Development and scale-up of an enrichment step for a difficult-to-produce recombinant AAV serotype

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Abstract

Recombinant adeno-associated viral (AAV) vectors present great promise for treating systemic and localized genetic disorders. Sensorion is developing AAV-based gene therapy products for the treatment of congenital hearing loss, and as such need to develop a manufacturing process capable of producing a safe and high-quality therapeutic product. Whilst the use of affinity resins allows the capture of a large panel of AAV serotypes, one persistent product-related impurity that remains following AAV capture is capsids that are devoid of the necessary genetic material. Decreasing the proportion of these "empty capsids" is necessary to ensure an efficacious product with low immunogenic potential, and requires significant fine tuning of downstream process development, especially for a difficult-to-produce recombinant AAV serotype, as is used here. A case-study using different ion-exchange chromatographic supports, such as POROS™ 50 HQ resin (Thermo Fisher Scientific) and CIMmultus® monoliths (Sartorius), to target enrichment of full AAV particles will be presented. The use of a novel chimeric serotype for this gene therapy product presents additional challenges for the development of the polishing step, and this will also be discussed. Whilst CIMmultus® PrimaS and PrimaT supports were determined not to be suitable for our process, both the POROS™ 50 HQ resin and CIMmultus® QA permit the generation of distinct peaks of empty and full particles. Due to the requirement for low product dilution and faster operating time, the monolith support was selected for further evaluation. The impact of the elution conditions (elution buffer e.g., salt screening, gradient vs isocratic elution) on the peak separation, step recovery and enrichment factor were assessed at small-scale (1 to 2 L-scale), followed by scale-up to 10 L production. The product recovery was greater than 100% for all elution buffers tested. Both elution buffers X and Y generated distinct peaks, however the enrichment factor was superior when using buffer X (3.5-fold for buffer X vs 3-fold for buffer Y). Gradient elution was preferred to isocratic elution as it overcame the challenge of variability in the starting material. Following optimization of the process conditions, scale-up at 10L-scale production (n=2) was performed; a 4.5-fold enrichment in full particles was achieved, however a decrease in peak resolution and product recovery was observed at the larger scale (78% at 2 L scale vs 52% at 10 L scale). Target protein expression was confirmed by Western-Blot following transduction of cells with Drug Substance that had been enriched to different ratios of full particles.

