A comparison of UPLC and CE-based approaches for glycan profiling of biotherapeutic proteins

- Challenges for the fast characterization of new biological entities (NBEs) and biosimilar candidates

Thomas Schröter[1], Eva Ennemann[1], Michaela Wachs[1], Roland Moussa[1], Martin Blüggel[1]

[1]Protagen Protein Services GmbH, Germany

PROTAGEN Protein Services

Abstract

The characterization of biopharmaceuticals such as new biological entities (NBEs) and their biosimilars is a challenging field due to their structural complexity. When implementing a manufacturing process, variations in the protein structure or post-translational modifications occur. Therefore, a characterization program is required according to the guidelines of national authorities. When characterizing proteins from scratch, glycosylation is a central modification that has to be taken into account, as it can affect the half-life and the stability of the protein.

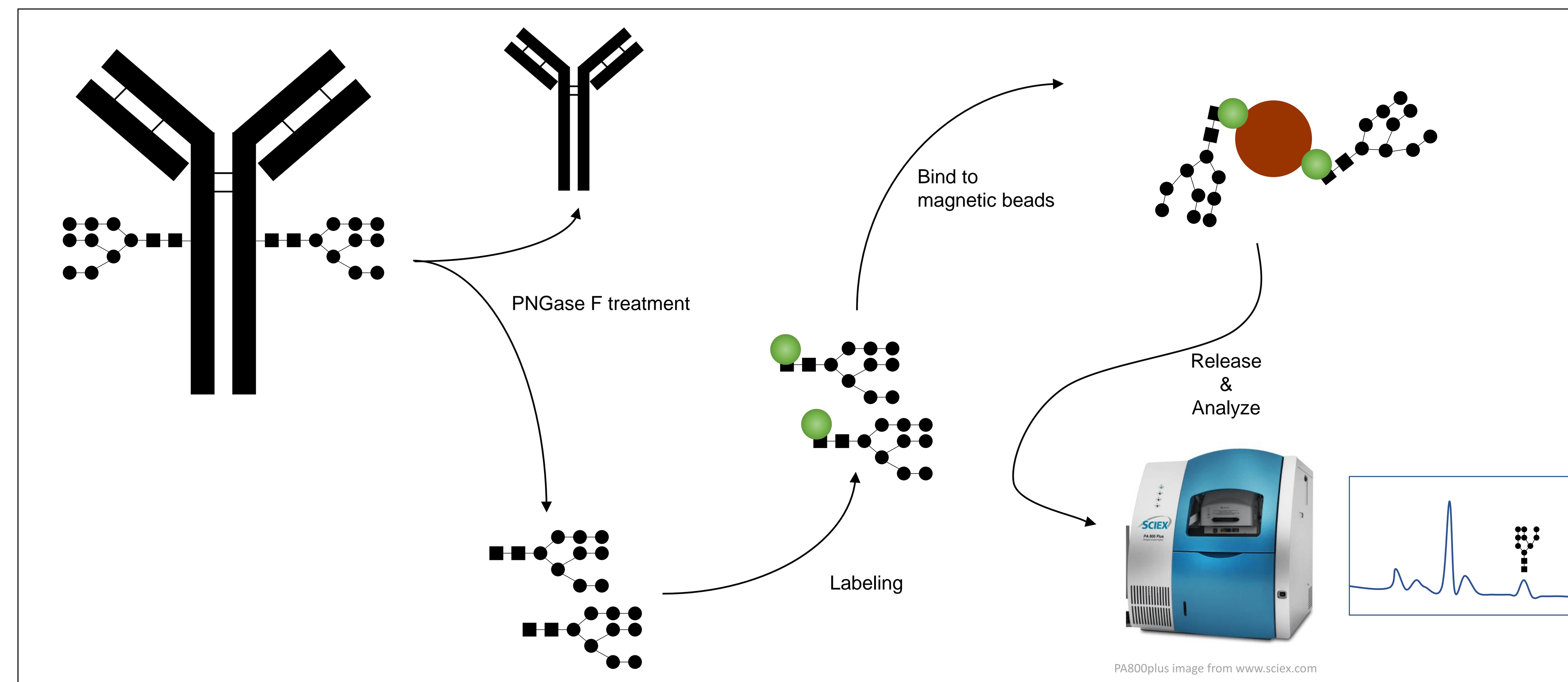
As established standard UHPLC methods, such as HILIC with IPC or 2AB labeling are commonly used to detect different glycans in biopharmaceuticals. However, as sample preparation takes up a large amount of the total analysis time, the method is not suitable for high-throughput analysis. In addition to that, sample analysis time takes up to 90 min per sample. In particular, for time sensitive development such as clone selection processes or assessing comparability of biosimilars to originators, those methods do not qualify for a quick screening process.

