A Streamlined Workflow for the Characterisation and Relative Quantification of Recombinant Adeno-Associated Viruses using Charge Detection Mass Spectrometry

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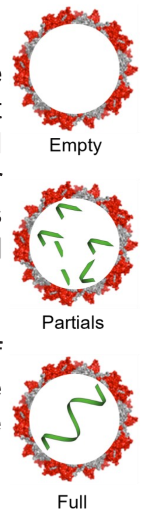
INTRODUCTION

- Recombinant adeno-associated viruses (rAAVs) are key vehicles for gene therapy, delivering therapeutic genes to target cells. Viral particles are packaged with a gene of interest that is delivered to specific tissue to impart a therapeutic effect.
- During manufacture, a range of capsid species are produced: empty (no payload), truncated or contaminant genomes (partials), intact gene of interest (full), and larger than anticipated genomes (overfull). Species other than fully packaged capsids are typically considered as product related impurities and may impact on clinical efficacy, safety, and immunogenicity.
- Analytical tools are required to quantify relative levels of empty, partial, full, and overfull species in order to guide product/process development, and to quantify the relative purity of clinical/commercial material.
- Charge detection mass spectrometry (CDMS), using an electrostatic linear ion trap (ELIT), is an ultra-high mass analytical technique which provides direct mass measurement of individual ions through simultaneous determination of their mass-to-charge ratio (*m/z*) and charge (*z*).



Herein, we demonstrate our dedicated CDMS software which offers a streamlined workflow for the assignment and relative quantification of AAV capsids, ensuring accuracy and efficiency. In addition, we compared AAV empty/intermediate/full analyses between CDMS, mass photometry (MP), multi angle light scattering (MALS), and analytical ultracentrifugation (AUC).

CDMS EXPERIMENTAL SETUP

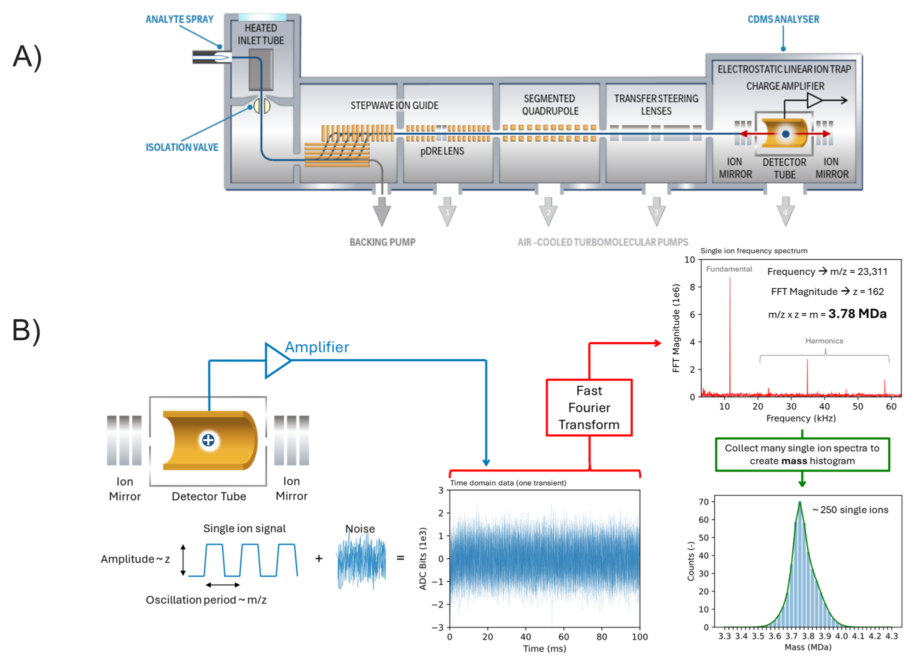


Figure 1. a) Schematic representation of the prototype ELIT-based CDMS instrument b) Diagram of the CDMS mass analyser and how m/z and z information is obtained.

