Fast, selective and quantitative protein profiling of adenovirus-vector based vaccines by ultra-performance liquid chromatography

UPLC method development

Tom Branson, Scientist Analytical Development
14Mar19 | Janssen Vaccines, Leiden, The Netherlands

Melinda, *Harmony*
Melinda's artwork reflects her journey living with HIV.

Janssen Infectious Diseases & Vaccines
PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Virus protein profiling

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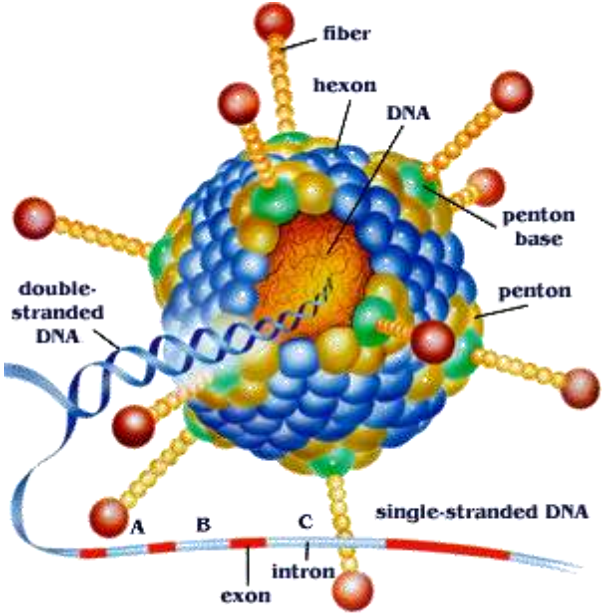
Adenoviruses

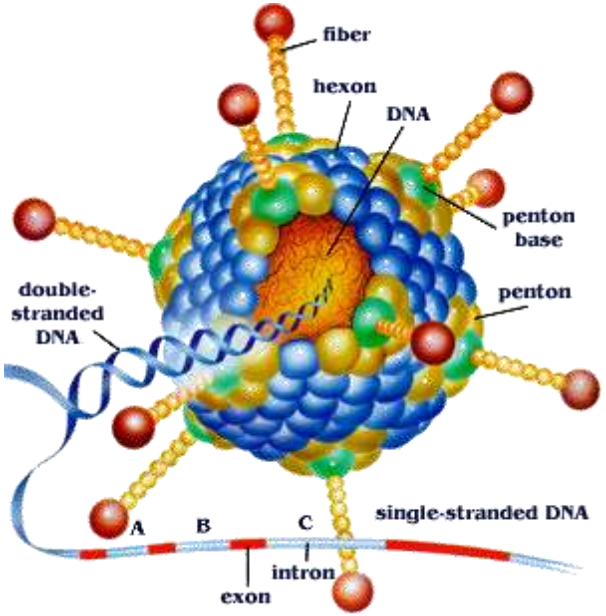
- 90 nm diameter
- Total mol. weight: 150 MDa
- Non-enveloped, icosahedral
- Molecular weight of proteins: ~5 – 120 kDa
- Core: DNA
- Subtypes: Ad26



Adenoviruses at Janssen

- Adenoviruses → transgene carriers
- Transgene inserts:
  - Ebola
  - HIV
  - RSV
  - Influenza
- Intracellular delivery of DNA
- Trigger immune response

= Vaccine!



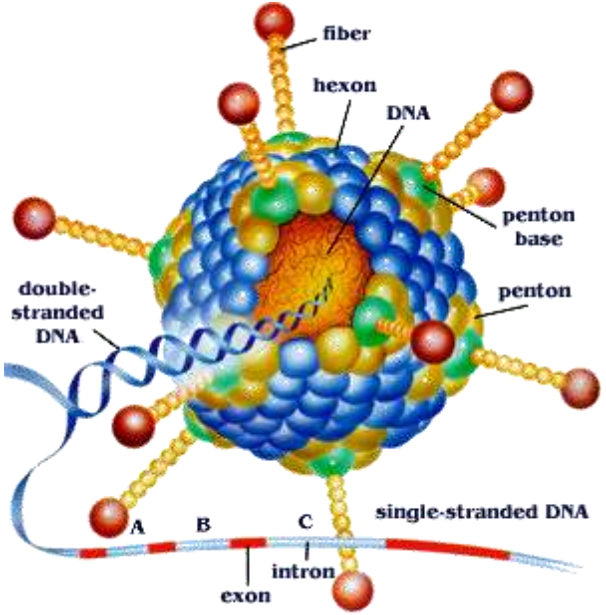
Why do we need a method?

