Poster #1499

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Understanding the factors that influence capsid-column affinity and peak profile in AEX-HPLC to measure empty:full ratio

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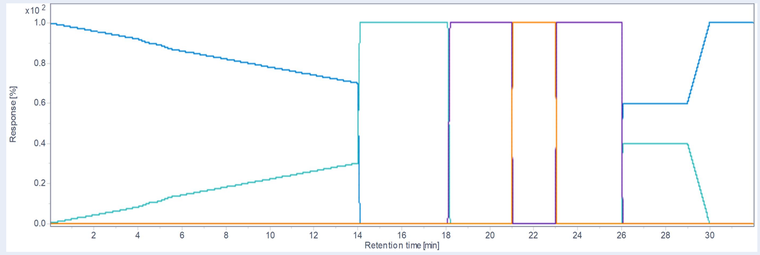
ABSTRACT

Anion exchange chromatography by HPLC is an analytical technique that can be used to determine the empty and full capsid content of adeno-associated viral (AAV) vector drug products. AEX columns have a positively charged resin which has a high affinity for negatively charged ions (anions). Under certain conditions, AAV capsids will bind to the column and the introduction of a salt gradient will alter the ionic strength, causing the bound empty capsids to elute first from the column shortly followed by full capsids. This order of elution of the empty and full capsid is due to their slightly different isoelectric points and affinity to the column. Full and empty capsid isoelectric points differ by a pI of approximately 0.2 and so baseline separation of the capsids is a challenge. In addition, the AEX method is sensitive to small changes in chemistry, sample serotype, and environmental conditions which can make developing a reproducible empty:full method difficult. Factors such as sample preparation, mobile phase components, pH, conductivity, salt concentration, and temperature all influence the binding efficiency of AAV capsids onto the column at initial injection and the elution of the empty and full capsids during the salt gradient. Data collected during the development of an AEX empty:full method demonstrates the effects of small method changes on capsid-column affinity and peak profile. AEX results are also compared to results of other orthogonal analytical techniques such as VG/VP ratio, AUC, cIEF, CryoEM, and mass photometry.[1]

1. Experiment Parameters

Method Parameter	Value
Column	CIMac™ AAV Empty,Full 0.1 mL Analytical Column (1.3 µm)
HPLC System	Agilent 1260 Bio-inert
Flow Rate	0.8 mL/min
Injection Volume	100 µL
UV Detection	260 nm, 280 nm
FLR Detection	280 nm Excitation, 350 nm Emission
Buffer A (binding mobile phase)	50 mM Tris, 2 mM $MgCl_2$, 0.01 % poloxamer, pH 9.0
Buffer B (eluting mobile phase)	50 mM Tris, 2 mM $MgCl_2$, 0.01 % poloxamer, 1 M NaCl, pH 9.0
Buffer C (equilibrating buffer)	1 M Ammonium acetate
Buffer D (strip buffer)	1 M NaOH, 2 M NaCl



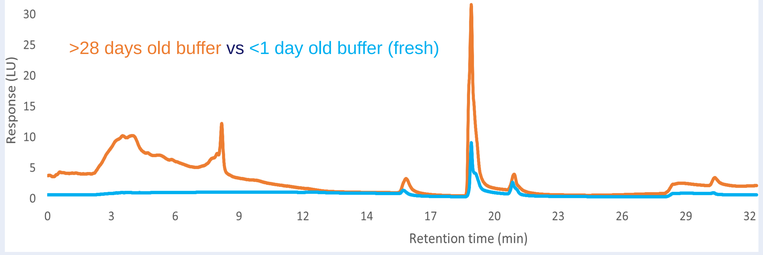
3. Effects of Buffer Conditions

Challenge: Baseline noise and capsid peak interference

Cause: Mobile phase degradation and accumulation of impurities

Reason: Degradation can change the buffer pH and ionic strength which influences column-capsid interactions and impurities interfere with the detection

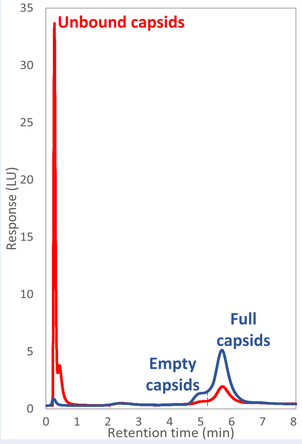
Solution: Give buffers, especially buffers containing sugar, an expiry of < 7 days



Reference

1.Werle AK, Powers TW, Zobel JF, Wappelhorst CN, Jarrold MF, Lyktey NA, Sloan CDK, Wolf AJ, Adams-Hall S, Baldus P, Runnels HA (2021) Comparison of Analytical Techniques to Quantitate the Capsid Content of Adeno-Associated Viral Vectors. Molecular Therapy - Methods & Clinical Development. 23:254-262. DOI: 10.1016/j.omtm.2021.08.009.