- The CDMS mass analyser houses a conductive cylinder with two end caps, which reflect the ion back and forth.
- When an ion enters 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.
- $m/z \times z \rightarrow$ **m** for each ion

CDMS WATERS_CONNECT™ APP: AAV ANALYSIS WORKFLOW

Easily calculate Empty/Intermediate/Full/Full(+) percentages for AAVs

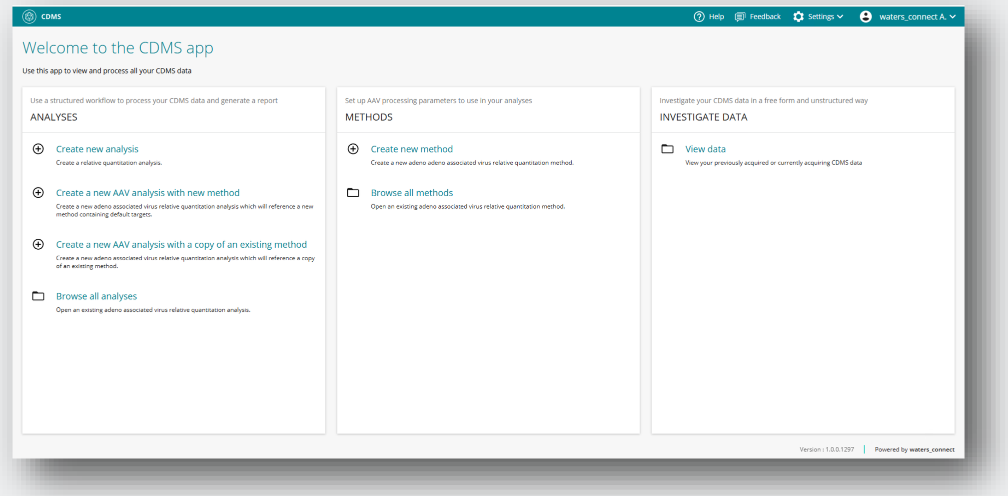
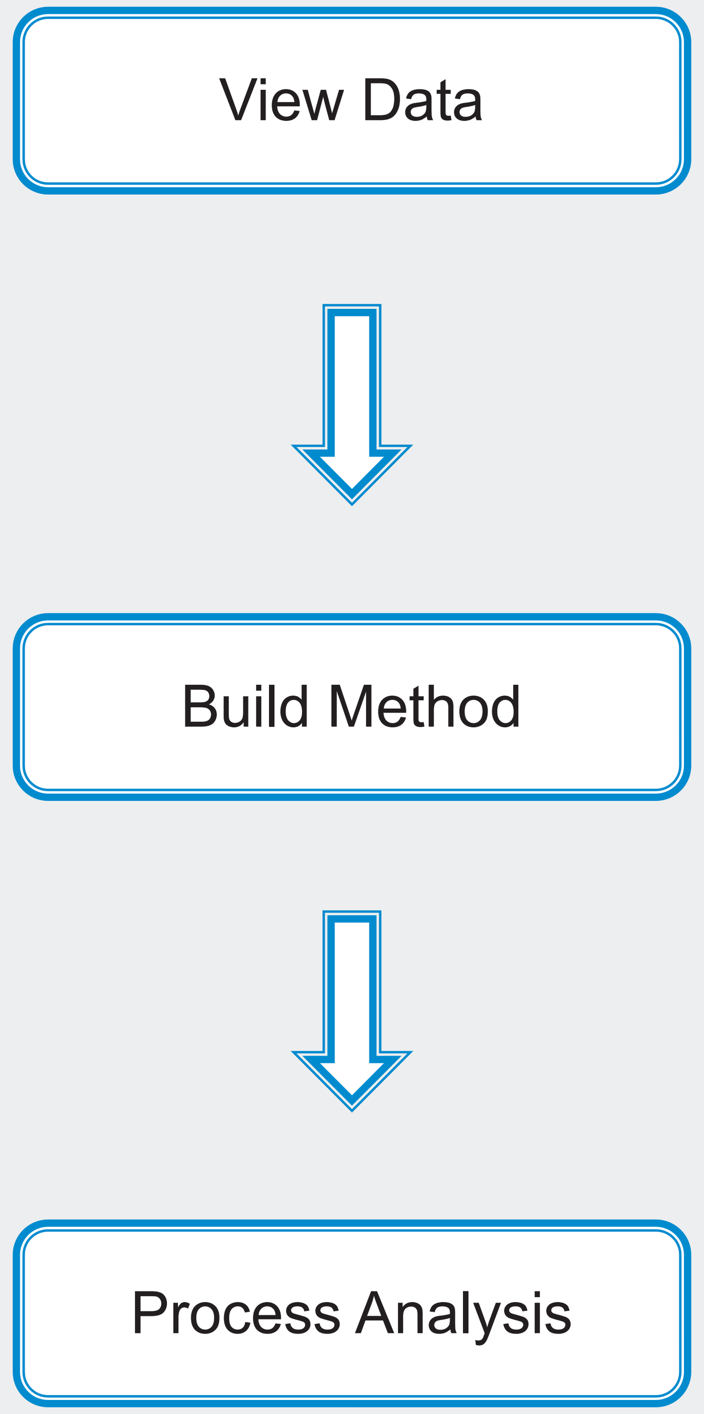


