A Dilute-and-Shoot Method for Monitoring mAb PQAs from Spent Media Enhances the Process Analytical Technology (PAT) Toolbox

Application Note

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Highlights

Simple Sample Prep: The ZipChip® CVA method uses a "dilute-and-shoot" approach, requiring only simple dilution to analyze mAb PQAs directly from spent media.

High Matrix Tolerance: ZipChip CVA maintains separation resolution and MS data quality across a wide range of media dilutions, effectively handling interfering media components without buffer exchange.

Broad Titer Sensitivity: The method supports monitoring of PQAs from early to late culture stages with high sensitivity, allowing flexible dilution adjustments based on process titer to capture accurate product quality data.

Introduction

There is an increasing demand for user-friendly analytics that provide insights into product quality attributes (PQAs) of biotherapeutic proteins throughout the development pipeline. While the ZipChip Charge Variant Analysis (CVA) assay has been widely used for downstream characterization of purified drug products,[1–5] this work demonstrates its application in upstream bioprocessing by measuring monoclonal antibody (mAb) PQAs directly from cell culture media sampled from active bioreactors. Compared to other process analytical technology (PAT) methods for product characterization, ZipChip CVA has the added benefit of providing information on charge heterogeneity and glycoform heterogeneity in a single method. The ZipChip separation effectively removes interfering media compounds from the mAb, maintaining both separation performance and mass spectrometry (MS) data quality, enabling a "dilute-and-shoot" sample preparation method. The sensitivity of the method allows for analysis of a wide range of titers with high quality MS data obtained for titers less than 0.1 mg/mL. Antibody PQAs can thus be monitored throughout cell culture to confirm product quality, assess process performance, and support process optimization.

Materials and Methods

Sample preparation

The ZipChip Charge Variant Analysis (CVA) Kit (PN: 850-00052, 908 Devices Inc.) was used for sample preparation and analysis. NISTmAb Reference Material (NIST RM 8760) system suitability test (SST) samples were prepared by diluting NISTmAb to 0.25 mg/mL with ZipChip CVA sample diluent. Four types of cell culture medium were used for method development: EX-CELL CD CHO Fusion (Millipore Sigma); Gibco CD CHO and Gibco Medium 199 (Thermo Fisher Scientific); and Modified Eagle's Medium (Sigma Aldrich). Fresh cell culture medium was diluted 0x to 100x with CVA sample diluent to vary the concentration of medium components in the sample matrix. These dilutions were then used to prepare NISTmAb at 0.25 mg/mL. To mimic bioreactor samples with a range of protein titers, NISTmAb was buffer-exchanged into full-strength Gibco CD CHO cell culture medium at concentrations of 10 mg/mL, 2.0 mg/mL, 0.4 mg/mL, 0.08 mg/mL, and 0.016 mg/mL. Each of these samples was then diluted 10x or 50x with CVA sample diluent before analysis.

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Data collection and analysis

Samples were analyzed using a ZipChip interface coupled to an Exploris 240 Biopharma Orbitrap mass spectrometer (Thermo Scientific). For charge variant analysis an HRN chip (PN: 810-00227, 908 Devices Inc.) and the ZipChip Charge Variant Analysis Kit were used (908 Devices Inc.) The ZipChip and MS acquisition methods are provided in Table 1 and Table 2. Data were visualized and peak areas generated using QualBrowser (Thermo Scientific). Mass spectra were processed using BioPharma Finder 5.0 (Thermo Scientific). Glycoform time traces were generated using UniDec Universal Deconvolution of Mass Spectra software[6] (University of Oxford).

