# High-Throughput Workflows to Accelerate Development of Chromatographic Purification of rAAV Viral Vectors

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## Abstract

Establishing a robust and scalable chromatographic full capsid enrichment step for adeno-associated virus (AAV) gene therapies presents an evolving challenge as novel combinations of capsids and genes of interest are continually developed. Traditional chromatographic development systems often require large quantities of feed-stream material, costly consumables, and a significant personnel commitment to plan, setup, and execute these experiments. Thus, high-throughput screening workflows at small-scale that provide directionally relevant performance data can be instrumental to the rapid, efficient, and successful development of a chromatographic process step. Here, we discuss development and application of a high-throughput workflow to screen and optimize an anion exchange (AEX) chromatography step for enriching full AAV capsids using an automated liquid handling system and micro-scale columns that delivers comparable process performance in terms of chromatographic profiles, step yield, and product quality as traditional bench-scale columns.

## Translating Methods to Micro-Scale

The instrumentation and programming interfaces of bench-scale FPLCs and liquid handling systems differ, but many of the key features of FPLCs (in-line gradient mixing, online multi-wavelength UV/Vis spectroscopy, automated fraction collection) can be emulated on liquid handlers while also leveraging their 8x higher throughput. Screening the binding behavior of a molecule relative to its in-process impurities using an extended, low-slope gradient on an ion exchange resin is a classic technique in downstream process development. We translated a typical salt gradient for FPLC into a high-throughput format by writing a script as follows:

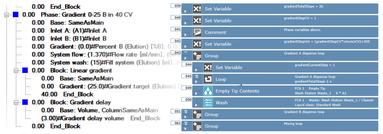


**Figure 1.** Example of adapting UNICORN lines into high-throughput script blocks.

Pipette "B" solution stepwise into 36 wells, linearly from 0 to 100% → Pipette "A" solution stepwise into 36 wells, linearly from 100 to 0% → Pipette mix each well sequentially in order of increasing gradient % → Aspirate and dispense from sequential wells to achieve linear gradient

The online UV/Vis spectroscopy of FPLC systems used to locate fractions of interest within a gradient can be replicated by pairing a high-throughput system with a 96-well optical plate reader. In Figure 2, the UV260 and UV280 (or A260 and A280) traces of a gradient on the FPLC (30,000 data points) are compared to that of the high-throughput system (36 data points). The same conclusions may be drawn from either data set, with the high-throughput set requiring 8 – 10x less feed material.

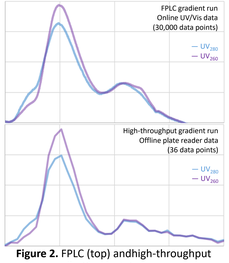
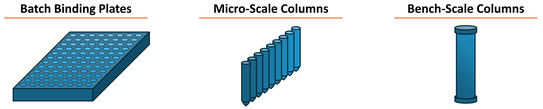



**Figure 2.** FPLC (top) andhigh-throughput (bottom) UV traces.

## Experimental Design Advantages

Early-stage programs often present the challenge of requiring screening work while also having limited material supply. As such, experimental work is often curtailed to speed up the time to *in vivo* study execution.




| | | |
|---|---|---|
| ~2 mL resin/run | 4.8 mL resin/run | ~3.0 mL resin/run |
| 96 conditions/run | 8 conditions/run | 1 condition/run |
| Static/batch binding | Pressure-driven flow | Pressure-driven flow |
| UV/Vis plate reader | UV/Vis plate reader | Online UV data |
| Typically too low volume | Moderate PQ testing | Substantial PQ testing |

**Figure 3.** At-a-glance comparison of attributes of typical downstream PD chromatography scales.

Whether adapting an existing platform or screening novel conditions for a pipeline molecule, investing resources into developing a micro-scale chromatography workflow can advance both objectives. Micro-scale columns exhibit the dynamic binding and pressure-driven flow of bench-scale columns and can reliably produce enough sample to enable product quality testing (e.g. PCR, ELISA, HPLC, cell-based assays).

**Table 1.** Design of Experiments applied to micro-scale and mL-scale columns.

| Runs | Full Factorial | Fractional Factorial | | micro-scale | | bench-scale | |
|---|---|---|---|---|---|---|---|
| | | | | Total vg | Days | Total vg | Days |
| 8 | $2^3$<br>3 factors<br>no aliasing | $2^{4-1}_{IV}$<br>4 factors<br>A = BCD | $2^{5-2}_{III}$<br>5 factors<br>A = BC | 5E13 | 1 | 2E14 | 2 – 4 |
| 16 | $2^4$<br>4 factors<br>no aliasing | $2^{5-1}_{V}$<br>5 factors<br>A = BCDE | $2^{6-2}_{IV}$<br>6 factors<br>A = BCD | 1E14 | 1 – 2 | 5E14 | 4 – 8 |
| 32 | $2^5$<br>5 factors<br>no aliasing | $2^{7-2}_{IV}$<br>7 factors<br>A = BCD | $2^{8-3}_{IV}$<br>8 factors<br>A = BCD | 2E14 | 2 – 4 | 9E14 | 8 – 16+ |

Assuming average experiment loading of 1E13 vg per mL of resin, 0.5 ID × 15 cm mL-scale column, 2 – 4 FPLC runs per day. Illustrative purposes only.

