Simplifying Progress

A PATfix® Analytical Method for Adenovirus Sample Characterization in Upstream Process Development

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Introduction

Adenovirus is a well-known gene therapy tool that has gained attention as a promising vehicle for vaccine delivery, especially during the COVID-19 pandemic, where it was used to deliver the sequence for protein S (S). Multiple serotypes have been tested in clinical trials for various applications, the most common one being human adenovirus serotype 5 (Ad5). With this in mind, we chose an Ad5-S construct with a GFP tag as a model vector to develop an upstream process (USP) and supporting analytical tools.

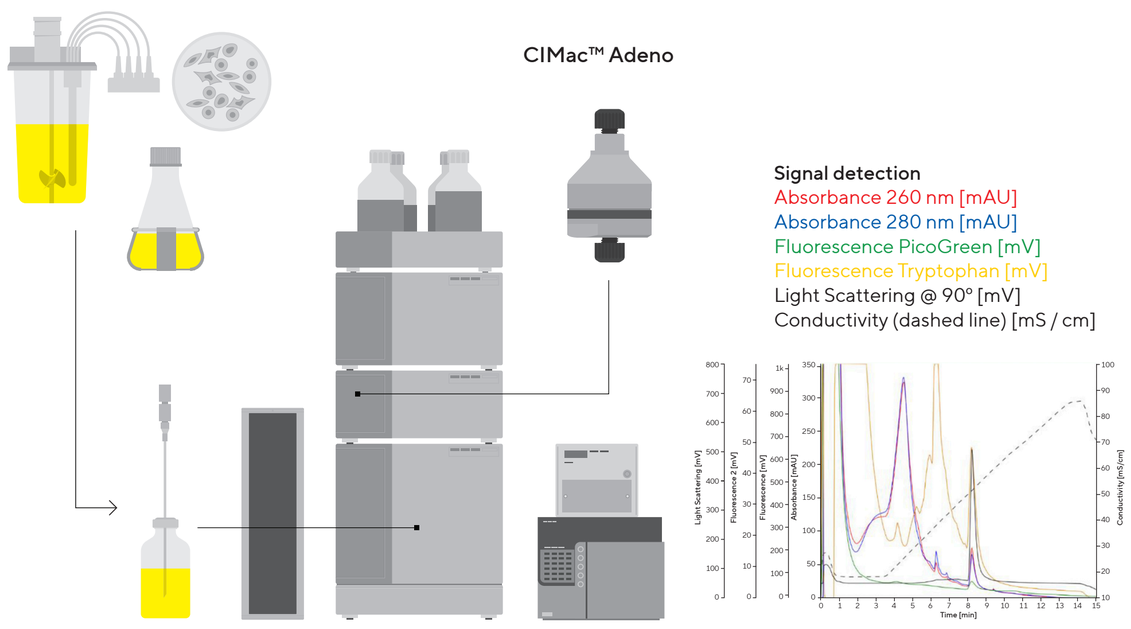
Analytical methods available on the market usually address either the product (droplet digital PCR [ddPCR] and infectivity assay) or sample impurities (quantification of host cell protein and DNA), each of them with different specificity and sensitivity. Additionally, differences in sample preparation can introduce variability into the output. Therefore, interpretations of such data can contain a variable degree of error, limiting the applicability of the results.

To address this challenge, we developed a method - based on anion-exchange (AEX) PATfix® high-performance liquid chromatography (HPLC) coupled with multiple detectors - that detects adenovirus and records impurity profiles at the same time. The method is performed using CIMac™ Adeno-0.1 mL (2 µm) monolithic analytical columns and utilizes multi-angle light scattering (MALS) to quantify adenovirus, with generated data being well aligned with ddPCR. The impurity profile is recorded at the same time as the product using UV and fluorescent detectors. In our experiments, the PATfix® Adeno profile was a good predictor of harvest performance in the downstream process (DSP).

PATfix® Adeno Analytical Tool

The PATfix® Adeno HPLC analytical method is shown schematically in Figure 1. Each sample (500 µL) was injected to CIMac™ Adeno-0.1 (2 µm) monolithic analytical column at a 1.0 mL / min flow rate. Multiple-detector PATfix® technology enables simultaneous detection of absorbance (monitored at 260 nm and 280 nm), two different fluorescence signals: the samples own tryptophan fluorescence for protein detection (Ex: 280 nm / Em: 348 nm) and | or PicoGreen fluorescence for nucleic acid detection (Ex: 485 nm / Em: 520 nm), and light scattering emitted / scattered from the sample (monitored at a 90° angle).

