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series	cap color	membrane	pore size	part #
standard	●	PES	0.2µm	35535

Simplified Sample Prep for Open Access SEC-HPLC Detection using the Thomson Filter Vials

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Abstract

Routine analysis of samples containing cell culture media and/or supernatant by HPLC can lead to performance issues and can require frequent purchasing of new columns and increased HPLC maintenance. Filtering or centrifuging samples can help reduce the impact of analyzing cell culture samples, however, traditional syringe filtering or centrifuging samples often requires large volumes, extends sample preparation time, and/or can lead to product loss. Here, we demonstrate that using Thomson Standard 0.22µm PES (P/N: 35535) filters during sample preparation results in minimal impact preparation time, no detectable loss in product, and improved column performance leading to extended column lifetimes.

Introduction

HPLC methods are often used to support product analysis of in-process cell culture samples. In the absence of any purification, host cell proteins and other process related impurities are also injected onto the HPLC system. Process related impurities with large hydrodynamic radii or that contain aggregates can lead to HPLC performance issues including rapid degradation of column or clogging of needle seats and lines. Often times these samples are filtered using syringe filters or centrifuges to remove large debris. Centrifuging and syringe filtering is inconvenient as this can significantly impact sample preparation time and lead to product loss. Syringe filtering presents an additional problem, in that it requires significantly larger volumes (>1 mL). We sought to investigate the use of Thomson filter vials for the preparation of upstream samples prior to HPLC analysis by SEC or Protein A Affinity Chromatography. Thomson Standard Filter Vials are suitable for sample volumes ranging from 10µl to 300µl, and add minimal sample preparation time.

Methods

Cell culture harvest containing product was collected on an Agilent 1200 HPLC and evaluated using Waters Empower 3™ Software. Method conditions are outlined in Table 1. Plates, tailing, and peak width were evaluated between bracketing reference standards (prepared in the absence of harvest material) after 10 injections of samples containing harvest material using Method A. 20 simultaneous injections of sample containing cell culture were evaluated using Method B and analyzed for total product content.

Table 1

Condition	Method A (Protein A Affinity)	Method B (Protein A Affinity)	Method C (SEC)
Mobile Phase A	50 mM Phosphate, 150 mM NaCl, pH 7.0	50 mM Phosphate, 300 mM NaCl, pH 7.0	20 mM Phosphate, 300 mM NaCl, pH 7.4
Mobile Phase B	50 mM Phosphate, 150 mM NaCl, pH 3.0	50 mM Phosphate, 500 mM NaCl, pH 2.8	N/A
Gradient	Time (minutes) / % Mobile Phase A / % Mobile Phase B 0.00 / 100 / 0 0.50 / 100 / 0 0.70 / 0 / 100 2.70 / 0 / 100 2.80 / 100 / 0 4.00 / 100 / 0	Time (minutes) / % Mobile Phase A / % Mobile Phase B 0.00 / 100 / 0 0.50 / 100 / 0 0.70 / 0 / 100 2.70 / 0 / 100 2.80 / 100 / 0 4.00 / 100 / 0	Isocratic 100% A
Column Temperature	35°C	35°C	20°C
Autosampler Temperature	5°C	5°C	5°C
Wavelength	5°C	5°C	5°C
Injection Volume	25µL	10µL	20µL
Column	POROS A/20 1-5024-12	POROS A/20 1-5022-24	TOSOH G3000SWXL 808541

Results

Method A was used to evaluate bracketing reference standards after injecting 10 samples containing cell culture supernatant. Sixty samples were injected either filtered or unfiltered, and each set of 10 contained bracketing reference standards. Peak width (baseline), USP plates, and Tailing were evaluated in bracketing 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).

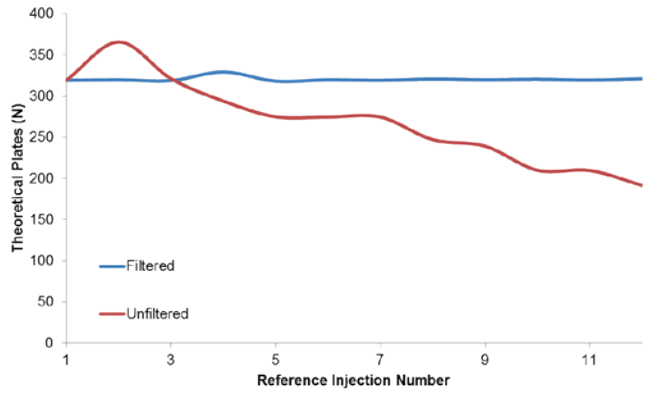

Fig 1. Theoretical plates in filtered (blue) vs unfiltered (red) bracketing reference standards

