Rapid Analysis of Adenoviruses with Novel Bio-Monolith Anion-Exchange HPLC Columns to Support the Development of a High-Titre Manufacturing Platform

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On behalf of:

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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, technical troubleshooting/diligence, clinical logistics

- Operate the UK's National Biomanufacturing Centre

- US subsidiary located in Research Triangle Park, NC

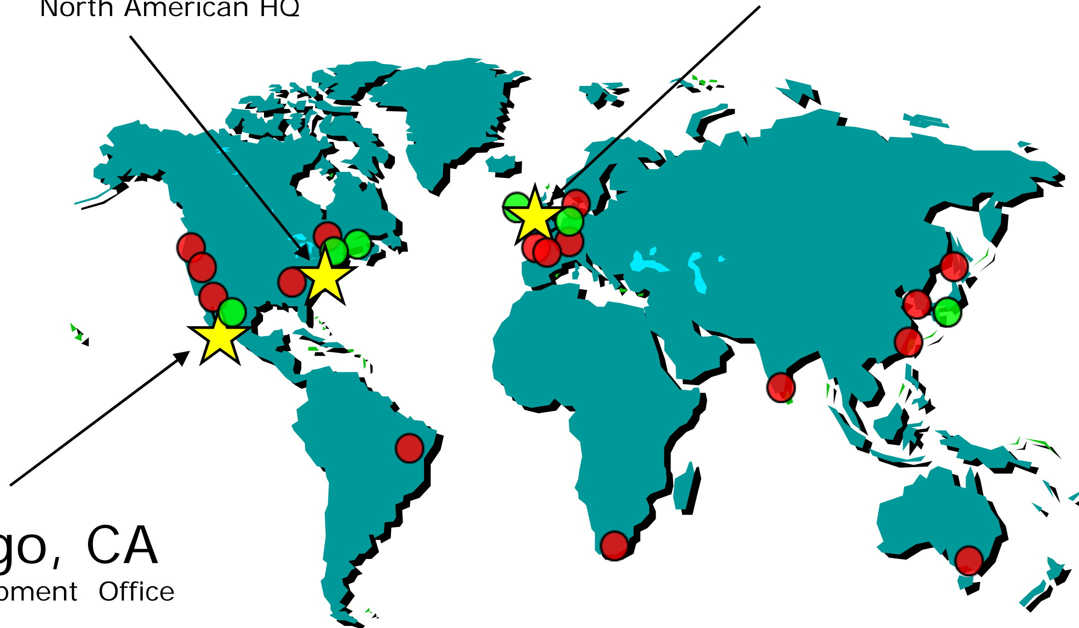
Eden Biodesign

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

Eden Biodesign Maintains a Globally Integrated Biopharmaceutical Network

Research Triangle Park, NC
North American HQ

Liverpool, UK
Global HQ & cGMP Operations



San Diego, CA
Business Development Office

Clients on all five continents

● *Client Assignments* ☆ *Eden Presence* ● *Strategic Partners*

Vaccine Experience

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Dramatic upswing in projects related to novel vaccine products

Diverse vaccine product types
- Live, attenuated, rec. protein, VLPs, whole cell microbial, viral etc.

Equally diverse production systems
- Cell culture, microbial fermentation, etc.

Low doses permit multiple production technologies
- Bioreactors (stainless steel and disposable), cell cubes, hollow fibers etc.
- Possibly even for commercial production

There is clearly no common production platform

Vaccines are difficult to characterize and the “process = product” regulatory paradigm is still in-place for vaccine products

Towards a Platform Approach

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Pandemics, bioterrorism, third world markets etc. require a more "platform like" approach that:

- Is robust
  - Fewer unit operations, ambient processing, hold steps, etc.
- Is easily transferred
  - Rapid global production
  - Must have a realistic supply chain
- Is reproducible
- Is scalable
- Is economically viable
- Makes wide use of disposable technologies

Potential Platform Approach

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Eden Biodesign and collaborators are working towards platform approach(es) for vaccine production

- Well characterized cell types with appropriate provenance
  - e.g. BHK, Vero, HEK, CHO
  - e.g. *Pichia* and *E. coli* strains for VLPs
- Serum-free high density suspension cell culture
- Stirred tank bioreactor systems (including disposables)
- Chromatographic purification
  - High recovery

But any such platform(s) must be supported by…..

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Rapid and robust analytical techniques for support of process development, in-process and release testing

Today's Presentation

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- Current methods for detection of adenovirus during process development or for in-process testing
- CIM® QA Monolith technology
- Comparison with other anion exchange HPLC methods
- Method development
- Method qualification
- Application of the method during process development for a type V adenoviral vaccine product
- Conclusions

Quantitative analysis of adenoviruses

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Eden Biodesign has considerable experience in developing processes for the production of adenoviral products and cGMP manufacture of products for clinical use

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

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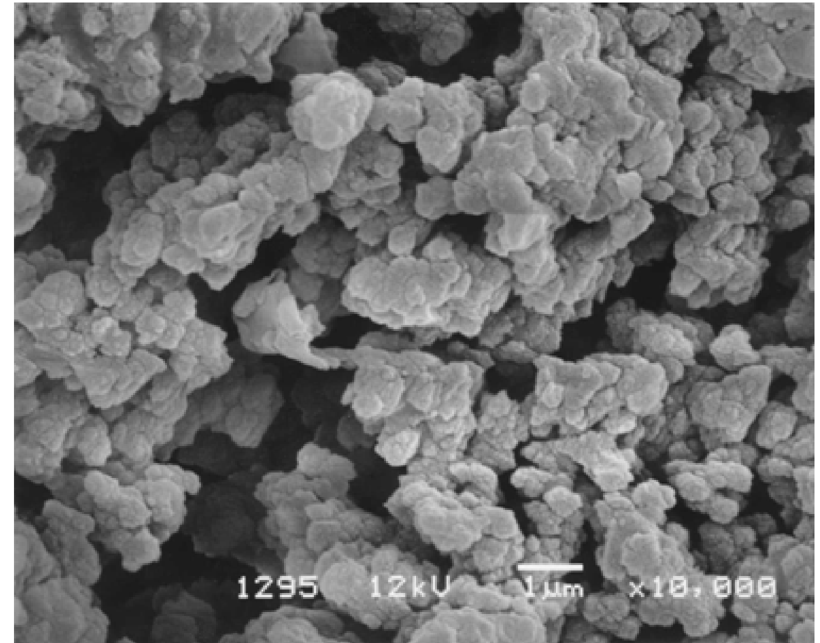
- UNO Q - BioRad
  - Ref: Transfiguracion, J *et al* (2001) Validation of a high-performance liquid chromatographic assay for the quantification if adenovirus type 5 particles. *Journal of Chromatography B,* 761, 187-194.
- Q Sepharose XL - GE Healthcare
  - Ref: Kuhn, I *et al* (2007) High-performance 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 (1.5 μm)
- - Uniform channel connectivity in 3D (homogeneous structure)

