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LC columns

SurePac Bio 550 SEC MDi columns

Product manual

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Contents

Introduction

Introduction to SurePac Bio 550 SEC MDi columns

The Thermo Scientific™ SurePac™ Bio 550 SEC MDi Columns are well-suited for the analysis of adeno-associated viruses (AAVs) monomeric capsids and high molecular weight species high molecular weight species (HMWS). The monodispersed silica particles are covalently modified with a proprietary diol hydrophilic layer. This proprietary process brings an extremely low level of non-desired interaction sites. Compared to traditional polydisperse particles, the monodisperse particles have a consistent size distribution (Figure 1) facilitates precise control over media synthesis and column packing, significantly improves column-to-column and lot-to-lot reproducibility. The columns are housed in state-of-the-art hydrophilic-coated stainless-steel hardware. The hydrophilic coating reduces secondary interactions, ensuring optimal performance during the initial injection process. Films are conformal to the hardware with a minimum thickness of 400 nm, ensuring no sample interactions with the underlying stainless-steel hardware. Variations in film thickness over 10 of nanometers may result in color differences ranging from yellow to purple and are not indicative of incomplete coating. Variation in color may also be observed depending on the viewing angle of the hardware. The reproducible particle size and chemistry, combined with controlled synthetic and packing manufacturing processes, make the SurePac Bio 550 SEC MDi column capable of analyzing AAV samples with high resolution and excellent reproducibility.

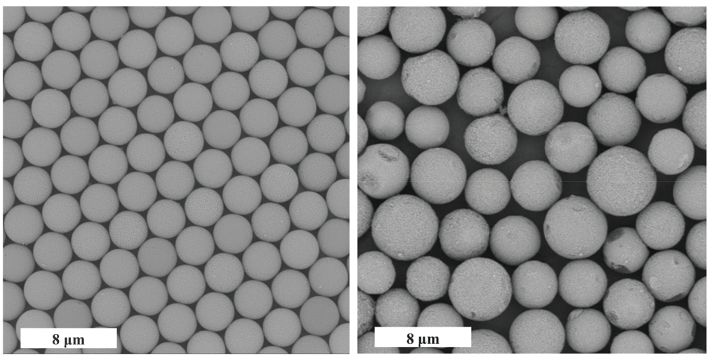


Figure 1: SEM image of 3 µm monodisperse silica particles (left) vs. 3 µm polydisperse particles (right)

Size-exclusion chromatography applications

Size-exclusion chromatography (SEC) has emerged as a pivotal technique in the nuanced analysis of AAV vectors and nucleic acids. In the context of AAV, SEC facilitates the purification and characterization of these vectors by effectively separating monomeric AAV capsids from aggregates, ensuring the attainment of a highly pure and homogenous vector population. The technique's ability to exploit size-based selectivity makes it necessary for achieving precise and reproducible results in AAV production for gene therapy. Furthermore, SEC proves instrumental in nucleic acid analysis, offering a robust method for the size-based separation of DNA and RNA molecules. This analytical approach holds considerable significance in diverse research and biotechnological arenas.

This manual demonstrates an approach to designing methods for the evaluation of AAV and nucleic acid samples through the utilization of a SEC column. Practical illustrations are presented specifically addressing distinct AAV serotypes separations.

Operation: System requirements and column use and care

Getting started

Prior to using the SurePac Bio 550 SEC MDi columns, review all the information in this section on column operation regarding flow rate, maximum pressure, mobile phase composition, temperatures, etc. Following these specifications for your column will help to ensure the column performs as it is intended and maximize its lifetime.

A biocompatible system is strongly recommended

A biocompatible system is highly recommended for mobile phases containing halide salts that can cause corrosion of metallic components. Avoid using stainless steel tubing, ferrules, and bolt assemblies and replace them with MP35N tubing, PEEK, or other equivalent biocompatible alternatives. Thermo Scientific™ Viper™ Fingertight Fitting Systems with biocompatible MP35N capillaries are strongly recommended.

System void volume and extra-column dispersion

Use appropriate tubing I.D. between the injection valve and detector to minimize dispersion. Minimize the length of all liquid lines, especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution. For best column performance, use low-dispersion UHPLC systems.

