TOSOH

Sensitive Quantification of AAVs and their Impurities

Your Challenge

- You need a method that detects AAVs at low titers.
- You need to separate AAVs from impurities.

Our Solution

LenS3™ MALS detector & TSKgel® GMPWXL column

- Sensitive detector and reliable separations

What was done?

- AAV5 and AAV8 samples were separated by size exclusion chromatography and detected by MALS

What was the result?

- Limit of detection for AAV5 is 8x10^9 vc & host-cell impurities are efficiently separated from AAVs

Determine down to 8x10^9 AAV capsids and separate impurities of AAV samples by combining the efficient separation by a mixed bed SEC column and the highly sensitive MALS detector LenS3.

Your Benefit

Reduce sample volume and detect lowest AAV titers

Analysis of AAVs

TOSOH BIOSCIENCE

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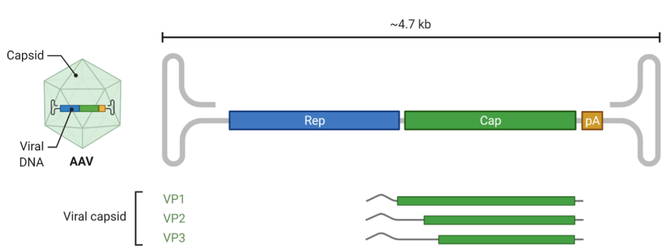
https://www.separations.eu.tosohbioscience.com/solutions/detectors

SEC-MALS Sensitively Quantifies Adeno-Associated Viruses and Detects Impurities

Cell and gene therapy in medicine relies on efficient and specific delivery of therapeutic genetic material to target cells. These delivery devices are called vectors, and many have been developed from viruses[1]. The parvovirus family of viruses called adeno-associated viruses (AAVs), have emerged as the vector of choice in therapeutic gene delivery due to their optimized tissue and cell specificity and their ability to edit the genome precisely. AAVs are non-enveloped viruses with an icosahedral structure belonging to the parvovirus family and containing single-stranded DNA (ssDNA) (~4.7 kb in length) as their genome (*Figure 1*). To date, 13 AAV serotypes and >100 variants have been identified with variable tissue tropism[2-4].

Figure 1. Graphic representation of a wild-type AAV and its genome.



The viral ssDNA cassette contains replicating genes (Rep) for virus multiplication, capsid genes (Cap), and poly(A) tail (pA). Cap encodes three structural viral proteins VP1, VP2, and VP3, needed for the capsid assembly[3-4].
The figure was created using BioRender.com from the original source: https://www.dynotx.com/introduction-to-aav-as-a-gene-therapy-vector-part-1/

Development of clinically desirable AAV capsids with optimal genome design requires rapid, accurate, and robust analytical methods to assess AAV purity, capsid titer, DNA content, and structure-activity relationships[5]. Size exclusion chromatography (SEC), when coupled with multi-angle light scattering (MALS), offers a powerful analytical method for AAV characterization. In contrast to the traditional method of AAV analysis using the UV 260/280 nm absorbance ratio, the advantage of MALS detection is the high sensitivity for AAVs due to their considerable capsid mass (~3.7 MDa), inherently providing a strong light scattering response for analytical characterization. Here, we describe the use of SEC-MALS to quantify AAV capsid titers and to determine sample impurities.

Experimental Conditions

Samples

Purified AAV5 (2.0 x 10^{13} viral capsids (vc)/mL) was purchased from Virovek (Hayward, CA). AAV8-containing human embryonic kidney (HEK) cell culture supernatant (titer 1.5 x 10^{12} vc/mL) was from ExcellGene SA (Monthey, Switzerland). Bovine serum albumin (BSA) standard was purchased from Thermo Scientific (Rockford, IL).