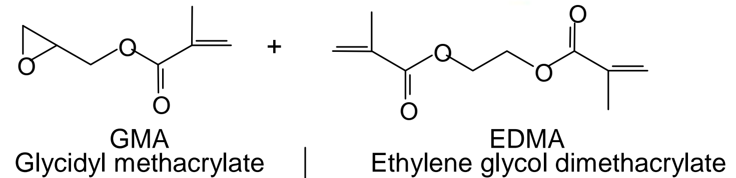


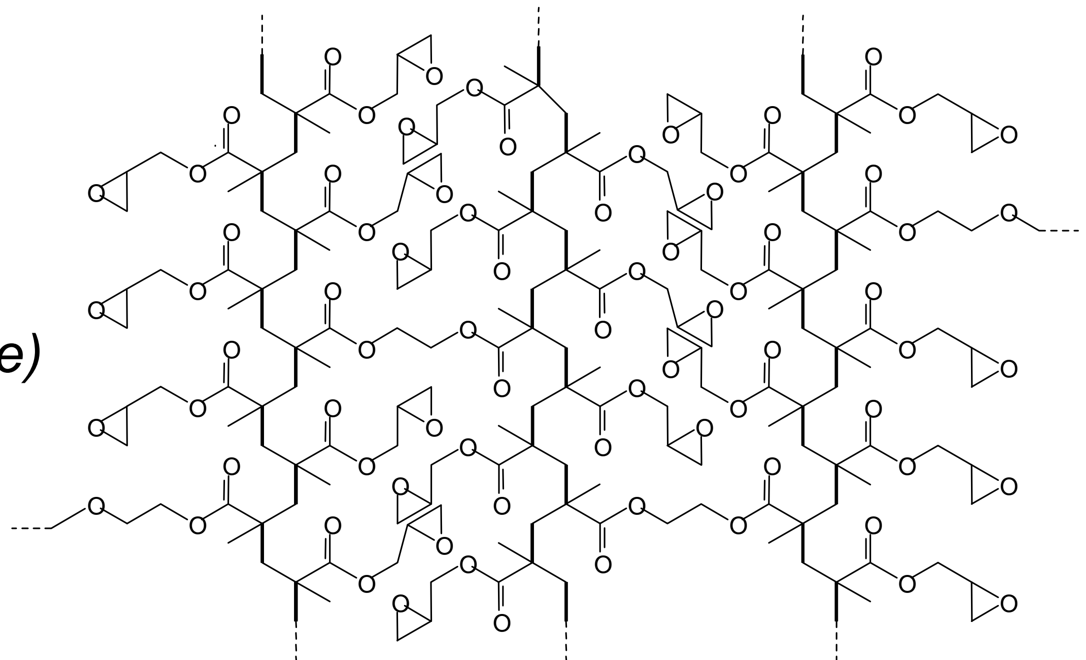
Courtesy BIA Separations

CIM® Chemical Structure

Made of highly cross-linked porous rigid monolithic polymers

poly(glycidyl methacrylate-co-ethyleneglycol dimethacrylate)
or
poly(styrene-divinylbenzene)





Poly(glycidylmethacrylate-*co*-ethyleneglycoldimethacrylate)

Courtesy BIA Separations

Macromolecule Separation

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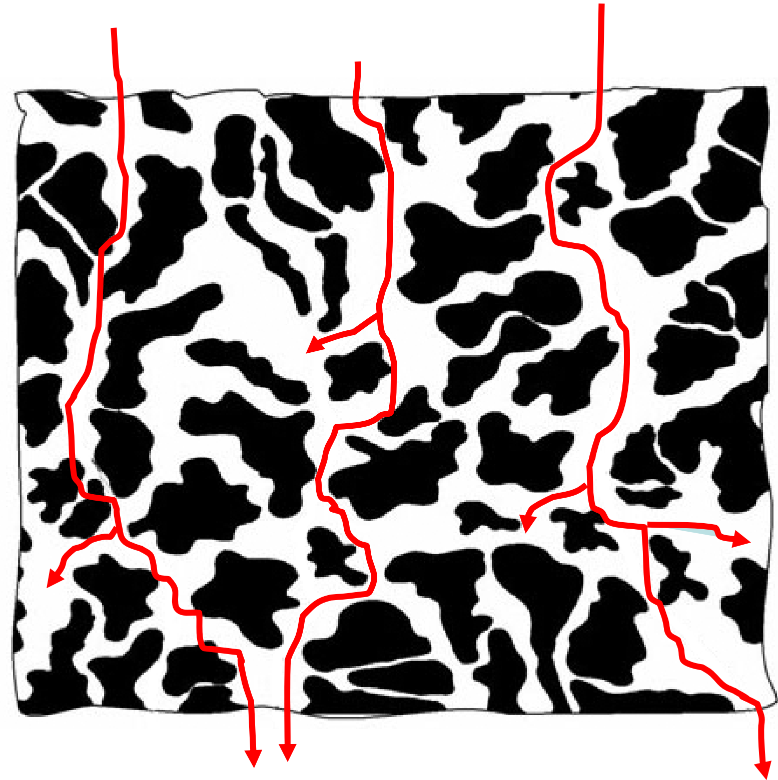
Convective mass transfer
- only one flow path exists

~1500 nm channel size

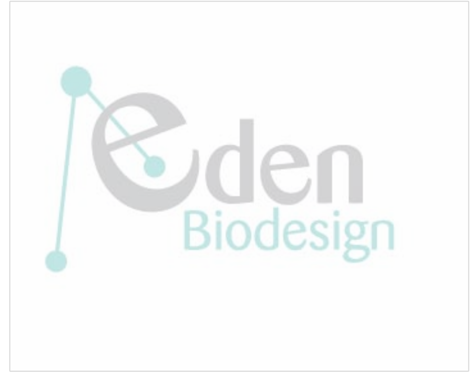
Zero void volume

- Flow unaffected loading
- Flow unaffected elution
- Flow independent dynamic binding capacity
- Flow independent resolution



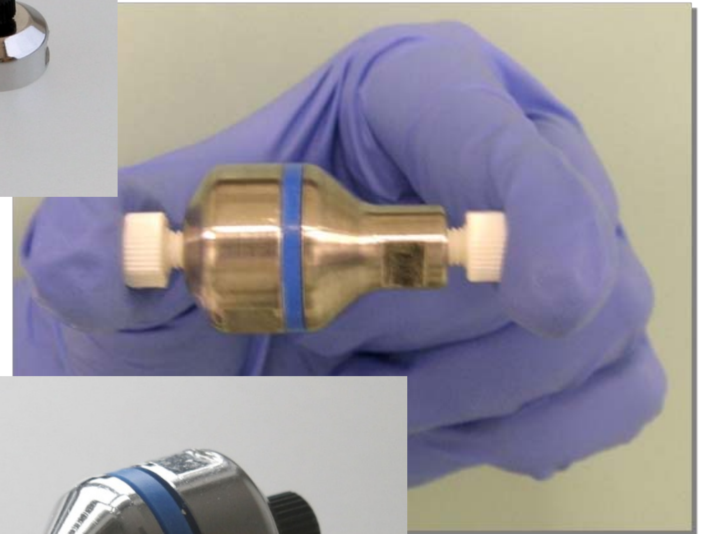
Courtesy BIA Separations

CIM® QA analytical HPLC column



- Strong Anion Exchanger
- Column size = 76 µL
- Max pressure = 150 bar

- Column temperature = 22ºC
- Injection volume = 25 µL
- Flow rate = 1 mL/min



Initial chromatography conditions

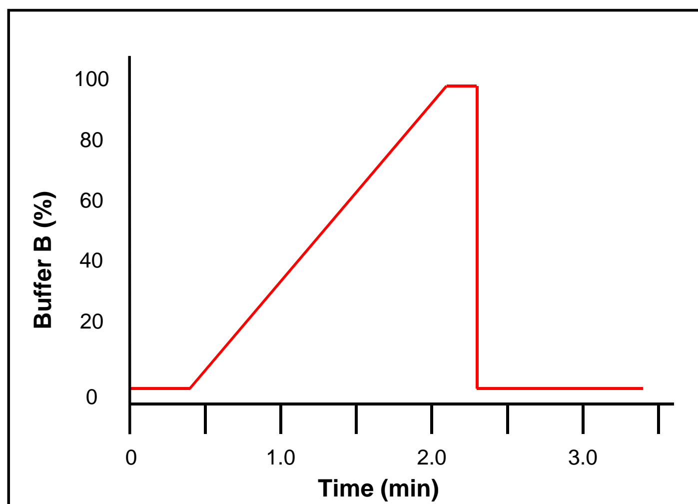
Stage	CV	Buffer B (%)
Equilibration	4	0
Gradient	19	100
Hold	2	100
Re-equilibration	9	0

