Novel HPLC Method to Accelerate the Development of Adenoviral Vaccines

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Eden Biodesign, Inc

CONSULTING • DEVELOPING • MANUFACTURING

Questions

Questions are encouraged throughout the presentation and can be asked by using the link provided within your webcast viewer.

Eden Biodesign

"Designing and developing valuable biopharmaceutical medicines by the application of good science from day one"

Eden Biodesign

- CMO offering: expression system development, cell banking, process/analytical development, cGMP production services
- Offering all major biologics cGMP production platforms
  - Mammalian cell culture
  - Microbial fermentation
  - Viral production
- Consultancy specializing in CMC issues, regulatory support, cGMP training, clinical trial supply logistics
- EMEA inspected cGMP facilities in Liverpool, UK
- US subsidiary located in Research Triangle Park, NC

Presentation overview

- Development and qualification of a HPLC method for the analysis of adenoviruses
- Application of the method during process development for a type V adenoviral vaccine product

Quantitative analysis of adenoviruses

Effective process development requires high quality analytics

Currently available analytical techniques are frequently unreliable and/or slow:

- Plaque assays (IU/mL)
  - Time consuming (> 3 days)
- OD_{260} (TP/mL)
  - Can only be applied on purified product
  - Assay variability
- Anion Exchange-High Performance Liquid Chromatography

Anion exchange HPLC

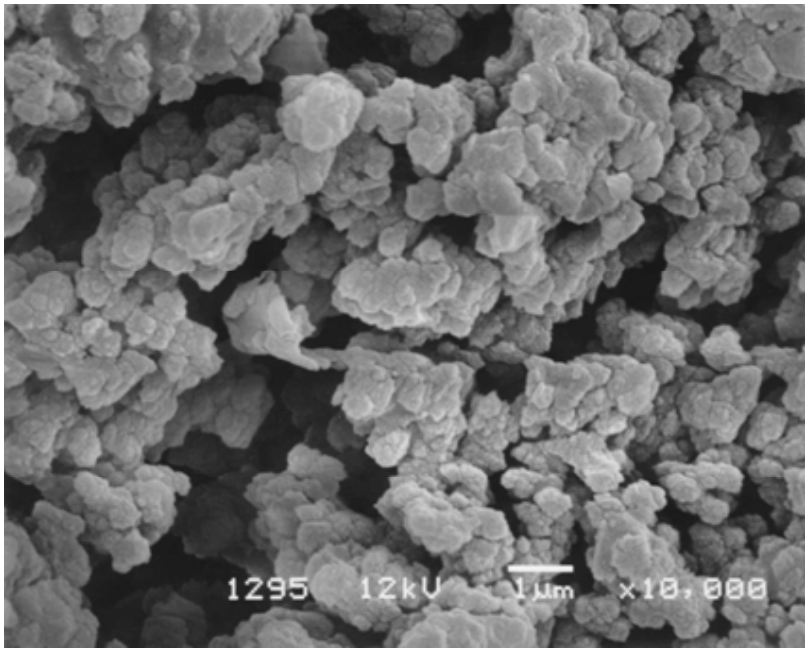
- UNO Q - BioRad
  - Ref: Transfiguracion, J *et al* (2001) Validation of a high-performance liquid chromatographic assay for the quantification if adenovirus type 5 particles. *Journal of Chromatography B,* 761, 187-194.
- Q Sepharose XL - GE Healthcare
  - Ref: Kuhn, I *et al* (2007) High-performance liquid chromatography method for rapid assessment of viral particle number in crude adenoviral lysates of mixed serotype. *Gene Therapy*, 14, 180-184.
- Resource Q - GE Healthcare
  - Ref: Klyushnichenko, V *et al* (2001) Improved high-performance liquid chromatographic method in the analysis of adenovirus particles. *Journal of Chromatography B*, 755, 27-36.
- *CIM® QA Monolith Columns - BIA Separations*

CIM® Monoliths

Convective Interaction Media Technology

Highly porous rigid polymers

- High porosity (over 60 %)
- Flow-through channels (“pores”) having large diameter (1 to 5 μm)
- Uniform channel connectivity in 3D (homogeneous structure)



Courtesy BIA Separations

CIM® QA analytical HPLC column

- Column size = 76 µL
- Max pressure = 150 bar
- Column temperature = 22ºC
- Injection volume = 25 µL
- Flow rate = 1 mL/min

Initial chromatography conditions

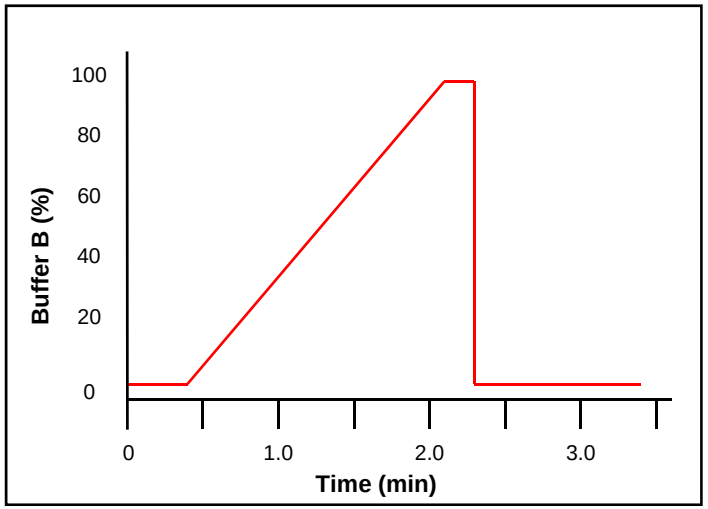
Stage	CV	Buffer B (%)
Equilibration	4	0
Gradient	19	100
Hold	2	100
Re-equilibration	9	0

Buffers:

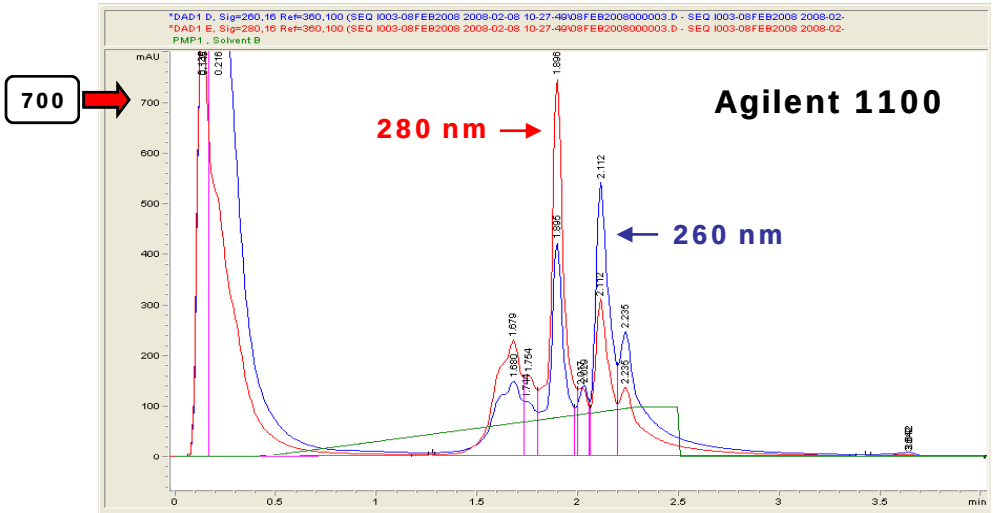
Buffer A 20 mM Tris, pH 7.5
Buffer B 1.5 M NaCl, 20 mM Tris, pH 7.5

