Method Qualification of Mass Photometry for the Determination of Full-to-Empty Capsid Ratio for rAAVs using the Refeyn System

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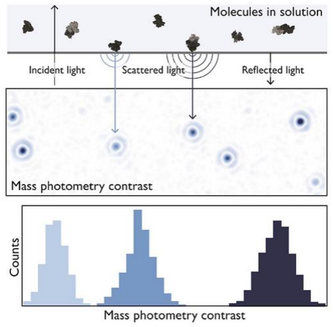
Abstract

The heterogeneity of packaging in the production of recombinant adeno-associated viruses (rAAV) leads to capsids that can contain DNA fragments of different lengths. Methods for calculating full-to-empty ratios often place capsids into two broad categories: capsids containing DNA of approximately the size of the full-length vector ('full') and capsids which contain little to no DNA ('empty'). Manufacturing processes should deliver consistent full-to-empty ratios, and methods to monitor this critical quality attribute are needed, because it may affect both safety and efficacy of drug product. Using mass photometry (MP) represents a straightforward platform for analysis of full-to-empty-ratios in an rAAV sample in just minutes with very little sample. Because MP measures the mass of individual biomolecular particles but is not sensitive to particle size or shape, it seems to be an ideal tool for quantifying rAAV capsid loading independent of the serotype.
MP builds on the principles of interference reflection microscopy and interferometric scattering microscopy. It enables the accurate mass measurement of single molecules in solution, in their native state and without the need for labels.

To demonstrate that this method is fit-for-purpose in monitoring the full-to-empty ratio of rAAV samples, we performed method qualification according to the principles of ICHQ2 (R1) using rAAV samples in matrices and buffers commonly used in the production process.

Mass Photometry



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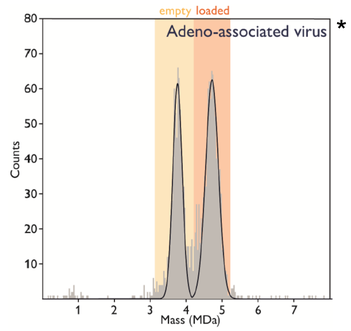
The light scattered by a molecule interferes with reflected light on a measurement surface. The interference signal scales linearly with mass.

Measuring AAV molecules with light



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Empty capsids (~3.7MDa) and DNA-loaded capsids (~1 MDa mass increase for an approx. 3K bp vg).

Refeyn Two™

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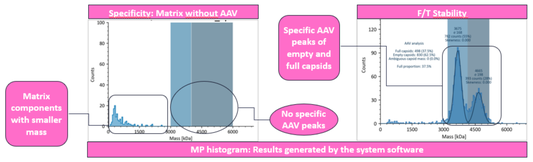
The second-generation mass photometry provides an improved differentiation of empty and DNA-loaded capsids.

*Image source: "Refeyn Two™ Mass Photometer" data sheet.

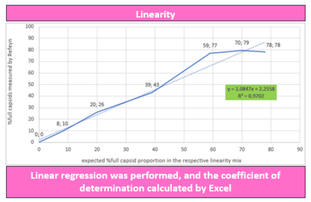
Qualification parameter based on ICHQ2 (R1)

Robustness	Repeatability	Interm. Precision	Stability	Linearity	Specificity	Accuracy
Robustness during sample processing	Precision of multiple measurements in one assay	Precision across four independent assays and 3 operators	Stability of results after FT- cycles	Identification of the linear range	Testing for non-specific signals	Inferred if the method is precise, linear and specific

Qualification results of specificity and linearity

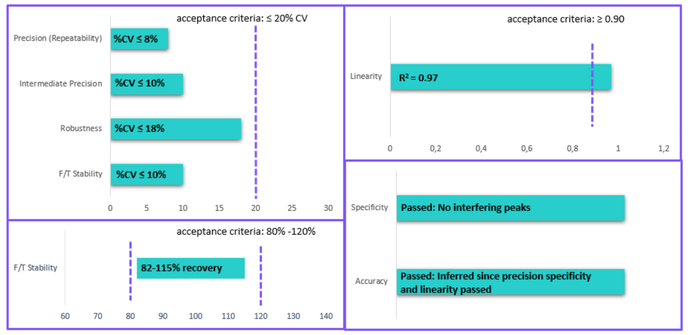


No interfering peaks were detected in the AAV-specific areas when matrix alone was measured.



Two samples (1: 0% full; 2: 78% full) were mixed in 7 different ratios. Measured results were plotted against the theoretical %full capsids proportion. Linearity passed the acceptance criteria of $R^2 \geq 0.90$.

Qualification results summary



All tested parameters are within the acceptance criteria. Method is qualified for samples purified by the Ascend commercial DSP process.

Summary

This in-house qualification was carried out by three different operators on the Refeyn Two™ system within two weeks.
During this method qualification all tested parameters were verified for the determination of the proportion of full and empty capsids in the samples of interest.
The method for quantifying the proportion of full and empty capsids by mass photometry is considered suitable for its intended purpose.

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