Assessment of adeno-associated virus (AAV) purity by capillary electrophoresis-based western

Poster #1814

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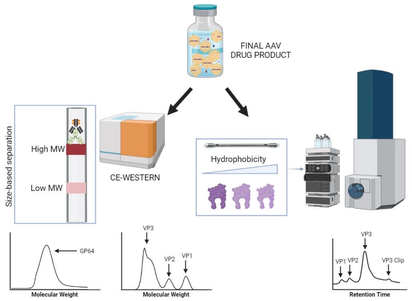
Introduction

- A rigorous analytical assessment of recombinant adeno-associated virus (rAAV)-based drug products is critical for their successful development as clinical candidates.
- It is important to determine product purity to ensure low levels of impurities in drug product.
- One approach to evaluate the purity of rAAV drug products is to determine the relative stoichiometry of the three viral proteins (VPs) that comprise an rAAV capsid, and the levels of impurities in the final drug product.
- We present two capillary electrophoresis-western (CE-western) assays for quantifying (1) the relative stoichiometry of VP proteins and (2) residual levels of a baculovirus protein impurity, GP64.
- In each assay, various purified samples from diverse AAV serotypes were analyzed.
- The ratio of VP3/VP1 in rAAV samples was correlated with biological activity, and the clearance of GP64 from the manufacturing process was demonstrated.
- The results obtained from both assays were compared to liquid chromatography-mass spectrometry analyses.

Methods

In this study[1] we explored the use of CE-Western and LCMS methods to assess protein distribution and purity of the viral capsid in rAAV6 products.

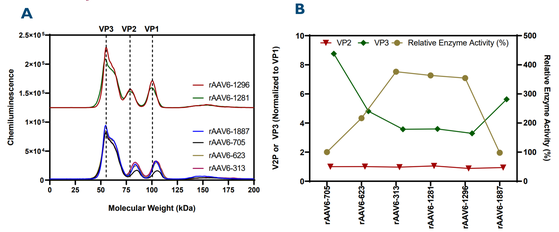
- CE-Western was used to develop two assays for VP stoichiometry (VP ratio) and GP64 protein quantitation. The VP ratio assay employs an anti-AAV (B1) monoclonal antibody capable of detecting all three viral proteins (VP1,VP2, VP3). The GP64 assay utilizes an anti-GP64 (AcV5) monoclonal antibody.
- LC-MS was used to determine VP ratio. The assay was developed using reverse-phase liquid chromatography with a Bruker ESI-QTOF Impact II MS for detection.



Results

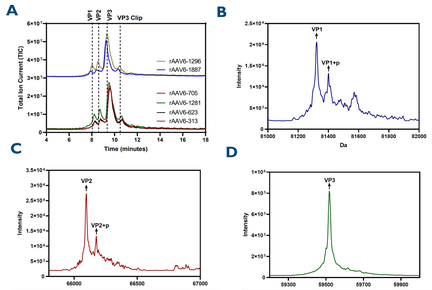
AAV VP ratio determination by CE-Western

- (A) Optimized assay conditions lead to near baseline separation of VP proteins for multiple lots of rAAV6 drug product.
- (B) Biological activity of these samples was measured and inversely correlated to VP1/VP3 ratios.



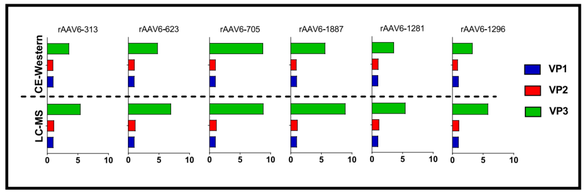
AAV VP ratio determination by LC-MS

- Optimization of the LC-MS assay was able to determine the relative ratio of viral proteins in rAAV6 samples.
- The assay was used to confirm the identity of all three major AAV6 viral proteins and was also capable of detecting additional VP variants and phosphorylation previously seen by other groups.
- (A) LC-MS chromatograms of multiple lots of rAAV6 drug product. (B) VP1and Phosphorylation (C) VP2 and Phosphorylation (D) VP3



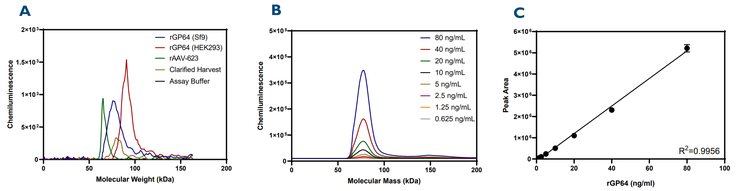
Comparison of VP ratios by CE-Western and LC-MS methods

- AAV capsids are composed of 60 individual protein subunits of three viral proteins with a theoretical ratio of 1:1:10.
- Results by the two methods show close agreement to the expected 1:1:10 ratio.



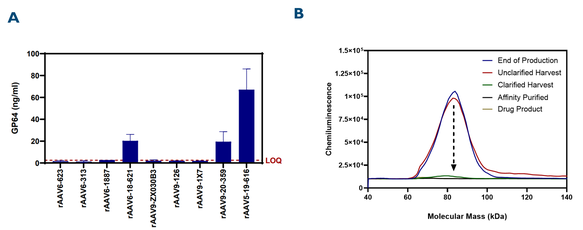
Assay for quantitation of purity of rAAV products by CE-Western

Sf9-produced recombinant GP64 protein (rGP64) was used to generate a standard curve and an anti-GP64 monoclonal antibody was used for the detection and quantification of GP64 in test samples. (A) Selection of rGP64 protein source for assay (B) Electropherograms of rGP64 protein (C) Linear regression of rGP64 Area under the curve



Quantification of residual GP64 and demonstration of clearance

- (A) Quantification of residual GP64 levels in several rAAV products from different serotypes (produced in Sf9 and HEK293 cells). Quantitation Limit (QL) is 2.5 ng/mL.
- (B) The clearance of GP64 protein impurity levels from the manufacturing process was demonstrated by measuring GP64 levels for samples from different steps within the manufacturing process. The in-process sample shows major reduction of GP64 protein at the clarified harvest step (indicated by the arrow).
- SWATH LC-MS/MS orthogonal approach corroborates results with no signal observed at LOQ of 1ng/mL.
- *selected group of purified rAAV6 (623, 313, 1887) and rAAV9 (ZX030B3, 126, 1X7)



Conclusion

- In general, the ratios of VP1,VP2, and VP3 determined for rAAV samples by either CE-Western or LC-MS methods were in close agreement with the theoretical ratio of 1:1:10 in AAV capsids.
- The variation in VP3 and VP1 content in AAV capsid was shown to significantly impact the biological activity of these AAV samples, supporting previous reports by others[2,3] where VP stoichiometry was shown to impact the infectivity and biological activity of rAAV samples.
- The clearance of GP64 protein impurities from rAAV products arising from the host cell expression system is important and analytical tools such as CE-Western are critical for quantitative assessment and subsequent verification of the clearance of such impurities from the final product.
- CE-Western and LC-MS approaches can be used orthogonally in the characterization of rAAV drug products.

References

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3. Bosma, B., et al. (2018). Optimization of viral protein ratios for production of rAAV serotype 5 in the baculovirus system. Gene Ther. 25, 415–424.

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