Buffers:

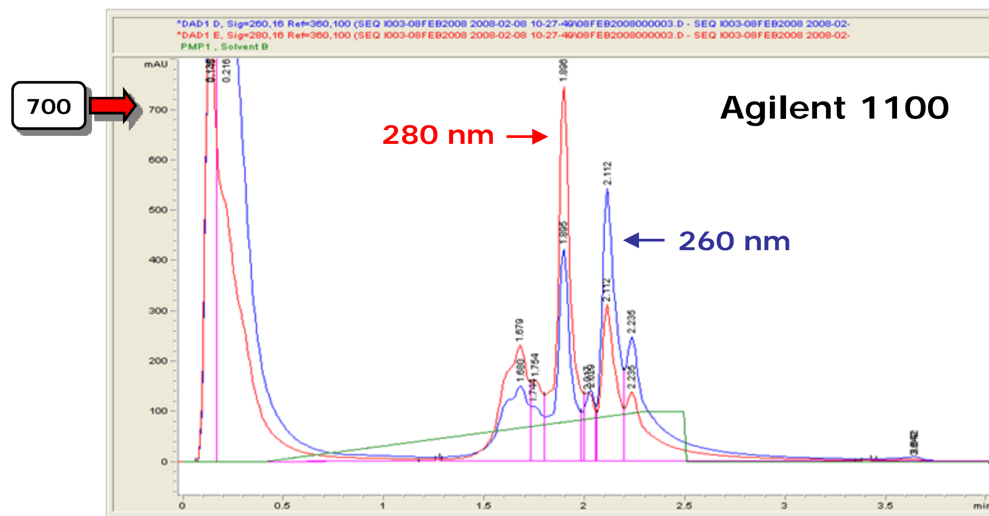
Buffer A 20 mM Tris, pH 7.5
Buffer B 1.5 M NaCl, 20 mM Tris, pH 7.5

Total run time: 4 minutes

Gradient profile



Crude lysate injection (2.3×10^8 IU; 9.26×10^9 IU/mL)



Comparison to Q Sepharose™ XL

- GE Healthcare
- Anion exchanger
- 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

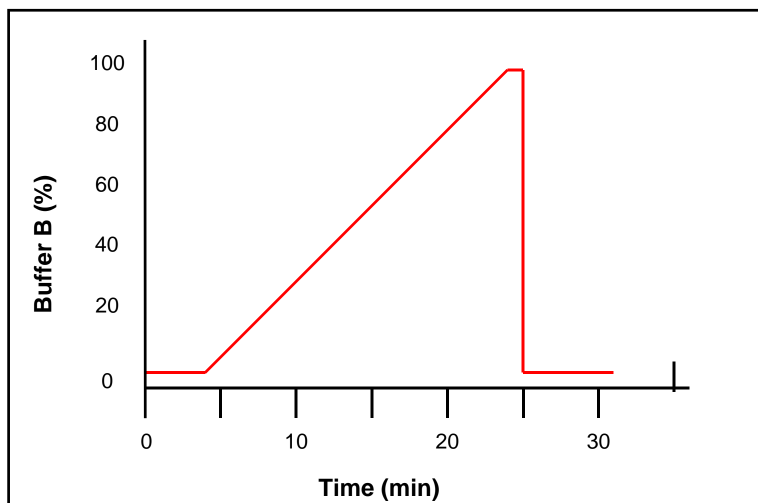
Stage	CVs	Buffer B (%)
Equilibration	4	0
Gradient	19	100
Hold	2	100
Re-equilibration	9	0

Buffers:

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

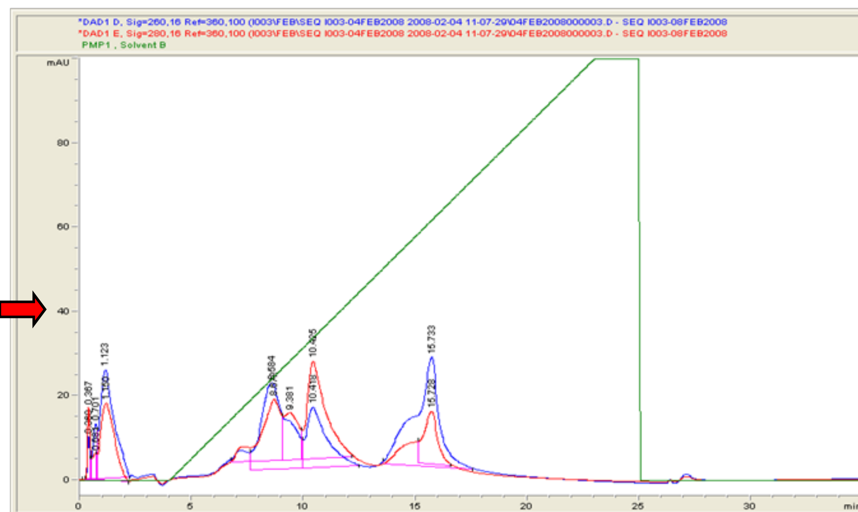
Total run time: 40 minutes

QXL gradient profile



Crude lysate injection (2.3×10^8 IU; 9.26×10^9 IU/mL)

40



Comparison to Q Sepharose XL

700

280 nm

260 nm

3

40

30

Comparison to Q Sepharose XL

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

Further method development

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

Method development

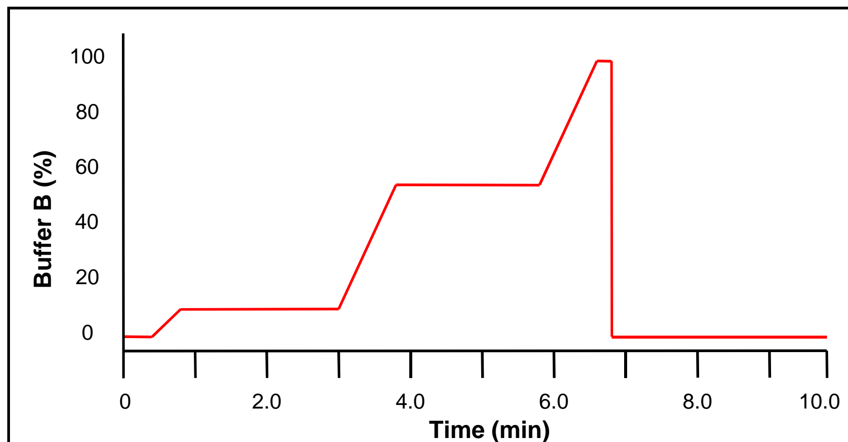
Stage	CV	Buffer B (%)
Equilibration	4	0
Step 1	4	20
Hold 1	22	20
Step 2	7	57
Hold 2	22	57
Step 3	8	100
Hold 3	2	100
Re-equilibrate	10	0

