ANALYSIS OF N-LINKED GLYCANS FROM RECOMBINANT AND HUMAN PLASMA DERIVED COAGULATION FACTOR IX, USING HILIC UPLC/FLR/QTof MS

Weibin Chen, Martin Gilar, Ashok Kumar, Cary Sutherland and Leland Paul
Waters Corporation, MA 01757; *CNC ICOS Biologics, Bothell WA 98021

RESULTS
Glycosylation of therapeutic protein drugs is of particular importance because it plays vital roles in the clinical performance of these drugs. In this work, we studied the N-linked glycans from two Coagulation Factor IX biologics that are used for Hemophilia B treatment; one is recombinant (rFIX, BeneFIX) and the other one is derived from human plasma (pd-FIX, Mononine). Both Factor IX proteins are heavily glycosylated. Previous findings on their glycoforms were done using analytical techniques other than mass spectrometry (MS).

Two analytical workflows were applied for rFIX N-linked glycan profiling: one is HILIC/HILIC-Anion/UPLC with off line fractionation and MALDI TOF MS, and the other one uses an UPLC/HILIC/FLR/QToF MS platform. Results from both workflows were shown here. 

CONCLUSION
N-linked glycan profiling using UPLC-HILIC/FLR/QToF MS was performed for two coagulation factor IX protein drugs. More glycans were observed comparing to HILIC/HILIC-Anion/UPLC/ Fraction Collection/MALDI TOF MS method, and other conventional HPLC methods.

UPLC-HILIC glycan separation improved the peak resolution, and enhanced the separation of isomeric glycans. For example, positional sialic acid and fucose separation was achieved, also the separation of sulfated and sialyated glycans were observed.

MSMS fragmentation and database search using Simulynx software helped the glycan structure elucidation. Adding more confidence to the glycan structure assignment.

Workflows:

- HILIC/Anion Exchange HPLC
- UPLC/FLR/QToF MS
- UPLC/FlR/QTof MS
- MALDI-TOFMS

- UPLC/FLR/QToF MS
- MALDI-TOFMS

Table 1. Comparison of 2AA labeled glycan dataset from UPLC/FLR/QToF. Proposed structures listed here were supported by MSMS data and Simulynx software.

References

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