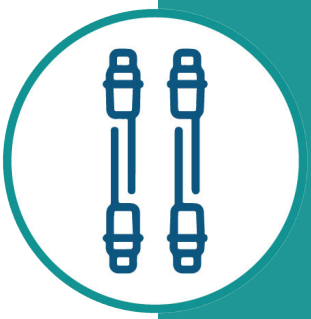
TOSOH



Cell line screening for mAb glycan profiles during USP

Your Challenge

- You suffer from lengthy and tedious glycosylation analyses in multiple mAb samples.
- You need an easy and rapid estimation of ADCC activity in multiple mAb samples.

Our Solution

TSKgel FcR-IIIA-NPR

- Quick ADCC & glycan estimates in complex matrices.

What was done?

- FcγRIIIa affinity-based separation was compared to HILIC glycan analysis using two sets of mAb samples.

What was the result?

- The conventional HILIC method correlated with the new FcR-IIIA HPLC method.

FcR-IIIA affinity HPLC is a quick method to estimate mAb glycan profiles and ADCC activity. Due to compatibility with complex matrices like cell culture fluid, it is valuable for cell-line screening.

Your Benefit

Save time and effort when assessing mAb glycoforms and ADCC

Analysis of Antibodies

TOSOH BIOSCIENCE

SEPARATION & PURIFICATION

CONNECTING MINDS.
TOUCHING LIVES.

https://www.separations.eu.tosohbioscience.com/products/hplc-columns-uhplc-columns/affinity/tskgel-fcr-iiia

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Application Note

FcR Affinity Chromatography - A new approach to assess glycan profiles during cell line screening and upstream development

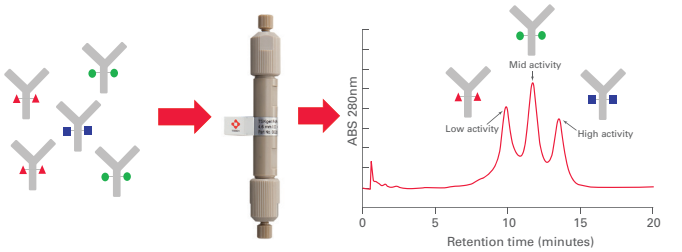
In cooperation with UGA Biopharma

Fc-glycans of therapeutic antibodies are known to play an important role in the recognition of antibodies by the FcγIIIa receptor. Accordingly, Fc receptor affinity-based separation is also correlated to the N-glycan profile. The TSKgel® FcR-IIIA-NPR affinity chromatography column uses an Fc receptor modified by site-directed mutagenesis to create a ligand with greater stability.

A thirty-minute analysis (Figure 1) gives valuable first information on the distribution of glycoforms and expected ADCC (antibody-dependent cell-mediated cytotoxicity)of analyzed mAb samples. This fast and efficient method can be applied to purified samples and supernatant alike and can therefore be used in many phases of development and production: cell line screening in early R&D, biosimilar/originator comparison, upstream development and optimization, monitoring of glycoengineering, or lot-to-lot comparison in QC.

This application note discusses the correlation of TSKgel FcR-IIIA-NPR elution profiles and mAb glycan structures determined by hydrophilic interaction liquid chromatography (HILIC) during a monoclonal antibody upstream process optimization. The glycan profiles that are mentioned throughout this application note are shown in Figure 2.

Figure 1. Separation of mAb glycoforms according to their affinity to Fc Receptor / ADCC activity.



Correlation of glycan profiles with FcR affinity profiles for purified and unpurified samples

An advantage of TSKgel FcR-IIIA-NPR (P/N 0023513) is, that it requires no specific sample preparation and can be applied to clarified cell culture supernatant and purified protein alike. Table 1 shows the relative area of each peak of the FcR chromatogram for an antibody in cell culture supernatant and for the Protein A purified target. The relative peak areas are very similar for both samples (raw samples versus purified antibody). Only peak 3 shows a higher deviation which is related to the small peak area and variation of the peak integration.

Figure 2. Nomenclature of glycan structure.

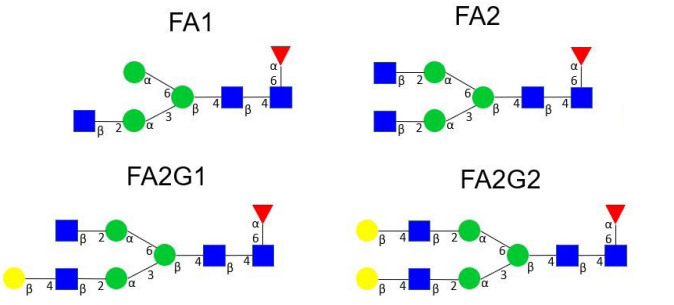


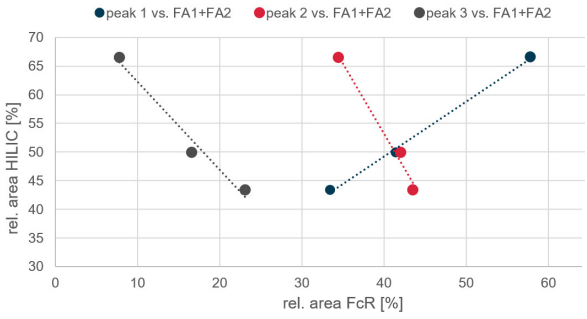
Table 1. Comparison of cell culture supernatant and purified antibody.