1. AAV Process Overview

2. Chromatographic support

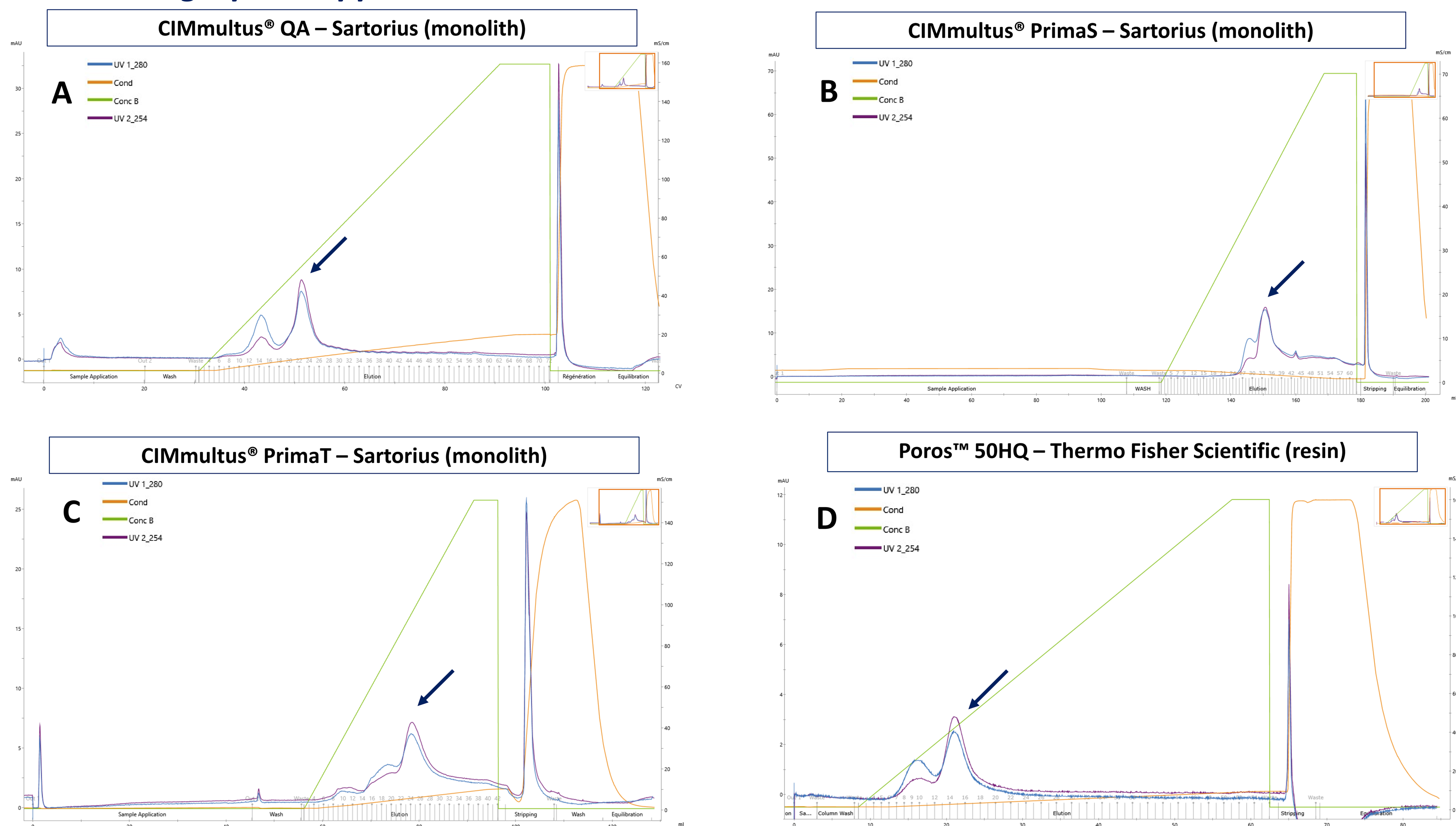

Figure 1: **A.B.C.** Chromatographic profile on CIMmultus® monolith (V=1mL) **D.** Chromatographic profile on Poros™ 50HQ resin (V=1mL, H=5cm, ID=5mm). – Sample loaded: Poros AAVX eluate diluted in appropriate equilibration buffer, 2^E+12 - 1^E+13 VG/mL support. The fractions enriched in full particles are indicated by the blue arrow.

- CIMmultus® PrimaS and PrimaT monoliths were not demonstrating a distinct separation of empty and full particle peaks. Supports not suitable for the enrichment of the used recombinant serotype
- POROS™ 50 HQ resin and CIMmultus® QA monolith permitted the generation of distinct peaks of empty and full particles.
- CIMmultus® QA monolith was selected for further evaluation based on the requirement of low product dilution and the operating time.

