# Improved Method for Biosimilar Comparison of Aggregate Profile With Originator mAb

NOVILYTIC

## BACKGROUND AND INTRODUCTION

Monoclonal antibody (mAb) biosimilars are being more widely used to make therapeutic treatments more affordable and available. The testing process for biosimilars differs from the original biologic in that it must demonstrate clinical "sameness" or equivalence to the reference medicine rather than safety and effectiveness as is required in the qualification of the originator mAb[1,2]. The originator Rituximab, marketed as MabThera by Roche, is a chimeric anti-CD20 mAb with indications for the treatment of non-Hodgkins lymphoma and chronic lymphocytic leukemia[3]. In 2017 Truxima, the first biosimilar for rituximab, was approved by the European Union (EU). This application note presents a new method for analyzing biosimilars that significantly reduces sample preparation thereby reducing method variability.

## PRINCIPLE

During the development of biosimilars, it is essential to carefully characterize the mAb. This includes monitoring quality structural attributes and impurities, such as aggregates, deamidation, and oxidation[4]. The structural attribute profiles of biosimilars must highly correlate with the reference mAb to be approved for use as a therapeutic agent. A research-use-only (RUO) biosimilar is not required to meet specification criteria for *in-vitro* use, therefore a different structural attribute profile is common. Using an RUO biosimilar of rituximab, we demonstrate the application of the Proteometer-L Kit to qualify biosimilar mAbs. This method detects differences in aggregate content between the biosimilar and the originator mAbs directly in clarified fermentation broth (CFB), eliminating the need for Protein A purification and other sample preparation requirements.

## RESULTS

Rituximab biosimilar (research grade) and Rituximab reference product (MabThera, Roche) were formulated at a concentration of 1 mg/mL in CFB of untransformed CHO cells and injected onto an HPLC system equipped with a fluorescence detector for the determination of mAb titer. The Proteometer-L Kit was utilized for all data generation. Peak area responses from triplicate injections ($n$ = 3) were plotted against the amount (µg) of each Rituximab injected, and the response factors of each mAb were compared (Figure 1). Linearity from the mAb load from 0.5 to 16 µg for both sourced mAbs was excellent ($R^2$ = 0.999), however the response factor was greater for the Rituximab RUO than for the originator (Figure 1). The Proteometer-L assay relies on the ability of the molecular recognition agent to bind to a functional Fc region. Therefore, this difference is likely due to subtle structural differences between the originator and the biosimilar mAb, and it highlights an added value accrued by use of the Proteometer-L assay.

The Proteometer-L assay also detected differences in aggregate content between the originator and RUO biosimilar samples directly in the CFB matrix (Figure 2). The average aggregate to total area percent for the RUO biosimilar was 2.44% ± 0.081 ($n$ = 10), whereas the originator aggregate content was only a third of the RUO biosimilar content at 0.55% ± 0.054 ($n$ = 10).

