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Impact on Glycan profile caused by media components as a critical quality attributes on in-house produced mAb

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Introduction

The glycan structure contributes to the protein half-life in plasma and also possesses an ability of the mAb to trigger the immune response, which is required for efficacy. Regulatory authorities consider glycosylation to be one of the critical quality attributes of biomolecules. Therefore, it must be characterized and quantified, with acceptable ranges determined, as part of the development process for a glycoprotein innovator, biosimilar, or biobetter pharmaceuticals. Any changes in glycan profile has shown to be associated with various inflammatory diseases and cancer. One of the most common PTM's related to protein glycosylation that involves in controlling of various biological processes like such as molecular recognition, cell adhesion, fertilization and signal transduction. These variations in glycosylation pattern affect the therapeutic proteins in board biological process, therefore regulatory approvals are closely monitored for observing consistency in glycosylation pattern. Hence, the acceptable limits for mAb glycosylation variability are provided by the regulatory bodies, for High Mannose 3-10%, Afucosylated 2-13%, Galactosylation 10-40%, Sialic Acid 0-2%.

Vitamins are essential for growth and maintenance of cells and hence are integral part of cell culture media.

Experimental

Methods and Materials

We have used 6 different vitamins in this study to investigate the effect of vitamins on glycosylation. The control in this experiment is Trastuzumab Innovator Batch1 and Standard refers to the prelabelled glycan from mAb provided with the GX96-IPCGly-X™ N-Glycan Rapid Release and InstantPC™ Kit . Herein,17 different runs were performed having different levels of vitamins under investigation. Higher and lower levels were determined by analyzing the concentration of vitamins in basal media. During the culture process the different concentration of vitamins based on DOE (design of experiment) were supplemented. N-linked glycans were released from the protein backbone using peptide-N-glycosidase F (PNGase F) in a single replicate. The released glycans were derivatized with instant mass tag (InstantPC from Prozyme Inc, now Agilent Technologies) that permits detection using both fluorescence and mass spectrometry (MS) at the reducing terminal N-acetylglucosamine (GlcNAc). The glycoprotein samples of concentration 50µg was used in 50mM of HEPES buffer with pH 7.9. The deglycosylation protocol was provided with the kit and was modified based on the nature of study. This unique strategy of the dye enhances the labelling speed and also improves the sensitive identification by UHPLC-FLD-QToF. The labeled oligosaccharides were separated by HILIC column using Agilent 1290 Infiniti II UPLC coupled to Agilent 6546 LC/Q-TOF. The bound oligosaccharides were eluted, and relative area under the curve of the oligosaccharides were calculated. Vitamins were supplemented at three levels wherein the lower level corresponds to basal media and upper level corresponds to 10 –100 times higher than lower level. The middle level was used as per design developed by software JMP.