Figure 2: Landing page of the prototype waters_connect™ CDMS app



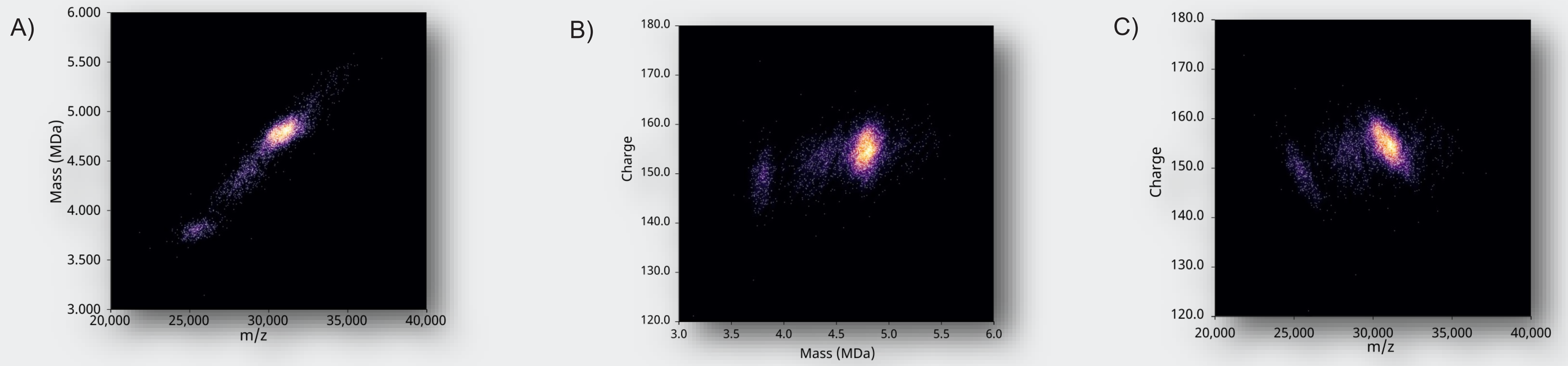


Figure 3: AAV8 Full Capsid in three different two-dimensional views A) mass vs mass/charge (m/z); B) mass vs charge (z); C) m/z vs charge (z)