Buffers:

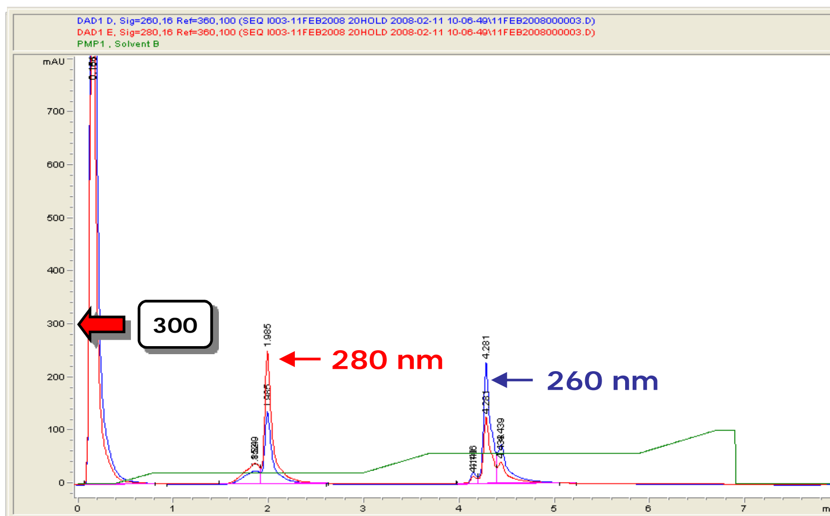
Buffer A 20 mM Tris, 0.1 M NaCl, pH 7.5
Buffer B 2.0 M NaCl, 20 mM Tris, pH 7.5

Total run time: 10 minutes

CIM QA Monolith gradient profile



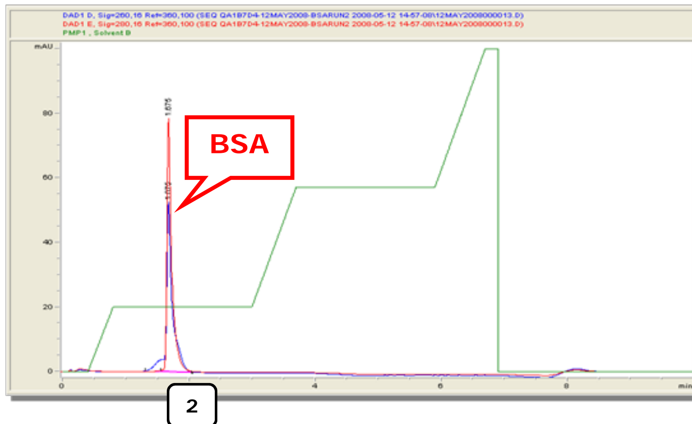
Crude lysate injection (1.15x10^8 IU; 4.63x10^9 IU/mL)