SEC-MALS Analysis

AAV samples were analyzed on a TSKgel $GMPW_{XL}$ SEC column coupled with the $LenS_3$ MALS detector. Capsid titers during analyses were as follows: AAV5 at 2.0 × 10^{11} vc/mL was used to compare light scattering signals (right, low, and high angles) to UV absorbance at 280 nm. For LOQ/LOD determination, AAV5 dilutions from 1.0 × 10^{12} vc/mL to 1.6 × 10^{9} vc/mL were used. AAV8-containing supernatant was tested at 1.5 × 10^{12} vc/mL and 1.5 × 10^{11} vc/mL titers. The SEC-MALS system was calibrated with BSA prior to sample analysis to determine the detectors' offsets, band-broadening parameters, and response factors. All data were processed and analyzed using the SECview software.

Instrument:	Thermo Fisher Scientific UltiMate™ 3000 U/HPLC
Column:	TSKgel $GMPW_{XL}$ (7.8 mm ID × 30 cm, 13 µm particles, pores 125-1000 Å)
Detectors:	(1) Ultimate 3000 variable wavelength detector (VWD) at 280 nm (2) Tosoh $LenS_3$ MALS detector
Mobile phase:	50 mmol/L HEPES, 150 mmol/L NaCl, pH 7.1
Flow rate:	0.5 mL/min
Temperature:	25 °C
Injection vol.:	40-50 µL

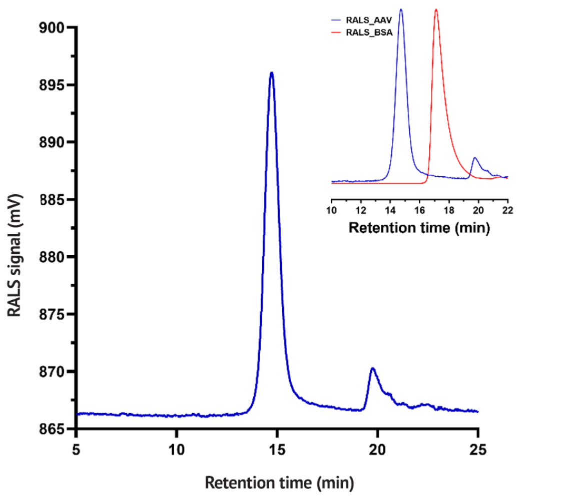
Results and Discussion

SEC-MALS Analysis

For the 22-25 nm sized AAV capsids, a TSKgel $GMPW_{XL}$ analytical column was chosen for separation due to its mixed pore size distribution up to 1000 Å. This SEC column enables excellent resolution for the analysis of AAVs, and their separation from accompanying impurities (cell culture media components, aggregated or degraded capsids, etc.).

Figure 2 shows the right-angle light scattering (RALS) signals for AAV5 and BSA. Characterization of AAV5 by SEC-MALS using the TSKgel GMPWXL column revealed a major AAV5 monomer peak, with a known capsid size of ~3.7 MDa for AAVs6, eluting at 14-15 min retention time. Some unassembled capsid proteins and other components were detected at ~20 min retention time. BSA (66 kDa), which was used for system calibration, eluted significantly later than AAV5 due to its much smaller size (insert in *Figure 2*).

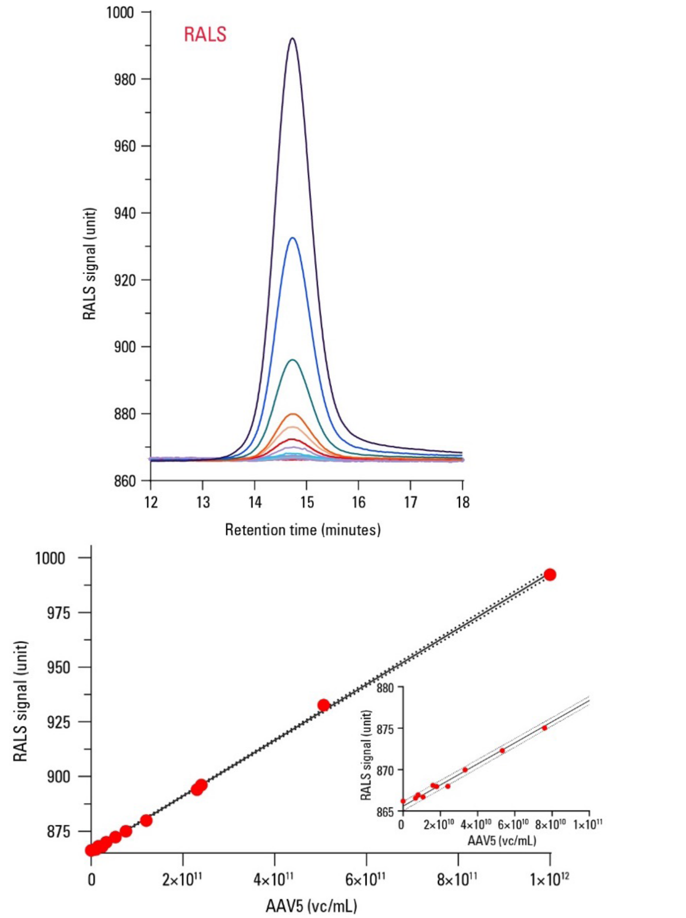
Figure 2. SEC-MALS detection (RALS) of AAV5 and BSA.



