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 biosimilar mAbs. They also concluded that lowering developmental costs and utilizing methods that allow rapid comparisons for "similarity assessment" could make the biosimilar market more attractive to new entrants. Furthermore, with the increased availability of generics in the market, the affordability of mAbs may increase.

Monitoring changes in mAb concentration (titer) and aggregate content during the development and manufacture of a mAb is of critical importance. Variations in aggregate and titer may be an indication of lower efficacy and/or higher toxicity of the drug. Rapid detection of these changes is possible with the Proteometer-L Kit. Samples may be analyzed without purification from interfering components such as host cell proteins present in fermentation broth.

PRINCIPLE

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 $2.28B in 2023 to $6.07B in 2031 at a CAGR of 12.98%[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 (research grade) 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 showed an average aggregate to total area percent of 2.47 ± 0.06 for 36 injections and a coefficient of variation (CV) 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 CV of 0.91% (Figure 2). The average aggregate content was 2.39% ± 0.12 for 30 injections, with a CV of 5.09% (Figure 3).

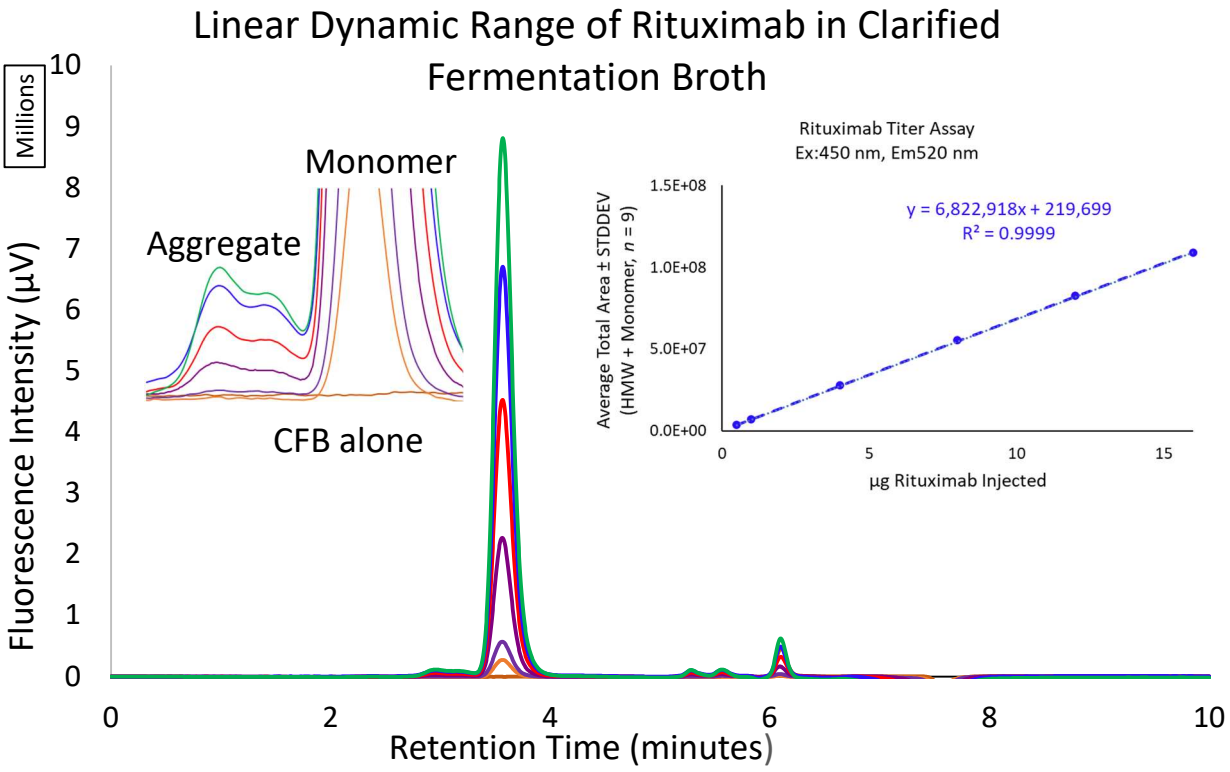

Figure 1. Linear dynamic range of Rituximab in CFB.

