Integrated Analysis of N-glycans from Innovator and Biosimilar Monoclonal Antibodies Using HPLC Chips with QTOF-MS

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Shiaw-Lin Wu[1], Yi Wang[1], Ning Tang[2], Dayin Lin[2], William S. Hancock[1], and Barry Karger[1]

[1]Barnett Institute and Department of Chemistry and Chemical Biology, Boston, MA 02115. [2]Agilent Technologies, Waldbronn, Germany 76337

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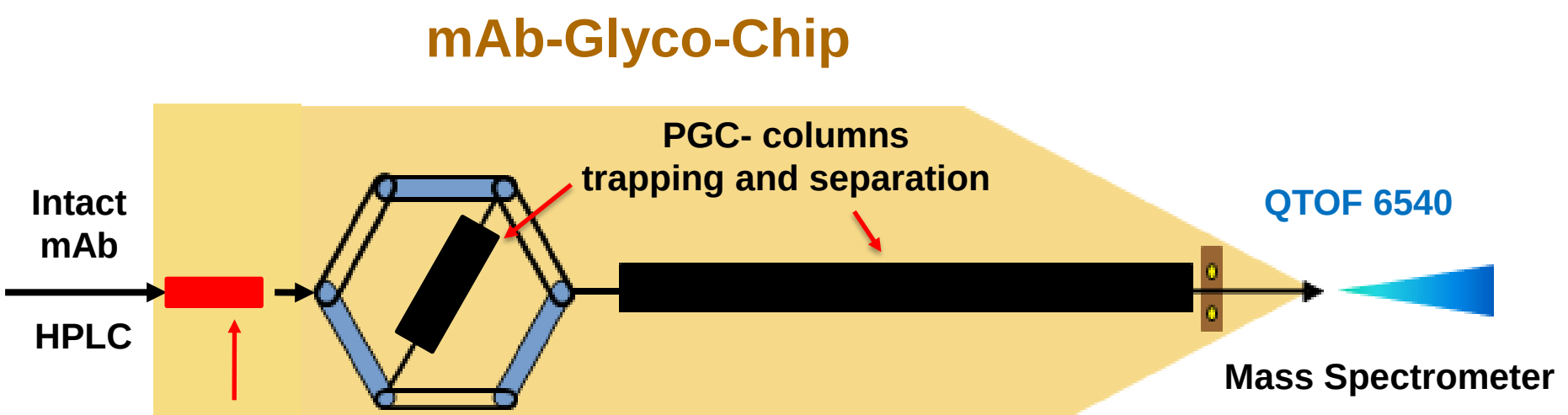
The N-linked glycans in the Fc region of therapeutic monoclonal antibodies are important to maintain drug efficacy. A robust LC-MS method with high throughput capability is needed to analyze these N-glycans. In this work, we used a chip-based LC-MS approach with immobilized PNGase F coupled to a high resolution QTOF mass spectrometer for analysis of the released glycans. Innovator and biosimilar monoclonal antibodies were studied by this approach to examine similarities and differences.

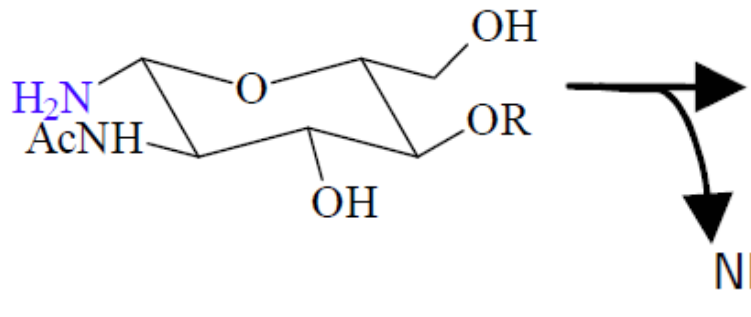
Methods

Intact monoclonal antibodies (100 ng) were directly loaded on a Agilent mAb-Glyco-Chip consisting of immobilized PNGase F and a porous graphitized carbon (PGC) column. After 4 min incubation with the immobilized PNGase F, the eluent (released glycans) was captured by the subsequent segment packed with porous graphitized carbon (PGC trap column). After washing, the trap column is then switched online with the analytical column (also packed with porous graphitized carbon), which was coupled to an Agilent QTOF 6540 mass spectrometer. A formic acid/acetonitrile linear gradient of 12 min duration was used for analysis. Separately, the glycans were released from the proteins by offline PNGase F treatment (37 °C, 4 hrs). These released glycans were then analyzed by an on-line PGC-Chip (porous graphitized carbon column) coupled to the QTOF 6540 mass spectrometer, using the same formic acid and acetonitrile gradient.

