Simplifying Progress

Implementing Chromatographic Methods for Evaluating Large-Scale Monolithic Columns for AAV Capsid Separation

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Introduction

Quaternary amine (QA)-modified monolithic columns are frequently used for the purification of therapeutically-relevant adeno-associated virus (AAV) vectors, used in gene therapy. Besides process- and sample-derived variability, the variability of chromatographic material can influence the efficiency and scalability of downstream AAV processing. This study presents the evaluation of highly reproducible (HR) QA-modified CIM® monolithic columns. The aim was to elute AAV capsids in a very narrow conductivity range, regardless of the batch and size of the column used.

We developed a chromatographic test method for demonstrating the intra- and inter-batch homogeneity of the material through different column sizes. The method is based on the separation of AAV2 | 8 capsids in an ascending KCl gradient on CIMmultus® QA 1 mL columns and specimen 0.2 mL units, taken from large-scale CIMmultus® QA monoliths up to 8,000 mL in size.

1. Experimental Approach

Test Columns: CIMmultus® QA 1 mL columns and specimen 0.2 mL units from 80 mL, 800 mL, and 8,000 mL CIMmultus® QA monoliths
System: PATfix® HPLC and column thermostat at 23 ± 1 °C
Sample: internal AAV2 | 8 standard sample
Buffer A: 25 mM BTP, 50 mM KCl, 2 mM $MgCl_2$, 1% sucrose, 0.1% poloxamer 188, pH 9.0
Buffer B: 25 mM BTP, 150 mM KCl, 2 mM $MgCl_2$, 1% sucrose, 0.1% poloxamer 188, pH 9.0
Method: linear KCl gradient from 100% buffer A to 100% buffer B over 30 column volumes (CV) at 2 mL/min (1 mL columns) or 1 mL/min (specimen)
Detection: intrinsic protein fluorescence (Ex/Em: 280/348 nm) and conductivity

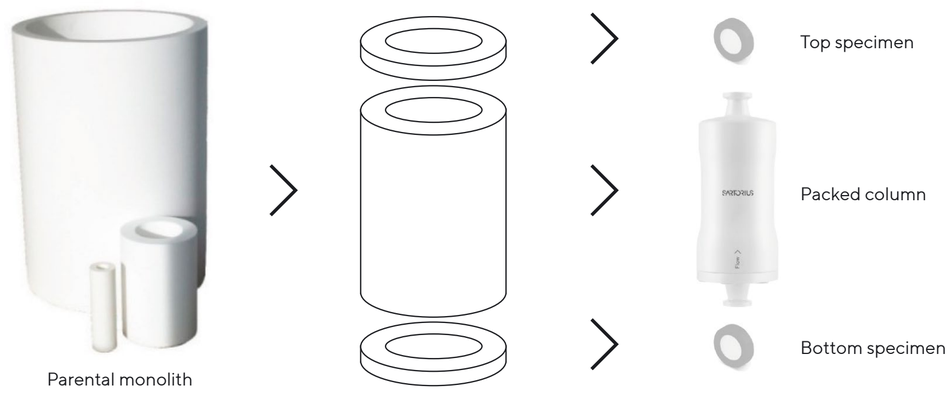
Retention times and elution conductivity values of empty and full capsids were obtained from chromatograms. KCl concentration at elution of empty capsid was calculated from the conductivity gradient for each evaluated column and compared for all evaluated columns.

Controlling the following parameters was crucial for method reproducibility:
- Consistency of buffer preparation and buffer stability after preparation
- Consistency of column equilibration
- Separation temperature

2. Results

The main challenge after establishing a reproducible method was analyzing the homogeneity of the material from the new HR chromatographic line from 1 mL to 8,000 mL column formats. Specimen 0.2 mL units enabled the evaluation of chromatographic material from parental monoliths (Figure 1 and 2). We evaluated the performance of four sizes of CIMmultis® QA monoliths (1, 80, 800, and 8,000 mL), with five batches for each size. The KCl concentration at the point of empty AAV capsid elution was within the range of 91.0 mM ± 1.7 mM (Figure 3), indicating excellent batch-to-batch material homogeneity. The results from specimen testing enable very precise comparison of monolith material between different column batches. Furthermore, specimen units enable testing of each individual column batch for a desired separation prior to release, while maintaining cGMP-compliance of the packed large-scale columns.

Figure 1: *Schematic Representation Showing How We Obtain Specimen Units and Packed Columns From the Large-Scale Parental Monolith*



Note. After monolith production, excess material is cut from the parental monolith and the central material is packed as a CIMmultus® column. Specimen 0.2 mL testing units are obtained from the excess material from above and below the packed column material. Because of their origin, specimen are composed of the same material as the packed column.

