ANALYTICAL REPEATABILITY AND PRECISION OF THE XEVO^{TM} CDMS SYSTEM FOR AAV CAPSID CHARACTERIZATION

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INTRODUCTION

Recombinant adeno-associated virus (AAV) vectors are widely used for gene therapy delivery; however, intrinsic capsid heterogeneity remains a critical analytical and manufacturing challenge. Only genome-containing (full) capsids contribute to therapeutic potency, while empty, partially filled, and overfilled particles represent product-related impurities that can dilute effective dose and increase immunogenicity risk. Accurate and reproducible quantification of these capsid populations is therefore essential for process development, lot comparability, and Chemistry, Manufacturing, and Controls (CMC) decision-making.

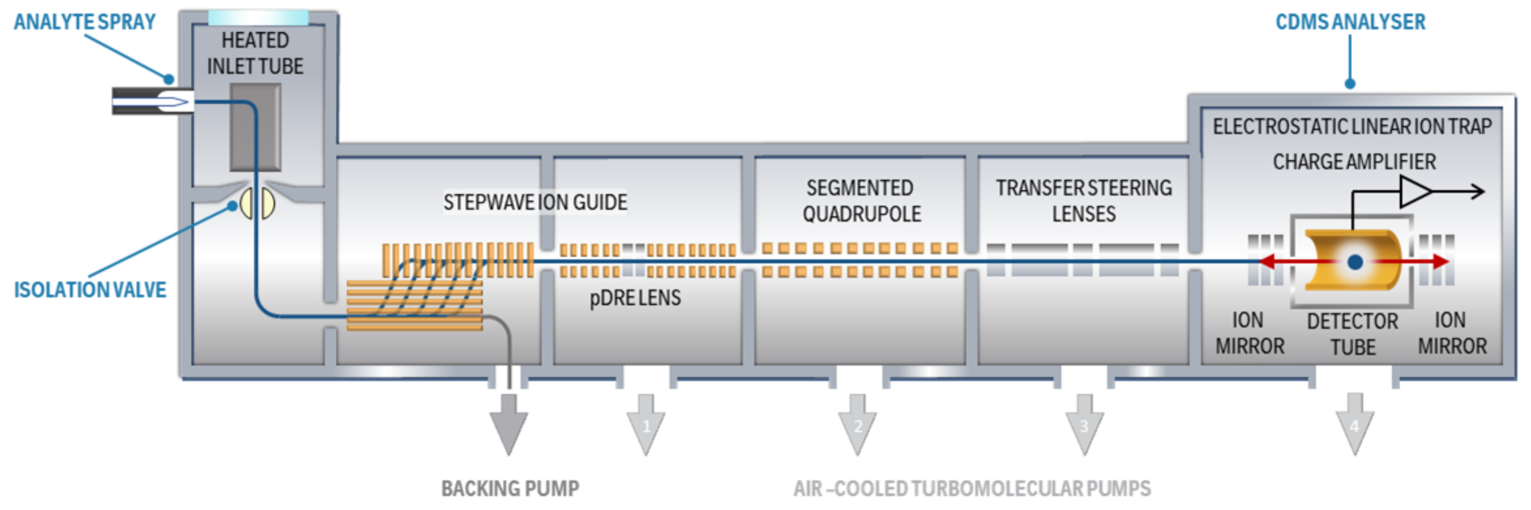
Charge detection mass spectrometry (CDMS) enables direct, single-particle mass measurement, providing unbiased resolution and relative quantification of intact AAV capsids without charge-state deconvolution. In this study, we evaluate the analytical repeatability, intermediate precision, and operational robustness of the Xevo™ CDMS system for AAV capsid characterization under high-throughput, multi-day operation and across process-relevant viral titres. These data establish the suitability of CDMS for routine application in AAV process monitoring and quality assessment.

METHODS

Samples: Standard empty and full CMV-GFP AAV8 capsids were sourced from Virovek Inc. at 2 × 10^{13} viral particles (vp)/mL and buffer-exchanged into 200 mM ammonium acetate containing 0.01% Pluronic F-68. Empty and full capsids were mixed 1:1 and diluted to 1 × 10^{13} vp/mL for repeatability and reproducibility assessment, consisting of 12 injections per day over five consecutive days. The same 1:1 mixture was further diluted to 1 × 10^{11} vp/mL and 5 × 10^{12} vp/mL to represent upstream- and downstream-equivalent process concentrations, respectively. Alternating injections of the low- and high-concentration samples were performed within a single day to evaluate robustness to concentration changes and injection order.