Results and Discussion

Method Assessment

Cell culture media are complex mixtures of salts, small molecule nutrients, and sometimes peptides. Analyzing proteins in a spent medium matrix can be difficult, as the media components are often at high concentration and can interfere with analysis of the biotherapeutic protein by causing distorted peaks or ionization suppression and adducts in the mass spectra. Buffer exchanging samples is an option, but this approach adds time and complexity to the workflow and risks sample loss. When assessing the effect of the sample matrix on ZipChip CVA, two things must be considered: impact on the capillary electrophoresis (CE) separation and impact on the MS data quality. To assess the matrix tolerance of ZipChip CVA, NISTmAb was prepared with media diluted 0x-100x with CVA kit sample diluent to mimic different dilution levels of spent media. Four different media types were used to assess whether there are significant differences in performance for different media. The samples were then analyzed using standard ZipChip CVA and MS method settings outlined in Table 1 and Table 2.

Table 1. ZipChip Settings for CVA

Setting	Value
Global Settings	
Sample Volume	20 µL
BGE Refresh Rate	Every 1 line
Method Settings	
Field Strength	500 V/cm
Chip	HRN
BGE	CVA
Injection Volume	1 nL
Pressure Assist Start Time	On | 0.0 min.
Analysis Time	14 min

Table 2. Thermo Scientific Exploris 240 (BioPharma) Orbitrap Mass Spectrometer

Setting	Value
Method Settings and Global Parameters	
Application Mode	Intact Protein
Method Duration	14 min
Ion Source Type	ESI
Gas Mode	Static
Sheath Gas (Arb)	2
Ion Transfer Tube Temp (°C)	300
Pressure Mode	Standard Pressure
Expected Peak Width (s)	5
Advanced Peak Determination	FALSE
Full Scan	
Orbitrap Resolution	30k
Scan Range (m/z)	2000—8000
RF Lens (%)	150
AGC Target	100%
Max. Inj. Time Mode	Auto
Microscans	3
Data Type	Profile
Polarity	Positive
Source Fragmentation	Enabled
Energy (V)	135

First the separation performance was assessed. NISTmAb RM separates into 4 primary peaks due to differences in C-terminal lysine clipping and deamidation. The variants in migration order are the following: 2 C-terminal lysines (Basic variant, 2K), 1 C-terminal lysine (Basic variant, 1K), 0 C-terminal lysines (Main variant, 0K), and deamidation (Acidic Variant). The separation resolution between the main variant and the 1K basic variant was calculated for each dilution level. An example of the results for each dilution level for a representative medium are shown in Figure 1. The SST sample corresponds to NISTmAb prepared with neat CVA sample diluent only. The separation resolution for all media and dilution levels tested is provided in Figure 2. Separation performance is maintained from 100x to 25x dilution but begins to degrade for media diluted less than 25x. Some separation of the charge variants is still achieved at 5x and 10x dilution, but by 2x no significant separation is obtained.

The impact of the media matrix on the MS data was then assessed. While it is expected that the CE separation should be highly effective at electrophoretically separating matrix components from the protein peak, it is necessary to confirm that this is the case since interfering species may cause ionization suppression or adducts on the protein during electrospray. The raw and processed mass spectra from the NISTmAb SST sample and the samples with media matrix were compared. The quality of the MS data was comparable down to 0x media dilution. As shown in Figure 3, no adducts due to the media components were observed. The deconvoluted masses of the main variant were matched to NISTmAb glycoforms with <10 ppm mass accuracy. This indicates that interfering media compounds effectively migrate away from the mAb peaks during the separation even with full strength media.

