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Online LC/MS Method for Measurement of Monoclonal Antibody Glycosylation from a Bioreactor

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Introduction

Monoclonal antibodies are glycosylated in the cell during production. Glycosylation is an important attribute for a final antibody drug product. Glycosylation affects the pharmacokinetic properties of monoclonal antibodies and consequently the final drug product quality and safety. Glycoforms of monoclonal antibodies can vary over the course of a bioreactor run. This variation can lead to undesirable properties in the final product lot. Monitoring of monoclonal antibody glycoforms directly from a bioreactor is a key step to controlling the degree of galactosylation over the course of a production run. An online monitoring system was developed using the MilliporeSigma MAST system and an Agilent 2D-LC/TOF MS system to monitor the glycosylation on the reduced heavy chain using Flash Characterization to rapidly reduce the antibody.

Figure 1. Online 2D-LC and 6230 TOF

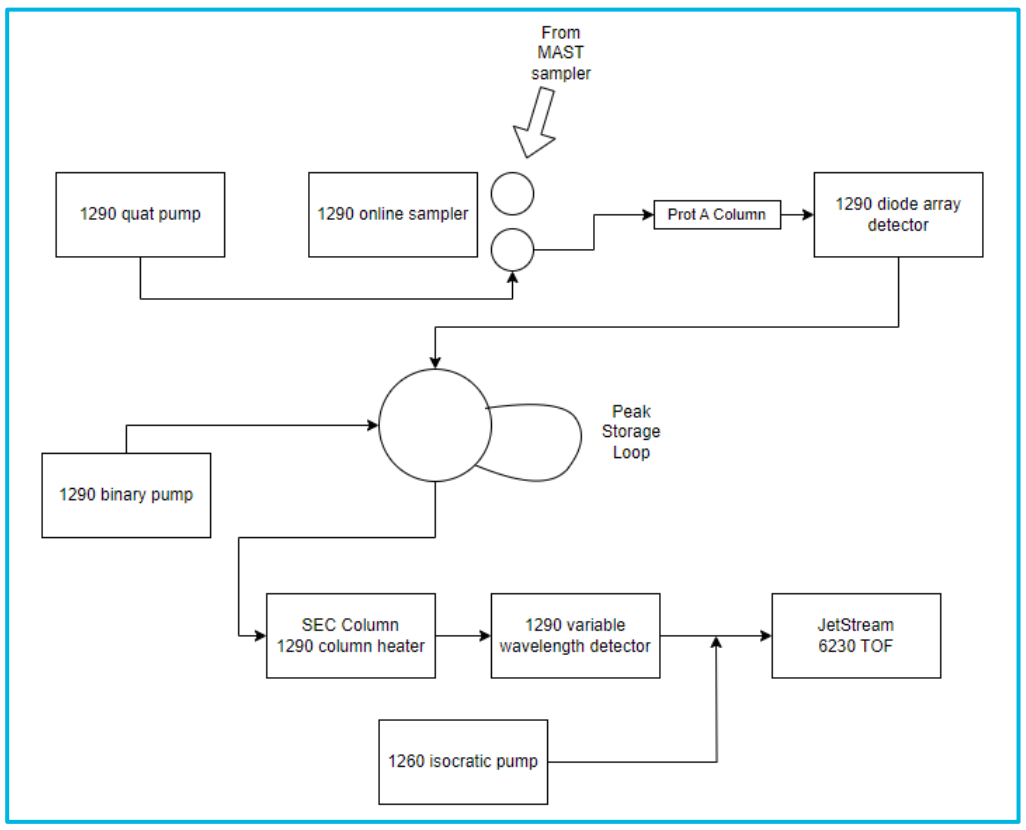

Figure 2. Initial setup of the 2D-LC/TOF system

Experimental

Method

The monitoring system was constructed with the MilliporeSigma MAST autosampling solution taking an aseptic sample from the bioreactor and transferring it to the Agilent 1260 Infinity Online Sample Manager. The process was controlled with the Agilent Online LC Monitoring Software connected with a prototype software bridge to MassHunter 11 for control of the 2D-LC/TOF system. The 2D LC system was run with a protein A column (Agilent Bio-Monolith Recombinant Protein A, 4.95 x 5.2 mm, PL 5190-6903) in the first dimension with a flowrate of 500 µL/minute, 50 mM ammonium acetate pH 7.4 for loading, and 50 mM acetic acid for elution. The eluted peak was collected in a storage loop and injected on to a SEC column (Agilent Advanced Bio SEC 200 Å, 4.6 x 150 mm, 1.9 µm, PL1580-3201) running with a flowrate of 300 µL/minute made up by flowing 200 µL/ min of 50 mM ammonium acetate pH 7.4 and teeing in 100 µL/min of 5 mM TCEP in 50 mM ammonium acetate pH 7.4. The SEC column was warmed to 40°C in the column oven. The outlet of the second dimension was connected to a JetStream source on a 6230 TOF MS. For method development the scan range was set from 100 to 3200 *m/z* to observe low mass ions and the reduced heavy and light chains. The MS was run in positive mode and additional source settings are listed in Table 1. The data analysis was done using