Limitations of Various Analytical Techniques in Characterizing Recombinant Adeno-associated Virus Empty Capsids

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Adeno-associated viral vector (AAV) has demonstrated its efficacy for gene transfer in vitro and in vivo, establishing its prominence as a safe viral vector for clinical applications. Nevertheless, a recent tragic incident in the Duchenne muscular dystrophy clinical trial has highlighted concerns regarding the safety of AAV gene therapy. Adverse events in this trial could be linked to high-dose AAV, triggering an elevated immune response. Empty capsids, which lack vector genomes and therefore lack efficacy, are considered product-related impurities. Detecting these empty capsids in final AAV products has become a focal point of interest. Various methods such as HPLC-anion exchange column (AEX), transmission or cryo-electron microscope (EM), size-exclusion column-multi-angle light scattering (SEC-MALS), analytical ultracentrifugation (AUC), mass photometry, and charge-detection mass spectrometry have been explored for this purpose.

In a previous study, we compared the ratio of empty capsid to full capsid in highly purified AAV8 using HPLC-AEX, EM, AUC, and SEC-MALS. HPLC-AEX, EM, and AUC failed to detect empty capsids, whereas SEC-MALS identified 6.5% empty capsids. Notably, empty and full capsids are eluted simultaneously in SEC-MALS, indicating the challenge of differentiation. Further analysis using AXE-MALS yielded similar results, suggesting that the separation matrix did not significantly impact the variation in the empty capsid ratio.

Recognizing the known variability in AAV capsid content, we isolated high-density AAV8 (HD-AAV8) by the CsCl ultracentrifugation. Surprisingly, all methods except AEX-MALS detected no empty capsids. AEX-HPLC detected 1.6% empty capsids, emphasizing the limitations of individual methods in accurately determining empty capsids. The findings underscore the necessity of employing multiple orthogonal methods to independently confirm Critical Quality Attributes, ensuring the precise determination of empty capsids.