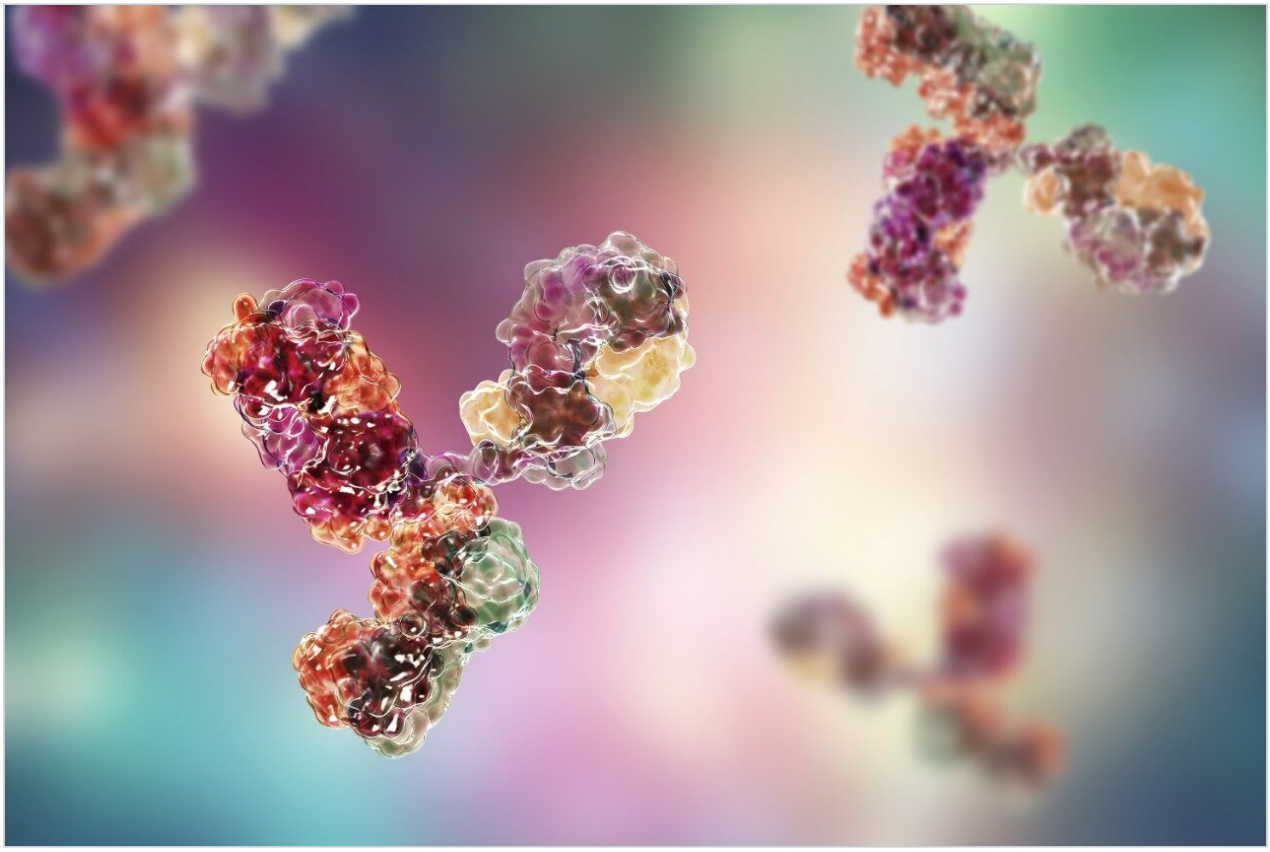
Waters™

Nota de aplicación

Analysis of Biomolecules by Size-Exclusion UltraPerformance Liquid Chromatography

Kenneth J. Fountain, Paula Hong, Susan Serpa, Edouard Bouvier, Damian Morrison

Waters Corporation



This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application note will demonstrate the use of the new ACQUITY UPLC SEC solution for the improved detection and/or faster analysis of protein aggregates in biopharmaceuticals.

Introduction

In the production of biopharmaceuticals, there may be different analytical requirements for groups performing clone section, formulations and stability, and quality control (QC). Depending on the goal of the separation, methods may be optimized for fast analysis time, highest possible resolution, and/or reproducibility. Size exclusion (SEC) chromatography is often used throughout the biopharmaceutical production process for the analysis of proteins and their aggregates. While SEC has traditionally been used in conjunction with low pressure HPLC instrumentation, the advent of UPLC Technology and new sub-2 µm packing materials allows for substantial improvements in chromatographic resolution and throughput. This application note will demonstrate the use of the new ACQUITY UPLC SEC solution for the improved detection and/or faster analysis of protein aggregates in biopharmaceuticals.