3. Elution buffer screening

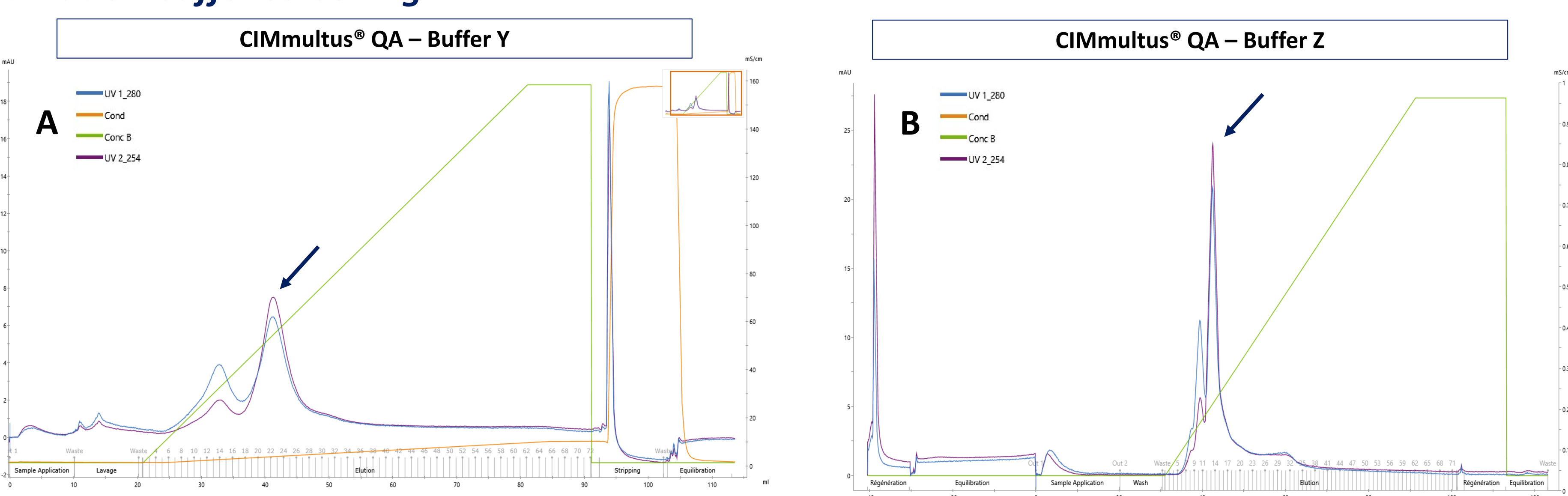

Figure 2: Chromatographic profile on CIMmultus® QA monolith (V=1mL) – Sample loaded: Poros AAVX eluate buffer diluted in equilibration buffer, 1^E+13 VG/mL support – The same equilibration buffer was used, and two different salts were assessed for performing the elution in comparison to the one used in Figure 1. A. The fractions enriched in full particles are indicated by the blue arrow.

