Rapid analysis of adenoviruses with prototype CIM® QA analytical columns to support the development of high titre manufacturing platform

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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 utilised in clinical applications such as suicide gene therapy, gene based immunotherapy, gene replacement strategies and vaccine platforms. To support the development of a high titre purification process, various anion exchange methods have been evaluated. A method was designed and developed to quantify adenoviral particles using a prototype CIM® QA analytical HPLC column. This method was validated according to performance criteria of repeatability, intermediate precision and linearity. We also report on conditions that efficiently regenerate the column, extending its functional life. Our results demonstrate efficient separation of intact adenovirus type 5 particles from contaminating proteins and DNA. This method was used to analyse samples taken throughout a downstream process. These samples were detected at 260nm and 280nm using a photodiode array detector (PDA).

Method Development

The prototype CIM® QA column was provided by BIA Separations (column size = 76 µL; estimated maximum pressure = 150 bar). 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 column.

Initial gradient: Buffer A: 20 mM Tris, pH 7.5; Buffer B: 20mM 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). Total run time = 4 minutes. Good 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: 20mM Tris, 2.0 M NaCl, pH 7.5. The step gradient has two 22 CV holds at 20% and 57% Buffer B (Figure 2). Total run time = 10 minutes. This step gradient increased separation of Ad5 particle peaks from the earlier eluting proteinaceous peaks.

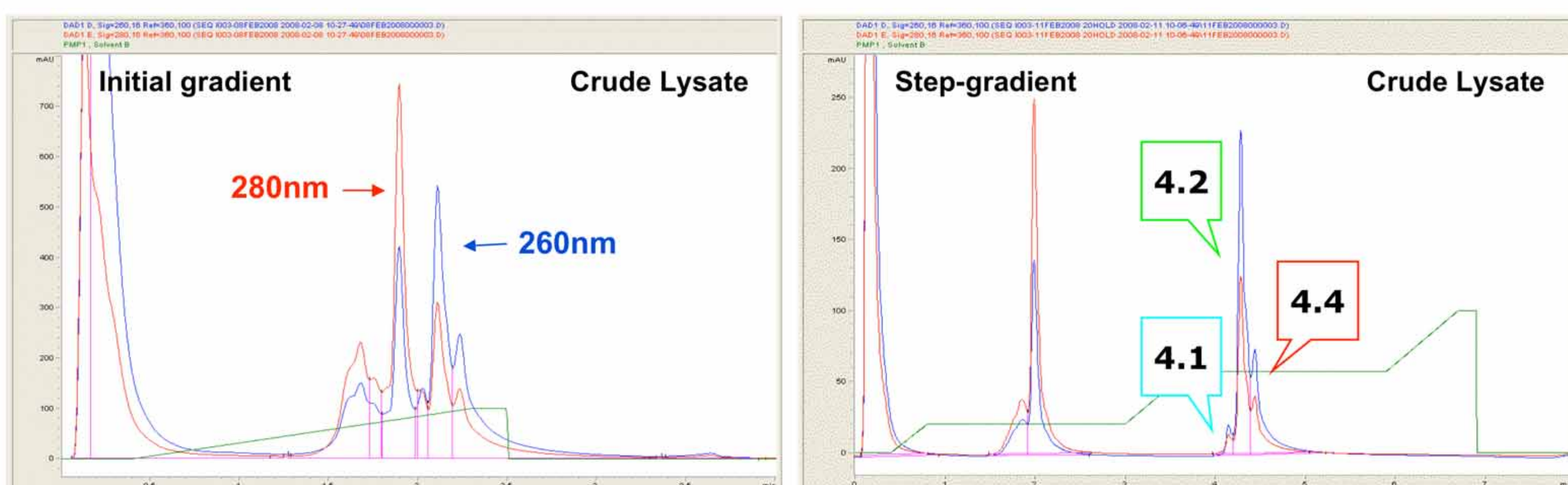

Figure 1: 25 µL injection of Crude Lysate (2.3×10^8 IU) - linear gradient.

Figure 2: 25µL injection of Crude Lysate (1.15×10^8 IU) - step gradient.

