Modernization of Compendial Size-Exclusion Chromatography Aggregation Analysis Methods in Compliance with Updates to USP General Chapter <129>

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PURPOSE

Aggregation in monoclonal antibody (mAb) drugs is considered a critical quality attribute that must be monitored throughout the drug product lifecycle. Aggregate formation can occur during the fermentation, purification, and storage steps of the manufacturing process and may lead to immunogenic reactions or loss of drug efficacy. Size-Exclusion Chromatography (SEC) is the gold standard for monitoring mAb aggregation. The United States Pharmacopeia (USP) provides instruction on the size-exclusion analysis of aggregates in mAbs through USP General Chapter <129>. Traditionally this chapter has only been provided for High-Performance Liquid Chromatography (HPLC) separation methods using 7.8 mm Internal Diameter (ID) columns. On May 1st, 2025 the USP introduced updates to General Chapter <129> detailing the use of Ultra-High Performance Liquid Chromatography (UHPLC) systems and 4.6 mm ID columns for mAb aggregate analysis. In this work an HPLC SEC method is modernized to UHPLC following the requirements in USP General Chapter <129>.

METHODS

The originator system used was a bioinert quaternary HPLC system. Receiver systems included the same bioinert HPLC and a bioinert binary UHPLC system. Each isocratic separation used a buffered mobile phase of 60.3 mM K_2HPO_4, 140.3 mM KH_2PO_4, and 249.5 mM KCl at pH 6.2. HPLC analysis used a 7.8 mm X 300 mm, 250Å, 2.5 µm column with a 0.5 mL/min flow rate. UHPLC analysis used a 4.6 mm X 300 mm, 250Å, 2.5 µm column with a 0.3 mL/min flow rate. Columns were kept at room temperature during analysis. A protein standard mix was reconstituted to 500 uL in mobile phase and injected at 10 µL and 5 µL for HPLC and UHPLC respectively. Infliximab was diluted to 10 mg/mL with water, injected at 20 µL and 10 µL for HPLC and UHPLC respectively, and analyzed past expiry. Samples were kept at 6˚C in an autosampler. UV detection was performed at 10 Hz at 280 nm.

RESULTS & DISCUSSION

System dispersion (4σ) was measured as 21 µL for the HPLC system and 8 µL for the UHPLC system. Looking at the standard, chromatographic resolution declines when moving to the 4.6 mm ID column, however, retention time repeatability and peak tailing remain comparable. In terms of aggregate determination of the mAb sample, modernization of the method to the 4.6 mm ID column achieved equivalent results to that produced on the 7.8 mm ID column. This was true when using the 4.6 mm ID column on both the HPLC and UHPLC systems.

But why modernize your method in the first place? Modernization of methods offers several benefits including reduced run times which require less solvent and lower sample consumption. Looking at the total amount of mobile phase (MP) consumed over the course of a single injection, the 7.8 mm ID column consumes 15 mL MP/injection while the 4.6 mm ID column consumes just 6 mL, a reduction in MP consumption of 60%. Additionally, analysis time is reduced by 33% and the amount of sample consumed is reduced by 50%.

CONCLUSIONS

- Successful modernization of a compendial SEC method from a 7.8 mm ID column to a 4.6 mm ID column using both an HPLC and a UHPLC platform
- 60% reduction in mobile phase consumption
- 33% reduction in analysis time
- 50% reduction in sample consumption

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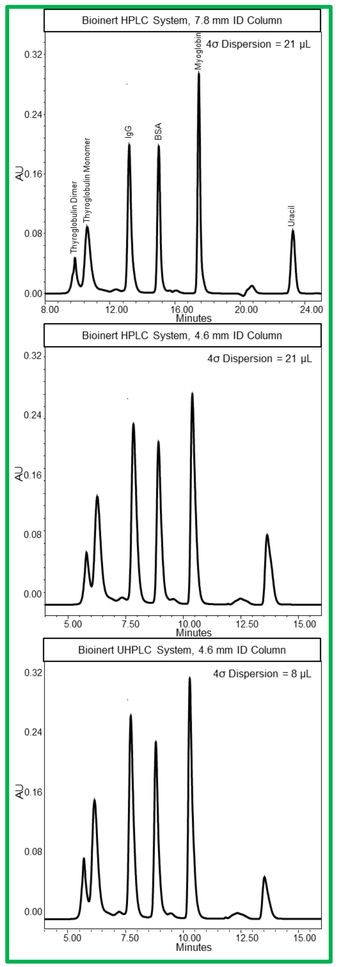
Modernization of compendial methods to smaller ID columns moves labs towards a greener more sustainable future.