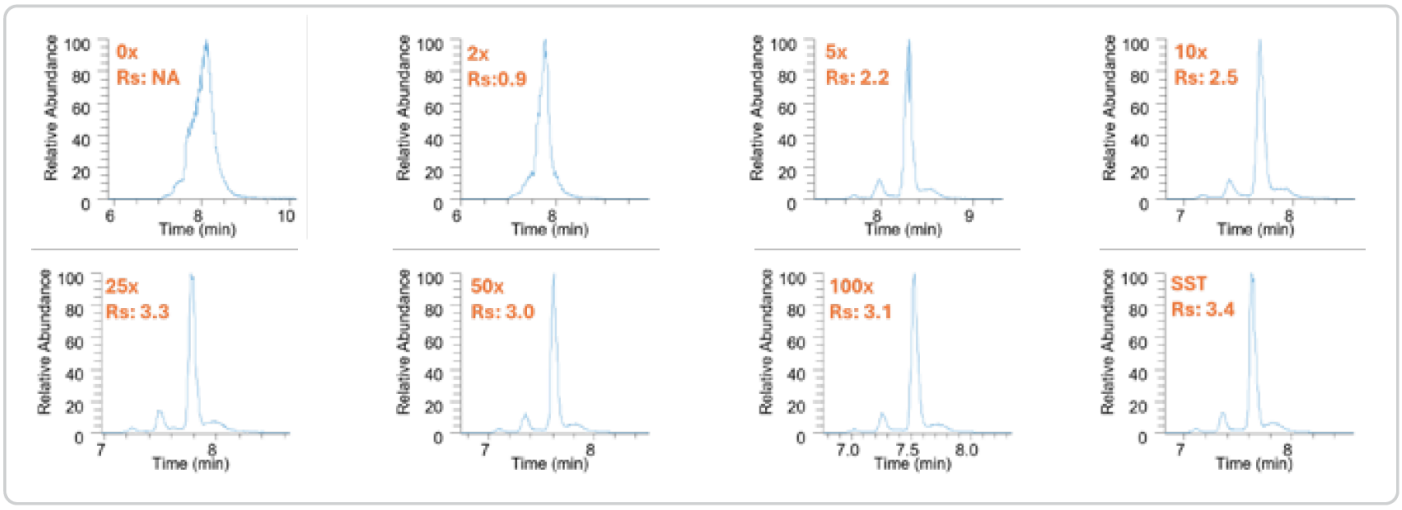

Figure 1. ZipChip CVA of 0.25 mg/mL NISTmAb in each media matrix dilution of Gibco CD CHO growth media.

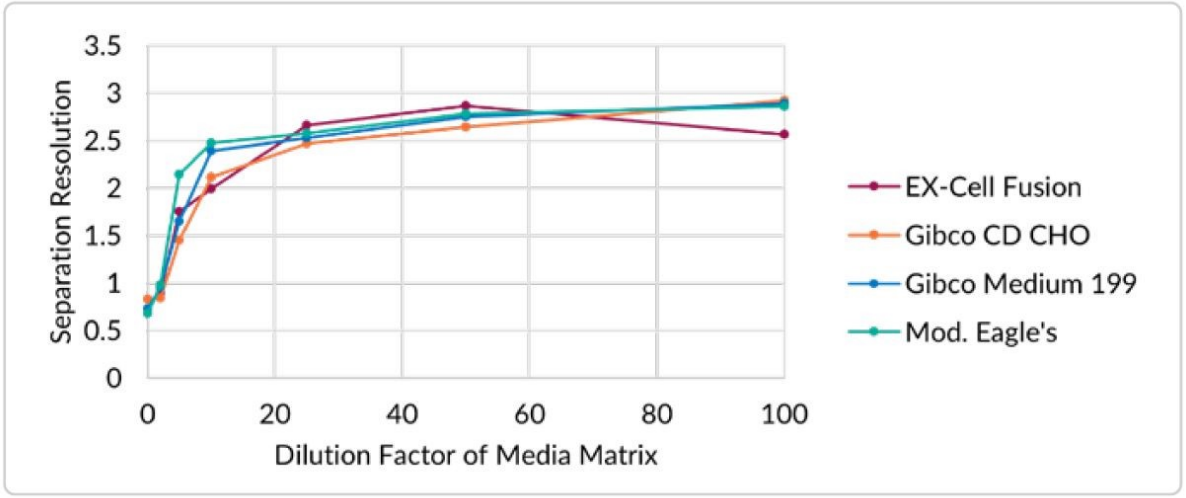

Figure 2. Separation resolution between the 0K and 1K variant of NISTmAb from each matrix dilution level of EX-Cell Fusion (red), Gibco CD CHO (orange), Gibco Medium 199 (blue), Mod. Eagle's (green).