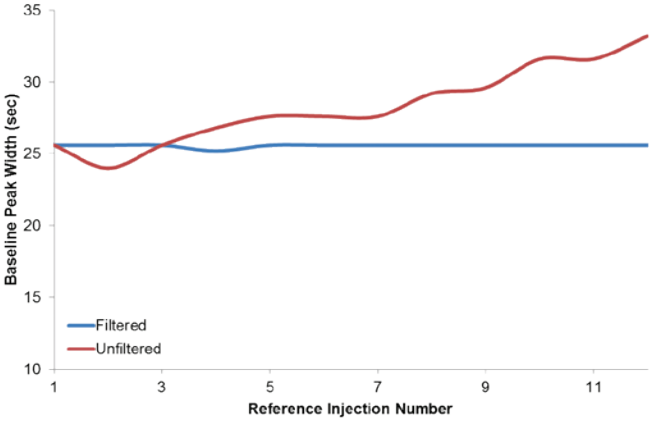

Fig 2. Peak width in filtered (blue) vs unfiltered (red) bracketing reference standards

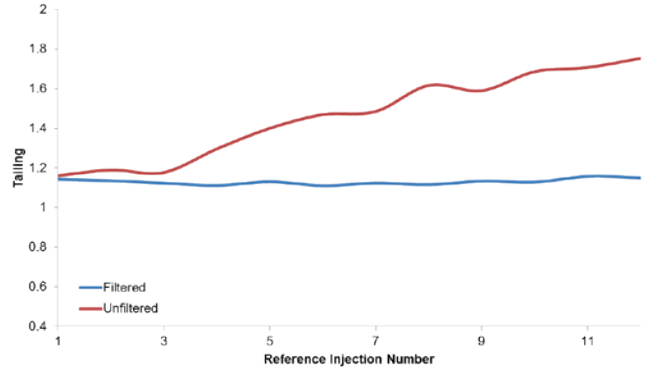

Fig 3. Tailing factor in filtered (blue) vs unfiltered (red) bracketing reference standards

Method B was used to evaluate replicate injections of the same sample containing cell culture supernatant. Peak area was plotted as a function or injection number (Figure 4).

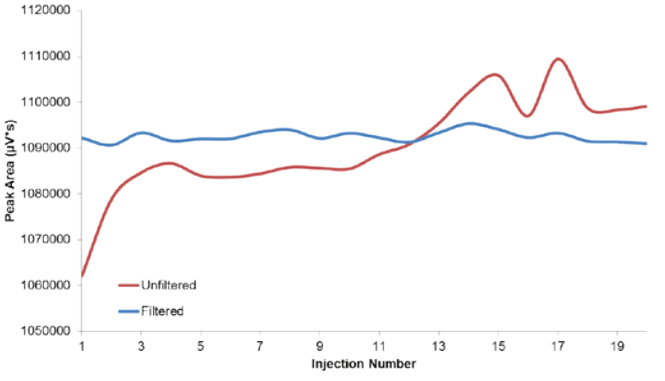

Fig 4. Peak Area in filtered (blue) vs unfiltered (red) samples

Finally, Method C was used to evaluate aggregate levels in filtered and unfiltered samples. Figure 5 shows the SEC-HPLC chromatograms for filtered (blue) and unfiltered (red) material.

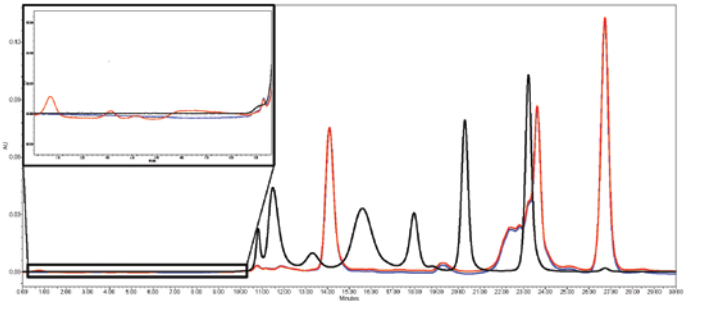

Fig 5. Full view (top) and zoomed view (bottom) SEC-HPLC chromatograms for filtered (blue) and unfiltered (red) cell culture supernatant overlaid with the gel filtration standard (black; Bio-Rad C/N: 151-1901).

Discussion and Conclusions

When evaluating bracketing reference standards in unfiltered samples, we observed significant increases in peak tailing and peak width for Protein A Affinity HPLC methods. Correspondingly, we observed significant loss in theoretical plates. Furthermore, after the first 40 injections of unfiltered samples (references 7 to 8) we observed the appearance of a shoulder in the chromatogram (data not shown), indicating this column should be replaced. When evaluating consecutive injections of identical samples (Method B), significant variation in peak area was observed for unfiltered samples (increasing with each injection), whereas samples that were filtered 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 SEC chromatograms observed in Figure 5. The unfiltered samples, shown in red, contain extremely high molecular weight species >670 kDa not present in the filtered samples.

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 HPLC filter vials directly extends the column lifetime and improves method performance.