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Quantitation of Biosimilar Monoclonal Antibody Glycans Using Microfluidic-based Chip with QQQ Mass Spectrometer

Ning Tang
ThP 641
Agilent Technologies
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Introduction

Generation of therapeutic monoclonal antibodies requires a range of manufacturing process, such as from cell culture, downstream purification, formulation, and final lot release. Fc-glycans in monoclonal antibody drugs have important therapeutic functions. Thus, the ability to precisely and rapidly determine these glycans could feedback the quality of the drugs timely in different manufacture steps. The ability to characterize glycans rapidly has been limited by the sample preparation steps and structural complexity of the glycoproteins. Previously, we developed a microfluidic chip to perform rapid on-line cleavage of glycans from monoclonal antibodies for detection by either TOF or Q-TOF mass spectrometers. The entire run time is 12 minutes. A glycan accurate mass database was established allowing quick assignment and identification of the glycans.

To further improve the versatility for various customer applications, we implemented a QQQ strategy with the same chip platform. This approach included the desired collision energy for each transition, and the optimized acquisition and tune for glycan detection in QQQ.

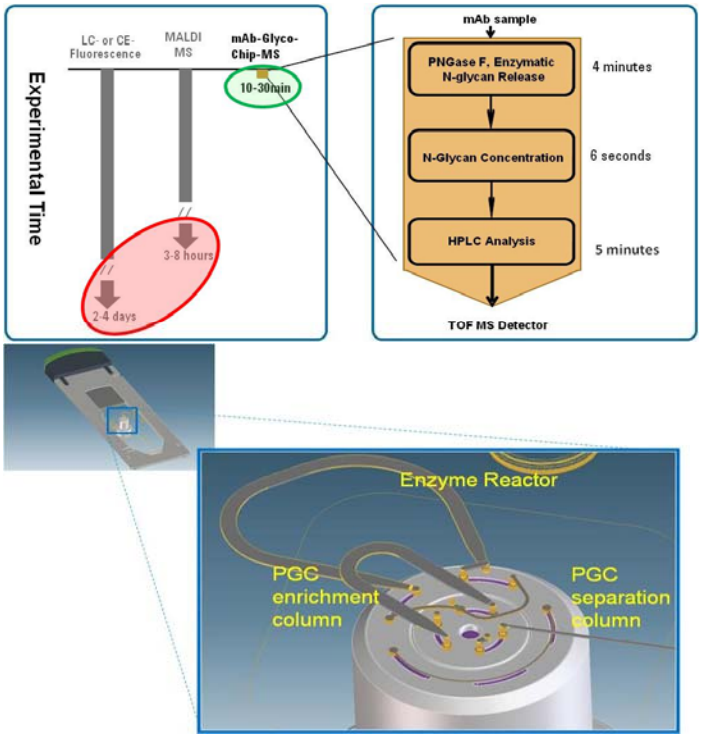

Figure 1. The mAb-Glyco chip consists of 3 columns: (a) a 310 nl enzyme reactor (ER), packed with immobilized PNGase F beads, (b) a 160 nl porous graphitized carbon enrichment column (PGC-EC) and (c) a 43 mm long porous graphitized carbon separation column (PGC-SC).

Experimental

Instruments

The instrument consisted of an Agilent Technologies 1200 Series nanoflow and capillary HPLC pumps, microdegassers, micro wellplate autosampler with thermostat, HPLC-Chip/MS interface, 6520 Q-TOF or 6460 QQQ mass spectrometers.

HPLC-Chip: mAb-Glyco Chip

LC conditions: Flow: 500 nL/min analytical pump, 3 µL/min loading pump. Loading pump mobile phase: deglycosylation buffer (Agilent). Analytical pump mobile phases A for analytical pump: 0.1% formic acid (FA) in water B: 90% Acetonitrile, 0.1% FA. Gradients: 2%B to 32%B at 2 min Stop time: 12 min.

Q-TOF MS conditons: Drying gas: 5L/min, 325°C; Auto MS/MS collision energy 15V. Optimization of the collision energy for each product ion was done using a prototype software. The collision energy was ramped automatically from 1V to 50V with 3V intervals.

