Analysis of Rituximab, an IgG1 subclass mAb, with Proteometer-L assay

NOVILYTIC

BACKGROUND AND INTRODUCTION

The demand for biologics is increasing and it is projected that the manufacture of both new therapeutic monoclonal antibodies (mAbs) and biosimilars will continue to grow at a healthy rate to meet this demand. Biosimilars help to lower treatment costs. In a recent analysis by McKinsey & Company[1], they concluded that speed to launch and early entry are critical for the success of a biosimilar mAb. They also concluded that newer and faster analytical approaches in general, and methods that allow rapid comparisons for "similarity assessment" between the originator and biosimilar mAbs especially, could make the biosimilar market more attractive to new entrants and may even make mAbs more affordable.

PRINCIPLE

Monitoring changes in mAb concentration (titer) and aggregate content during the development and manufacture of a mAb is of critical importance as these can indicate changes in quality attributes of the molecule that can result in lower efficacy and/or higher toxicity of the drug. Rapid detection of these changes is possible with the Proteometer-L Kit without purification from interfering components such as host cell proteins present in fermentation broth. About three-quarters of all therapeutic mAbs in the market and, therefore, the majority of biosimilars that will be developed, will likely be of the IgG1 subclass[2]. We demonstrate here the utility of the Proteometer-L Kit in rapid analysis of titer and relative aggregate content of Rituximab, a mAb of the IgG1 subclass[3], in clarified fermentation broth. Rituximab was approved for use by the Food and Drug Administration in 1977 and is used to treat a variety of cancers and autoimmune diseases[4,5]. The Rituximab biosimilars market is expected to grow from $1.60B in 2020 to $3.47B in 2025 at a rate of 16.7% and continue to grow at a CAGR of 10.5% until 2030[6]. The Proteometer-L Kit will be an invaluable time and labor-saving tool in the development of Rituximab biosimilars as well as the majority of upcoming mAbs.

RESULTS

Rituximab biosimilar (ichorbio, UK) was formulated at a concentration of 1 mg/mL in clarified fermentation broth (CFB) and injected in varying amounts onto an HPLC system equipped with a fluorescence detector. The Proteometer-L Kit was utilized for the system setup. Peak area responses from triplicate injections, performed on three different days (n = 9) were plotted against the amount (µg) of Rituximab injected (Figure 1). Excellent linearity was observed from the range of 0.5 to 16 µg of mAb injected (R^2 = 0.9999). Despite the presence of host cell proteins in the CFB sample matrix, no interference from these components is observed and aggregate content is determined without any sample cleanup prior to analysis. Compilation of data from injections ≥ 4 µg level showed an average high molecular weight (HMW) to total area percent of 2.47 ± 0.06 for 36 injections and a percent RSD of 2.36%. Repeatability was evaluated by performing 10 consecutive injections of 4 µg Rituximab in CFB in three assay runs, on three different days. Total area, representing the titer for the mAb showed excellent repeatability with an average area of 27,140,670 ± 246,731 for 30 injections and a percent RSD of 0.91% (Figure 2). The HMW percent was also consistent with an average of 2.39% ± 0.12 for 30 injections, and a percent RSD of 5.09% (Figure 3).