In this study, we evaluated a CE-based fast glycan analysis kit as a fast screening method to identify samples for subsequent detailed HILIC analysis. To assess the comparability, the UHPLC method and CE method have been performed using the same samples.

We analyzed an IgG market product and compared the obtained results with respect to identified glycans and the obtained relative peak areas. As for the glycans identified by both methods, the relative areas were in a comparable value range.

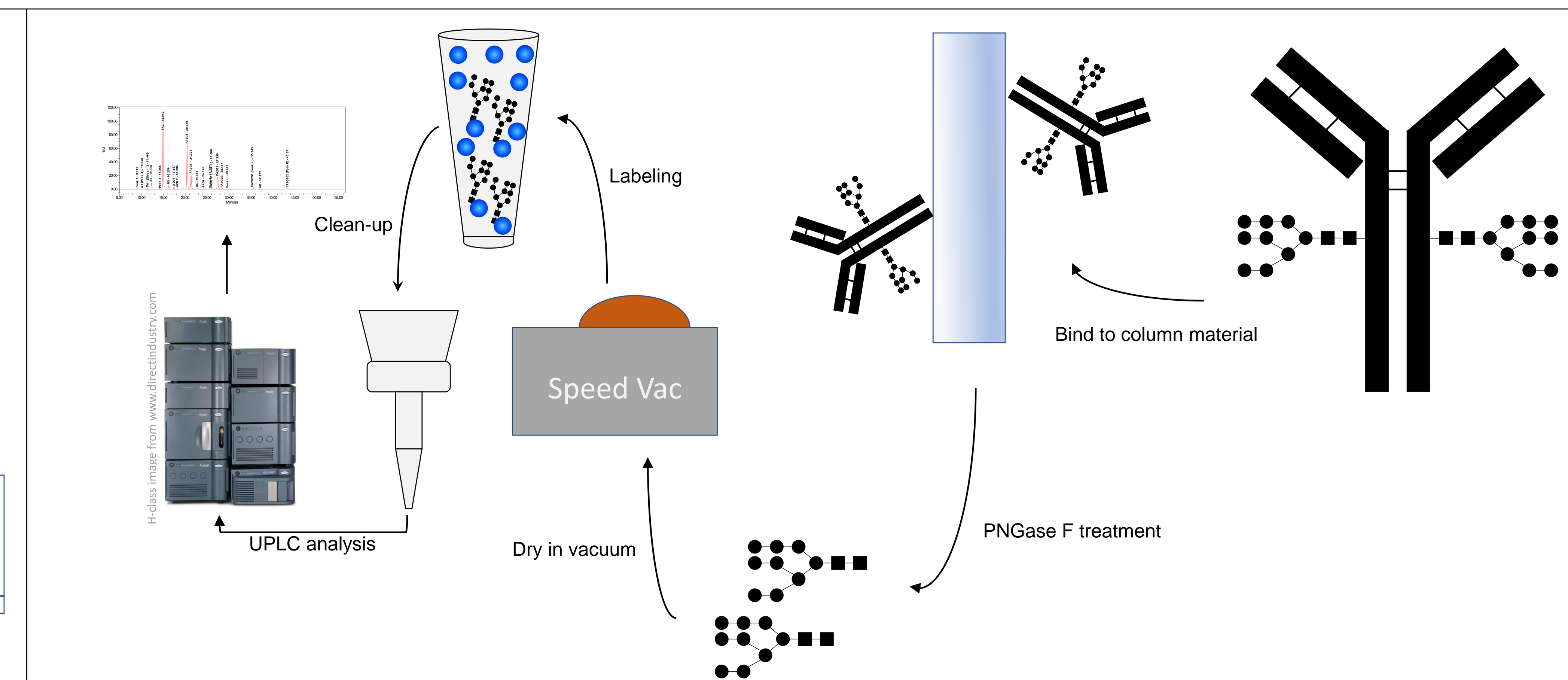
Introduction of the workflow

Fast Glycan Analysis Kit



The workflow of the Fast Glycan Analysis Kit comprises denaturation of the sample, followed by PNGase F treatment to release the N-linked glycans from the protein. The glycans are subsequently labeled with a dye and bound to magnetic beads. This enables a fast purification of the labeled glycans and easy handling. After release from the magnetic beads, the solution can readily be analyzed using capillary electrophoresis. The resulting electropherogram is automatically annotated using a special software and database, reducing manual efforts to annotate the glycans.

