Introduction

Protein biologics now represent a significant share of pharmaceutical sales and future growth potential, particularly in an era of increasing patent expirations. A range of analytical methods is required to determine the purity, identity and integrity of protein biologics at multiple points along the manufacturing process, from cell culture to downstream purification, product characterization and lot release. Characterization of glycans from antibodies is fundamentally important in biotherapeutics design and disease progression and detection. The ability to characterize glycans rapidly has been limited by the sample preparation steps and structural complexity of the glycoproteins. To address this problem, we have developed a microfluidic chip that performs rapid on-line cleavage of glycans from monoclonal antibodies, captures the released glycans and then separates them prior to nanospray ionization in the mass spectrometer. The entire run time is 12 minutes. A glycan accurate mass database was established allowing quick assignment and identification of the glycans.

Experimental

The instrument consisted of an Agilent Technologies 1200 Series nanoflow and capillary HPLC pumps, microdegassers, micro wellplate autosampler with thermostat, HPLC-Chip/MS interface, and 6520 QTOF mass spectrometer.

mAb-Glyco Chip Kit

The 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

Step 2: Data analysis, search database and identify glycans -- 5minutes

Step 3: Quantitation of glycans and report generating -- 2minutes

Conclusions

• mAb-Glyco chip workflow automated on-line deglycosylation, glycan enrichment, separation and MS analysis. The entire workflow takes 12 minutes.
• mAb-Glyco chip kit provides all chemicals needed for chip operation and system checkout.
• Integrated comprehensive data analysis offers automated glycan identification, quantitation and reporting.