Online UPLC Applications for Biotherapeutic Development

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Overview

• Process Analytical Technologies (PAT) at Merck
  – Continuous Processing and Facility of the Future
  – Real Time Release Testing

• BioProcess Case Studies w/ Online UPLC
  – RP-UPLC for Titer
  – UP-SEC
    ▪ Multi-Attribute Method (Titer, Purity, Excipients)
    ▪ UF/DF for High Concentration Formulation Development
    ▪ Parallel Sampling

• Summary and Future Work
Vision

**PAT is engineered into our manufacturing processes, fully integrated in our business processes, part of the technical fabric of our people so that PAT provides a clear competitive advantage for Merck.**

Mission

**PAT will provide value to Merck Manufacturing Division through innovative technical solutions to further manufacturing science resulting in efficient commercialization and minimized in-line manufacturing cost.**

**PAT Tools**
- On-line HPLC
- In-Line FTIR
- On-line Mass Spec
- FTIR
- RAMAN

- In-line NIR
- On-line PSD
- FBRM
- Sensors
  - pH, Temp, etc
Merck’s Global Presence:
Active PAT Implementations

- Italy
- Netherlands
- Ireland
- Singapore
- Puerto Rico
- Durham
- Elkton
- Singapore API
- Singapore Pharm
- NJ/PA pilot plants
- UK site RTRT
Transition to Future Manufacture Concepts

INTENSIFICATION

Batch Stainless

Batch Stainless / Single Use

Continuous Single Use Enabled

Public
Continuous Processing:
**PAT, Automated Control & Real Time Release**

- End Product Testing transition to Real Time Release Testing
- Real time automated control: process responds to variability & disturbances
  - End to end prediction models for complete process
  - RM control $\Rightarrow$ Process input $\Rightarrow$ Product quality & yield
### Continuous Processing: Purification PAT Examples

#### PAT On-line & At-line

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Inline UV280 or Waters UPLC</td>
</tr>
<tr>
<td>Purity</td>
<td>IEX &amp; SEC: Waters Patrol</td>
</tr>
<tr>
<td>Quality Bioburden</td>
<td>BioVigilant</td>
</tr>
<tr>
<td>Quality Endotoxin</td>
<td>At line</td>
</tr>
<tr>
<td>Quality: n-glycans</td>
<td>At line</td>
</tr>
<tr>
<td>Impurities</td>
<td>At line (Gyrolab ELISA’s)</td>
</tr>
<tr>
<td>Conductivity</td>
<td>Parker / Pendotech sensors</td>
</tr>
<tr>
<td>pH</td>
<td>Senova SU pH</td>
</tr>
<tr>
<td>Flow</td>
<td>Transonics SU ultrasonic flow</td>
</tr>
</tbody>
</table>

- **Titer, SEC, IEX**: Waters Patrol UPLC
- **Process Residuals**: Microfluidic ELISA
- **Senova Solid State pH**
- **Flow**: Clamp on Transonics flow
- **Pressure & Conductivity**: Parker Pendotech
Online UPLC Case Studies

• Case Study 1
  – Online Titer (RPLC)
    ▪ 3L bioreactor
    ▪ Continuous perfusion

• Case Study 2
  – Multi Attribute Method
    ▪ UPSEC for Titer

• Case Study 3
  – UPSEC for Process Understanding
    ▪ UF/DF for high concentration formulation development
      » Concentration, purity, & excipient monitoring
    ▪ Parallel Sampling
Dilution Range is 1-100x
PSM System Volume: 104 µL
PEEK Tubing Volumes:

0.040” @ 36” → 741 µL (Natural)
0.030” @ 36” → 417 µL (Green)
0.020” @ 36” → 185 µL (Orange)

At-Line and On-Line Sampling
Fixed Loop Injection
Case Study #1 Bioreactor Real-time Titer Monitoring

- 3L Bioreactor
- RP-UPLC for Online Titer
- Flownamics FISP Filter
  Probe:
  - 200mm
  - Autoclave for probe sterilization

The HPLC Tubing, or second, configuration uses a PEEK nut and ferrule for connecting PTFE, PEEK or other plastic HPLC tubing. In this case, the PEEK nut and ferrule are used in lieu of the PEEK female luer connector. Figure 2-4 shows the P-Series FISP sampling probe with the PEEK nut and ferrule connector.