2. Effects of Sample Preparation



Challenge:
Unsuccessful initial binding of the capsids onto the AEX column

Causes:
- Salt present in the sample/buffer
- Sample pH and conductivity not matching the mobile phase

Reasons:
- Salt competes for binding sites on the AEX resin
- pH and conductivity affects the charge state of the capsids

Solution:
Buffer exchange or dilute the sample in the mobile phase before injection

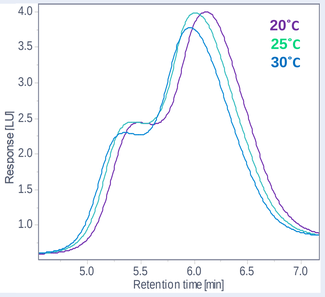
4. Effects of Temperature & pH

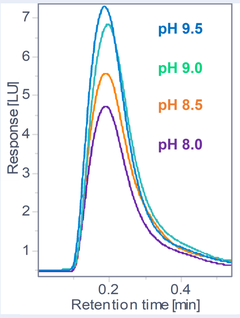
Challenge: Peak profile shift

Cause: Fluctuating temperature

Reason: Temperature has an impact on the strength of the binding of the AAV capsids to the AEX column

Solution: Find the optimal column temperature during development (serotype dependent) and keep it constant for all runs





Challenge: Inefficient initial binding of AAV capsids onto the AEX column

Cause: Unoptimised mobile phase pH

Reason: pH of the mobile phase affects the ionisation state of the stationary phase and the charge state of the AAV capsids which influences the capsids binding and elution from the column

Solution: Find the optimal mobile phase pH during development (serotype dependent) and monitor it before and during a run

Empty:Full Orthogonal Method Comparison

Method	Full %	Empty %	Intermediate %
AEX	85.1	14.9	Unknown
AUC	81.9	11.6	6.5
cIEF	79.9	20.1	Unknown
CryoEM	80.0	20.0	Unknown
Mass Photometry	82.6	10.7	6.7
VG/VP Ratio	55.8	44.2	Unknown

Empty:full separation by:

- **AEX:** Charge
- **AUC:** Gravitational force
- **cIEF:** Isoelectric point
- **CryoEM:** Particle image processing
- **Mass Photometry:** Mass
- **VG/VP ratio:** qPCR and ELISA

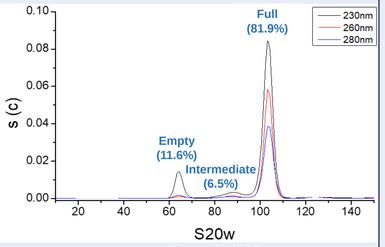


Figure 1: AUC

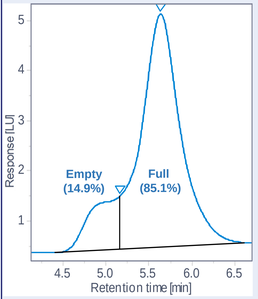


Figure 2: AEX

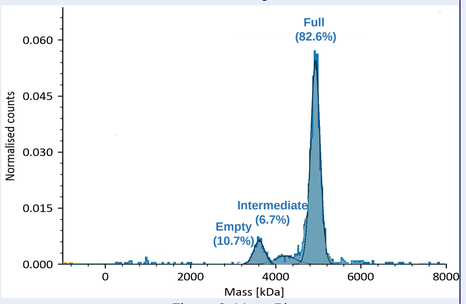


Figure 3: Mass Photometry

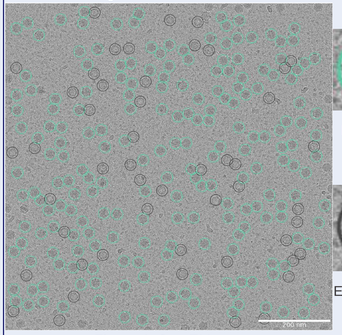
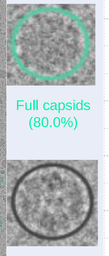


Figure 4: CryoEM



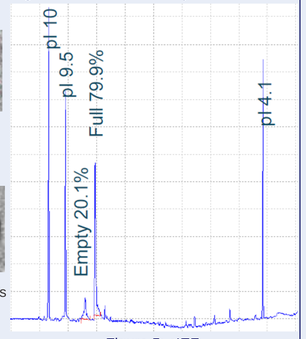


Figure 5: cIEF