A Fast, Reliable O-glycan Analysis Workflow

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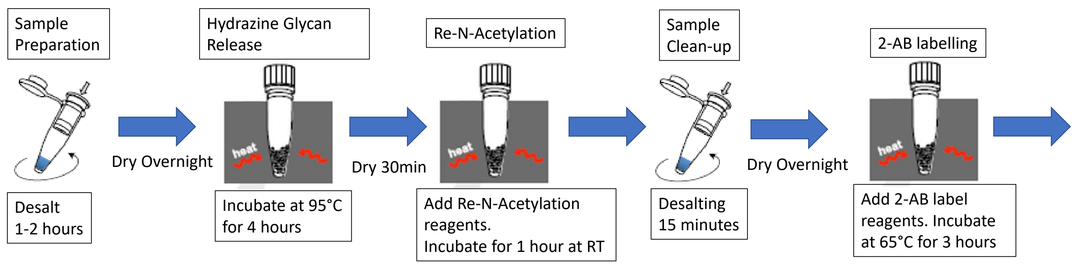
Introduction

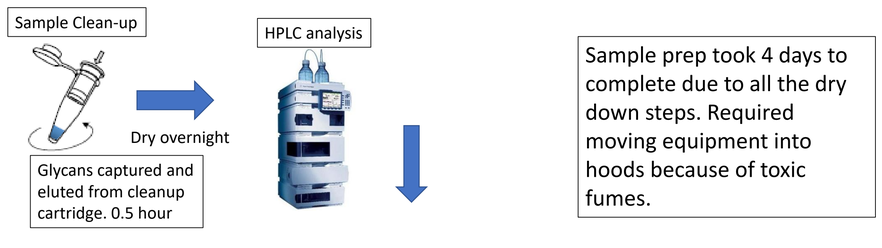
At Zoetis, we have a need to provide fast and reliable O-glycan analysis in support of our biopharma and vaccine projects. There are already several high-throughput N-glycan analysis kits on the market, but none for O-glycans. The O-glycan prep kits currently on the market require long sample preps (3-4 days), use toxic reagents (hydrazine) and cause significant peeling of the glycans. The EZGlyco™ O-Glycan Prep Kit from S-BIO, Sumitomo Bakelite Co.,Ltd. (www.s-bio.com) can cut the sample prep time down to 5 hours, doesn't require the use of toxic reagents and significantly reduces the amount of peeling.

In this poster, we compare the traditional Hydrazine O-glycan sample prep to the new EZGlyco™ O-Glycan Prep Kit.

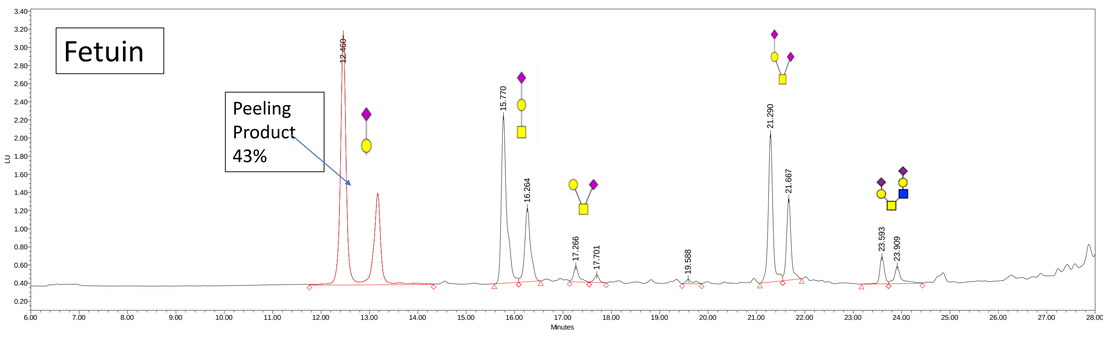
Hydrazine Release O-Glycan Workflow Overview

A commercial O-glycan Hydrazinolysis kit was used on a Fetuin standard. The use of Hydrazine required great care be taken to avoid exposure and sample preps were done in the hood. Sample prep took 4 days due to the multiple dry down steps.