Total run time: 4 minutes

Gradient profile



Crude lysate injection (2.3x10^8 IU; 9.26x10^9 IU/mL)



Comparison to Q Sepharose™ XL

- GE Healthcare
- Column size = 1 mL
- Max pressure = 3 - 4 bar
- Column temperature = 22ºC
- Injection volume = 25 µL
- Flow rate = 1 mL/min

Comparison to Q Sepharose™ XL

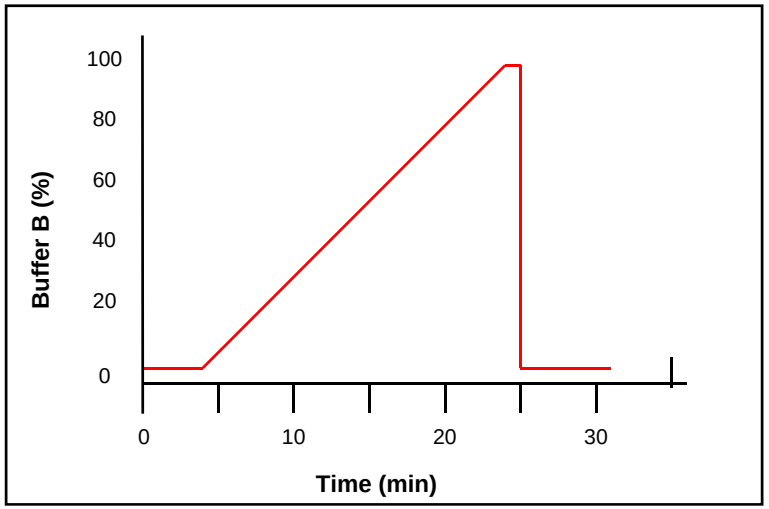
Stage	CVs	Buffer B (%)
Equilibration	4	0
Gradient	19	100
Hold	2	100
Re-equilibration	9	0

Buffers:

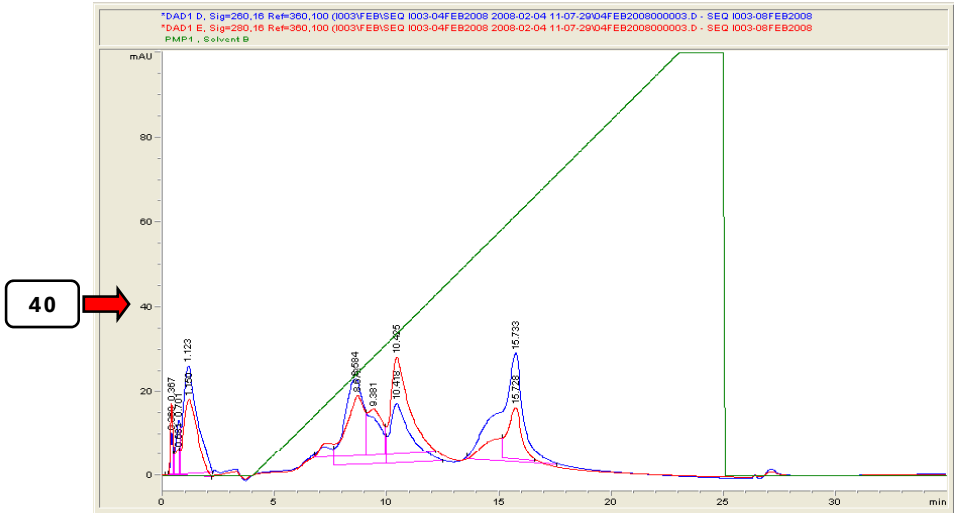
Buffer A 20 mM Tris, pH 7.5
Buffer B 1.5 M NaCl, 20 mM Tris, pH 7.5

Total run time: 40 minutes

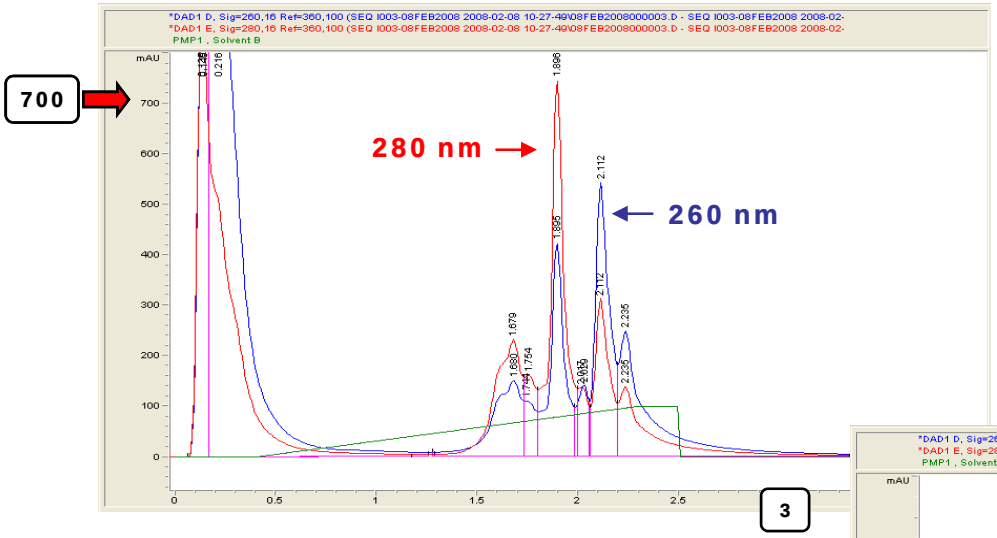
QXL gradient profile

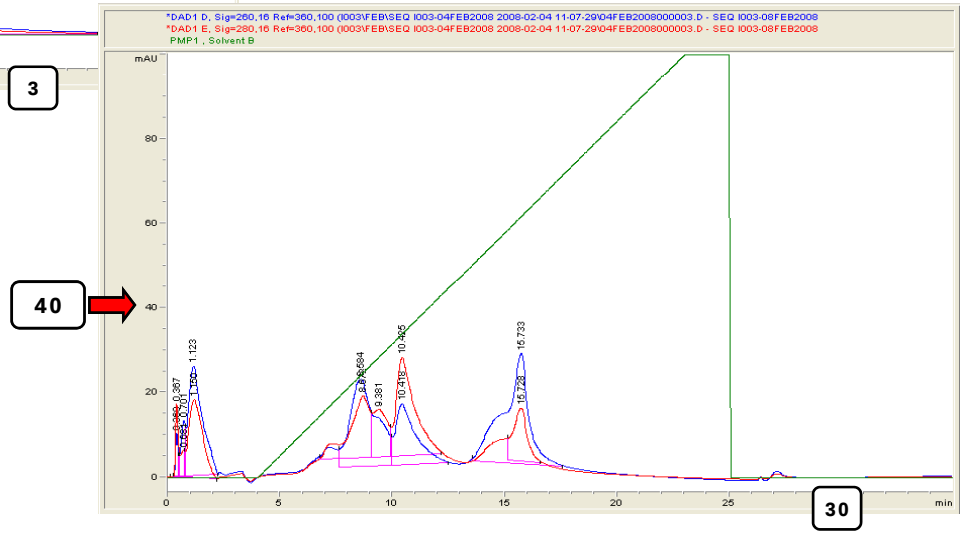


Crude lysate injection (2.3x10^8 IU; 9.26x10^9 IU/mL)