Method qualification

Repeatability: Demonstrated by 6 x 25 µL (intra-day) injections of purified Ad5 at (2.4×10^{10} VP; Figure 3). 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 (Table 1).

Intermediate Precision: Ad5 samples at 66% and 33% of the normal test concentration were in injected 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 (Table 2). 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 2 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 4).

Column performance: Performance was maintained and monitored by regular regeneration (5 x 25 µL 1M NaOH injections) and running AEX protein standards daily (Figures 5 & 6).

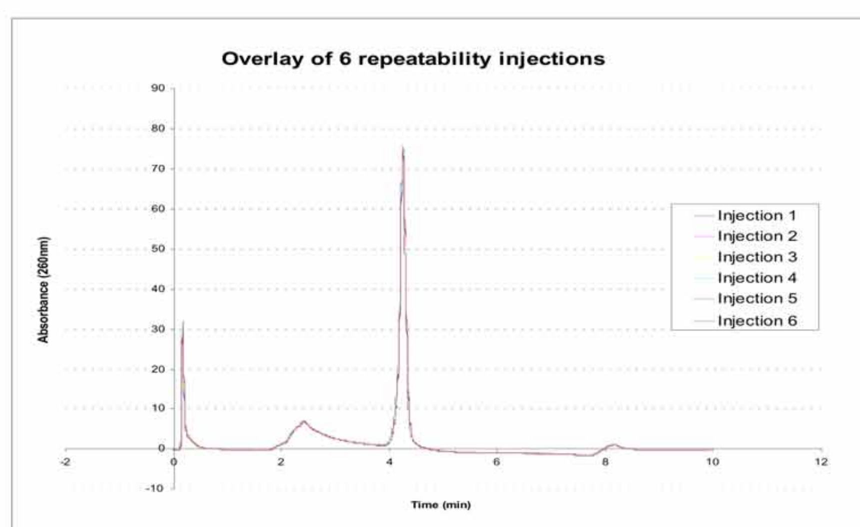

Figure 3: Overlay profile of 6x Ad5 injection (2.4×10^{10} VP)

6x25µL	R T (min)	Peak Area	Peak Height
Mean	4.23	629.5	74.8
SD	0.001	5.244	0.669
RSD %	0.02	0.83	0.89

Table 1: Repeatability data recorded at 260nm

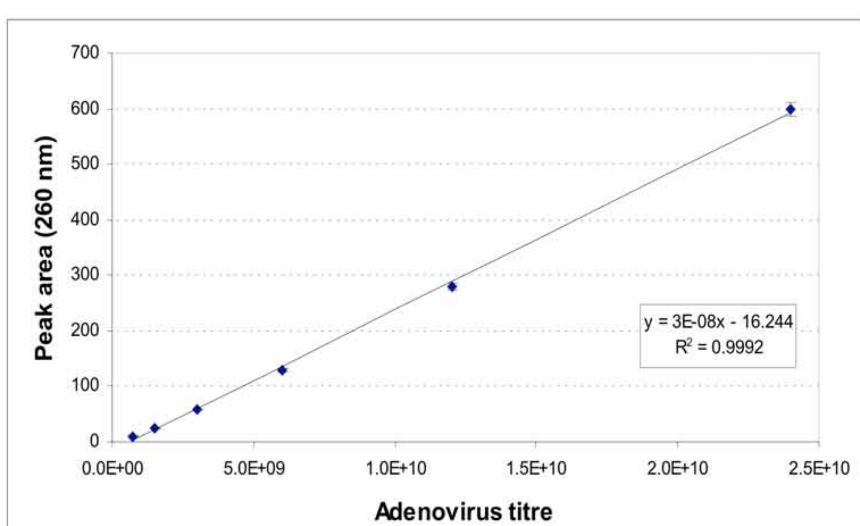

Figure 4: 2-fold serial dilution of Ad5 (4.7×10^8 to 2.4×10^{11}VP)

		RT (min)	Peak Area	Peak Height
66%	Mean	4.24	405.6	40.51
	SD	0.007	8.199	5.837
	RSD %	0.16	2.02	14.41
33%	Mean	4.24	195.8	18.79
	SD	0.006	2.262	2.618
	RSD %	0.14	1.16	13.93