CDMS: Ions were generated in positive ion mode using nano-electrospray ionisation and mass analysis was performed using the Xevo CDMS system . Signal processing and data visualization were performed using the Waters_Connect^{TM} software. Ions were trapped for 100 ms, and acquisition times of ~30mins were used to collect ~5000 ions. Detected time-domain signals were Fourier transformed; the measured frequency and the magnitude correspond to an individual ions' *m/z* and *z* respectively, enabling direct calculation of mass values.

XEVO CDMS EXPERIMENTAL SETUP



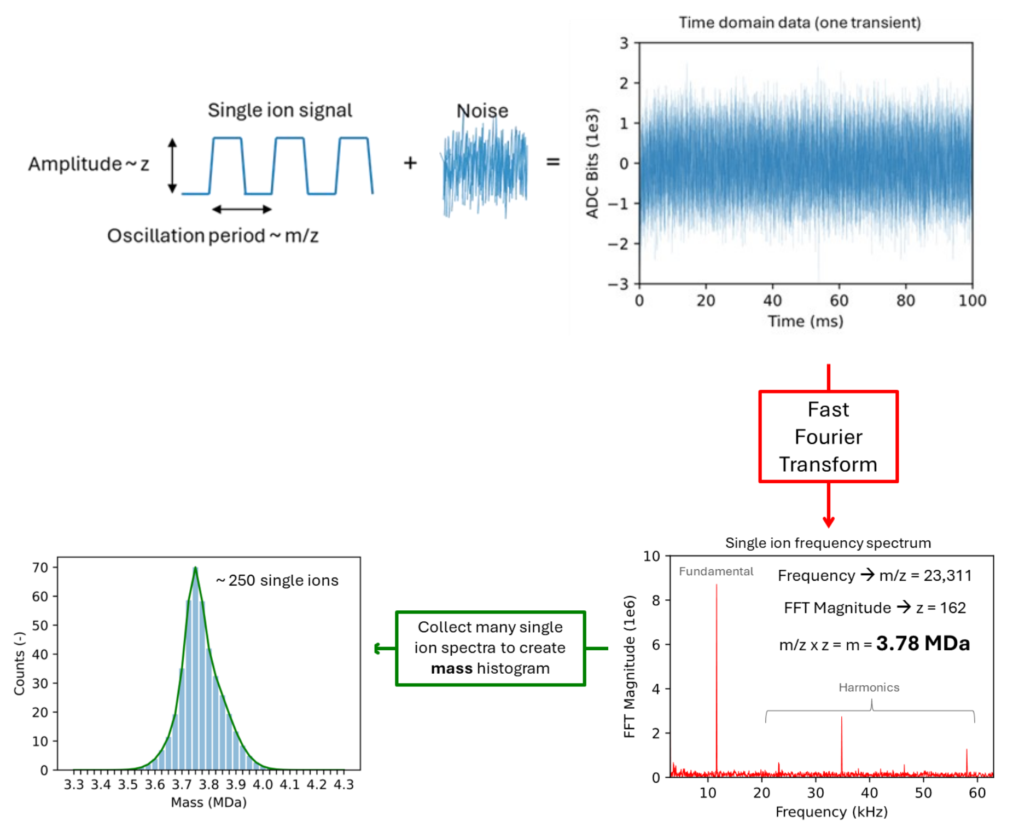


Figure 1: A schematic representation of the Waters ELIT-based CDMS instrument with diagrams showing how m/z and z information is obtained, ultimately providing mass information.

