Evaluation of Critical Attributes on Different Lots of Adalimumab Using BPV Flex Software 1.0.1

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It is important to characterize biotherapeutics and monitor their quality to ensure patient safety and drug efficacy. It is common during the development and particularly following commercialization and lifecycle management of protein therapeutics that raw materials, production scale, production site, and many other factors are changed, which can potentially impact the quality of the final product. There are many approaches to characterize and monitor product attributes, and these results are correlated to activity assays.

Intact mass and subunit analysis are among the most common assays used during the development of protein therapeutics. These assays provide molecular weight, glycoform distribution, and other heterogeneity information. Subunit analysis, including those leveraging an IdeS digest, can provide domain-specific information and more detailed glycoform composition. Due to limited sample preparation steps, intact mass analysis and subunit analysis are often implemented as platform assays. The results from each study are compared to previous analysis to ensure consistency. Ideally, comparisons are accomplished without the need for re-acquiring or reprocessing data.

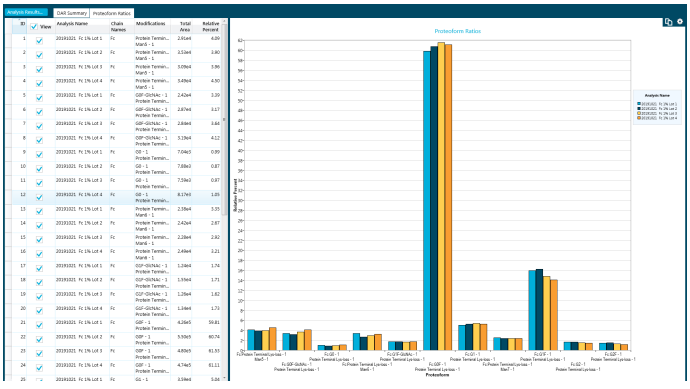

Figure 1. Comparison of glycoform distribution across , lots of Fc region of adalimumab.

SCIEX X500B QTOF system

In this technical note, we highlight the use of BPV Flex software 1.0.1 for high throughput analysis of intact and subunit data. Discussed is the ability to monitor and track quality attributes across different studies and determination of the relative abundance of targeted post-translational modifications. In the study, different lots of adalimumab were used to highlight the workflow. Presented here is an overview of how this solution enables high throughput data processing and subsequent comparison of results to track targeted quality attributes at the intact and subunit level.

Key Feature of X500B QTOF system and BPV Flex software 1.0.1

- High-resolution mass spectrometer for a wide range of biopharmaceutical applications
- Easy to use data processing with definable target masses
- Flexible batch processing with different sequences and customized processing parameters on each sample
- View and compare across different analyses to guide method development and optimize processing parameters
- Directly compare raw and reconstructed data to rapidly investigate similarities and differences in samples
- Easy to use hardware and software accessible for a wide range of users

Table 1. LC Conditions for intact

Time (min	%A	%B	Flow Rate ml/min
Initial	*85*	*15*	*0.5*
5.0	*85*	*15*	*0.5*
9.0	*5*	*95*	*0.5*
11.4	*5*	*95*	*0.5*
11.5	*85*	*15*	*0.5*
15	*85*	*15*	*0.5*

Table 2. LC Conditions for subunit

Time (min	%A	%B	Flow Rate ml/min
Initial	*75*	*25*	*0.5*
3.0	*75*	*25*	*0.5*
12.0	*25*	*75*	*0.5*
12.5	*10*	*90*	*0.5*
15.0	*10*	*90*	*0.5*
17.0	*75*	*25*	*0.5*
18.0	*75*	*25*	*0.5*

Methods

Sample Preparation:

Intact mass: Adalimumab samples were diluted to a concentration of 0.1 ug/ul using deionized water.

Subunit: Adalimumab samples were digested with IdeS and further reduced with TCEP.

Chromatography: Separation was accomplished using an ExionLC™ system fitted with an Agilent PLRP-S column (2.1mm X 50mm, 300Å, 5µm) at 80°C using the gradient shown in Table 1 and 2. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile.

