

Analysis of Rituximab Biosimilar With Novilytic's Proteometer-CV Kit



ABSTRACT

Novilytic's Proteometer-CV Kit enables rapid analysis of titer and charge variant (CV) composition of biologics containing a human Fc region, including human, humanized, or chimeric monoclonal antibodies (mAbs), bispecific antibodies, antibody-drug conjugates (ADCs), and Fc-fusion proteins directly in clarified fermentation broth (CFB). This application demonstrates the utility of the Proteometer-CV Kit using Rituximab research-grade biosimilar as a model therapeutic monoclonal antibody (mAb). Rituximab is a mAb of the IgG1 subclass for the treatment of B-Cell cancers and autoimmune diseases¹. Charge variant characterization of a therapeutic mAb is a critical quality control step in its development and manufacturing². These variants arise from post-translational modifications (PTMs) and can affect the drug's efficacy, stability, and immunogenicity. The Proteometer-CV method provides exceptional repeatability, linearity, and robustness for determining the titer and charge variant composition of a rituximab biosimilar formulated in CFB. Novilytic's Proteometer-CV is proven to be a viable solution for detecting unfavorable proteoforms of mAbs that can manifest as charge variants.

INTRODUCTION

Titer and charge variant composition are routinely measured during the development and manufacturing of a therapeutic antibody. Titer correlates with product yield, while changes in charge variant composition indicate structural differences. These changes can be associated with lower efficacy, reduced stability, or increased toxicity of the biologic. Undesirable charge variants must be removed from the final product by purification, which lowers yields and is costly for the antibody manufacturer². Similarity assessment by charge variant analysis is used to ensure process control during manufacturing of different lots of a therapeutic antibody. It is commonly used for quality comparisons between a biosimilar and the originator mAb throughout development and manufacturing.

The Proteometer-CV Kit monitors the quality of human, humanized, or chimeric mAbs, bi-specific antibodies, antibody-drug conjugates, and fusion proteins with a human Fc region, regardless of their stage in the development continuum. It accelerates upstream process optimization by rapidly comparing samples' titer and charge variant composition in CFB. This novel method combines titer and CV analyses without Protein A purification. It has an analytical run time of 25 minutes, which includes column regeneration. Specific detection of the biologic analyte proteoforms is achieved after fractionation by using a fluorescently labeled affinity selector that binds with high specificity to the Fc domain of human IgGs.

RESULTS

A Rituximab biosimilar was selected to demonstrate the capabilities of Proteometer-CV. Several biosimilars of Rituximab have received FDA approval based on comparison to the originator, including extensive structural and functional product characterization^{1,3}.

Rituximab biosimilar (research grade, IgG1, pI 9.3) was formulated at a concentration of 1 mg/mL in CFB and injected in varying amounts into an HPLC system with a fluorescence detector. The Proteometer-CV Kit was utilized for the system setup, following the Proteometer-CV for mAb Charge Variant Analysis Quaternary Pump Configuration Instructions for Use⁴. The suggested gradient of 7-14% CV-MPB was used for each injection, as it provided optimal separation. The analyses were performed over three days on three unique lots of Proteometer-CV Reactors by two different analysts using two LC systems.

Repeatability

Repeatability testing was performed with ten injections of rituximab (8 µg) per run. The composition of acidic variants, main species, and basic variants from each injection was calculated, and the data from all injections ($n = 90$) were plotted against the injection number (Figure 1). Excellent reproducibility was observed between the three Proteometer-CV Reactor lots. The highest coefficient of variance, %CV = 7.83, was observed for % acidic



variants. For data collected on any reactor on a single day, the highest coefficient of variance was only 7.85% ($n = 10$) for the % acidic variants.