QQQ MS conditions: Drying gas: 5 L/min, 325°C; Fragmentor: 125 V; Collision energy: optimized for each transition; Dwell time: 20ms; Delta EMV: 200V.

mAb-Glyco Chip Kit

The mAb-Glyco Chip Kit incorporates the mAb-Glyco Chip, the Reagent Pack, the mAb-Glyco Chip Content Disk, and a Quick Start Guide. The reagent pack provides all chemicals needed for ready chip operation: System Conditioning Reagent for flow path deactivation and carry over minimization, Glycan Standards for chromatographic checkout and method development, Antibody Standard for functional checkout and troubleshooting, and Deglycosylation Buffer for dilution of standards, samples and for loading the mAb samples onto the chip's enzyme reactor. The Content Disk has optimized methods for HPLC-Chip/MS analysis and data processing, including efficiency tools such as glycan accurate mass and structure database for ease of characterization, and reporting templates.

Results and Discussion

Using mAb-Glyco Chip on Q-TOF for fast glycan profiling

Three mAb glycans were analyzed using mAb-Glyco Chip on Q-TOF mass spectrometer. The data was searched against the mAb glycan accurate mass database. Twenty five glycans were identified.

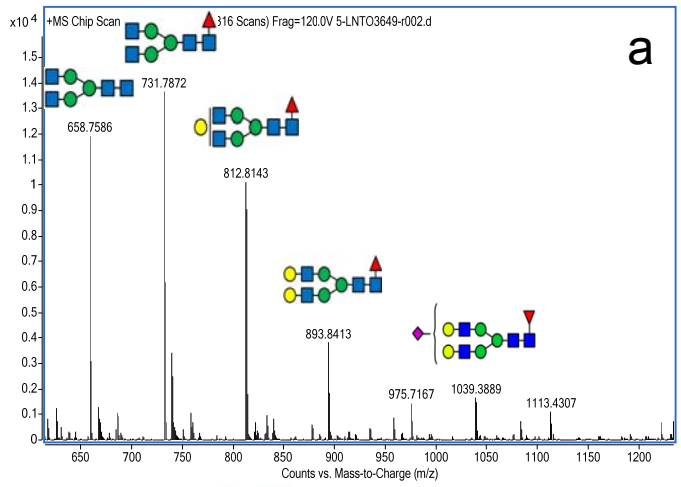

Figure 2. a) Average mass spectra between 9 - 10.5 minutes with major glycans annotated. b) Overlaid extracted compound chromatogram with major glycans annotated.

Using PCA to look at similarities and differences

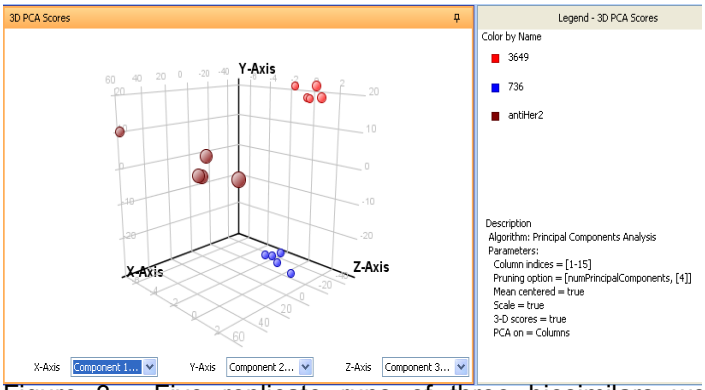

Figure 3. Five replicate runs of three biosimilars were analyzed using a statistical software Mass Profiler Pro (Agilent). PCA plot showed each mAb clustered together and three biosimilars clearly separated.