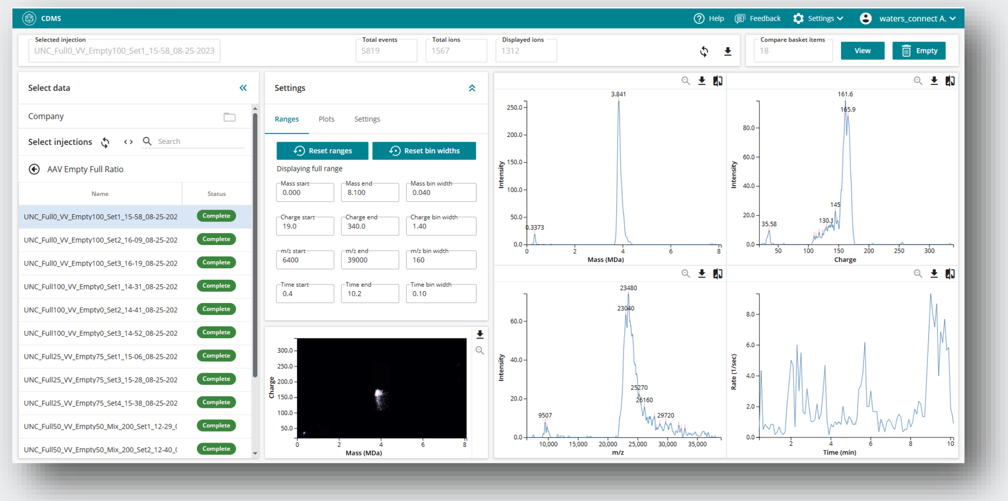


Figure 4: Investigate CDMS data in a free form and unstructured way

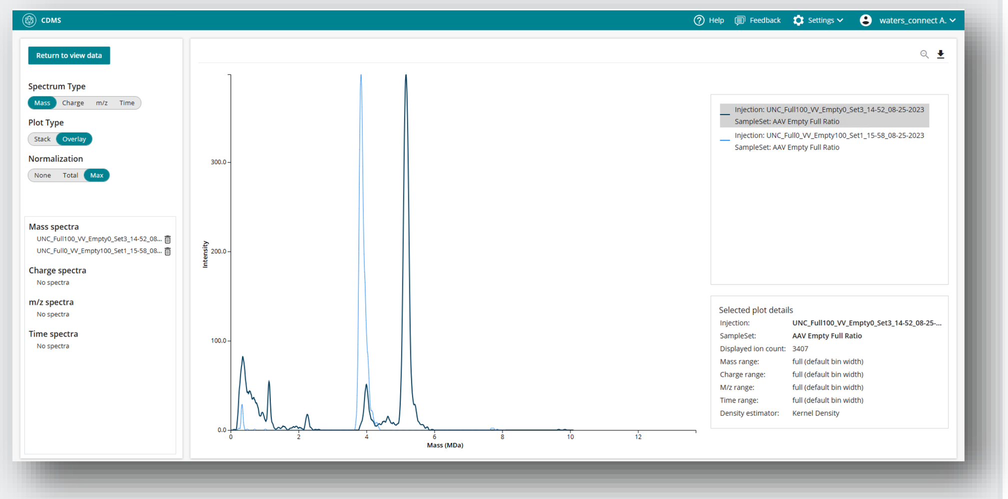


Figure 5: Compare data in any dimension. Adjust view (overlay or stacked) and normalize for direct comparisons