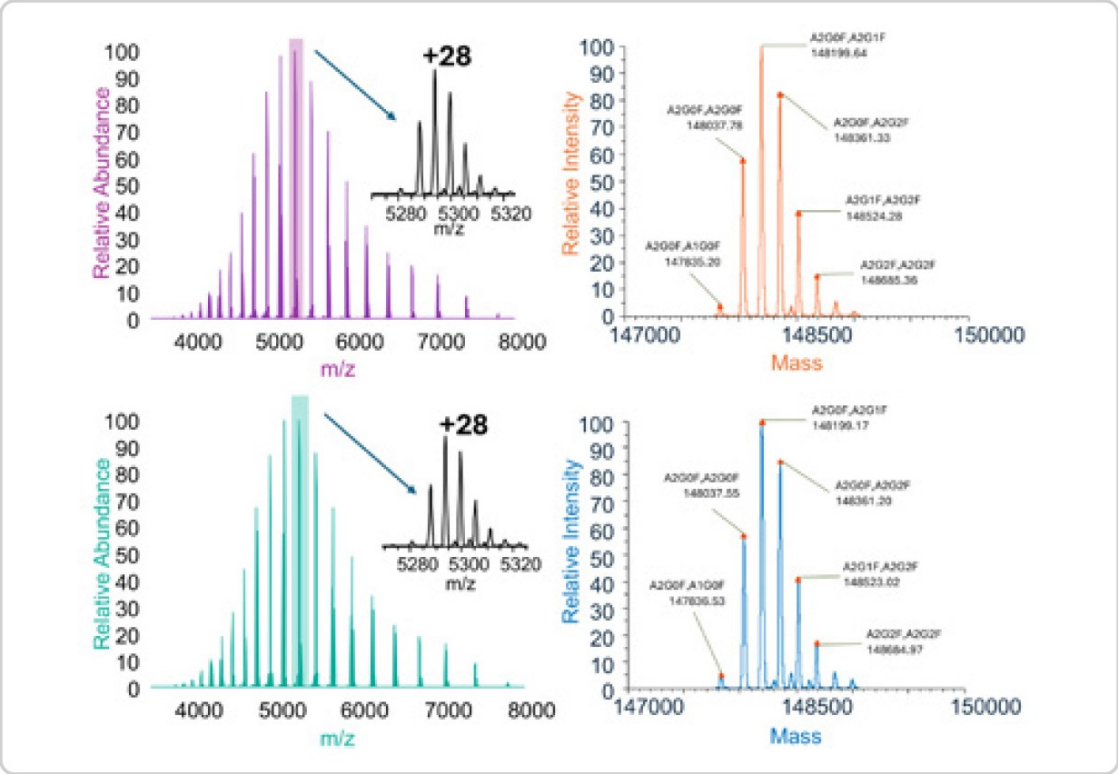

Figure 3. Raw and processed mass spectra from the main variant of the NISTmAb SST (top) and the 0x dilution (full-strength) growth media matrix (bottom). No adducts due to the media components were observed in the full-strength media matrix indicating effective separation of the mAb from interfering components.