Mass Spectrometry: A SCIEX X500B QTOF mass spectrometer with a TwinSpray Ion source was used for data acquisition. Data was acquired using TOF-MS mode with intact protein mode (IPM) turned off. MS instrument conditions are listed in Table 3.

Data Processing: Data were processed using BPV Flex software 1.0.1 using the settings defined in Table 4.

Table 4. Reconstruction Parameters

Parameter	Setting
Mass Range Selection	*Mass Range automatically set by defined molecule*
Peak Threshold	*5%(intact) 1%(Subunit)*
Step Mass	*1.0 Da*
Iterations	*20*
Signal to Noise Threshold	*≥20*
Gaussian Smoothing	*0*
Resolution	*5000*

Table 3. MS Parameters

Parameter	Setting
Scan Mode	*Positive*
GS1	*60*
GS2	*60*
Curtain Gas	*30*
Temperature	*550°C (intact), 500 °C (subunit)*
Ion Spray Voltage	*5500 V*
Time Bins to Sum	*80*
Accumulation Time (ms)	*0.5 sec*
TOF Start Mas (Da)	*400*
TOF Stop Mas (Da)	*4000*
Declustering Potential	*250 (intact), 150 (subunit)*
Collision Energy	*10*

Results and Discussion

To evaluate the variability in glycoform distribution in various lots of adalimumab, intact mass analysis, and subunit analysis with mass spectrometric detection was used. Data were processed and reviewed using BPV Flex 1.0.1. The molecule was defined in the project with information on primary sequence, disulfide bond