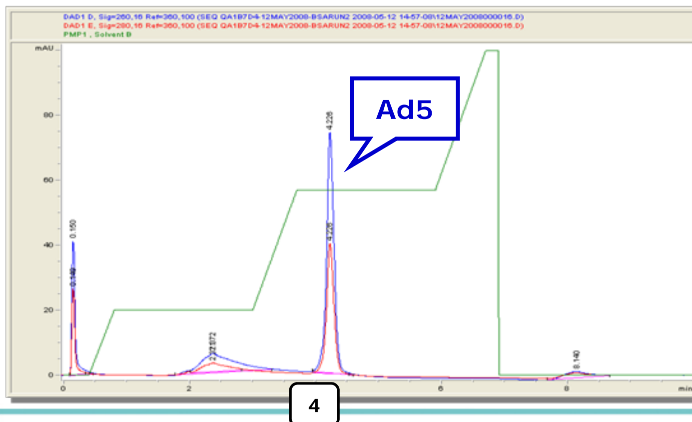
Method development

BSA spiked into an a purified adenovirus sample

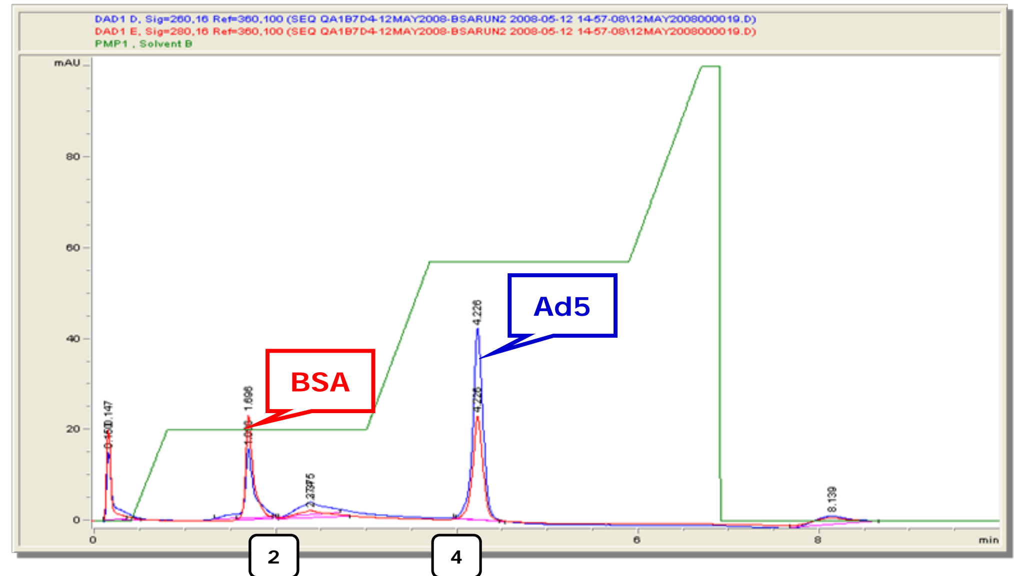
Bovine Serum Albumin



Partially purified Ad5



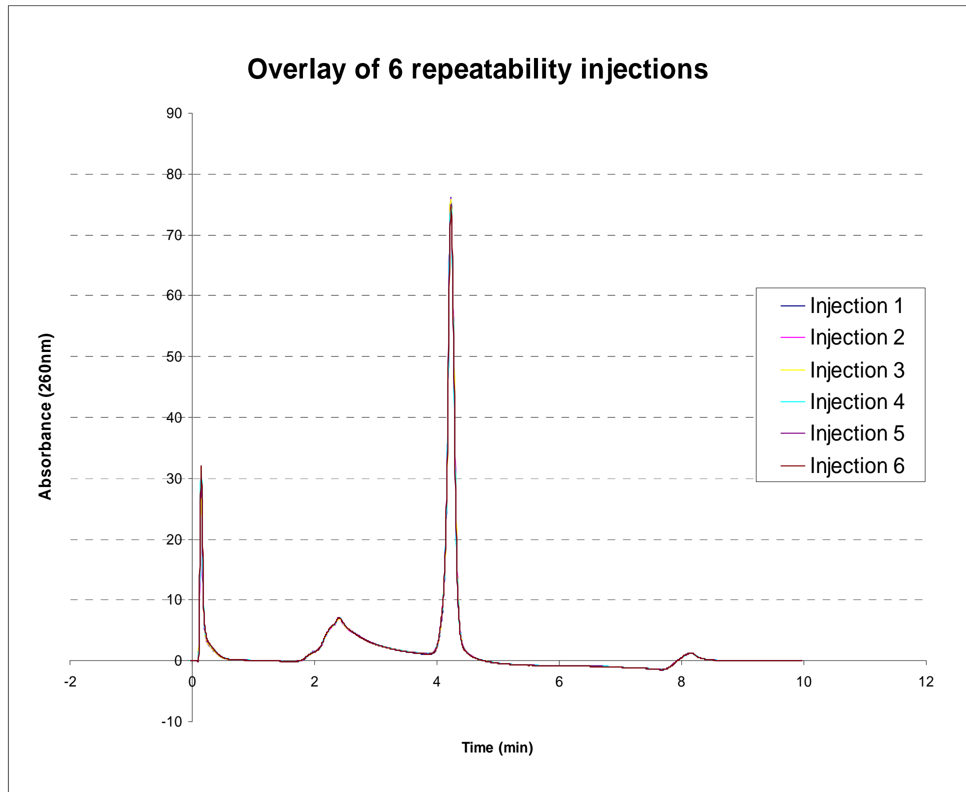
1:1 BSA : Ad5



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

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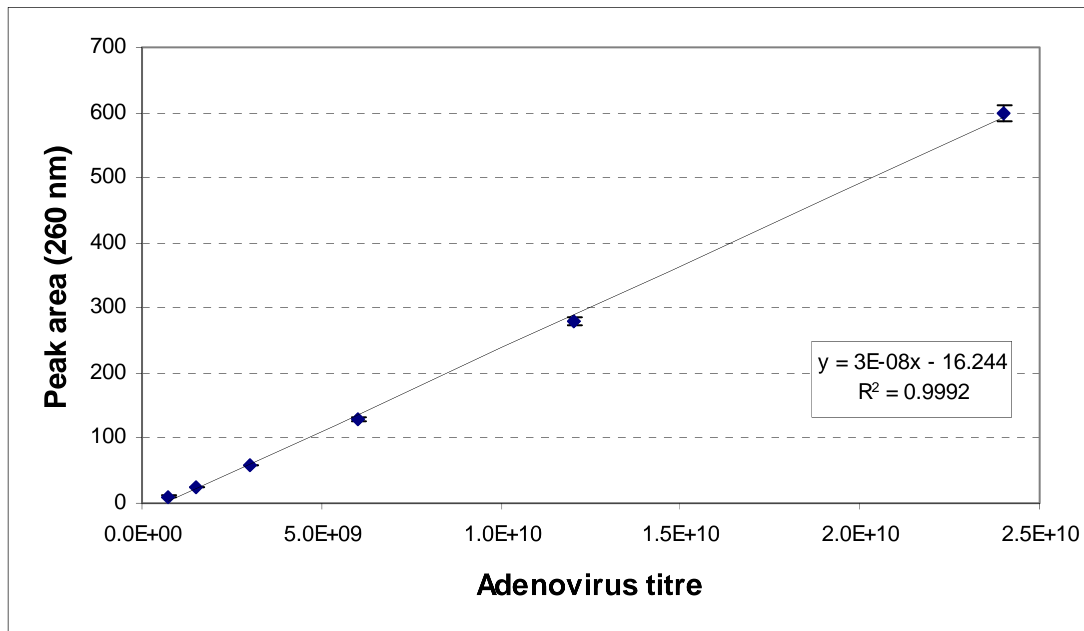
Linearity:

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



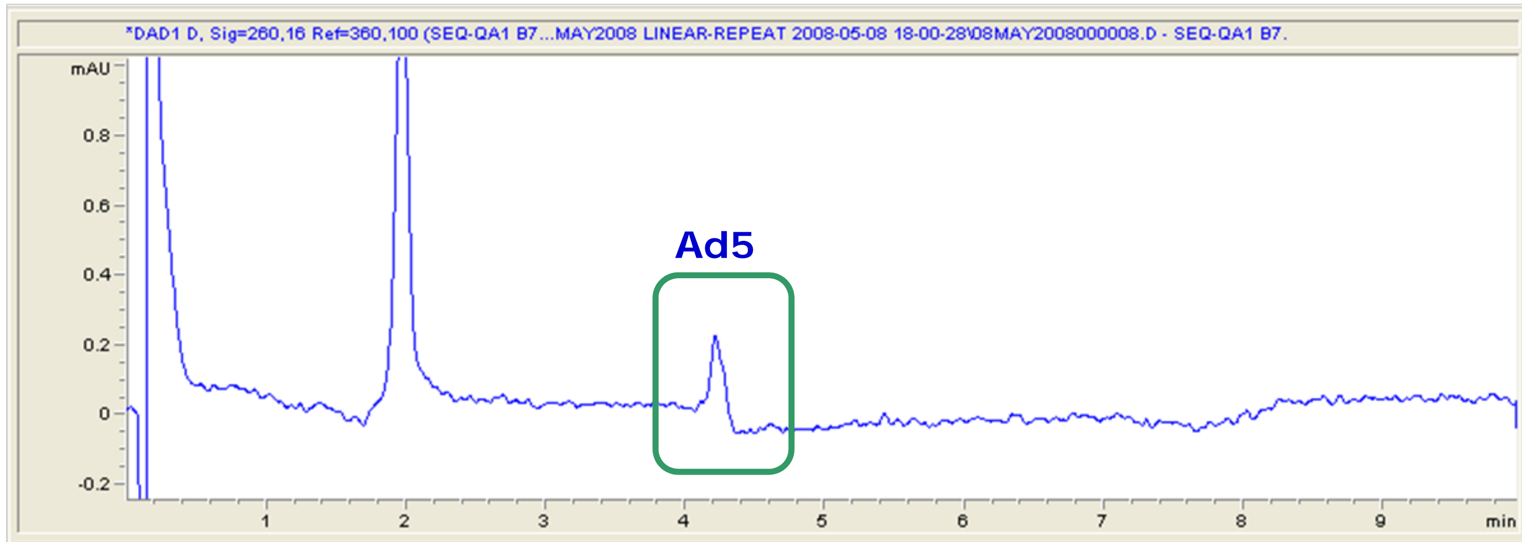
Working linear range = 7.5×10^{8} to 2.4×10^{10} VP

Method qualification

Limits of detection and quantitation

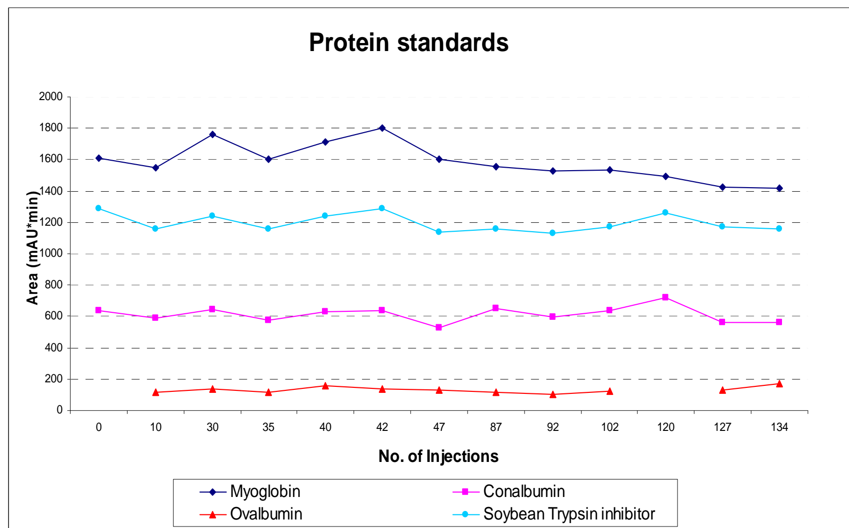
25 µL injection of $1.8x10^8$ VP ($7.5x10^9$ VP/mL)

