ADVANCES IN ULTRAPERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY FOR THE ANALYSIS OF BIOMOLECULES

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Paula Hong, Kenneth J Fountain, Damian Morrison
Waters Corporation, 34 Maple Street, Milford, MA 01757

INTRODUCTION

Complete characterization and analysis of biopharmaceuticals includes size exclusion chromatography (SEC) to measure protein aggregates and other size variants. Current silica-based HPLC-SEC methods can be time-consuming and unreliable. We will demonstrate how UltraPerformance Liquid Chromatography (UPLC) can now be used in conjunction with sub-2 µm size-exclusion packing materials for improved chromatographic separations of biological macromolecules.

While size-exclusion chromatography has traditionally been performed on low pressure instrumentation, the introduction of low dispersion, high pressure instruments and sub-2 µm packing materials allows for improvements in size-based separations. In the following poster, the benefits in throughput, and robustness for UPLC-SEC separations will be shown for biomolecules, including monoclonal antibodies. These studies will demonstrate the improvements in impurity detection and/or faster analysis of biomolecules achievable with UPLC-SEC separations.

METHODS

UPLC SEC Chromatographic Conditions

LC System: ACQUITY UPLC® H-Class Bio System with PDA detector
Detection: PDA @ 280 nm
Column: ACQUITY BEH200 SEC,1.7 µm, 4.6 x 300 mm
Injection Volume: 5.0 µL
Flow Rate: 0.4 mL/min
Mobile Phase: 25 mM Sodium Phosphate, pH 6.8, 0.15 M NaCl
Wash and Purge Needle Washes: Mobile Phase
Seal Wash: 80/20 H_2O/MeOH
Temperature: 30°C
Sample Diluent: 25 mM Sodium Phosphate, pH 6.8, 0.15M NaCl

RESULTS AND DISSCUSION

METHOD DEVELOPMENT

Size exclusion chromatography is an isocratic technique. Resolution can be affected by a number of factors including, system dispersion. To further optimize resolution, particularly of monomer and dimers, a number of factors can also be adjusted. These parameters include flow rate and column length. Typical ideal flow rates are lower than other modes of chromatography, as resolution decreases with increasing flow. Resolution also increases with column length, as is typical of other modes of chromatography.

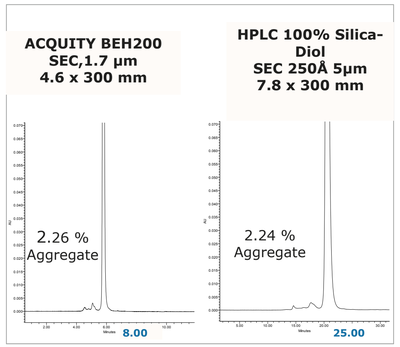

Figure 1. Comparison of traditional HPLC and UPLC for the separation of a murine monoclonal antibody. Equivalent aggregate quantification in significantly shorter run times are possible with the ACQUITY UPLC SEC System Solution as compared with traditional SEC. Injection volumes: 10 µL for HPLC; 5 µL for ACQUITY UPLC.

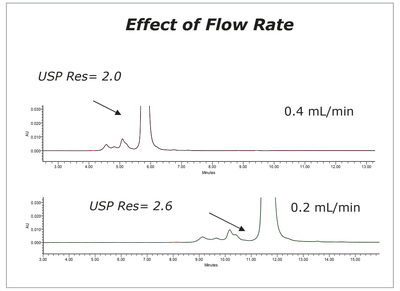

Figure 2. Effect of flow rate on monomer/dimer resolution. A murine monoclonal antibody was analyzed on an ACQUITY BEH200 SEC 4.6 x 300mm column at varying flow rates. For each flow rate triplicate injections are overlaid. USP Resolution increases with decreasing flow rate.