From analyzing NISTmAb in different media matrix strengths it is evident that the ZipChip CVA method can work with a wide range of dilution levels without sacrificing data quality. The ideal dilution level is, however, tied to the titer of the mAb in the spent media samples and sensitivity of the MS being used. Titer from a well optimized bioprocess can be as high as 10 mg/mL at the end of culture, but titers from earlier time points or unoptimized bioprocesses could be much lower. NISTmAb stock at 10 mg/mL was buffer exchanged into Gibco CD CHO medium to mimic a high titer spent media sample. This stock was serially diluted with Gibco CD CHO growth medium to mimic a broad titer range from 10 mg/mL down to 0.016 mg/mL. Each titer mimic was diluted 50x and 10x with CVA diluent. The resulting charge variant separations are shown in Figure 4. For the 50x dilution all 4 variants were detected down to the 0.08 mg/mL titer sample (0.16 µg/mL analysis concentration). There is evidence of the 2K variant (the least abundant basic variant) at the lowest titer sample, but the intensity is not sufficient for confident deconvolution. In contrast, a 10x dilution of the lowest titer mimic at 0.016 mg/mL gives sufficient signal to detect all variants even though some separation resolution is lost. For well optimized, higher-titer bioprocesses, a 50x dilution is a “fool-proof” strategy for preparing spent media samples for ZipChip CVA. In instances where titer is low or MS sensitivity may be a concern, a 10x dilution can be used. Given the impact on separation quality, we would not recommend diluting samples less than 10x.

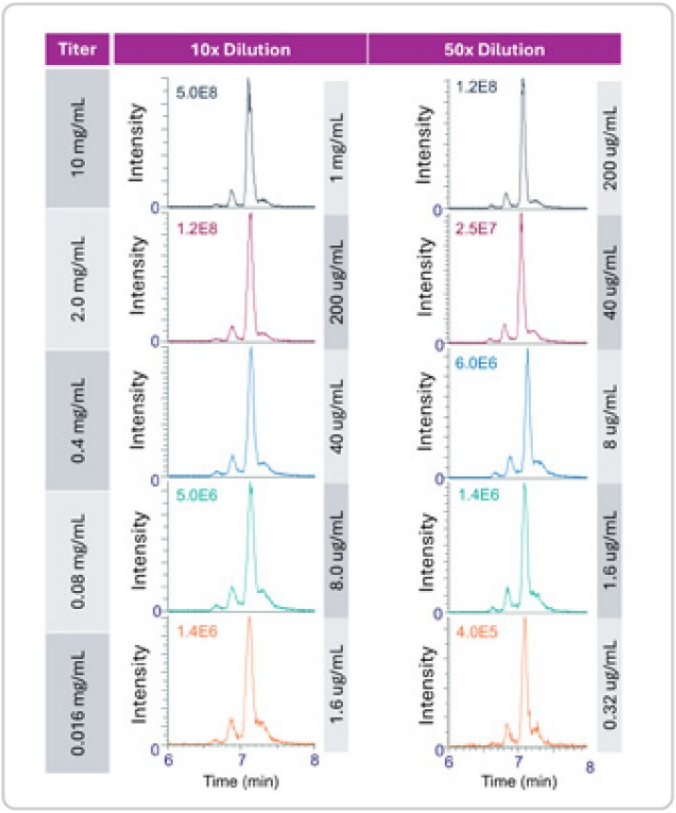

Figure 4. ZipChip CVA analysis of titer mimics after a 10x and 50x dilution with CVA kit sample diluent.

Analyzing Spent Media from a CHO Bioreactor

Spent media samples were collected twice daily from a bioreactor culture of NISTCHO expressing NISTmAb. Off-line measurements from day 6–14 reported a titer range of 0.3–1.67 mg/mL. A 50x dilution of the spent media samples was performed using the Charge Variant Analysis sample diluent. Figure 5a shows the charge variant profile for the day 3.5 timepoint, which corresponds to peak charge heterogeneity. Seven variants are detected: 5 basic variants, the main variant, and an acidic variant. Mass spectra for each charge variant peak in the ZipChip separation were deconvoluted to obtain masses. Mass shifts between the charge variants and shifts in migration times are used to identify the post-translational modification(s) (PTMs) causing the charge heterogeneity. Identified PTMs are shown in Table 3. Mass shifts within the deconvoluted spectra of the main variant were used to identify glycoforms as shown in Figure 5b. Note that sialylated glycans would migrate as an acidic variant due to the reduction in net charge, but sialylated glycans were not detected on this molecule. Figure 6 tracks the fractional abundance of the identified charge variants throughout the course of the cell culture. The most heterogeneous charge profiles were observed from days 2–5 with 6 different species detected. However, by the end of the culture the charge variant profile is more homogeneous with the main variant, accounting for 62% of the detected variants. Figure 7 tracks the fractional abundances of the main glycoforms throughout the culture. A higher degree of afucosylated glycans (G0,G0 and G0,G0F) was observed in earlier time points and more extensive galactosylation is observed by the end of the culture. This method shows that key product quality attributes can be detected and monitored even at early stages of the culture providing valuable insight for process optimization or biomanufacturing support.