Experimental

Chromatographic Conditions

UPLC System:	ACQUITY UPLC System with TUV (with stainless steel flow cell)
HPLC System:	Waters 2796 Separations Module with 2487 dual λ detector
UPLC Column:	ACQUITY UPLC BEH200 SEC, 4.6 x 150 mm, 1.7 µm

HPLC Column:	Traditional silica diol-coated SEC, 7.8 x 300 mm, 5 µm
Mobile Phase A:	25 mM sodium phosphate, pH 6.8, 0.15 M NaCl
Flow rate:	0.4 mL/min
Temperature:	30 ˚C
Detection:	UV 280 nm (sampling rate 10 Hz, 0.2 s filter time constant)
Sample Diluent:	25 mM sodium phosphate, pH 6.8, 0.15 M NaCl

Other conditions are specified in the figure captions.

Results and Discussion

Figure 1 shows the chromatographic comparison of traditional HPLC and ACQUITY UPLC for the separation of a humanized monoclonal antibody. Equivalent aggregate quantification in significantly shorter run times is possible with the ACQUITY UPLC SEC solution as compared to traditional SEC. T his is especially important for those scientists performing clone selection who may need increased throughput for large numbers of samples.

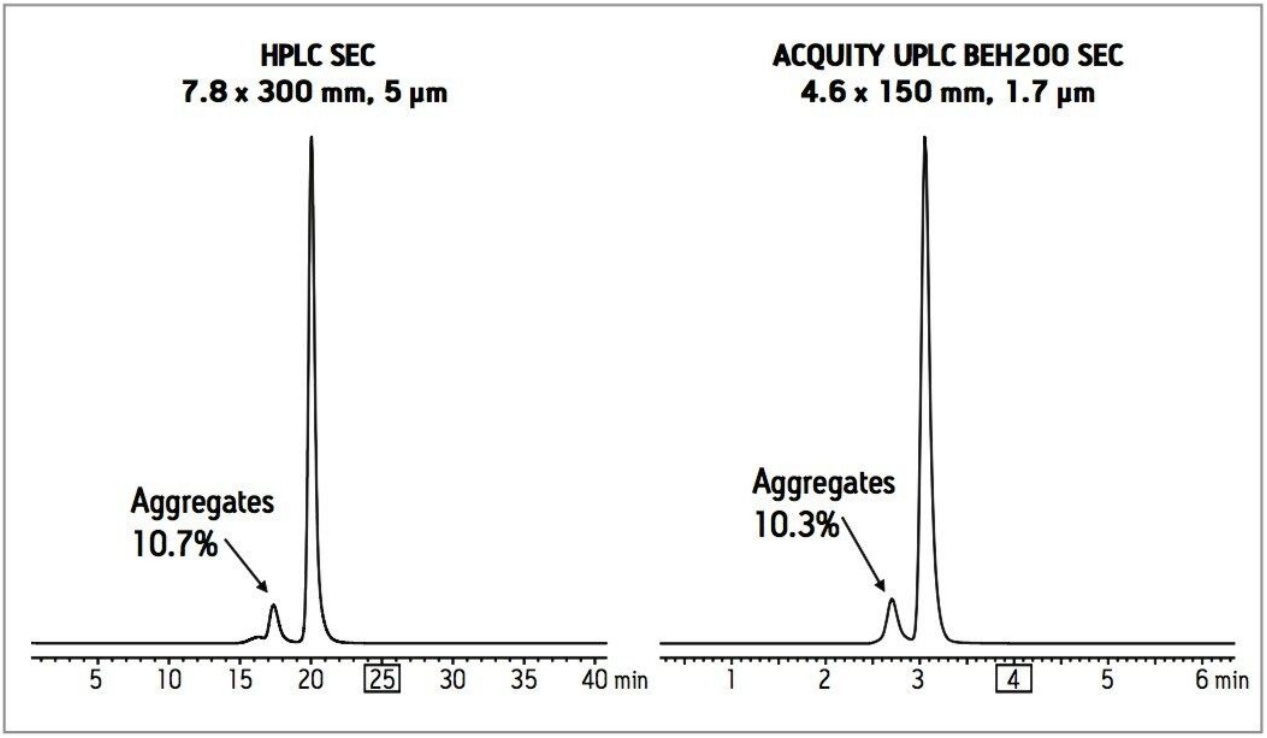


Figure 1: Comparison of traditional HPLC and ACQUITY UPLC SEC for the separation of a humanized monoclonal antibody (IgG). Injection volumes: 20 µL for HPLC and 5 µL for ACQUITY UPLC.

