protein subunits (heavy chain, and light chain), and peptide fragments were included in experimental design. To aid in the quantitative assessment of Bevacizumab in biological matrix, the Shimadzu nSMOL™ (nano-surface and molecular orientation limited proteolysis) technology was utilized for increased sensitivity by selectively digesting the antibody's FAB region and reducing matrix interference. MRM transitions offer an increased limit of detection for quantifying the amount of mAb in human serum or blood for PK/PD determination and therapeutic drug monitoring (TDM)

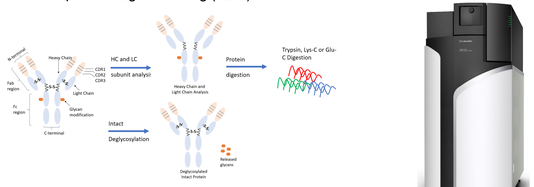

Figure 1: Typical mAb structure

2. Experimental

Materials and Methods

For intact protein analysis, NIST mAb was diluted to 1mg/mL in 50mM ammonium bicarbonate. 100µg of the intact protein was reduced to its HC and LC subunits by adding 8M Urea, 50mM Tris-HCl, and 50mM DTT. HC and LC subunits were alkylated with 500mM IAA followed by digestion using trypsin gold (Promega Corp, Madison WI), Lys-C (Promega Corp, Madison WI), or Glu-C (Promega Corp, Madison WI) at a 1:25 enzyme:substrate ratio. Intact protein was also treated PGNGase F (Promega Corp, Madison WI) to remove glycosylation on the heavy chain region. Protein Metrics Byos software was used to characterize all components. For quantitative work, MRM transitions were found using nSMOL™ (nano-surface and molecular orientation limited proteolysis[3]) to selectively digest the FaB region of the antibody (Figure 2)

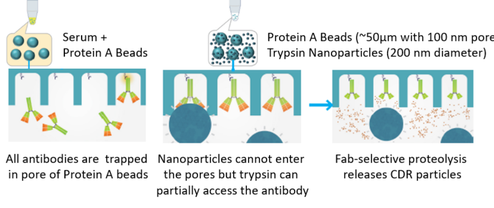

Figure 2: nSMOL enables selective proteolysis of the Fab region of monoclonal antibodies

Instrumentation

All data was obtained on a Shimadzu Nexera X2 UHPLC in conjunction with a Q-TOF Mass Spectrometer, LCMS-9030. The specific configurations include LC-30AD x2 solvent delivery pumps, DGU-20A5R online degassing unit, SIL-30ACMP autosampler, CTO-20AC column oven, CBM-20A system controller, LCMS-9030 QTOF and LabSolutions Ver. 5.95 chromatography workstation.

3. Results

Intact NIST mAb

The mass spectrum and deconvoluted spectrum of NIST mAb intact protein is shown in Figure 3. The found masses were consistent to the theoretical NIST mAb masses (Table 1). The intact mass was deconvoluted using Protein Metrics Intact Mass Workflow.

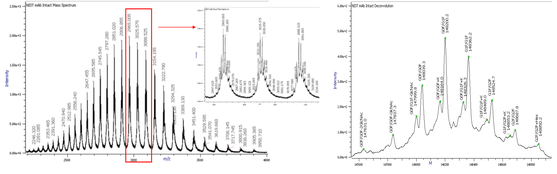

Figure 3: NIST mAb mass spectrum (left) and deconvoluted spectrum (right)

Table 1: Theoretical vs Observed Mass for Intact NIST mAb on the Shimadzy LCMS-9030