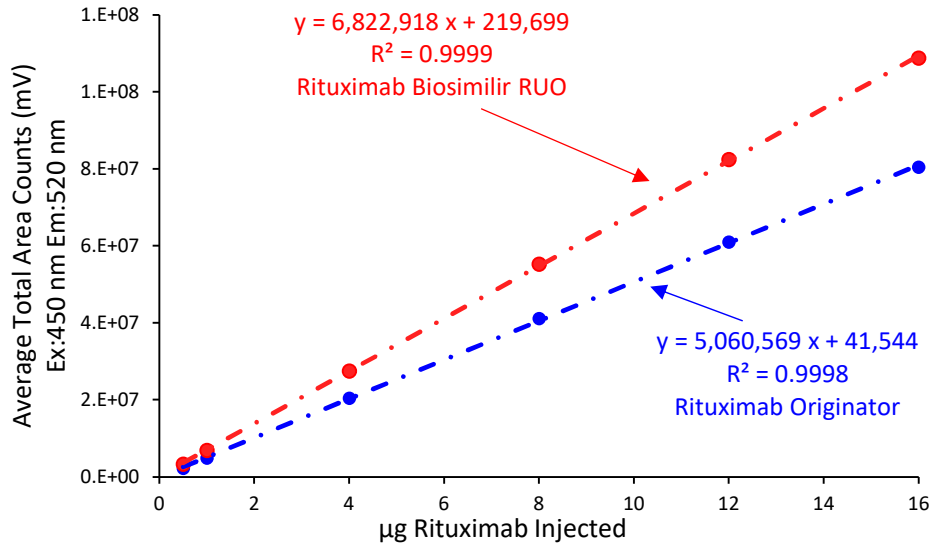


**Figure 1.** Linear dynamic range of rituximab RUO biosimilar vs. rituximab originator

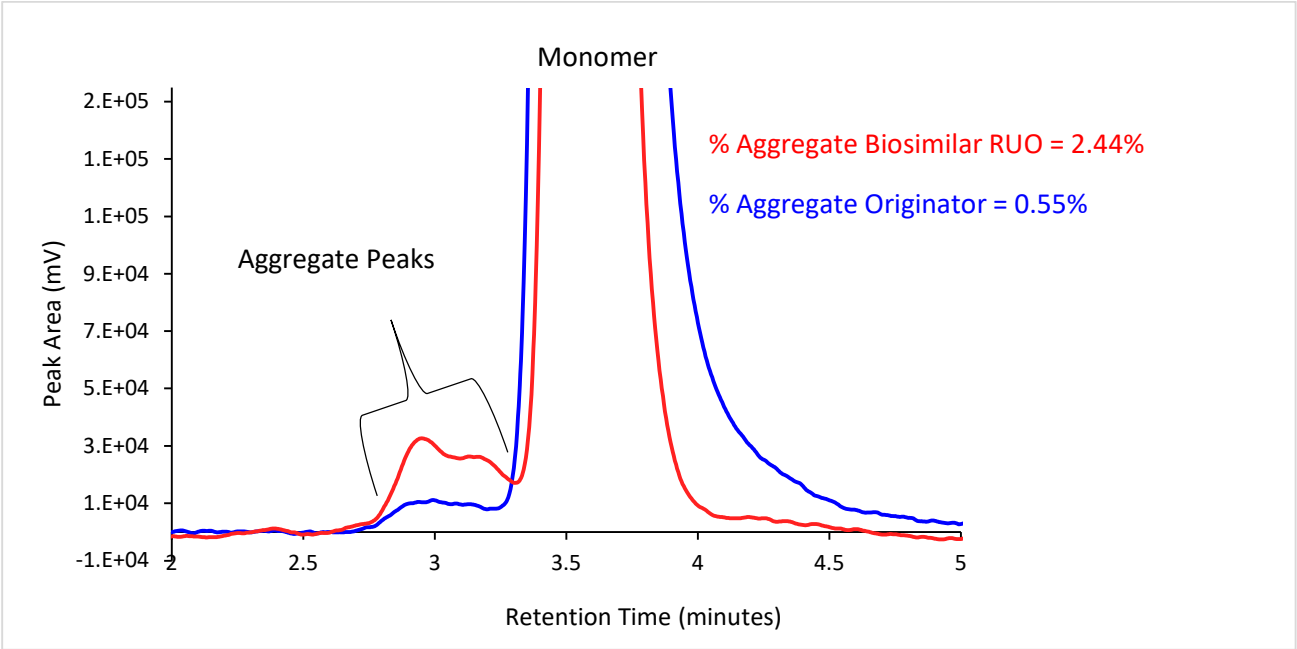


**Figure 2.** Rituximab RUO & originator comparison (8 ug injections), expanded view

## CONCLUSIONS

By eliminating interference from non-mAb components in the sample, the Proteometer-L assay can directly quantify titer and relative aggregate content in crude mAb samples. An additional value of the Proteometer-L assay is indicated by the data in Figure 2 which reveals a difference in the chromatographic profiles of the aggregate species contained in the originator and biosimilar mAb. Such differences in the chromatographic profile of mAb aggregates have been observed by size exclusion chromatography in pure mAb samples[5,6]. They are attributable to differences in aggregate structure and composition and have been correlated with differences in biological activity.

Given the tight timelines for biosimilar development, rapid detection of differences in titer and aggregate profiles early and often during product development is critical. Both time and labor savings ensue using the Proteometer-L assay due to circumvention of the Protein A purification step. These two critical quality attributes can be measured with Proteometer-L at all steps during product development, process R&D, and through production – from bioreactor samples and downstream processing steps, making it an effective quality assurance and quality control tool for biosimilar mAb producers.

## REFERENCES


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