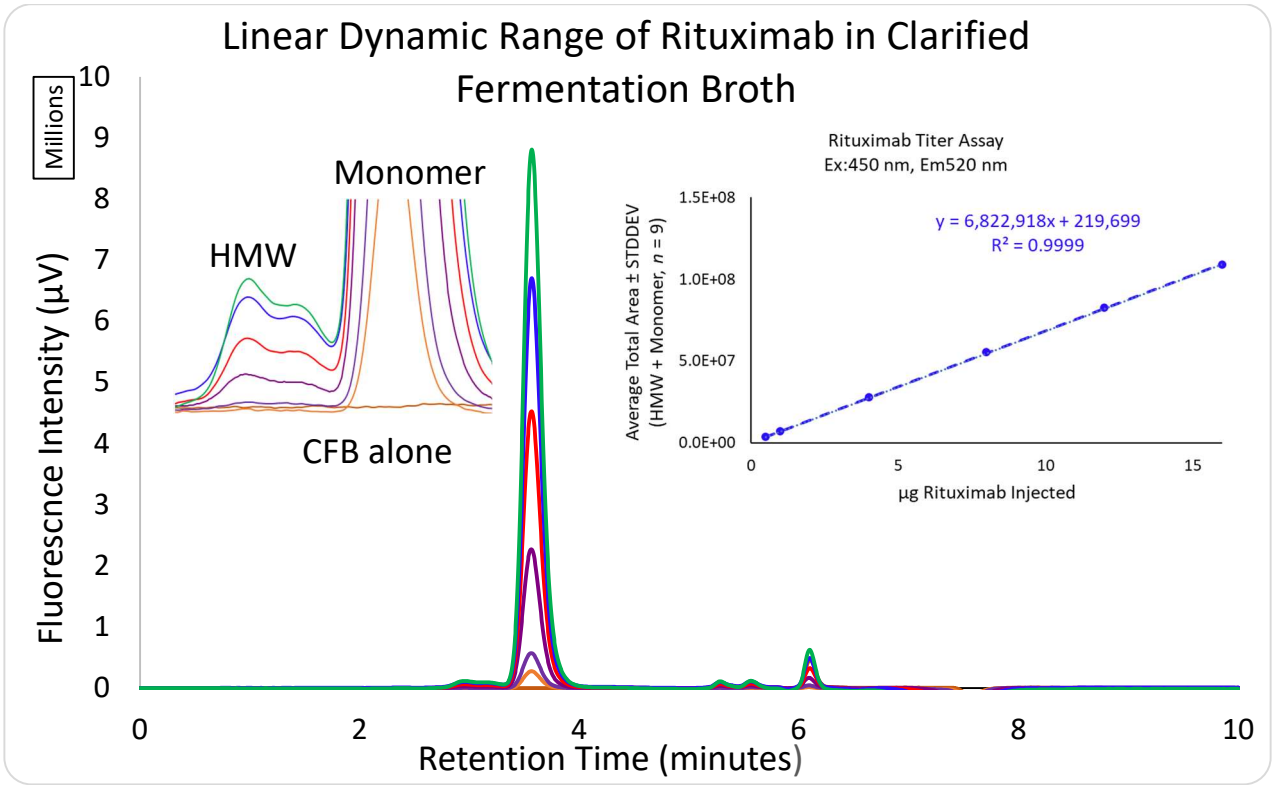

Figure 1. Linear dynamic range of Rituximab in CFB. Data is the average of three individual experiments with triplicate samples that were performed on different days using Proteometer-L assay.

