A SCALABLE ADENOVIRUS PRODUCTION PROCESS, FROM CELL CULTURE TO PURIFIED BULK

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Adenovirus (AdV) vectors are commonly used in cancer gene therapy trials, evaluated in gene therapy and used as vaccines for various diseases. AdV vectors are well studied and are suitable as vaccine vectors due to their ability to infect different cell types, remain episomal and produce stable high titer material. Manufacturing of safe and efficacious clinical-grade virus relies on a scalable and cost-effective production process. In this study, we have combined experimental work and process economy calculations, from AdV production in cell culture to purified bulk product up to 10L scale. An efficient and scalable process for AdV production was developed by evaluation of each process step. The most studied vector is serotype 5, making this a suitable system for process development of AdV vectors. Human AdV5 expressing the green fluorescent protein (GFP) was used for process development. First, suspension HEK 293 cells adapted to serum-free cell culture medium were optimized for AdV production and evaluated in different single use bioreactor systems. Tween 20 was used for cell lysis as a replacement for the traditionally used Triton X-100 (now on the Authorization list (Annex XIV) of REACH, the regulation on Registration, Evaluation, Authorization and restriction of Chemicals). A residual Tween 20 assay with low detection limit was set-up. Filters and conditions for clarification, concentration and buffer exchange by tangential flow filtration were optimized. Anion exchange based capture step alternatives were compared, including different chromatography resins and membrane formats. Finally, core bead technology was evaluated as an alternative to size exclusion chromatography for the polishing step before the final formulation. Analytical methods for virus titer are challenging and depend on purity and quality of the sample. For total virus titer, qPCR and HPLC methods were used. Furthermore, a method based on surface plasmon resonance (Biacore) was developed for analysis of adenovirus titer. For infectious virus titer, we have used a cell based assay with automatic image analysis. Based on analytical data different downstream process alternatives were compared regarding load capacity, recovery and purity and we propose a robust and scalable process with a favorable process economy.

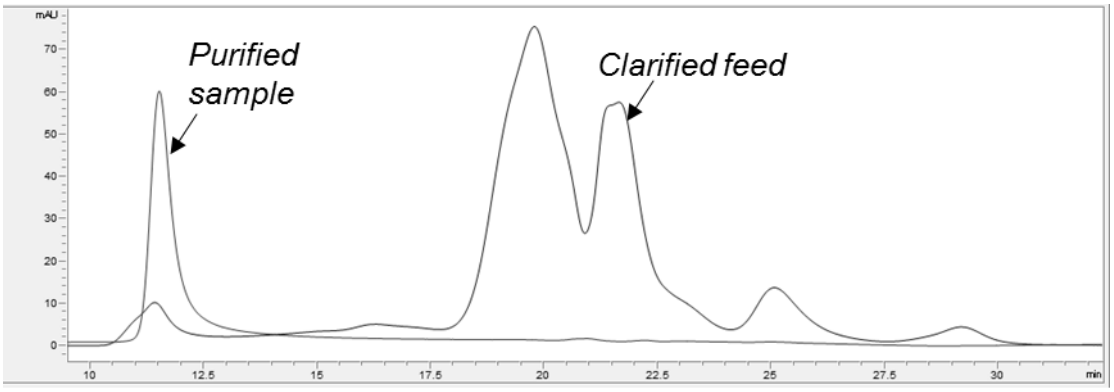

Figure 1 – Size exclusion HPLC analysis of start clarified material and final purified sample from the selected process variant.