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
Virus protein profiling

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Melinda, Harmony

Melinda’s artwork reflects her journey living with HIV.
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

AQbD

Stage 1: Method Understanding
- ATP
- Technology selection
- Critical Parameters
- Risk assessment
- Method optimization

Stage 2: Method Performance
- Method validation

Stage 3: Life Cycle
- 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)

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>Adenovirus type 26 protein profile</td>
</tr>
<tr>
<td></td>
<td>• Identity</td>
</tr>
<tr>
<td></td>
<td>• Modifications</td>
</tr>
<tr>
<td>Sample matrix</td>
<td>Drug product formulation</td>
</tr>
<tr>
<td>Accuracy</td>
<td>80 – 120% recovery</td>
</tr>
<tr>
<td>Precision</td>
<td>&lt; 10% CV</td>
</tr>
<tr>
<td>Range</td>
<td>$0.5 \times 10^{11} – 3.0 \times 10^{11}$ VP/ml</td>
</tr>
<tr>
<td>End users</td>
<td>• Product characterisation</td>
</tr>
<tr>
<td></td>
<td>• Formulation development</td>
</tr>
<tr>
<td></td>
<td>• Quality control</td>
</tr>
</tbody>
</table>
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
• TFA concentration
• Column temperature

Responses:
• Run time
• Resolution
• Robustness

>10%

>45%

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

Final conditions:
- Column: Acquity BEH 300, C4, 300 Å, 1.7 µm, 2.1 mm x 150 mm
- Gradient: 20 – 50% ACN
- TFA: 0.175%
- Inj. vol.: 30 µL
- Flow rate: 0.6 ml/min
- Temp: 50 °C

Adenoviruses with different transgenes

Separation and quantitation of 15 proteins!
ATP Technology selection Critical Parameters Risk assessment Method optimization Method validation Method verification

Stage 1: Method Understanding

Stage 2: Method Performance

Stage 3: Life Cycle

Request Based on ATP Prior knowledge → mindmap Parameters impacting ATP 1.Screening 2.Optimisation 3.Robustness Total error analysis Design space Control strategy Trending

AQbD
Method performance

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<td></td>
<td>• Identity</td>
</tr>
<tr>
<td></td>
<td>• Modifications</td>
</tr>
<tr>
<td>Intermediate Precision</td>
<td>RRT: ( \leq 2% )</td>
</tr>
<tr>
<td></td>
<td>Peak Area%: ( \leq 14% ) (protein V 26%)</td>
</tr>
<tr>
<td>Accuracy</td>
<td>79 – 108% recovery</td>
</tr>
<tr>
<td>Linearity (dilutional)</td>
<td>( R^2 \geq 0.98 )</td>
</tr>
<tr>
<td>Range</td>
<td>1.0 – 2.5 ( \times 10^{11} ) VP/mL</td>
</tr>
</tbody>
</table>
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...

Thank you

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