

Development of full capsid enrichment step in AAV purification process with CIMmultus® PrimaT monolith

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Background and Objectives

Gene therapies treatments are promising for rare diseases, especially at Genethon, for muscular dystrophies and metabolic disorders. However, while producing AAV vectors for these gene therapies, empty capsids are also produced in a certain amount. Clinical effect of empty capsids is unclear.

CIMmultus® PrimaT monolithic column developed by Sartorius was evaluated for the full capsid enrichment of the product. Parameters like viral loading, residence time and conductivity of loading and elution were tested on AAV9. Based on the full capsid enrichment rate and the viral genome recovery, conditions were defined and applied on AAV8.

1. CIMmultus® PrimaT monolithic column Technology

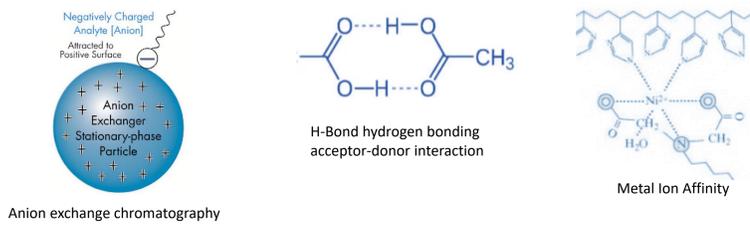


Fig.1: CIMmultus® PrimaT technology

CIMmultus® PrimaT is a mixed-mode monolithic column based on anion exchange, hydrogen bonding interaction and metal ion affinity.

2. Impact of viral loading on AAV9

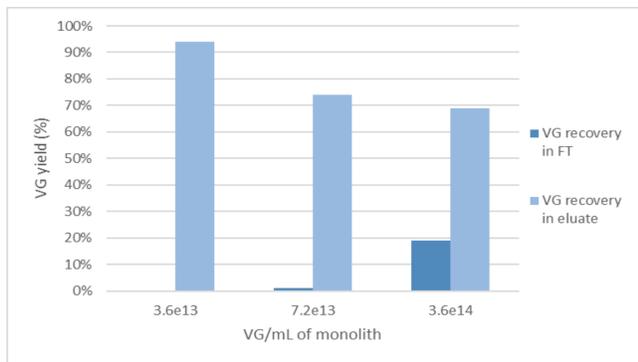


Fig.2: Viral loading evaluated on PrimaT 1 mL on AAV9

The viral loading values were chosen according to the range of monolith available. The best VG yield is obtained with the lower viral loading, corresponding to the loading of a 200L AAV9 production on an 800mL PrimaT column.

3. Impact of residence time on AAV9

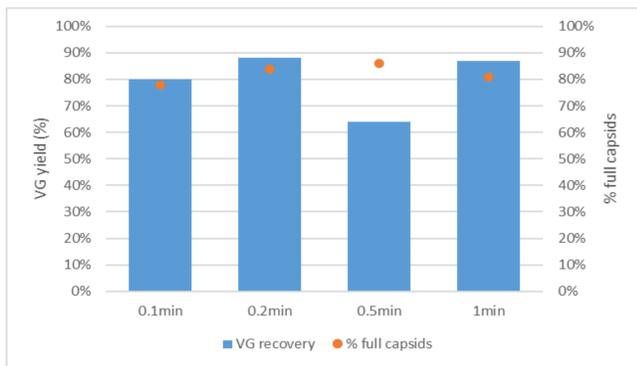


Fig.3: Residence time on PrimaT on AAV9

The residence time of 0,2min ensures a good yield/enrichment ratio without significantly increasing the purification process time. This residence time is recommended by the supplier.

4. Impact of salt on AAV9

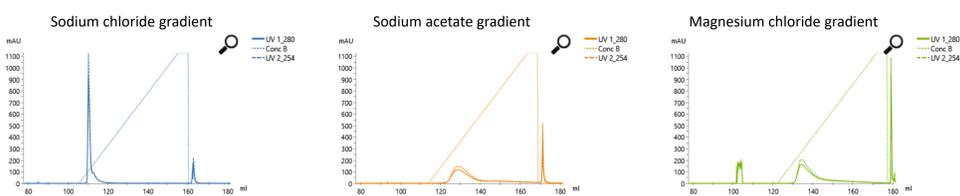


Fig.4: Chromatography profiles obtained on PrimaT 1mL with different salts on AAV9

Sodium chloride does not permit to enrich the product in full capsid. Sodium Acetate and Magnesium chloride both ensure equivalent enrichment with the same VG recovery. However, magnesium chloride is preferable for reducing residual DNA.