Figure 1: *Schematic Diagram of the PATfix® Adeno Analytical Method*



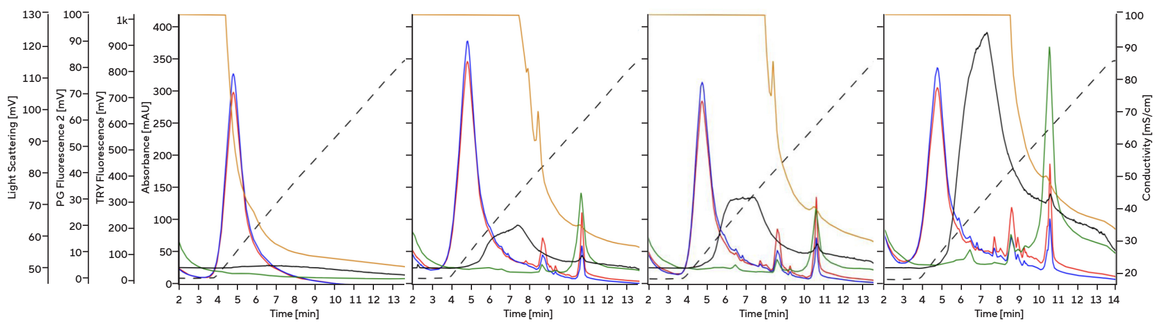
Note. Example of recording is shown on the right and is labeled with a different color (as noted in the legend above).

Recording the Impurity Background of the Upstream Setup

Before starting USP development, we wanted to address bioburden generated by components used in Ad5 production, (i.e. media, supplements, and cells). For this purpose, suspension HEK293 cells were seeded at different cell densities in chemically defined complete medium. All samples were collected and frozen 96 hours later. For PATfix® Adeno analytics, samples were thawed, lysed for 1 hour with 0.5M NaCl, and filtered. To provide proper binding conditions, samples were diluted and | or buffer-exchanged to a running buffer. In the case of PicoGreen fluorescence, Quant-iT™ PicoGreen® dsDNA Reagent (Invitrogen) was added to each sample. Samples were then incubated for at least 2 hours at 4° C in the dark.

PATfix® Adeno analytics of background impurities from components used in Ad5 process development are given in Figure 2.

Figure 2: *HPLC Chromatograms – Zoomed in to the Elution Gradient.*



Note. (A) Media and supplements without cells. (B), (C), (D) Media nd supplements inoculated with HEK293 suspension cells at different densities, collected after 96 hours and lysed.

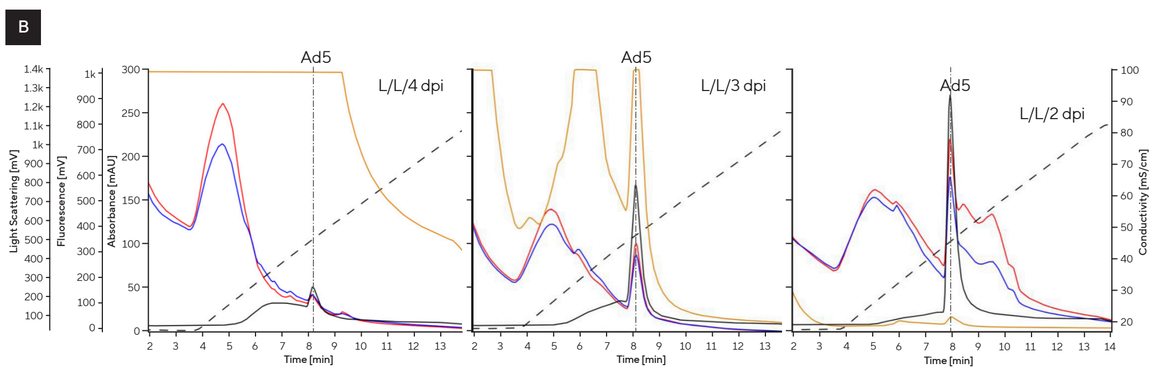
Results – Comparing Different Upstream Strategies

For upstream Ad5 process development, an Ad5-S-GFP construct (Vigene) was amplified in adherent HEK293 cells, and frozen as a viral bank. Larger quantities of this vector were prepared by infecting suspension HEK293 cells; the harvest was purified and concentrated using a combination of tangential flow filtration (TFF) and CIMmultus® QA chromatography. Aliquots were stored at -80 °C and used for optimizing USP parameters. These experiments included HEK293 cells growing in suspension, infected with Ad5-S-GFP construct at various cell densities and MOI, collected at different timepoints. Results of experiments are given in Figure 3.