Table 1. Recommended column operating conditions for optimal performance and extended column lifetime

Column format	Cat. no.	Max. flow rate	Max column pressure drop[1] psi (bar)	Temperature	pH
2.1 × 150 mm	43903-152131	0.125 mL/min	3500 (241)	Ambient – 60°C	2-8
2.1 × 30 mm guard	43903-032131				
4.6 × 150 mm	43903-154631	0.6 mL/min			
4.6. × 30 mm guard	43903-034631				
7.8 × 150 mm	43903-157831	1.4 mL/min			
7.8 × 30 mm guard	43903-037831				

[1]The column pressure drop for a given flow rate is calculated as the pressure of the system with column minus the pressure of system with union in place of column.

Additional requirements for column care and operation:

- Always set up the mobile phase flow direction as indicated on the column tag. Flowing mobile phase opposite the intended direction will cause irreversible column damage.
- Avoid exposing the column bed to sharp pressure fluctuations that may disrupt the column bed.
- When starting, stopping, or changing the flow rate, a flow ramp rate (mL/min/min) of ~1/3 of the maximum flow rate for the specific column format is recommended.

Operation: System requirements and column use and care (continued)

Recommended buffers

Please consult Table 2 below for recommended buffer conditions to achieve optimal separations and maintain good column performance throughout its lifetime.

Table 2. Recommend buffers and mobile phase requirements

Parameter	Recommended
Typical buffer	• Phosphate buffer with NaCl, e.g. 50 mM phosphate buffer (pH 6.5) + 0.3 M NaCl • Good's buffer with NaCl, e.g. 20 mM MES buffer (pH 6.1) + 0.3 M NaCl • Ammonium formate or ammonium acetate solutions, pH 5 – 7
Solvents compatibility	• Compatible with 100% organic solvents
Detergent compatibility	• Compatible with 0.1% SDS, though binding will be irreversible and it is recommended that the column be dedicated to this application
Storage solution	• 75% acetonitrile in deionized water

Column conditioning

Your column has been designed to minimize secondary interactions and for low carryover. Depending on the nature of your sample, column conditioning may be required prior to achieving optimal performance. To quickly condition your column, we recommend performing 1-2 sample overload injections of 10x your standard sample loading and standard method.

For MALS experiments, columns should be conditioned with mobile phase at 0.1 mL/min for at least 12 hours prior to starting runs to minimize signal that may interfere with MALS detection

Column storage and extended care

To maintain the performance of your column between uses, always store the column filled with the recommended buffers as detailed in Table 3. Use the plugs the column was shipped with to seal the ends of the column to prevent evaporation of the buffer and drying of the stationary phase. The formation of salt crystals in a dried bed may result in column clogging or reduced column performance in subsequent uses.

The column can be stored in the mobile phase for short-term storage. For long-term storage (more than 5 days), it is recommended to store the column in a solution containing 75% acetonitrile in deionized water.

Column washing procedure

The following procedure is designed for the 4.6 mm I.D. column. Please adjust the flow rate for different I.D. columns. Particulates in the sample or the mobile phase will plug the column inlet frit. If solvent flow appears to be restricted (high column back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the column has the restriction, there may be particulate matter on the inlet frit. An attempt should be made to remove any inlet debris by back-flushing 25 to 30 mL of mobile phase through the column (at 0.3 mL/min). If this fails to return the column to near its original operating pressure, consider replacing the column.

Make sure that the mobile phases and samples are free of such particulates by filtering the eluents and samples with a 0.2 µm filter prior to use. In the event that column washing/cleaning is needed, the following procedure can be used as a guideline:

1. Wash the column with 100% DI water at 0.3 mL/min for 30 minutes.
2. Wash the column with 80% methanol at 0.3 mL/min for 30 minutes.
3. Wash the column with 0.1 M ammonium acetate pH 5.0 buffer at 0.3 mL/min for 30 minutes.
4. Wash the column with the mobile phase at 0.3 mL/min for at least 30 minutes.

Column performance verification

Perform the column QA test using the conditions described in the QAR and compare the result with the reported values.

The column should be fully equilibrated before any injection. At least three injections should be made to assess the reproducibility. Once you are satisfied with the column performance report result, proceed to the next step.