HILIC



The workflow of the HILIC analysis of glycans comprises a multi-step sample preparation. First, denatured proteins are bound to a protein-binding surface. The glycans are then released by an on-column treatment with PNGase F. Eluted glycans are subsequently dried in vacuum and then labeled with fluorescent dye. After clean-up of excess dye, the labelled glycan sample will be separated by UHPLC and the resulted chromatogram is evaluated.

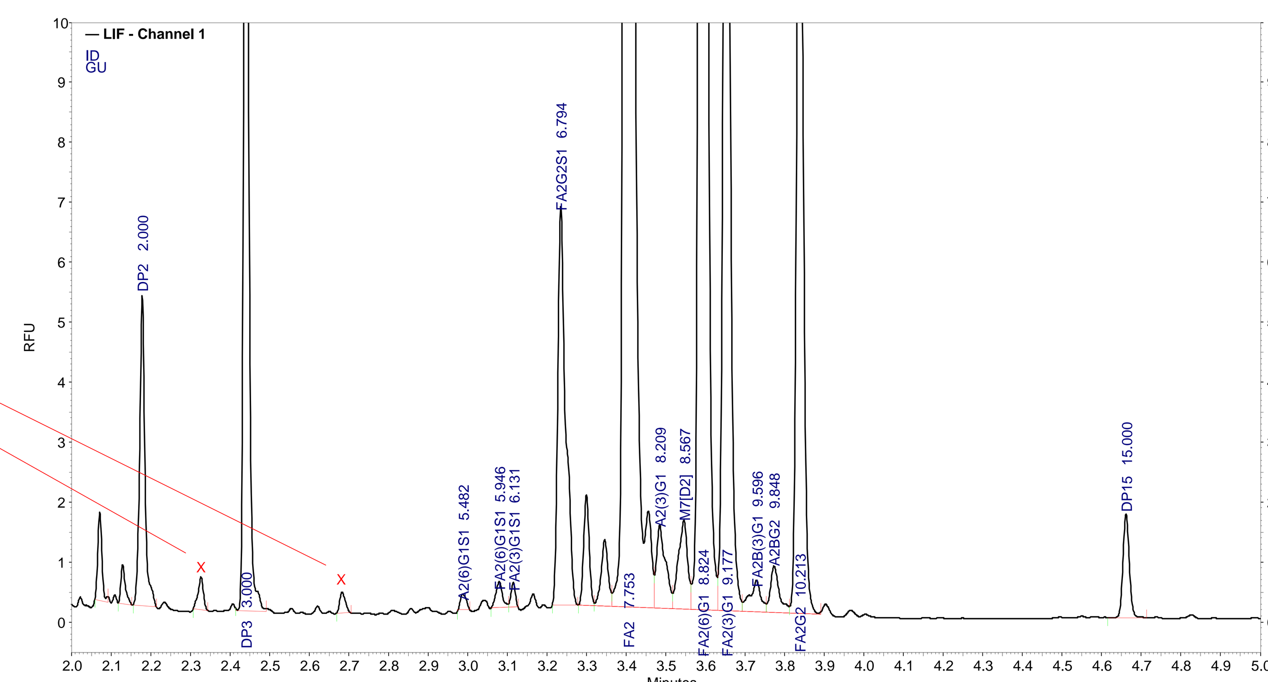
Sample measurement using HILIC and CE

Glycan	HILIC	Fast Glycan Kit
FA2	40.6	46.7
FA2G2	8.7	6.2
FA2G2S1	1.2	3.8
A2G1' / A2(3)G1	0.6	0.9
FA2G1 / FA2(6)G1	27.3	29.4
FA2G1' / FA2(3)G1	9.5	9.0
A1 (Peak A)	0.1	X
A2	2.9	X
A2(6)G1S1	n.d.	0.1
A2BG2	n.d.	0.4
A2G2	0.3	X
FA1 (Glycan 1)	0.6	X
FA2(3)G1S1	n.d.	0.1
FA2(6)G1S1	n.d.	0.2
FA2B(3)G1	n.d.	0.4
FA2G1B (Peak F)	0.4	X
FA2G2B	0.6	X
FA2G2S2 (Peak K)	0.4	X
M5	2.2	X
M6	0.4	X
M7[D2]	n.d.	0.9
M8	0.3	X
A2G1	1.4	X