	Relative Area (%)			Relative Area (%)				Ratio		
	Cell Culture Supernatant			Purified Protein				Purified /Supernatant		
Sample	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3	no.	Peak 1	Peak 2	Peak 3
1	72	25.3	2.7	71.1	25.6	3.3	1	0.99	1.01	1.22
2	71.1	25.9	3	72.4	25	2.6	2	1.02	0.97	0.87
3	73.5	23.8	2.7	72.4	24.7	2.9	3	0.99	1.04	1.07
4	71.5	25.7	2.8	70.8	25.8	3.4	4	0.99	1	1.21
5	72	25.5	2.5	72	25.5	2.4	5	1	1	0.96
6	72.4	24.9	2.7	72.5	25.1	2.4	6	1	1.01	0.89

In order to evaluate the potential of FcR affinity chromatography to predict glycan pattern, the glycan species determined by conventional HILIC glycan profiling were correlated with the results of FcR affinity chromatography for three different lots of an originator-drug.
In general, a low content of galactosylated species indicates a low ADCC, which is reflected in low retention on the FcR column. Figure 3a shows the correlation for the non-galactosylated species FA1 and FA2. The higher the content of these non-galactosylated species the lower the ADCC and the retention on the FcR column.

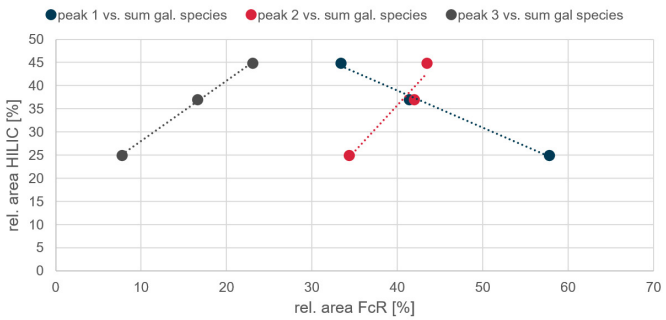
Figure 3b shows the correlation for the galactosylated species for these three antibody lots. The higher the content of the galactosylated species, the higher the ADCC and the retention on the FcR column. The antibody lot with the highest content of galactosylated species shows the highest rel. area on the 3rd. peak and the lowest rel. area for peak 1 and 2.

Figure 3a. Correlation of non-galactosylated species with FcR peak profile.



The purification of the target and tedious sample preparation that is required for the glycan analysis (cleaving the glycans, 2-AB labeling and HILIC analysis) can be avoided when using affinity chromatography on TSKgel FcR-IIIA-NPR.

Figure 3b. Correlation of galactosylated species with FcR peak profile.



Since the proof of concept was successful, this method was used for the upstream process optimization for two clones expressing a biosimilar.

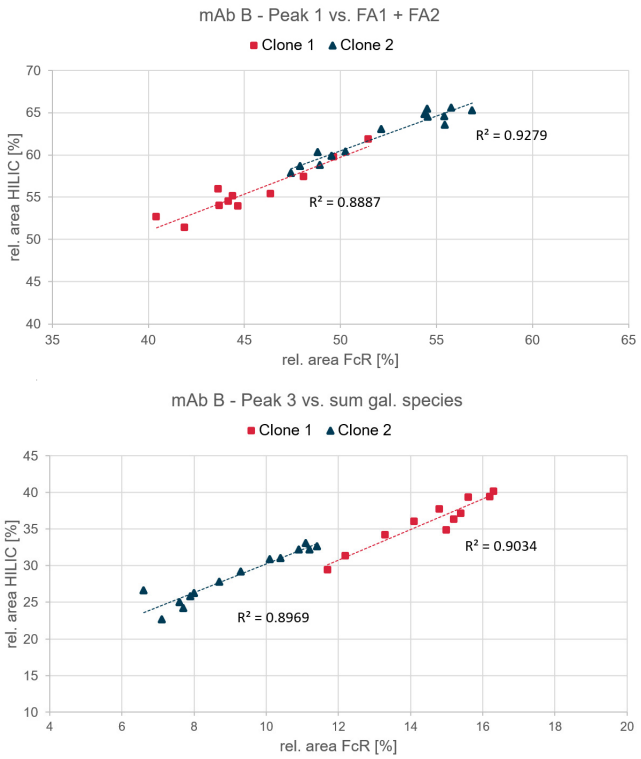
BIOSIMILAR UPSTREAM DEVELOPMENT – SCREENING OF PROCESS CONDITIONS

Two different clones of a Ready-to-Use Biosimilar Cell Line* were investigated with different process conditions in First CHOice® media and feeds (Figure 4). Media, feed, feed strategy and feed volume differed between the samples. A clear correlation of the rel. area of glycan species identified by HILIC with the results of FcR chromatography could be obtained for both non-galactosylated as well as galactosylated species.

CONCLUSION

We have shown that the TSKgel FcR-IIIA-NPR affinity chromatography column is very well suited for scientists working in upstream development. It can be used instead of HILIC, which requires tedious sample preparation and a purified sample, to determine the glycan structure of antibodies produced by different clones, cell lines and process conditions. By switching to FcR-IIIA-NPR affinity chromatography, purified and non-purified samples can be analyzed without prior sample preparation. Compared to currently used methods, this method offers significantly less cost, work and time while providing similar information.

Figure 4a & b. Correlation of relative area of peaks in HILIC vs. FcR chromatography – Process optimization.



First CHOice® is a registered trademark of UGA Biopharma
**Material and data was kindly provided by UGA Biopharma*