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Aggregate analysis on Adeno-Associated Virus and Virus-Like Particle Analysis using newly developed novel Size Exclusion Chromatography Columns

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Introduction

High-resolution size exclusion chromatography (SEC) is a powerful tool for determining the aggregation of viral vectors and LNPs, a critical quality attribute, to ensure safety and purity.

Here we demonstrate two new AdvanceBio SEC columns designed specifically for analyzing adeno-associated viruses (AAV) and virus-like particles (VLP) aggregate, respectively.

The new columns use small particles with a size of 2.7 µm for fast mass transfer, with pore sizes tuned to include the different classes of viral vectors and provide robust baseline separations of aggregates from their respective monomer peaks.

Experimental Details

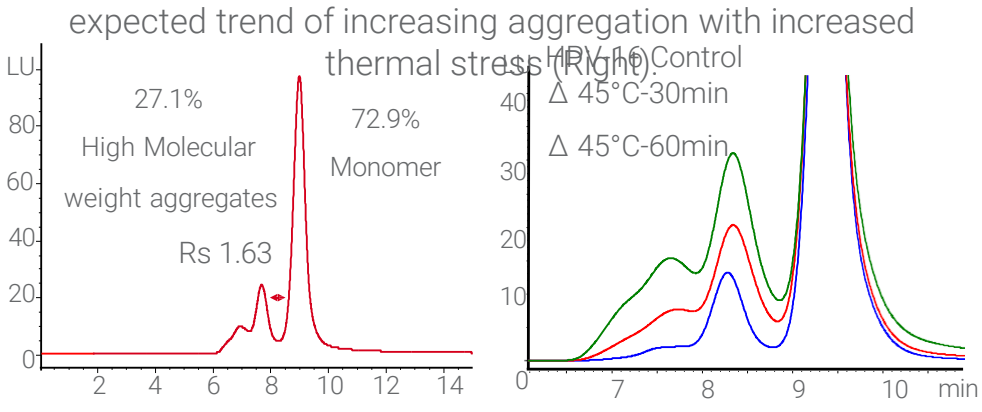
Instrument	1290 Infinity II Bio LC system with binary high-speed pump
Column	• Agilent AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 300 mm (p/n PL1580-5325) • Agilent AdvanceBio SEC 1000 Å, 2.7 µm, 4.6 × 300 mm, (p/n PL1580-5302) • Agilent AdvanceBio SEC 500 Å, 2.7 µm, 4.6 × 150 mm (p/n PL1580-3325)
Mobile Phase	50 mM phosphate buffer, 400 mM NaCl, pH 7.2
Flow rate	0.35mL/min
Samples	• Recombinant human papilloma virus type 16 L1 protein (HPV-16) VLP, 1 mg/mL, ~50 nm diameter • AAV (full capsid) reference standards AAV1 3.42 × 10^{11} GC/mL AAV2 1.27 × 10^{13} GC/mL AAV5 2.6 × 10^{11} GC/mL AAV6 4.1 ×10^{11}GC/mL AAV8 3.53 × 10^{11} GC/mL AAV9 6.07 × 10^{11} GC/mL
Detection	1260 Infinity II fluorescence detector (FLD), λex = 280 nm , λem = 348 nm

Results and Discussion

Chromatographic separation of VLP sample & Forced Degradation Study

HPV-16 showed well resolved monomer and aggregate peaks on the 4.6 x 300 mm AdvanceBio SEC 1000Å column (Left).

Thermal forced aggregation experiments of HPV-16 showed good resolution of the monomer vs aggregate species, with the expected trend of increasing aggregation with increased thermal stress (Right).