Comparison to Q Sepharose™ XL



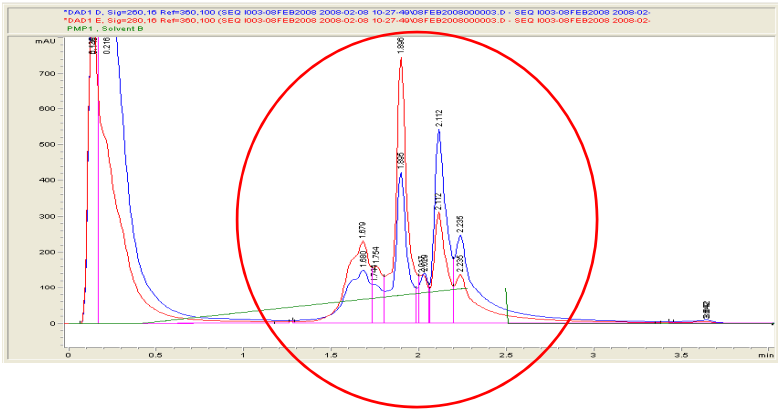


Comparison to Q Sepharose™ XL

Column	Repeatability	Carryover	Sensitivity	Analysis time
Q Sepharose XL	✓	✓	✗	✗
CIM QA Monolith	✓	✓	✓	✓

Note – Q XL column subject to resin compaction

Further method development



- Good preliminary data, but all bound proteins elute over a 1.5 minute duration
- Attempted to increase separation by investigation of:
  - Alternative buffer compositions
  - Increasing the gradient length
  - Step gradient

Method development

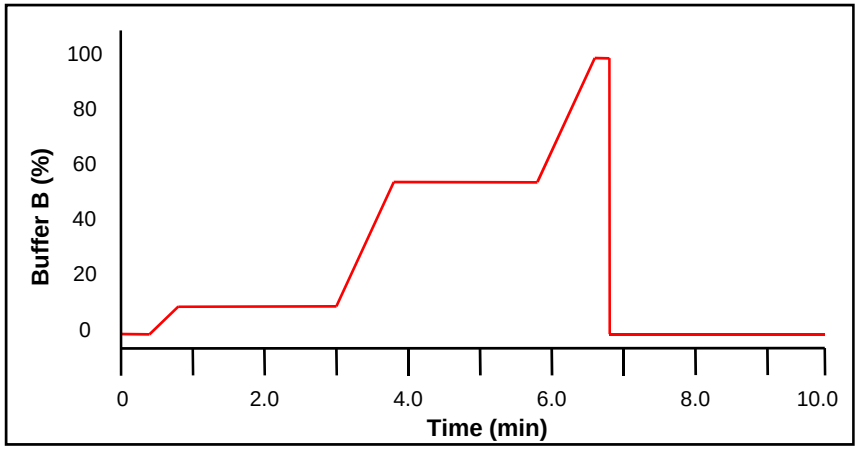
Stage	CV	Buffer B (%)
Equilibration	4	0
Step 1	4	20
Hold 1	22	20
Step 2	7	57
Hold 2	22	57
Step 3	8	100
Hold 3	2	100
Re-equilibrate	10	0

Buffers:

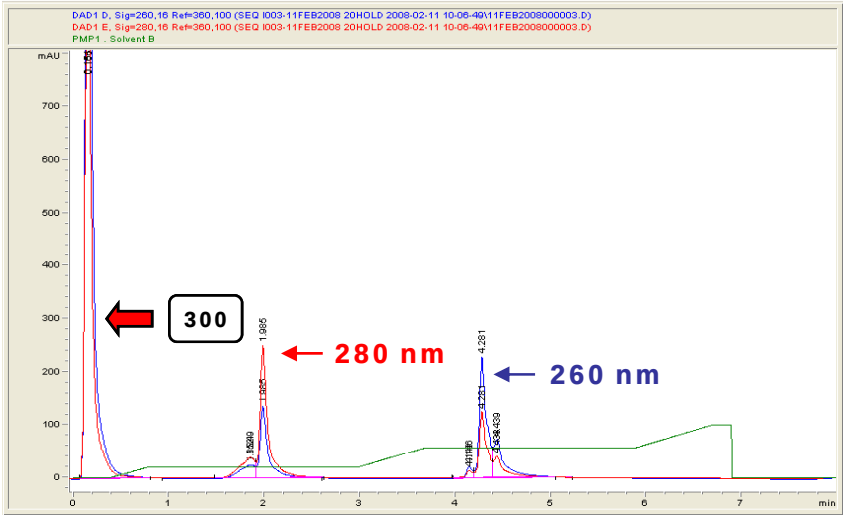
Buffer A 20 mM Tris, 0.1 M NaCl, pH 7.5
Buffer B 2.0 M NaCl, 20 mM Tris, pH 7.5

Total run time: 10 minutes

CIM QA Monolith gradient profile



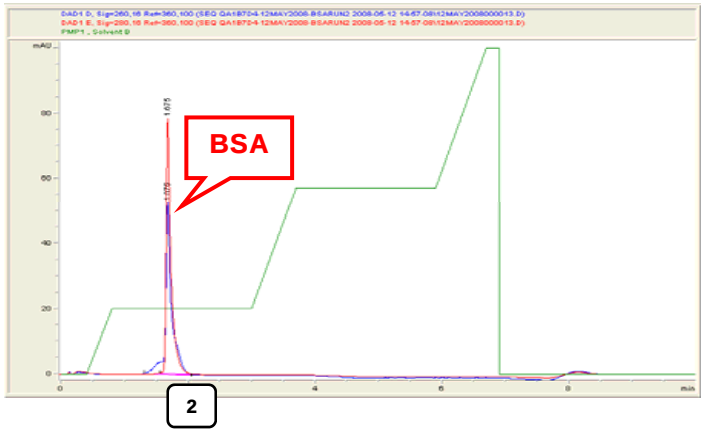
Crude lysate injection (1.15×10^8 IU; 4.63×10^9 IU/mL)