Identified by both methods

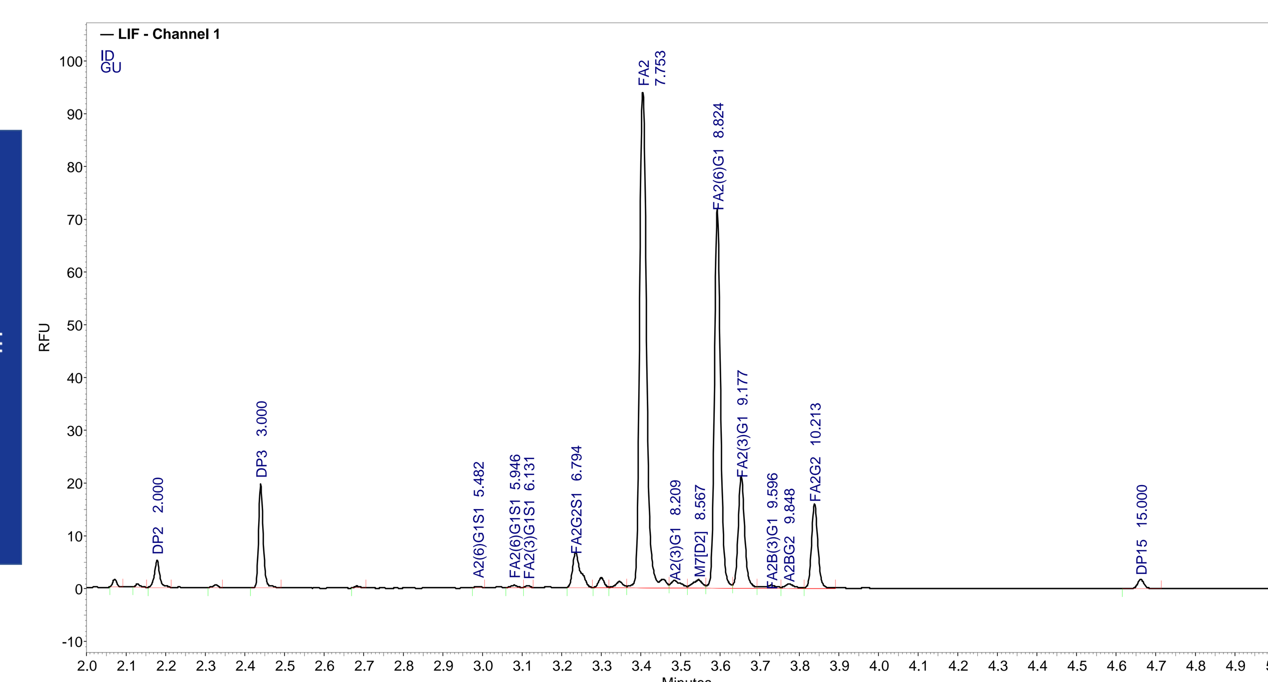
X Not annotated or detected by Fast Glycan Kit evaluation

The glycan modification of a monoclonal antibody has been analyzed by capillary electrophoresis using the Fast Glycan Analysis Kit and by HILIC. Differences in the relative peak areas of glycans that were identified by both methods may be accounted to different methods that were used to obtain the data and different labeling technologies that were used by HILIC and the Fast Glycan Kit.