![Image of a bioreactor and probe](image)

**Probe Volumes**

- 120mm = 0.585 mL
- 200mm = 0.625 mL
- 310mm = 0.685 mL
- 410mm = 0.745 mL
Case Study #1: mAb 1 Calibration

1, 2, 3, 6, & 30x dilutions
0.1-3 mg/mL

Column: Poros R2/10, 2.1 x 30 mm
Mobile phase: A: 0.1% TFA in Water, B: 0.1% TFA in ACN
Flow rate: 2 mL/min
Gradient: 0-0.5 min: 28.8 %B, 0.5 → 5.0 min: 28.8 → 54 %B, 5.0-6.0 min: 54 → 90 %B, 6.0-6.1 min: 90 → 28.8 %B, 6.1-7.0 min: 28.8 %B
Column Temp.: 70 °C
Injection Volume: 2 µL (full sample loop injection)
Case Study #1: 24 Hour Real-time Titer Monitoring

On-line RP-UPLC provides both content and quality data → Multi Attribute Method Capabilities

Added new mobile phase, slowed down the flow rate to 0.2 mg/mL between runs
Case Study #1: Online UPLC and Continuous Processing

Online UPLC Sample Locations

- #1- BR
- #2- Permeate
- #3- PAP
- #4- VI

Sampling Distance and Volume
- BR- 30” and 345 µL
- Permeate- 36” and 417 µL
- PAP- 6’ and 834 µL
- VI- 15’ and 925 µL

- Sequential Injection on Patrol
Case Study #1: Online UPLC and Continuous Processing

Bioreactor
- Online Titer for Perfusion
  - 14 days of continuous data
  - Bioreactor probe fouling at day 9
    - Decreased product sampling
    - Fouling characterization ongoing

Continuous Downstream Processing
- 4 sequential sampling locations
- Good online and offline titer correlation
  - Permeate, PAP, and VI

Online UPLC is a valuable tool for continuous processing
Case Study #2: UPSEC as Multi Attribute Method

mAb 2 Clarified Cell Culture from 3L Batch

Filtered with smart polymer 0.3%, pH=6.5

RP Titer- 4.9 mg/mL

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Waters BEH200 SEC, 4.6x150 mm, 1.7 µm</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>100 mM sodium phosphate and 100 mM NaCl, pH 7.0</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.5 mL/min</td>
</tr>
<tr>
<td>Run Time</td>
<td>5 minutes</td>
</tr>
<tr>
<td>Column Temp.</td>
<td>25 ºC</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>2 µL (full sample loop injection)</td>
</tr>
</tbody>
</table>
Case Study #2: UPSEC Automated vs Manual Dilution

**Automated Dilution**  
On-line Sampling

**Manual Dilution**  
At-line Sampling

**UPSEC Summary**
- Excellent linearity ($R^2$)
- Comparable slope (difference < 2%)
- Manual dilution has less carryover (smaller intercept)
- UPSEC preforms as a Multi Attribute Method (MAM)
  - Purity and Titer information
Case Study #3: Online UPSEC for Multi Attribute Monitoring of High Concentration UF/DF

Sampling Position #1 - Post Pump
Case Study #3: mAb3 Arginine Diafiltration

<table>
<thead>
<tr>
<th>DV #</th>
<th>UPSEC [mg/mL]</th>
<th>A280 [mg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.0</td>
<td>44.2</td>
</tr>
<tr>
<td>2</td>
<td>40.2</td>
<td>40.5</td>
</tr>
<tr>
<td>3</td>
<td>40.3</td>
<td>41.7</td>
</tr>
<tr>
<td>4</td>
<td>39.9</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>40.4</td>
<td>37.4</td>
</tr>
<tr>
<td>6</td>
<td>39.7</td>
<td>37.2</td>
</tr>
</tbody>
</table>