SEC applications

UV vs. fluorescence detection

The application of SEC in the analysis of various AAV serotypes stands as a critical endeavor in biopharmaceutical research. This section employs both ultraviolet (UV) and fluorescence detection (FLD) methods to comprehensively assess AAV3 sample as an example. UV detection at 280 nm and 260 nm proves instrumental in elucidating the capsid content of AAV particles, distinguishing between empty and full capsids, thereby confirming peak identity. This is because the absorbance at 280 nm primarily corresponds to the presence of proteins, which are abundant in the capsid, while the absorbance at 260 nm corresponds to nucleic acids, which are present only in full capsids. Simultaneously, the heightened sensitivity of fluorescence detection offers a robust alternative, allowing for substantial sample conservation as lower sample volumes can be employed without compromising signal strength. This integrated approach not only advances the precision of AAV serotype analysis but also underscores the versatility of SEC in facilitating insights into viral vector characteristics for biopharmaceutical applications.

In Figure 2, the distinction between the first and second peaks is discerned through the larger 260 nm absorbance relative to 280 nm. This disparity indicates that the first peak corresponds to the HMWS while the second peak represents the monomeric AAV3. The 3rd peak, exhibiting greater 260 nm absorbance without FLD signal, is indicative of potential extracellular DNA. The baseline separation of AAV monomer from HMWS underscores the efficacy of the 550 Å pore size in the column. The sharpness of the monomer peak further attests to the high efficiency facilitated by the 3 µm monodispersed particles in the column.

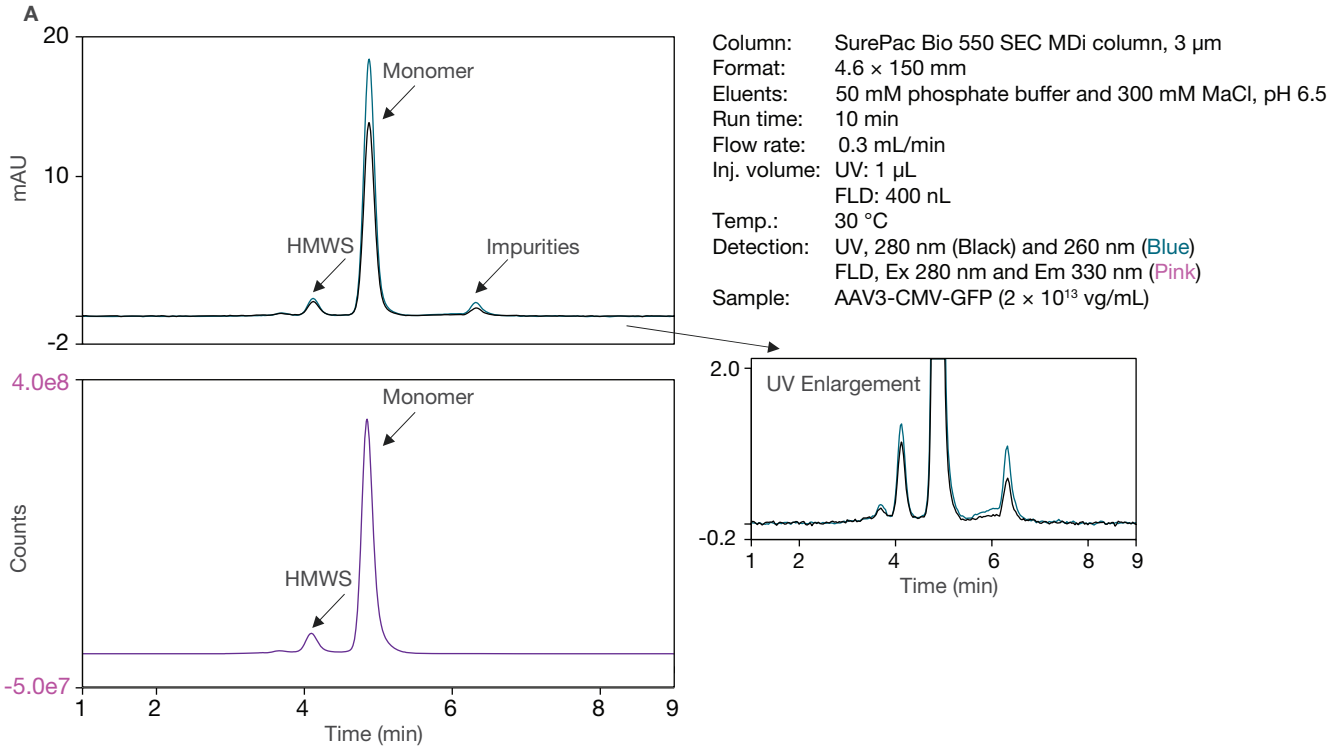
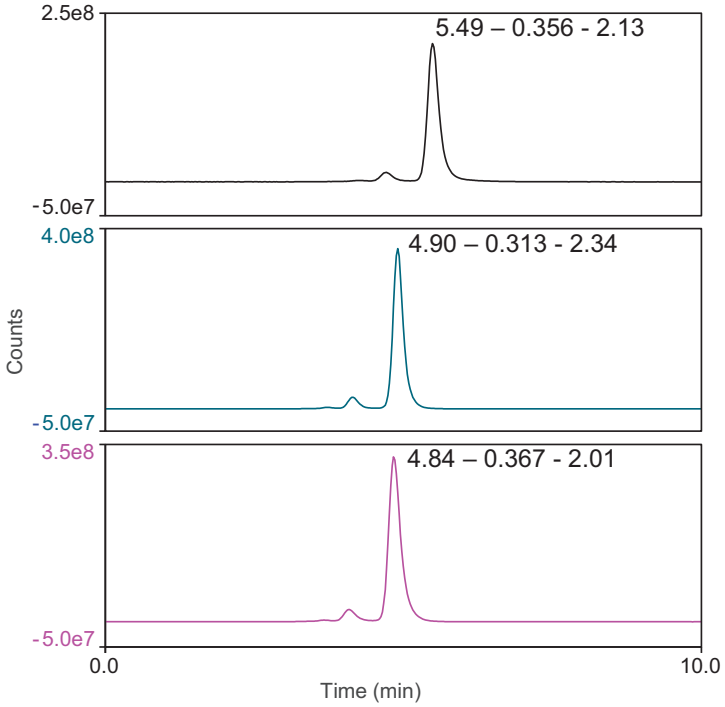