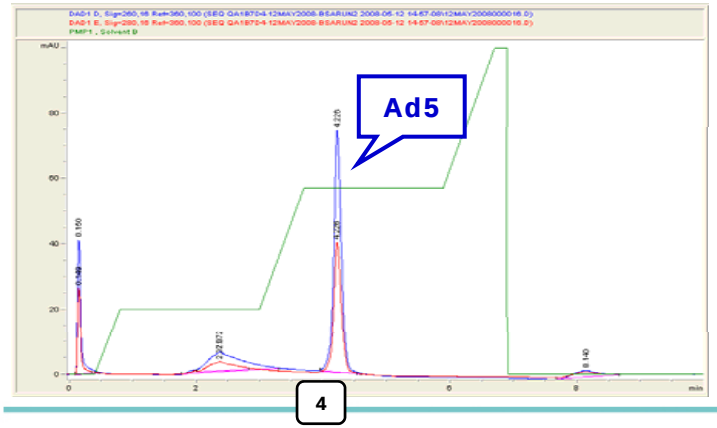
Method development

BSA spiked into an a purified adenovirus sample

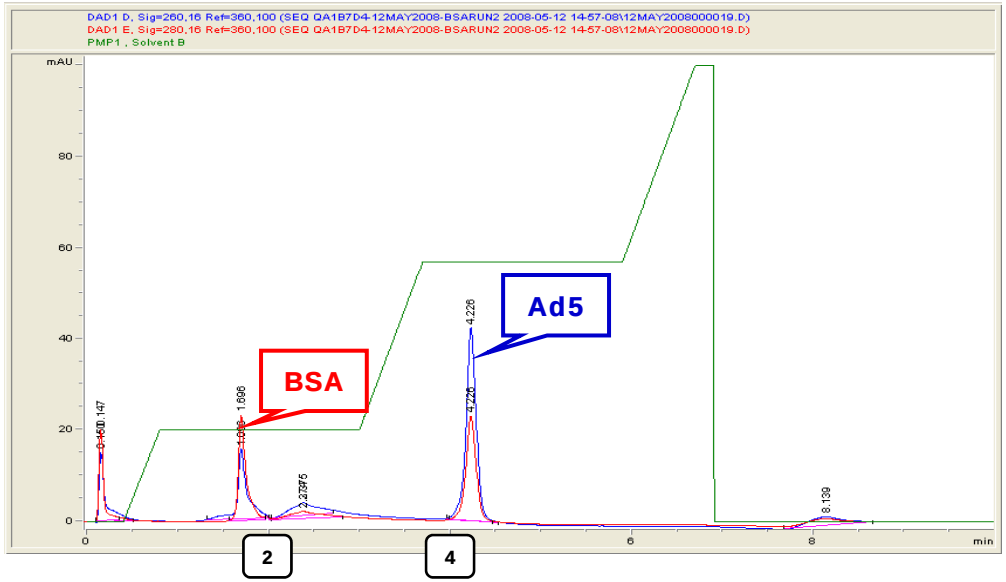
Bovine Serum Albumin



Partially purified Ad5



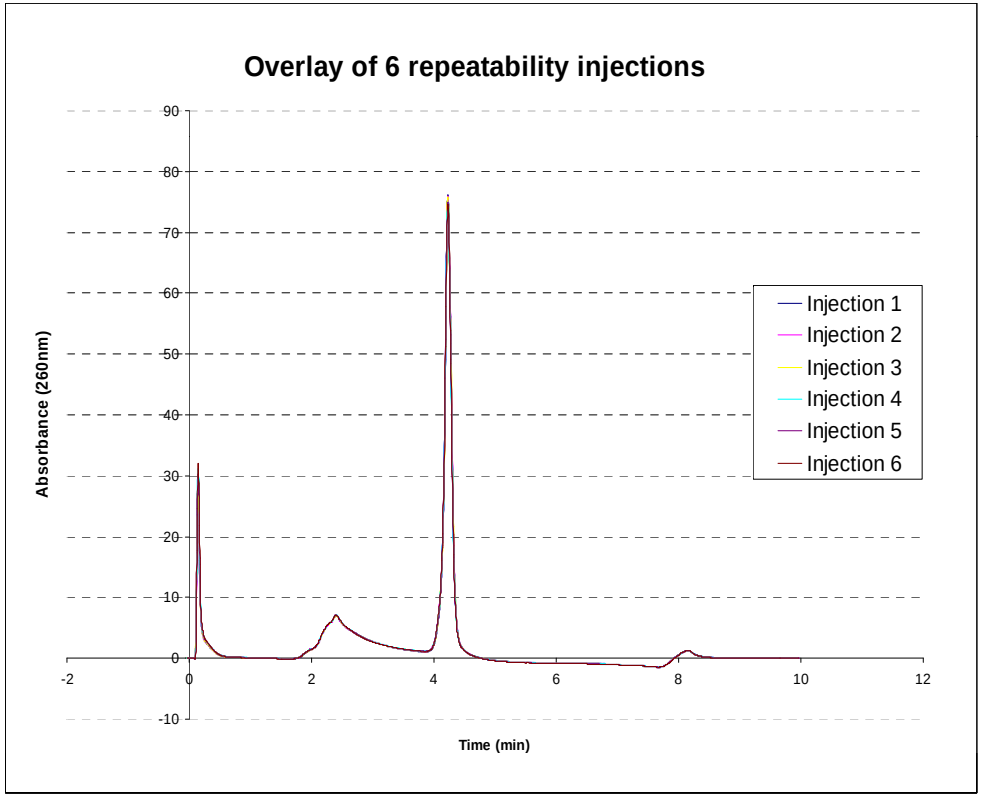
1:1 BSA : Ad5



Method qualification

Repeatability:

6x 25 µL injections of Ad5 standard ($2.4x10^{10}$ VP; $9.6x10^{11}$ VP/mL)



	Peak at 260 nm		
Injection	**RT**	**Peak Area**	**Peak Height**
1	4.23	629.87	75.57
2	4.23	635.41	75.38
3	4.23	635.75	75.35
4	4.23	622.54	74.19
5	4.23	627.27	74.35
6	4.23	626.34	74.15
Mean	4.23	629.53	74.83
Std Dev.	0.001	5.244	0.669
RSD %	0.02	0.83	0.89

RSD values for retention time, peak height and peak area < 1%

Method qualification

Intermediate Precision:

3x 25 µL injections of Ad5 standard (66% and 33% normal test concentration)
3x “events”; 2x analysts

		Peak at 260 nm		
		RT	Peak Area	Peak Height
66%	Mean	4.24	405.63	40.51
	SD	0.007	8.199	5.837
	RSD %	0.16	2.02	14.41
33%	Mean	4.24	195.86	18.79
	SD	0.006	2.262	2.618
	RSD %	0.14	1.16	13.93

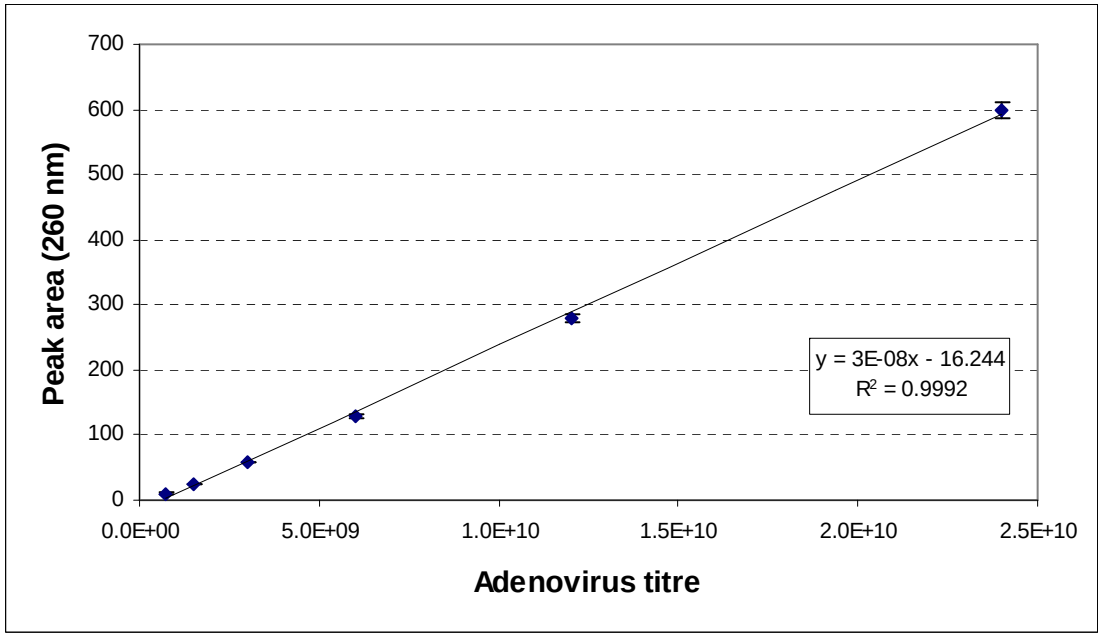
Load (%)	Event #	Peak Height (mAU)		
		Mean	SD	RSD%
66	1	46.47	0.603	1.30
	2	40.27	0.751	1.86
	3	34.80	0.917	2.63
33	1	21.37	0.153	0.71
	2	18.87	0.379	2.01
	3	16.13	0.153	0.95