Regulatory expectations:

- Guidelines (ICH Q6B, USP <1047>, etc):
  - Product-related impurities controlled.
  - Fingerprint and quantitation of selected proteins
  - Control consistency, quality, comparability

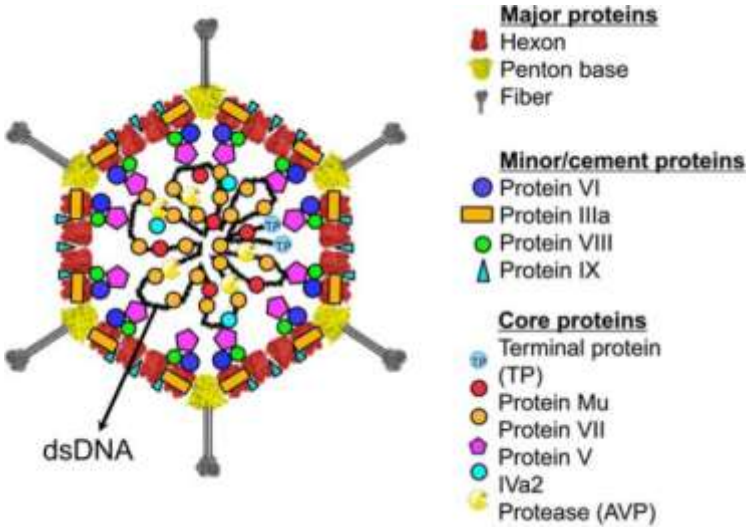
Internal assessment:

- Critical quality attributes
  1. Correct expression of the adenovirus proteins
  2. Viral protein degradation products



