Rapid Analysis of Adenoviruse Type 5 Particles with Bio-Monolith Anion-Exchange HPLC Columns to Support the Development of a High-Titre Manufacturing Platform

Application Note

Biopharmaceutical

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Abstract

Rapid analytical methods that can identify and quantitate adenoviral particles throughout the manufacturing process, from cellular lysate through to purified adenovirus, is necessary to support effective process development . A method was designed and developed to quantify adenoviral particles using a strong anion-exchange Bio-Monolith HPLC column. This method incorporates regeneration conditions to extend the functional life of the column. Results demonstrate efficient separation of intact adenovirus type 5 (Ad5) particles from contaminating proteins and DNA. This method was used to analyze samples taken throughout a downstream process. The samples were detected at 260 nm and 280 nm using a photodiode array detector (PDA). The method was validated according to performance criteria of repeatability, intermediate precision and linearity. The linear working range of analysis was established between 7.5×10^8 to at least 2.4×10^{10} viral particles (3×10^{10} to 9.6×10^{11} viral particles/mL), with a correlation coefficient of 0.9992. Relative standard deviations (RSDs) for intra- and inter-day repeatability and precision for retention time and peak area were less than 1 and 2.5%, respectively.

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Introduction

Adenoviruses are among the most commonly used vectors for the delivery of genetic material into human cells. Adenoviral vectors are currently being used in clinical applications such as suicide gene therapy, gene-based immunotherapy, gene replacement strategies and vaccine platforms. They are the most commonly used vectors for the delivery of genetic material into human cells, and there is demand for high-titre manufacturing processes for this application.

Successful development of biopharmaceuticals depends on high quality analytics to interpret process design and development studies. Analytical methods that can be applied not only to final purified samples but also throughout production are of particular benefit because this allows a process to be more thoroughly evaluated.

Many conventional methods for quantitative analysis of adenoviruses are labour and time-intensive. The biological activity of material within crude cellular lysates can be determined by a plaque assay. This cell-based assay method can take up to seven days to perform, is prone to error and will only report the number of infectious and not total viral particles. Total viral particle (VP) concentration is commonly quantitated by measuring the optical density at 260 nm with spectrophotometry, however, this method can only be applied to relatively pure viral samples.

The resolving power of the high-performance liquid chromatography (HPLC), however permits separation of intact virus particles from other cellular contaminants or virus particle fragments. Anion-exchange chromatography has already been applied to analyze various adenovirus preparations. The results of the anion-exchange HPLC methods can be obtained within minutes, allowing a faster evaluation of different process steps.

The objective of this method development was to enable rapid analysis of both cellular lysate and purified Ad5 preparations, to support the development of a purification program.

Experimental

Conditions

Column	Agilent Bio-Monolith Column QA (5.2 mm id × 4.95 mm) p/n 5069-3635
Mobile phase A:	see Figure legends
Mobile phase B:	see Figure legends
Gradient:	see Figure legends
Detection:	UV at 260 and 280 nm
Flow rate:	1 mL/min
HPLC system	An Agilent 1100 series HPLC system fitted with a diode array detector (DAD)

Results and Discussion

Method Development

The assay was developed on an Agilent 1100 HPLC system running at 1 mL/min. For each run 25 µL of adenovirus type 5 (Ad5) was loaded onto the Bio-Monolith QA column.

Initial gradient: Buffer A: 20 mM Tris, pH 7.5; Buffer B: 20 mM Tris, 1.5 M NaCl, pH 7.5. Linear gradient of 0 to 100% Buffer B over 19 column volumes (CV), followed by a 2 CV hold at 100% B (Figure 1 a). Total run time = 4 minutes.

Excellent efficiency was observed, however all bound analytes eluted over a 1 minute duration. Therefore, to increase separation the buffer conditions were altered and a step gradient was introduced.

Step gradient: Buffer A: 20 mM Tris, 0.1 M NaCl, pH 7.5; Buffer B: 20 mM Tris, 2.0 M NaCl, pH 7.5. The step gradient has two 22 CV holds at 20% and 57% Buffer B (Figure 1 b). Total run time = 10 minutes.

