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Comprehensive Quantitative Study of mAb by Q-TOF/MS and Ion Mobility Q-TOF/MS

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Overview

This work describes the utilization of the Agilent's 6550 Q-TOF and the 6560 IM Q-TOF systems to rapidly determine the accurate concentration of mAb sample.

Introduction

Monoclonal antibodies (mAbs) and their derivative products such as Antibody-Drug Conjugate (ADC) and Biosimilars comprise a very important class of biopharmaceutical molecules with a wide range of therapeutic and diagnostic applications. Regulatory guidelines mandate the establishment of the analytical methods to ensure accurate measurement of such molecules. LC-MS techniques have been widely used to provide the high resolution and high mass accuracy for protein quantitation. In the past, we have also reported the use of LC-Q-TOF for the intact mAb quantitation and the LC-QQQ for quantifying mAb at the peptide level (the MRM approach). However, both of these quantitative methods posted some limitations: Intact mAb quantitation by TOF/Q-TOF is simple and fast, but with limited sensitivity in the low ng (fmol) range. Peptide quantitation using QQQ offers great sensitivity in the low amol range, but needs multiple steps sample preparation and time consuming. Here we present quantifying mAbs subunits after rapid IdeS digestion and deglycosylation with fg (amol) sensitivity on Q-TOF.

Figure 1. Agilent's 6550 Q-TOF and 6560 IM Q-TOF system.

Experimental

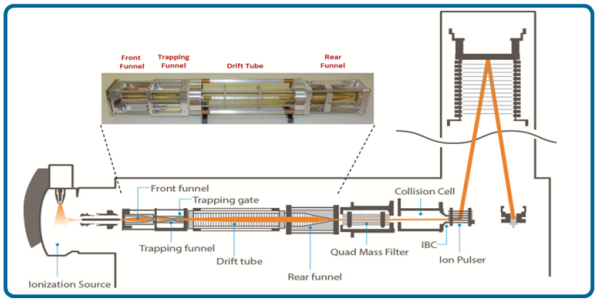


Figure 2. Schematic diagram of Agilent's 6560 IM Q-TOF system.

Sample Preparation:

IdeS digestion: IdeS protease (Genovis Inc) was added at 1 unit enzyme per 1 ug of mAb. The digestion was carried out in 50 mM sodium phosphate, 150 mM NaCl (pH 6.6) buffer at 37°C for 30 min.

Protein deglycosylation: Deglycosylation of IgG-2 was achieved by incubating with the Rapid PNGase F (NEB) in the Rapid Buffer at 37°C for 20 min.

mAb reduction: The completed IgG-2 reduction was done by incubation of mAb with 20 mM DTT at 57°C for 30 min.

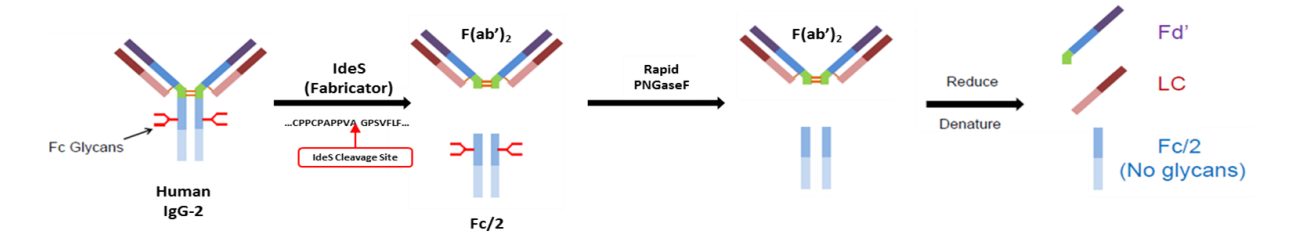
LC/MS Analysis:

Agilent 1290 Infinity UHPLC with Agilent Poroshell 300SB-C8 Column (2.1x 75 mm) was coupled to either the 6550 Q-TOF/MS or the 6560 IM Q-TOF/MS system with the dual Agilent Jet Stream (dual AJS) source. Sample concentrations ranging from 50 fg/µL to 100 ng/µL were introduced onto the column at a flow rate of 0.5 mL/min using the 20 min HPLC gradient.

Data Analysis:

The data obtained from LC/MS were analyzed using Agilent MassHunter BioConfirm, Quantitative Analysis and IM-MS Analysis software. The top most abundant peaks in targeted molecule's MS charge profiles were selected for quantitation. Four qualifiers (four other m/z next to the most abundant peaks) were also selected for accurate quantitation analysis.

Results and Discussion



Scheme 1: Sample Preparation Workflow: Enzymatic digestions (IdeS and PNGaseF) and reduction on mAb.