MALS vs. UV Absorbance at 280 nm

Due to the high molecular weight (MW) of AAVs, the MW-dependent response of light scattering provided much higher sensitivity than the regular UV absorbance at 280 nm, as illustrated in *Figure 3*. When analyzing AAV5 at 2 x 10^{11} vc/mL (40 µL injection, 8 x 10^{9} vc total loaded), no UV signal was detected at 280 nm, whereas strong MALS signals (signal/noise ratio of 10 to 60) were obtained at three different light scattering angles: RALS (90°), LALS (10°), HALS (170°).

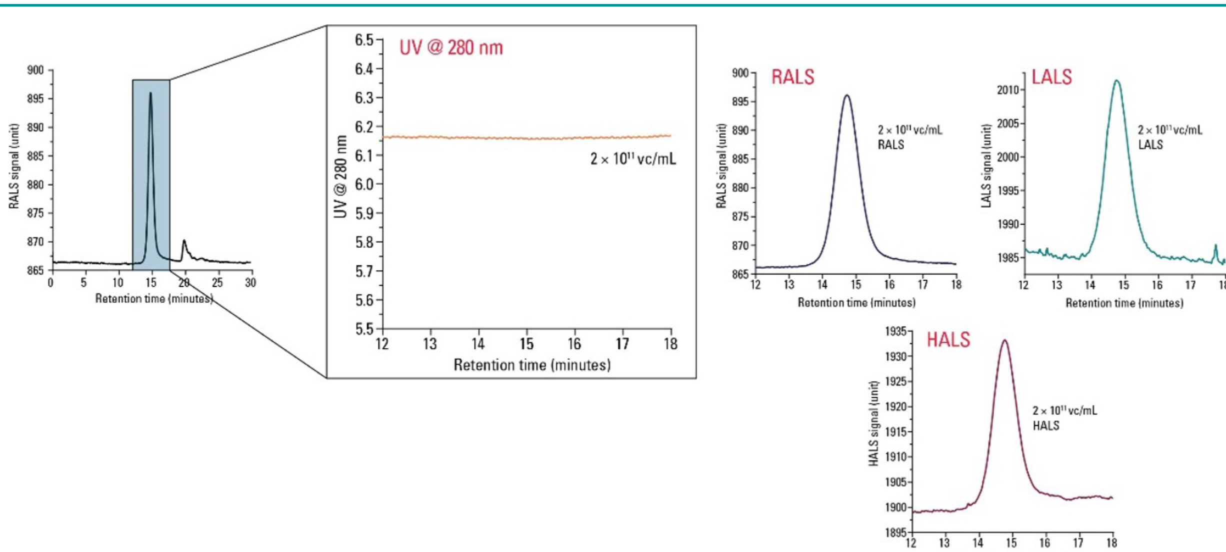
Figure 4. Limit of detection by RALS signal.



Linearity and Sensitivity

The linearity and sensitivity of AAV detection by MALS were characterized by determining the limit of detection/limit of quantitation (LOD/LOQ) using serial dilutions from the starting AAV5 concentration of 1.0 x 10^{12} vc/mL and loading 40 µL injections onto the TSKgel GMPWXL column (*Figure 4*).