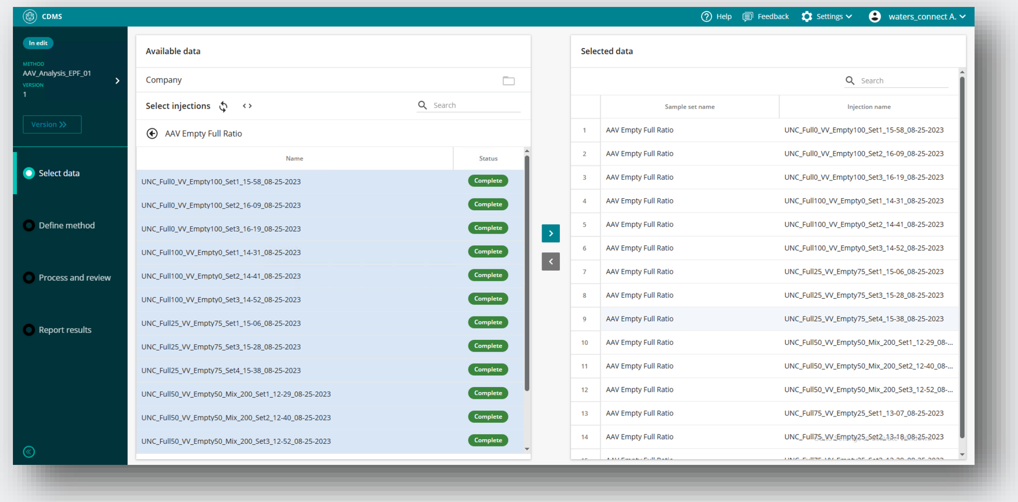


Figure 6: Select example data to build an analysis method

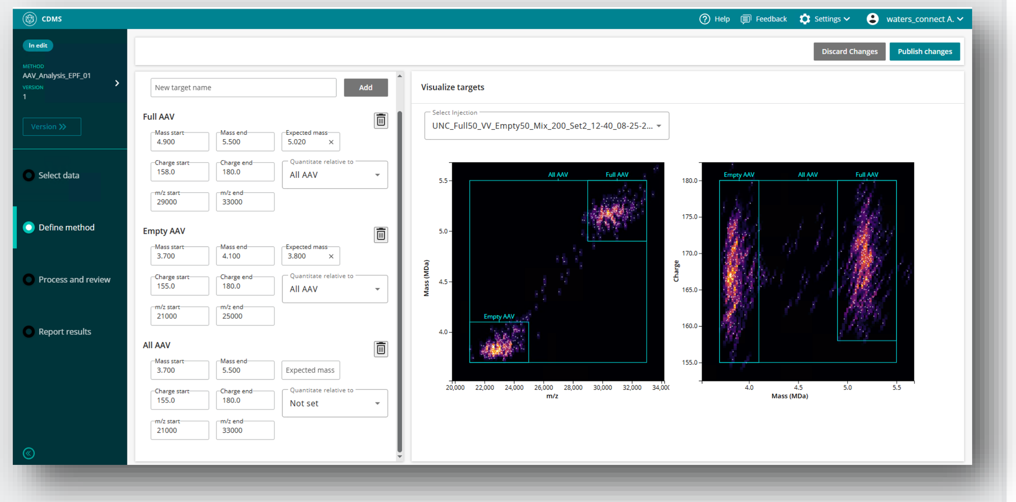


Figure 7: Define targets using mass, charge, and m/z ranges. Set up relative quantitation (calculated by ion count) and add expected masses.