Retention time RSD < 0.2%
Peak area RSD < 2.5 %
Peak height RSD < 15%
- *Evidence of peak flattening*

Method qualification

Linearity:

2 fold dilution series, 25 µL injections in duplicate (Ad5 standard)
Range = 2.4×10^{11} to 4.7×10^{8} VP



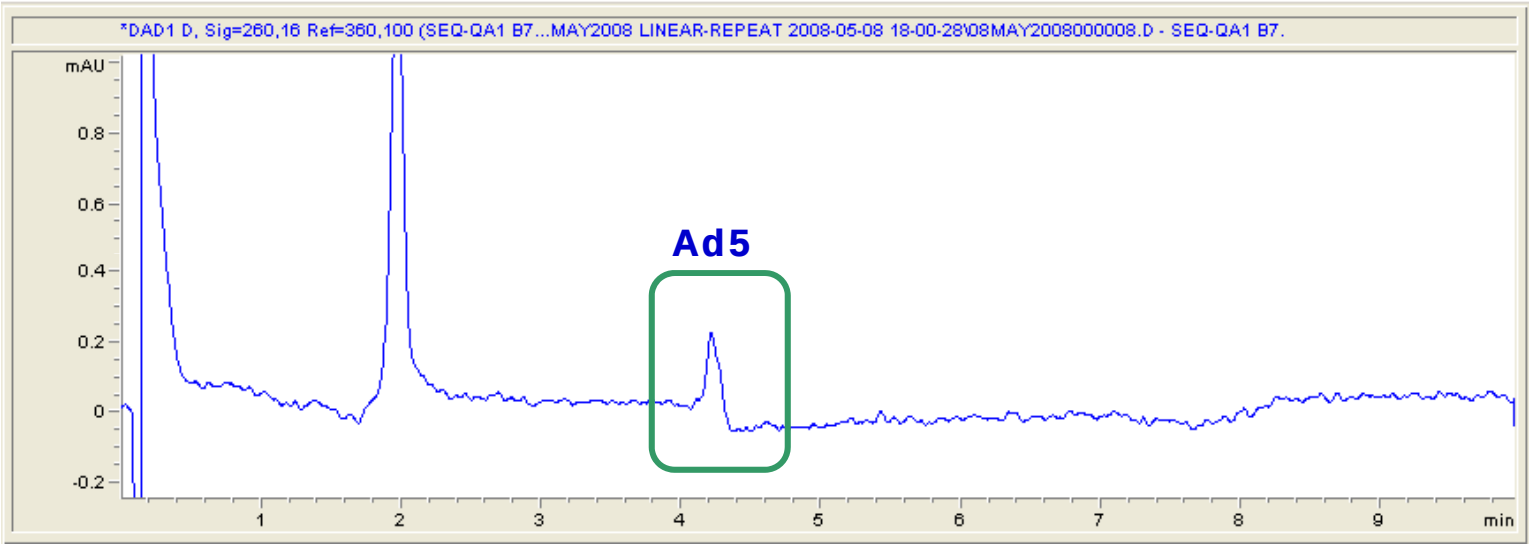
Working linear range = 7.5×10^{8} to 2.4×10^{10} VP

Method qualification

Limits of detection and quantitation

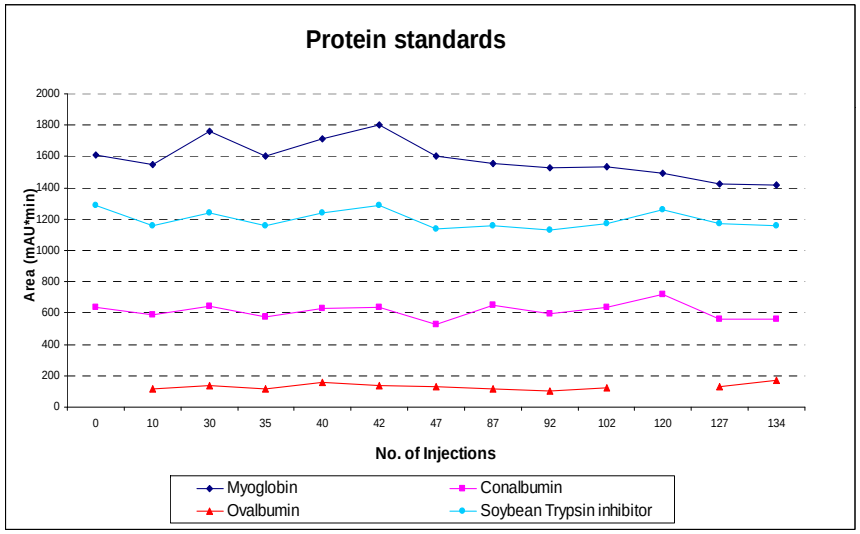
25 µL injection of 1.8×10^8 VP (7.5×10^9 VP/mL)

