Development of a Rapid and Robust N-Glycan Profiling Method for Therapeutic Antibodies with UHPLC and Multi-Capillary CE

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ABSTRACT

Sample preparation for glycoprofiling of glycoproteins has been a cumbersome, multi-day process with long overnight labeling, multiple lengthy centrifugation and vacuum drying steps. Most sample preparation protocols also involve toxic reagents during the labeling step of glycans. Here we present feasibility for a rapid and robust workflow with 8-aminopyrine-1,3,6-trisulfonic acid (APTS), a well-established dye in regulated environment with no centrifuge step, no vacuum drying step and no toxic reagents. The new method is also scalable based on the user need from 400 µl to 50 ml volume, making it adaptable to both capillary electrophoresis (CE) and liquid chromatography (LC). When a protocol is used during final lot release, it needs to be very robust to not fail the acceptance criteria due to errors originating from low volume-pipette limitations. Here we provide specific attention to develop a workflow that circumvents the variation due to these errors as dealing with them can be very time consuming and costly. The results generated from this workflow, demonstrated low variation with >95% RSD for the majority of peaks with relative area >1% and unbiased profiling of glycans from hIgG samples, matching closely the profile and abundance of glycans from published reference profiles with 2-AB and 2-AA, the common dye used for LC platform. This allows the flexibility in use of existing libraries for tentative structure assignments upon use of similar analytical conditions. Also the feasibility of this workflow on both LC and CE provides orthogonal methods to resolve glycans peaks that can not be resolved in one system or the other.

RESULTS

For orthogonal analysis with Capillary electrophoresis (CE), 21 replicates of purified hIgG were prepared per the workflow described here and analyzed on HILIC-UC-capillary system. Primary peak assignments were performed using co-elution time with labeled standards, spiking and GU values. As it can be observed, all major glycans in hIgG were well resolved from each other (See Table 2). Also a similarity profile was observed between APTS labeled glycans with 2-2A labeled hIgG glycans from reference. A comparable profile was also obtained with standards that were labeled with APTS obtained at Thermo Fisher (A) and 21 replicates of APTS labeled glycans from GU profile obtained at CE3500xL Protein Quality Analyzer (B).

Abundance of Major Glycans & Repeatability of Sample Prep. (UHPLC)

Comparative abundance of major glycans compared to reference with low variation between replicates. Two replicates of hIgG were prepared according to the workflow developed in this work, >50% of all major glycan peaks with relative area >1% demonstrated a %RSD of <5%. Additionally, 84% and 16% of major glycans were shown to be neutral and sialylated species, respectively which agree with published references with 2ABCA.

Comparative GU Values of APTS & 2-AB Labeled Standards (UHPLC)

Comparison of Ladder facilitates the tentative identification of peaks using existing libraries. Glycobiology (Ce) were used to facilitate glycan assignments. A ladder de-labeled shown below is analyzed typically with a sample for primary identification of unknown peaks. GU value can be obtained for unknown glycans which is basically a normalized glycan retention time relative to a ladder analyzed on HILIC. We demonstrate a good comparability between the GU values of glycans standards labeled with APTS found in our lab with that of reported reference values (with APTS) and 2-AB. Differences in GU values result from the use of different gradient profiles. Comparative results presented here were analyzed at similar, but slightly different analytical gradient than that of reference.

ORTHOGONAL ANALYSIS ON LC AND CE

Flexible Workflow (Scalable from 40 to 800 µl Enzymatic Reaction)

To reduce the analytical effort, workflow can be scaled up to 20X without any changes in final results.

Due to poor reproducibility many workflows may not be adaptable for lot release of a final drug product. Based on international organizational for standardization (ISO), the lowest systemic error due to mechanical and instrumental pipettes is reduced for precision and accuracy when volume is 30 µl. Therefore we have developed a flexible workflow that allows users to scale up as needed from 30 µl in micro plate up to 50 ml volumes using DynaMag™ 96 bottom to DynaMag™ 2, 4 or 50 bottom shown below. Our results demonstrated a strong correlation (R-Sq 0.999) between relative areas from two scales tested (DynaMag™ 2 ml vs. 5ml).

CONCLUSIONS

GlycanAssure™ Sample preparation workflow for LC provides a rapid and simple workflow with minimal hands-on and sample preparation time (<10min) and low variability between replicates (<5% for majority of peaks with >1% relative area). No centrifugation and vacuum drying or toxic sodium cyanobiphenylhydroxide is used in this workflow. The intrinsically feasible workflow for this workflow is considered a critical quality attribute (CQA). Changes in efficacy, immunogenic responses and circulation half-life of therapeutic proteins are complex traits with macro and micro environment and glycans is considered a critical quality attribute (CQA). Changes in glycans is considered a critical quality attribute (CQA). Changes in glycans is considered a critical quality attribute (CQA).

REFERENCES

2. ISO8065-2:2002, pg 6

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