Targeted MS/MS to further confirm the glycan specificity

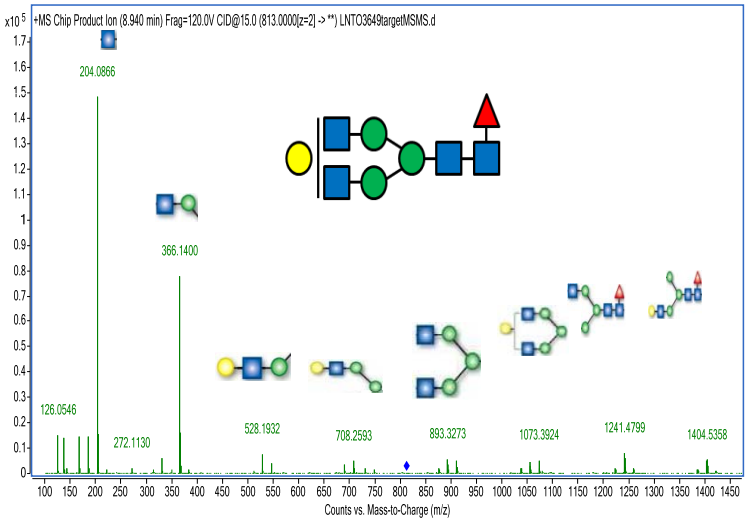

Figure 4. MS/MS spectrum of G1 acquired with collision energy of 15V. The major fragments are annotated by the glycan structure.

Determine the optimum collision energy for each product ion

To determine the optimum collision energy for each product ion, MS/MS was acquired again using automatic ramping of the collision energy from 1V to 50V with 3V intervals. The intensity of four product ions were extracted and normalized by the total intensity. The normalized intensity was plotted against the collision energy. The optimum CE for each product ion was deduced from the plot.

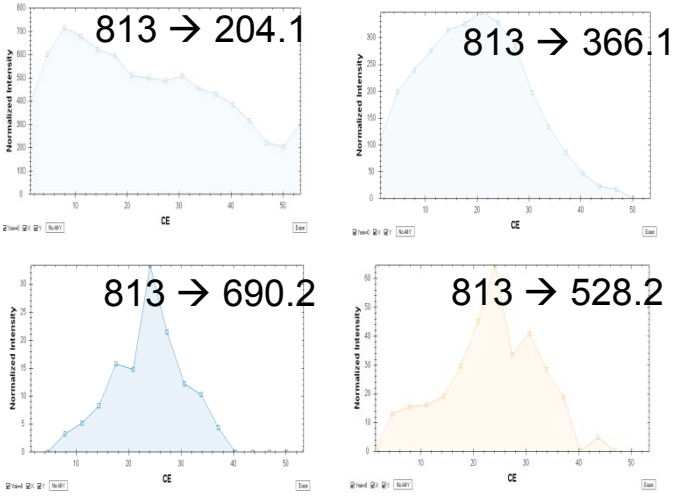

Figure 5. Normalized product ion intensity profiles from G1 as the function of collision energy (CE). The optimum collision energy was used to develop MRM assays on QQQ mass spectrometer.

Results and Discussion

Different optimum collision energies for N-acetyl-glucosamine (204.1) from different glycans

Optimization for different glycans were performed using the automated CE ramping. For G0, the optimum CE for 204.1 lies around 10V, while for G2+SA, the optimum CE is around 50V. The wide difference in CE for the complex glycan could be due to the sialic acid is attached at the terminal end, which requires higher CE for the core N-acetyl glucosamine to fragment.

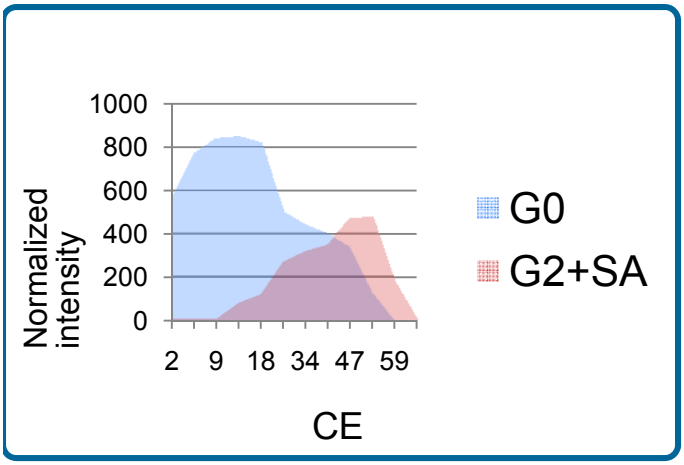

Figure 6. Comparison of optimized CE for N-acetyl-glucosamine fragments from two glycans G0 and G2+SA.