Table 2: Intermediate precision data recorded at 260nm

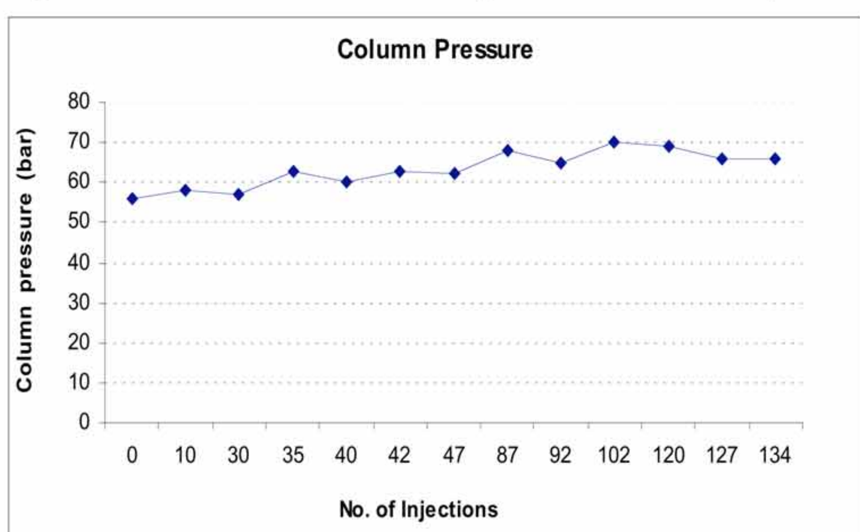

Figure 5: Column pressure over increasing sample injections.

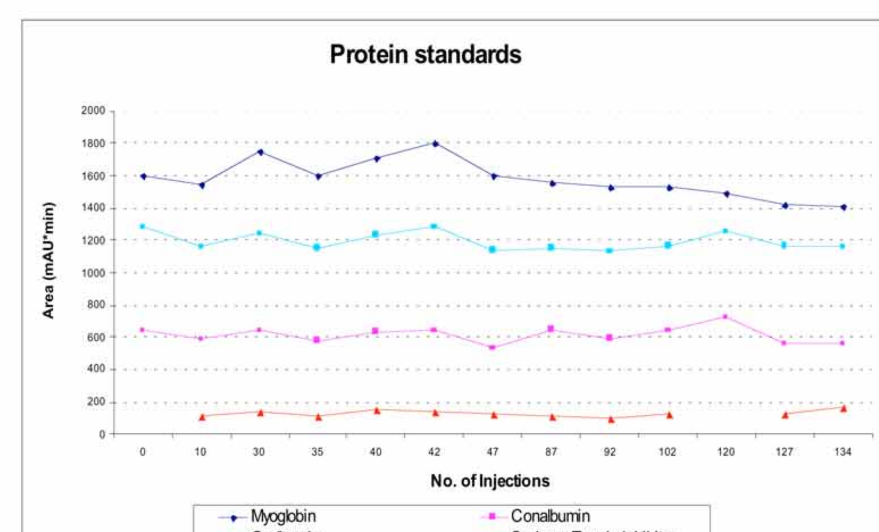

Figure 6: Column performance over increasing sample injections.

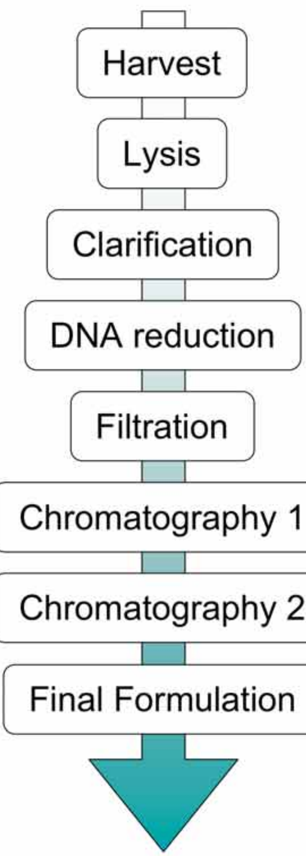

Figure 7: DSP Flowchart.