- The CDMS mass analyser houses a conductive cylinder with two ion mirrors, which reflect the ion back and forth.
- When an ion enters and progresses through the detection cylinder, the charge on the ion is induced on to the cylinder.
- The induced charge is then detected by a low-noise charge sensitive amplifier, which results in a periodic signal that can be analysed using fast Fourier transform (FFT).
- The *m/z* of an ion is determined from the oscillation frequency and the charge from the signal amplitude.
- *m/z* × *z* → **m** for each ion

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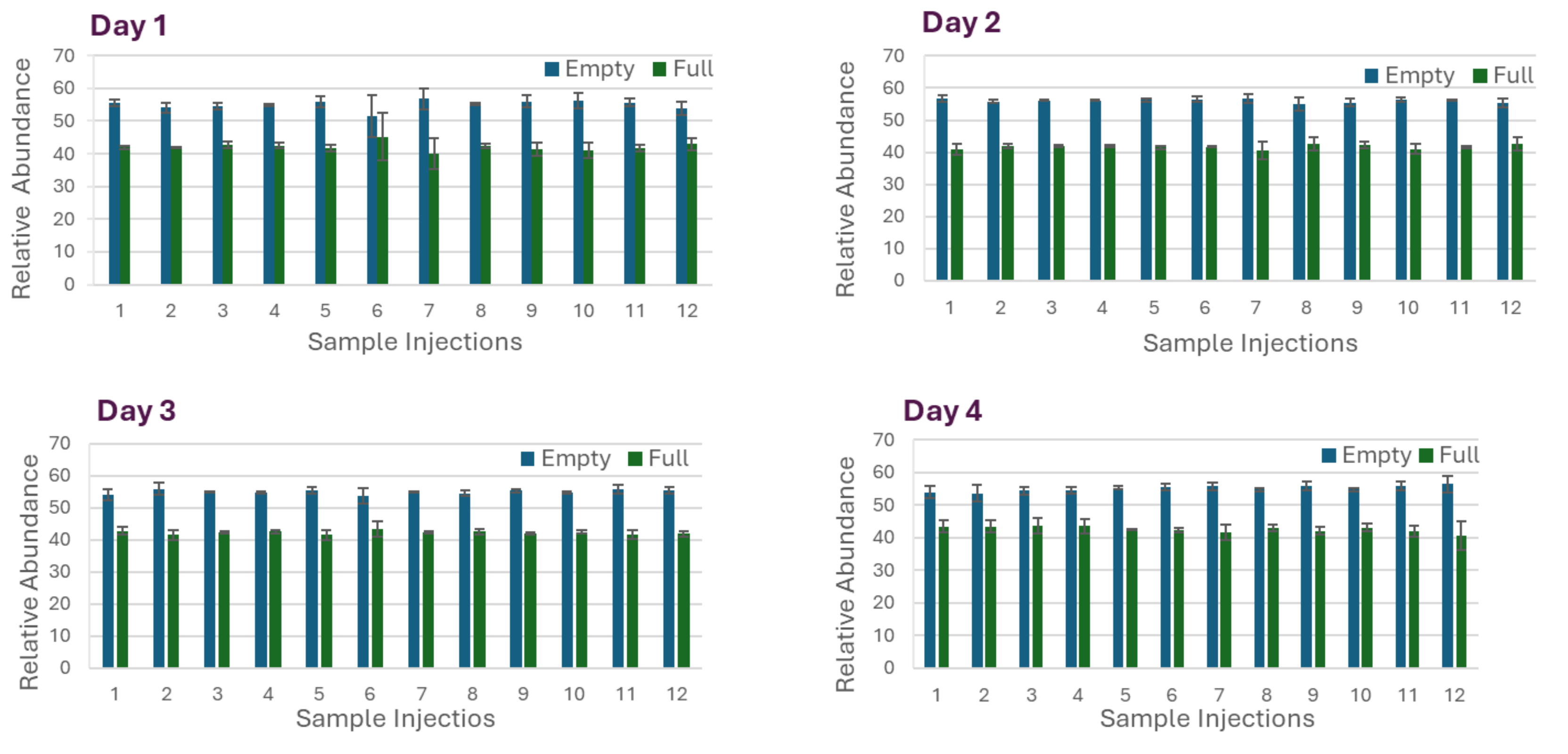


- Relative peak abundance shows <2% RSD, demonstrating highly reliable instrument performance.
- All injections across the five days remain within ±3% of the mean, ensuring consistent results across runs.

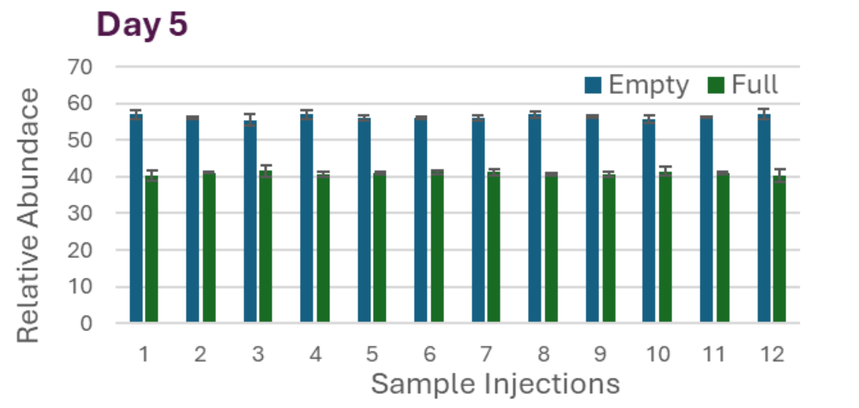
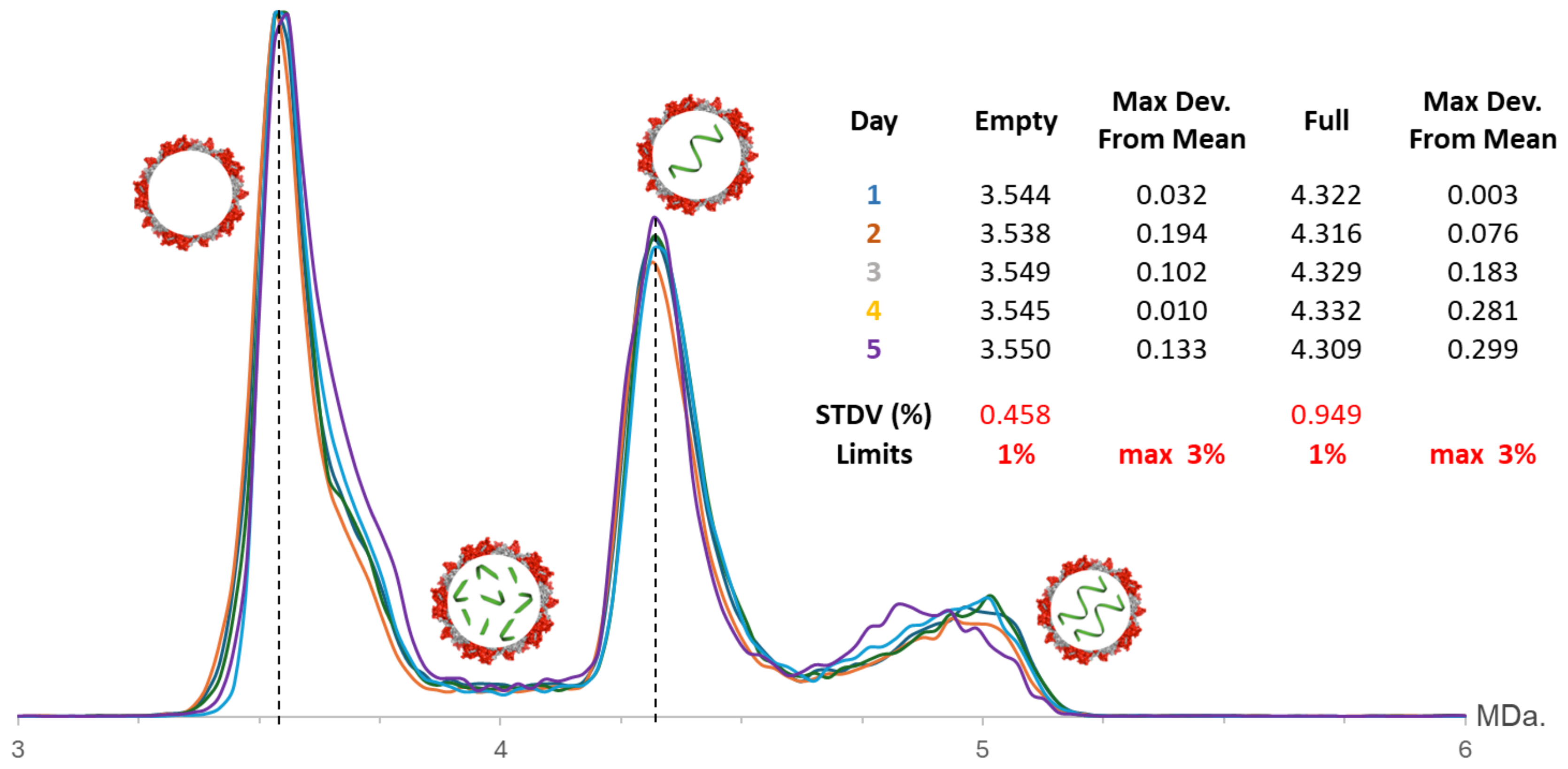


Figure 2: Relative quantification stability across five consecutive days. Relative abundance of a 1:1 mixture of Empty and Full (CMV-GFP) AAV8 capsids measured across 12 injections per day over five days. Empty capsids are shown in blue and Full capsids in green. Signals demonstrate <2% RSD, with all injections within ±3% of the mean.