BioConfirm 12.1 in an automated method called from the worklist. The deconvolution was done from 20,000 to 160,000 Da to capture all possible species from the intact antibody to the reduced heavy and light chains. The masses were searched against the target antibody and its glycoforms as part of the BioConfirm method. The resulting assigned peaks were automatically exported to a CSV file. For bioreactor monitoring and control the CSV data file was fed into a SCADA platform and feed media pumps connected to the bioreactor were adjusted automatically by PID feedback for galactosylation control.

Table 1. JetStream Source Settings

JetStream Source Settings		
Fragmentor:	380	Volts
Drying Gas:	12	l/min
Gas Temp:	365	°C
Nebulizer:	60	psig
Skimmer:	65	Volts
Sheath Gas Flow:	12	l/min
Sheath Gas Temp:	400	°C
VCap:	5000	Volts

Results and Discussion

Method optimization

The system method was initial tested with a purified monoclonal antibody sample to work out the optimized conditions. The initial runs demonstrated good capture and elution of the antibody on the protein A column as monitored by the UV detector. The eluted peak was captured in the storage loop on the 2D system. After collection of the peak, the storage loop was automatically placed in line with the second-dimension pumps and directed into the SEC column. The eluting peak from the SEC column was monitored by the second UV detector. The reducing TCEP buffer was teed into the flow post UV detector and was directed into the JetStream source for mass analysis on the 6230 TOF.

The mass spectra of the eluted reduced peak is shown in figure 3. The light chain has good signal, but we were unable to see signal for the heavy chain. There were some broad peaks on the baseline and manual deconvolution indicated species at ~110,000 Da. This mass could indicate two heavy chains together. While this could be disulfide linked heavy chains, given the strong reducing environment, the heavy chains were expected to be reduced. The heavy chains may still be associating together non-covalently after reduction. The reduced products were formed spraying from a neutral buffered solution without denaturant that could have led to this outcome.

System modification

The TCEP introduction was moved from post column to a pre column position, and the SEC column was warmed up to 40°C. The pre column introduction enabled warming up the antibody, more reaction time, and better mixing with the TCEP. Figure 5 shows the resulting mass spectra that shows clear light and heavy chain spectra.

