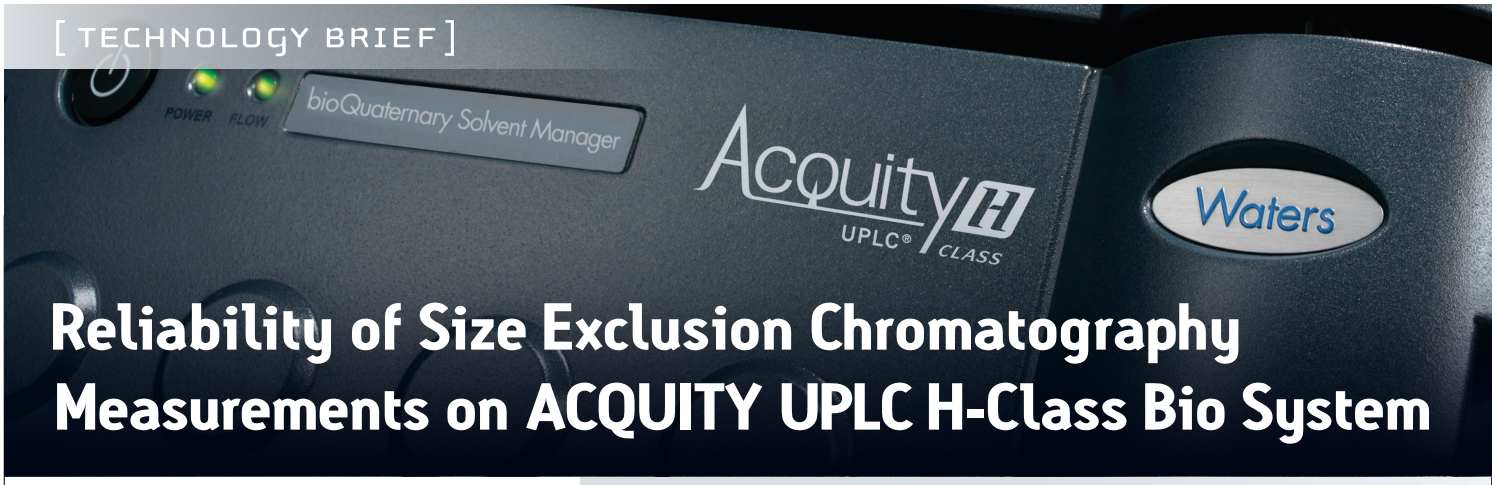
[TECHNOLOGY BRIEF]



Reliability of Size Exclusion Chromatography Measurements on ACQUITY UPLC H-Class Bio System

GOAL

To demonstrate reliability of the quaternary-based ACQUITY UPLC® H-Class Bio System and the ACQUITY UPLC BEH200 SEC Column for the analysis of proteins by size exclusion chromatography (SEC).

BACKGROUND

The complete characterization and analysis of biopharmaceuticals includes the application of size exclusion chromatography (SEC) to measure protein aggregates and other size variants. Soluble protein aggregates, in particular, can contribute to immunogenicity; accurate analysis and quantitation of biotherapeutic protein aggregates is, therefore, often required.

Current HPLC/silica-based SEC methods can be time-consuming and unreliable. These uncertain results may be due to changes in retention time, peak shape, or spacing between peaks as well as irreproducibility between columns and changes in columns within a few runs.

With the introduction of the ACQUITY UPLC H-Class Bio System and sub-2-µm ACQUITY UPLC BEH200 SEC Column chemistry, SEC separations can be obtained reproducibly, reliably, and in shorter analysis time with minimal development. Methods can be easily developed with the system's quaternary solvent manager utilizing Auto•Blend Plus™ Technology. This new implementation of instrument control functions removes the need for buffer pH adjustment and reduces time spent in buffer preparation.

The ACQUITY UPLC H-Class Bio System along with an ACQUITY UPLC BEH200 SEC Column deliver reliable and reproducible SEC separations for biomolecules.