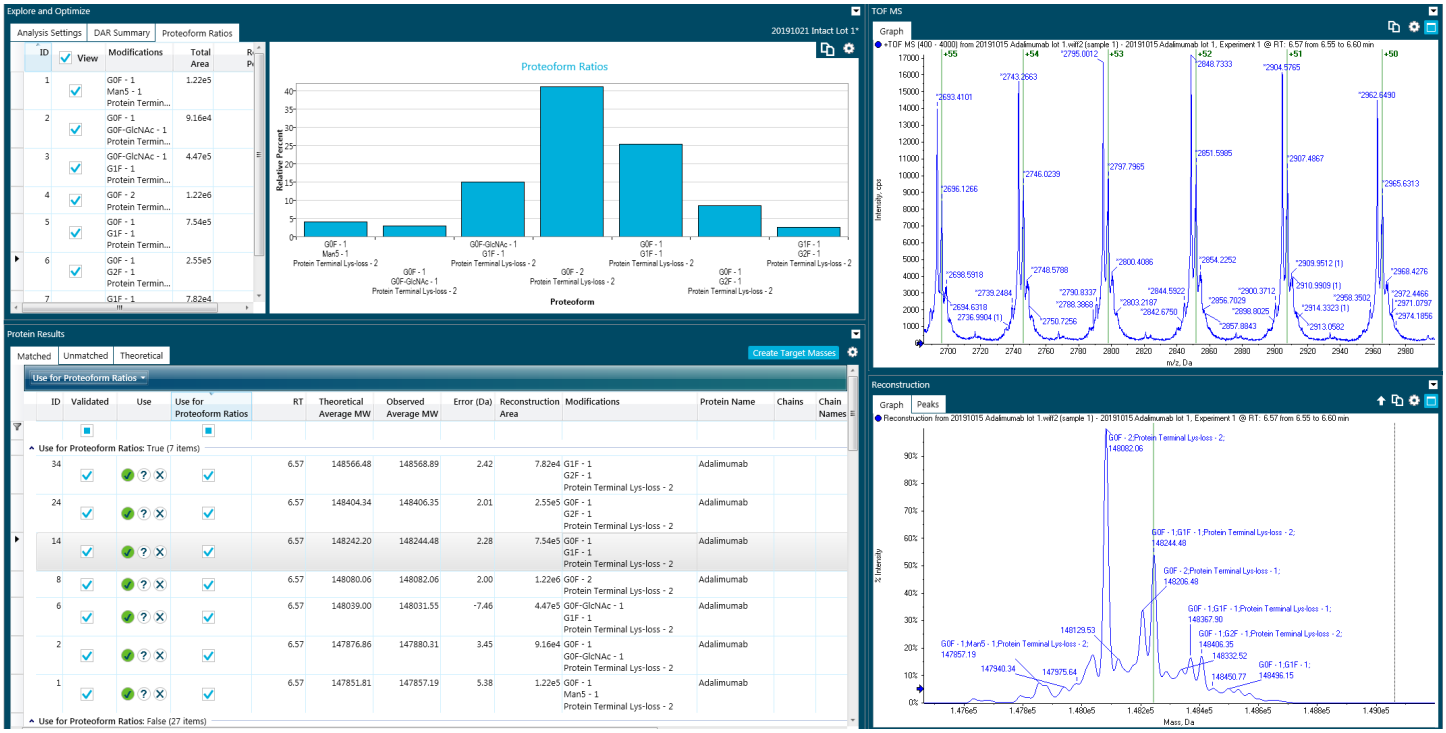

Figure 2. Analysis of Adalimumab lot 1 intact mass in BPV Flex software 1.0.1. Each window may be rescaled, and the data are linked to expedite review.

arrangement, and expected modifications. It is worth noting that modifications may be positioned if desired, particularly if the defined molecule will be used in additional studies. If desired, BPV Flex also allows for non-sequence-based data analysis of an expected target mass, which is useful for high throughput screening studies. One lot of adalimumab was used to optimize the acquisition and processing parameters to achieve an accurate result, including reconstruction resolution, matching tolerance, and input spectrum mass range.

The processed results of the first lot of adalimumab are shown in Figure 2. For each matched component, the underlying raw data and reconstructed data can be viewed simultaneously. To expedite review, the protein results, raw data, and reconstructed data may be undocked from the review window to allow for evaluation of constituents and their corresponding underlying data. From the results of lot 1 characterization, the identified major glycoforms are G0F, G1F, G2F, G0F-GlcNAc, G1F-GlcNAc, and Man5. G0F/G0F is the most abundant glycoform observed, which accounts for 41% of total proteoforms.