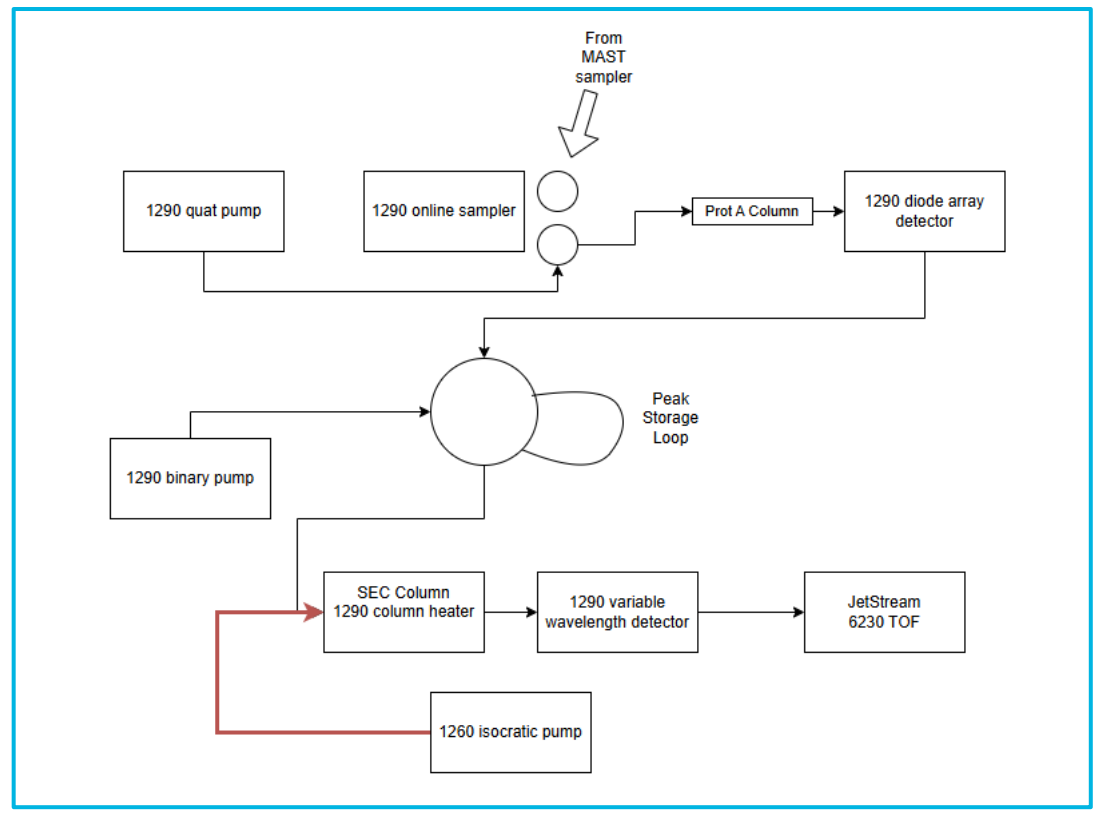

Figure 4. Modification of the of the 2D-LC/TOF system

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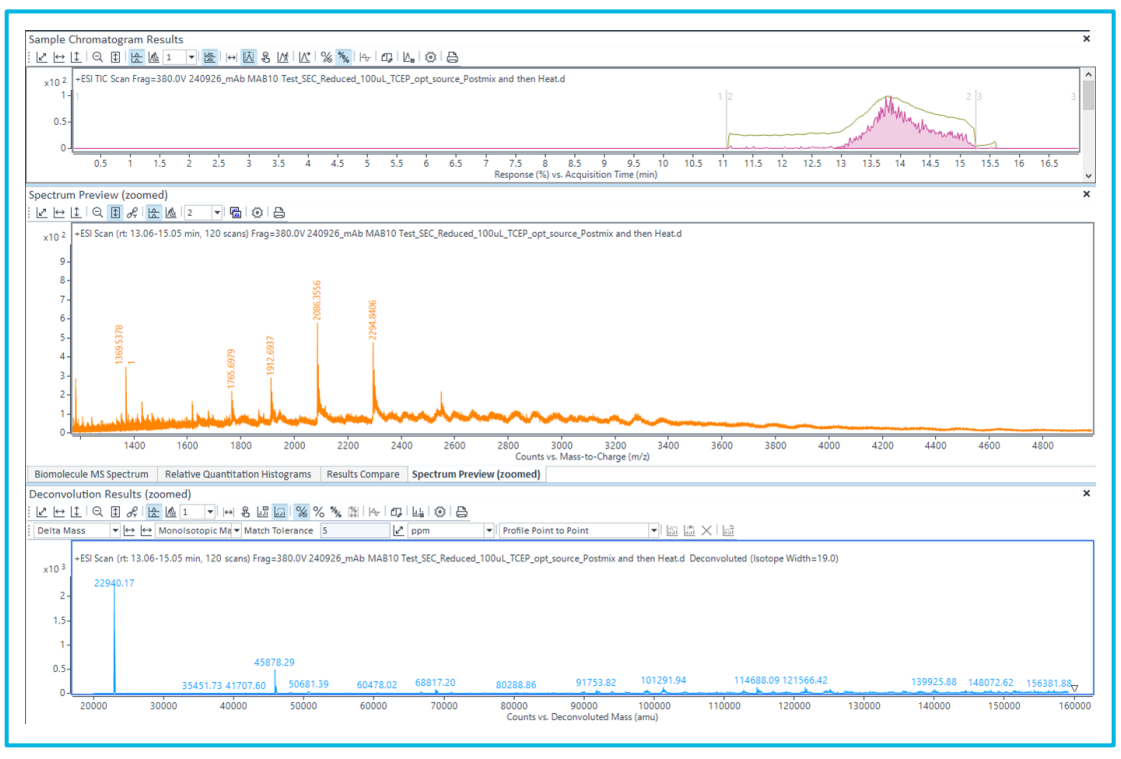