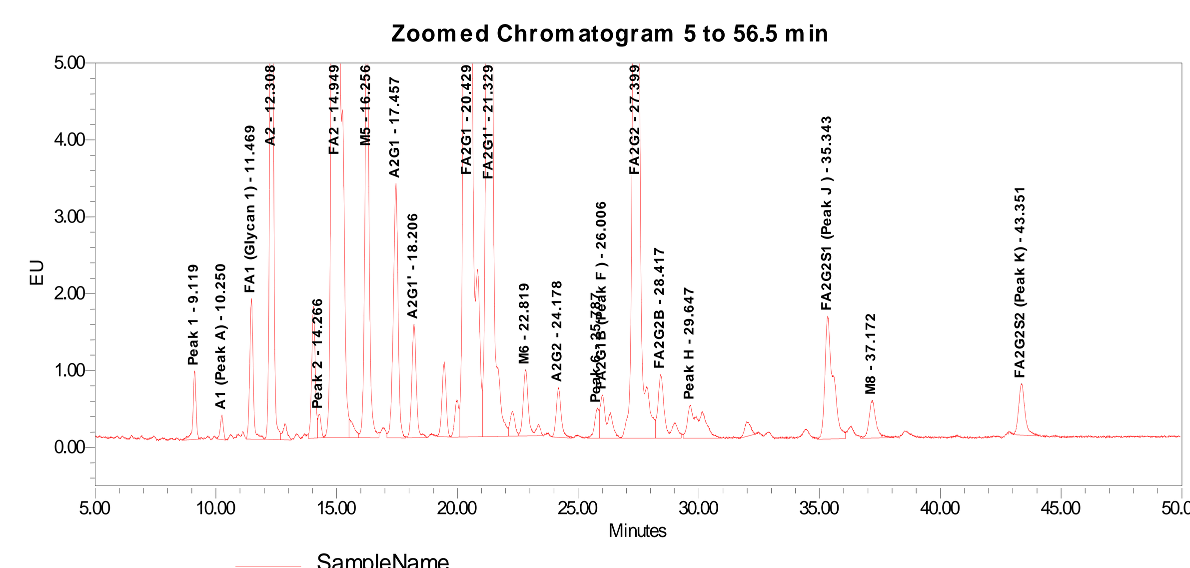


Zoom-in into the region of interest of the Fast Glycan Analysis Kit profile of antibody A. Some peaks are not annotated due to non-existing reference glycans in the GU database. This means that glycans marked with X in the table are not necessarily undetected but possibly not annotated. Putative assignments are shown with red lines.

Zoom -in

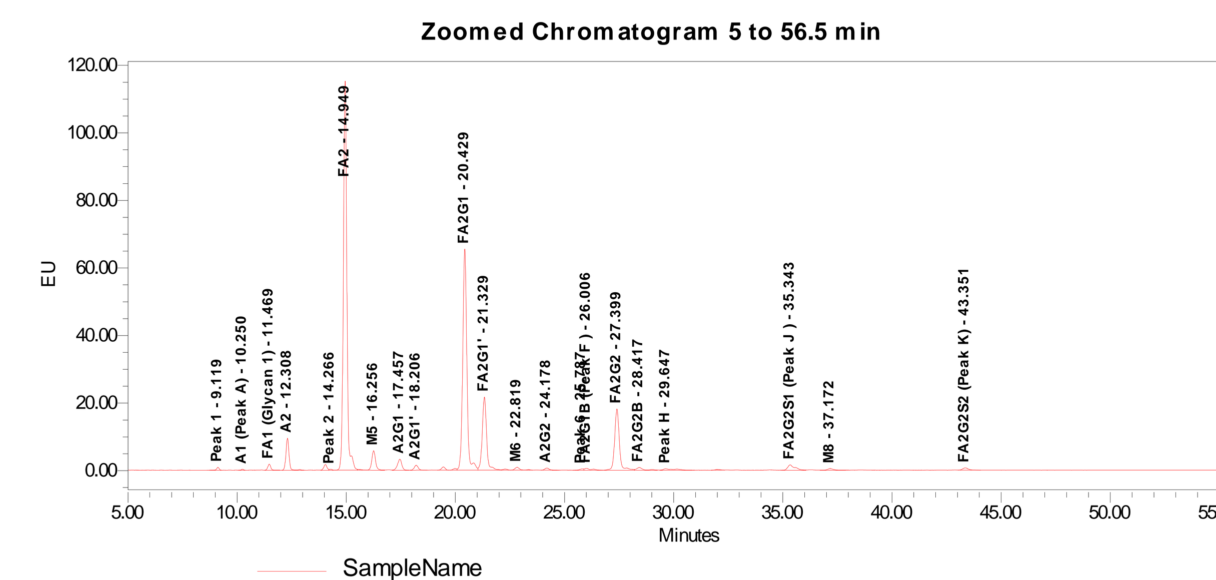


Analysis of a monoclonal antibody A using the protocol of the Fast Glycan Analysis Kit. Glycans present in the internal database were automatically annotated in 32 Karat software according to their GU values (Glucose Units, measure of retention time).