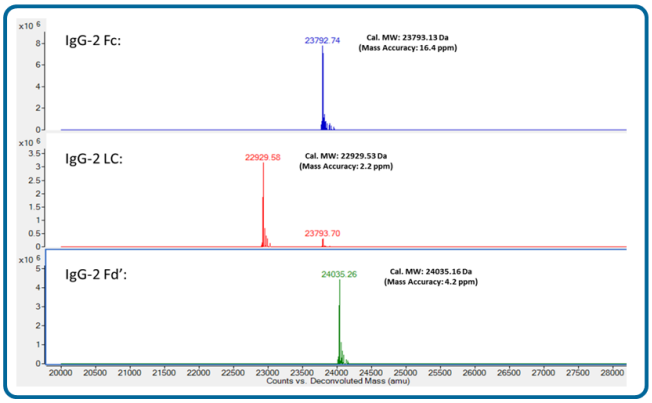


Figure 3. MS deconvolution spectrum of various enzymes-treated IgG-2 fragments. Top panel: IgG-2 Fc, Middle: IgG-2 light chain, Bottom: IgG-2 Fd'. Excellent mass accuracy (2 – 16 ppm) was achieved on these IgG-2 fragments.

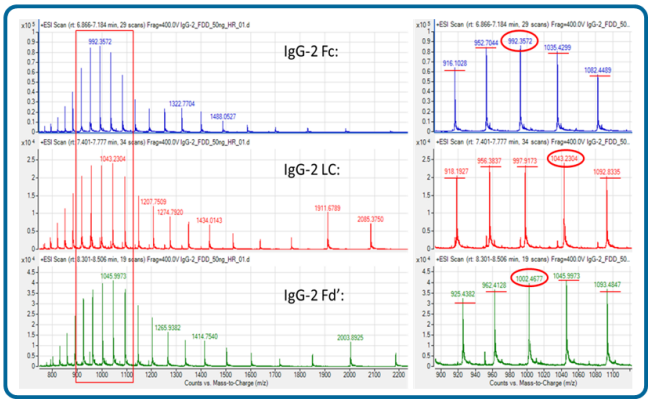


Figure 4: Mass spectrum of three IgG-2 fragments (LC, Fd' and Fc) at 50 ng on column (Poroshell SB-300 C8, 5µm, 1.0 x 75 mm) using the 6550 iFunnel QTOF system (left). For IgG-2 quantitation (right), the top most abundant peaks of each fragments (circled) were selected as the quantifiers and the four adjacent peaks (underlined) were also selected as the qualifiers.

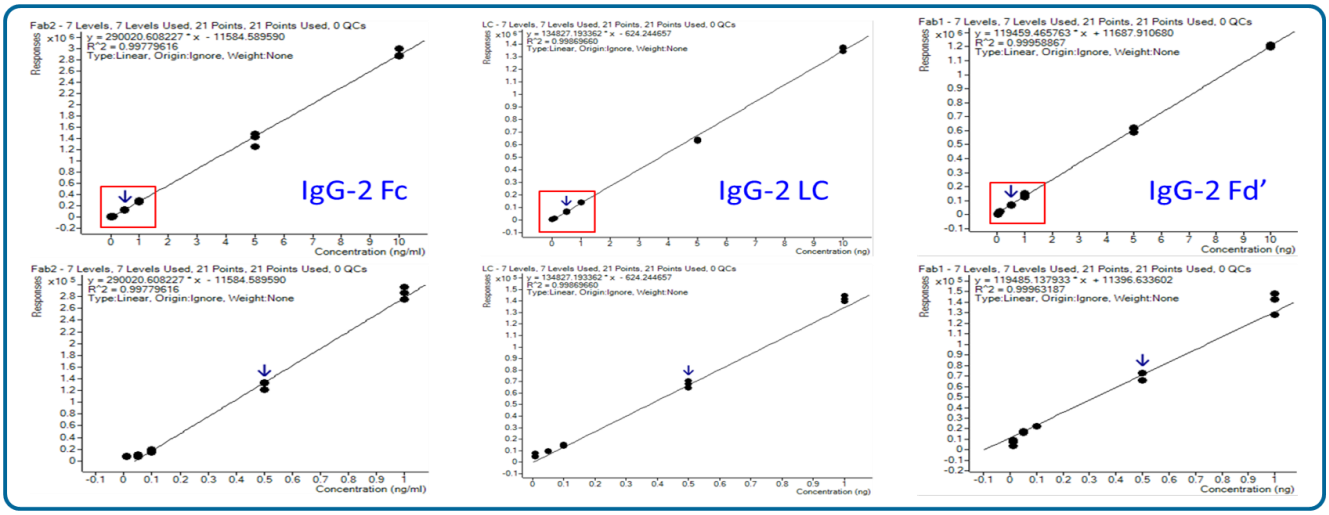


Figure 5: Calibration curves of Enzymes-treated IgG-2 from 50 fg to 10 ng on column (Poroshell SB-300 C8, 5µm, 1.0 x 75 mm) using the 6550 Q-TOF/MS system.

