ABSTRACT
We have developed a magnetic bead based sample prep that improves the N-glycan analysis process for both Ultra High Performance Liquid Chromatography (UHPLC) and Capillary Electrophoresis (CE) analytical platforms. We have simplified sample preparation by reducing the hands on time, eliminating lengthy centrifugation and vacuum drying steps, and avoiding the use of toxic chemicals. The magnetic bead-based procedure provides a streamlined workflow for glycoprotein denaturation and deglycosylation, APTS labeling of released glycans, and excess free dye clean up. This workflow is automated on a cartridge based platform making the overall sample prep a hands-free, walkaway solution. Samples from both manual and automated workflow can be analyzed by high throughput 3500 multi-capillary CE or Vanquish UHPLC. This provides an end-to-end single sample prep solution that can be used from high throughput clone selection applications to low throughput drug product QC release applications. N-glycan data generated from multi-capillary CE and UHPLC instruments is presented.

INTRODUCTION
Glycans or polysaccharides attached to proteins after protein post-translation modification play critical roles in eukaryotic cell protein functions, such as protein assembly and folding, stability, signal transduction, ligand binding, protein interaction, etc. In the therapeutic immunoglobulin, the N-glycosylation on asparagine of asparagine is a critical quality attribute in pharmacology, affecting immunogenicity, pharmacokinetics and pharmacodynamics. A challenge for glycan analysis is to have a fully automated sample preparation and analysis platform that can not only generate high quality data but also in a high throughput manner. We have developed a fully automated platform and workflow for N-glycan sample preparation using Thermo Fisher GlycanAssure reagents. Glycan profiles of NIST mAb and human serum IgG using GlycanAssure AutoXpress kits are compared to the GlycanAssure HyPerformance APTS manual kits.

MATERIALS AND METHODS
Glycoprotein
GlycanAssure AutoXpress kits
(Prefilled Cartridges and magnetic beads)
GlycanAssure HyPerformance APTS kits

Sample preparation automation method
1. Add 10 µl of glycoproteins to a 1.5-ml micro-tube.
2. Place the cartridges, tips, elution tubes, and the tube of magnetic beads on the instrument.
3. Start the run on the instrument. The run takes 1 h and 45 min.

Sample preparation manual method
1. Add water, glycoprotein, denaturant, and denaturant buffer to a 1.5-ml micro-tube.
2. Incubate 80°C for 5 minutes.
3. Add PNGase F enzyme and enzyme buffer.
4. Incubate at 50°C for 10 minutes.
5. Add APTS dye and reductant.
6. Incubate 50°C for 60 minutes.
7. Add magnetic beads and wash 2x with wash buffer.
8. Elute with elution buffer and let sit for 10 minutes and then place on magnetic stand.

Glycan analysis on UHPLC or CE
1. Mix 15 µl eluted glycans with 45 µl Acetonitrile, and analyze on UHPLC (Thermo Fisher Vanquish or Waters Acquity).
2. For 3500xL CE analysis, dilute glycans 1:40 or 1:80 with HPLC water before loading on a 96-well plate.

RESULTS

Figure 1. The GlycanAssure HyPerformance APTS kit

Figure 2. Design of the glycan prep workflow

Glycan analysis can be done on the 3500xl, Genetic Analyzer (CE) or UHPLC instruments such as the Thermo Fisher Vanquish Horizon

Figure 3. Comparing manual and automated glycan profiles for Human IgG (UHPLC)

Both automated and manual method show similar Human IgG profiles when analyzed on UHPLC.

CONCLUSIONS

- N-glycan analysis on high performance APTS labeling kits are capable for both automated and manual workflows.
- Both manual and automated sample prep methods have samples analyzed on a UHPLC or a 3500xL CE instrument capable of high throughput.
- Similar glycan profiles and relative area percentage when performing either manual or automated sample prep methods for NIST mAb or Human IgG.
- The magnetic bead based procedure provides a streamlined workflow for excess dye removal and can be automated.

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