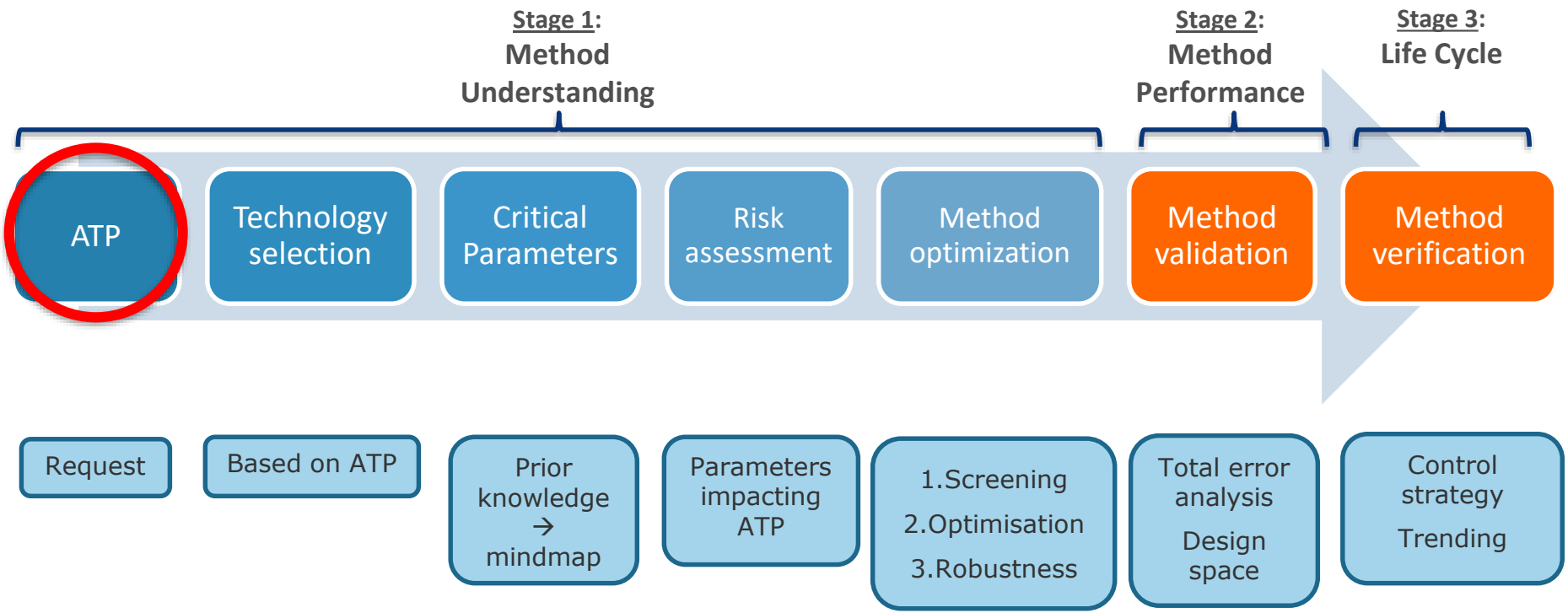
Challenges

- > 10 proteins
- Plus precursors / modifications
- Differences
  - Size
  - Hydrophobicity
  - Copy number
  - Mass
  - Charge
- DNA
- Buffer



J Biol Chem. 2014 Apr 18; 289(16): 11421–11430

AQbD

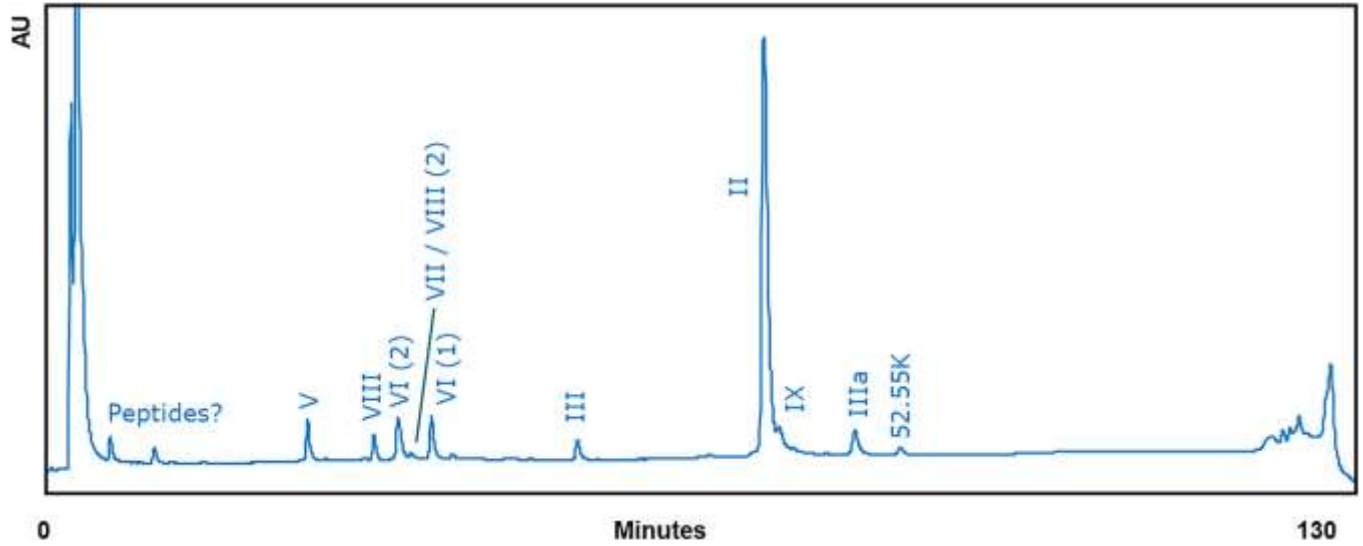

Stage 1:
Method Understanding
Stage 2:
Method Performance
Stage 3:
Life Cycle
ATP
Technology selection
Critical Parameters
Risk assessment
Method optimization
Method validation
Method verification
Request
Based on ATP
Prior knowledge → mindmap
Parameters impacting ATP
1.Screening
2.Optimisation
3.Robustness
Total error analysis
Design space
Control strategy
Trending