High-throughput chromatography also presents the opportunity to apply Design of Experiments concepts to early-stage programs that would otherwise have to wait until late-stage process characterization activities to generate statistical models of unit operations. Table 1 provides a snapshot of how a single operator can execute DoE studies with a fraction of the material and time that bench-scale would require.

## High Experimental Throughput

The preceding sections highlighted the translatability of results, material savings, and increased throughput of this workflow. These concepts were applied to development of an anion exchange step intended to purify full (gene of interest containing) AAV capsids from empty capsids.

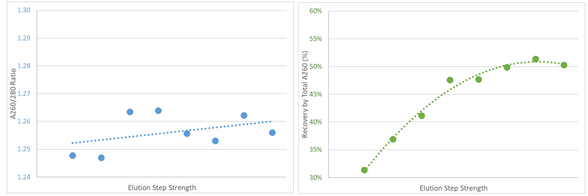



**Figure 4.** A260/280 and yield vs. anion exchange elution strength.

Two experiments were run in succession: a univariate analysis of anion exchange elution strength, followed by a univariate analysis of wash step strength using the elution setpoint from the first experiment. Figure 4 shows the anion exchange elution pool A260/280 ratios (analogous to full capsid content) and load-to-eluate recovery. The elution A260/280 ratio was stable across the evaluated range of elution strengths while yield increased, eventually reaching a plateau. This data suggested selecting a moderate elution strength to achieve a satisfactory yield while preserving the potential for impurity removal and virus clearance.

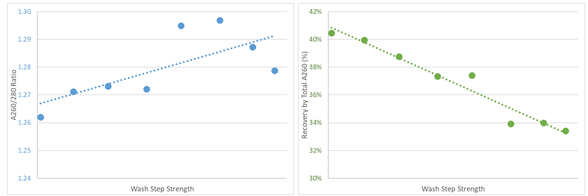



**Figure 5.** A260/280 and yield vs. anion exchange wash strength.

Figure 5 shows the anion exchange elution pool A260/280 ratios and total A260 as functions of the anion exchange wash step strength. The A260/280 ratio and yield exhibited positive and negative correlations with wash step strength, as expected. Empty capsids are more thoroughly removed with increasing wash step strength, increasing the elution pool A260/280 ratio. However, if the wash is too strong, full capsid loss occurs, and in some cases can even lead to depressed eluate pool A260/280 ratios due to late-eluting species (such as aggregates) increasing in proportion relative to the full capsid species.

## Translating Back to Bench-Scale

Generating chromatograms (such as Figure 2) using lower material requirements is immensely helpful for determining retention of species of interest. Variable pathlength absorbance instruments such as the SoloVPE allow for accurate UV/Vis measurements without sample dilution. Table 2 and Table 3 compare the load-to-eluate recovery and the eluate A260/280 ratios when measured by SoloVPE and plate reader.

**Table 2.** Recovery by A260 on SoloVPE and plate reader.

| SoloVPE | Plate | Difference |
|---|---|---|
| 56% | 45% | -11% |
| 37% | 31% | -7% |
| 62% | 52% | -10% |
| 64% | 53% | -11% |
| 41% | 38% | -4% |
| … | … | … |
| Mean ± 1SD (*n* = 50) | | -9 ± 2% |
| $R^2$ (SoloVPE, Plate) | | 0.91 |

**Table 3.** A260/280 ratios on SoloVPE and plate reader.

| SoloVPE | Plate | Difference |
|---|---|---|
| 1.25 | 1.24 | -0.01 |
| 1.32 | 1.30 | -0.02 |
| 1.30 | 1.27 | -0.02† |
| 1.20 | 1.18 | -0.02 |
| 1.29 | 1.27 | -0.01† |
| … | … | … |
| Mean ± 1SD (*n* = 50) | | -0.01 ± 0.01 |
| $R^2$ (SoloVPE, Plate) | | 0.89 |

† Artifact of rounding for display.

Load-to-eluate recovery and eluate A260/280 ratios were highly correlated when measured by SoloVPE and by plate reader. An offset was observed between the two methods; the plate method did not implement pathlength adjustment or dilution and thus was more vulnerable to signal suppression in high-concentration samples. This data set suggested the relevance of both directional conclusions and absolute absorbance values when accounting for a fixed offset.

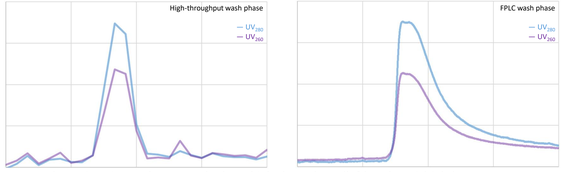



**Figure 6.** Anion exchange wash phase high-throughput (left) and FPLC (right) UV traces.

An experimental wash condition discovered using the high-throughput screening method was scaled up to a bench-scale FPLC system. Chromatograms of the empty capsid removal wash step for both scales are presented in Figure 6 above. Table 4 shows A260/280 ratios for the wash and eluate pools as well as load-to-eluate recovery. The data was comparable, supporting our proposal – that investing in training downstream scientists in the art of high-throughput workflows can pay dividends in process development.

**Table 4.** Comparison of micro-scale and bench-scale AEX method.

| Parameter | micro-scale | bench-scale |
|---|---|---|
| Wash A260/280 Ratio | 0.68 | 0.61 |
| Elution A260/280 Ratio | 1.32 | 1.34 |
| Load to Elution Recovery by Total 260 | 38% (by SoloVPE) | 38% (by SoloVPE) |