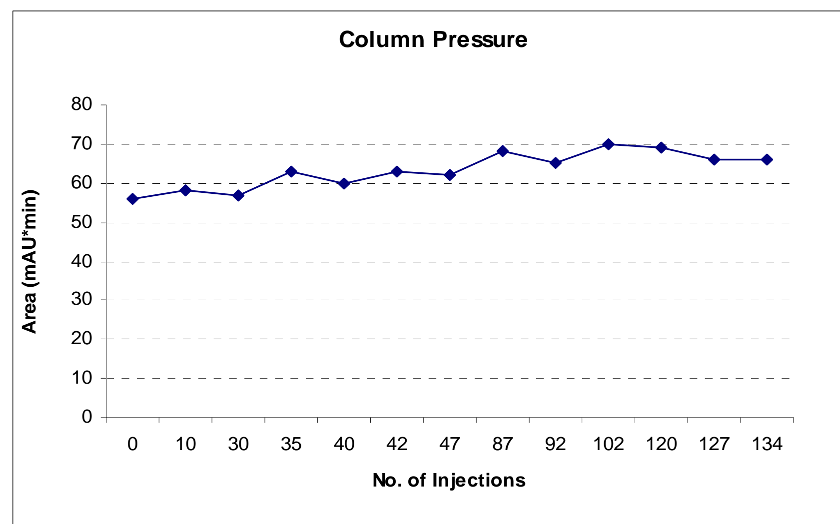
Signal to noise > 10:1



Column performance

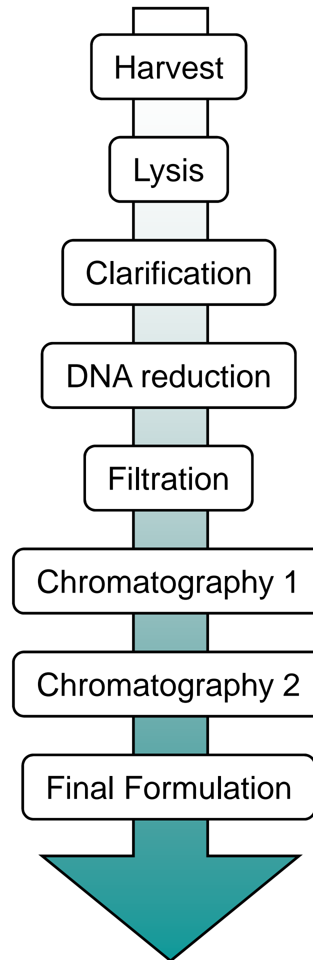
- Included regular column regeneration; 5 x 25 μL injections of 1M NaOH every 5 -10 injections.
- Analyse protein standard every run to monitor column performance



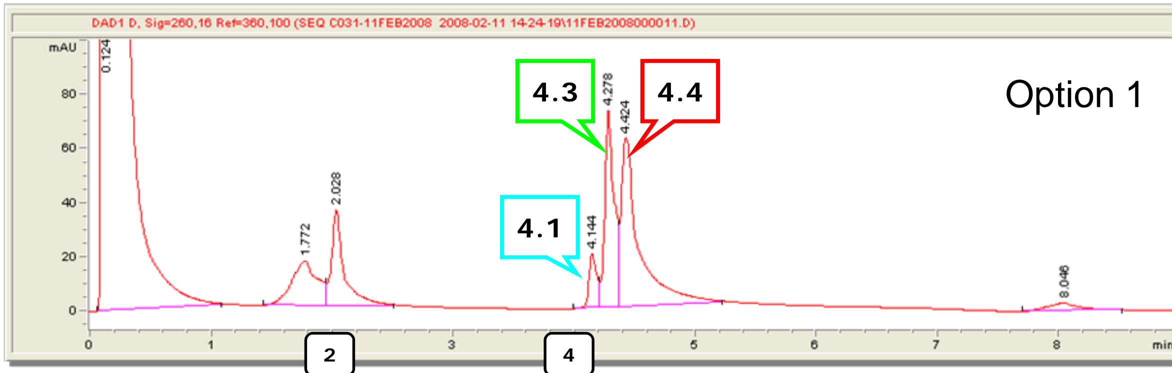


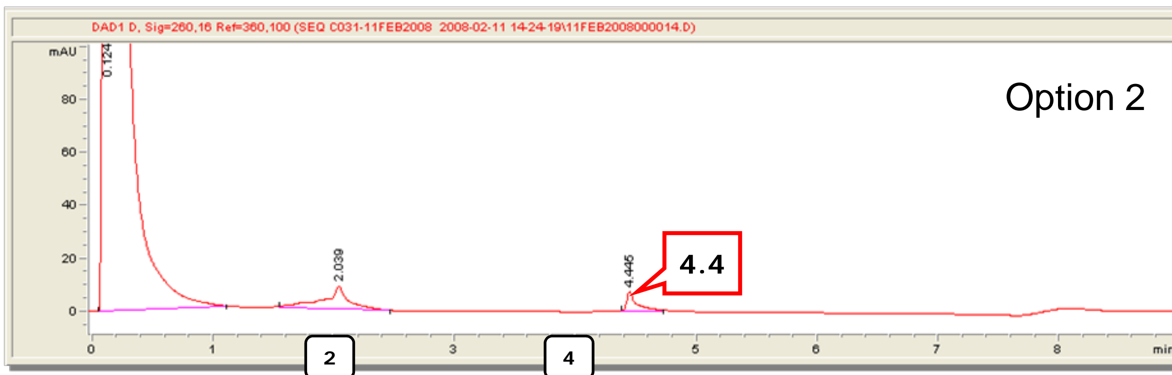
Note: Blank injections not included

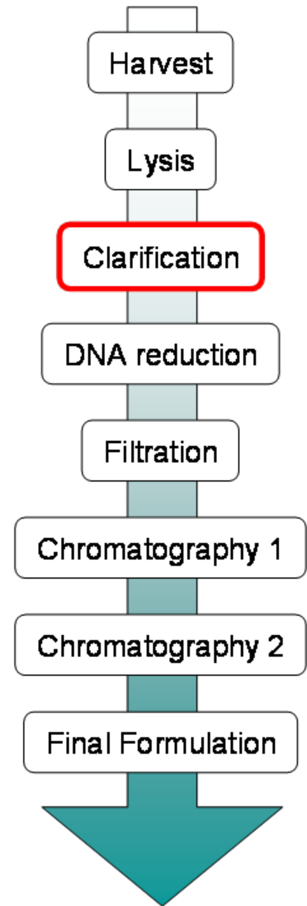
Application of CIM QA HPLC during DSP of an adenoviral product



