Simplified sample preparation of antibodies for purity determination by SEC-HPLC using Thomson Filter Vials

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Protein aggregation in therapeutic products has become a major concern for the pharmaceutical industry and regulatory agencies. Protein aggregates can cause adverse patient immune responses and therefore are typically monitored throughout the formulation and production of bio-therapeutics. Monitoring aggregates by HPLC during production, storage and shipping is a part of the process of optimizing early formulations to minimize risk in clinical applications. Many clean-up techniques used to prepare cell culture samples prior to HPLC/UPLC provide incomplete clean-up, are time consuming, and waste precious sample. The presence of cellular debris remaining after sample preparation can lead to analytical instrument performance issues and will shorten column life. Here, we demonstrate how the use of Thomson Filter Vials for sample preparation allows for the analysis of low volume samples, processed in less time, with minimal sample loss and fewer transfer steps while extending column lifetimes and reducing HPLC/UPLC downtime.

Experimental

Method

The use of Thomson Filter Vials was investigated for the preparation of upstream cell culture samples prior to HPLC analysis by SEC or Protein A Affinity Chromatography. Theoretical plates, tailing, and peak width were evaluated using reference standards in samples containing harvest material.

Equipment

- Agilent 1260 Infinity HPLC
  - Column: 4.6x300 mm Bio-SEC3
  - Buffer: 1x PBS (Phosphate Buffered Saline)
  - Flow Rate: 0.2 mL/min
  - Injection: 2 µL
- Thomson nano|Filter Vials, p/n 15535
- 48 Position Multipress, p/n 35015-476

Results

The method was evaluated by bracketing the reference standards for 10 samples containing cell culture supernatant. Sixty samples were injected either filtered or unfiltered. Peak width (baseline), USP plates, and tailing were evaluated using bracketed reference standards. Theoretical plates were calculated using the USP formula for calculating theoretical plates:

$$N = 16\left(\frac{tr}{w}\right)^2$$

Where W is the width of the peak at the baseline. Figures 1, 2, and 3 compares the number of theoretical plates, tailing factor,
and peak width, respectively in bracketing reference standards when evaluating filtered (blue) and unfiltered samples (red).

Fig 1. Theoretical plates in filtered (blue) vs unfiltered (red) bracketing reference standards

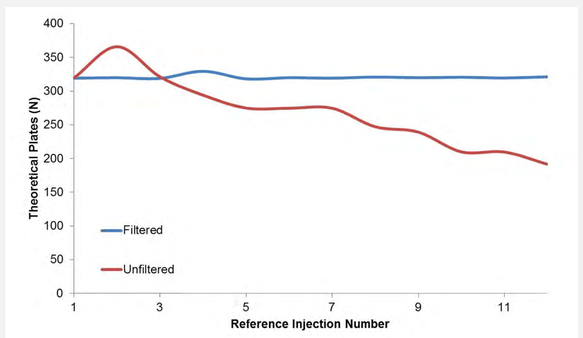


Fig 2. Peak width in filtered (blue) vs unfiltered (red) bracketing reference standards

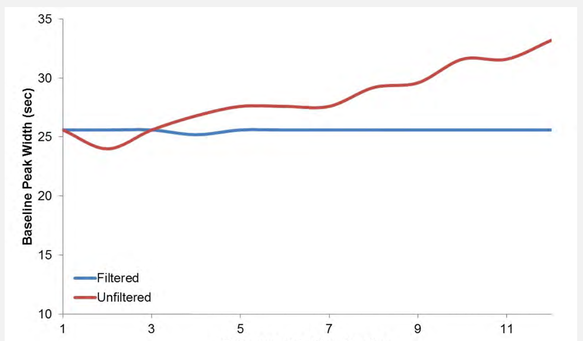


Fig 3. Tailing factor in filtered (blue) vs unfiltered (red) bracketing reference standards

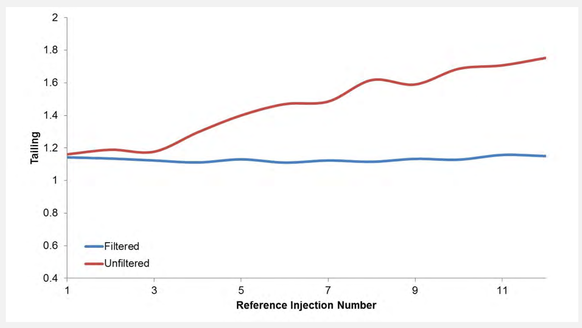


Fig 4. Peak Area in filtered (blue) vs unfiltered (red) samples

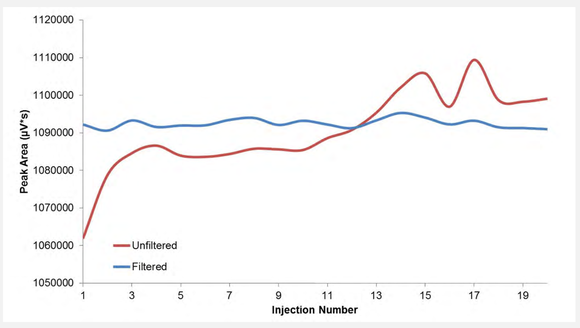


Fig 5. shows the SEC-HPLC chromatograms for filtered (blue) and unfiltered (red) material.