Figure 3. The top panel show the TIC overlaid with the EIC of the light chain. The middle panel shows the summed mass spectra of the peak from 13.5-14.5 minutes. The bottom panel shows the deconvoluted spectra from 20,000 Da to 160,000 Da. Only the light chain and its dimer are present. Ripples in the baseline indicate a broad mass at ~110,000.

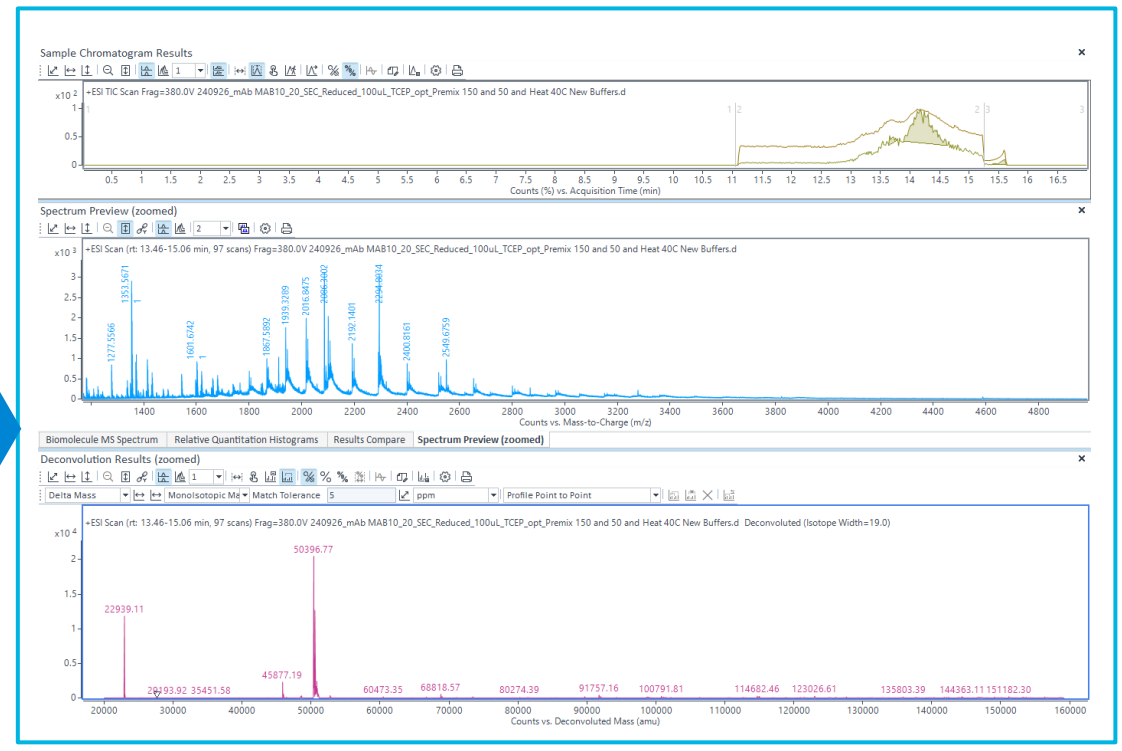

Figure 5. The top panel show the TIC overlaid with the EIC of the light chain. The middle panel shows the summed mass spectra of the peak from 13.5-14.5 minutes. The bottom panel shows the deconvoluted spectra from 20,000 Da to 160,000 Da. After the pre-column addition of the TCEP, the glycosylated heavy chain is now the dominant peak in the deconvoluted spectra.

Results and Discussion

Bioreactor samples

The optimized method was tested with stored samples from three prior bioreactor runs that had the glycosylation profile established over the run with an offline 2AB LC method. During each of those runs, the feed was modulated to obtain different glycosylation profiles, with the unmature glycoform species percentage ranging between 40 and 70 percent.

