Simplifying Progress

Implementing Chromatographic Methods for Evaluation of Large-Scale Monolithic Columns for AAV Capsids Separation

S. Rotar[1], T. Simčič[1], R. Miklavčič[1], I. Bergoč[1], D. Kukanja[1], M. Makarovič Hrvatin[1], D. Pavlovič[1], J. Vidič[1], U. Černigoj[1], A. Štrancar[1]

[1]Sartorius BIA Separations d.o.o., Mirce 21, 5270 Ajdovščina, Slovenia

Introduction

Quaternary amine (QA) modified monolithic columns are frequently used for purification of therapeutically relevant adeno-associated virus (AAV) vectors, used in gene therapy. Beside process- and sample-derived variability, chromatographic material variability can influence the efficiency and scalability of AAV downstream processing. This study presents the evaluation of highly reproducible (HR) QA-modified CIM monolithic columns, where the goal is to elute AAV capsids in a very narrow conductivity range, regardless of the batch and size of the the column used.
We have developed a chromatographic test method for proving the intra- and inter-batch homogeneity of the material through different column sizes. The method is based on separation of AAV2/8 capsids in ascending KCl gradient on CIMmultus QA 1 mL columns and specimen 0.2 mL units, taken from large-scale QA monoliths up to 8000 mL in size.

1. Experimental approach

Test columns: CIMmultus QA 1 mL columns and specimen QA 0.2 mL units (from 80 mL, 800 mL and 8000 mL parental 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 MgCl2,, 1% sucrose, 0.1% poloxamer 188, pH 9.0
Buffer B: 25 mM BTP, 150 mM KCl, 2 mM MgCl2,, 1% sucrose, 0.1% poloxamer 188, pH 9.0
Method: linear KCl gradient from 100% buffer A to 100% buffer B over 30 CV at 2 mL/min (1 mL columns) or 1 mL/mL (specimen)
Detection: intrinsic protein fluorescence (Ex/Em: 280/348nm) 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 reproducible method utilization:
- consistency of buffer preparation and buffer stability after preparation,
- consistency of column equilibration,
- separation temperature.

2. Results

The main challenge after establishing a reproducible chromatographic method was analysing the homogeneity of the chromatographic material from new HR chromatographic line from 1 up to 8000 mL column format. Specimen 0.2 mL units enabled the evaluation of chromatographic material from parental monoliths (see Figure 1). Four QA monoliths sizes (1, 80, 800 and 8000 mL) and 5 analysed batches from each size show excellent material batch-to-batch homogeneity, as the KCl concentration at empty AAV capsid elution was within 91.0 mM ± 1.7 mM range. 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.

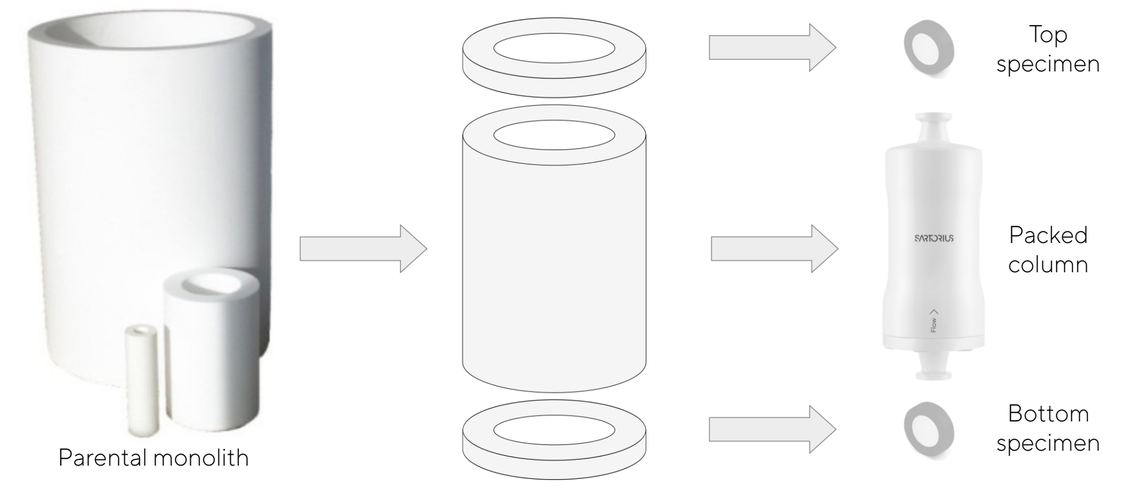

Figure 1: Schematic representation of obtaining specimen units and packed columns from the large-scale parental monolith. After monolith production, excess material is cut from the parental monolith and the central material is packed as a CIMmultus column. specimen, 0.2 mL testing units, are obtained from the excess material from above and below the packed column material. Because of their origin, specimen are composed of the same exact material as the packed column.

