FASTFACTS

Adenovirus vector manufacturing platform using CIMmultus™ QA assures the supply of safe vaccines

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Downstream processing remains one of the main bottlenecks in adenoviral vector manufacturing. At BIA Separations, a Sartorius company, we offer a platform for purification of adenoviral vaccines using market-leading CIM® monolithic chromatographic columns and an analytical toolbox for process monitoring in adenoviral vaccine production.

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ADENOVIRAL VECTORS PURIFICATION PROCESS

Simplified purification of adenovirus consists of typical downstream steps, including lysis, clarification, tangential flow filtration (TFF), and a chromatographic capture on pre-packed monolithic columns from CIMmultus™ line. Lysis is performed by adding sodium chloride and detergent. DNA is digested by adding a salt-tolerant nuclease, Kryptonase, to the mixture. After incubation, clarification is done with coarse and fine filtration and subsequently, TFF is performed to prepare the sample for anion exchange chromatography on CIMmultus QA column. After the capture step, the polishing step of ultrafiltration and diafiltration, or another chromatography step can be used for formulation of the final adenovirus product.

CAPTURE

Capture using CIMmulus QA column is designed in bind-elute mode, to facilitate the concentration of virus and removal of impurities in flow-through and CIP **(Figure 1)**. TFF Retentate is prepared for chromatography with a fine filtration step in order to remove aggregates formed during TFF. The sample is then loaded on the column and eluted in a salt gradient.

ANALYTICS OF IN-PROCESS FRACTIONS

The in-process samples are analyzed using a fast and high-throughput chromatographic approach on PATfix™ HPLC system with analytical CIMac™ Adeno column. The analytical method was developed to track adenovirus, control adenoviral harvests, as well as follow the reduction of impurities through the manufacturing processes **(Figure 2)**. An additional feature of such HPLC analytics is a multi-angle light scattering detector, which serves as an orthogonal quantification method. Another important tool we are exploiting is PicoGreen fluorescence detection. PicoGreen reagent binds to DNA and helps us follow the removal of chromatin and DNA residuals, known to cause side effects of vaccines.

CONCLUSION

We offer an approach to tackle adenovirus production bottlenecks and improve the purity and quality of adenovirus vaccines. Chromatographic capture on CIMmultus QA is an effective tool for adenovirus vector purification. Due to high operational flow rates these columns enable production of virus at high concentrations and with outstanding purity in a very short time. CIMmultus QA columns provide higher yields at lower manufacturing costs and are readily available due to increased production capacity, overcoming the shortage of raw material supply on the market. Accompanying HPLC analytics using CIMac Adeno column is a reliable method offering rapid in-process insight for upstream and downstream applications.

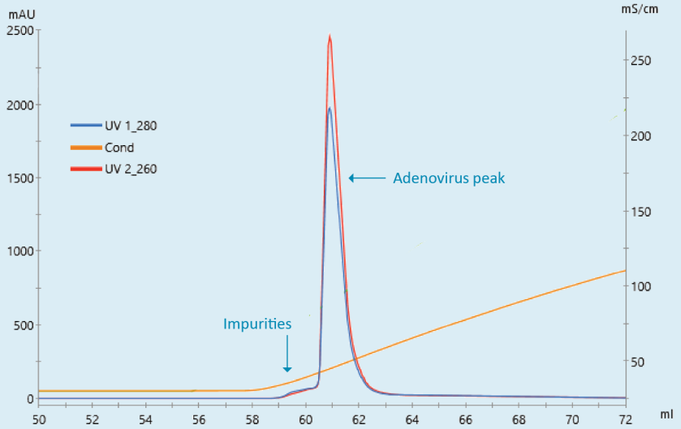

Figure 1. Example chromatogram of adenovirus elution from CIMmultus QA. Adenoviral harvest was prepared and loaded to CIMmultus QA column. Elution in salt gradient shows highly concentrated virus, already substantially purified of host cell contaminants.

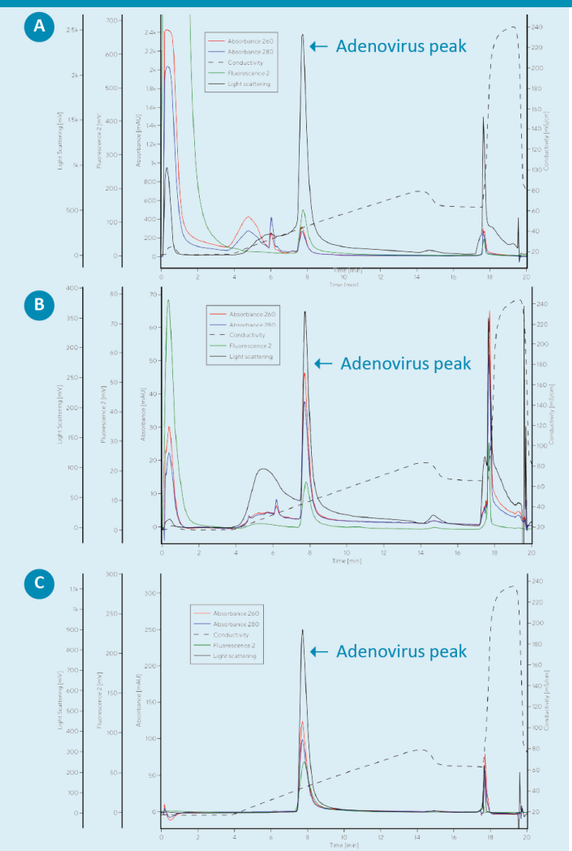

Figure 2. Analytical chromatograms using CIMac Adeno analytical columns showing the composition of in-process samples. Lysate **(A)** contains a high level of impurities, subsequently reduced with clarification and TFF **(B)**, that are well separated by CIMac Adeno column. Adenovirus eluted from CIMmultus QA **(C)** is already pure and aggregate-free.