BEH200 SEC PROTEIN MIX CHROMATOGRAMS

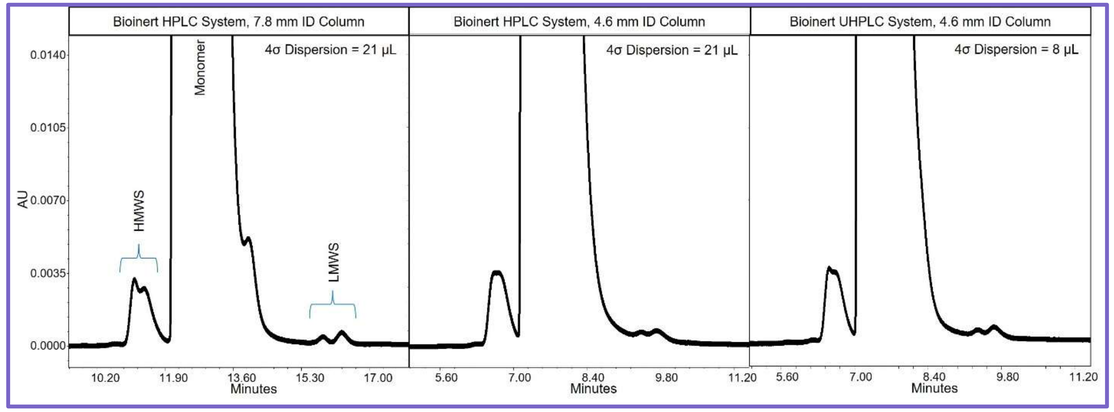
Overlaid duplicate injections of a protein mixture. Peaks are labeled in the top chromatogram.



SAMPLE CHROMATOGRAMS

Infliximab

Single Infliximab injections with peaks labeled in the left frame. HMWS = high molecular weight species. LMWS = low molecular weight species.



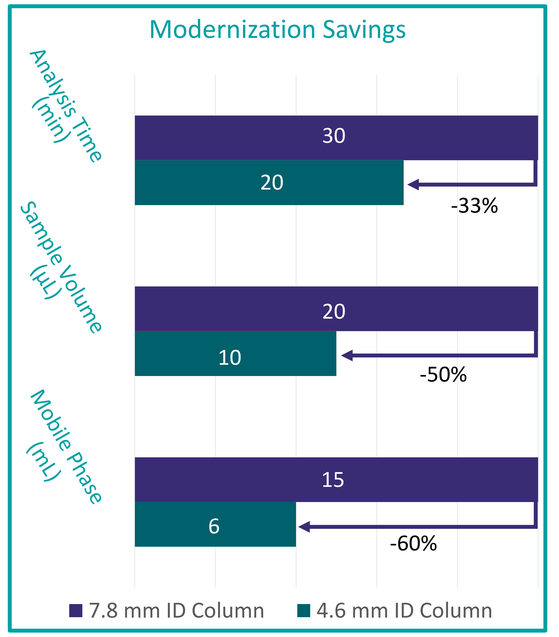
SAMPLE RESULTS

Infliximab

Relative area comparison of Infliximab. HMWS = high molecular weight species. LMWS = low molecular weight species

Relative Area (%)

Component	Bioinert HPLC System, 7.8 mm ID Column	Bioinert HPLC System, 4.6 mm ID Column	Bioinert UHPLC System, 4.6 mm ID Column
HMWS	0.5	0.5	0.5
Monomer	99.4	99.5	99.5
LMWS	0.1	0.1	0.1



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