Figure 3: *Comparing Different Upstream Strategies*

A

Strategy	Cell density [viable cells/mL]	MOI [IFU/cell]	Production duration (days post-infection)	Modality (batch/fed batch)
L/L/4dpi	0.3E+06	0.5	4 dpi	Fed batch (5% feed at 1 dpi)
M/M/3dpi	0.5E+06	1.8	3 dpi	Batch
H/H/2dpi	1.0E+06	3	2 dpi	Batch

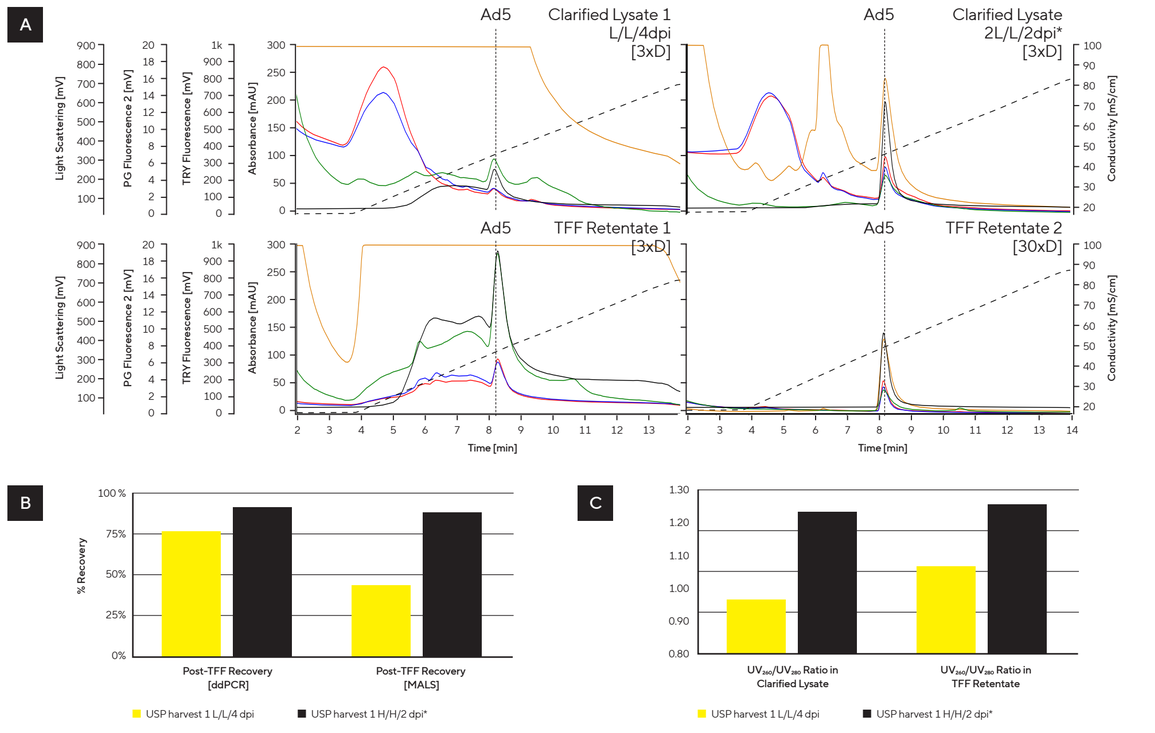


Note. (A) Different upstream strategies to produce Ad5-S- GFP harvest. (B) HPLC chromatograms – zoom in to the elution gradient of lysate samples. (C) Cell density and viability at harvesting point (Y-axis is given in log scale). (D) Physical and functional Ad5 titer in lysates determined by ddPCR and infectivity assay (Y-axis is given in log scale). *sample was not measured in infectivity assay. dpi = days post-infection, IFU = infectious units

Results – DSP Performance of Upstream Materials

To determine how different harvests perform in the DSP, we compared two upstream strategies for their recovery and purity after TFF step. Harvests were lysed using NaCl and Tween 20 and, treated with salt-tolerant nuclease. Samples were then subject to coarse and fine filtration and TFF for buffer exchange and concentration.

Figure 4: *Different Upstream Strategies in Early Downstream Purification*



Note. (A) HPLC chromatograms – zoom in to the elution gradient of diluted lysates and their respective TFF retentates of two upstream materials. (B) Recovery from TFF in the DSP, calculated from MALS signal and ddPCR titer. (C) Quality of Ad5-S-GFP material indicated by UV_{260}/UV_{280} ratio, calculated using PATfix® software. *harvest was produced separately, under the same conditions as stated in Figure 3 (A).

Conclusions

- In this work we demonstrate the application of the PATfix® Adeno HPLC analytical method in sample characterization during USP development.
- This method records the product and impurities at the same time, and is suitable for samples of various complexity.
- It is well aligned with common analytical approaches, such as ddPCR and IFU, and complements them.
- PATfix® Adeno HPLC output can be used for further optimizaction of adenovirus production strategies to reduce the burden on the DSP and improves overall process yields.