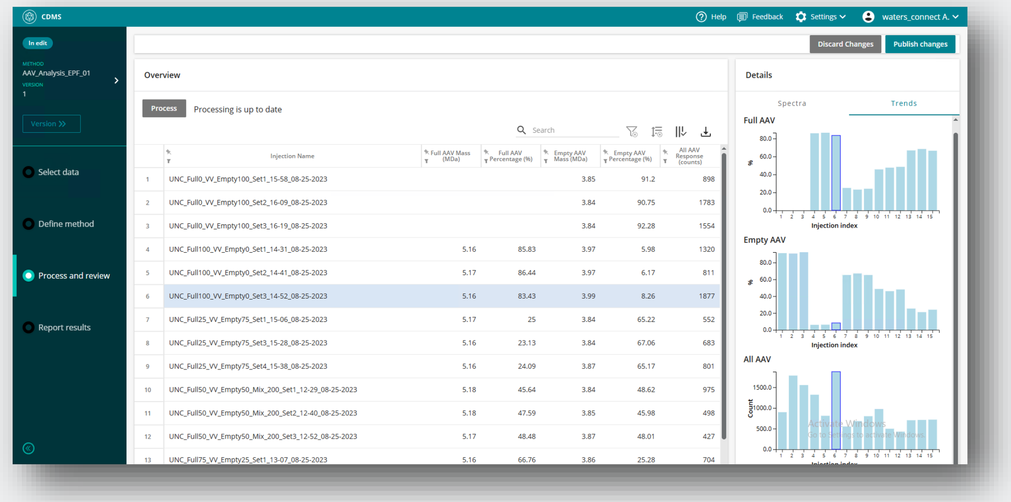


Figure 8: After processing data, review results in table format. Trends are visualized over injections for each target defined in the method.

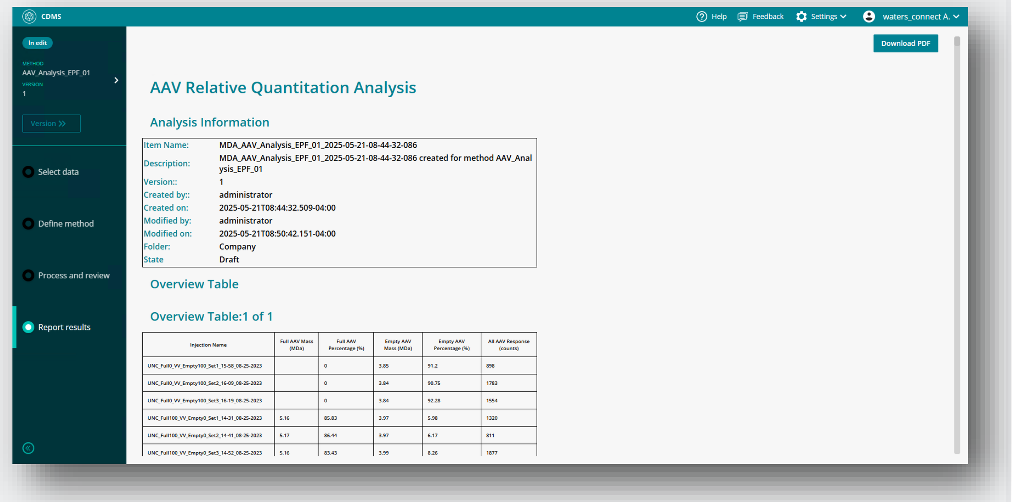


Figure 9: An analysis report is generated and can be downloaded in PDF format. The report includes info on each injection, a table of results , trend plots and plots for each individual injection based on target.

RESULTS

AAV8 Empty / Full Ratios series (by volume)