Analytical Target Profile (ATP)

Requirements	Targets
Specificity	Adenovirus type 26 protein profile • Identity • Modifications
Sample matrix	Drug product formulation
Accuracy	80 – 120% recovery
Precision	< 10% CV
Range	0.5×10^{11} – 3.0×10^{11} VP/ml
End users	• Product characterisation • Formulation development • Quality control

Starting point

- HPLC method already in QC



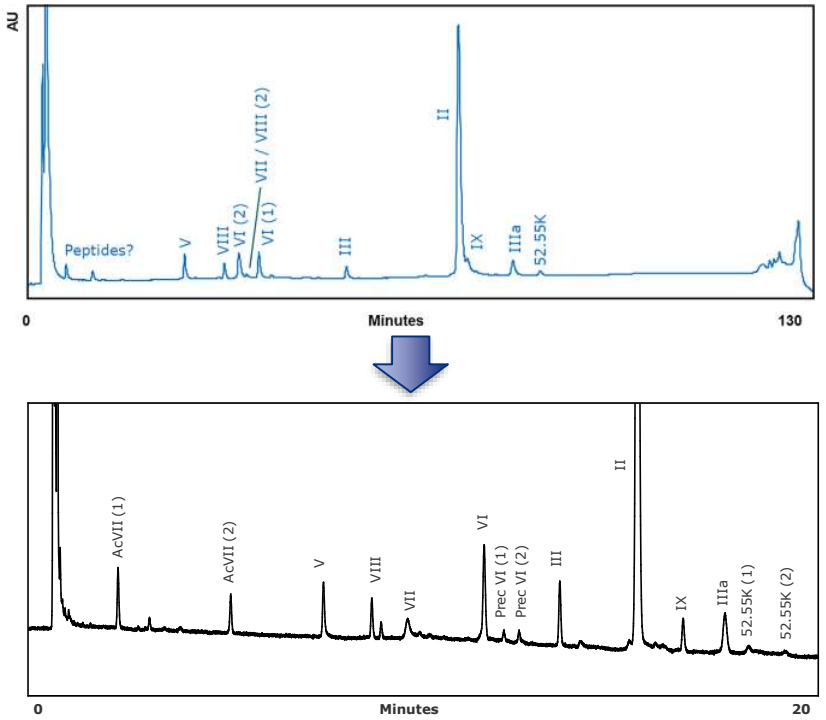
Transfer to UPLC

New column

- Acquity BEH300 C4 (1.7 µm, 2.1 × 150 mm).

New conditions

- 110 → 22 min
- 0.2 → 0.6 ml/min
- Single gradient
- Sample prep → no improvement



Critical parameters → Screening

Conditions:

- Gradient start
- Gradient end

>10%

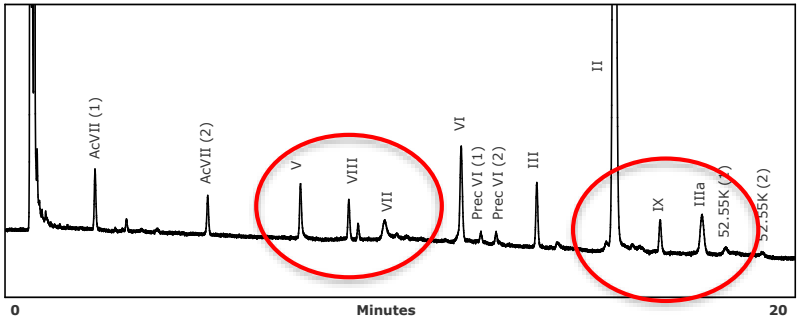
>45%

- TFA concentration
- Column temperature

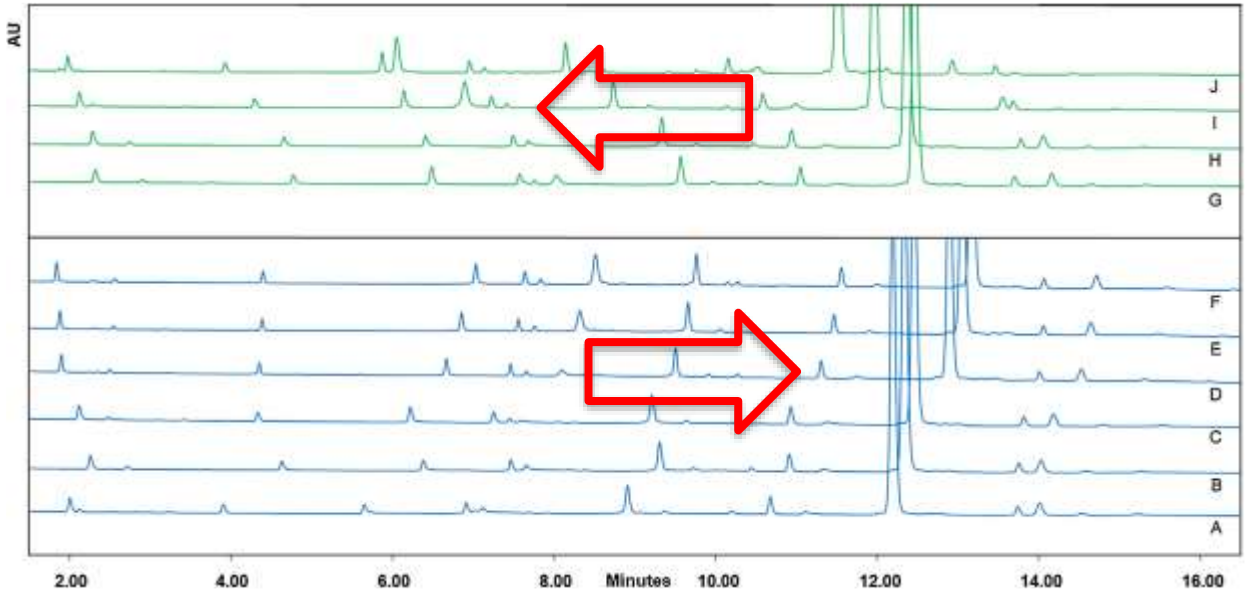
Responses:

- Run time
- Resolution
- Robustness

} of key proteins



More screening



Increasing Column temp.

Increasing TFA conc.

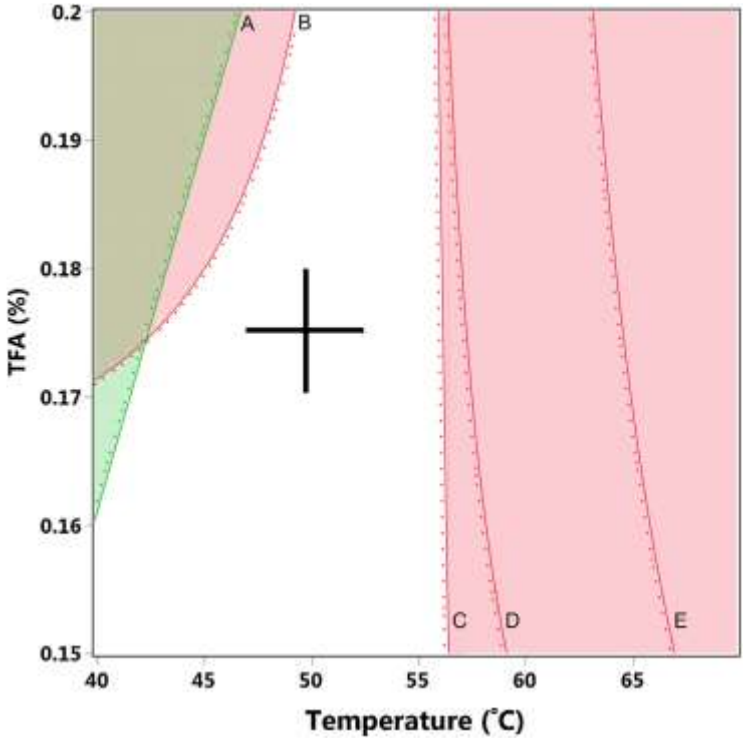
Full factorial design

TFA: 3 levels
0.150, 0.175, 0.200%

Column temp: 4 levels
40, 50, 60, 70 °C

Responses:

- Run time (A)
- Resolution of key proteins (B – E)



Robustness

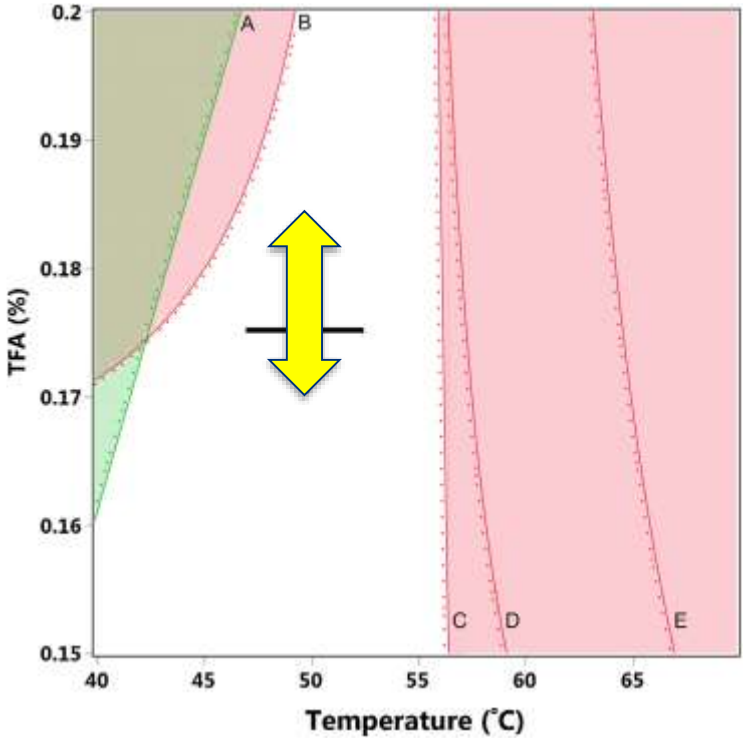
TFA: 3 levels
0.170 – 0.185%

Responses:

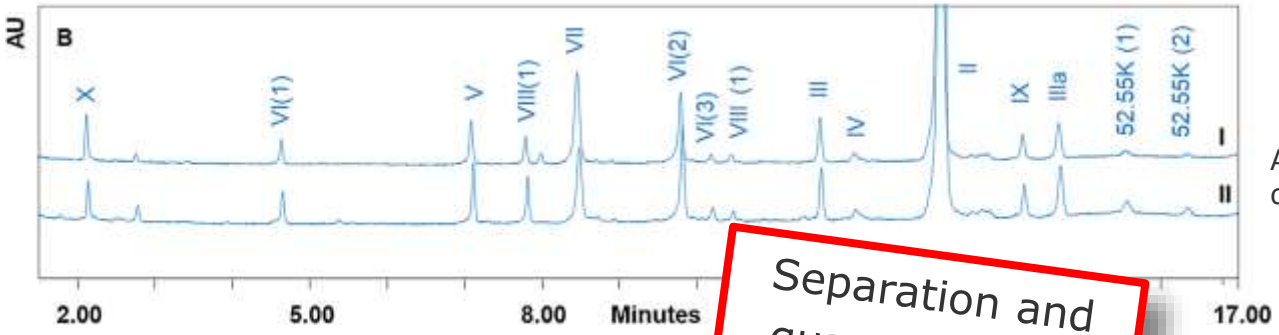
- Resolution
- Peak areas

} of key proteins

<5% variation found ✓



Optimal conditions



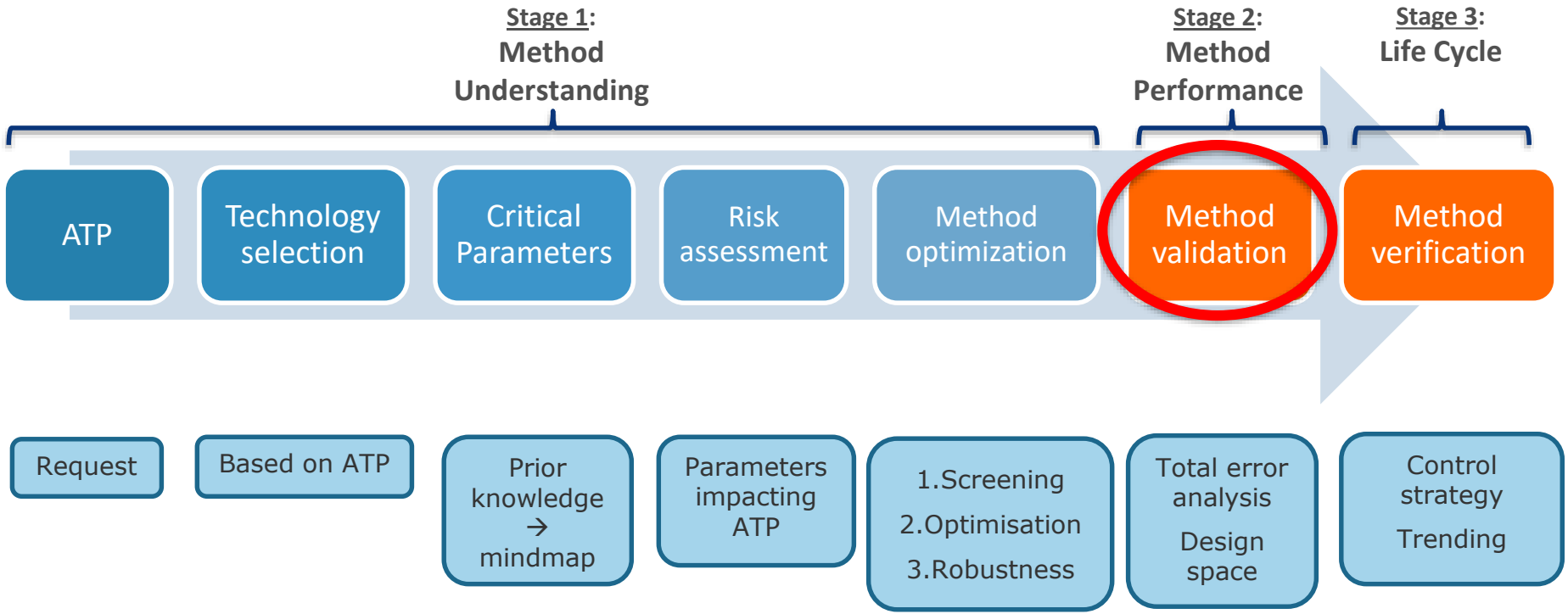
Adenoviruses with different transgenes

Separation and quantitation of 15 proteins!

Final conditions:

- Column: Acquity BEH 300, C4, 300 Å, 1.7 [illegible] m
- Gradient: 20 – 50% ACN
- TFA: 0.175%
- Inj. vol.: 30 µL
- Flow rate: 0.6 ml/min
- Temp: 50 °C