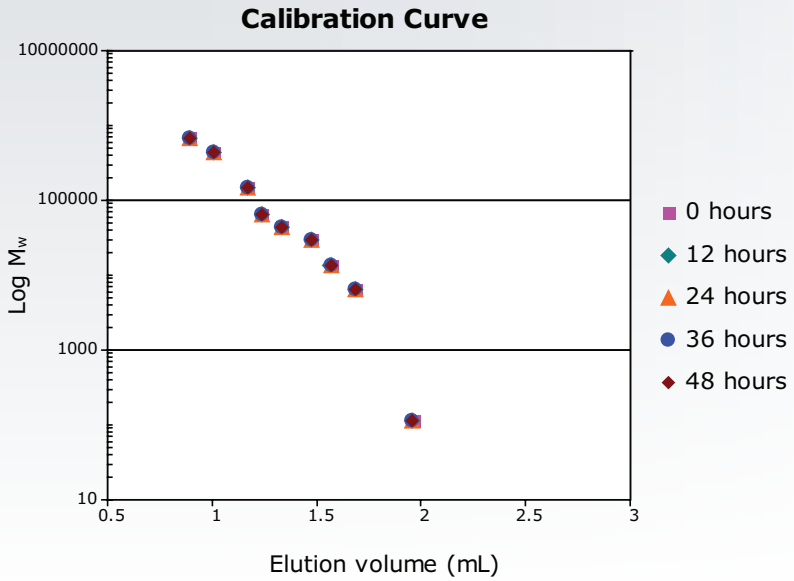


Figure 1. Protein calibration curve, ACQUITY UPLC BEH200 SEC 1.7-µm, 4.6 x 150 mm Column. Recommended molecular weight range is 10,000 to 450,000. Overlay of five calibration curves over 48 hours. Buffer: 20 mM Sodium Phosphate, 0.15 M NaCl, pH 6.8. Flow rate: 0.4 mL/min.

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The superior performance of this UPLC® SEC method relies on both the inert, low-dispersion system and the chemically-stable BEH column. The combination of these components allows users to obtain more accurate and reproducible results over a larger number of samples than is observed with current SEC methodologies.

THE SOLUTION

The SEC separation of biomolecules combines the ACQUITY UPLC H-Class Bio System with a 1.7-µm ACQUITY UPLC BEH SEC Column that provides the biochemist with a reliable separation. The low-dispersion, high-pressure system contains an inert flow path, that, when combined with four-solvent mixing and Auto•Blend Plus Technology, facilitates easy buffer preparation without pH adjustment.

The ACQUITY UPLC BEH200 SEC particle has an effective diol coating that provides a stable particle with minimal secondary interactions. The packing material is more resistant to chemical and mechanical degradation over time. These attributes combine to provide an SEC column stable more than 600 injections and requiring lower buffer concentrations than traditional silica-based columns.

In a series of experiments, protein standards and monoclonal antibody biotherapeutics were analyzed with UPLC-based SEC. Repeated analysis of the same sample was performed at regular intervals over a two-day period. Reproducibility of the calibration was tested by analysis of proteins standards over the molecular weight range of 10,000 to 450,000 Da.

The elution volume for each protein standard was found to be within 0.2% RSD. The calibration curve points do not fall on a perfect straight line because the elution volume reflects both size and shape of protein standard. The consistency of the calibration curve is, however, indicative of both the column life and instrument control of flow rate and injection volume.

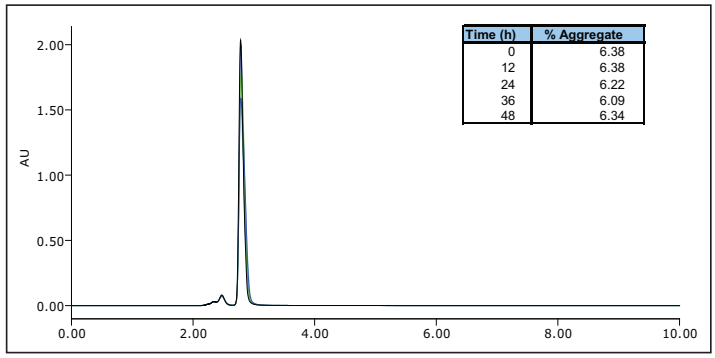

Figure 2. SEC separation of humanized IgG, 20 mg/mL. Injection of undiluted humanized IgG over 48 hours showed aggregate quantitation relative to the monomer of 6.09% to 6.38% with a RSD of 0.2%. Buffer: 20 mM Sodium Phosphate, 0.15 M NaCl, pH 6.8. Flow rate: 0.4 mL/min.

To test the reliability of quantitation, a humanized monoclonal antibody was analyzed. The sample shown was found to have an average aggregate quantitation of 6.82% +/- 0.3% of the monomeric species over the time period. The reliability of this analysis is demonstrated by the reproducibility of this measurement. The SEC separations demonstrate the accuracy and reproducibility of UPLC SEC technology, which, in turn, ensures accurate identification and aggregate determination.

SUMMARY

The ACQUITY UPLC H-Class Bio System with an ACQUITY UPLC BEH200 SEC Column combine to provide reliable separations of proteins and their aggregates. As previously described, the analysis of both protein standards and monoclonal antibodies demonstrates the reliability of the calibration over a period of days. This reproducibility ensures accurate identification and quantitation of proteins and their aggregates, which can minimize analysis delays due to irreproducible results or incorrect peak identification. This, in turn, can increase throughput, thereby saving time and money. With the introduction of the ACQUITY UPLC H-Class Bio System and the new ACQUITY UPLC BEH200 SEC Column, reliable and reproducible SEC separations can be obtained for biomolecules.

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