Table 3. Intensified PTMs from the expressed NISTmAb

Variant	PTM	
Global Settings		
B5	2x pyroE, 2x C-am	pyroglutamic acid, C-terminal amidation
B4	1x pyroE, 1x C-am	pyroglutamic acid, C-terminal amidation
B3	0x pyroE	pyroglutamic acid
B2	2x pyroE, 2x C-am	pyroglutamic acid, C-terminal amidation
B1	1x pyroE	pyroglutamic acid
Main	2x pyroE	pyroglutamic acid
Acidic	deamidation	

Summary

This work evaluates the performance of ZipChip Charge Variant Analysis for analyzing biotherapeutic proteins from spent cell culture media. By using a dilute-and-shoot strategy, the sample prep required for analysis is greatly simplified. NISTmAb was used as a model antibody to evaluate the

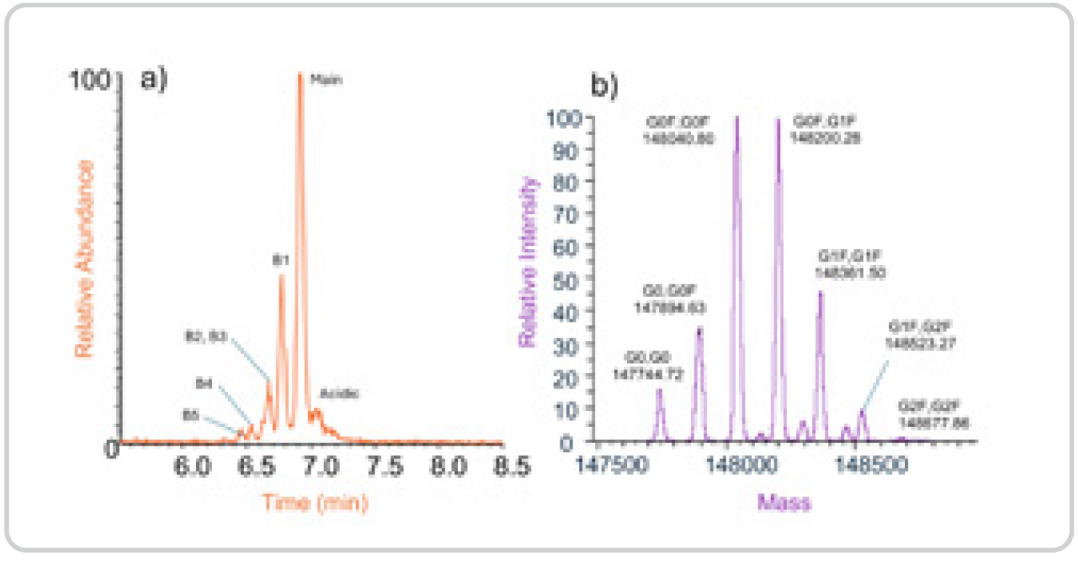

Figure 5. a) Charge variant profile from the day 3.5 timepoint corresponding to peak charge heterogeneity. b) Processed mass spectrum of the main variant with identified glycoforms.