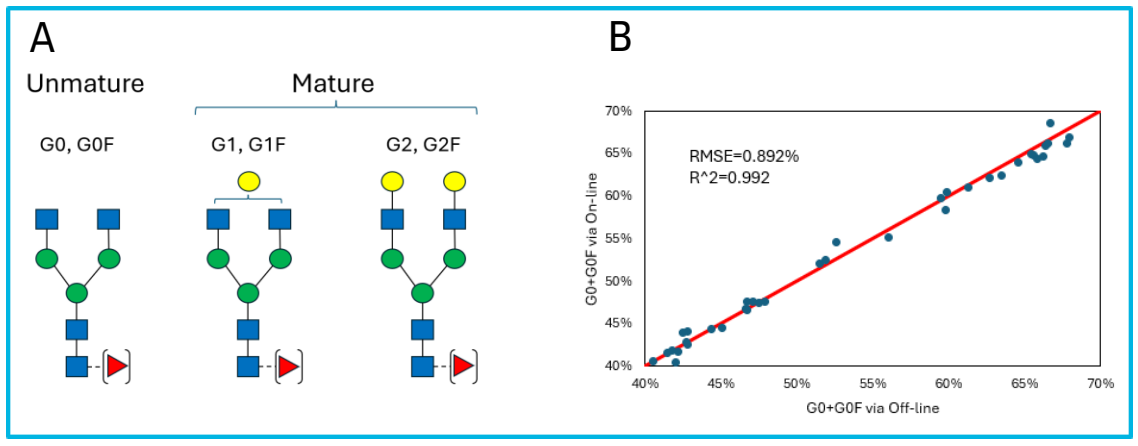

Figure 6. (A) Mature and unmature glycans on IgG. (B) Correlation of online LC/MS with offline 2-AB galactosylation.

Each sample was run in duplicate, with an average taken of the area and height percentages obtained through BioConfirm deconvolution for each species in a glycosylation profile consisting of G0, G0F, G1F, and G2F which were the most consistent glycoforms that were measured with BioConfirm. The percent sum of unmature species, G0 and G0F, were compared to the 2-AB percent sum of unmature species recorded previously. It was found that 2D-LC/TOF measurement generally agreed with the 2-AB method. Height percent was found to be closer to 2-AB compared to area percent, with the maximum difference of the percent sum being only 2 percent with height vs 4 percent with area.

Bioreactor monitoring

A 3L bioreactor run producing IgG was run over 35 days with the real-time glycosylation monitoring and control system. The 2D-LC/TOF system results were converted to percent galactosylation which was calculated by summing the mature glycans divided by the sum of the total glycans. The results were sent to a digital twin that controlled feed pumps to the bioreactor. The first few days were monitored with 100% basal feed. A series of setpoints were tested over the length of the run. The results are plotted in Figure 8. Overall, it demonstrates that the glycoforms were responding as expected to the setpoints in the bioreactor.

