Särö Conference 2009

Peptide purification strategies

Ulf Altenhöner
Lonza Exclusive Synthesis R&D
Outline

- Introduction
- Integrated process development
- Model-based process development
- Inspiration
- Conclusions
The leading supplier to the life-science industries

- **Production facilities worldwide**
  - Europe (CH, UK, CZ, BE, SP, F, D)
  - USA
  - Asia

- **Business activities**
  - LES - APIs & Intermediates, Synthetic and recombinant Peptides
  - LBP - Biopharmaceuticals
  - LSI - Agrochemicals & organic intermediates
  - LBS - Therapeutic Cells
Lonza’s History in Peptides and Oligos

- **1980** First peptide production in Braine-l’Alleud, Belgium.
- **1997** First peptide production in Visp, Switzerland (recombinant technology).
- **1999** Synthetic peptide production in Visp (solid phase synthesis).
- **2002** First oligonucleotide production in Visp.
- **2002** Dedicated Tides team in Visp formed (R&D, QC, QA).
- **2005** $20 million Tides investment in Visp (capacity enlargement, mid-scale plant, lyophylization, infrastructure).
- **2006** Lonza acquires UCB Bioproducts.
Custom Manufacturing:  
Focused on Late Stage Development

- **Products**
  - Early intermediates
  - Advanced intermediates
  - **Bulk actives/drug substance**

- **A Broad Range of Production Scales**
  - Assists lifecycle management
  - Increases flexibility
  - Enables seamless scale-up
Requirements for an Industrial API manufacturing process

- **Quality by Design Concept**
  - Structured Process Development ensures consistent delivery of *quality*, *safety* and *efficacy objectives*
  - Ensures process robustness
  - Process scalability
  - Continuous improvement

- **Competitive process, determined by:**
  - Yield
  - Productivity
  - Number of unit operations
  - Cost of goods
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„The sum of the optimal process steps is not equal to the optimal process!“

From Prof. Dr. rer. nat. K. H. Simmrock, Dortmund around 1990
Customer requirements

- Typical customer request:
  - product is a peptide/protein (10 to 50 amino acids)
  - delivery 1 kg 95 to 99% pure within 6 months after request for proposal
  - eventually single impurities specified
  - lyophilized as salt (e.g. acetate)
  - established laboratory process
Process Sketch

Structure formation

Purification

Concentration / Buffer exchange

Isolation

Product acc. Specifications
Integrated process development

- Multi step approach:
  - step 1: run a best process structure formation
  - step 2: run a best process purification
  - step 3: identify **critical impurities** in purification
    - use structure elucidating techniques (LC-MS, NMR etc.)
  - step 4: give feedback for structure formation
  - step 5: run improved structure formation
  - ...
  - step X: stop when:
    - a: no improvement is achieved anymore
    - b: proces performance targets are achieved
Example:
Structure formation by **Solid-Phase Peptide Synthesis**

1. **Coupling of the first amino-acid**
2. **Fmoc deprotection**
3. **Amino-acid coupling**
4. **Fmoc deprotection**
5. **Amino-acid coupling**
6. **Repeat n times**
7. **Cleavage + deprotection**

Schema by Prof. F. Albericio, Institut de Recerca Biomedica, Barcelona, IRB-PCB
Impurities!

During the peptide assembly in SPPS the following impurities will be formed:

- Impurities in starting materials
- Impurities formed during peptide elongation
  - Isomers
  - Double hits
  - Deletion products
  - Side products from reactions with coupling reagents, deprotection reagents or solvents
  - Side products from other reactions on side chain functionalities
Example 1: Impurities!

- double hit AA, deletions, diastereomers
Evaluation of impurity influences

- Score 1: Yield loss in structure formation
- Score 2: Yield loss in purification
- Score 3: Productivity limiting in purification
Example 2: Integrated Process Development

- Purification by RP-HPLC

  *Crude peptide*

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  *Pure peptide*

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Peptide Area%: 58.9%
RRT 0.99: 8.9%

Peptide Area%: 94.9%
RRT 0.99: 3.5%

- RRT 0.99 was identified via MS/MS: AA double hit
Example 2: Integrated Process Development Results

<table>
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<tr>
<th>Reaction #</th>
<th>Time (h)</th>
<th>Coupling reagent</th>
<th>Temperature °C</th>
<th>Conversion %</th>
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<td>1</td>
<td>72</td>
<td>TCTU</td>
<td>20</td>
<td>92.2%</td>
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<tr>
<td>2</td>
<td>7</td>
<td>DIC / 6-CI-HOBt</td>
<td>40</td>
<td>99.6%</td>
</tr>
</tbody>
</table>

**Peptide Area%:** 75.3%  
RRT 0.99: 0.9%

**Peptide Area%:** 98.8%  
RRT 0.99: 0.3%
Outline

- Introduction
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- Model-based process development
- Inspiration
- Conclusions
Model-based Process Optimization

Example

- The Peptide:
  - Lonza Code XX-003 - 4 kDa polypeptide (39 AA)
  - Produced by solid-phase synthesis