Figure 2: *Representative Chromatograms of AAV2 | 8 Empty | Full Separation Performed on Specimen 0.2 mL Units Taken From Parental Monoliths of Three Different Scales: 80 mL, 800 mL and 8,000 mL.*

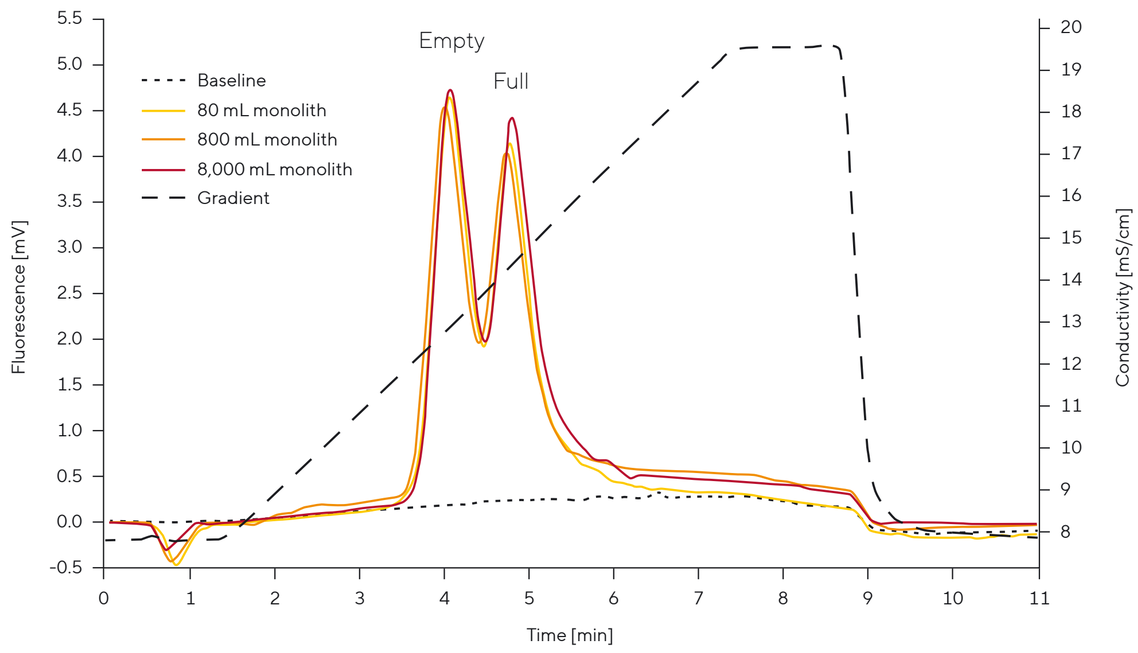
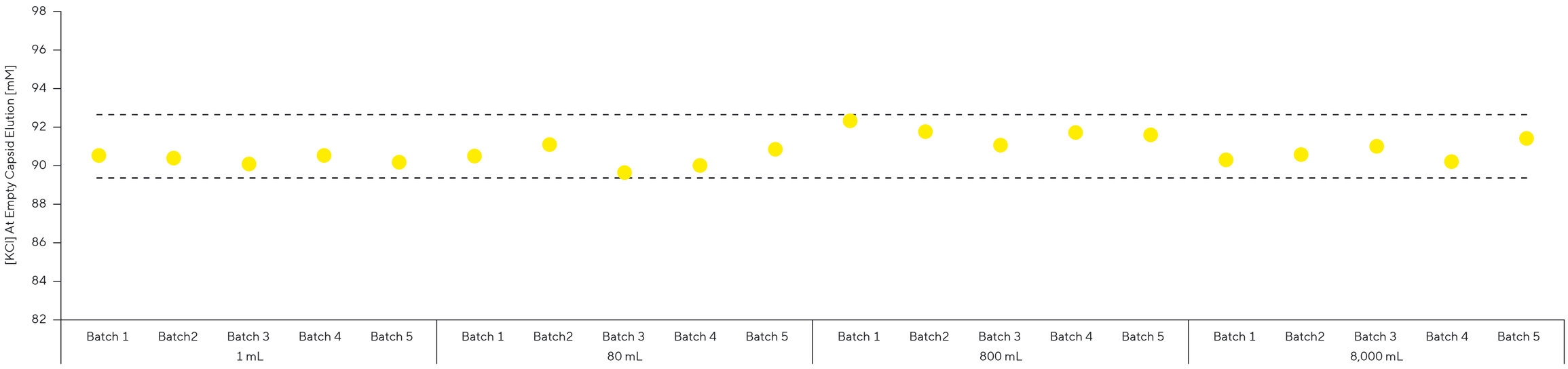


Figure 3: *Material Homogeneity of Five Batches of CIMmultus® QA Monoliths From 1 mL to 8,000 mL Column Size*



Note. AAV2 | 8 empty | full separation was performed on CIMmultus® QA 1 mL columns and specimen 0.2 mL units obtained from 80 mL, 800 mL, and 8,000 mL parental QA monoliths. Specimens contain the same material as the packed chromatographic columns and, therefore, represent the characteristics of the large chromatographic units. KCl concentration at empty capsid elution was in the range of 91.0 mM ± 1.7 mM for all twenty evaluated batches of QA monoliths.

3. Conclusions

Batch-to-batch reproducibility of the chromatographic material is crucial to achieve enhanced robustness of AAV downstream processing. For this purpose:

- The CIMmultus® QA HR chromatographic monolith line was developed to provide intra intra- and inter-batch material homogeneity
- AAV empty | full separation was implemented as a column | material quality control test
- KCl concentration at empty capsid elution was 91.0 mM ± 1.7 mM for twenty batches of 1 – 8,000 mL CIMmultus® QA monoliths, confirming excellent reproducibility of the chromtographic material