Results and Discussion

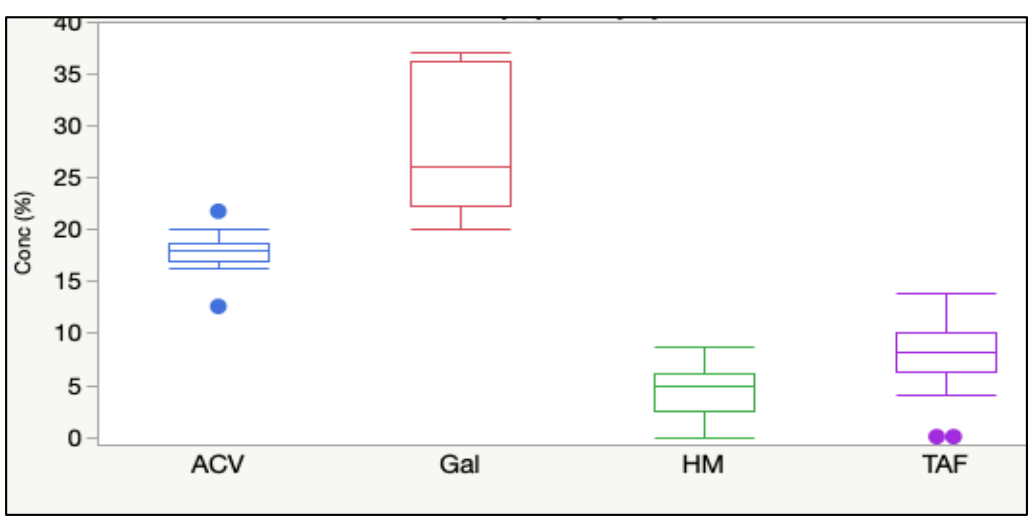


Figure 1a: Total glycan distribution profile from DOE.

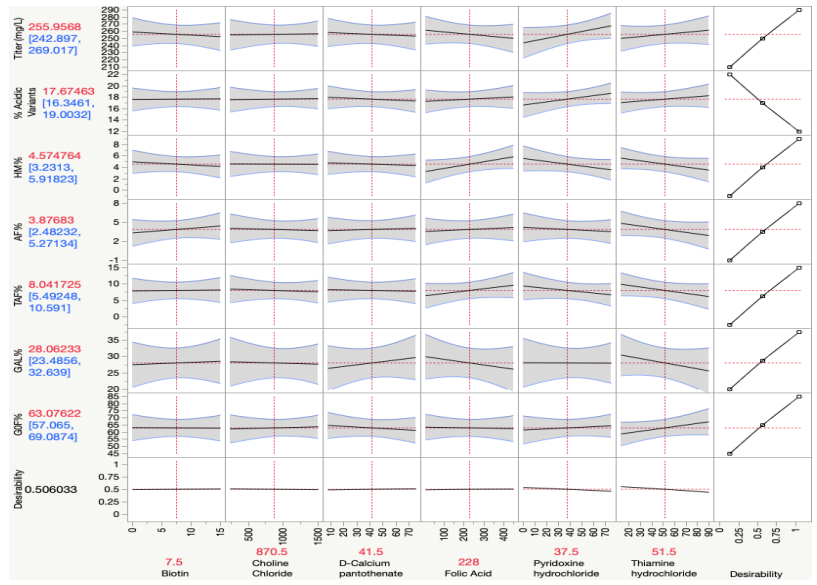
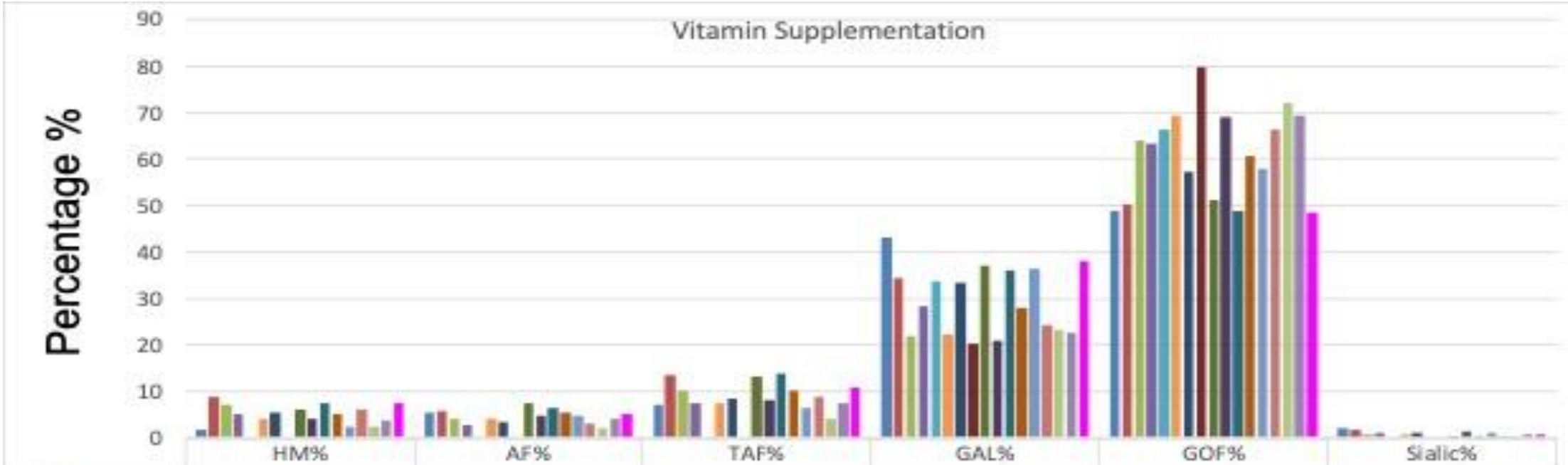


