3 in 1: Increasing Efficiency of Potency Assay Optimization via DoE

CASSS, April 16th, 2018
Overview

- Principle of DoE vs. One Factor at a Time
- "Classic" DoE vs. "how we set up" DoE
- Which response to assess the quality of a bioassay?
- