Enhancing AAV8 Analysis With Automated ELIT-CDMS Sample Delivery

Jakub Ujma[1] Rosie Upton[1], Emily Christofi[1], Anisha Haris[1]
[1]Waters Corporation, Wilmslow SK9 4AX, United Kingdom

INTRODUCTION

- **Charge Detection Mass Spectrometry (CDMS) is a powerful tool for analyzing adeno-associated virus (AAV) vectors, which often exist as heterogeneous mixtures of full, empty, overfull and partially packaged capsids.**
- **By measuring the mass-to-charge and charge of individual particles, CDMS with an Electrostatic Linear Ion Trap (ELIT) can overcome the limitations of conventional mass spectrometry to provide direct, high-resolution insight into genome packaging levels and product quality.**
- **Coupling CDMS with an autoloader further strengthens AAV workflows by enabling automated, consistent introduction of multiple samples. This improves throughput, minimizes handling variability, and reduces contamination risk which is critical when working with limited or high value AAV material.**
- **Here we present the results across five days for the characterization of alternating AAV8 Full and Empty injections to demonstrate injection-to-injection reproducibility and effective inter-injection flushing to prevent carryover.**

METHODS

SAMPLES: Empty AAV8 and full (CMVGFP) AAV8 capsid standards were sourced from Virovek Inc. at 2 × 10^{13} viral particles (vp)/mL. Samples were buffer exchanged into 200 mM ammonium acetate containing 0.01% Pluronic acid (F-68) using Biospin P-6 size-exclusion columns (Bio-Rad). Each sample was diluted down to 5 × 10^{12} vp/mL to represent the higher concentration of AAV sample typically analyzed by CDMS to fully assess the presence of any carryover.

CDMS: A prototype nESI Autoloader was coupled to the Waters Xevo™ CDMS system for delivery of samples and post-injection flushing of the nano-electrospray emitter. Ions were generated in positive ion mode, with a 100 ms trapping time and the data was processed using the Waters_Connect™ CDMS Toolkit app to generate relative quantification of the capsid ratios. Wash solutions were A) deionized water, B) magic mix (1:1:1:1 H_2O:IPA:MeOH:ACN), and C) 200 mM AmAc + 0.01% F-68 with a sequence of A, B, A, C performed between each AAV injection.

CDMS EXPERIMENTAL SETUP

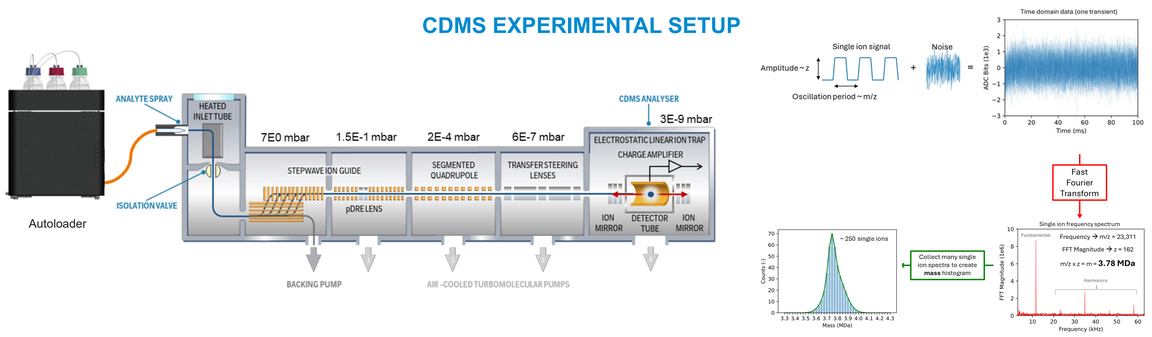

Figure 1. A schematic representation of the Waters ELIT-based CDMS instrument coupled to an autoloader with diagrams showing how m/z and z information is obtained, ultimately providing mass information.

CDMS Data Acquisition:

When an ion enters and progresses through the detection cylinder, the charge on the ion is induced on to the cylinder. A single ion will oscillate within the detector tube for the duration of the trapping event. 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 is proportional to the signal amplitude. Multiplying these two measurements together gives us the mass for each ion; *m/z* × *z* → **m.**

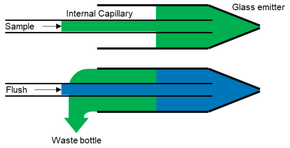

Figure 2. A schematic representation of the flushable nano-ESI capillary design. The capillary loads both sample and wash solvent into the emitter and the back pressure forces the previous sample backwards out of the tip towards waste, ready for the next sample.