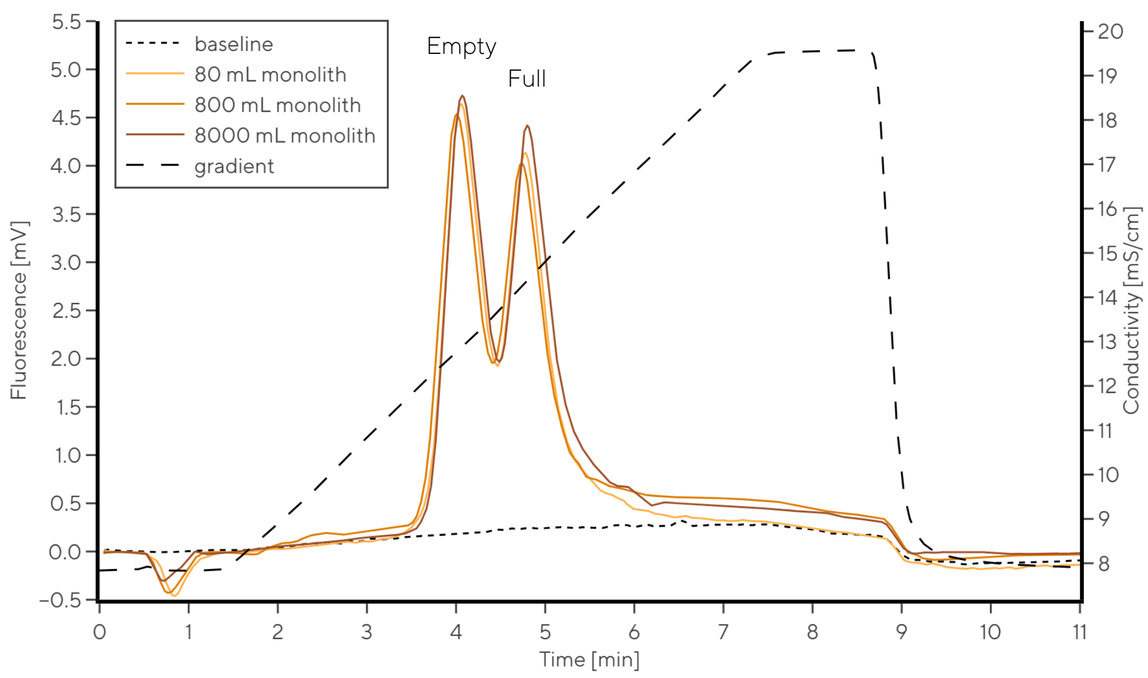

Figure 2: Representative chromatograms of AAV2/8 empty-full separation performed on specimen 0.2 mL units taken from parental monoliths of three different scales: 80 mL, 800 mL and 8000 mL..

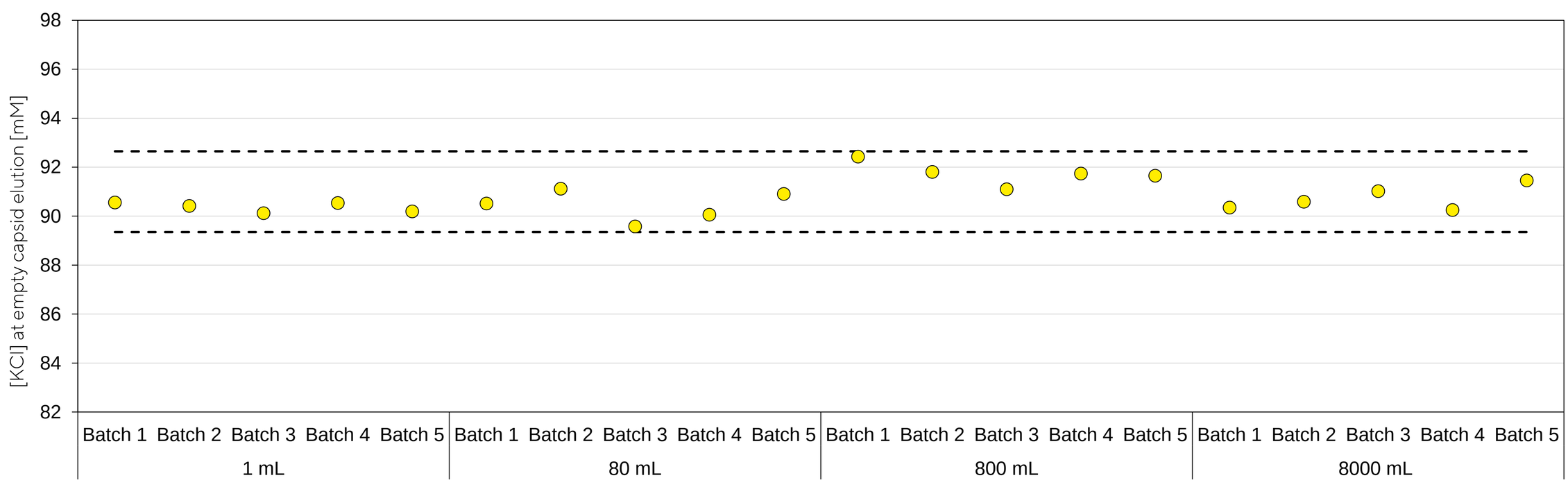

Figure 3: Material homogeneity of five batches of QA monoliths from 1 mL up to 8000 mL column size. AAV2/8 empty-full separation was performed on CIMmultus QA 1 mL columns and specimen 0.2 mL units obtained from 80 mL, 800 mL and 8000 mL parental QA monoliths. Specimen contain the same material as the 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:
- QA HR chromatographic monolith line was developed to provide intra- and inter-batch homogeneity of the material,
- AAV empty-full separation method 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 1-8000 mL QA monoliths, confirming excellent reproducibility of CIM QA material.