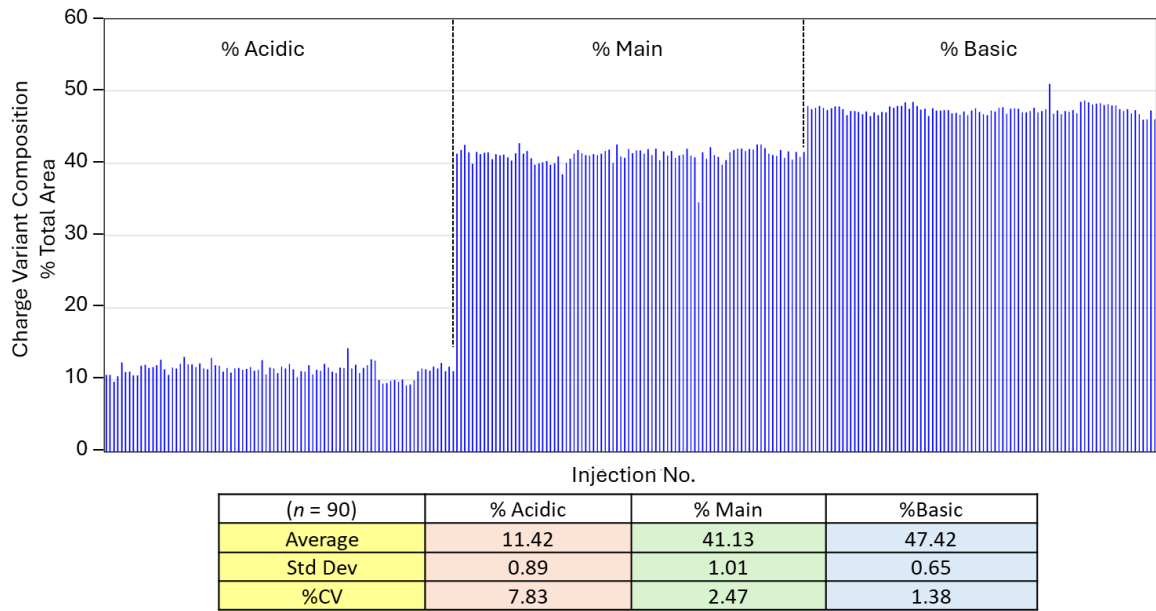


Figure 1. Repeatability testing of rituximab in CFB using the Proteometer-CV ($n = 90$)

Linearity

Triplicate injections of 8, 12, 16, 20, and 24 µg of the Rituximab formulation in CFB were employed for each linearity analyses. An overlay of chromatograms from a linearity analysis is shown in Figure 2. No signal was observed for the CFB control. This indicates that the Proteometer-CV achieves the same specificity-objective of Protein A affinity chromatography by using the fluorescently labelled mobile affinity selector.

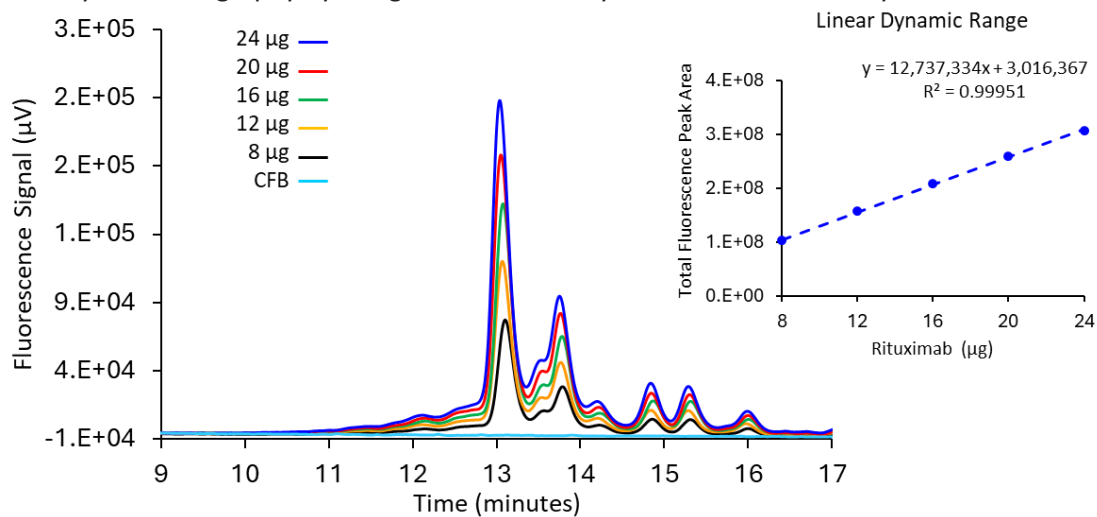


Figure 2. Linearity response of rituximab biosimilar in CFB. Inset on the right shows linear regression of average total fluorescence peak area ($n=3$).

Figure 2 (inset) also shows the linear regression of the total peak area as a function of mAb injection amount. A total of nine linearity analyses were performed on the three different Proteometer-CV reactors. Linear regressions of these data returned $R^2 \geq 0.999$ in all nine data sets, which exceeds the acceptable level of $R^2 > 0.990$ for impurity analysis methods⁵.