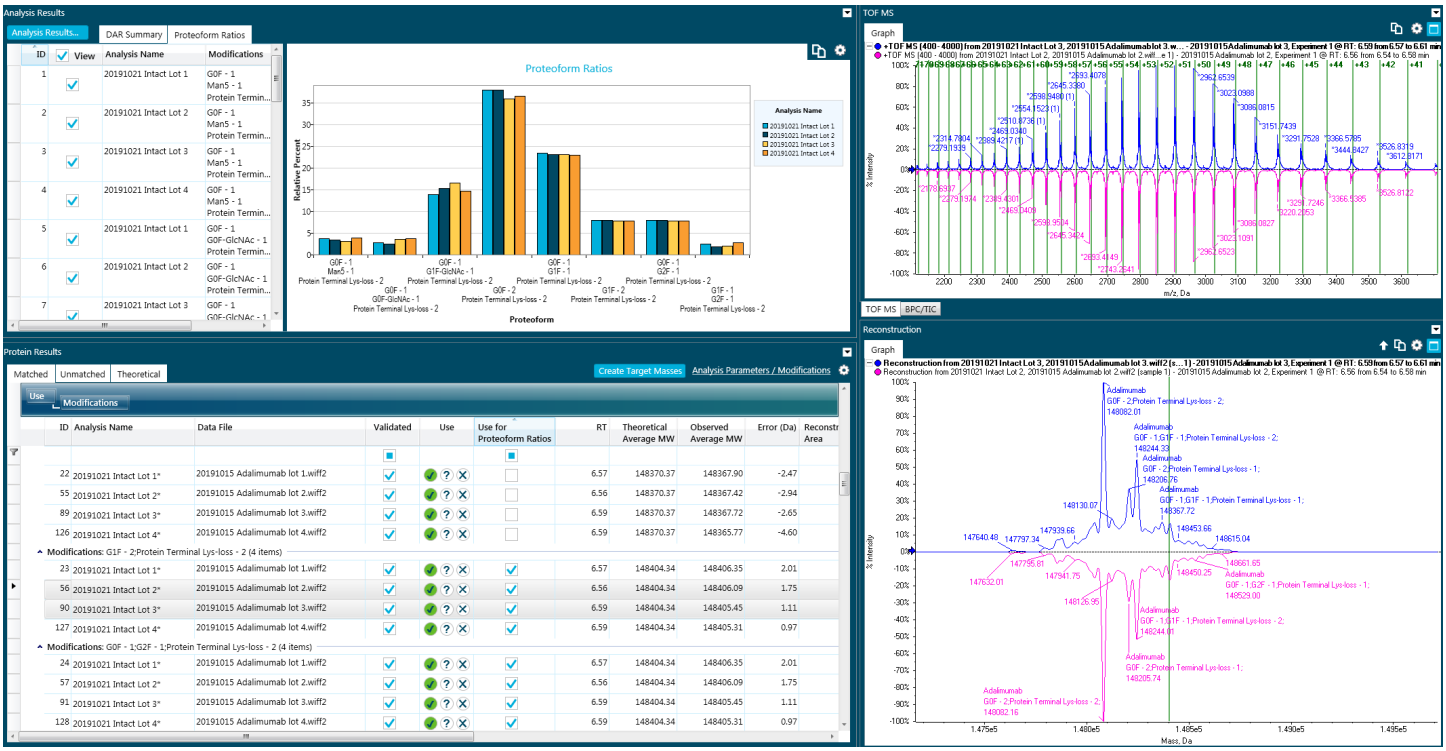

Figure 4. Summary of selected proteoforms and relative percentage plot based on reconstructed peak area in 4 lots of Adalimumab.

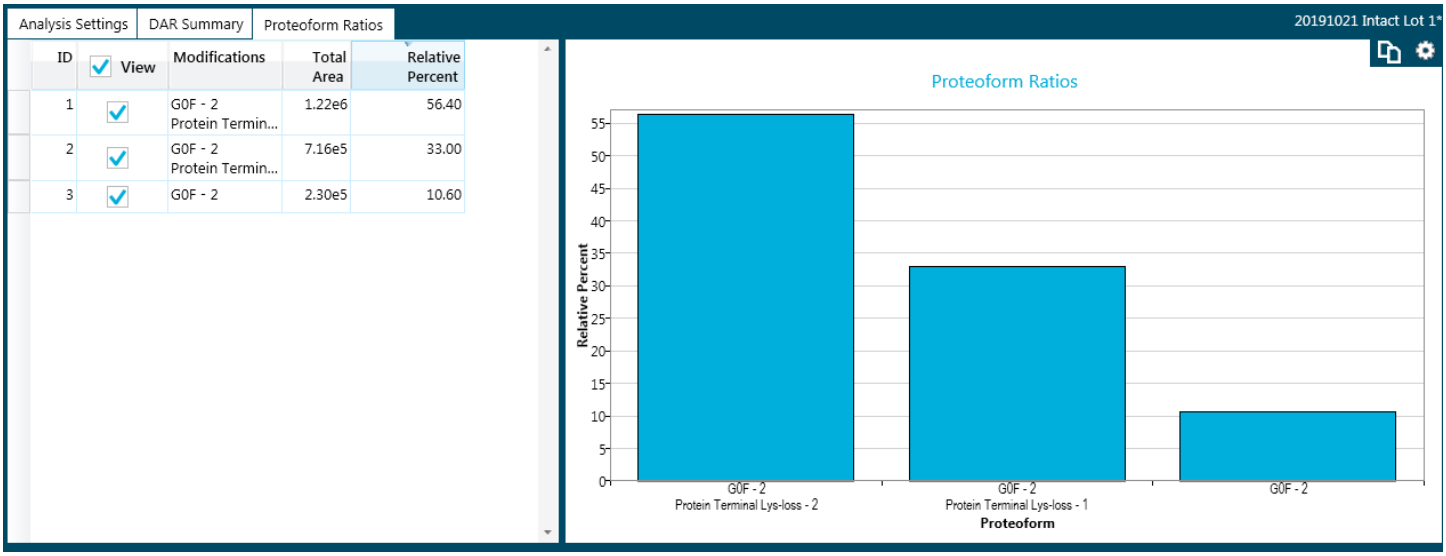

Figure 3. Summary of different number of C-terminal lysine and relative percentage plot based on reconstructed peak area in adalimumab lot 1.