Figure 2: Separation of AAV3 sample. UV (top) and FLD (bottom)

SEC applications (continued)

Column format comparison

In this section, we have evaluated the performance of three different formats for analyzing AAV3 samples: 2.1, 4.6, and 7.8 x 150 mm. For the 2.1 mm I.D. column, we utilized a 50 µm I.D. Thermo Scientific™ nanoViper™ Fingertight Fitting to minimize extra-column volume, while 100 µm I.D. tubing was used for the 4.6 and 7.8 mm I.D. columns. Flow rates and sample injection volumes are scaled according to each column's I.D. to ensure optimal separation conditions. Fluorescence detection is performed using an 8 µL flow cell. The 4.6 mm and 7.8 mm columns provide very similar retention times for the AAV3 main peak. The resolution between the main peak and the HMWS peak is also comparable with values of 2.34 and 2.01 respectively. The 2.1 mm column shows a retention time for the main peak that was approximately half a minute longer than the other formats. The observed differences between the 2.1 mm I.D. column and the other two formats are due to the increased surface area-to-volume ratio in the smaller column, which can enhance mass transfer resistance.



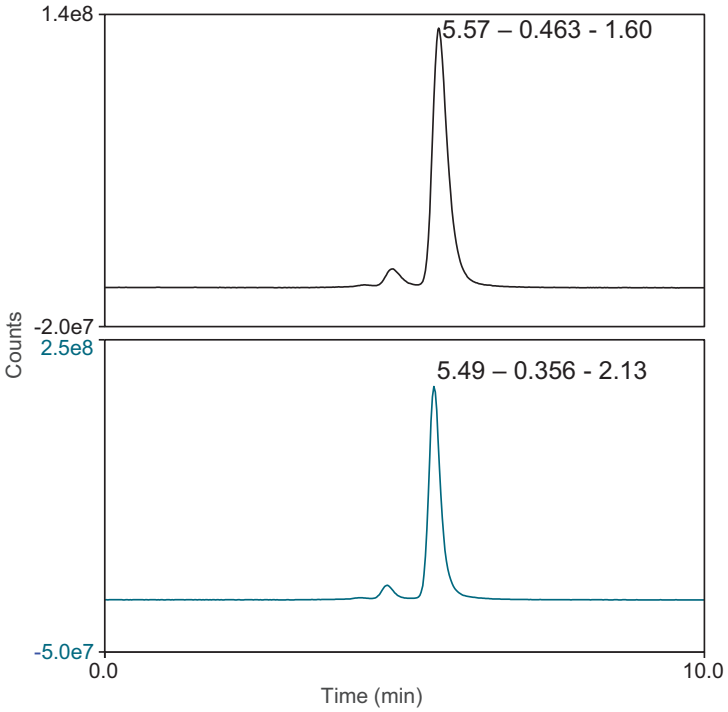
Column:	SurePac Bio 550 SEC MDi column, 3 µm
Format:	2.1, 4.6 (Blue) and 7.8 (Pink) × 150 mm
Eluents:	50 mM phosphate buffer and 300 mM MaCl, pH 6.5
Run time:	10 min
Flow rate:	0.06, 0.3 (Blue) and 0.86 (Pink) mL/min
Inj. volume:	100 nL, 400 nL (Blue) and 1150 nL (Pink)
Temp.:	30 °C
Detection:	FLD: Ex 280 nm and Em 330 nm
Sample:	AAV3-CMV-GFP (2×10^{13} vg/mL)