- The Process:
  - 2-step chromatographic process
  - 1st step: H₂O-AcN, TEAP buffer
  - 2nd step: H₂O-AcN, AcOH buffer
  - Overall Yield: 51.3% (no recycled fractions)
  - Overall Productivity: 0.012 g/L/min

- Specifications:
  - Overall purity 97%
  - Max. single impurity <1%
Model-based Process Optimization

The Strategy

- Model calibration for pure component
- Impurity characterization
- Pareto optimization of gradient & load
- Optimal batch process
Pure Component Parameter Estimation

First Chromatographic Step (TEAP)

- Henry coefficient

![Graph showing Henry coefficient vs concentration AcN (g/L)]
Pure Component Parameter Estimation
First Chromatographic Step (TEAP)

Full Isotherm Estimation
- Linear Conditions
- Overloaded Conditions
Pure Component Parameter Estimation

First Chromatographic Step (TEAP)

- Anti-Langmurian behavior with gradient elutions

Moreau Isotherm
- Account for adsorbate-adsorbate interactions

Gradient: 20-70%B in 30min
Bi-Moreau Isotherm

The Equations

\[ q_{i}^{eq} = \frac{c_i H_{i,i} + q_{i,i}^e \Omega_{i,i}}{1 + \sum_j \left( c_j \frac{H_{i,j}^e}{q_{i,j}^e} + \Omega_{i,j}^e \right)} + \frac{c_i H_{\Pi,i} + q_{i,i}^\infty \Omega_{\Pi,i}^e}{1 + \sum_j \left( c_j \frac{H_{\Pi,j}^e}{q_{\Pi,j}^\infty} + \Omega_{\Pi,j}^e \right)} \]

\[ H_i = \alpha_1 \left( c_M \right)^{\alpha_2} \]
\[ H_{\Pi,i} = \alpha_3 \left( H_i \right)^{\alpha_4} \]
\[ H_{i,i} = H_i - H_{\Pi,i} \]

\[ q_{i,i}^e = \frac{\alpha_5 H_{i,i}}{1 + \frac{\alpha_5}{\alpha_6} H_{i,i}} \]
\[ q_{\Pi,i}^\infty = \frac{\alpha_7 H_{\Pi,i}}{1 + \frac{\alpha_7}{\alpha_8} H_{\Pi,i}} \]

\[ \Omega_{i,i} = \alpha_9 \left( H_{i,i} \right)^{\alpha_{10}} \left( c_i \frac{H_{i,i}}{q_{i,i}^e} \right)^2 \]
\[ \Omega_{\Pi,i} = \alpha_{11} \left( H_{\Pi,i} \right)^{\alpha_{12}} \left( c_i \frac{H_{\Pi,i}}{q_{\Pi,i}^\infty} \right)^2 \]

Equilibrium Adsorption Surface

Langmuir behavior

Anti-Langmuir behavior
Model-based Process Optimization

The Strategy

- Model calibration for pure component
- Impurity characterization
- Pareto optimization of gradient & load
- Optimal batch process
Impurity Parameter Estimation

First Chromatographic Step (TEAP)

- Characterization of key impurities
Model-based Process Optimization

The Strategy

- Model calibration for pure component
- Impurity characterization
- Pareto optimization of gradient & load
- Optimal batch process
Model-based Process Optimization

Pareto Curve (95% purity)

- Productivity vs Yield
Model-based Process Optimization

Pareto Curve (95% purity)

- Loading vs Yield
Model-based Process Optimization

The Strategy

- Model calibration for pure component
- Impurity characterization
- Pareto optimization of gradient & load
- Optimal batch process
Optimal Batch Process
Experimental Validation

- current method
- experimental result
- chosen point
Optimal Batch Process
Experimental Validation

- Simulation of recommended gradient
Optimal Batch Process
Experimental Validation

- Experimental verification: UV + fraction analysis
## Process Summary
### Chromatography 1 + 2

#### Experimental validation

<table>
<thead>
<tr>
<th></th>
<th>Old Process</th>
<th>New Process</th>
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<tr>
<td><strong>1st purification</strong></td>
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<tr>
<td>column load [mg/ml]</td>
<td>5.6</td>
<td>3.44</td>
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<td>yield [%]</td>
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<tr>
<td>productivity [mg/mL/min]</td>
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<td>0.054</td>
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<tr>
<td>process time [min]</td>
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<td>main runs per recyle</td>
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<table>
<thead>
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<td>productivity [mg/mL/min]</td>
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<td>process time [min]</td>
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Inspiration

- 0.2 % TFA
- 0.05 % TFA
Summary

- Integrated Process Development:
  - easy to use
  - no “tools“ needed
  - all about communication

- Model based Process Development:
  - Current process has been largely improved
    - Higher process yield
    - Higher productivity
  - Quality by design can be applied
    - Flexible optimization of production conditions
    - Simpler validation and qualification procedures
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Thank you for your attention!