TOSOH

Safer AAV Analysis with Non-toxic AEX Method

Your Challenge

- You need to analyze AAV empty/full ratio.
- You may be using toxic compounds in your current analytical method.

Our Solution

TSKgel® Q-STAT

- Non-toxic AEX method

What was done?

- We developed a choline chloride-based AEX method.

What was the result?

- We achieved safer and efficient AAV full/empty capsids separation.

This innovative AEX method replaces toxic tetramethylammonium-Cl (TMAC) with non-toxic choline-Cl, ensuring safety and efficiency in AAV capsid analysis, applicable across multiple AAV serotypes.

Cell and Gene Therapy

Your Benefit

Enhanced safety and **accuracy** in **AAV vector analysis.**

https://www.separations.eu.tosohbioscience.com/solutions/multi-column-chromatography/octave-bio-system

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Application Note

Improved Safety in Ion Exchange Chromatography for Empty/Full Adeno-Associated Virus Analysis

Introduction

The Cell and Gene Therapy industry relies heavily on Adeno-Associated Viruses (AAVs) as a therapeutic delivery platform. AAVs carry immense potential for the treatment of genetic disorders due to the optimized tissue and cell specificity of various AAV serotypes and their ability to edit the genome precisely. Other AAV benefits include their generally low immunogenicity, long-term gene expression, and non-pathogenic behavior. As illustrated in the BioPhorum whitepaper "An Industry Perspective on Understanding AAV Capsid Content Variants", analytical scientists need efficient and accurate analytical methods to assess the quality of AAV vectors[1].

Anion-exchange (AEX) chromatography is a powerful method for determining the proportion of empty and filled AAV capsids. The empty/full ratio is a critical quality attribute for AAV biotherapeutic characterization. However, traditional AEX methodologies heavily rely on toxic eluents such as tetramethylammonium chloride (TMAC) for separation.

We developed a novel AEX method utilizing a non-toxic choline-based alternative mobile phase for effective and safer AAV capsid separation[2].

Experimental Conditions

Column:	TSKgel® Q-STAT (4.6 mm ID × 10 cm, 7 µm particles)
Mobile phase:	A: 20 mmol/L Tris-HCl, pH 9.0 B: 20 mmol/L Tris-HCl, pH 9.0, with 1.0 mol/L test salt (tetramethylammonium-Cl (TMAC), choline-Cl, acetylcholine-Cl, carnitine-Cl, sodium chloride, or trimethylglycine (betaine))
Gradient:	10 - 35 % B linear in 20 min, 100 % B for 5 min, 10 % B for 5 min
Flow rate:	1 mL/min
Detection:	UV @ 260 nm & UV @ 280 nm, Fluorescence Excitation 280 nm; Emission 350 nm
Samples:	Purified AAV2, AAV5, AAV6, and AAV8 serotypes (Virovek, Hayward, CA) (+/- 4.7 kb ssDNA payload) at the concentration of 2.0×10^{13} vc/mL. For non-linear AEX gradient testing, AAV8 was in-house affinity purified from the human embryonic kidney (HEK) cell culture supernatant (titer 1.5×10^{12} vc/mL) (ExcellGene, Monthey, Switzerland). All sample vials were held at 4 °C and analyzed within 72 h of thawing.

Data acquisition was performed using the Chromeleon™ software. Resolution (R_s) values for the separated empty and full capsid peaks were calculated using the EP method in the Chromeleon software. Retention time differences (ΔRT) were calculated from the elution peak chromatograms for empty and full capsids.

Results and Discussion

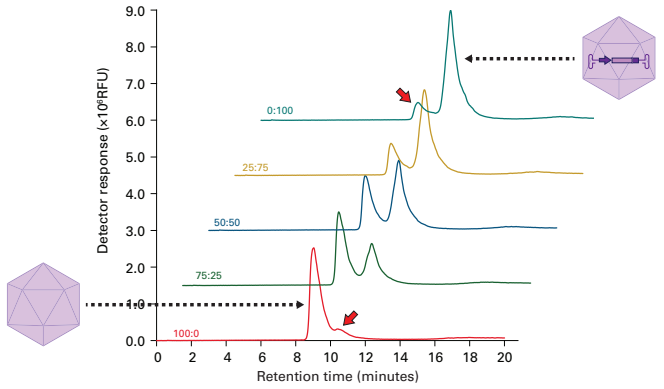
Screening of Novel Compounds in AEX Chromatography

Initial experiments evaluated the TSKgel Q-STAT column for separating empty and full AAV5 capsids using a tetramethylammonium-Cl (TMAC) gradient. While an efficient separation was achieved with TMAC, it is well known to be acutely toxic by ingestion. TMAC is easily absorbed by the skin and exposure may also harm the central nervous system. All these toxicity concerns prompted us to investigate other suitable compounds with less deleterious effects for use in AEX chromatography. These salts included, for example, choline chloride, acetylcholine chloride, and carnitine hydrochloride. Among these, choline chloride demonstrated the best separation efficiency, comparable to TMAC, and was selected for further characterization.