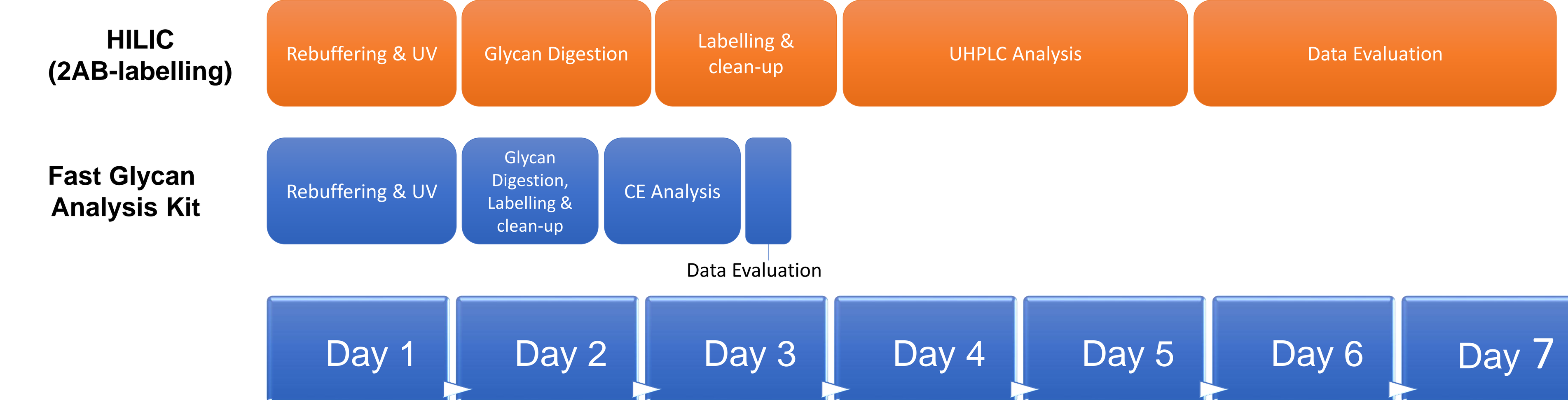
Zoom in into the region of interest of the HILIC profile. Glycans were annotated according to glycan markers used in calibration runs as well as mass spectrometry.

Zoom -in



Analysis of a monoclonal antibody A using the HILIC approach. Glycans were annotated according to glycan markers used in calibration runs as well as mass spectrometry.

Time consumption of Analysis – Fast Glycan Analysis Kit vs. HILIC



The fast glycan preparation and labelling workflow as well as the short analysis time of glycans on a Capillary Electrophoresis (CE) device provides a good advantage in terms of analysis time for the analysis of high amounts of samples in an adequate time frame. Time frames are calculated for 25 samples to be analyzed in parallel.

Conclusions

- Peak areas determined for a certain glycan are mostly in a comparable range throughout the two methods.
- Fast Glycan Kit Analysis method sometimes struggles to clearly identify glycans, resulting in multiple possible hit results. Therefore, the Fast Glycan off-the-shelf method needs to be optimized regarding resolution and annotation.
- Fast Glycan Analysis Kit benefits form a faster separation time compared to HILIC approaches (approx. 15 minutes on a CE device vs. 90 min on a UHPLC device).
- Costs per analyzed sample are about three times as much for HILIC as for the Fast Glycan Kit.
- Automated annotation and report generation of the Fast Glycan Analysis Kit software for each analyzed sample is a great time advantage in the data evaluation phase.
- HILIC method in the presented approach identified more glycans than the Fast Glycan Analysis Kit, but in principle, the detection set can be extended relatively easy by use of additional marker runs for the databasea of both methods.

The analysis of glycans using a CE device is suitable for a fast analysis of a set of many sample and as a tool to pre-select sample for an in-depth HILIC analysis if internal standards can be applied to close the annotation-gap problem