The monoclonal antibodies generated from three different manufacturers (Biosimilars) are analyzed by mAb-glyco chip with QQQ (MRM mode)

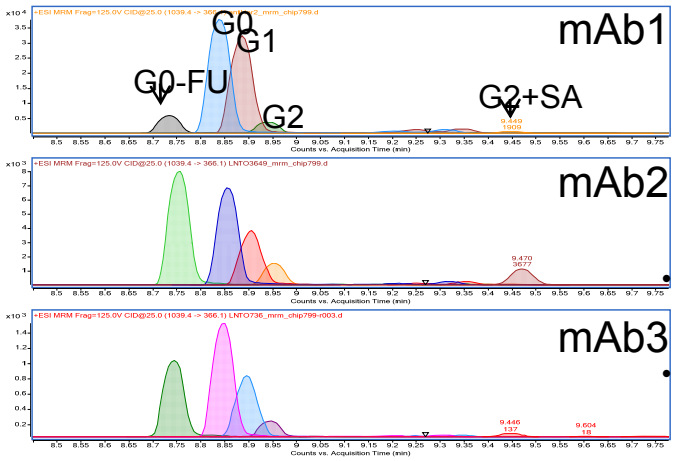

Figure 7. The glycan profiles of each mAb are compared. The relative ratio of the glycans from each mAb can be calculated from the peak area of each glycan.

Quantitation curves for glycans from mAb-Glyco-Chip

Quantitation curve was acquired using four different amounts of mAb on-chip, 50ng, 100ng, 500ng and 1000ng. Three replicates for each concentration. R^2 ranged from 0.973 -0.993.

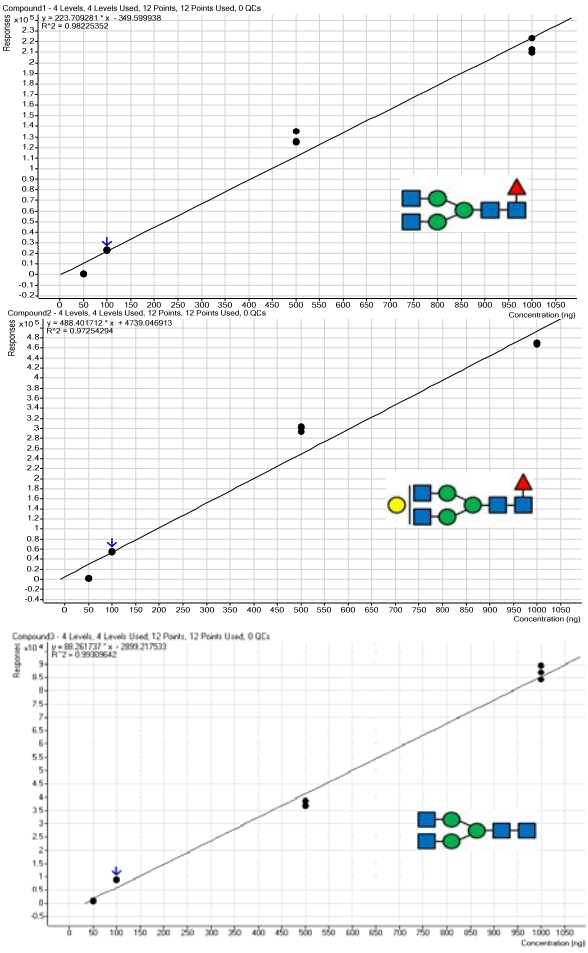

Figure 8. Quantitation curves for three glycans using MRM assay.

Conclusions

The mAb-Glyco-Chip is an integrated microfluidic chip, which can be easily transferred from one type of mass spectrometer to another.

The analysis of mAb glycosylation is reproducible and quantitative by either precursor ion (QTOF) or product ion (QQQ) monitoring.

The targeted MS/MS in QTOF can be used to further confirm the glycan identity if needed. Similarly, the use of MRM in QQQ obtains the glycan specificity and sensitivity for quantitation.