Glycoform	Theoretical Mass (Da)	Observed Mass (Da)	Δmass
G0F/G0F –2GlcNAc	147630.8	147631.0	0.2
G0F/G0F –GlcNAc	147834.0	147837.3	3.3
G0F/G1F –GlcNAc	147996.1	147999.8	3.7
G0F/G0F	148037.2	148039.3	2.1
G0F/G0F + K	148165.3	148164.0	1.3
G0F/G1F	148199.3	148200.2	0.9
G0F/G1F + K	148327.5	148326.3	1.2
G1F/G1F	148361.4	148362.2	0.8
G1F/G1F +K	148489.6	148489.0	0.6
G1F/G2F	148523.6	148524.7	1.1
G1F/G2F + K	148651.8	148653.2	1.4
G2F/G2F	148685.7	148687.8	2.1
G2F/G2F + Hex	148847.7	148850.2	2.5

Light chain and heavy chain

The mass spectrum and deconvoluted spectrum of the LC and HC subunits for NIST mAb are shown in Figure 4. The subunits' masses were deconvoluted using Protein Metrics Intact Mass Workflow.

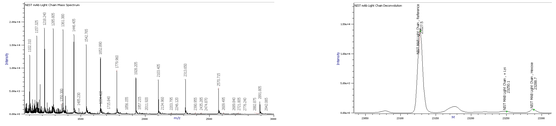

Figure 4a: Heavy Chain mass spectrum (left) and deconvoluted spectrum (right)

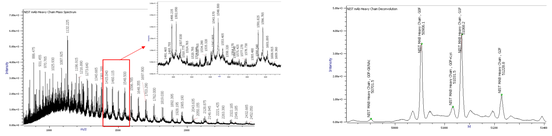

Figure 4b: Light Chain mass spectrum (left) and deconvoluted spectrum (right)

Peptide Mapping Digestion Results

HC and LC subunits were digested with either trypsin, Glu-C or Lys-C proteases. Using different enzymes can selectively cleave different amino acid residues of the protein giving more confidence in the protein sequence. The results were analyzed using Protein Metrics PTM Workflow (Figure 5).

Figure 5. NIST mAb peptide coverage using tryprin (blue), Lys-C (red) and Glu-C (black)

Deglycosylation

*N-linked glycosylation is a common post-translational modification (PTM) that imparts structural heterogeneity to mAb therapeutics. PNGase F (*Promega Corp, Madison WI) *was used to release the N-glycans from the HC component of intact NIST mAb (Figure 6a) and from the reduced NIST mAb subunit (Figure 6b).*

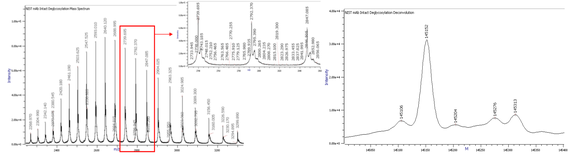

Figure 6a: NIST mAb intact deglycosylation mass spectrum (left) and deconvolution (right)

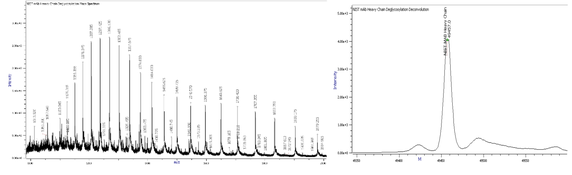

Figure 6b: NIST reduced deglycosylation mass spectrum (left) and deconvolution (right)

Quantitation

Serum spiked with Bevacizumab was subjected to proteolysis of the Fab region using nSMOL technology. Peptide FTFSLDTSK was used for quantitation with a custom stable labeled internal standard. Calibration range for quantitation was from 100ng/mL to 10,000ng/mL. The calibration curve is shown in Figure 7. Accuracy was less than 20% at all calibration levels and r^2 was 0.998. For more information on nSMOL technology, please visit www.totallabbio.com.

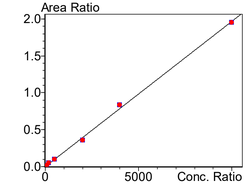

Figure 7: Peptide FTFSLDTSK calibration curve

Special thanks to Intertek Pharmaceutical Services for preparation of the nSMOL quantitation samples