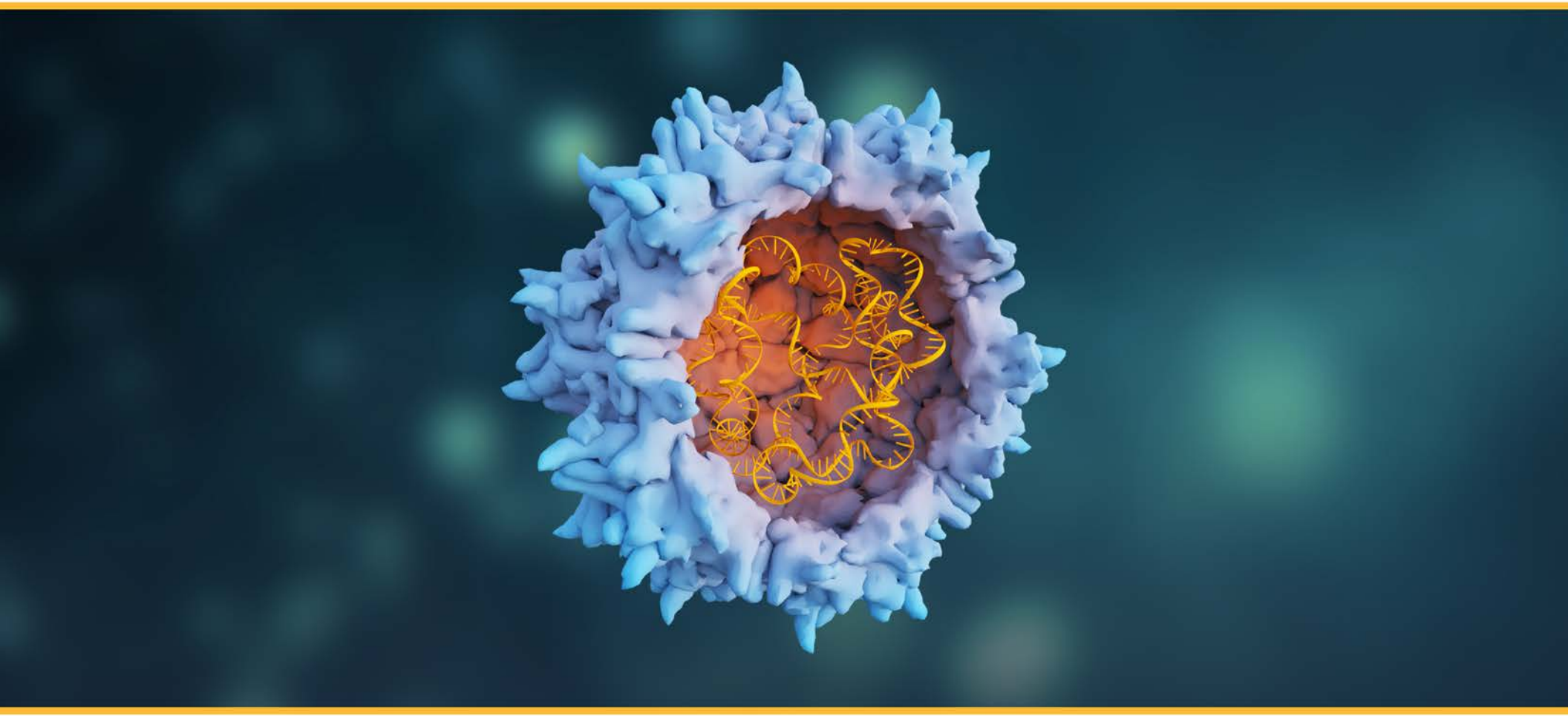
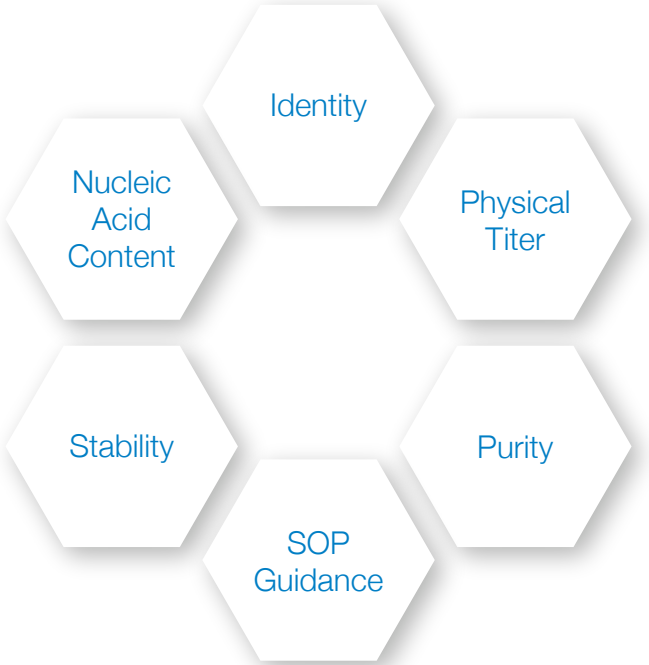
Solutions for AAV Characterization