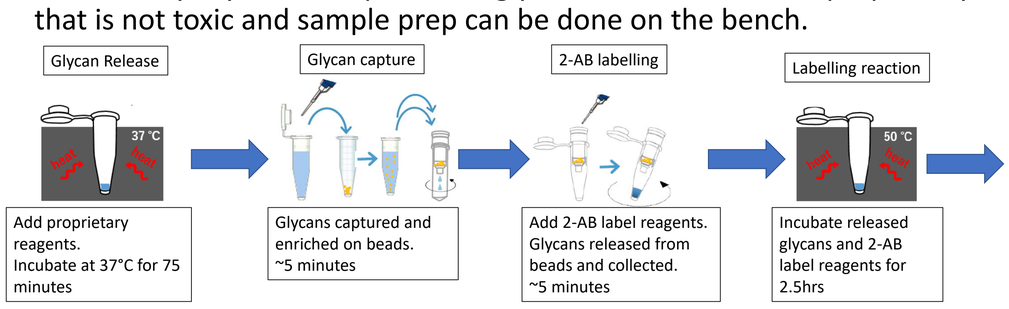
Sample prep took 4 days to complete due to all the dry down steps. Required moving equipment into hoods because of toxic fumes.

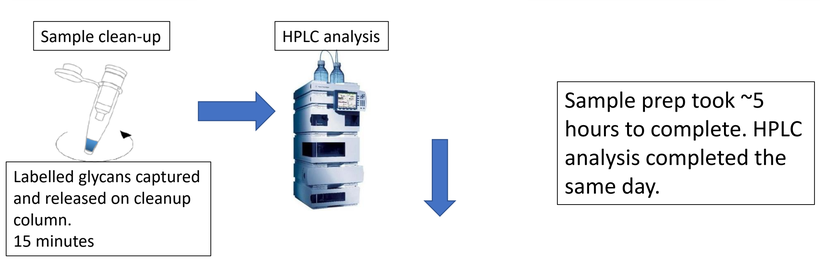


Major peeling of O-glycans observed using Hydrazine sample prep. The peeling peak was 43% by area (split peaks due to diluent mismatch on HILIC column).

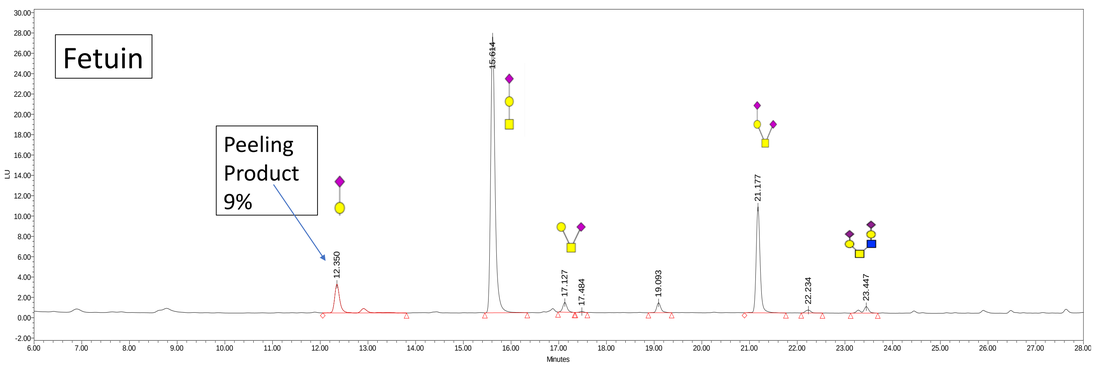
EZGlyco™ O-Glycan Prep Kit Workflow Overview

The EZGlyco™ O-Glycan Prep Kit streamlines the O-glycan sample prep down to 5 hours. The use of glycan capture beads means you can concentrate the glycans without any dry down steps. The O-glycans are released with proprietary reagent that is not toxic and sample prep can be done on the bench.