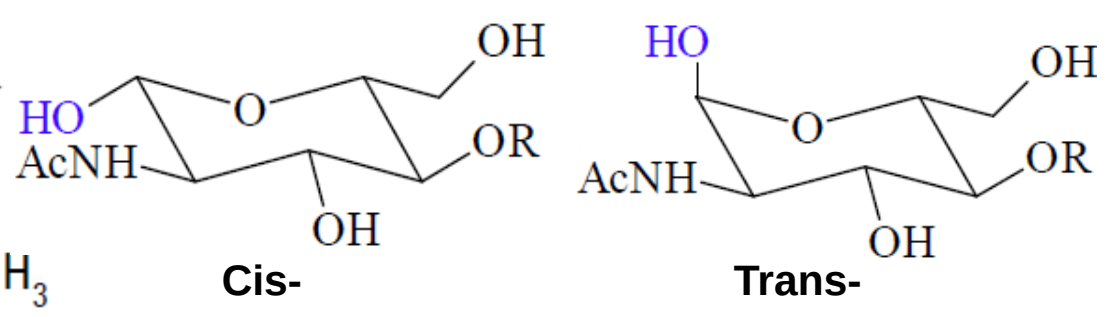


Deglycosylation by PNGase F

Online PNGase F (amine Form)



Offline PNGase F (hydroxyl form)