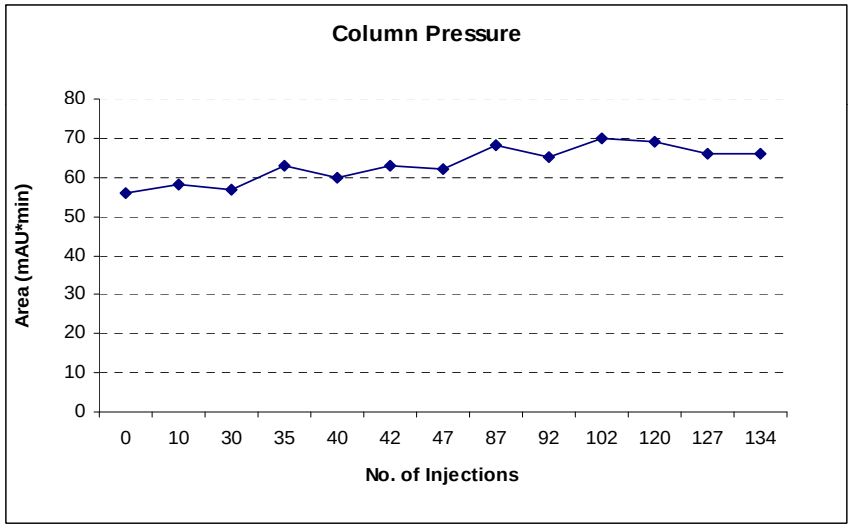
Signal to noise > 10:1



Column performance

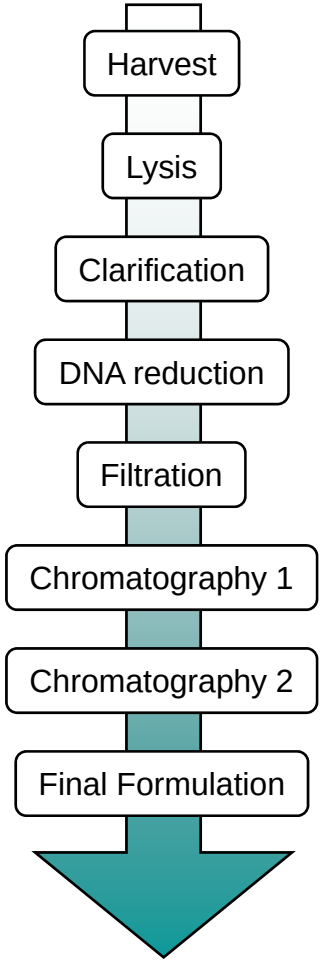
- Included regular column regeneration; 5 x 25 µL injections of 1M NaOH every 5 -10 injections.
- Analyse protein standard every run to monitor column performance



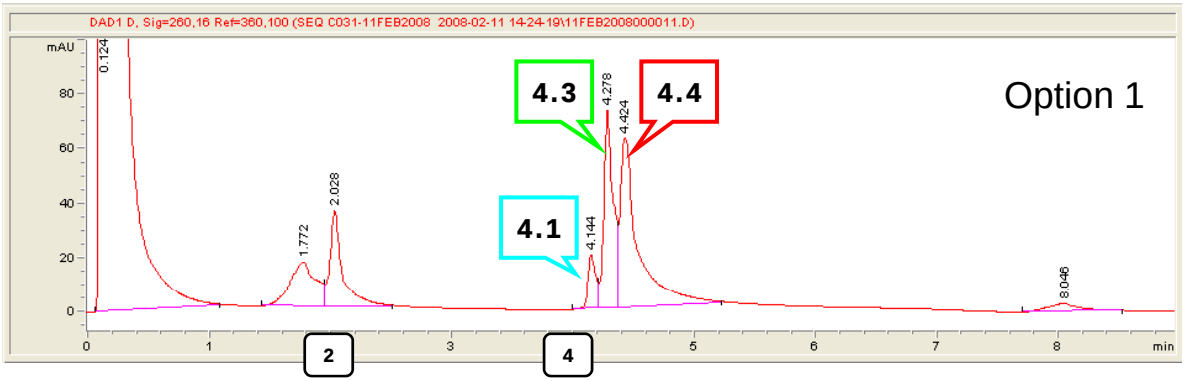


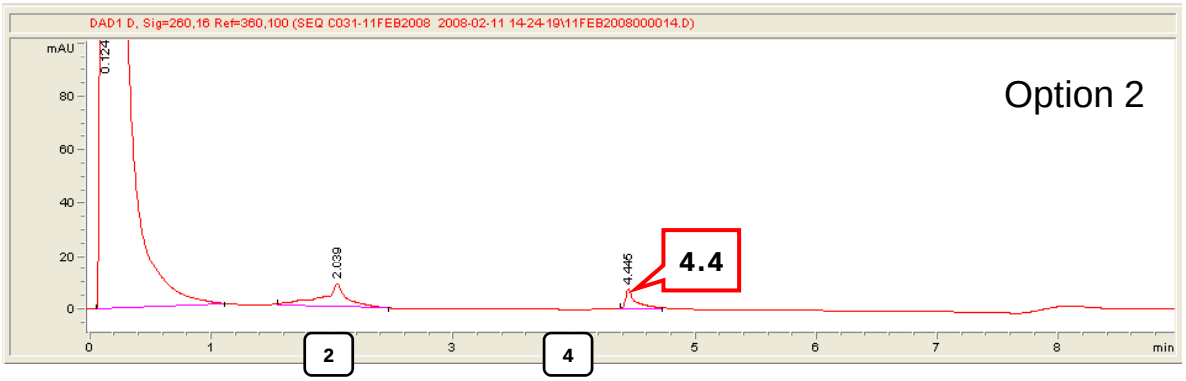
Note: Blank injections not included

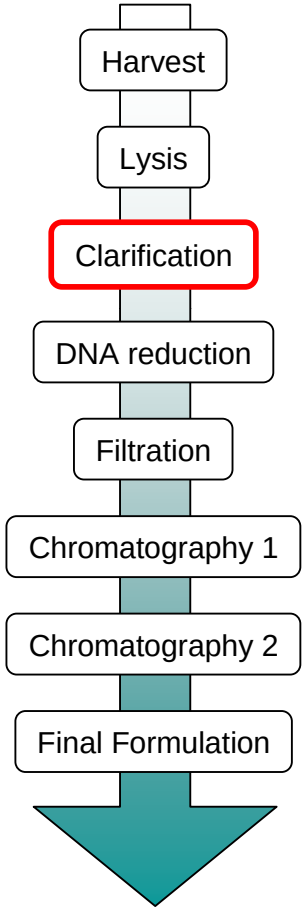
Application of CIM QA HPLC during DSP of an adenoviral product