Separation of Empty and Full AAV5 Capsids Using Choline Chloride-based Eluents

Fluorescence detection was used to quantify empty/full AAV5 capsid ratios (*Figure 1*). Various mixtures of empty and full capsids were tested, demonstrating a strong correlation between calculated and experimental ratio values. Choline chloride gradients enabled nearly complete separation of empty and full capsids, leading to enhanced AAV genetic payload quantitation by relative peak area.

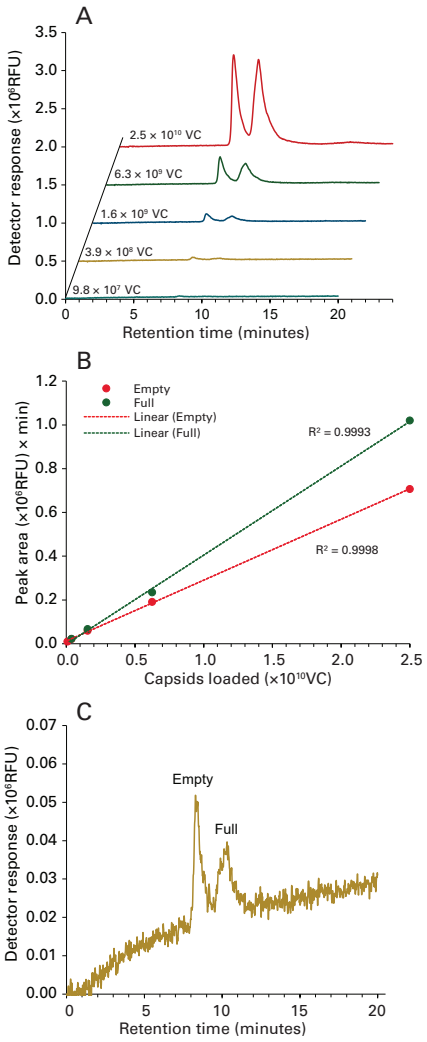
Figure 1. AEX chromatograms of various AAV5 empty and full mixtures using choline chloride-based mobile phases.



Assay Sensitivity

The study established a limit of detection (LOD) of ~3.9 × 10^8 virus capsids (VC) for both empty and full AAV5 capsids using fluorescence detection, in line with values using TMAC-based measurements (*Figure 2*). *Figure 2B* indicates a strong linear response across a wide concentration range, confirming the assay's sensitivity and effectiveness. *Figure 2C* shows the enlarged chromatogram for the empty and full AAV5 capsids at the lowest injection amount (3.9 × 10^8 VC) where both peaks are still visible.

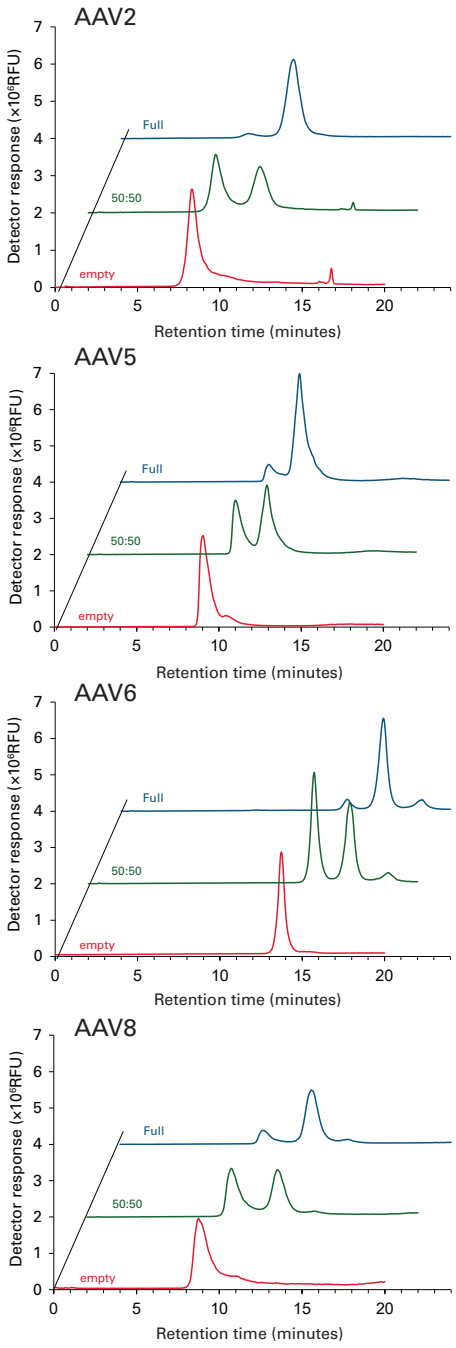
Figure 2. Limit of detection determination for AAV5 using a choline-Cl gradient.