Determine total concentration, capsid content, stability, purity and more with unmatched precision



Waters™ | WYATT TECHNOLOGY

End-to-end AAV Analytics



Wyatt Technology provides the instruments and methods you need for quantifying multiple critical quality attributes of AAV-based therapies throughout the product life cycle. Our solutions are simple to learn and operate, yet directly access the core biophysical properties that define these CQAs.

These techniques—size-exclusion chromatography with multi-angle light scattering (SEC-MALS), high-throughput dynamic light scattering (HT-DLS) and field-flow fractionation with MALS (FFF-MALS)—are rapid, fully automated and require little hands-on effort.

With SEC-MALS, HT-DLS and FFF-MALS you can:

- Quantify absolute particle concentration and capsid content without the need for error-prone reagents like primers or antibodies
- Identify aggregates and other impurities via complementary and orthogonal light scattering methods
- Perform high-throughput measurements of size, stability and quantity across process development, formulation development and QC

The DAWN™ multi-angle light scattering instrument determines molar mass, size and concentration of macromolecules and nanoparticles.

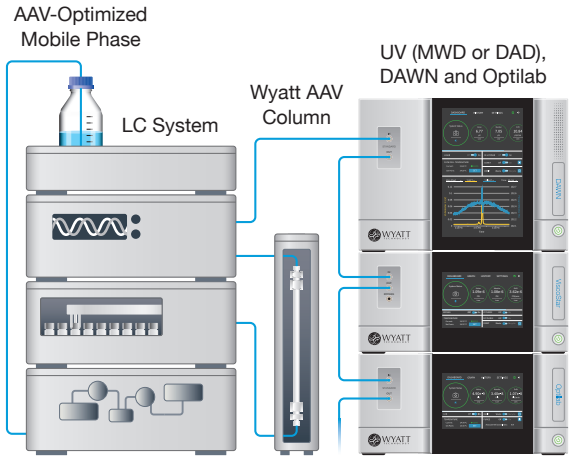
The Eclipse™ field-flow fractionation instrument separates particles, including AAV monomers and aggregates, beyond the capabilities of size-exclusion chromatography.

The DynaPro™ Plate Reader uses dynamic and static light scattering to measure size and concentration of molecules and particles from nanometers to microns.

Quantify CQAs with SEC-MALS

SEC-MALS is the premier technique for biophysical characterization of AAV. It is based on a standard HP-SEC setup with DAWN MALS and Optilab™ refractive index (RI) detectors from Wyatt plus an online multi-wavelength UV detector. Operation is similar to standard chromatography.

Wyatt provides a comprehensive package that includes instrumentation, application-specific software, an SEC column for AAV separation and a full complement of implementation with an optimized method, training and detailed guidance for constructing a standard operating procedure (SOP) for quality control.