Figure 3. Separation of AAV3 sample using columns of 2.1, 4.6 and 7.8 x 150 mm formats. Flow rates and sample injection volumes are adjusted based on the I.D. of each format

SEC applications (continued)

Column format comparison (continued)

A test using a 2.1 x 150 mm column is also performed with both 50 µm and 100 µm I.D. fittings. Remarkably, employing the 50 µm I.D. nanoViper fittings resulted in approximately an 23% sharper monomer peak compared to using the 100 µm I.D. Viper fittings. The resolution between the main peak and the HMWS peak is also significantly enhanced when utilizing the 50 µm I.D. nanoViper fittings, with a value of 2.13 compared to 1.60 observed with the 100 µm I.D. fittings. The improved resolution and peak area observed with the 50 µm and 100 µm I.D. fittings concludes that minimizing extra-column volume is critical for achieving best chromatographic performance. These findings emphasize the need for careful consideration and selection of tubing components to ensure optimal separation results in SEC analysis.



Column:	SurePac Bio 550 SEC MDi column, 3 µm
Viper:	100 µm, 50 µm (Blue)
Format:	2.1 × 150 mm
Eluents:	50 mM phosphate buffer and 300 mM MaCl, pH 6.5
Run time:	10 min
Flow rate:	60 µL/min
Inj. volume:	100 nL
Temp.:	30 °C
Detection:	FLD: Ex 280 nm and Em 330 nm
Sample:	AAV3-CMV-GFP (2×10^{13} vg/mL)

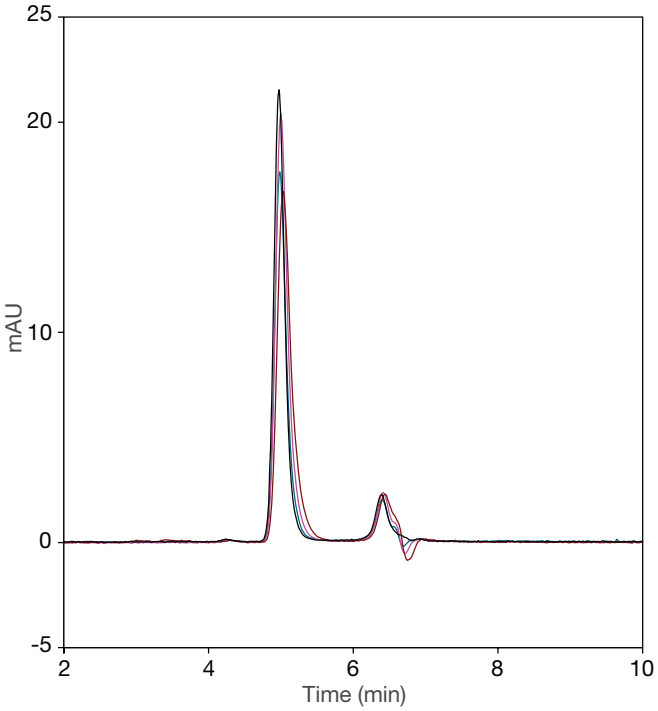
Figure 4. Separation of AAV3 sample using a 2.1 x 150 mm format column with both 50 µm and 100 µm I.D. fitting

SEC applications (continued)

Injection volume analysis

The injection volume significantly impacts the separation of AAV. We conducted loading experiments using increased volumes of diluted AAV8 samples. The injection volumes were as follows: 1 µL for undiluted AAV8, 10 µL for 10-fold diluted, 20 µL for 20-fold diluted, and 40 µL for 40-fold diluted AAV8 samples. Samples were diluted with 1 X PBS buffer. These adjustments were made to maintain a consistent amount of AAV8 injected onto the column and evaluate uniquely the peak broadening due to the injection volume.