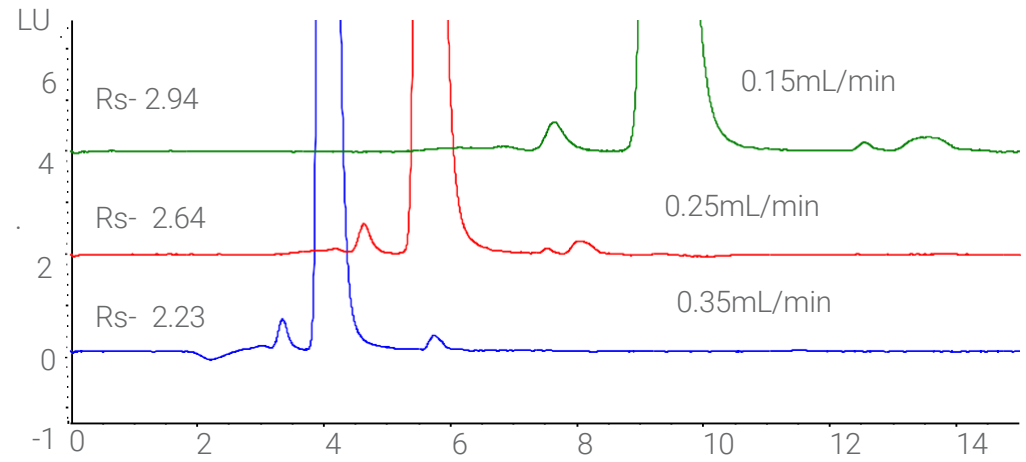


The columns also showed compatability with a light scattering and ESI-MS detection where low background signal is critical (data not shown).

Results and Discussion

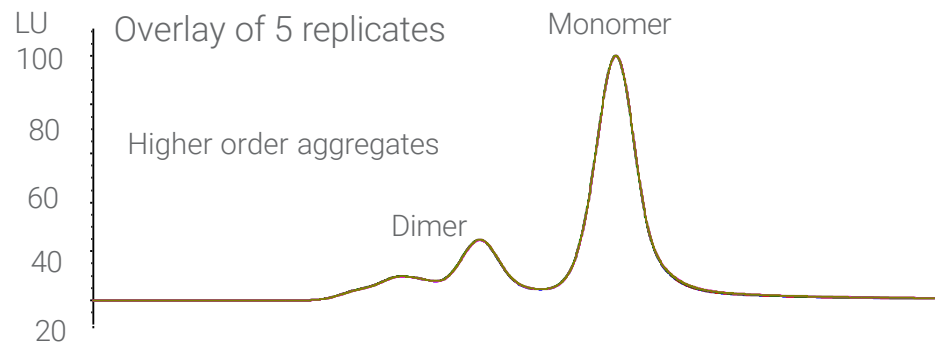
Fast Separation

AAV9 sample were run on a 4.6x150mm AdvanceBio SEC 500Å shorter length column and showed baseline separation with shorter runtime (<6min) at different flow rates.



Run to Run Reproducibility

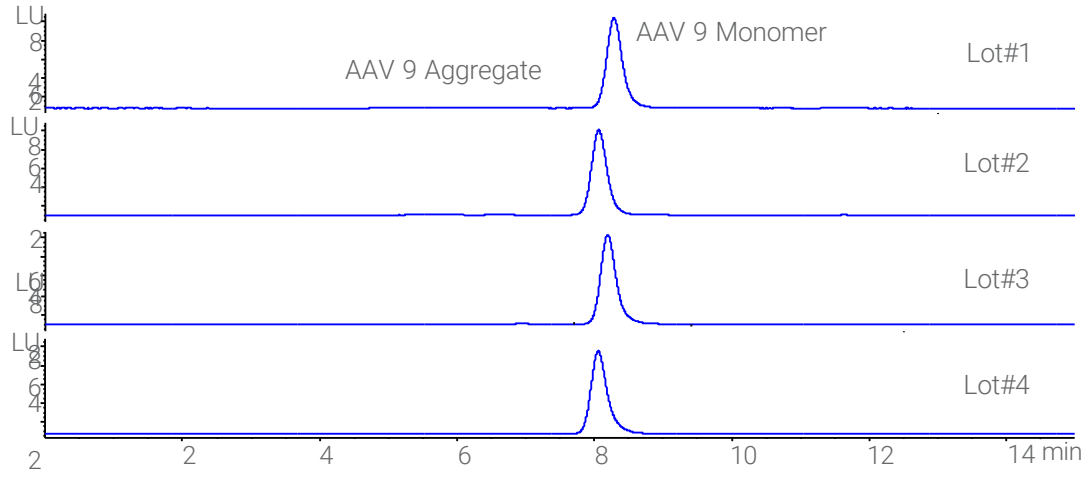
The columns showed excellent injection to injection reproducibility, with RSD for all metrics ≤ 1% (data shown below for HPV-16).



Injection to Injection Reproducibility HPV Type16, 4.6 x 300 mm AdvanceBio SEC 1000Å					
	Rt Monomer	% HMW	% Monomer	Resolution	Tailing Factor
Avg	8.99	26.93	73.06	1.63	1.12
Std Dev	0.00	0.22	0.19	0.01	0.01
%RSD	0%	1%	0%	0%	1%

Column Lot to Lot Reproducibility

The columns also showed excellent reproducibility when comparing across columns packed with four production batches of SEC particles.