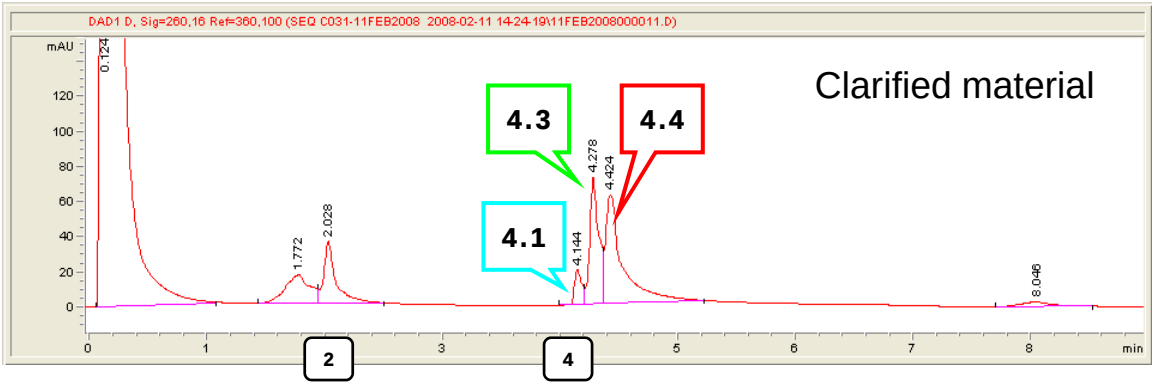
Clarification development

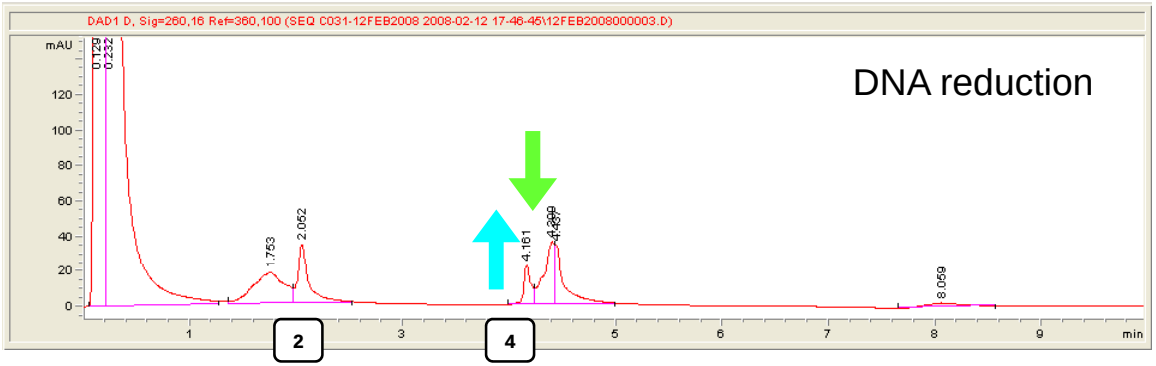


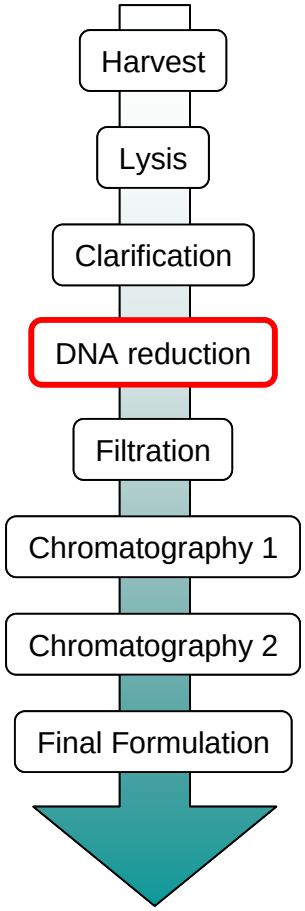




DNA reduction

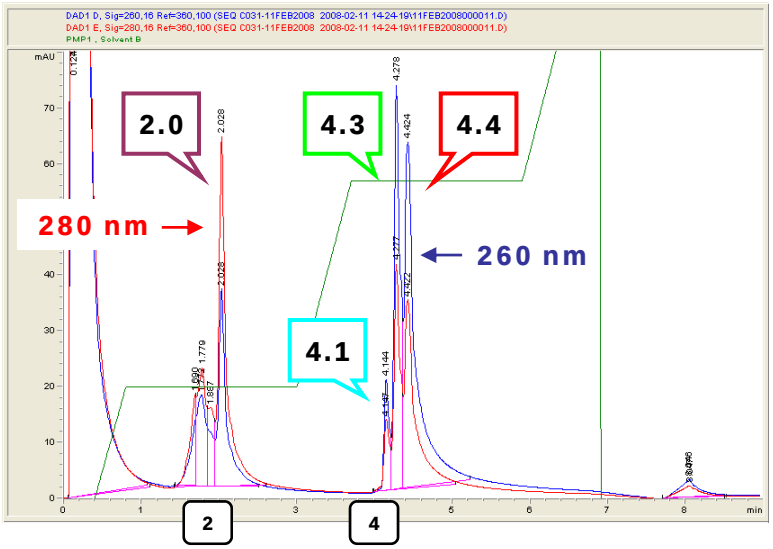




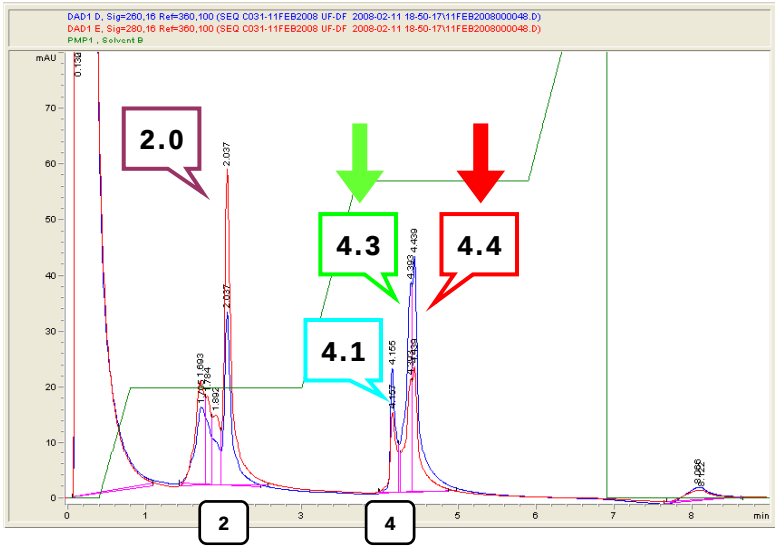


DNA reduction = peak ID?