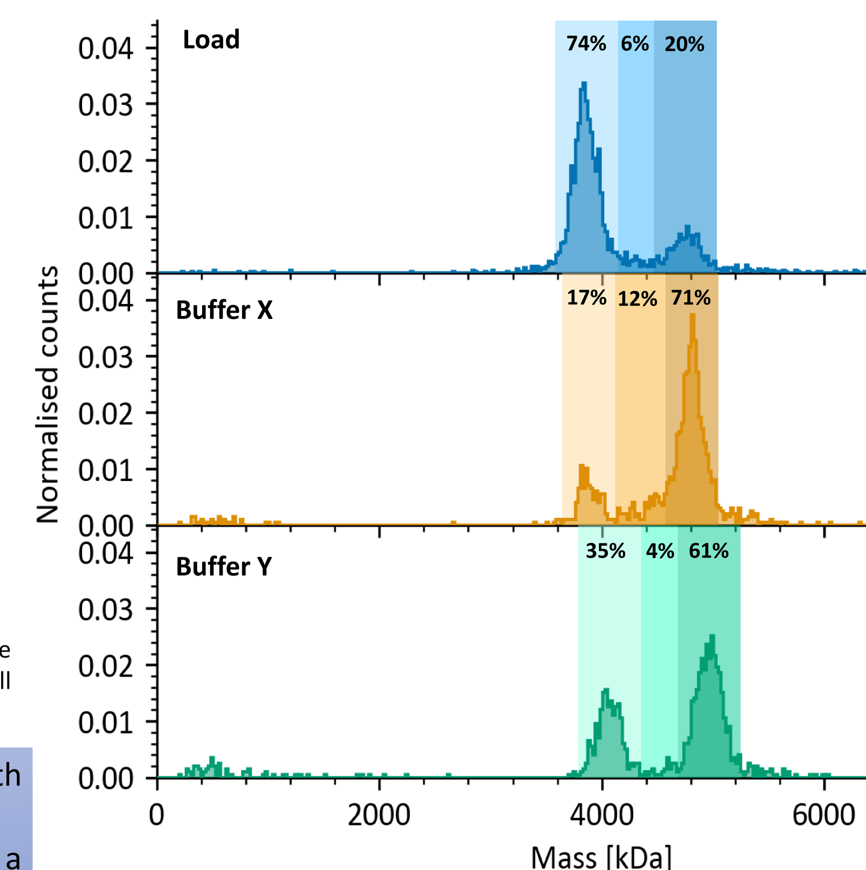

Figure 3: Mass Photometry analysis of the load and the fractions enriched in full particles using SamuxMP, Refeyn

- Whilst buffer Z (Fig 2.B) did not provide a separation of empty and full particles, similar satisfying profile was obtained for both buffers X (Fig 1.A) and Y (Fig 2.A).
- Isocratic elution was implemented using buffers X and Y. Enriched fractions were analysed by Mass Photometry (Fig 3.), showing a higher enrichment factor using buffer X (3.5-fold vs 3-fold) leading to ~70% full particles.
- Buffer X was selected for setting up the elution strategy.

4. Gradient vs isocratic elution

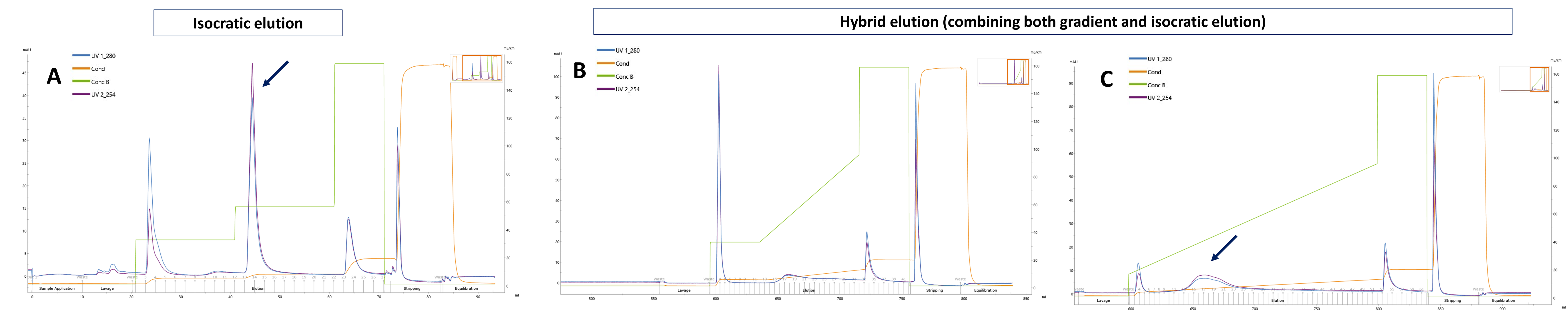

Figure 4: Chromatographic profile on CIMmultus® QA monolith (V=1mL A; V=4mL B & C) – Sample loaded: Poros AAVX eluate diafiltrated (A) or diluted (B & C) in equilibration buffer, 2.5^E+13 VG/mL support **A.** Isocratic elution: 20 CV at 20%, 20CV at 35%, 10CV at 100% **B.** Hybrid elution: 10 CV at 20%, 20% to 60% in 20 CV, 10 CV at 100% **C.** Hybrid elution: 10% to 60% in 50 CV, 10 CV at 100%. The fractions enriched in full particles are indicated by the blue arrow.

Table 1: Summary of the polishing step performance; data corresponding to the peak indicated by the blue arrow in Fig.4.

	Vector genome recovery	Full particles content	Enrichment factor
Run 4.A	93%	70%	3.5-fold
Run 4.C	78%	56%	3.5-fold

- Although isocratic elution led to highest vector genome recovery and enrichment performance, variability in starting material content could cause preliminary elution of full particles and therefore co-elution with empty particles.
- A hybrid elution including a gradient resulted in satisfactory vector genome recovery and enrichment performance and overcame the challenge of variability in the starting material.