Team originally planned 8 DV but made real time decision to stop after 6 DV

Additional Multi Attribute Method Capabilities for UPSEC \(\rightarrow\) Titer, Purity, and Excipients
Case Study #3: Patrol System Pressure

- Sample/wash
- Diluent
- Process pump
- Injection Mark

System pressure for all pumps are normal and consistent
Case Study #3: UF UP-SEC Overlay

6 injections were tried but only the first 4 were made due to increasing sample viscosity.
Case Study #3: Patrol System Pressure

Normal Profile (first 4 injections)

Pressure too high due to higher sample viscosity → injection failed

Sampling with slower pumping speed – investigation ongoing

Hi Viscosity Alternative Sampling Options
- Larger bore tubing
- Slower process pump sampling speed
- Shorter distance
Case Study #3 Summary: Correlation with Offline Data

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Conc. by Online UPSEC (mg/mL)</th>
<th>Conc. by UV280 w/ Dilution (mg/mL)</th>
<th>%HMWS by Online UPSEC</th>
<th>Process Pump Pressure Range (psi)</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before UF</td>
<td>39.7</td>
<td>37.2</td>
<td>0.41</td>
<td>108-118</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>51.1</td>
<td>47.9</td>
<td>0.46</td>
<td>108-118</td>
<td>1.778</td>
</tr>
<tr>
<td>2</td>
<td>91.9</td>
<td>84.8</td>
<td>0.41</td>
<td>120-215</td>
<td>3.031</td>
</tr>
<tr>
<td>3</td>
<td>138.0</td>
<td>155.5</td>
<td>0.44</td>
<td>212-530</td>
<td>9.919</td>
</tr>
<tr>
<td>4</td>
<td>144.1</td>
<td>198.4</td>
<td>0.41</td>
<td>548-778</td>
<td>50.900</td>
</tr>
<tr>
<td>5</td>
<td>247.8*</td>
<td>254.2</td>
<td>0.42</td>
<td>830-1003*</td>
<td>271.022</td>
</tr>
</tbody>
</table>

* Sample is too viscous. Tested at-line with dilution.

Good correlation between online and offline UV 280 concentration data
Case Study #3: Parallel Sampling with UPSEC for High Concentration UFDF

Sampling Position #1
- Post Pump

Sampling Position #2
- Post TFF Membrane
Case Study #3: Parallel Sampling

Parallel Patrol Online UPSEC
- 2 Patrol Systems testing in parallel
  - Good correlation with offline UPSEC data
- Position 2 is post TFF membrane
  - Concentration increase observed

UF/DF Pump Comparison

The 1st UF (Pump 1)

The 2nd UF (Pump 2)
Case Study #3 Summary: Online vs Offline Aggregation

- HMW Species Increase with Concentration
- Aggregation not observed in offline UPLC
- Reversible Aggregation tied to dilution and analysis time is suspected
Summary and Future Work

• Successful application of Online UPLC for BioProcess Development
  • Upstream monitoring of titer
    • Bioreactor
    • Continuous Processing
      • 4 positions for >14 days
  • Online UPSEC
    • Multi Attribute Method Capabilities
    • Downstream monitoring of concentration, aggregation and excipients
    • Parallel Sampling show value (Multiple Patrol Systems)
• Line of Sight to Continuous Processing
  • Facility of the Future and Real Time Release testing
• Expanded applications
  • Online Mass Spec, Admixture, and Forced Degradation
  • Expanded UPLC Methods (i.e. IEX and Oxidation)
On-line UPLC-MS
- Multi Attribute Methods (MAM)
  - Metabolites
  - Excipients
  - Purity
  - Quality

Waters QDA
- 30-1250 m/z
  - Mass accuracy of +/- 0.2 Da
- ESI Positive and Negative Ion Modes
  - 25ms switching time
- Empower Software
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