Pre-treatment

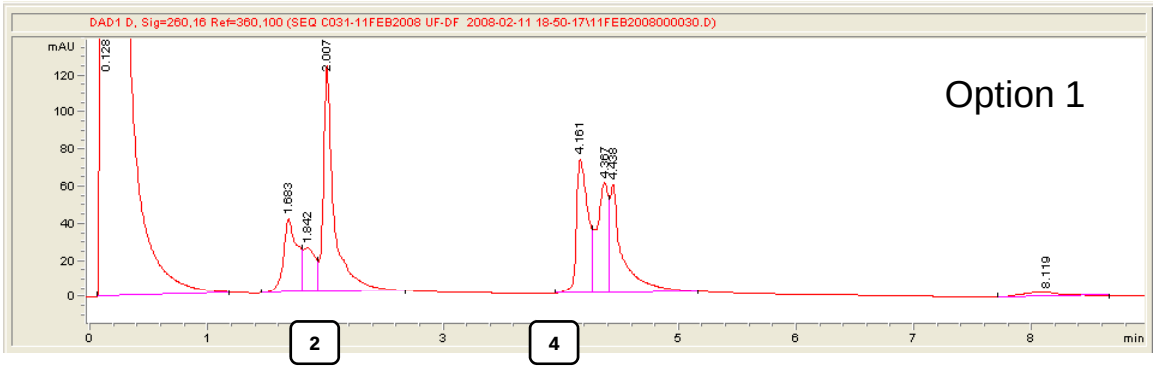


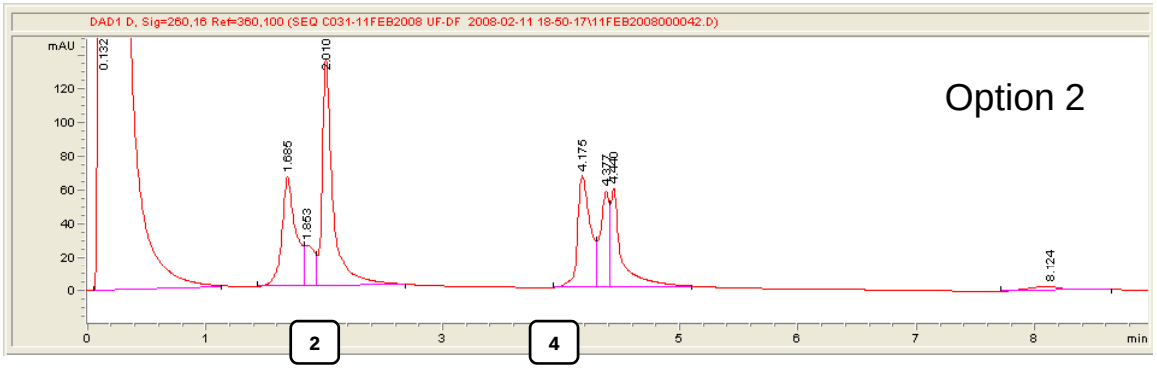
Post-treatment

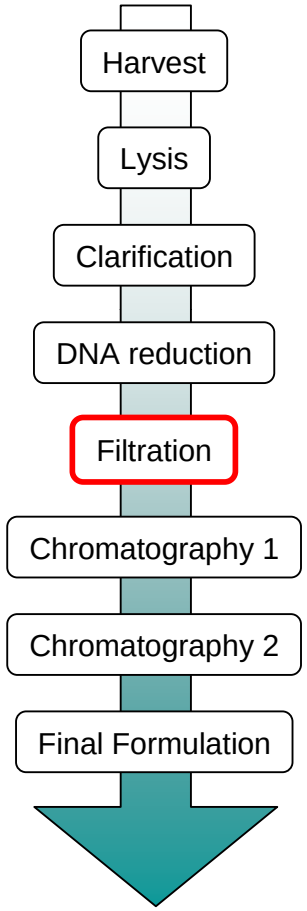


- Species eluting at 2 minutes are proteinaceous (280 nm > 260 nm).
  - Likely to be HCP or Ad5 proteins
- Species eluting at 4 minutes a mixture of Ad5 particles, possibly free DNA.

Filtration development

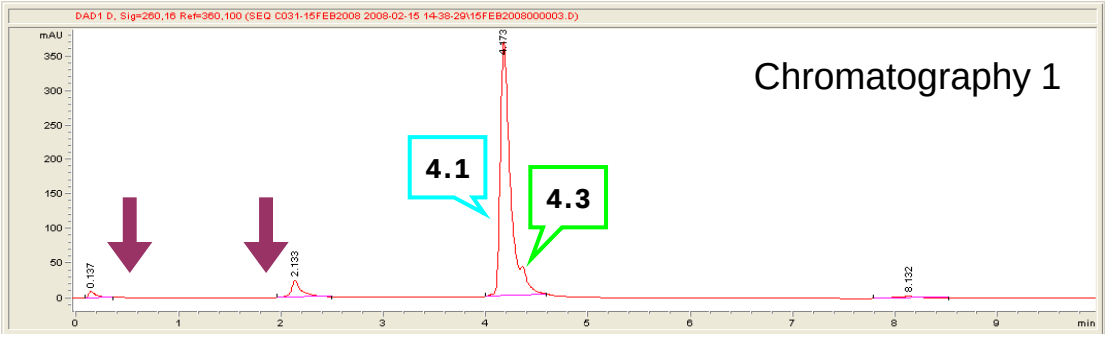


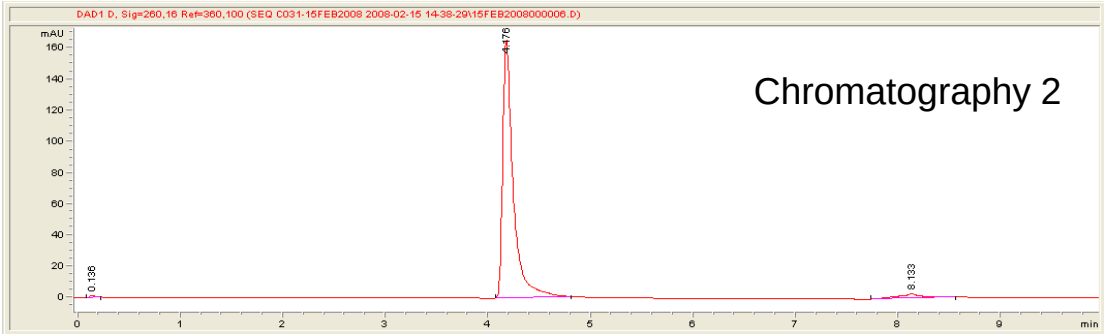


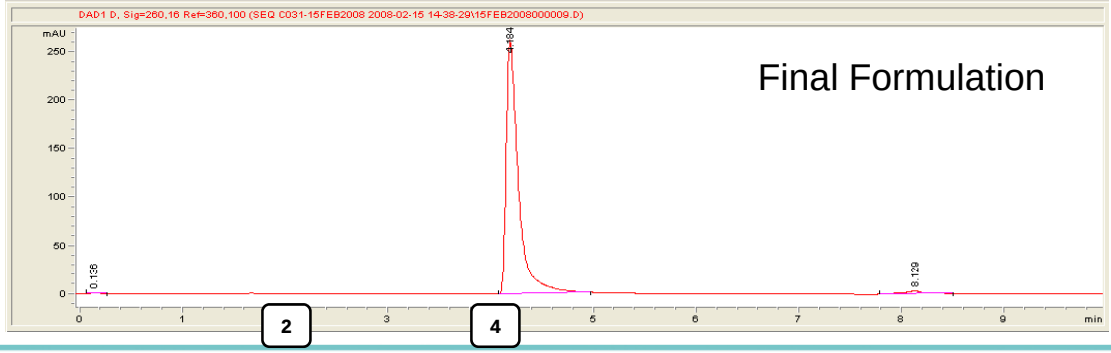


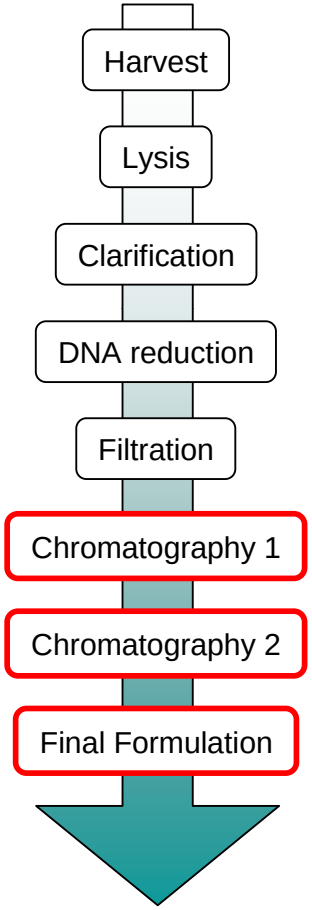
Option 1 chosen for speed of processing

Chromatography exemplification









Conclusions

- CIM® QA HPLC allows rapid analysis of adenovirus particles
  - Can be used to analyse crude lysate and purified preparations
  - Potential for application in absolute quantification
- Gradient method has been developed to separate adenovirus particles from host-cell proteins and particle fragments
- Method and column performance is consistent and robust
- Invaluable in the development and exemplification of a adenovirus purification platform
- Work accepted for publication
  - Whitfield RJ *et al*., *J Chromatography A*. 2009

Acknowledgments

Eden Biodesign:

- Rob Whitfield
- Suzy Battom
- USP and DSP teams

BIA Separations:

- Miloš Barut
- Aleš Štrancar

Questions

Questions are encouraged throughout the presentation and can be asked by using the link provided within your webcast viewer.

Thank You.