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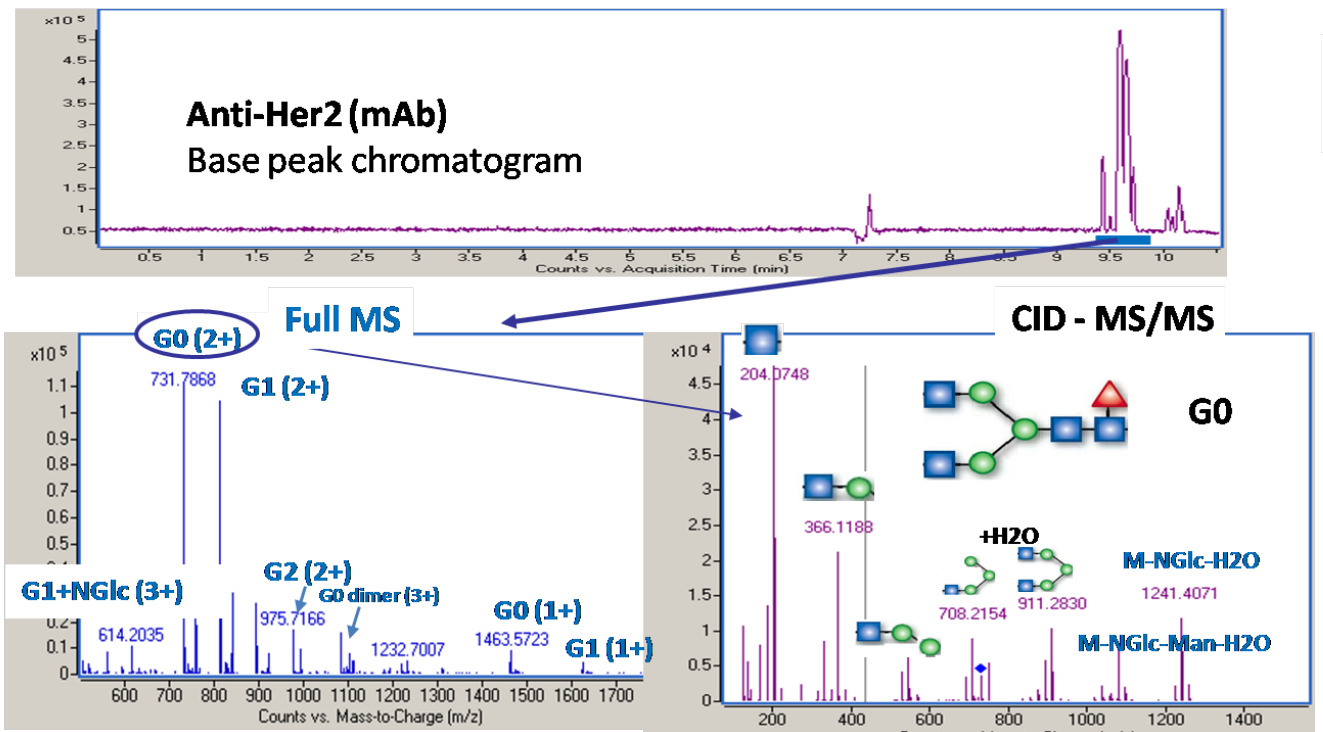
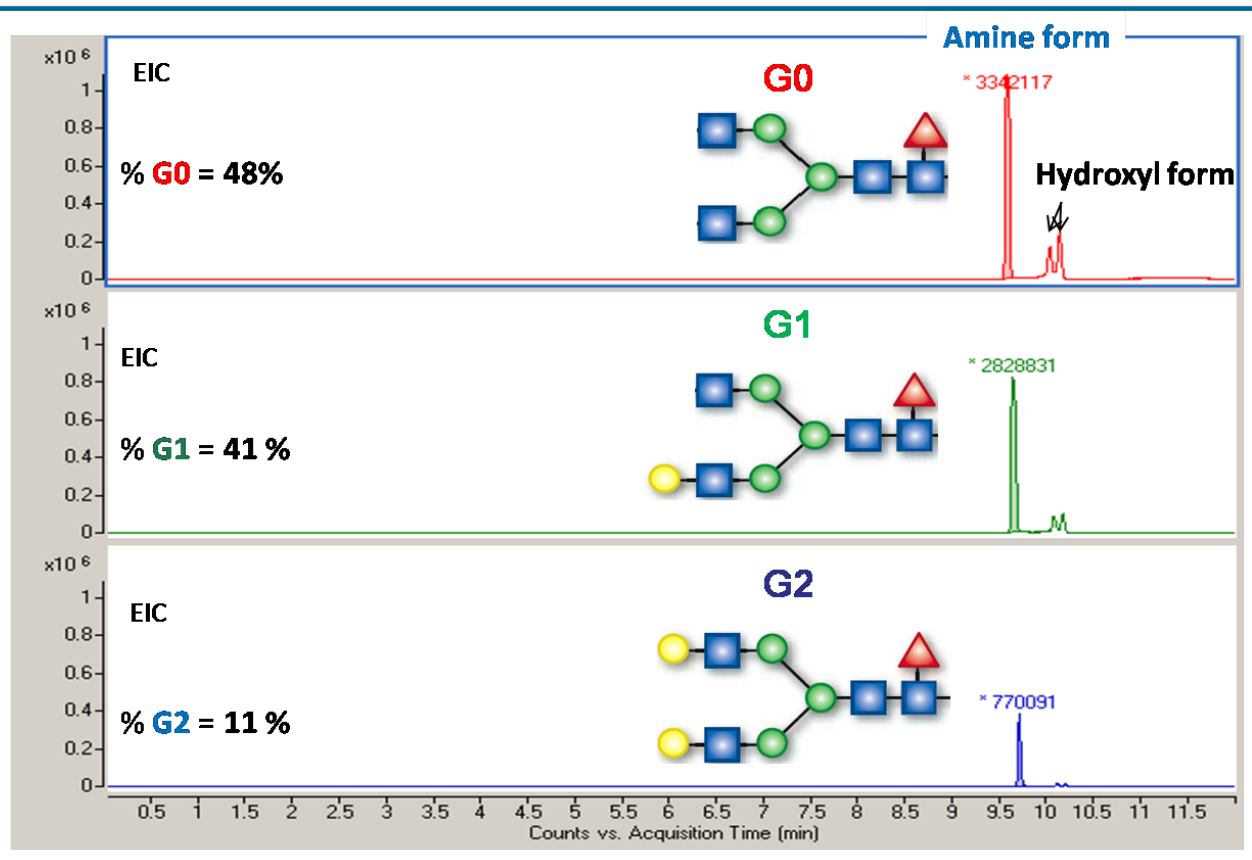
Results and *Discussion*



A

N-glycan Analysis (Online Chip MS and MS/MS)