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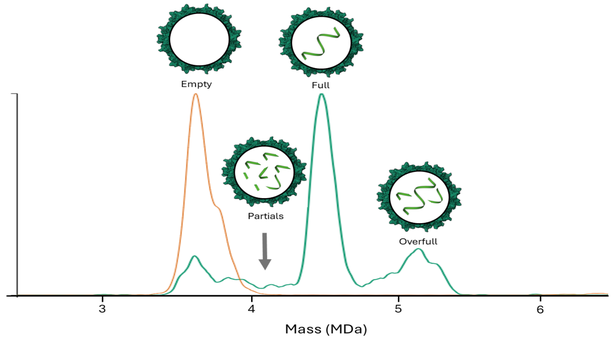
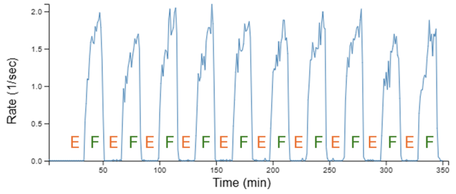


Figure 3. Example mass histograms for the AAV8 Empty (Green) and AAV8 Full (Orange) samples demonstrating the measured ratios of empty, partial, full and overfull capsids. AAV8 structure based on PDB structure 2QA0[1].

Figure 4. Extracted ion rate chromatogram for AAV8 Full, demonstrating the low levels of carryover in the AAV8 Empty injections.

***E** = Empty, **F** = Full*

Each sample was acquired for 15 minutes and all 20 injections were injected through a single nESI emitter.



DISCUSSION

Full AAV preparations inherently consist of a distribution of empty, partially-full, full, and overfull capsids (Figure 3), making it challenging to detect trace amounts of empty carryover within a full injection. In contrast, full capsids carried over into an empty injection produce a clear, easily identifiable signal. This asymmetry allowed us to sensitively quantify the level of residual full AAV8 in the following empty AAV8 injection. As shown in Figure 4 almost no carryover was observed and relative quantification levels of 0.07-0.13 % residual Full capsids were detected in the consecutive Empty injections. These trace levels demonstrate the effectiveness of the autoloaders wash routine (less than 1 minute) and fluid handling architecture in removing viral material between samples and therefore improves analysis throughput by automating these steps.

A key indicator of successful high-throughput operation is the preservation of expected population distributions without distortion or the appearance of anomalous intermediate masses; both of which could indicate contamination. Across injections, the expected AAV8 profiles remained clean and well resolved as shown in the examples in Figure 3. Tables 1 and 2 summarize the capsid ratios for Empty and Full variants across 50 injections of each, respectively. The low RSD across 5 days of analyses highlight the accuracy and reproducibility of the results which is essential for research, process development, and comparative studies requiring high analytical integrity.

The nature of nESI means native samples are prone to adduction in the gas phase, however this is consistently below 2.5% for AAV samples and so mass accuracy is good with little effect on relative quantification.

RESULTS

Table 1. Empty AAV8 capsid ratios measured from the average of 10 alternating injections of Empty AAV8 across 5 days. Average measured mass and mass error per day are also reported.

Day	Empty AAV8 Capsid Ratio Average R_1-R_{10} (%)	Measured Empty AAV8 Mass Average R_1-R_{10} (MDa)	Mass Error from theoretical (%)
1	98.27	3.6382	2.46
2	98.16	3.6610	1.85
3	97.51	3.6600	1.93
4	97.83	3.6485	2.18
5	98.09	3.6424	2.35
RSD	**0.31**	**0.27**	

Table 2. Full AAV8 capsid ratios measured from the average of 10 alternating injections of Full AAV8 across 5 days. Average measured mass and mass error per day are also reported.

Day	Full AAV8 Capsid Ratio Average R_1-R_{10} (%)	Measured Full AAV8 Mass Average R_1-R_{10} (MDa)	Mass Error from theoretical (%)
1	56.35	4.4606	1.32
2	53.44	4.4607	1.31
3	54.53	4.4665	1.18
4	56.37	4.4624	1.27
5	54.47	4.4691	1.13
RSD	**2.34**	**0.08**	

CONCLUSION

- **Multiple sample injections and wash sequences performed by a prototype nESI autoloader; increasing sample analysis throughput**
- **Negligible sample carry-over between injections**
- **Accurate intact mass determination for megadalton sized species and high degree of reproducibility across 5 days using a single emitter per day**

References
1. 2QA0: https://www.rcsb.org/structure/2QA0

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