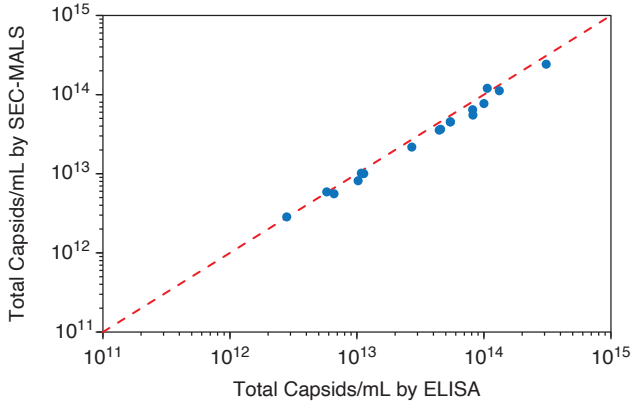


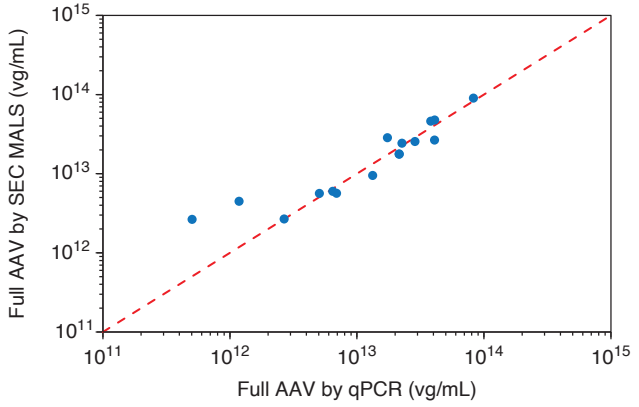
Replace two or more methods with a single, robust solution

Analysis of AAV with SEC-MALS provides all of these CQAs in a single assay:

- Total concentration
- Capsid content (full to total ratio, Vg/Cp)
- Identity (from molar mass)
- Purity (percent aggregate)

SEC-MALS replaces outmoded assays that suffer from low precision and sample preparation artifacts. Whereas qPCR results may vary within a factor of 1.5× to 3× from run to run and ddPCR precision is no better than 15%, SEC-MALS provides absolute molar mass and concentration to within 5%. Eliminating reliance on primers and antibody reagents creates an AAV quantitation method that can be easily transferred across departments and across sites while eliminating extensive hands-on time and sample preparation artifacts.





Figures: Data comparing measurements of total capsids by SEC-MALS and ELISA, and full AAV concentration by SEC-MALS and qPCR, were kindly provided by PTC Therapeutics.

Replace two or more methods with a single, robust solution

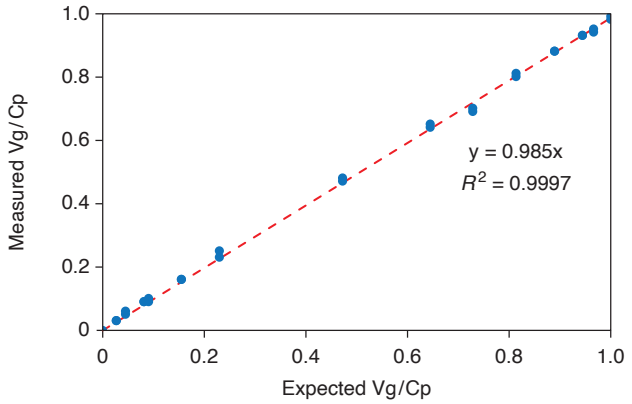
Measurements from MALS, UV and RI detectors, combined with Wyatt's ASTRA™ software for data collection, rigorous analysis and customizable reporting, provide reliable results independent of serotype. Sample consumption is minimal, typically 1 to 5 µL injected per run. SEC-MALS studies, performed with Wyatt's AAV separation column and AAV-optimized mobile phase, can successfully quantify and characterize samples containing as few as 10^9 capsids in the injection volume.

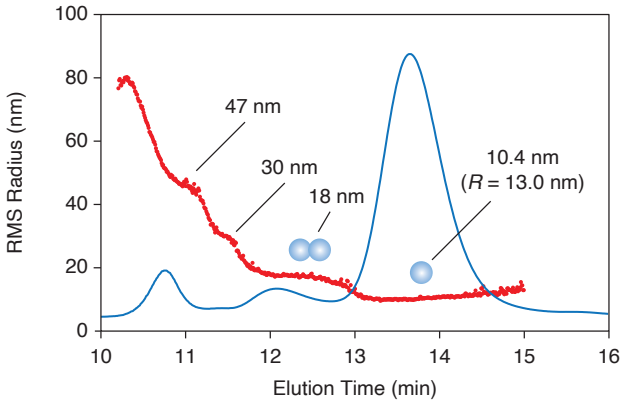
Light scattering's wide dynamic range makes these techniques suitable for early stage characterization, where sample quantity may be scarce, as well as later stage formulations with concentrations as high as 10^{14} particles/mL with no additional dilution. Even small changes to the Vg/Cp ratio are identified with high precision across the entire range.