5. Elution mode on AAV9

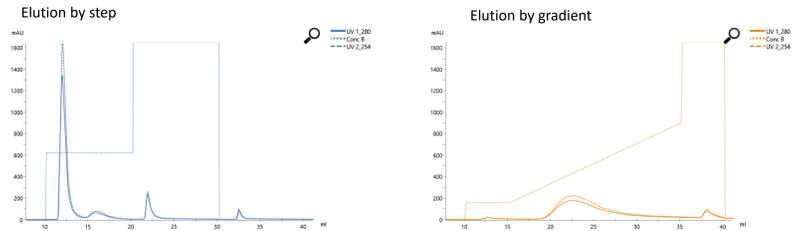


Fig.5: Chromatography profiles obtained on PrimaT 1 mL with different elution mode on AAV9

Both elution modes ensure good enrichment with a high VG recovery, but elution by gradient provides more robust repeatability.

6. Repeatability inter and intra batches on AAV9

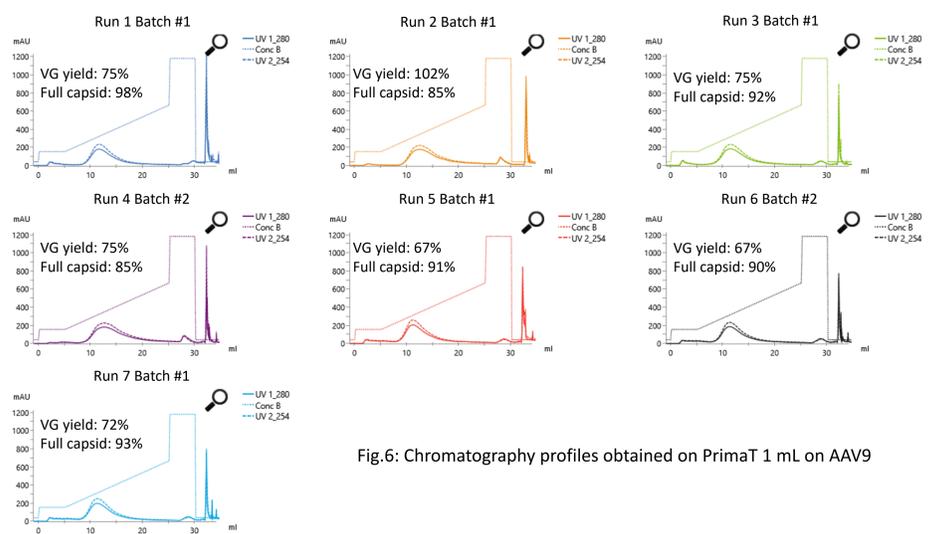


Fig.6: Chromatography profiles obtained on PrimaT 1 mL on AAV9

On Prima T 1 mL, similar chromatography profiles are obtained across 7 tests. The VG yield is 75% for a full capsid ratio of 90% starting from 60%.

7. Scale-up on AAV9

Prima T volume	VG yield ⁽¹⁾	% full capsid ⁽²⁾
1mL	75%	60% → 90%
8mL	79%	60% → 94%

(1) Obtained by qPCR (2) Obtained by SEC/MALS

Fig.7: Scale-up results on PrimaT

Conditions developed at a small scale of 1 mL are scalable to an 8 mL monolith scale.

8. Application on AAV8

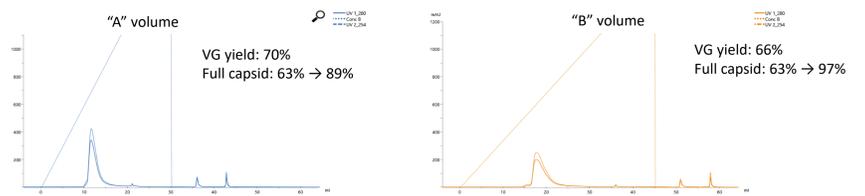


Fig.8: Chromatography profiles obtained on PrimaT 1 mL for two different volumes on AAV8

The same viral loading, salt and residence time were tested with the AAV8 serotype. Two different gradient volumes were tested, resulting in similar VG yield and final full capsid ratios. These findings are consistent with the results obtained for the AAV9 serotype.

Conclusion

Based on the generated data obtained at two different scales, conditions were defined on CIMmultus® Prima T monolith column for an industrial purification process of an AAV9 product. The final full capsid ratio determined by AUC is around 90% with a viral genome yield of 80% compared to the initial product containing 50% of full capsids before this enrichment step. Moreover, PrimaT ensures the removal of some impurities like residual DNA and Host Cell Proteins and so improves the quality of the final product. As for the AAV8 product, the conditions established for the AAV9 product enabled the execution of preliminary tests, yielding comparable results.