This step gradient increased separation of Ad5 particle peaks from the earlier eluting proteinaceous peaks.

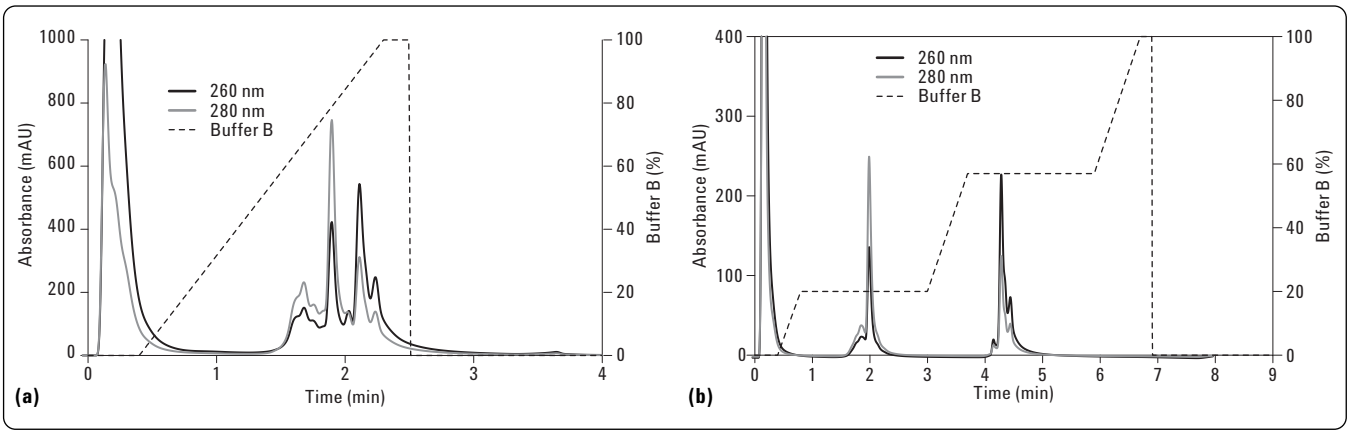


Figure 1 Analysis of Ad5 cellular lysate on a Bio-Monolith QA column. The solid blank line is absorbance at 260 nm and the grey line is absorbance at 280 nm.

Conditions:
(a) Buffer A: 20 mM Tris, pH 7.5; Buffer B: 20 mM Tris, 1.5 m NaCl, pH 7.5. Linear gradient of 0 to 100% Buffer B over 19 column volumes (CV), followed by a 2 CV hold at 100% B.
(b) Buffer A: 20 mM Tris-HCl buffer + 0.1 M NaCl, pH 7.5; Buffer B: 20 mM Tris-HCl buffer + 2 M NaCl, pH 7.5. Step gradient: a 22 CV hold at 20% buffer B and a 22 CV hold at 57% Buffer B. Flow rate: 1 mL/min. Sample: Ad5 cellular lysate. Injection volume: 25 µL.