Day	Empty	Max Dev. From Mean	Full	Max Dev. From Mean
1	3.544	0.032	4.322	0.003
2	3.538	0.194	4.316	0.076
3	3.549	0.102	4.329	0.183
4	3.545	0.010	4.332	0.281
5	3.550	0.133	4.309	0.299
STDV (%)	0.458		0.949	
Limits	1%	max 3%	1%	max 3%

Figure 3: Normalized mass distributions of a 1:1 mixture of Empty and Full (CMV-GFP) AAV8 capsids measured over five consecutive days. Each trace represents the daily average of 12 injections. Across the five-day study, measured masses exhibit <1% RSD, with all values remaining within ±3% of the overall mean, demonstrating highly reproducible mass precision suitable for reliable empty/full determination.

RESULTS

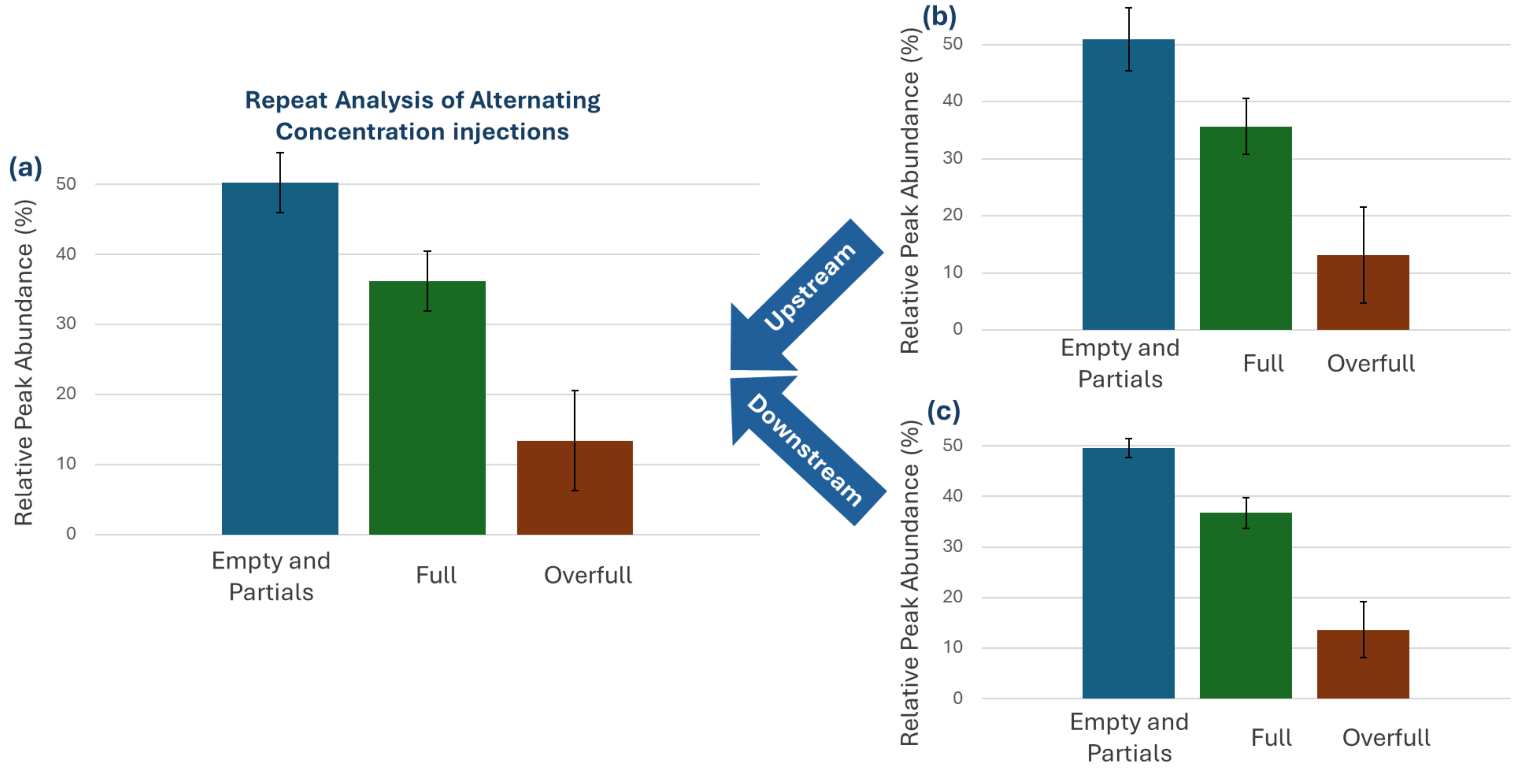


Figure 4: Reproducibility of relative peak abundance across alternating concentration injections. Average relative peak abundance of Empty/Partials, Full, and Overfull species measured during repeated alternating injections of low (1 × 10^{11} vp/mL; upstream-equivalent) and high (5 × 10^{12} vp/mL; downstream-equivalent) AAV concentrations. (a) Mean relative peak abundance across all alternating injections. (b) Individual species contributions at the downstream-equivalent concentration. (c) Individual species contributions at the upstream-equivalent concentration. Error bars represent relative standard deviation (RSD), which remains below the predefined 5% limit, demonstrating consistent and reproducible relative quantification across concentration and injection order.

