

# Aggregate Analysis of a Bispecific Antibody

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## Overview

Size Exclusion Chromatography (SEC) is a technique focused on separating biomolecules based on their size in solution and is particularly useful for quantitating size variants and high molecular weight aggregates. This is a common analytical method for monoclonal antibodies (mAbs), as aggregate can be quantitated by percentage of peak area.

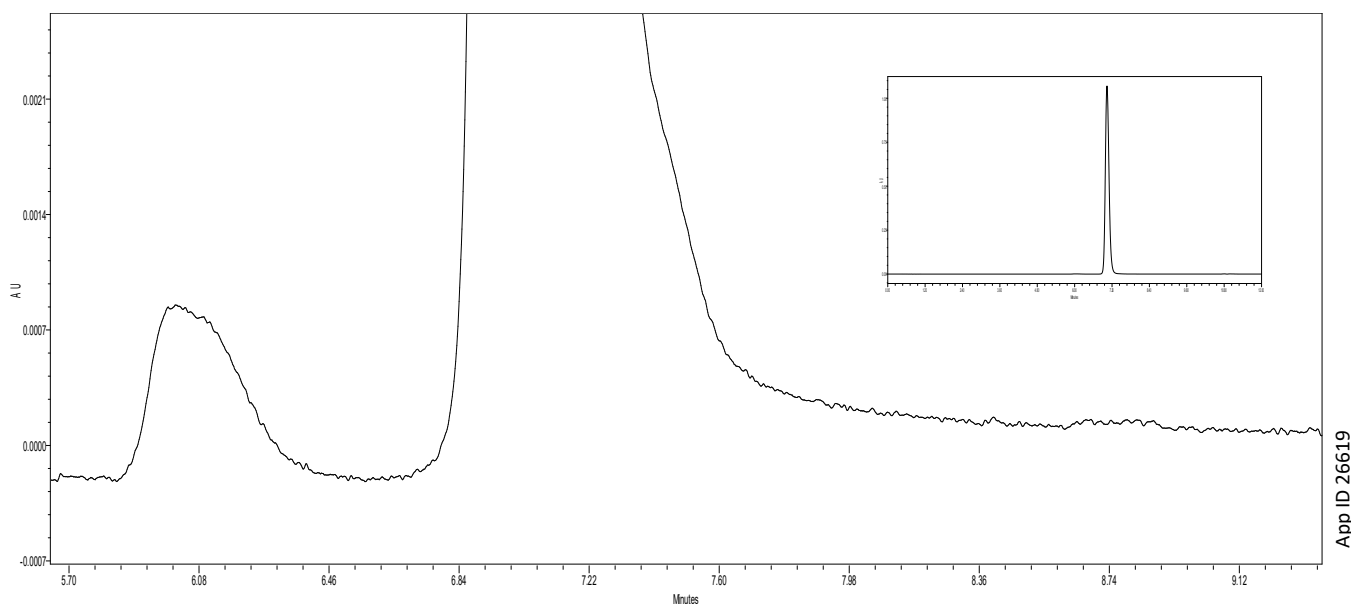
In this application note, we explore the use of standard phosphate containing buffers for aggregate analysis of a bispecific antibody, Emicizumab. Because aggregate is less than 0.2 % by peak areas, 100 µg of protein had to be loaded onto a 4.6 mm internal diameter (ID) SEC column to sufficiently quantitate. The use of a sub-2 µm column also was necessary as the additional improvements in efficiency help in detection.

## LC Conditions

**Column:** Biozen™ 1.8 µm dSEC-2, 200 Å  
**Dimensions:** 300 x 4.6 mm  
**Part No.:** [00H-4787-E0](#)  
**Mobile Phase:** 200 mM Potassium Phosphate + 250 mM Potassium Chloride, pH 6.2  
**Flow Rate:** 0.35 mL/min  
**Injection Volume:** 10 µL  
**Temperature:** 25°C  
**Detection:** UV @ 280 nm  
**Sample:** Emicizumab, 10 mg/mL

In summary, SEC is an effective method for quantitation of mAb aggregate. Peak areas below 0.2 % can be obtained with sufficient mass load. With aggregate this low, it is prudent to investigate other orthogonal methods in confirming no aggregate was lost due to adsorption.

**Figure 1. SEC chromatogram for Emicizumab. 100 µg load was necessary to quantitate aggregate by peak areas. Peak asymmetry of 1.2 indicates column is not overloaded. Aggregate is measured as 0.19 % by peak area.**



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