In regulated environments, the use of SEC is often required for the characterization of biopharmaceutical therapeutics. Given these demands, columns are expected to be reproducible from batch-tobatch and have long lifetimes. Figure 2 shows the batch-to-batch performance for three different batches of 1.7 µm, BEH200 SEC packing material. These data show the consistent performance of the BEH200 SEC columns regardless of the batch of material being used, which provides confidence for those performing aggregate determination in biopharmaceutical drugs. Figure 3 demonstrates the lifetime of the ACQUITY UPLC BEH200 SEC columns with the same humanized IgG sample shown in Figure 1. No deterioration in peak shape or retention was observed, which ensures accurate identification and quantitation of the antibody monomer and dimer over several hundred injections.

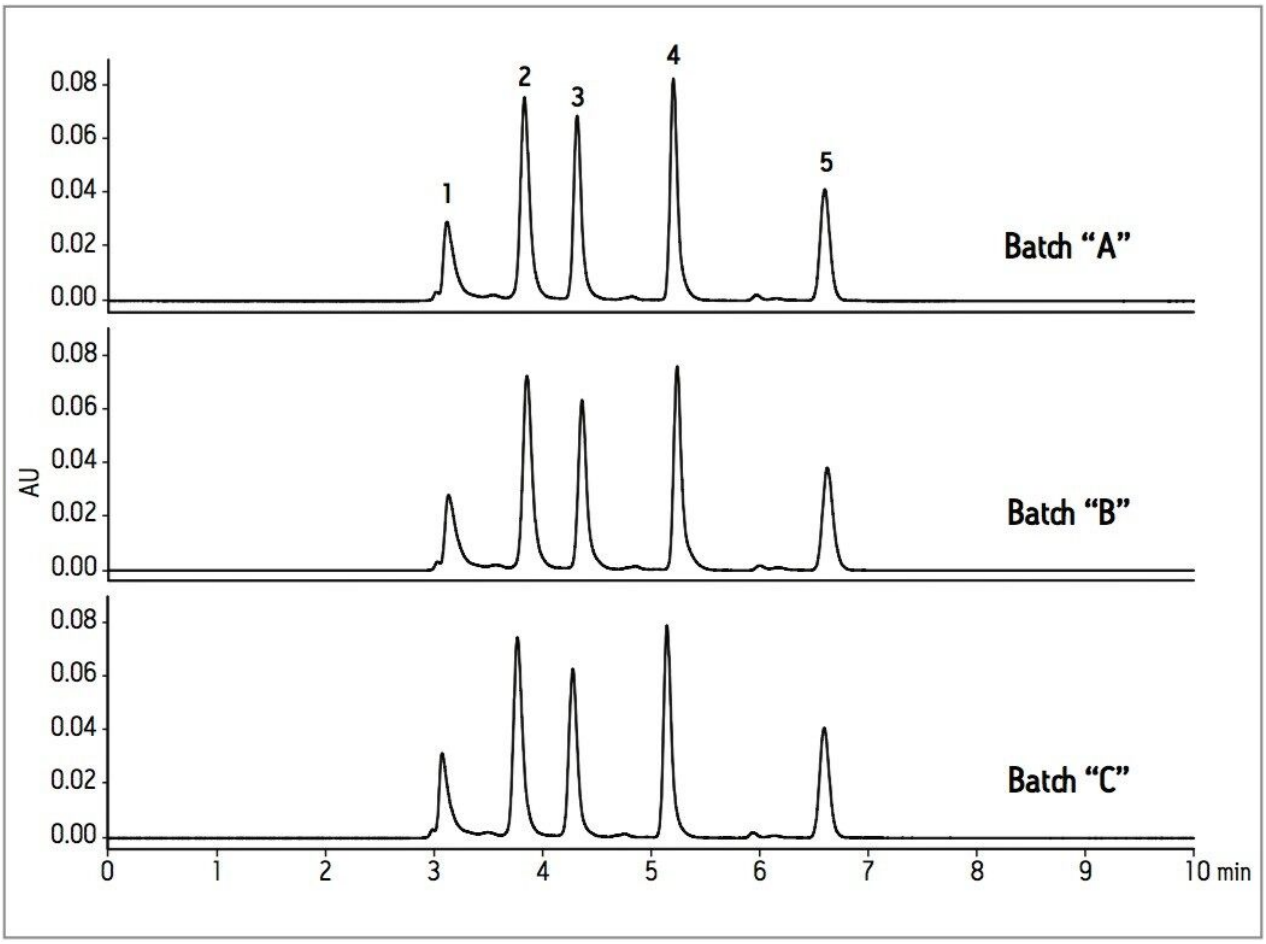


Figure 2: Batch-to-batch reproducibility for a mixture of protein standards. Buffer: 100 mM sodium phosphate, pH 6.8. Flow rate is 0.3 mL/min. Peaks: (1) thyroglobulin, (2) IgG, (3) bovine serum albumin, (4) myoglobin, (5) uracil.