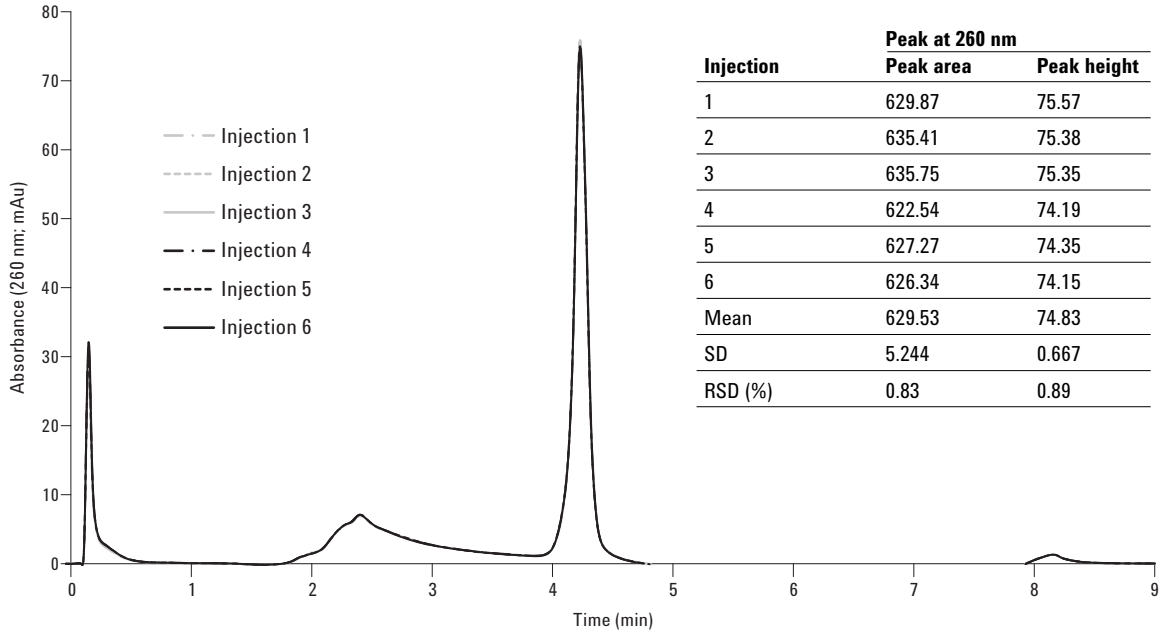
Method qualification

Repeatability: This was demonstrated by 6 × 25 µL (intra-day) injections of purified Ad5 at (2.4×10^{10} VP; Figure 2). The mean, standard deviation and RSD values of the retention time, peak height and peak area were calculated. Excellent repeatability with RSD values of <1% was demonstrated (see Table insert).

Intermediate Precision: Ad5 samples at 66% and 33% of the normal test concentration were injected in triplicate over three separate days. The data was combined and statistical analysis showed RSD values of <0.2% and 2.5% for retention time and peak area respectively. A higher RSD value of 15% was recorded for peak height. However, there was evidence of peak flattening, which may be indicative of product stability rather than decreased assay precision.

Linearity and working range: A two-fold serial dilution was applied in duplicate from 2.4×10^{11} to 4.7×10^{8} VP. The data was plotted and the correlation coefficient (r^2) was used to estimate the linearity of the standard curve ($r^2 = 0.9992$; RSD = <3%). The working linear range was determined as 7.5×10^{8} to 2.4×10^{10} VP (Figure 3).

Column performance: Performance was maintained and monitored by regular regeneration (5 × 25 µL 1M NaOH injections) and daily runs of AEX protein standards. The retention times of four protein standards (myoglobin, conalbumin, ovalbumin and trypsin inhibitor from soybean) remained constant over 100 injections. The pressure only slightly increased (from 55 to 65 bar).



Injection	Peak at 260 nm Peak area	Peak height
1	629.87	75.57
2	635.41	75.38
3	635.75	75.35
4	622.54	74.19
5	627.27	74.35
6	626.34	74.15
Mean	629.53	74.83
SD	5.244	0.667
RSD (%)	0.83	0.89

Figure 2. Intra-assay repeatability. 6 × 25 µL injections of purified Ad5 were injected onto the Bio-Monolith QA column. A two-step gradient with a 22 CVs hold at 20% Buffer B and a second 22 CVs hold at 57% Buffer B was applied at a flow rate of 1 mL/min. The peak areas and heights were determined, and means were calculated.