Figure 3. Comparison of RALS, LALS, and HALS signals to UV @ 280 nm for AAV5.



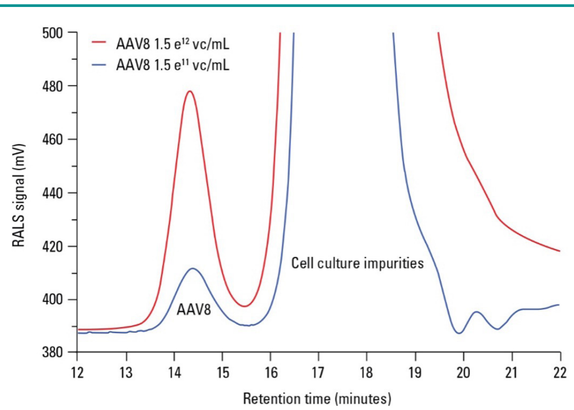
The left panel shows 40 µL injections of serially diluted samples from 1.0 x 10^{12} vc/mL (4.0 x 10^{10} vc loaded) to 1.6 x 10^{9} vc/mL (6.4 x 10^{7} vc loaded). The right panel shows the linearity of RALS signal from the same dilutions. The insert in the right panel magnifies the plot with the lowest AAV5 concentrations. As low as 7.0 x 10^{9} vc/mL AAV5 samples (2.8 x 10^{8} vc total loaded) were detectable by SEC-MALS using the LenS3 MALS detector's signal (RALS). The LOQ was then calculated from the LOD to be 2.3 x 10^{10} vc/mL (9.2 x 10^{8} vc total loaded). Additionally, RALS detection on the LenS3 provided a strong linear response across a large concentration range, allowing the determination of capsid titers.

AAV8-containing Cell Culture Supernatant

An additional challenge of AAV analysis is to achieve efficient separation of AAVs from the remaining impurities to allow the quantification of AAV capsids. To test the separation performance, we analyzed AAV8 directly from the HEK cell culture media without prior AAV8 purification using TSKgel GMPWXL column.

Figure 5 shows the chromatographic separation of AAV8 capsids with the elution peak at ~14.5 min retention time whereas the HEK cell culture impurities mainly eluted at 16-20 min retention time, with additional tailing at higher loading concentration. The injection of two different AAV8 capsid loads (50 µL of 1.5 x 10^{12} vc/mL and 50 µL of 1.5 x 10^{11} vc/mL) illustrates the concentration-dependent response of the RALS signal, allowing AAV separation and quantification from more complex sample matrices. AAV8 at both concentrations shows a clear peak whereas cell culture impurities saturated the RALS signal (upper part not shown).

Figure 5. SEC-MALS analysis of AAV8-containing cell culture supernatant.



Featured Products		
0008025	TSKgel GMPWXL	7.8 mm ID × 30 cm, 13 µm, 12.5-100 nm pore size
0040000	LenS3 MALS detector	

Conclusion

A rapid and reproducible analytical methodology is crucial for the timely development of high-quality tools for cell and gene therapy applications. This application note describes a powerful analytical SEC-MALS technique for the sensitive detection of AAVs using the LenS3 MALS detector and the TSKgel GMPWXL column. The limit of detection (LOD) for the method was determined to be as low as 7.0 x 10^{9} vc/mL (2.8 x 10^{8} vc loaded in 40 µL). The high sensitivity of SEC-MALS detection allows the injection of low-concentration AAV samples which is a highly sought attribute in the early development stages with often limited availability of material. In addition, AAV separation directly from HEK cell culture supernatant was possible enabling direct MALS detection without prior AAV purification. In summary, the SEC-MALS methodology described here provides a sensitive, powerful, and robust tool for the detection of AAVs during product development, viral vector production, and throughout quality control. Analytical characterization (virus size, molecular weight, empty/full ratio) based on SEC-MALS data will be fully described in a separate application note.

References

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