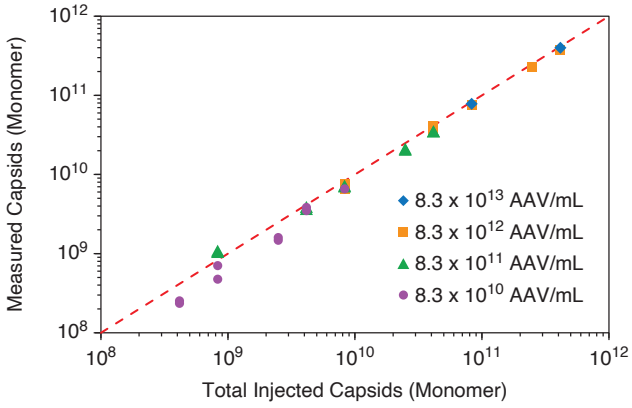
Advanced analytical capabilities

The Viral Vector Analysis method is a dedicated algorithm (patent pending) in ASTRA that provides serotype-independent measurement of total particle (capsid) concentration, capsid content, fraction of full and empty capsids, and even the molar masses of the protein and nucleic acid portions. Results from multiple injections and samples are automatically collated in one EASI Table, which also provides statistical analysis. Graphical data plots such as light scattering chromatogram with size calculations shown here may be overlaid in EASI Graph for a quick comparison.

An optional 21CFR11-compliant module in ASTRA means that SEC-MALS is readily positioned for at-line process analytics and final QC release assays, where MALS is combined with UV_{260} and UV_{280} concentration measurement for rock-solid CQA results.







Top figure: SEC-MALS measurements of Vg/Cp on samples prepared by mixing completely empty and completely full AAVs.

Middle figure: Identification of AAV monomer and oligomers by rms radius in a SEC-MALS run.

Bottom figure: SEC-MALS measurements of total capsid particles using samples prepared by diluting well-characterized AAV solutions.

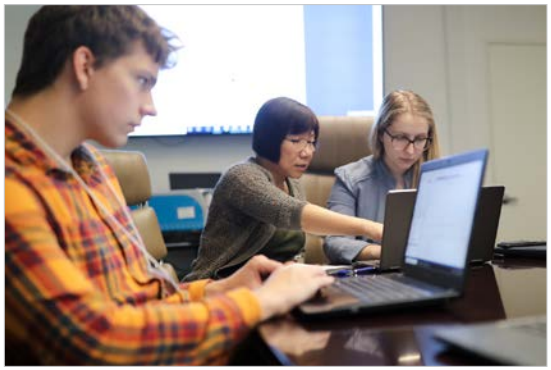
Adoption and Implementation

Additional resources to facilitate transition to productivity

AAV METHOD IMPLEMENTATION & TRAINING

Expert guidance for rapid deployment

Optimizing a method and determining suitability criteria for validation can be the biggest stumbling blocks when incorporating a new technology into your lab. We created the *AAV SOP Guidance Manual* and developed the on-site *AAV Method Implementation & Training Service* for SEC-MALS in consultation with AAV industry leaders. The complete service package provides a turnkey SEC-MALS method for quantifying titer, full:empty ratio and aggregate content. Personalized on-site instruction helps you quickly climb the curve towards method optimization, validation and productivity.



The sure route to implementing a robust SOP

SEC-MALS utilizing Wyatt's cutting-edge instruments, a fit-for-purpose SEC column and ASTRA software's *Viral Vector Analysis* method are the foundation for multi-CQA quantification. The AAV Method Implementation & Training Service and SOP Guidance Manual proceed step-by-step, beginning with the principles of the method and on to materials and reagents data analysis and interpretation, troubleshooting and suitability criteria. Upon completion you will be ready to implement your custom SOP in product and process development, all the way through QC.

