Simplifying Progress

Adenovirus Vector Manufacturing Platform Using CIMmultus® QA Column to Produce Safer Vaccines Cheaper

K. Vrabec, H. Jug, A. Mavri, I. Petrovic Koshmak, V. Fujs, M. Leskovec, A. Štrancar

Sartorius BIA Separations d.o.o., Mirce 21, 5270 Ajdovščina, Slovenia
Contact: monolith-purification@sartorius.com

Introduction

Adenovirus has after two decades gained new consideration and is now used as a COVID-19 vaccine delivery vehicle. To reduce side effects of the vaccine it's purity is of utmost importance. Constant enhancement of the vaccine purity and improvement of the impurity detection methods is therefore necessitated. In this work we present second generation adenoviral vectors purification procedure based on monolith chromatography using CIMmultus® QA to secure safer product, as well the accompanying analytical tools. The novel industrial process secures better purity at higher yields The robustness of the process was verified using different upstream materials. Manufacturing of the vaccines in large quantities due to pandemic represent great challenges, mainly in terms of production time and costs. Higher capacity of the CIMmultus® QA columns used in this process overcomes the raw material supply bottlenecks.

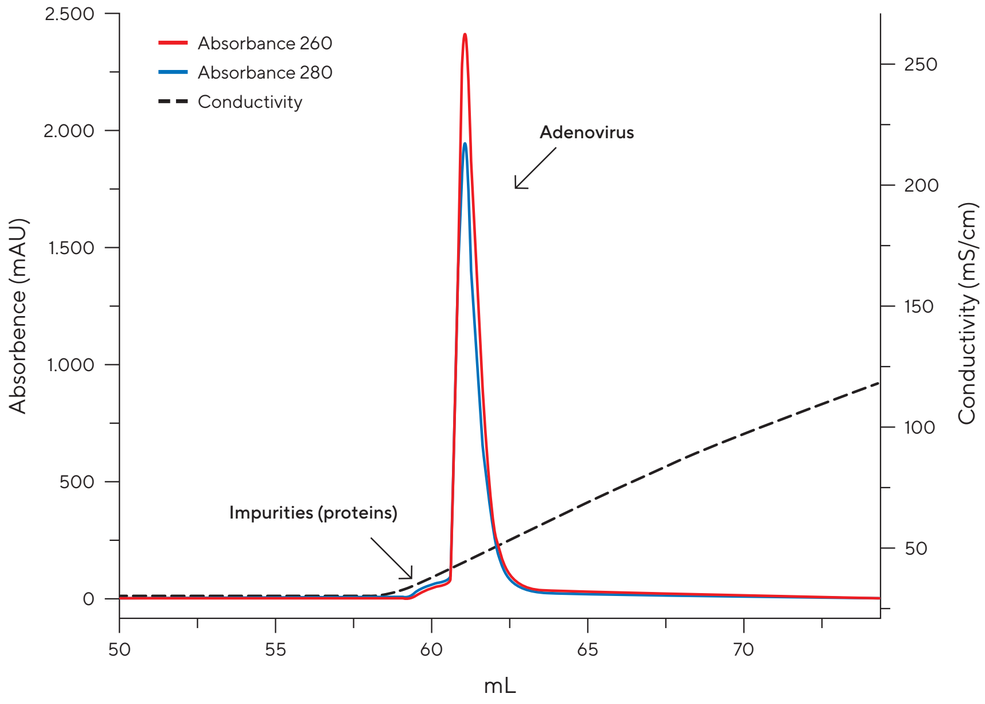
1. Capture of Adenovirus Using CIMmultus® QA

CIMmultus® monolithic columns are designed to separate and purify viral particles of any nature. The geometry of these columns enable higher flow rates without compromising capacity and separation performance. Due to laminar flow through the channels shear stress is eliminated, therefore virus integrity is not affected.

Simplified purification of Adenovirus consisting of typical downstream steps used in this process:

Figure 1: *Chart of Purification Process.*

Figure 2: *Chromatogram of Adenovirus Elution Using 1 mL CIMmultus® QA*



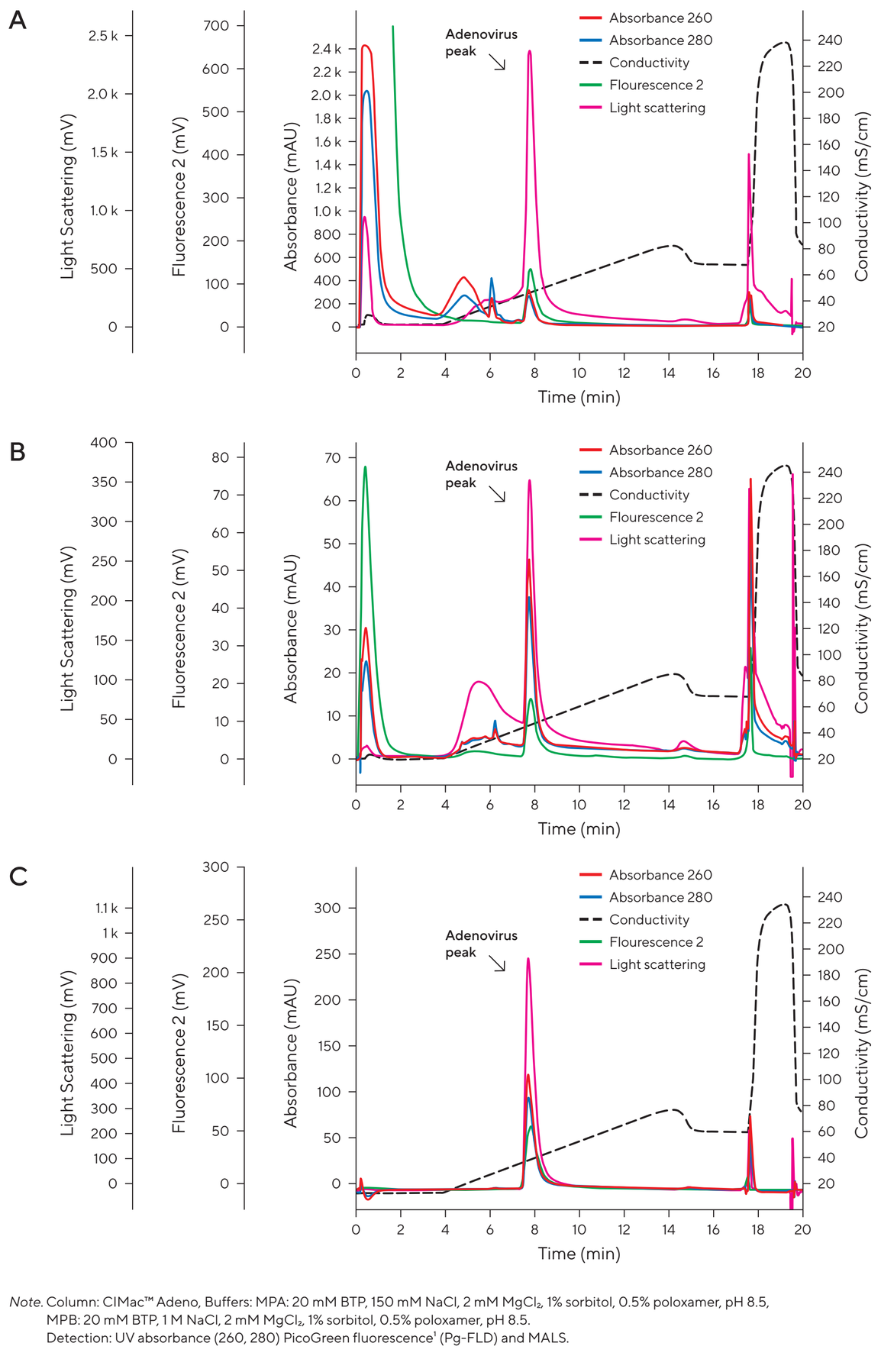
Note. Buffers: MPA: 50 mM Tris, 300 mM NaCl, 5% saccharose, 0.1% poloxamer 188, pH 8.5, MPB: 50 mM Tris, 2 M NaCl, 5% saccharose, 0.1% poloxamer 188, pH 8.5 | Sample: Adenovirus 5, TFF clarified retentate | Detection: absorbance (260, 280)

Capture step using CIMmultus® QA enables fast and efficient elution of Adenovirus of very good purity. Ratio of absorbance 260 and 280 indicates proteins in first peak and presence of virus in second peak.

2. Analytics of In-Process Fractions

In-process samples were analysed using PATfix® HPLC system with analytical CIMac™ Adeno column. Analytical method used allow for track Adenovirus through the process as well to follow the removal of impurities from the beginning to the end. In-line MALS detector serves as orthogonal Adeno virus quantification method.

Figure 3: *HPLC Chromatograms of In-Process Fractions, A - Lysate, B – TFF Retentate, C – QA Elution.*



Note. Column: CIMac™ Adeno, Buffers: MPA: 20 mM BTP, 150 mM NaCl, 2 mM $MgCl_2$, 1% sorbitol, 0.5% poloxamer, pH 8.5, MPB: 20 mM BTP, 1 M NaCl, 2 mM $MgCl_2$, 1% sorbitol, 0.5% poloxamer, pH 8.5.
Detection: UV absorbance (260, 280) PicoGreen fluorescence[1] (Pg-FLD) and MALS.

HPLC analytics of lysate (Fig. 3A, note: high Pg-FLD signal in flow-through corresponds mostly to the GFP protein) confirms presence of high levels of well separated impurities which were reduced during clarification, TFF and filtration (Fig. 3B). Effect is seen by comparing height of flow through and CIP peaks of in-process fractions. Once material is eluted from CIMmultus® QA Adenovirus is pure and free of complexes (Fig. 3C).

3. Polishing Step

Ultrafiltration and diafiltration or another chromatography step can be used to further purify and/or concentrate the virus as well exchange to formulation buffer.

4. Conclusions

Data presented in this study confirms CIMmultus® QA to be effective tool for Adenovirus purification. Due to high operational flow rates these columns enable virus of high concentration and outstanding purity in very short time. Furthermore, CIMmultus® QA columnsprovide higher yields at lower manufacturing costs. As well they represent an alternative for vaccine manufacturing constantly in lack of material supply. Accompanying HPLC analytics using CIMac™ Adeno column is reliable method offering rapid in-process insight for upstream and downstream applications.

1 B. Goricar, S. Peljhan, P. Gagnon, A. Strancar, BIA Separations,
Orthogonal characterization of AAV samples by simultaneous monitoring of multiple analytical parameters during chromatography