In-process analysis during Ad5 DSP development

The developed HPLC method was applied during development of a Downstream Process (DSP) for adenovirus production (Figure 7). Results from analysis were used to influence or demonstrate the purification process development.

1. DNA reduction.
$A_{260:280}$ ratio of Peak 4.2 and 4.4 indicated that it was of nucleic acid origin. Peak reduction following a specific DNA reduction step demonstrated effectiveness of the method (Figures 8 & 9).

2. Purification by primary capture (Chromatography 1).
HPLC analysis demonstrated the product purification achieved during chromatography 1 (Figures 10 &11).

3. Product polishing by Chromatography 2.
Further product purification was demonstrated following the second chromatography step (Figure 12).

4. Product Final Formulation.
Concentration and buffer exchange into final formulation (Figure 13).

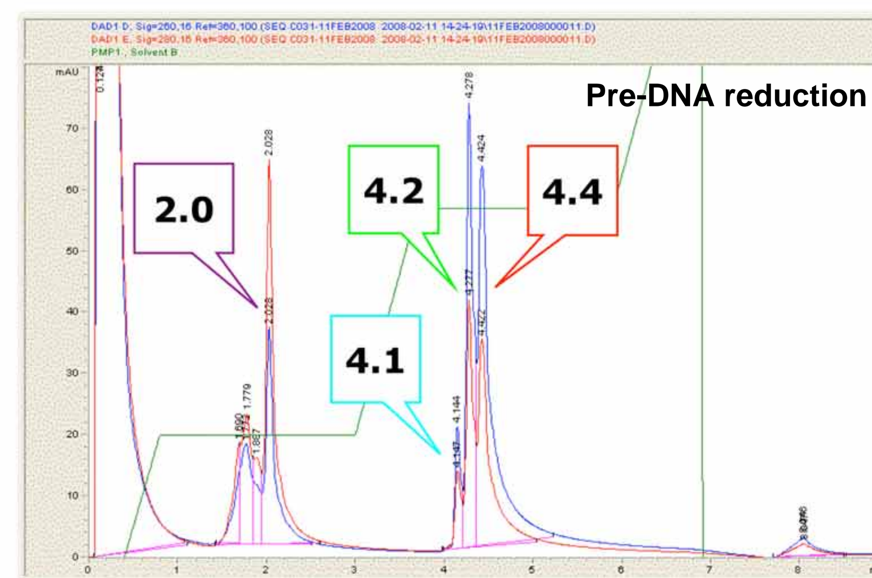

Figure 8: Elution profile of 25 µL pre-DNA reduction sample

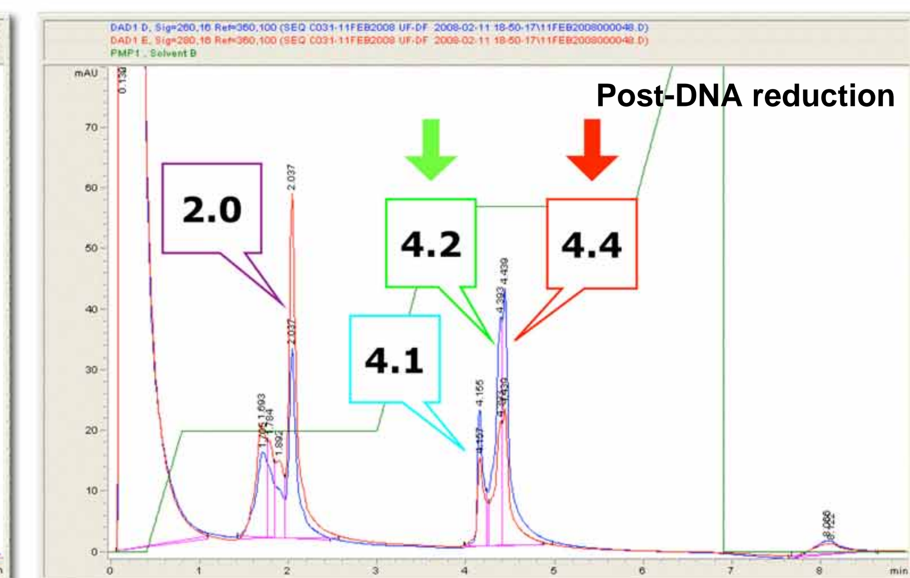

Figure 9: Elution profile of 25 µL post-DNA reduction sample

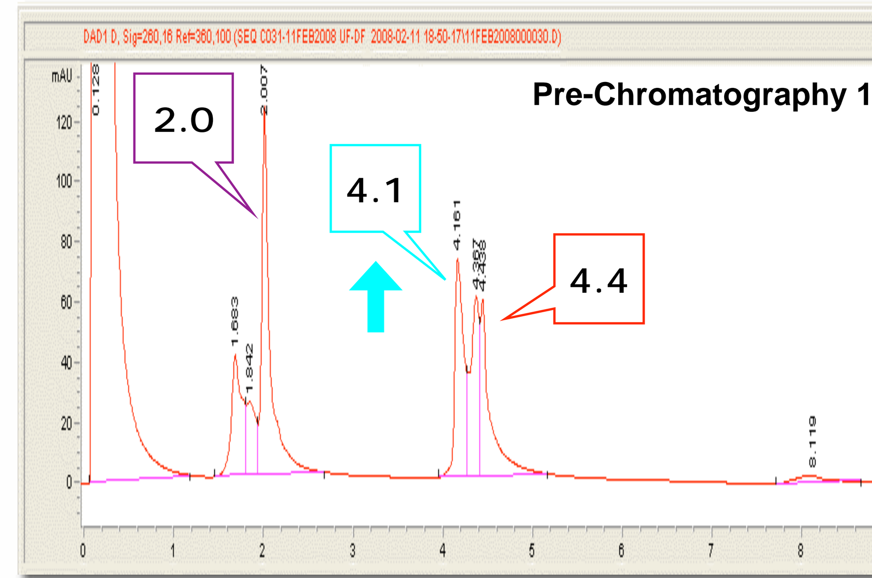

Figure 10: 260nm elution profile of 25 µL pre-chromatography 1 sample

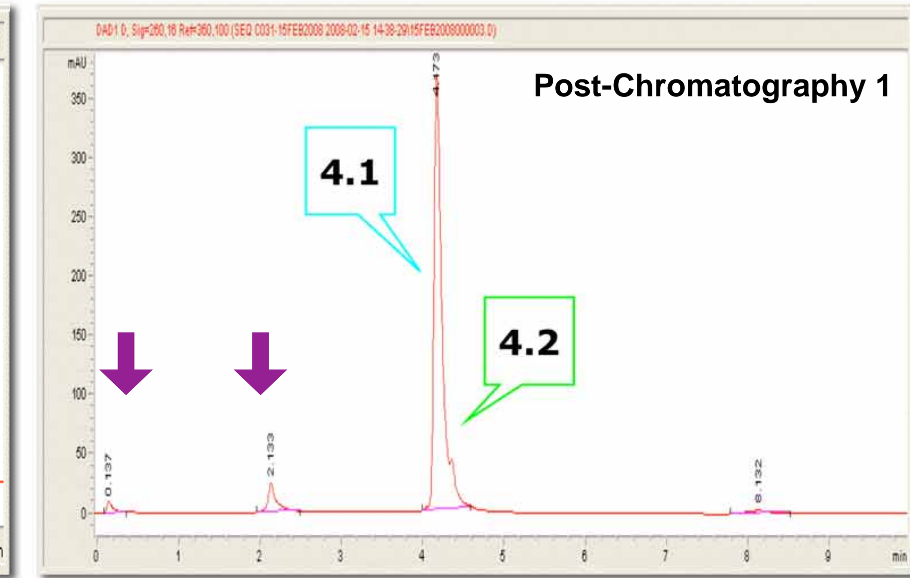

Figure 11: 260nm elution profile of 25 µL chromatography 1 sample

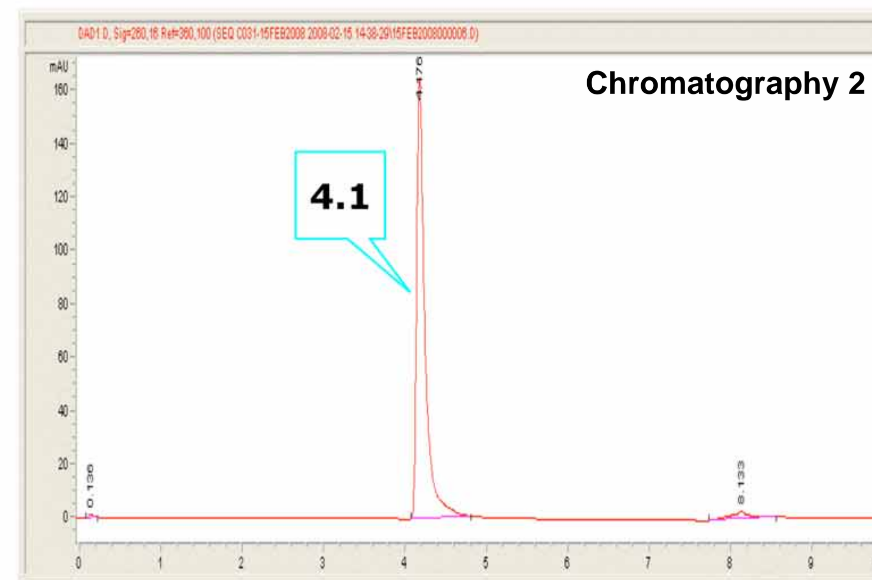

Figure 12: 260nm elution profile of 25 µL chromatography 2 sample

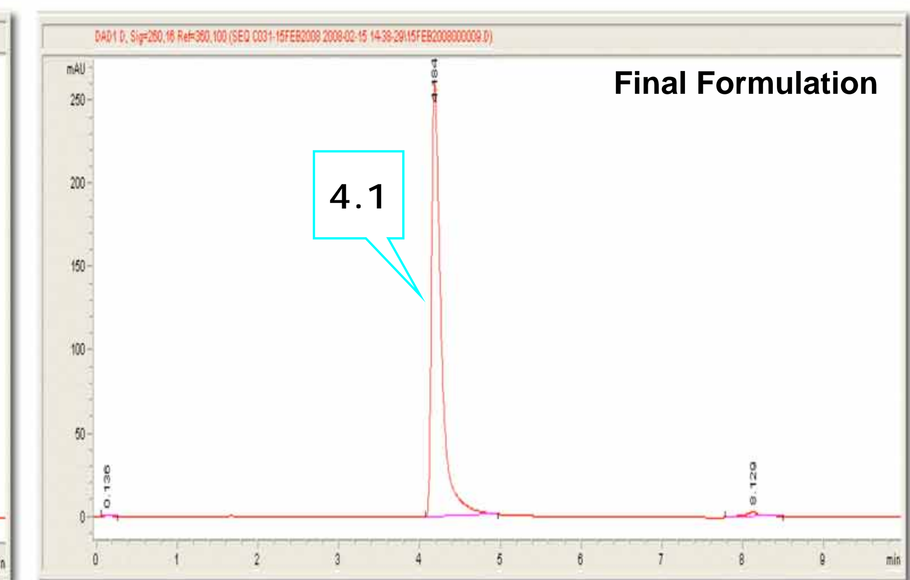

Figure 13: 260nm elution profile of 25 µL final formulation sample

Conclusions

- CIM QA HPLC allows the rapid analysis of both crude lysate and purified Ad5 preparations.
- The HPLC method can potentially be used to quantify adenoviruses over a linear working range of 7.5×10^8 to 2.4×10^{10}+ viral particles.
- The gradient method developed provides separation of intact Ad5 particles from other biomolecules.
- Method qualification demonstrated assay repeatability, precision and linearity.
- Performance is increased by routine column regeneration.
- The process was invaluable in the development and exemplification of an adenovirus purification platform.
- Further work is being conducted to increase separation of sample components.

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