Robustness and Charge Variance Repeatability

Charge variant composition, or charge heterogeneity, is monitored throughout development and manufacturing. It is expressed as a percentage of acidic variants, main species, and basic variants, which are

calculated by measuring their respective peak areas. Charge variant composition values over all injections of the three independent linearity analyses on a Proteometer-CV reactor were compared to examine the repeatability of charge heterogeneity values. The charge variant composition obtained from the three linearity analyses performed on one Proteometer-CV Reactor is shown in Figure 3. When the data from the 45 total injections (three runs, five load amounts with triplicate injections per run) were analyzed together, charge variant composition values were highly reproducible. Excellent repeatability data was also obtained for the other two reactors. The greatest coefficient of variance (%CV) observed over the data collected from the three reactor lots was 7.3% for the percent acidic variants.

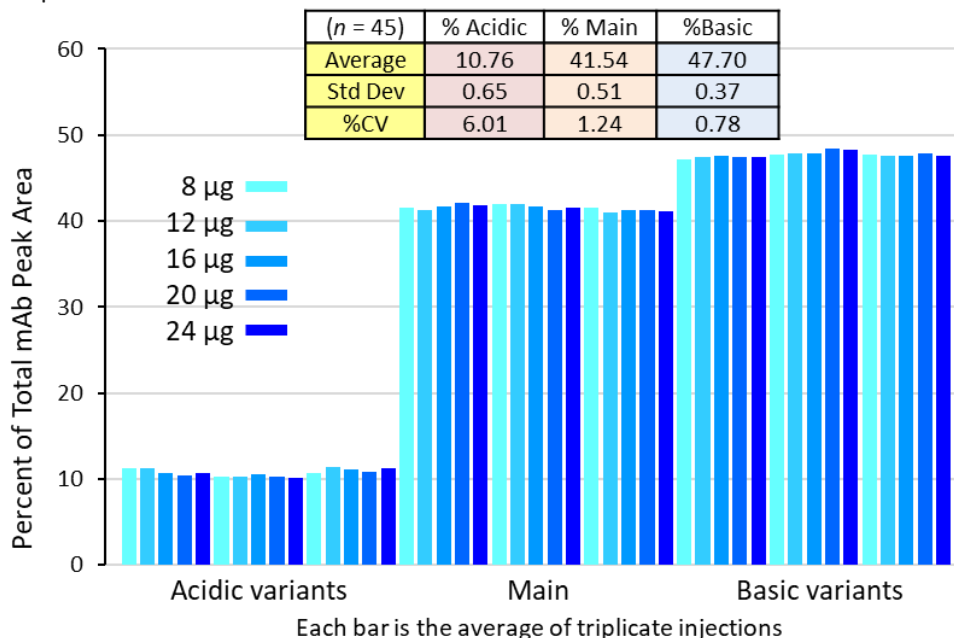


Figure 3. Percent Composition of variants obtained using the Proteometer-CV on rituximab biosimilar in CFB for various injection amounts (n=45). Each bar represents the mean of three injections.

The relationship between charge variant composition and sample load was investigated for the data depicted in figure 3. Trendlines of the sample µg load versus the % acid, main, and basis peak areas each have slopes within the range 0 +/- 0.02, indicating that the sample load does not affect the charge variant composition reported by Proteometer-CV. Furthermore, Analysis of Variance (ANOVA) of this data showed that there is no statistically significant difference ($p \geq 0.05$) between the charge variant composition values over the five sample loads tested. Specifically, p-values of 0.7956, 0.8789, and 0.3995 were obtained for the % acidic variants, % main species, and % basic variants. This shows that the charge variant composition results obtained from the Proteometer-CV Kit through direct analysis of Rituximab in CFB are highly reproducible across sample loads between 8 µg and 24 µg.

CONCLUSION

Novilytic's Proteometer-CV streamlines charge variant analysis in two ways: (1) sample purification is not required and (2) two quality attributes, titer and charge variant composition, are monitored simultaneously with high repeatability and linearity. The Proteometer-CV method provided exceptional precision, linearity, and robustness for determining titer and charge variant composition of a Rituximab biosimilar formulated in CFB. The results demonstrate the advantages of the Proteometer-CV Kit for detecting unfavorable antibody proteoforms that can manifest as charge variants. This method is suited for rapid comparative assessment of quality attributes throughout therapeutic antibody development. Eliminating the need for purification before charge variant analysis enables rapid sequential assays that provide results that are most representative of the mAb in a production environment.

REFERENCES

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