Figure 5 illustrates the overlay of chromatograms obtained from these four injection volumes. The slight difference in peak height may be attributed to errors introduced during sample dilution. Notably, despite the injection volume being 40 times that of the non-diluted sample, the increase in peak width at half height was only approximately 26%. This attribute of the SurePac column makes it particularly suitable for customers requiring large sample injection capacities. This attribute of the column is highly advantageous in applications where substantial sample injection volumes are often required like SEC-MALS.



Column: SurePac Bio 550 SEC MDi column, 3 µm
Format: 4.6 × 150 mm
Eluents: 50 mM phosphate buffer and 300 mM MaCl, pH 6.5
Run time: 10 min
Flow rate: 0.3 µL/min
Inj. volume: 1 µL for undiluted (black)
10 µL for 10-fold undiluted (Blue)
20 µL for 20-fold undiluted (Pink)
40 µL for 40-fold undiluted (Brown)
Temp.: 30 °C
Detection: UV, 280 nm
Sample: AAV8-CMV-Luciferase (2×10^{13} vg/mL undiluted)

Figure 5. Separation of undiluted, 10-fold diluted, 20-fold diluted and 40-fold diluted AAV8 sample. Sample is diluted with 1x PBS buffer

Table 2. Separation of undiluted, 10-fold diluted, 20-fold diluted and 40-fold diluted AAV8 sample, sample is diluted with 1x PBS buffer

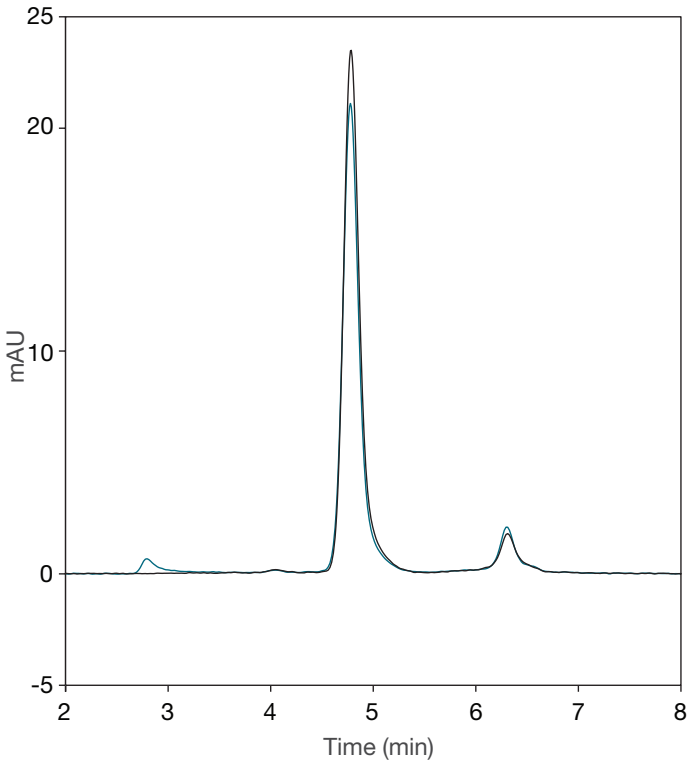
Injection volume (µL)	RT (min)	PWHH	Resolution	Asymmetry
1	4.97	0.165	2.85	1.25
10	4.99	0.176	2.84	1.33
20	5.00	0.182	2.42	1.38
40	5.03	0.208	2.32	1.47

SEC applications (continued)

Stressed sample evaluation

To demonstrate the utility of the SurePac Bio 550 SEC MDi column, we provide a practical example evaluating AAV8 in its native form against a AAV8 sample that has been stressed at 40° C for 24 hours to induce thermal stress to observe modifications.