AQbD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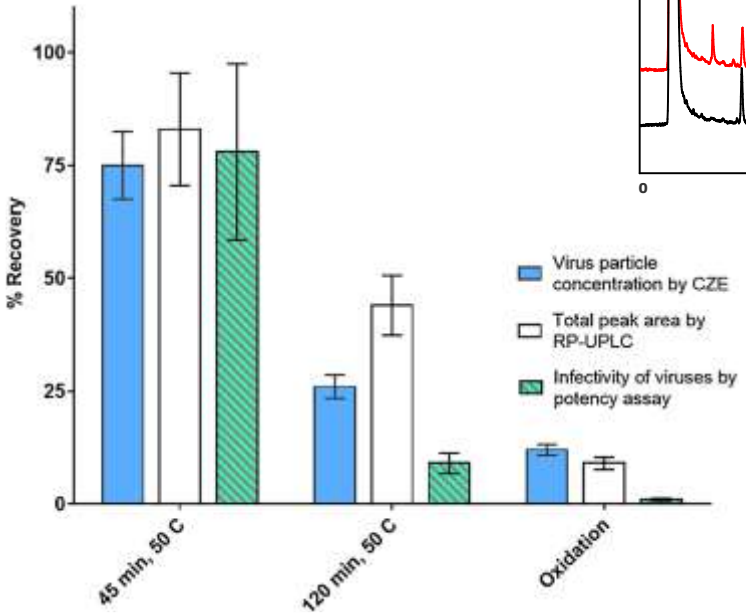
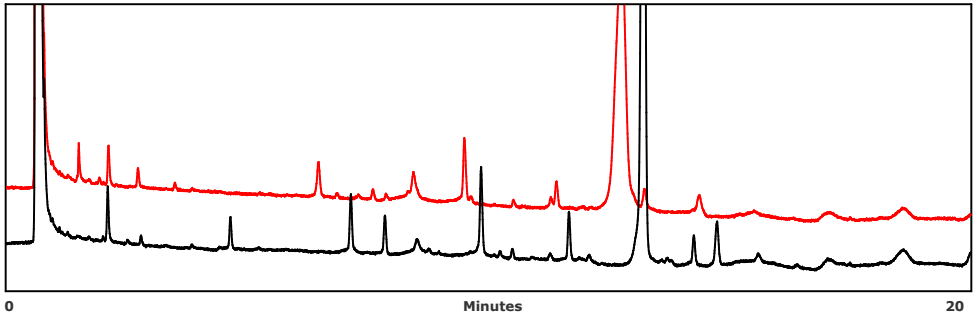
Method performance

Parameter	Targets
Specificity	Adenovirus type 26 protein profile • Identity • Modifications
Intermediate Precision	RRT: ≤ 2% Peak Area%: ≤ 14% (protein V 26%)
Accuracy	79 – 108% recovery
Linearity (dilutional)	$R^2 \geq 0.98$
Range	$1.0 – 2.5 \times 10^{11}$ VP/mL

Stability indicating power

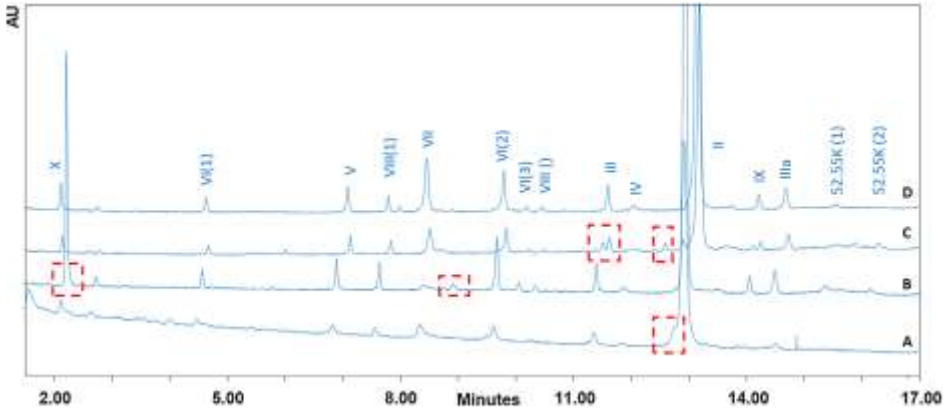




- 50 °C 45 min
- 50 °C 120 min
- 30% peroxide 4 h

Method lifecycle

- Continuous improvement
  - Robustness
  - Sensitivity
- Further use:
  - Characterisation
  - Formulation development
  - Process development
  - Leachables studies



The better the method gets
→ the more we want to know!

Conclusion

Virus protein profiling

- Separation and relative quantitation of >10 proteins ✓
- not all proteins equally reliable ✗

Method upgraded

- 130 → 17 min
- 10 → 50 samples a day
- Precision: 27% → 14% CV

Future outlook

- Analyse more → how far can we go...

Big thanks to…

Ewoud van Tricht
Pascal de Raadt
Annemiek Verwilligen

Plus many many more…

Van Tricht, Journal of Chromatography A, 1581–1582 (2018) 25–32

Thank you

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