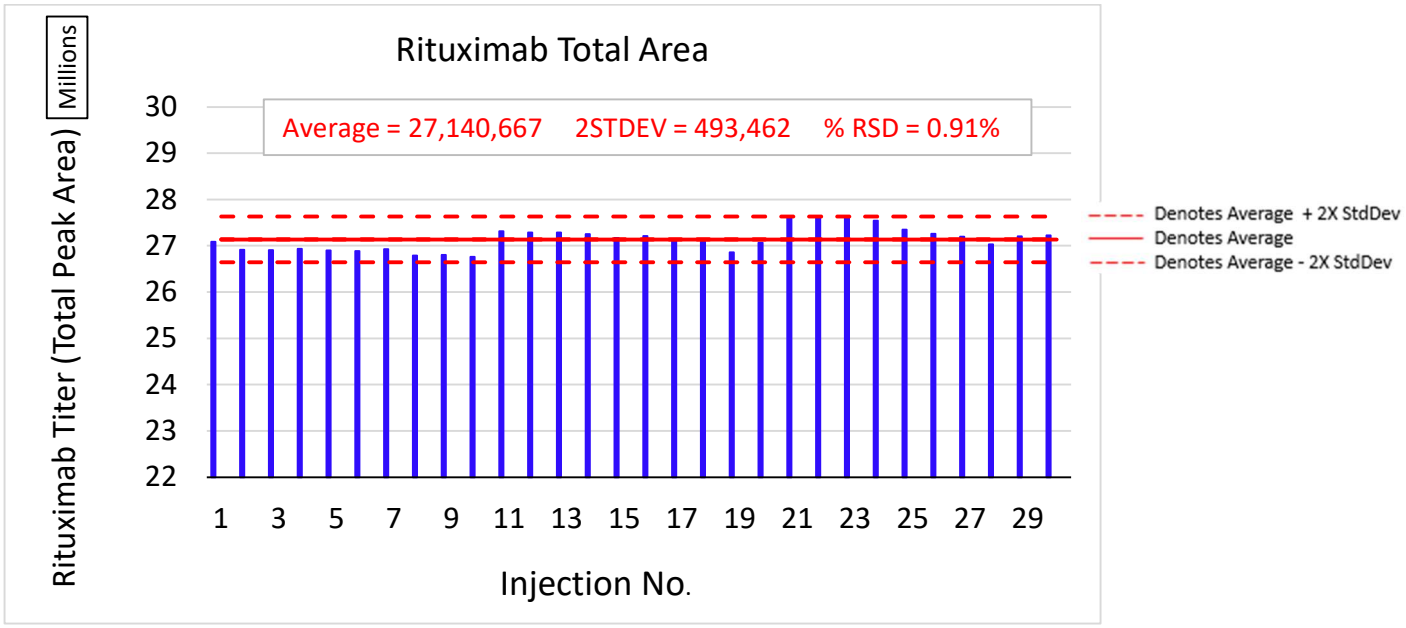

Figure 2. Repeatability of total peak area observed in Rituximab in CFB. Ten replicates of 4 µg injections were made on three different days for a total of 30 injections. Total area was calculated

using integrated peak areas of high molecular weight and monomer species. Rituximab titer (total peak area) is plotted against injection number.

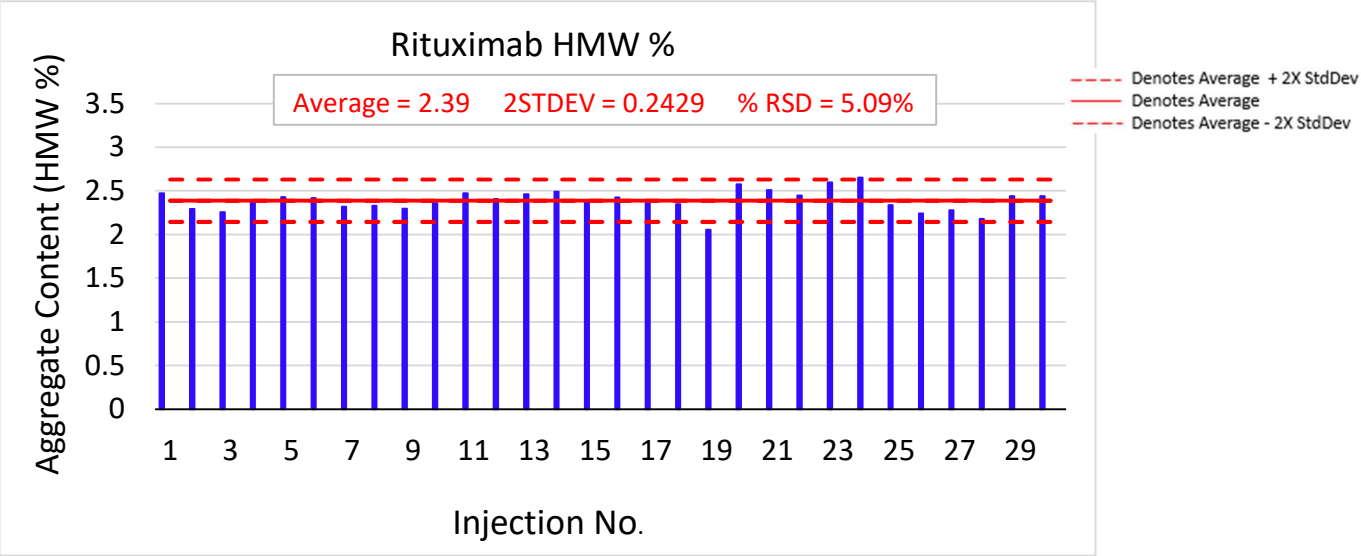

Figure 3. Repeatability of percent high molecular weight species of Rituximab in CFB. Ten replicates of 4 µg injections were made on three different days for a total of 30 injections. Aggregate content (HMW %) was calculated using integrated peak areas of high molecular weight and monomer species.

REFERENCES

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