In Figure 6, notable differences are observed between the untreated AAV8 and the heat-stressed sample. The HMWS peak for the untreated AAV8 appears at a retention time of 4.0 minutes. In contrast, the heat-stressed sample reveals an additional HMWS peak at a RT of 2.8 minutes. This suggests the formation of HMWS with an increased size, likely attributed to intensified aggregation of monomers under stressed conditions. This observation hints at a more complex profile following thermal stress. The AAV monomer peak is diminished in size after heat treatment, aligning with expectations of increased HMWS formation. Another noteworthy change is the significant enlargement of the peak at RT 6.3 minutes, suggesting a potential increase in extracellular DNA content, which becomes more pronounced after thermal stress. These findings offer a detailed insight into the structural alterations of AAV8 induced by heat stress. The separation profiles revealed distinctive peaks and variations in peak intensities, aiding in the identification of potential changes in AAV integrity or structure. This comprehensive analysis not only enhances our understanding of the stability of AAV under thermal stress, but also underscores the effectiveness of the SurePac Bio 550 SEC MDi column in discerning subtle alterations in viral vector characteristics.



Column:	SurePac Bio 550 SEC MDi column, 3 µm
Format:	4.6 × 150 mm
Eluents:	50 mM phosphate buffer and 300 mM MaCl, pH 6.5
Run time:	10 min
Flow rate:	0.3 µL/min
Inj. volume:	1 µL for undiluted (black)
Temp.:	30 °C
Detection:	UV, 280 nm and 260 nm
Sample:	AAV8-CMV-Luciferase (2×10^{13} vg/mL undiluted) (Black) Stressed AAV8-CMV-Luciferase (Blue)

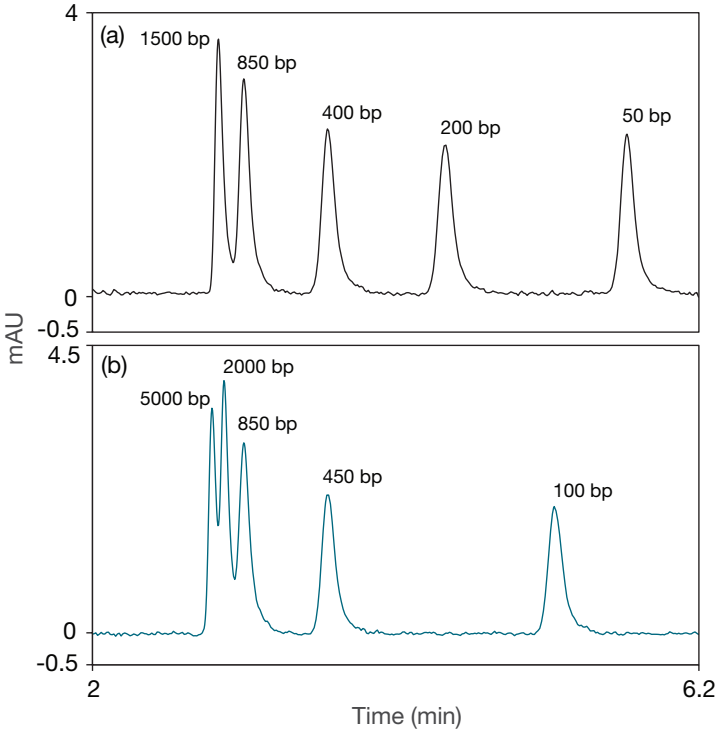
Figure 6. Chromatograms of non-stressed (black) and stressed (blue) AAV8 samples

SEC applications (continued)

Separation of DNA ladders

The implementation of this state-of-the-art SEC column in nucleic acid analysis represents a significant advancement in molecular characterization. With its meticulously designed 550 Å pore size, the column enables precise separation, allowing for the discrimination of nucleic acid fragments based on their sizes. This targeted approach facilitates the isolation and purification of DNA and RNA molecules, providing well-defined chromatographic profiles. The application of the SurePac Bio 550 SEC MDi column in nucleic acid analysis showcases its versatility and effectiveness, offering researchers a valuable tool for obtaining detailed insights into sample composition and structural characteristics, thereby contributing to the advancement of biotechnological research and applications.

In Figure 7(a), the chromatogram of the Thermo Scientific™ FastRuler™ Low Range DNA Ladder sample (1500, 850, 400, 200, and 50 bp) reveals exemplary baseline separation with remarkable resolution across all five peaks. Moving to Figure 7(b), the chromatogram of the Thermo Scientific™ FastRuler™ Middle Range DNA Ladder sample (5000, 2000, 850, 400, and 100 bp) demonstrates the capability of the 550 Å pore size to effectively separate larger components, particularly evident between the 5000 and 2000 bp fragments. This underscores the column's suitability for real-world nucleic acid samples with substantial sizes, offering not only superior separation performance but also ensuring great reproducibility and high efficiency. The observed chromatographic results reinforce the utility of the SurePac Bio 550 SEC MDi column for precise and robust nucleic acid analysis across a spectrum of sample sizes.