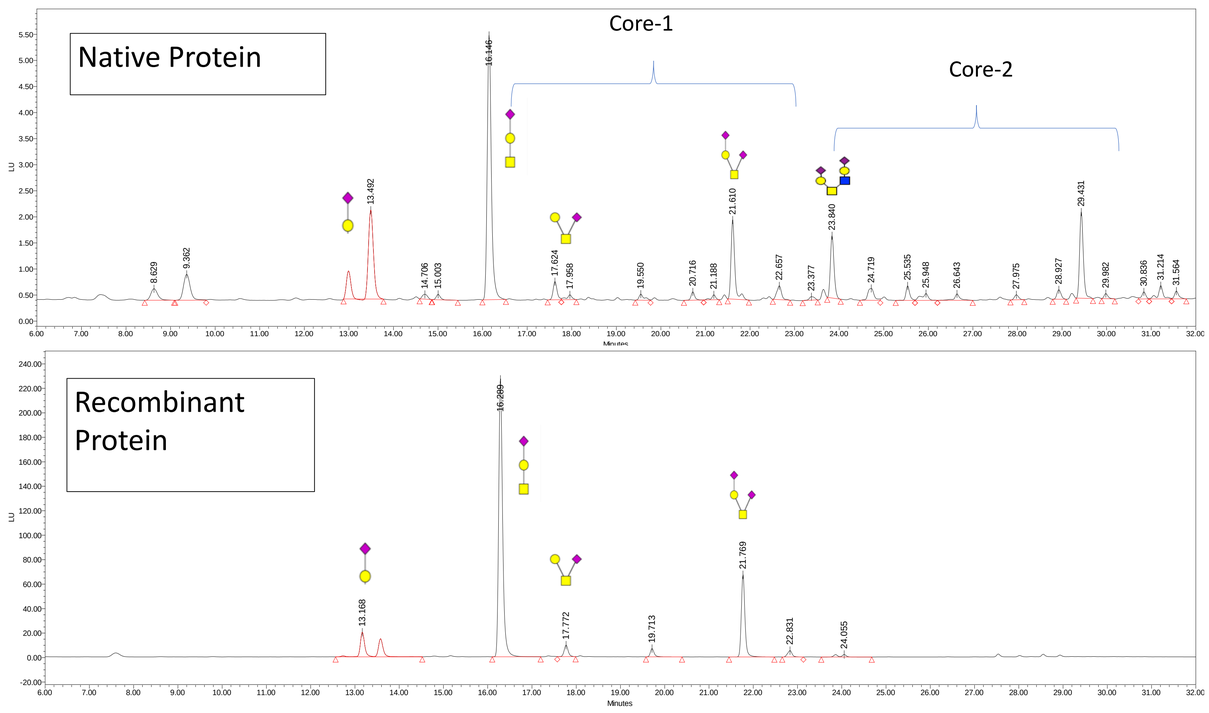
Sample prep took ~5 hours to complete. HPLC analysis completed the same day.