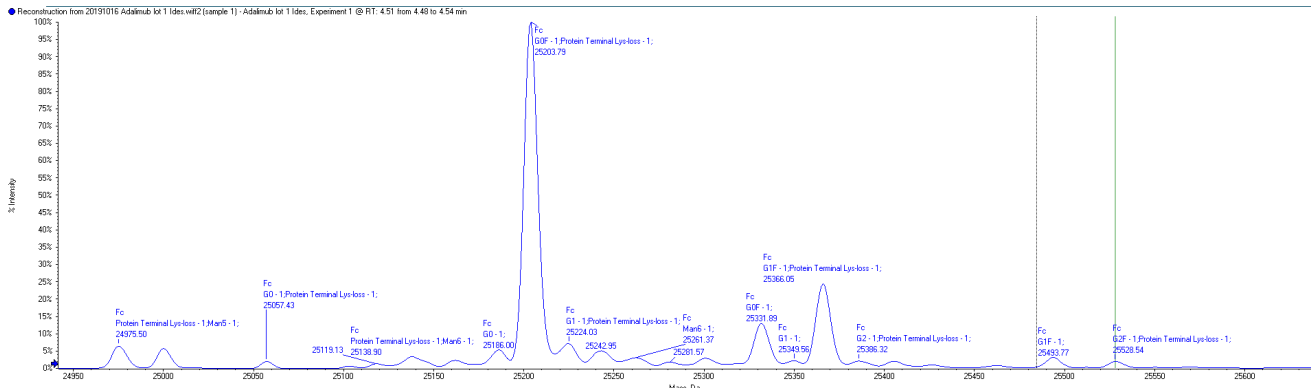

Figure 5. Identification of glycan distribution in Adalimumab Fc region.

A detailed glycoform distribution is displayed in the bar graph and in the table, Figure 2. G0F/G0F is also detected with a different number of C-terminal lysine attached, 0, 1, and 2. The percentage of each species is automatically calculated, and the results are displayed in BPV Flex software, Figure 3. The data shows majority of C-terminal lysine has been cleaved during the process, which is consistent with previous analysis [1].

An additional 3 lots of adalimumab were processed using the optimized parameters used for lot 1 characterization. After analysis, all the major glycoforms were detected in the other 3 lots with acceptable experimental precision, Figure 4. BPV Flex software provides a visual graph as well as a detailed percentage table, which enables rapid assessment and tracking of attribute levels.

Intact mass provides high-level information on glycosylation distributions; however, it is difficult to monitor low abundant glycans. Subunit analysis, particularly those of IdeS digested antibodies, requires simple sample preparation but provides much deeper insight into attributes, as shown in Figure 5. Highlighting the data quality, a much richer glycan profile was obtained, as shown in Figure 5. In addition to the common bi-antennary glycans, high mannose species such as Man 5, Man 6, and Man 7 were also detected using the IdeS approach, which provides the capability to track those critical quality attributes in a more efficient way.

Like the intact work, IdeS subunit analysis from the four lots of adalimumab was analyzed in parallel, and all the identified glycoform percentages were monitored. Figure 1 shows a detailed comparison (table and bar graph) on the attribute percentage across the 4 different lots for a subset of the monitored glycoforms. Consistent with the intact data, G0F is the most dominant glycoform in all samples, close to 60% (<10%CV). G1F is around 15%, while Man5 and G1 were observed at 4% and 5%, respectively. In addition, G2F, G0F - GlcNAc, G1F-GlcNAc, G0, G1, G2, Man6 and Man 7 were monitored. The identified glycoforms are highlighted and compared in mirror plot for a better visualization, Figure 6.

BPV Flex enables users to track targeted attributes and their changes in intact and subunit level. Data is easily visualized in an interactive and flexible manner with raw and reconstructed spectra tabulated results available simultaneously. The software provides the flexibility to define targeted proteoforms for determination of relative abundance calculations also it allows users to view and analyze the results from the different batches at the same time.