Column:	SurePac Bio 550 SEC MDi column, 3 µm
Format:	4.6 × 150 mm
Eluents:	50 mM phosphate buffer and 300 mM MaCl, pH 6.5
Run time:	10 min
Flow rate:	0.3 µL/min
Inj. volume:	3 µL
Temp.:	30 °C
Detection:	UV, 260 nm
Sample:	FastRuler Low Range DNA Ladder (Black) FastRuler Middle Range DNA Ladder (Blue)

Figure 7. Separation of (a) FastRuler Low Range DNA Ladder and (b) FastRuler Middle Range DNA Ladder

SEC applications (continued)

Lot-to-lot reproducibility

Ensuring consistent and reproducible performance from one column lot to another is important for the reliability of chromatographic analyses. The utilization of the SurePac Bio MDi technology platform, characterized by the use of monodisperse particles and precision-controlled chemistry, plays a pivotal role in achieving this goal. Figure 8 illustrates the analysis of AAV3 using three distinct lots of media, each created from a unique lot of monodisperse silica particles. The observed robust performance in this analysis showcases excellent lot-to-lot reproducibility, providing users with a consistent and dependable separation profile. This noteworthy consistency not only enhances the reliability of analytical results, but also underscores the effectiveness of the SurePac Bio MDi technology platform in ensuring uniform performance across different column lots, reinforcing its suitability for rigorous and reproducible chromatographic studies.

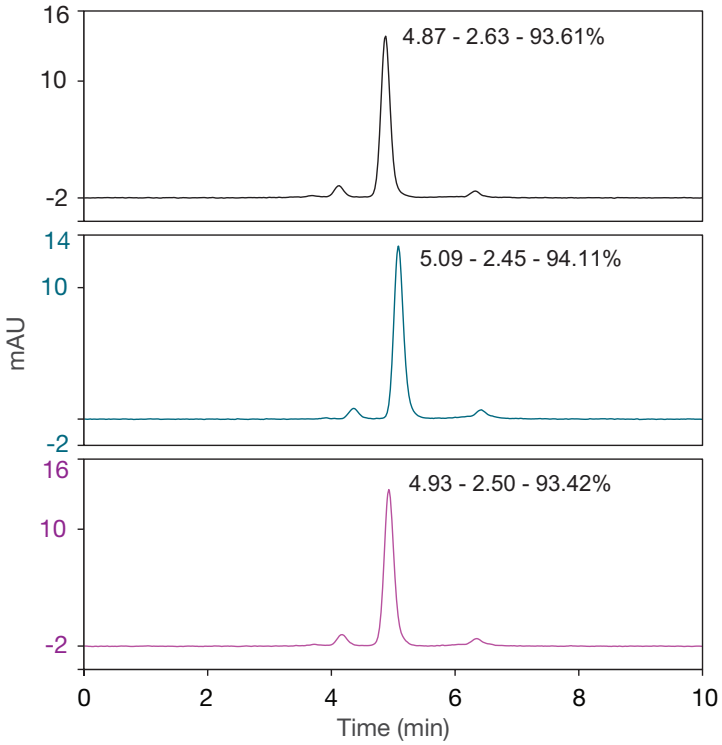


Figure 8. Chromatograms of AAV3 using 3 different lots of the SurePac Bio 550 SEC MDi columns using UV detection at 280 nm.
Legend: RT (min) – Resolution between monomer and HMWS – %area

Ordering information

Ordering information

Description	Particle size	Format (I.D. x L)	Cat. no
Analytical columns			
SurePac Bio 550 SEC MDi analytical columns	3 µm	2.1 x 150 mm	43903-152131
		4.6 x 150 mm	43903-154631
		7.8 x 150 mm	43903-157831
Guard columns			
SurePac Bio 550 SEC MDi guard columns	3 µm	2.1 x 30 mm	43903-032131
		4.6 x 30 mm	43903-034631
		7.8 x 30 mm	43903-037831

Learn more at **thermofisher.com/surepac**