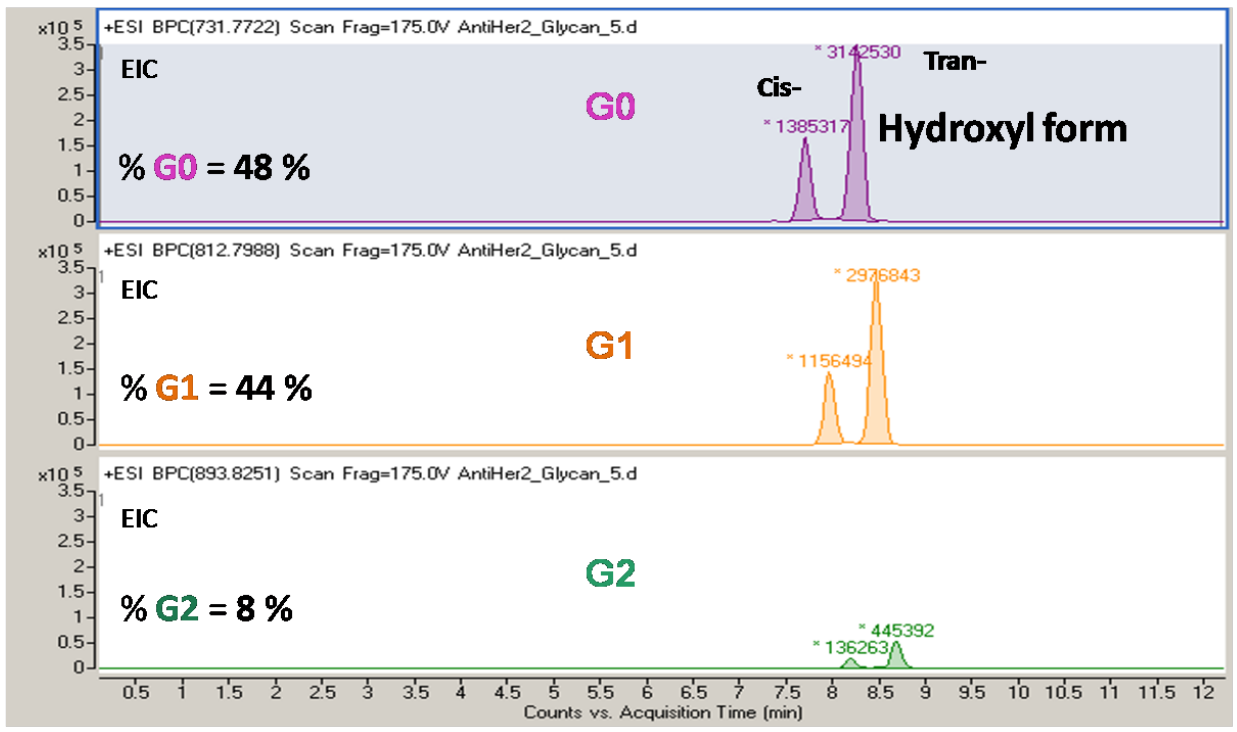
Released glycans, from the intact monoclonal antibody (anti-Her2), with different oligosaccharide compositions in the elution time window (indicated by the blue bar) were detected by the full MS spectrum, and a high abundant glycan (G0) was automatically fragmented by CID-MS/MS, further confirming the glycan structure from the corresponding product ions.



B

Anti-Her2 - mAb-Glyco-Chip (online PNGase F)

Analysis of the intact monoclonal antibody (anti-Her2) using mAb-Glyco-Chip. The released glycans with different oligosaccharide compositions (G0, G1, and G2) were displayed by the extracted ion chromatogram (EIC). The glycan distribution could be estimated by the peak area of G0 divided by the sum of all glycans (i.e. G0, G1, and G2). The detected glycans were mainly the amine forms (with minor hydroxyl forms) using the online PNGase F reaction (4 min).



C

Anti-Her2 – PGC Chip (off-line PNGase F)

Analysis of the intact monoclonal antibody (anti-Her2) using offline PNGase treatment, and the released glycans were detected by a porous graphitized carbon chip (PGC-Chip). As indicated in the inserts, the detected glycans were the hydroxyl forms by the offline PNGase F treatment (37 ºC, 4 hrs). Nevertheless, these glycan distributions are similar to the result of online PNGase F analysis.