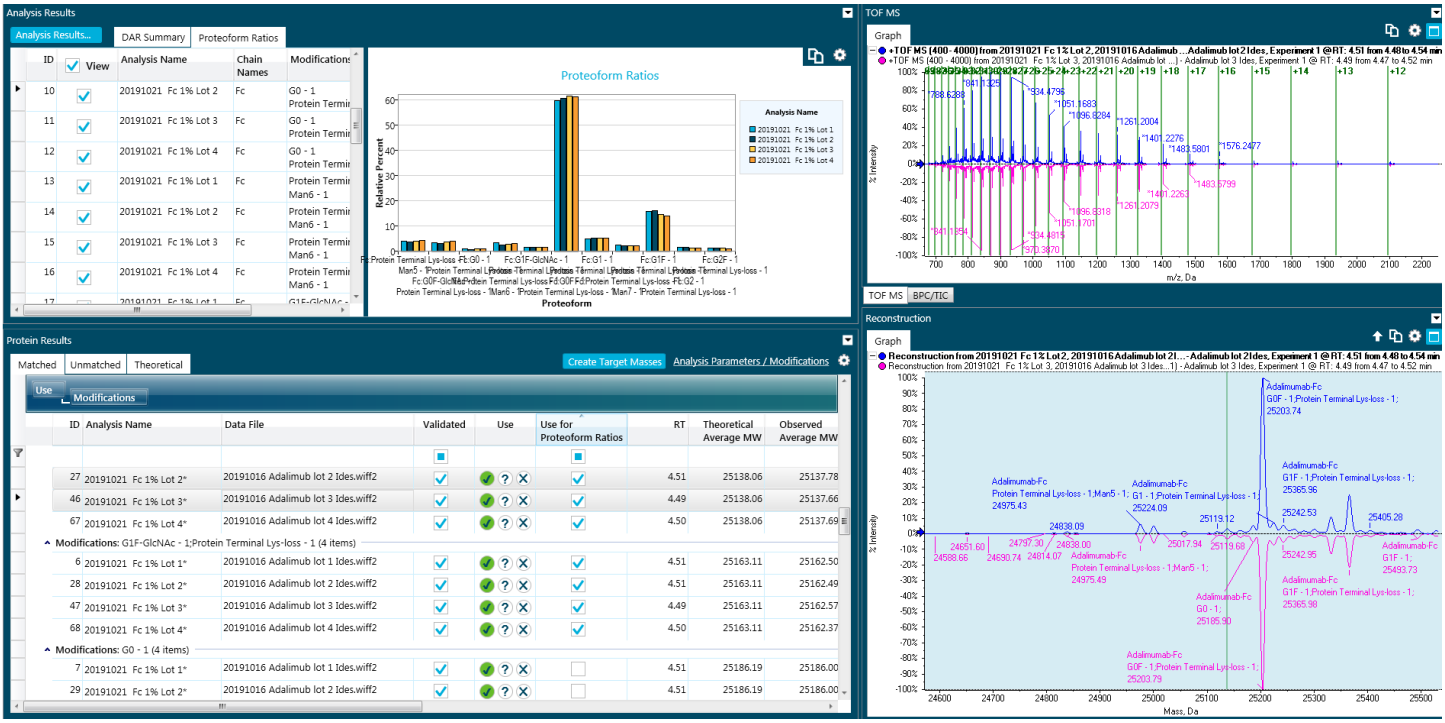

Figure 6. Comparison among four different lots of Adalimumab Fc region in BPV Flex software 1.0.1.

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Conclusions

- BPV Flex software 1.0.1 provides an efficient solution to refine and optimize processing parameters using an interactive interface
- BPV Flex provides a flexible way to define a molecule with or without sequence.
- Flexible and intuitive filtering and group to focus on the most important results
- Data review in BPV Flex software 1.0.1 easily accomplished using a customizable interface to focus on relevant information
- Monitor and track components across different studies with automatically generated plots of critical species

Reference

[1] Evaluation of similar quality attribute characteristics in SB5 and reference product of adalimumab. mAbs, Volume 11, 2019 Nayoung Lee, Gwangmin Park, etc.

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