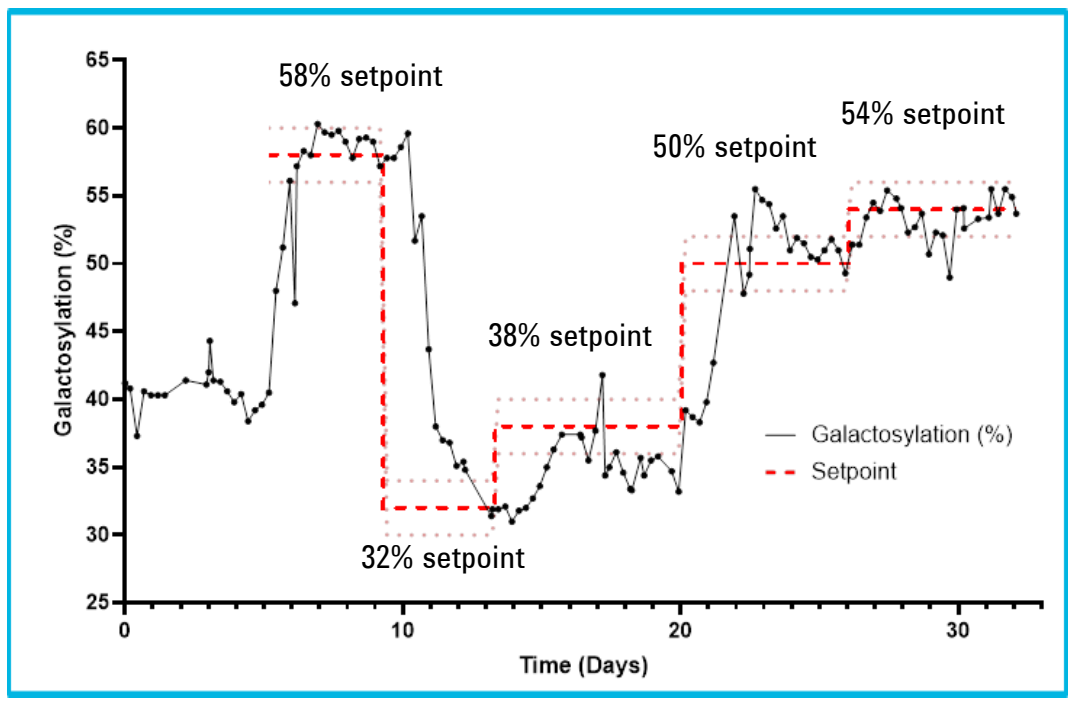

Figure 8. Response of glycan profile to bioreactor control setpoints monitored with 2D-LC/TOF system over 30 days

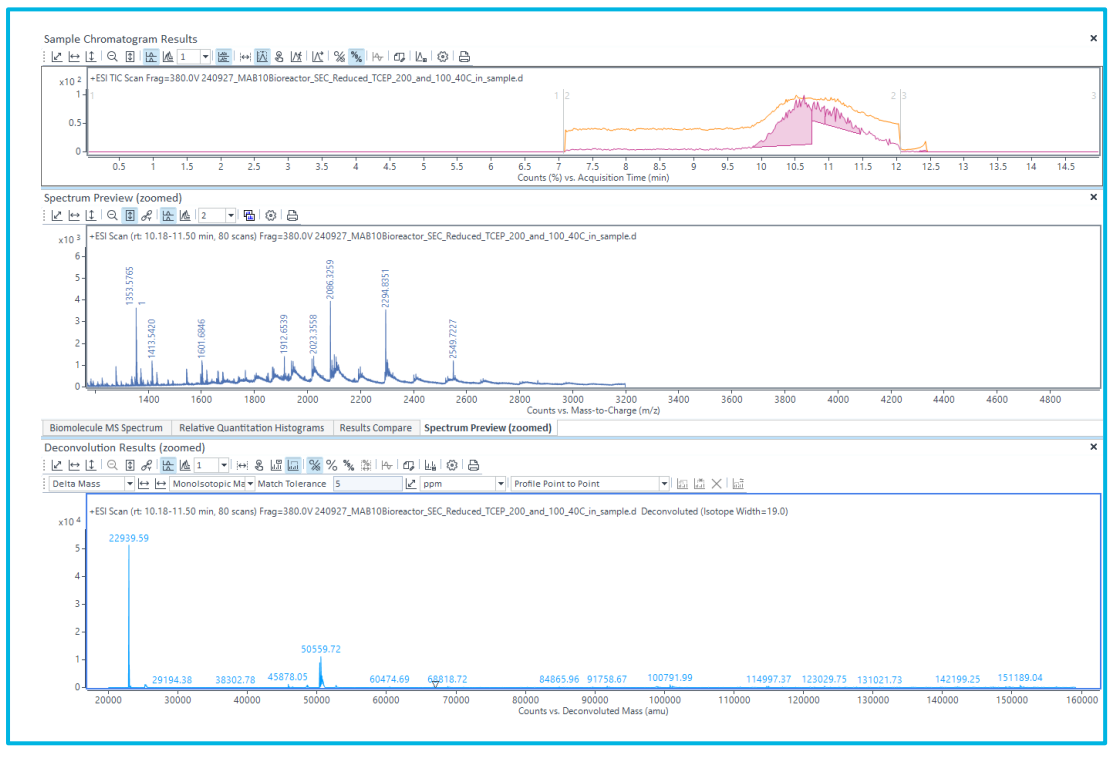

Figure 7. Bioreactor sample run

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Conclusions

- A successful online protein glycosylation monitoring system was developed that leveraged a 2D-LC/TOF and Flash Characterization to rapidly reduce the antibody without extra sample handling.
- Integration of the a 2D-LC/TOF into a closed loop bioreactor feedback control system was achieved
- Successful demonstration of control of protein glycosylation in a bioreactor over >30 days of runtime

The authors declare no competing financial interest.

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