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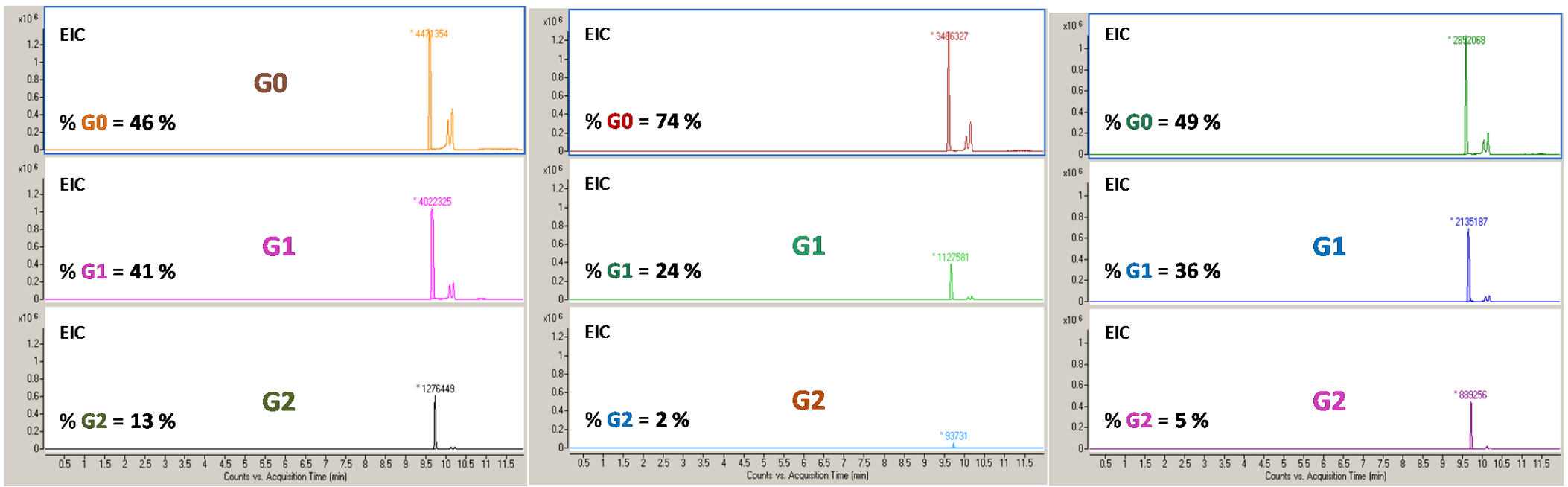
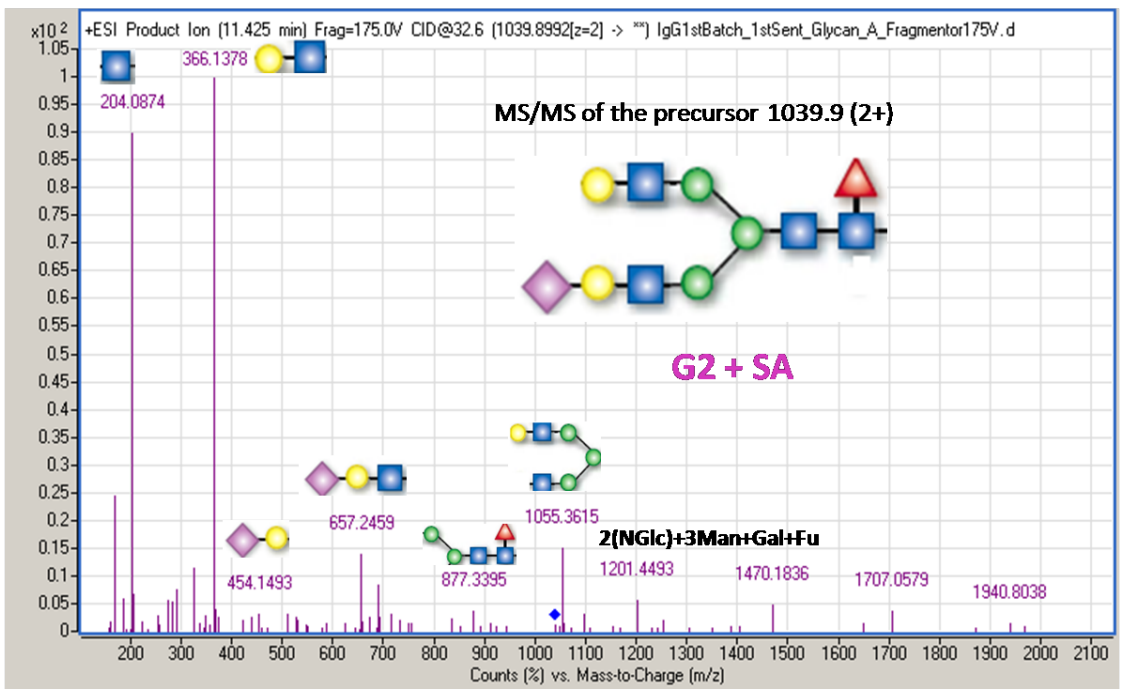
Conclusions