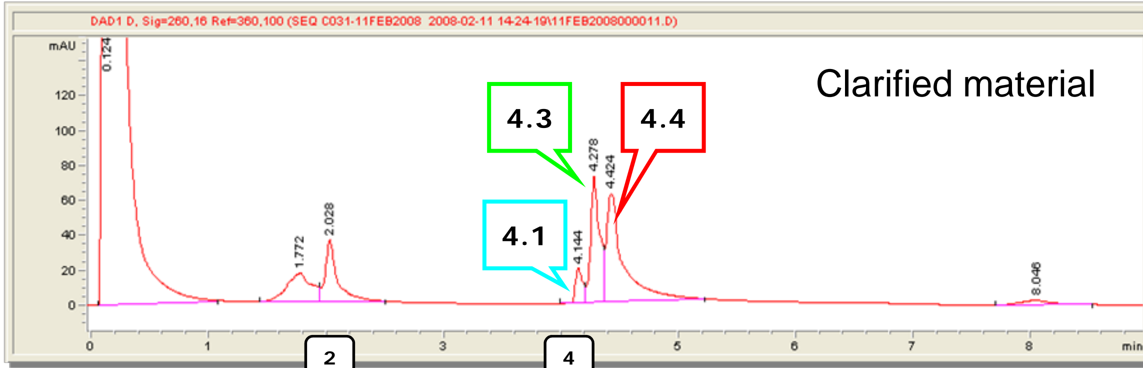
Clarification development

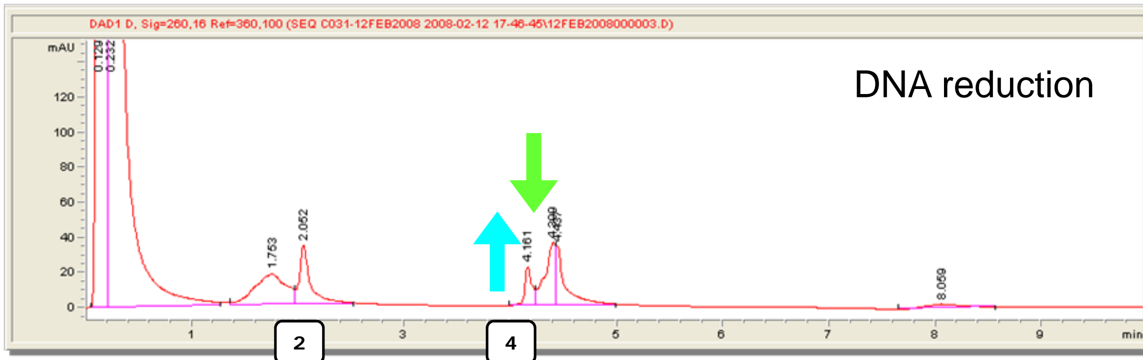


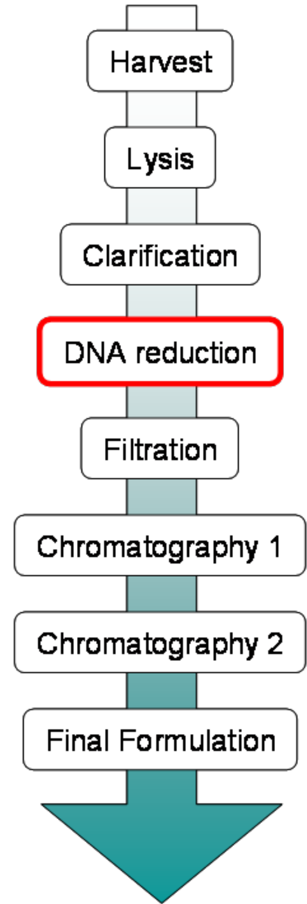




DNA reduction

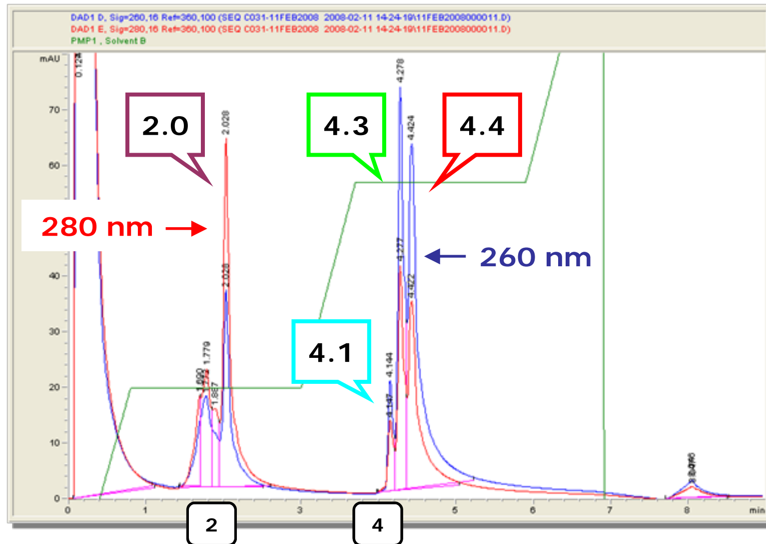




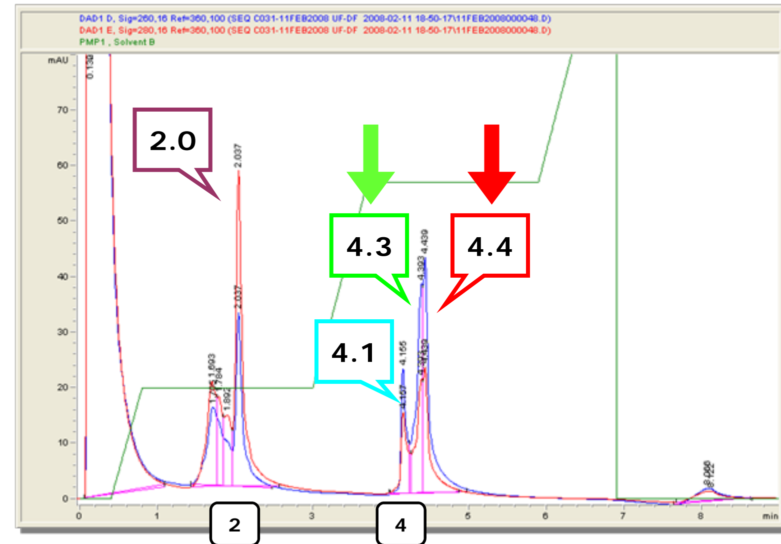


DNA reduction = peak ID?