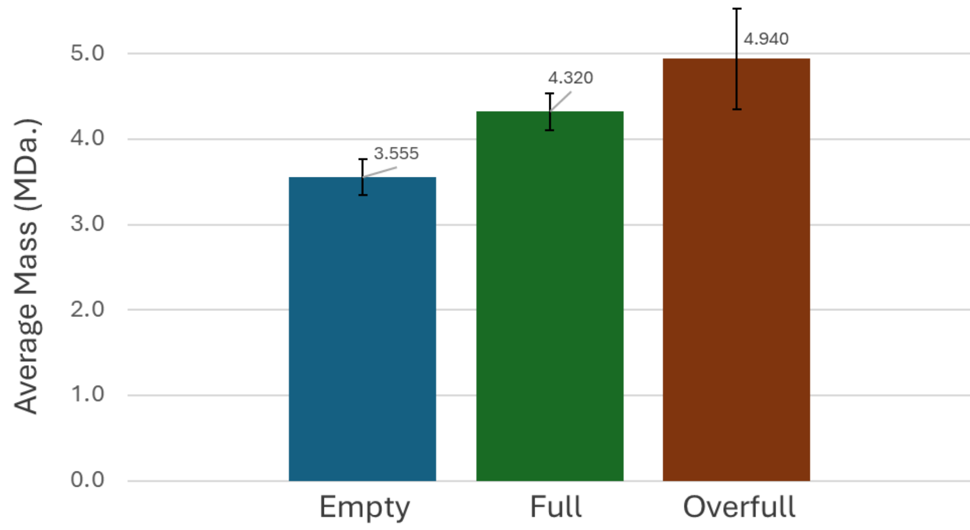


Figure 5: Average capsid mass across alternating concentration injections. Average measured mass of Empty, Full, and Overfull AAV capsid species obtained from repeated alternating injections of low (1 × 10^{11} vp/mL; upstream- equivalent) and high (5 × 10^{12} vp/mL; downstream-equivalent) concentrations. Bars represent mean mass values, with error bars indicating relative standard deviation (RSD). RSD values remain well below the 1% limit for all species, demonstrating high mass measurement precision across concentration and injection order.

CONCLUSION

- Direct charge and m/z measurements enable accurate, single-particle mass determination
- Mass precision below 1% RSD ensures highly consistent measurements across runs and days
- Peak abundance variability below 2% RSD demonstrates excellent signal stability and robust relative quantification
- Consistent relative quantification (<5% RSD) and mass precision (<1% RSD) are maintained across alternating upstream- and downstream-equivalent injections.
- A 95% first-pass success rate minimizes repeat injections and increases analytical throughput
- A 99% usable data rate across all runs delivers the reliability required for QC, comparability, and development studies

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