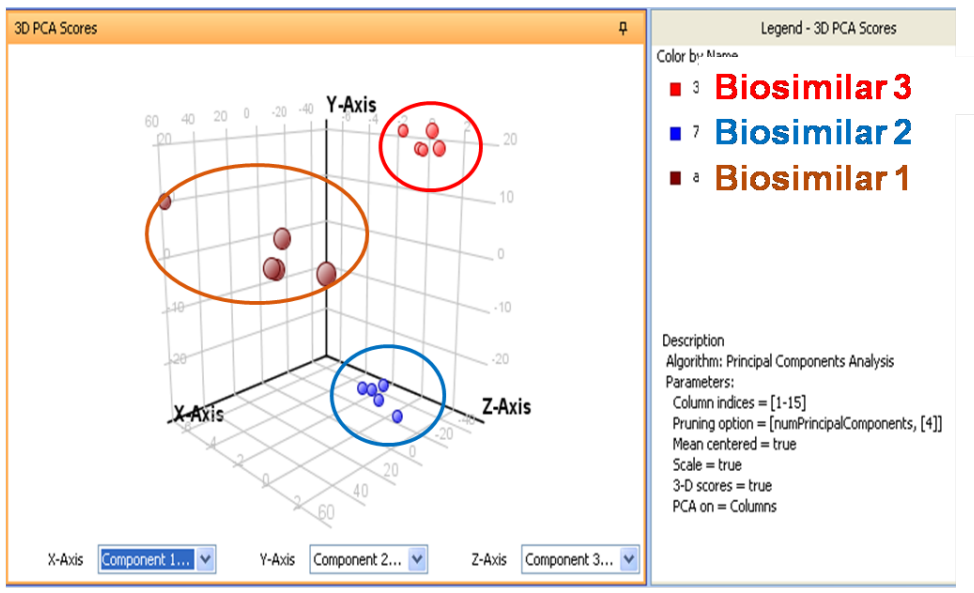


Analysis of the three biosimilar monoclonal antibodies (anti-Her2) using mAb-Glyco-Chip. As described previously, the relative ratio of these glycan distribution can be used to compare the samples from different manufacturers. The biosimilar 1 has the similar glycan distribution as compared to the innovator (See Box B to the left).



Analysis of the sialylated glycan from Biosimilar 3 monoclonal antibody by the mAb-Glyco-Chip with QTOF 6540. The glycan structure (G2+SA), fragmented by CID-MS/MS, was confirmed by the corresponding product ions, such as the arm contained N-acetyl glucosamine ( ) with galactose ( ) and sialic acid ( ) labeled as ( ).

Similarities and Differences



The results of the three biosimilar monoclonal antibodies were analyzed by a statistic software using principal component analysis (Mass Profiler Pro from Agilent). As indicated, the analysis results from the same manufacturer (5 replicates) were distributed in one cluster, while the three biosimilar products from three different manufacturers were distributed in three different clusters.

Conclusions

- *The mAb-Glyco-Chip (immobilized PNGase F with a porous graphitized carbon column) successfully released and separated glycans from intact monoclonal antibodies for detection. The offline PNGase F treatment with a porous graphitized carbon column also produced similar results with the detected glycans in the hydroxyl form instead of the amine form. Nevertheless, the automation of the on-line approach (mAb-Glyco-Chip) simplified the analysis procedure and time significantly.*
- *The glycans with different compositions (e.g. with or without sialylation) could be released by the mAb-Glyco-Chip and further characterized by online QTOF with MS and MS/MS measurements.*
- *The analysis of the three biosimilar monoclonal antibodies by this analysis platform displayed similar major glycan compositions but with different ratios of the glycans. The three monoclonal antibodies could be easily differentiated using a statistic software tool.*
- *In addition to the detection of different biosimilar products, this analysis platform could also be used for analysis of glycans from different manufacturing lots.*