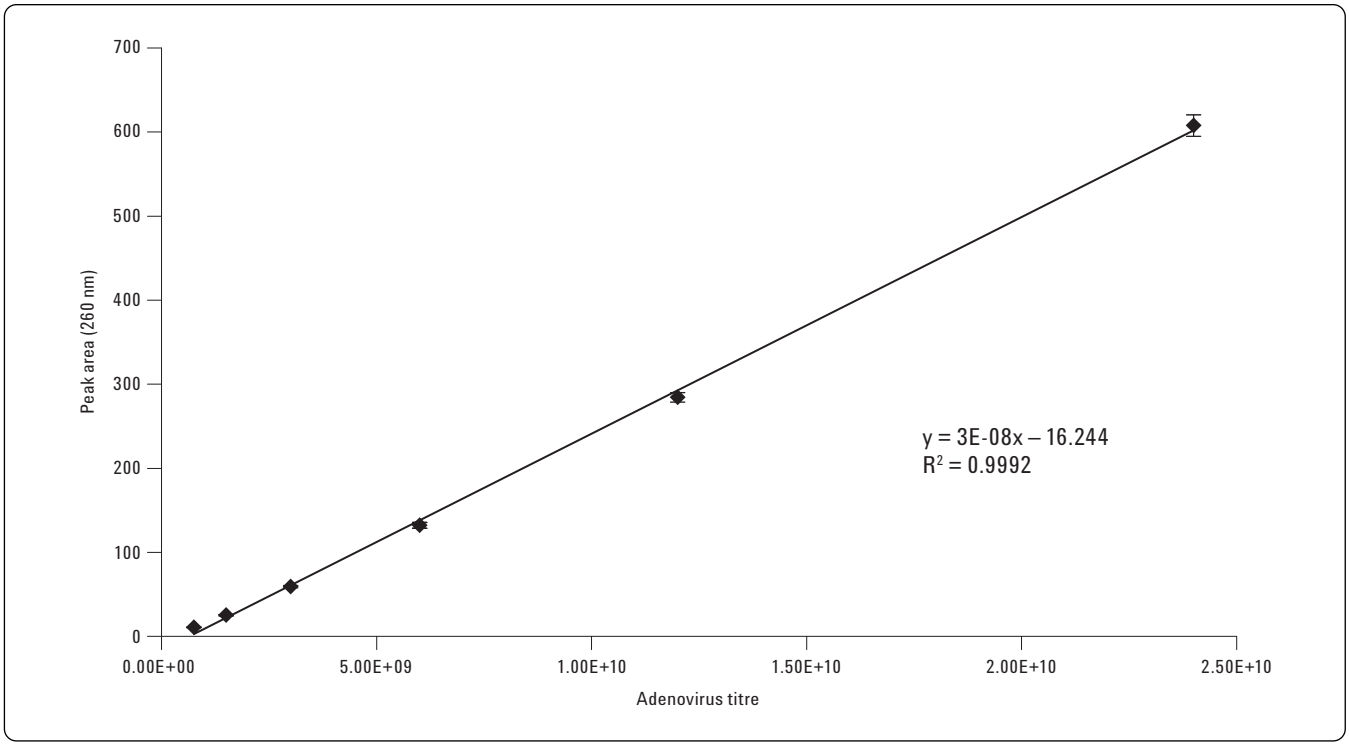


Figure 3 Linearity and working range for the Bio-Monolith QA assay. Two-fold serial dilution of Ad5 (4.7×10^8 to 2.4×10^{11}).

In-process analysis during Ad5 DSP

The Bio-Monolith QA HPLC analytical method was applied to enable rapid decisions during development of an Ad5 purification method.

Once developed, the method was applied as an in-process assay to evaluate product purity (Figure 4). The majority of contaminating protein or nucleic acid contaminants were removed by the primary capture chromatography step (Figure 4b). The remaining contaminating proteins, detected in the flow through or during the first hold step, were removed during the final, polishing chromatography step (Figure 4c). Product purity was demonstrated, because the Ad5 particles were detected as a single peak following concentration and exchange into the final formulation buffer (Figure 4d).