Pre-treatment

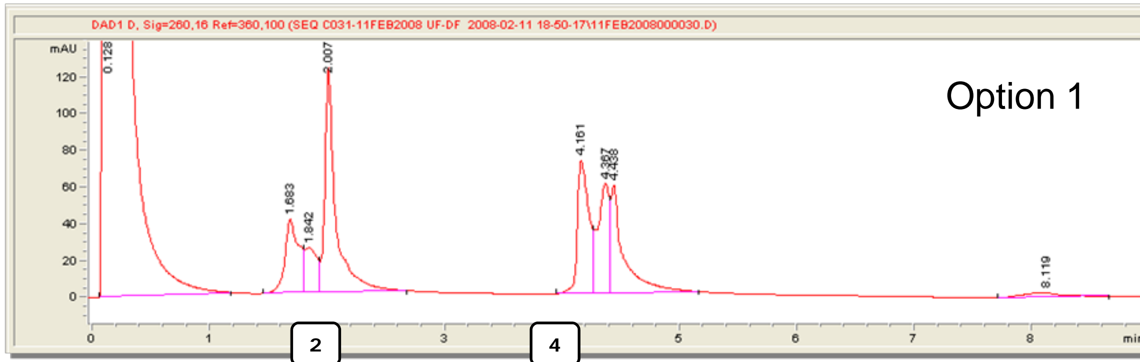


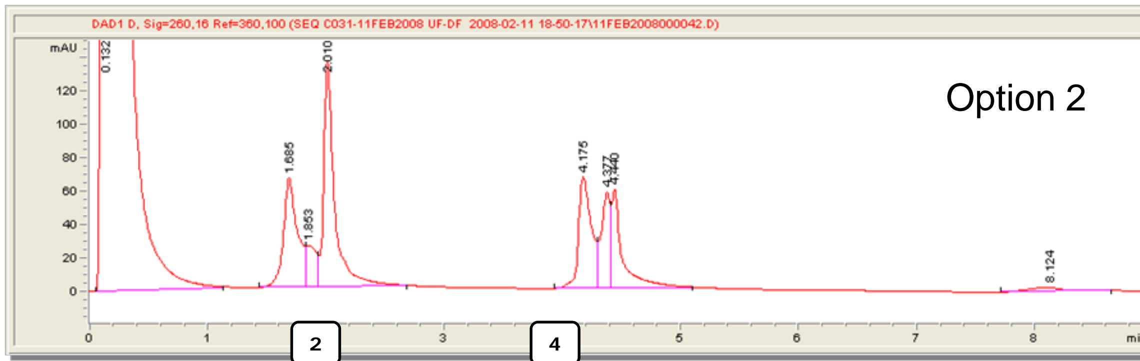
Post-treatment

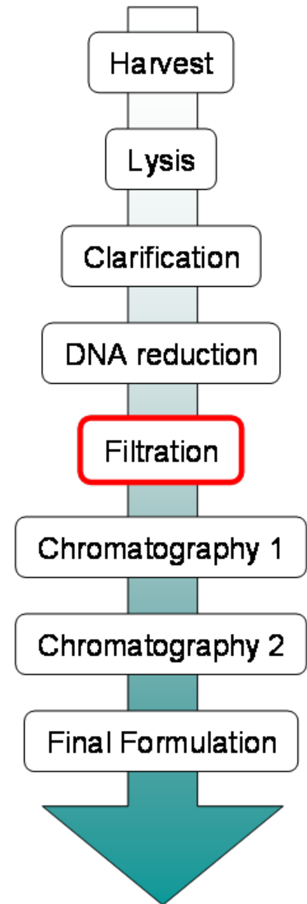


- Species eluting at 2 minutes are proteinaceous (280 nm > 260 nm).
  - Likely to be HCP or Ad5 proteins
- Species eluting at 4 minutes a mixture of Ad5 particles, possibly free DNA.

Filtration development

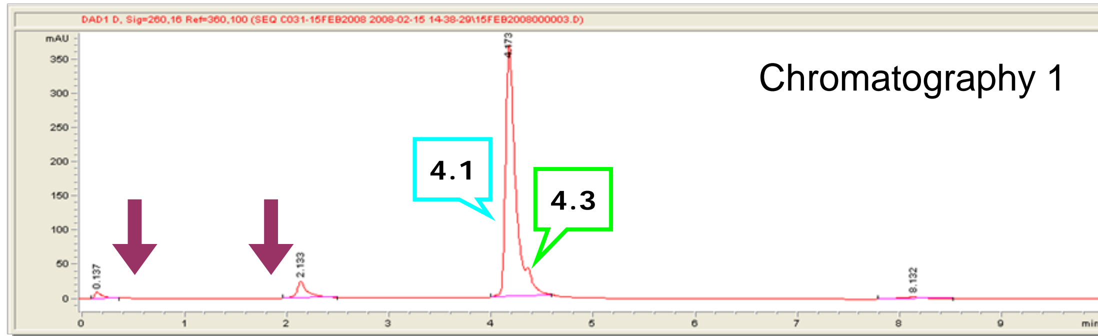


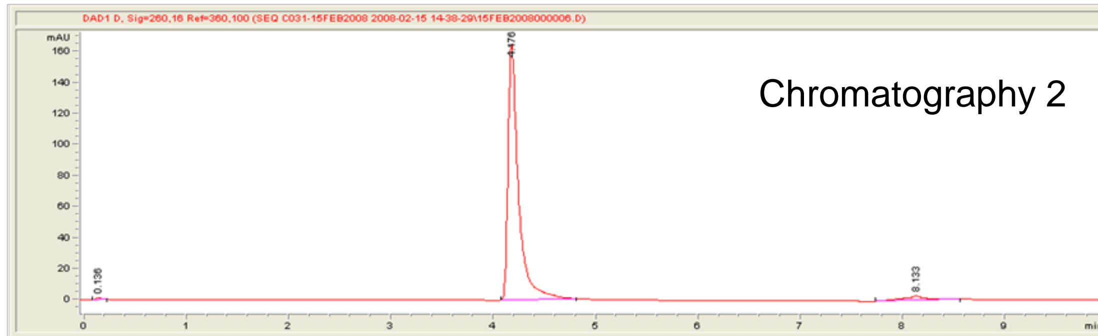


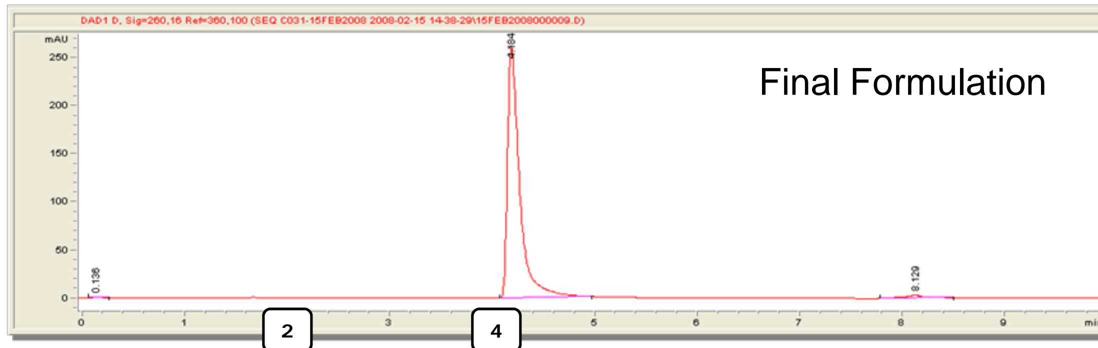


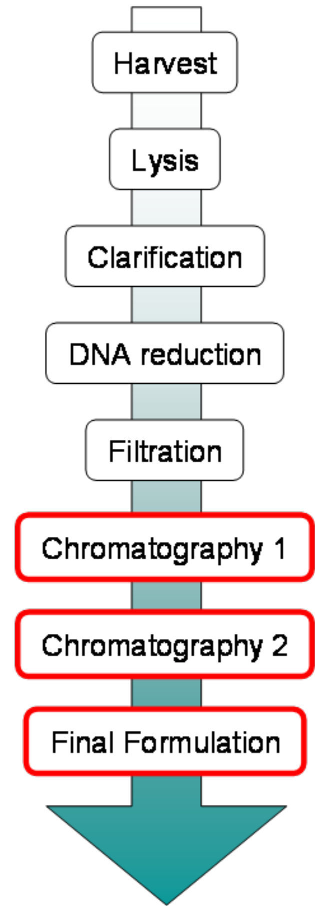
Option 1 chosen for speed of processing

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 develop 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

Acknowledgments

Eden Biodesign:

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- Rob Whitfield
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- USP and DSP teams

BIA Separations:

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