Figure 1b: Prediction profile for vitamin supplementation.

Impact of vitamin supplementation on glycan profile:

The Figure 1a and 1b refers to the prediction of total glycan distribution and the effect of vitamin on glycan. The glycoforms are compared between the innovator, vitamin supplemented sample and the Glycan library. The final area under the curve was grouped under AF%, GAL%,TAF%, G0F% and HM% is shown in Figure 2. The glycosylation pattern of the major abundant glycans, such as the G0F and AF% were comparable between the innovator and Vitamin samples (Figure 2). In the vitamin supplemented samples, differences in the HM% (Man5). of the glycoforms were observed, as shown in Fig. 2. A significant impact was observed in HM% upon the supplementation of vitamins in media during the mAb production. The Gal% can be improved to 40% as compared to innovator, which can be achieved with such supplementation. While, TAF% and AF% are in alignment with the innovator. More than 50% of variation is observed in prediction profile Figure 1b.



	HM%	AF%	TAF%	GAL%	GOF%	Sialic%
Control	1.75	5.26	7.01	43.08	48.99	1.88
Sample_V1	8.8	5.8	13.4	34.4	50.3	1.8
Sample_V2	7.0	4.0	10.1	22.0	64.1	0.9
Sample_V3	4.9	2.8	7.5	28.2	63.4	0.9
Sample_V4	0.0	0.0	0.0	33.6	66.4	0.0
Sample_V5	4.2	4.0	7.4	22.3	69.3	0.8
Sample_V7	5.3	3.5	8.6	33.3	57.3	1.2
Sample_V8	0.0	0.0	0.0	20.1	79.9	0.0
Sample_V9	6.0	7.3	13.3	37.0	51.1	0.0
Sample_V10	4.2	4.7	8.2	21.0	69.0	0.2
Sample_V11	7.5	6.4	13.9	36.1	48.8	1.3
Sample_V12	5.2	5.4	10.0	27.9	60.7	0.4
Sample_V13	2.3	4.9	6.3	36.5	58.0	1.1
Sample_V14	6.1	3.1	8.8	24.3	66.3	0.3
Sample_V15	2.5	2.2	4.0	23.1	72.3	0.3
Sample_V16	3.7	3.9	7.5	22.8	69.4	0.9
Standard	7.4	4.9	10.9	38.1	48.5	0.8

Figure 2. Glycan area percentage between innovator and vitamin supplemented samples. The percentage of glycoforms are showed for various supplemented vitamin samples. The Control represents the innovator of trastuzumab, V1-V16 different concentration of vitamin supplemented samples and Standard represents the Glycan library.

Conclusions

1. Vitamin supplementation can help to control the CQA to greater extent in achieving optimum DOE.
2. Different media supplementation needs to be added for establishing optimal media component and creating a list of components, which posses an significant impact on the glycan and HCP profile.
3. Different supplementation of media components are required to construct an optimal DOE for achieving higher product yield. While Vitamin supplementation has clearly shown a significant effect on HM% and Gal% this possess significant effect on CDC (Complement Dependent Cytotoxicity)

References

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