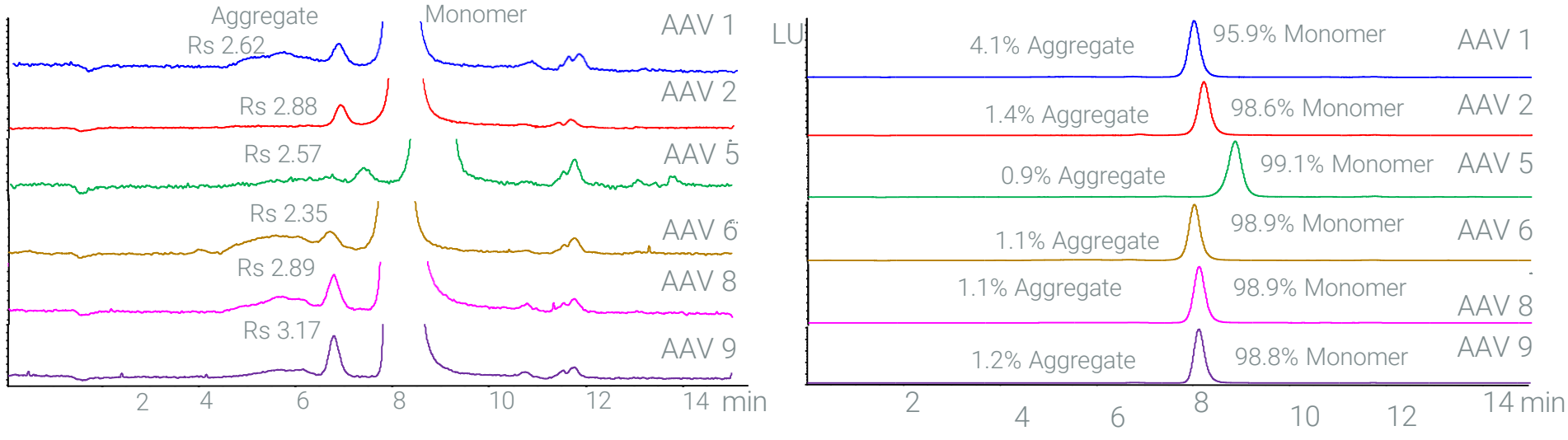


Batch to Batch Column Reproducibility AAV9, 4.6 x 300 mm AdvanceBio SEC 500 Å				
Different batches of column	Rt Monomer	Total Peak Area	Resolution	Recovery based on no column
Avg	8.14	156.33	3.27	99%
Std Dev	0.11	5.31	0.18	0.03
%RSD	1%	3%	5%	3%

Results and Discussion

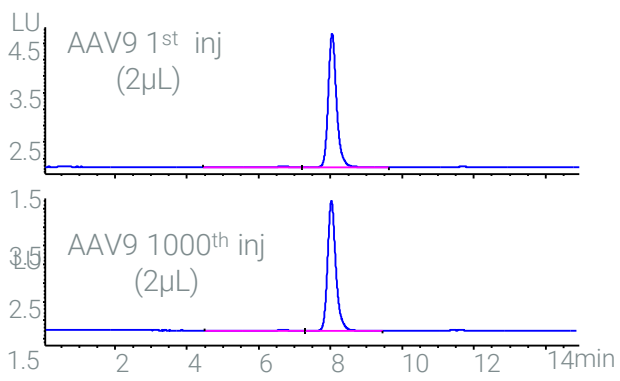
Chromatographic separation of different AAV serotypes

5 µL samples of six different AAV serotypes were injected onto the 4.6 x 300 mm Agilent AdvanceBio SEC 500Å column.The results are shown below at expanded scale (left) and full scale (right), showing excellent baseline separation between AAV aggregates and its monomer for every AAV serotype tested.