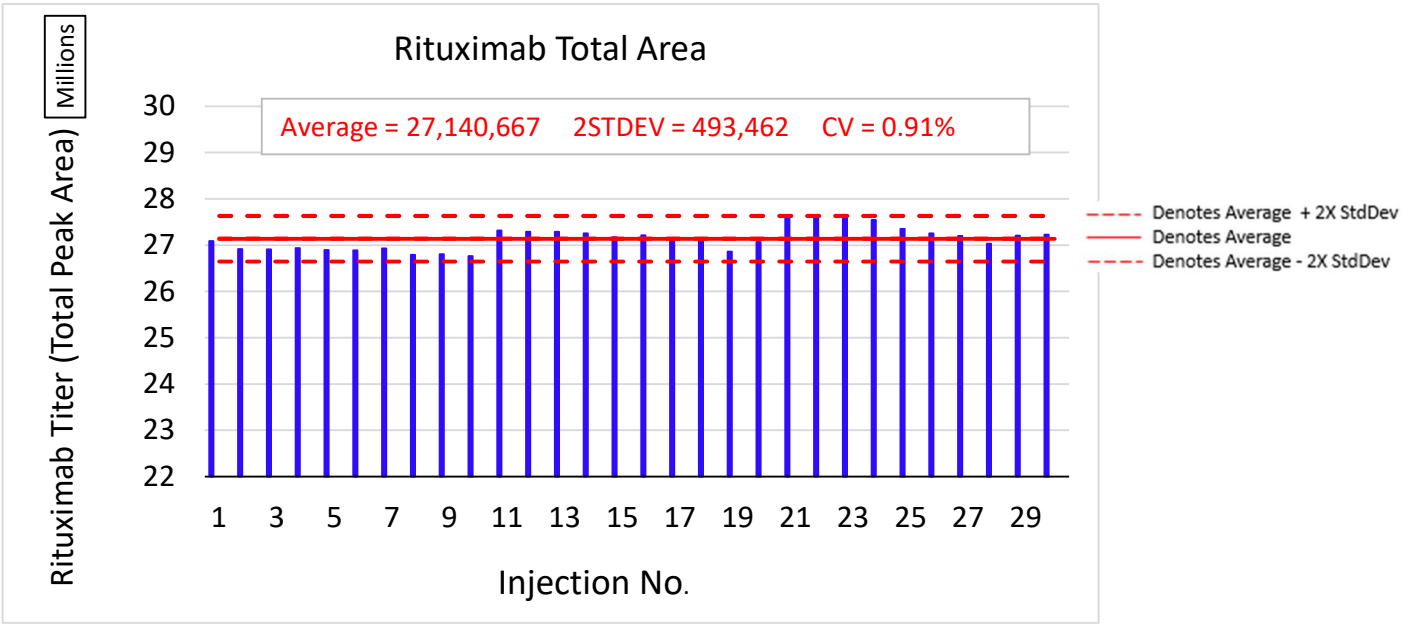

Figure 2. Repeatability of total peak area observed in Rituximab in CFB.

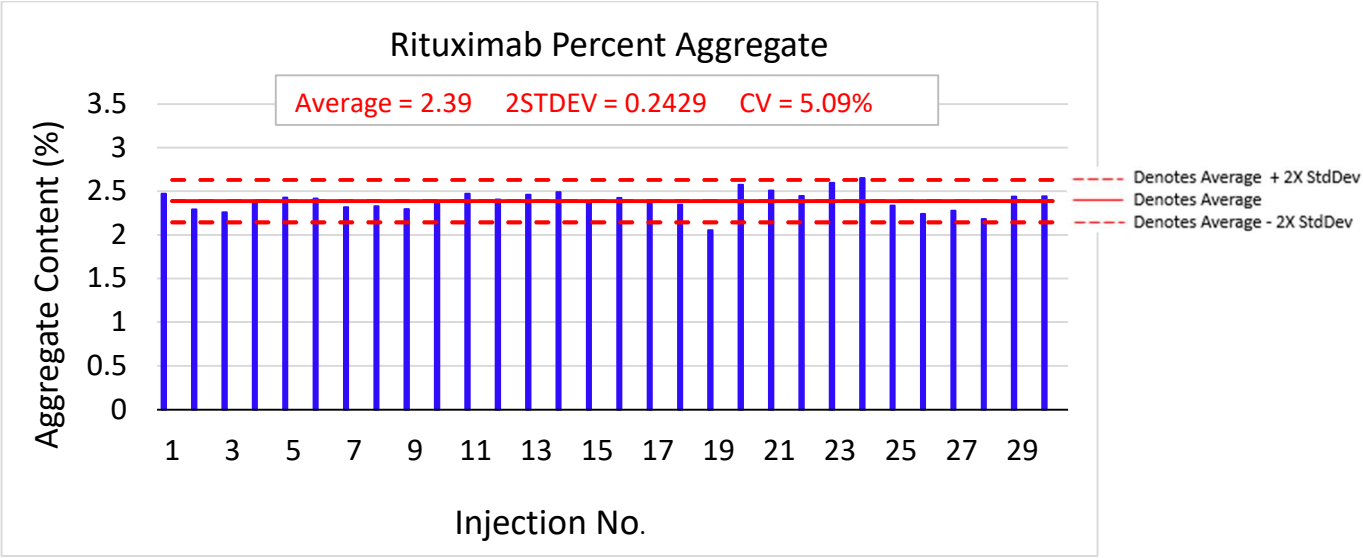

Figure 3. Repeatability of percent high molecular weight species of Rituximab in CFB.

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