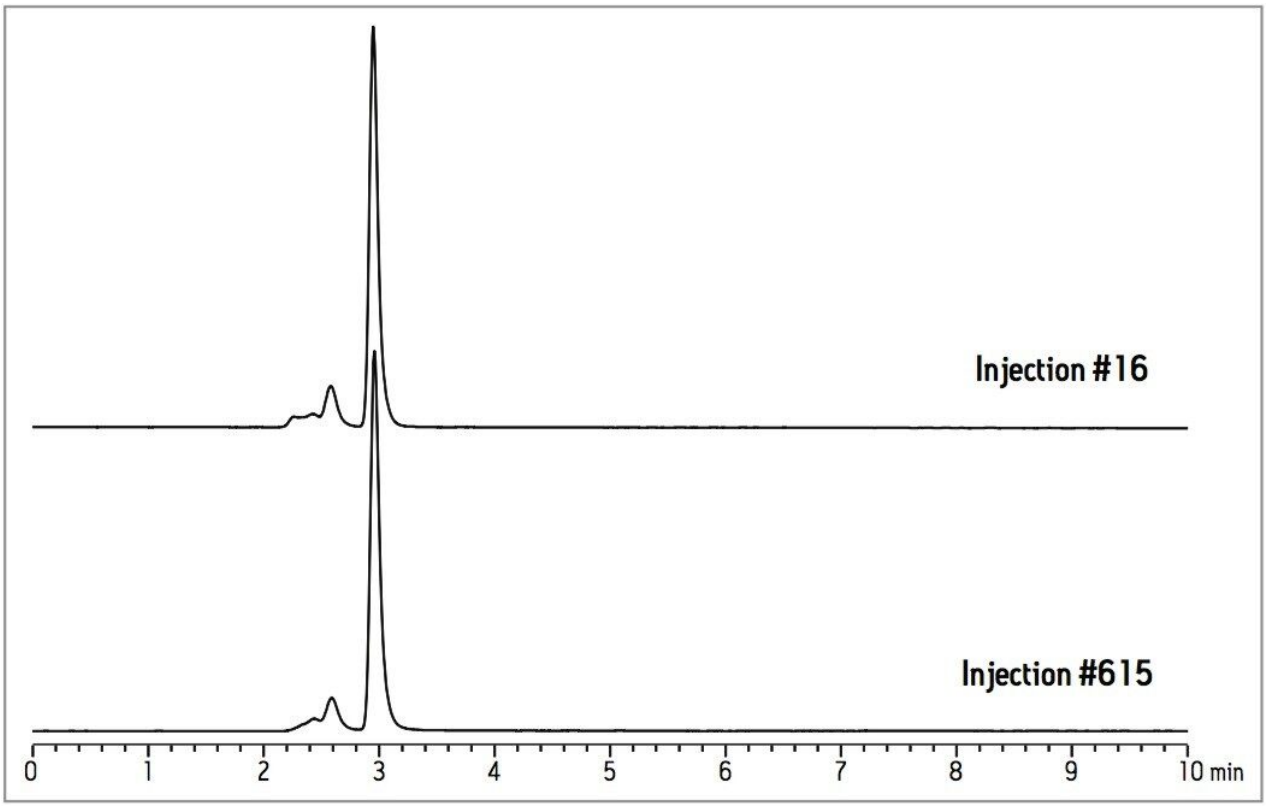


Figure 3: Lifetime study for a humanized IgG sample on the ACQUITY UPLC BEH200 SEC column.

Conclusion

The Waters ACQUITY UPLC SEC solution provides the speed, resolution, and sensitivity of UPLC for proteins (antibodies) in a molecular weight range of 10,000 to 450,000 Daltons. It can be adapted to varying requirements of run time and resolution that are often needed in clone selection and/or quality control testing. This work extends UPLC technology to a wider range of bioseparations.

Featured Products

ACQUITY UPLC System <https://www.waters.com/514207>

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

WA64266, April 2010