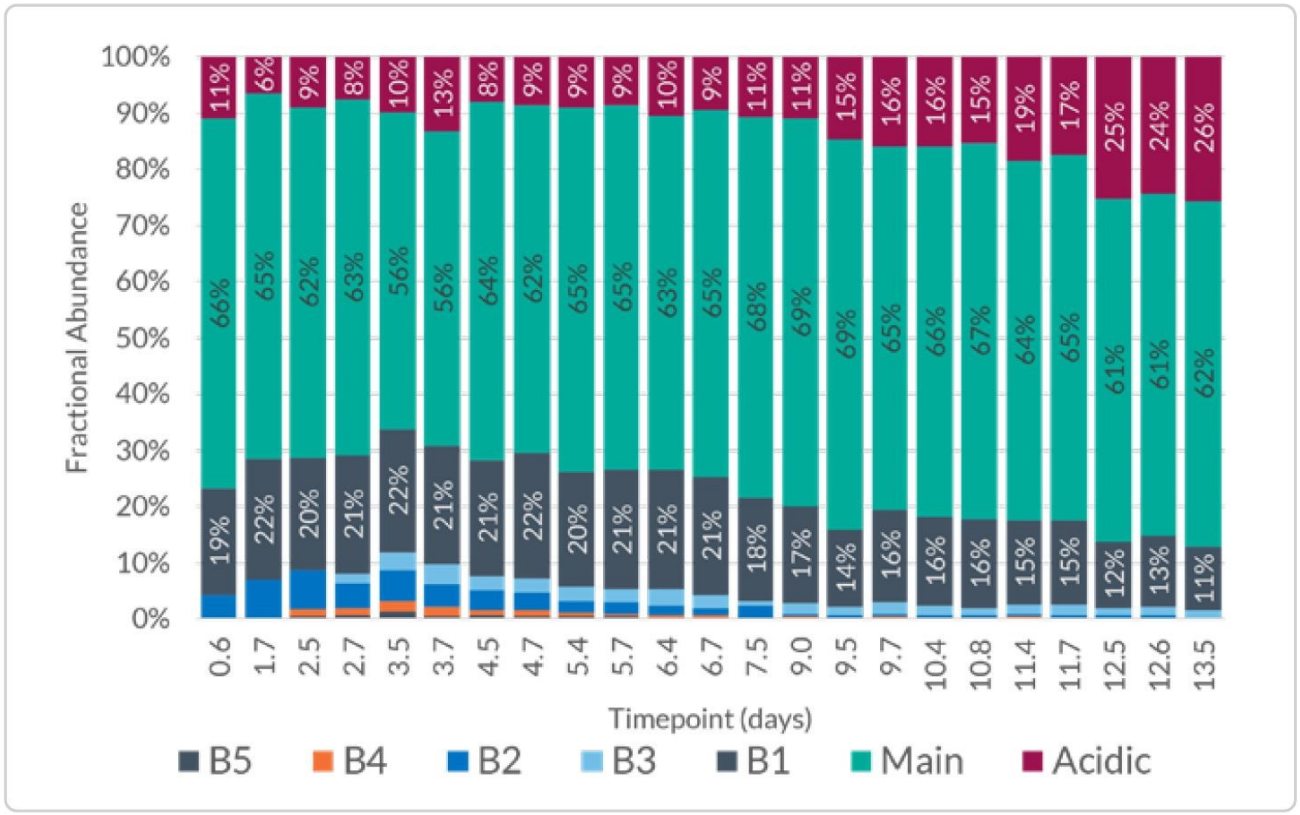

Figure 6. Fractional abundance of mAb charge variants from media samples collected throughout cell culture.