Applicability to Multiple AAV Serotypes

The method's applicability to four AAV serotypes (AAV2, AAV5, AAV6, and AAV8) was confirmed, with all showing clear separation of empty and full capsids (*Figure 3*).

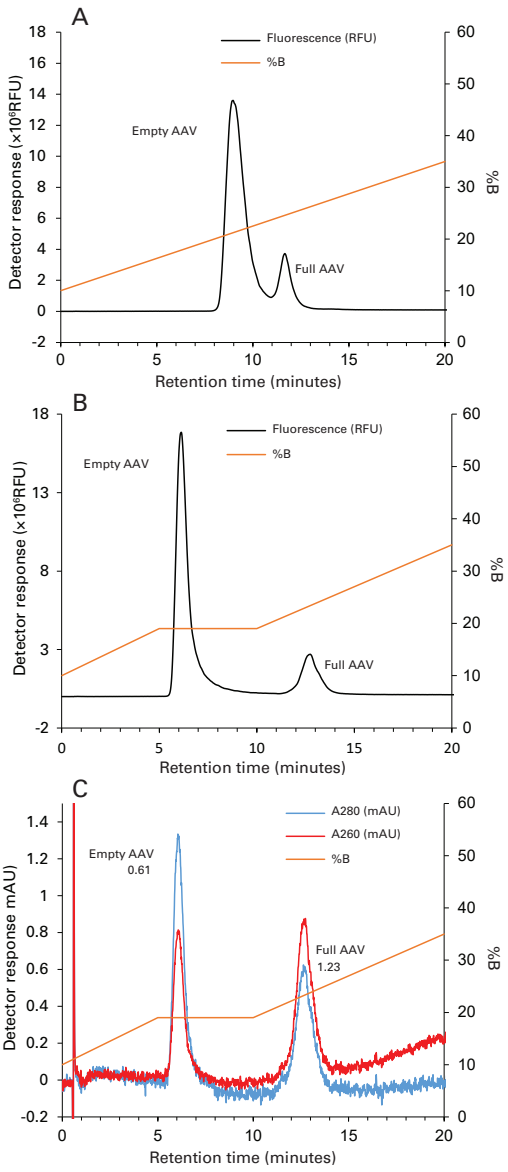
Figure 3. Separation of multiple AAV serotypes.



Improved Separation through Isocratic Hold Step

An isocratic hold step using choline-Cl was introduced to optimize baseline separation between in-house affinity-purified empty and full AAV8 capsids (*Figure 4B*). This modification effectively improved the separation, demonstrating the method's adaptability and potential for refining AAV purification processes for any desired AAV serotype. *Figure 4C* shows the corresponding UV chromatograms at 260 and 280 nm and the calculated AU 260/280 peak area ratios next to the peaks.

Figure 4. Comparison of linear (A) and non-linear (B) choline-Cl gradients for separation of empty and full AAV8 capsids.



Conclusion

This novel AEX method using a non-toxic choline chloride-based mobile phase eluent separates empty and full AAV capsids for several serotypes. It enhances the safety for AAV critical quality attribute analyses while preserving the desired separation performance exhibited by its toxic counterpart, TMAC. Further method development, such as isocratic hold steps, can be implemented to enhance serotype-dependent analytical quantitation and process development.

References

1) An industry perspective on understanding AAV capsid content variants - BioPhorum Operations Group
2) Kurth et al. Analytical Biochemistry, Vol. 686, 2024, 115421; https://doi.org/10.1016/j.ab.2023.115421

Featured Product

Part #	Description	Column dimensions
0021961	TSKgel Q-STAT	4.6 mm ID × 10 cm, 7 µm