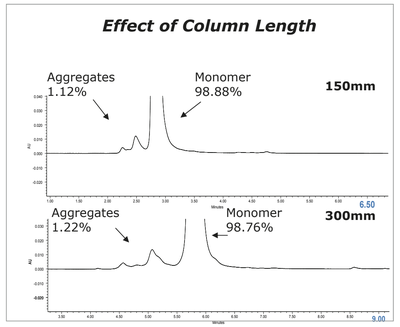

Figure 3. Effect of column length on resolution. A murine monoclonal antibody was analyzed on an ACQUITY BEH200 SEC 150 mm and a 300mm column. USP resolution increases with increasing column length. Aggregate quantitation for the same sample on both columns is comparable.

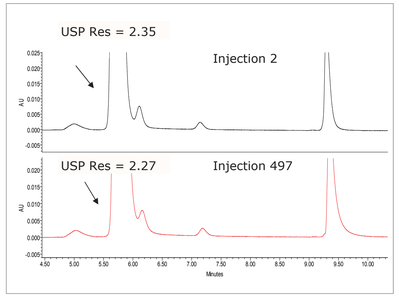

Figure 4. Overlay of injection 2 and 497 of humanized monoclonal antibody on an ACQUITY BEH200 SEC column. Minimal decrease in monomer/dimer USP resolution over more than 400 injections.

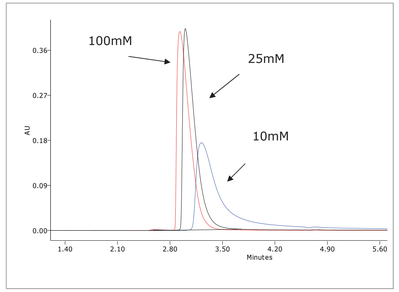

Figure 5. Effect of buffer concentration on chimeric IgG peak shape. At low buffer concentration, secondary interactions affect peak shape, i.e. tailing. Higher buffer concentration improves peak shape and improves resolution of IgG and aggregates. Buffer: XXM Sodium Phosphate, pH 6. 8.

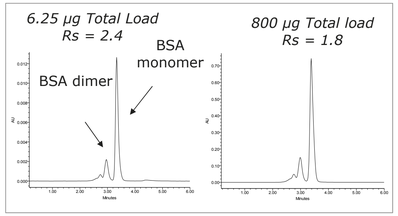

Figure 6. Bovine serum albumin loading on ACQUITY UPLC BEH200 SEC, 1.7 µm, 4.6 x 150 mm column. Quantification of dimer and higher order aggregates possible at loads of up to 800 µg. No significant deterioration of resolution.

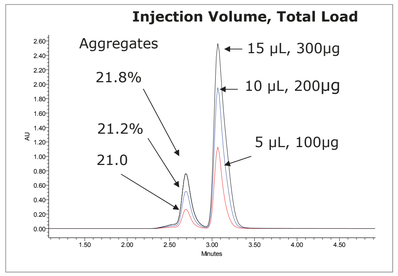

Figure 7. Injection of an undiluted humanized monoclonal antibody biopharmaceutical at 20mg/mL. Quantification of aggregates for injection volumes of 5 to 15 µL give similar results.

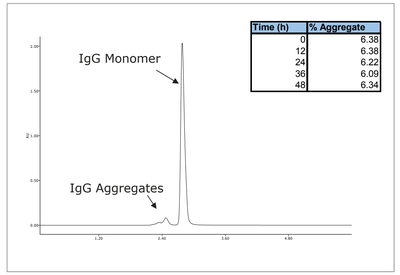

Figure 8. SEC separation of humanized IgG. Injection of undiluted humanized IgG over 48 hours showed aggregate quantitation relative to the monomer of 6.09-6.38% with a RSD of 0.3%.

CONCLUSION

- **UPLC SEC can provide improved resolution and comparable aggregate quantitation with higher throughput as compared to traditional HPLC SEC**
- **Method development requirements can be met by varying parameters such as flow rate and column length**
  - **Resolution in SEC can be increased by decreasing the flow rate or increasing the column length**
  - **15 cm length columns can be used for high throughput applications such as clone selection**
  - **30 cm length columns can be used for high resolution applications such as QC and release testing**

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