

Aggregate Analysis of an IgG2 Monoclonal Antibody

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Overview

Size Exclusion Chromatography (SEC) is a technique for the separation of 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 a standard phosphate containing buffer for aggregate analysis of an IgG2 mAb, Panitumumab, which has known recovery issues due to its hydrophobicity.¹ Using a moderately high ionic strength mobile phase, panitumumab exhibits good chromatography, as demonstrated by good USP tailing (0.99), and separation of dimer and pre-peak (putative full-length antibody + fragment). Percent dimer was determined to be 0.92 % by peak areas, with pre-peak determined 1.13 % by peak area.

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

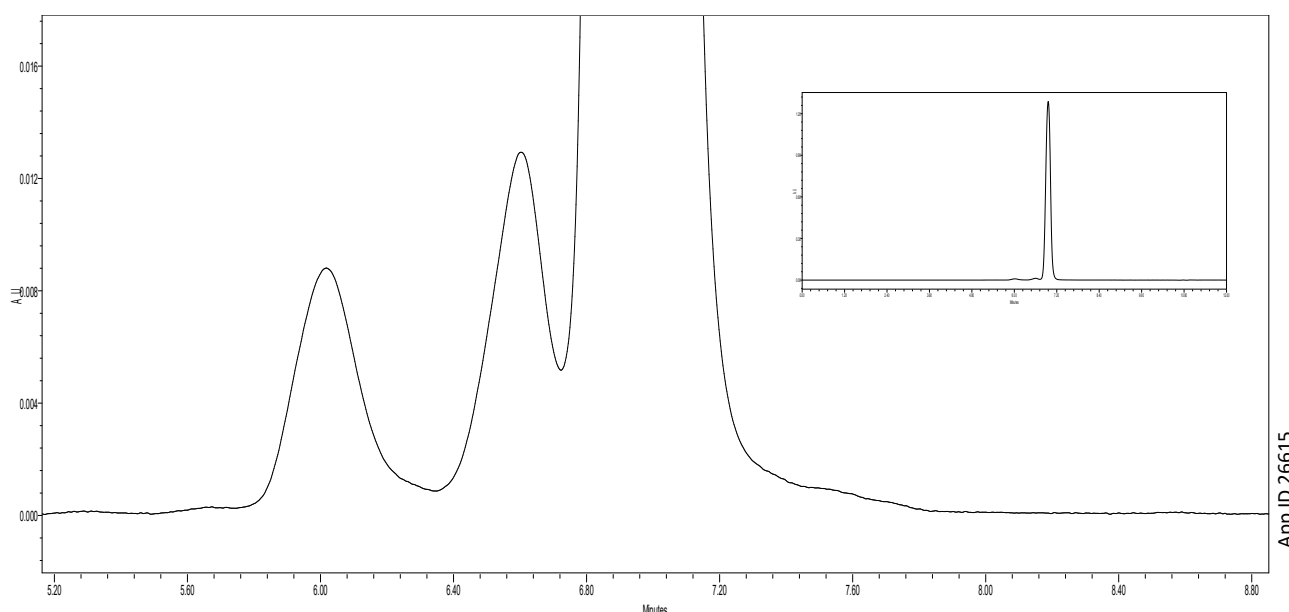
Detection: UV @ 280 nm

Sample: Panitumumab, 10 mg/mL

In summary, certain isotypes of antibodies (e.g. IgG2s) may present challenges when analyzing by a non-adsorptive separation modalities such as SEC. However, with optimal mobile phase conditions and high-resolution SEC columns, one can obtain good separation of dimer and other related high molecular weight impurities.

1. Fekete, Szabolcs et al. "Adsorption and recovery issues of recombinant monoclonal antibodies in reversed-phase liquid chromatography." *Journal of separation science* vol. 38,1 (2015): 1-8. doi:10.1002/jssc.201400996

Figure 1. SEC chromatogram for Panitumumab.



App ID 26615



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