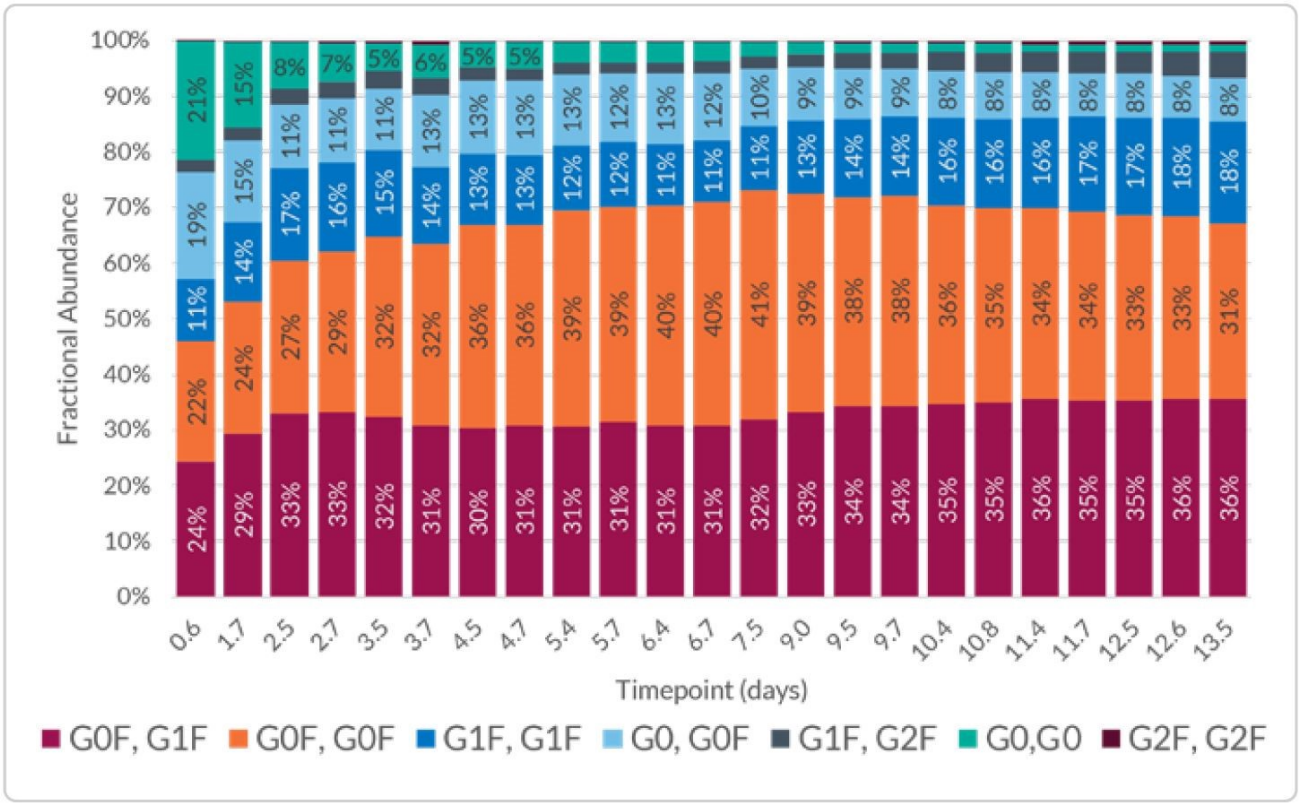

Figure 7. Fractional abundance of identified glycoforms from media samples collected throughout cell culture.

impact of the media components on the charge variant separation and MS data quality. Results indicate that dilution levels of 25x or greater effectively maintain separation resolution, while lower dilutions lead to reduced separation. MS data quality remained consistent, with minimal interference from media components, even at undiluted levels. An assessment of sensitivity revealed that higher titer processes can tolerate higher dilutions for analysis, while lower titer processes may benefit from lower dilutions for adequate detection. Additionally, samples from a CHO bioreactor culture were analyzed, tracking charge variants and glycoforms across time points. This method enables efficient monitoring of multiple product quality attributes during early and late stages of cell culture, providing insights useful for bioprocess optimization.

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