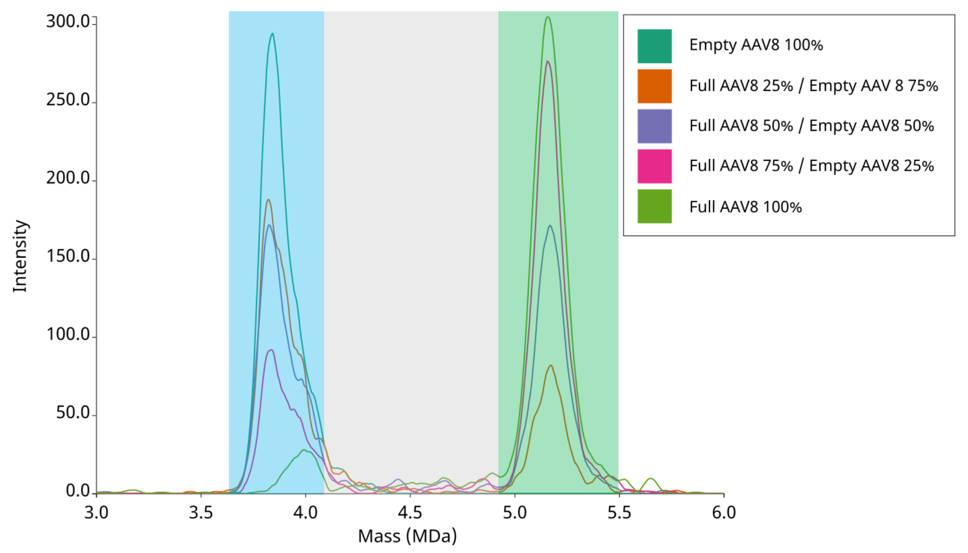


Figure 10: AAV8 empty/full dilution series using samples from Manufacturer #2. The colored overlays indicate where the boundaries were determined when defining targets in the analysis method. [Blue = Empty, Grey = Intermediate, and Green = Full]

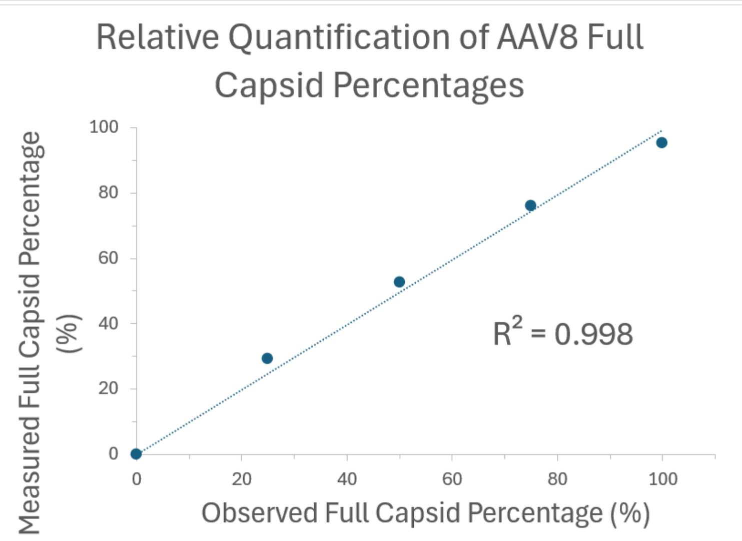


Figure 11: Relative quantification trend plot of the peak area of full capsid peaks from the CDMS mass peaks found in Figure 10