5. Scale-up to 10L-scale

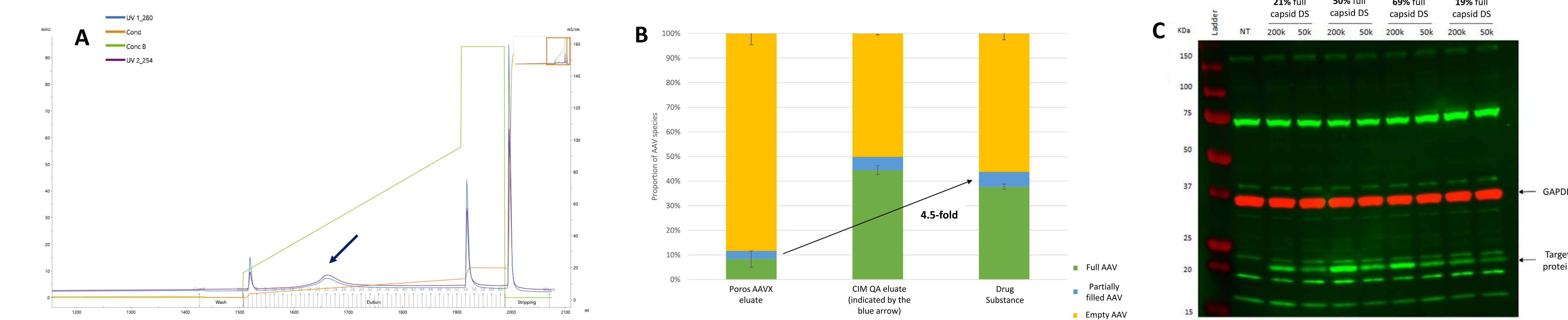

Figure 5: **A.** Chromatographic profile on CIMmultus® QA monolith (V=8mL) – Sample loaded: Poros AAVX eluate diluted in equilibration buffer, 1.3^E+13 VG/mL support – Elution buffer X – Hybrid elution: 10% to 60% in 50 CV, 10 CV at 100%. The fraction enriched in full particles is indicated by the blue arrow. **B.** Proportion of the AAV species in different in-process controls and Drug Substance, determined by Mass Photometry, SamuxMP, Refeyn. Results shown correspond to the average of two independent experiments. **C.** Western-blot analysis of cell lysate extracts obtained after transduction of adherent cells with non-enriched or enriched Drug Substance (DS) at two multiplicity of infection (50k & 200k); NT=non transduced control.

Table 2: Comparison of the process performance at 2 and 10L-scale

	2L-scale	10L-scale
Vector genome recovery upon polishing step	78%	52%
Global vector genome recovery from cell lysate to Drug Substance	26%	19%
Enrichment factor	3.5-fold	4.5-fold

- The chromatographic profile obtained at 10L-scale was similar to that obtained at 2L-scale.
- A lower vector genome recovery was observed at 10L-scale which is likely due to the lower saturation point of the support. However, the enrichment factor was maintained (>3.5-fold).
- Target protein expression was confirmed by Western-Blot following transduction of cells with Drug Substance that had been enriched to different ratios of full particles.

Conclusions

- The initial development and optimization of the polishing step for a difficult-to-produce recombinant AAV serotype demonstrated significant promise in the ability to achieve good recovery of highly enriched capsids for a therapeutic drug product required for the treatment of congenital hearing loss.
- Additional development activities are required to fine-tune the operating parameters to improve peak resolution and product recovery, and to translate the process to a relevant scale for clinical and commercial manufacture.
- The challenges associated with developing an isocratic elution strategy are magnified due to the necessity to use a specific serotype, selected for its favourable biological properties ; modifying the conductivity of the material loaded to prevent the binding of empty particles is currently being evaluated.