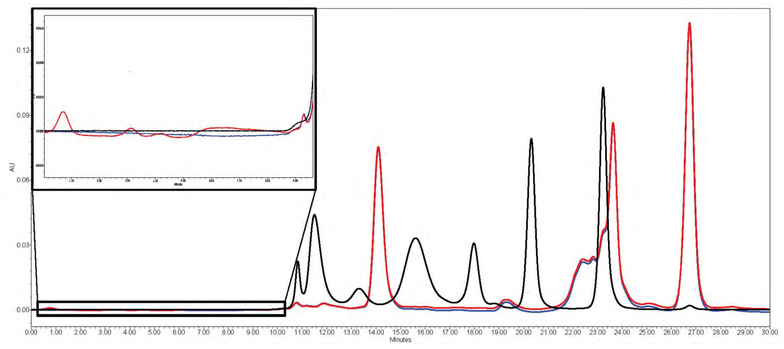


Fig 6. Shows analysis of a purified antibody including dimer peak

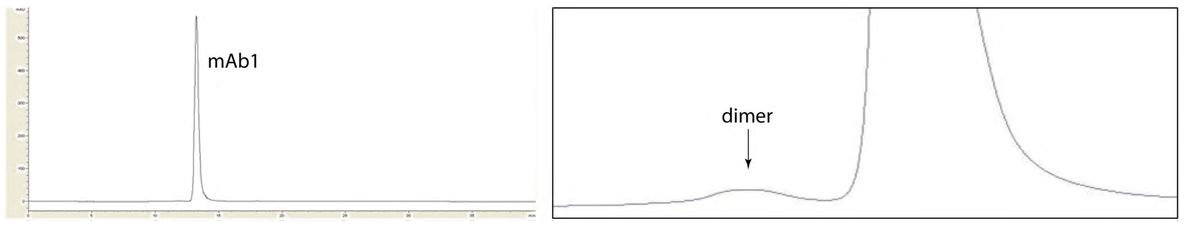
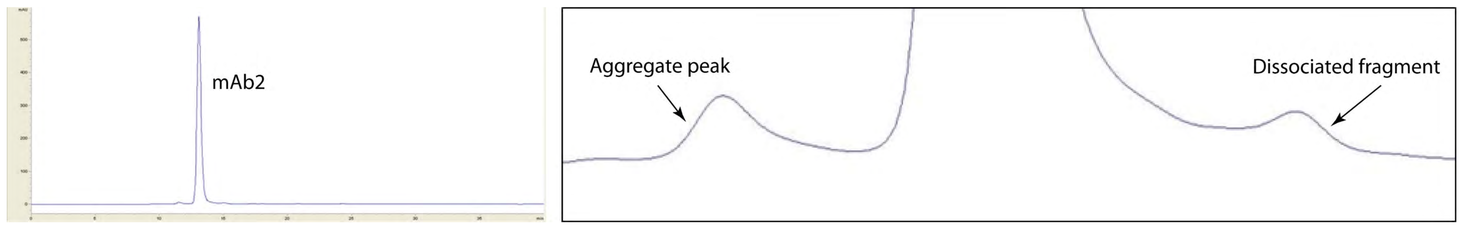


Fig 7. Shows a purified antibody with an aggregate peak and dissociate fragment



Conclusion

When evaluating bracketing reference standards in unfiltered samples, we observed significant increases in peak tailing and peak width and significant loss in theoretical plates. Furthermore, after the first 40 injections of unfiltered samples we observed the appearance of a shoulder in the chromatogram indicating that the column should be replaced. When evaluating consecutive injections of identical samples, significant variation in peak area was observed for unfiltered samples, whereas samples that were filtered with the nano|Filter Vial showed minimal change in peak area. This may be a result of higher order aggregates sticking to the column and eluting during the elution phase. This is evidenced by the chromatograms observed in Fig. 5. The unfiltered samples, shown in red, contain extremely high molecular weight species >670 kDa that is not present in the samples filtered with the Filter Vials. While small in abundance, repeated injection of unfiltered samples could be contributing to column fouling or could create other generic HPLC issues resulting in poor method performance. In conclusion, implementing use of Thomson nano|Filter Vials extended the column lifetime and improved method performance.