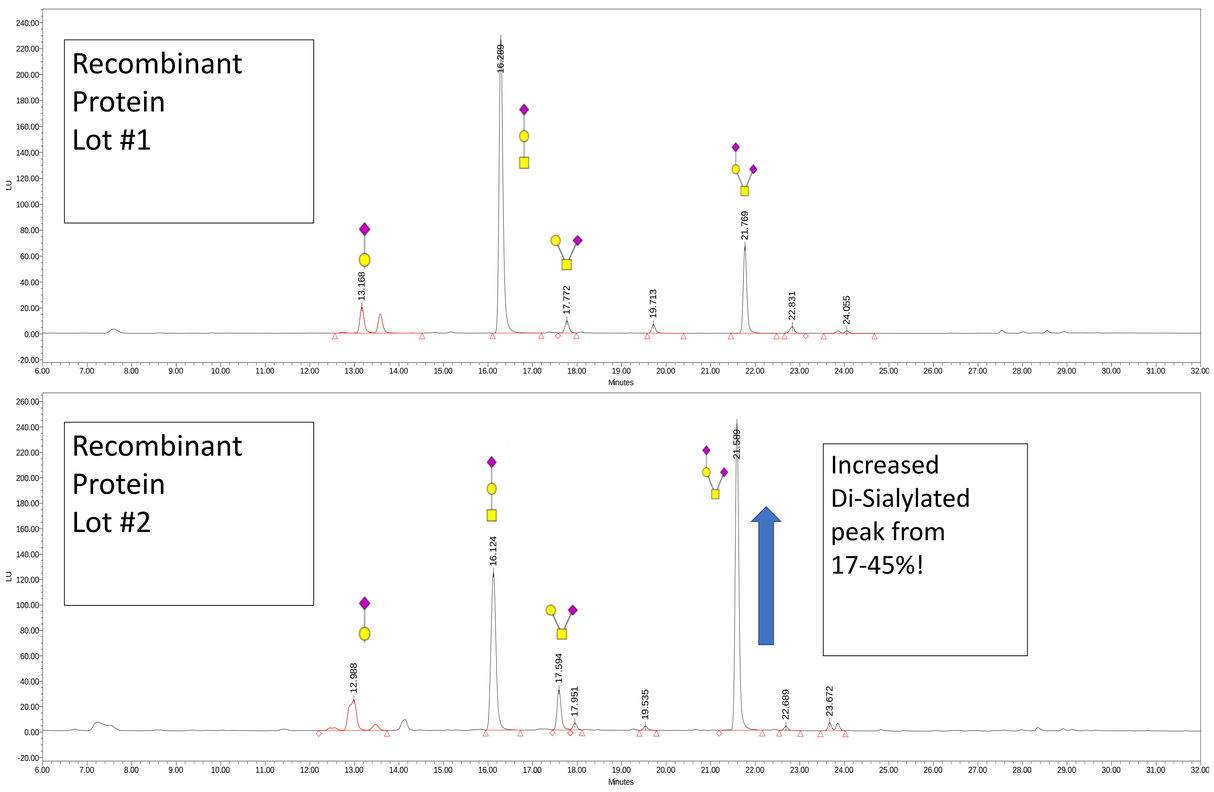
The peeling product was greatly reduced compared to the Hydrazinolysis method.

Recombinant Glycoprotein O-Glycan Analysis

We characterize a lot of glycoproteins and some of them contain O-glycosylation sites. The O-glycosylation has been reported to affect the pK profile for some glycoproteins. Until now, we have not had an easy way to monitor the O-glycosylation. We needed a fast O-glycan kit to routinely analyze for O-glycosylation profiles.



The O-glycan analysis revealed that the recombinant protein only contains Core-1 type O-glycans, but no Core 2 types. The native protein contains > 25% of the Core-2 types. An increased level in di-sialylated O-glycans could give us a better pK profile.



With the help of the new EZGlyco™ O-Glycan Prep Kit, we were able to quickly screen many different bioreaction conditions to help optimize our glycoprotein's O-glycan profile. The increase in the amount of di-sialylated O-glycans resulted in a greater half-life and potentially efficacy.

Discussion

The new EZGlyco™ O-Glycan Prep Kit from S-BIO allowed for fast, safe and reliable O-glycan analysis compared with the traditional Hydrazinolysis method. With lower peeling and high recovery of the O-glycans, the data was accurately and quickly analyzed for the project teams which enabled us to make more informed decisions and meet our aggressive project timeline.

Acknowledgements

We would like to thank S-BIO team for providing us with EZGlyco™ O-Glycan Prep Kit and for their helpful guidance.