AAV8 Full / Empty standards evaluated by CDMS, MP, AUC, and SEC-MALS

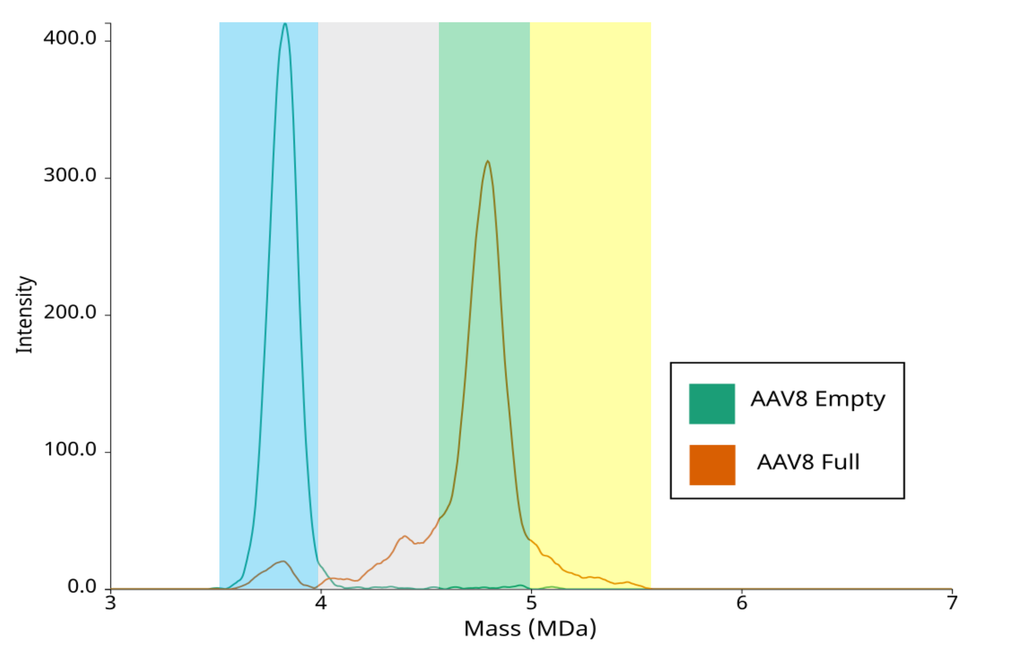


Figure 12: Mass spectra of the AAV8 empty standard and full standard sourced from Manufacturer #1; normalized to the total number of ions. The colored overlays indicate where the boundaries were determined when defining targets in the analysis method. [Blue = Empty; Grey = Intermediates; Green = Full; Yellow = Full (+)]

Empty/Full Ratios by CDMS, SEC-MALS, MP, and AUC

Attribute	CDMS (E/F)	SEC-MALS (E/F)	MP (E/F)	AUC (E/F)
Empty AAV8 %	99 / 4	98 / 2	95 / 9	88 / 3
Empty AAV8 MW (MDa)	3.82 / 3.	3.80 / -	3.67 / -	- / -
Full AAV8 %	<1 / 77	2 / 98	5 / 91	1 / 84
Full AAV MW (MDa)	- / 4.	- / 4.56	- / 4.	- / -
Intermediate AAV8 (%)	1 / 16	- / -	- / -	3 / 6
Full (+) AAV8 (%)	0 / 3	- / -	- / -	5 / 7

Table 1: Multiple analytical methods compared for the analysis of AAV8 standards (empty/full) from Manufacturer #1. Green triangles = AAV8 Empty Standard Values ; Blue triangles = AAV8 Full Standard. While initially, CDMS seems to deviate by almost 7% from AUC when calculating the full AAV8 percentage in the full AAV8 standard, if one were to sum the Full, Intermediate, and Full (+) contributions determined by each method, the values agree within 1% (97% by AUC, 96% by CDMS). (-) indicates that the value was not measured.

METHODS

AAV8 empty and full capsids were sourced from two manufacturers. SEC-MALS, MP, and AUC data were sourced from the Manufacturer #1's standard certificates. The samples were buffer exchanged into ammonium acetate solution with 0.01 % Pluronic™ F-68 (Gibco) using BioSpin® P-6 size-exclusion columns (Bio-Rad Laboratories, Inc). Ions were generated in positive ion mode using nano-electrospray ionisation and mass analysis was performed using a prototype ELIT-based CDMS. Signal processing and data visualization was performed using a prototype CDMS application for waters_connect™ software. Ions were trapped for 100 ms, and total acquisitions times were between 10 and 15 minutes. Detected time-domain signals were Fourier transformed, the measured frequency and the magnitude correspond to an individual ion's m/z and z respectively, enabling direct calculation of mass values. Data for individual ions were compiled in a histogram to generate m/z, charge and mass spectra as well as 2-dimensional heat-maps.

CONCLUSIONS

- With our dedicated CDMS waters_connect™ application software, we have developed a streamlined workflow for analyzing AAV capsid ratios.
- CDMS data is quite powerful providing several dimensions of data which helps give a complete picture of each injection.
- This software is not limited to AAV capsid analysis. Targets within the method can be ubiquitous and defined for any analyte of interest.
- Compared to other orthogonal methods, CDMS is more discerning to impurities (intermediate, full (+) capsids, high MW, low MW) in AAV products.

ACKNOWLEDGEMENTS

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