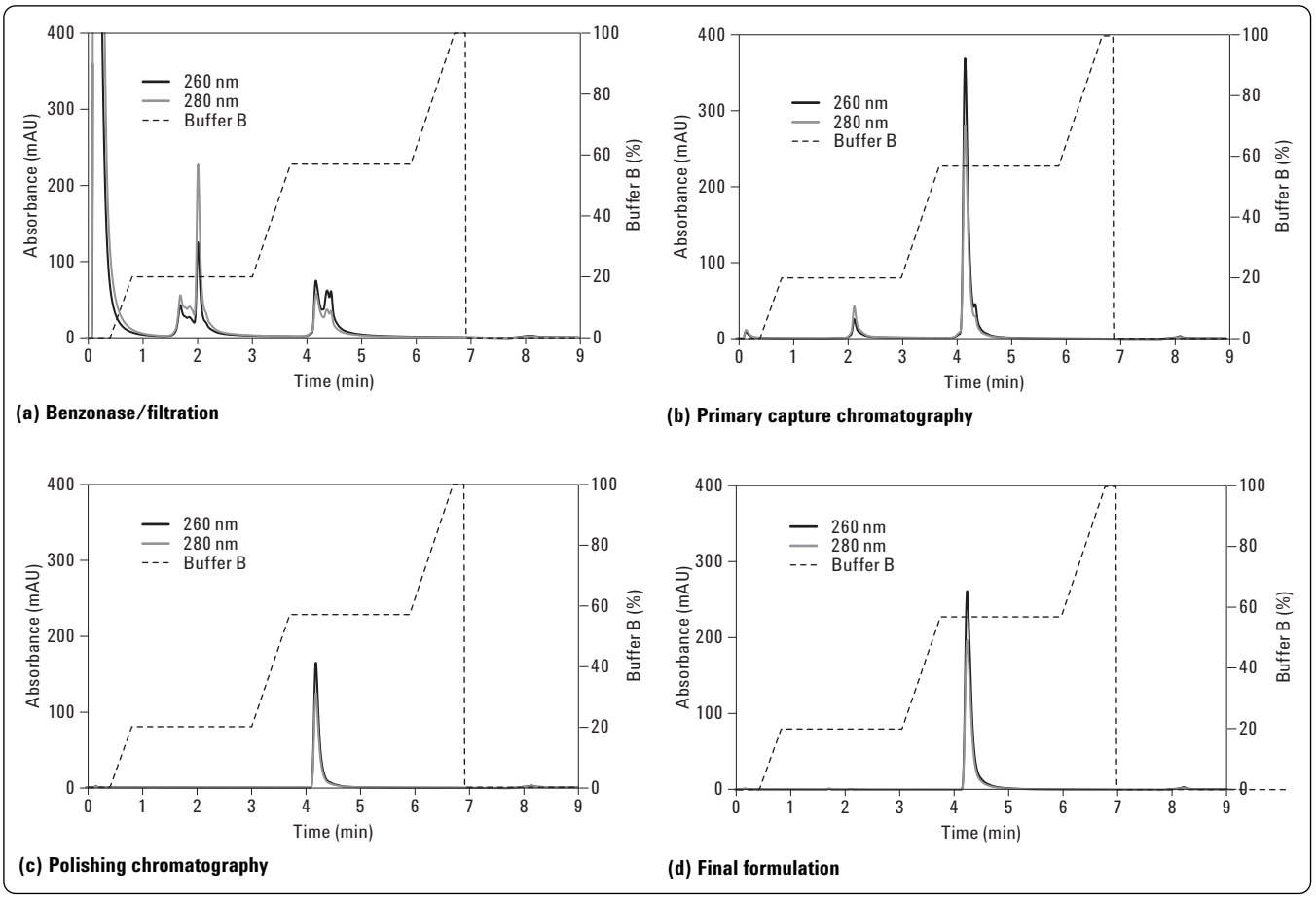


Figure 4: Ad5 purification process exemplification by high-performance anion-exchange chromatography on a Bio-Monolith QA column.

Conclusions

The Agilent Bio-Monolith QA HPLC method allows the rapid analysis of Ad5 particles throughout the entire production method. A two-step gradient method provides separation of intact Ad5 particles from other biomolecules, and is precise and linear in the range of 7.5×10^8 to at least 2.4×10^{10} viral particles. The method has been applied to develop and exemplify a purification strategy for the production of Ad5 particles for clinical applications.

Reference

Robert J. Whitfield, Suzanne E. Battom, Miloš Barut, David. E. Gilham, Philip D. Ball, "Rapid High-Performance Liquid Chromatographic Analysis of Adenovirus Type 5 Particles with a Prototype Anion-Exchange Analytical Monolith Column," *J. Chromatogr. A*, 1216, (2009), 2725 – 2729.

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