Results and Discussion

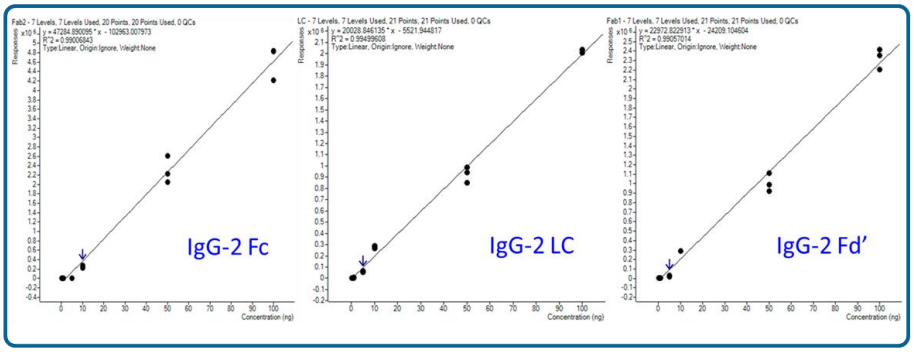


Figure 6: Calibration curves of Enzymes-treated IgG-2 from 500 fg to 100 ng on column (Poroshell SB-300 C8, 5µm, 1.0 x 75 mm) using the 6560 IM Q-TOF/MS system.

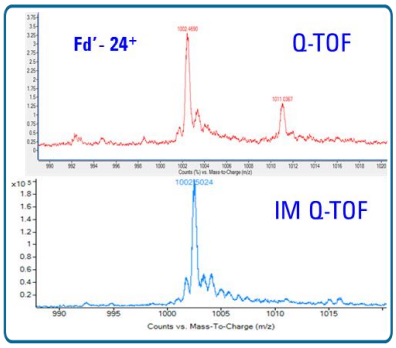


Figure 7: MS comparison of Q-TOF and IM Q-TOF data. With the additional drift separation, the IM data posted better signal-to-noise level.

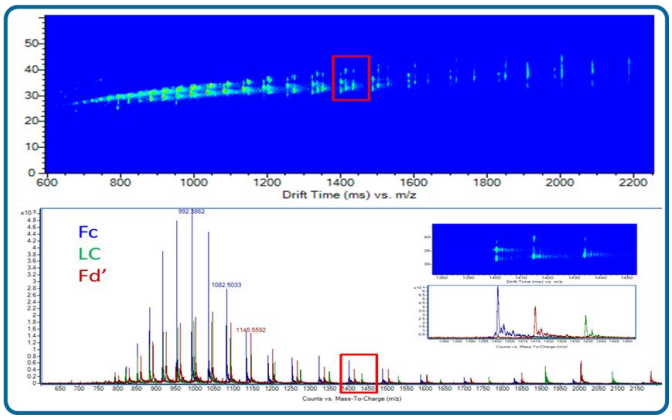


Figure 8: IM Q-TOF/MS analysis of IgG-2 fragments. Top: Ion Mobility heat map (drift time vs. m/z) of the mixture. Bottom: Mass Spectrum of the mixture. Insert: Various conformers were detected in these fragments with different charge states.

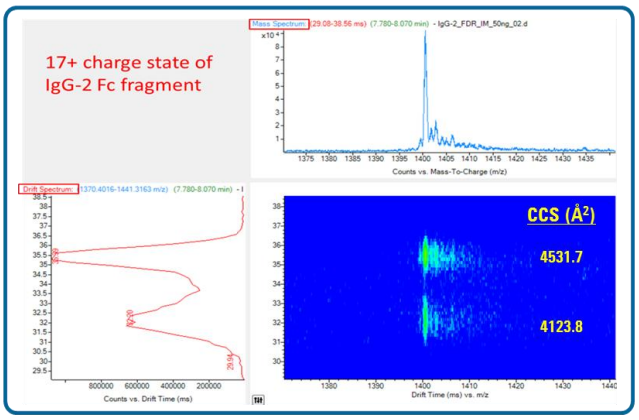


Figure 9: IM Q-TOF analysis of the IgG-2 Fc fragment demonstrated that its 17+ charge state molecule posted two major conformers with the Collision Cross Section (CCS) values of 4531.7 Å² and 4123.8 Å², respectively.

Conclusions

This work demonstrates the major advantages of using the Q-TOF or IM Q-TOF systems for rapid quantitation of the antibody samples:

- **Quantifying mAb subunits using Q-TOF has shown an improvement in the detection limit (LOD) at 100 fg – 500 fg (600 amol – 3 fmol). This is 10X more sensitive than quantifying the intact mAb using Q-TOF system. The dynamic ranges on quantitating all 3 target antibody fragments are about 2-3 orders.**
- **The IM Q-TOF system generated relatively higher LOD (500 fg – 1 ng) than the Q-TOF system. It posted about 2 orders of dynamic ranges in quantitating antibody fragments. However, the IM data showed better signal-to-noise as the background matrix effect can be significantly reduced.**
- **The 6560 IM Q-TOF system is an excellent analytical tool in separating and quantitating the biomolecules with various conformers (CCS values).**