Platform-ready

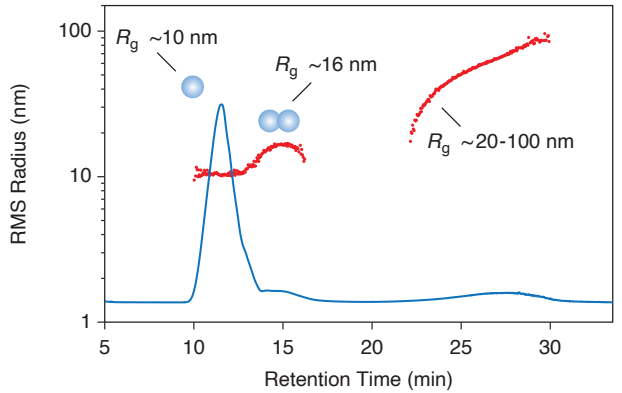
Utilizing standard analytical instrumentation, SEC-MALS requires no reagents or special sample preparation, is fully automated and GMP-compliant. These benefits along with Wyatt's full support for implementation, SOP development, training and cross-lab method transfer makes it an ideal method for your AAV platform technology.

Extended Characterization

Wyatt's solutions for AAVs include a set of orthogonal and complementary methods, to address analyses beyond the CQAs covered by SEC-MALS.

- FFF-MALS, performed with an Eclipse FFF system and DAWN MALS instrument, resolves and quantifies aggregates that may be removed by the SEC column.
- HT-DLS is essential to screen AAV quality and quantity in formulation and stability testing, as well as at-line process analytics.

These techniques reveal the effect of capsid mutations, accelerated stress conditions and more. The FFF-MALS analysis to the right highlights identification of aggregates that are too large to separate by SEC or that may dissociate due to column shear.

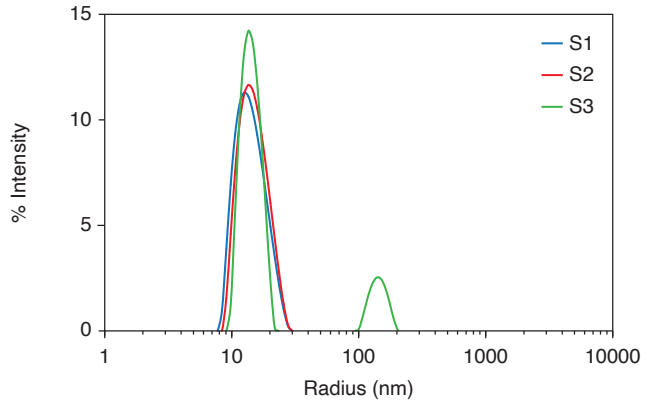


Extended quantification of AAV aggregates by FFF-MALS.

Screen quality and stability by HT-DLS

HT-DLS using a DynaPro Plate Reader provides nondestructive quantitation and characterization with concentrations as low as 6×10^{10} AAV/mL and volumes down to 4 µL, all in standard microwell plates. The size distribution and particle concentration are obtained for each well, automatically, in less than 30 seconds, making it feasible to screen dozens or hundreds of conditions. Temperature may be held constant to study isothermal aggregation rates, or ramped to identify the onset temperature of degradation.

DYNAMICS™ is a full-featured package for collecting, analyzing and reporting data acquired with a DynaPro DLS instrument. Analyses include particle concentration, thermal stability, colloidal stability, formulation development and aggregate screening. A 21 CFR 11-compliant version is available.



	Monomer (Main Species)		Aggregates	
	Radius (nm)	Particle Concentration (mL^{-1})	Radius (nm)	Particle Concentration (mL^{-1})
S1	14.7 ± 0.7	(8.2 ± 0.6) x 10^{13}	–	–
S2	15.6 ± 0.1	(2.7 ± 0.1) x 10^{13}	–	–
S3	14.2 ± 0.2	(6.9 ± 0.3) x 10^{13}	118.2 ± 4.2	(1.3 ± 0.1) x 10^{7}

Aggregation, capsid concentration and stability screening of AAV formulations by HT-DLS.

RT-MALS for PAT

Real-time MALS utilizes an ultraDAWN™ in-line or on-line with downstream processes such as chromatographic purification or UF/DF to monitor AAV product attributes including Vg/Cp, titer and molar mass. RT-MALS accelerates process development and improves yield and quality during production.

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