Robustness

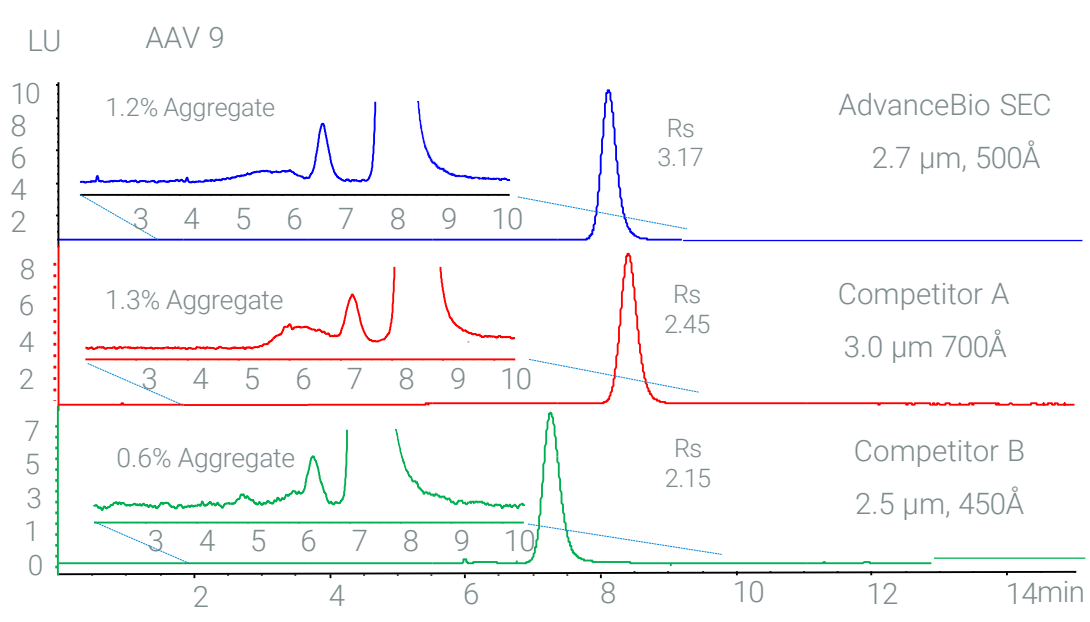
To evaluate the robustness of the new columns, we ran 1000 sequential injections on a new 4.6 x 300 mm AdvanceBio SEC 500Å column, following a repeating cycle of 49 injections of sample matrix ((49x 1XPBS+0.01%F-68) followed by 1 injection of AAV9 sample. Data below show AAV9 before and after 1000 injections (A), with tabulated results, where resolution is between the monomer and aggregate peak. Also shown is the RT, resolution and tailing factor over the course of the experiment.

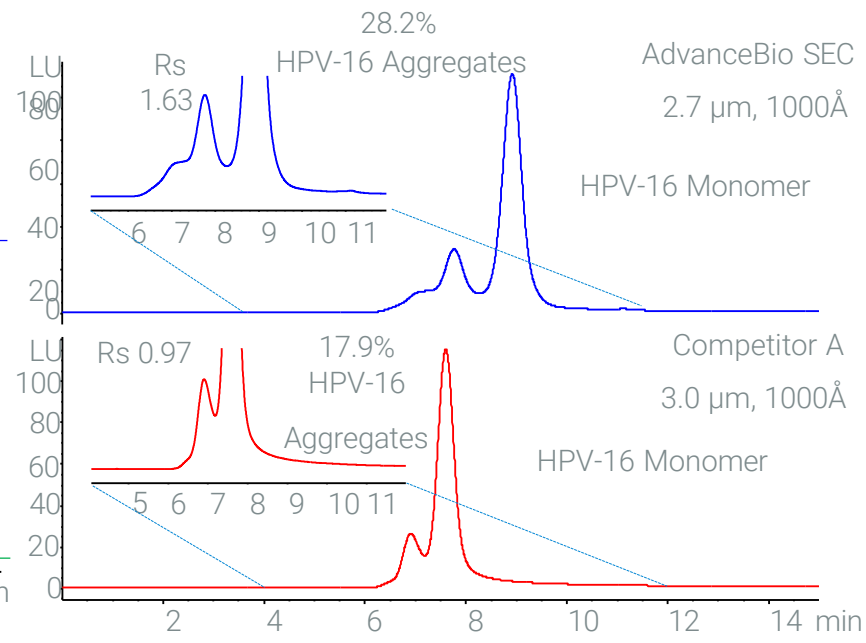


Performance Robustness AAV9, 4.6 x 300 mm **AdvanceBio SEC 500Å**					
AAV9	Monomer RT (min)	% Aggregate	% Monomer	Resolution	Total Peak area
Inj 1	8.1	1.1	98.9	3.3	65.5
Inj 1000	8.1	1.2	98.8	3.3	58.6

Comparison with Competitor Columns

AAV & VLP samples were also tested on 4.6 x 300 mm SEC columns from different vendors and showed higher resolution and recovery on AdvanceBio SEC columns





Conclusions

Both AdvanceBio SEC columns show robust column performance with baseline resolution between monomer and aggregate peaks for viral vectors. The column performance stays reproducible and consistent after 1000 injections. High throughput & shorter runtime (<6 min) with similar resolution could be achieved using shorter length column.

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This information is subject to change without notice.

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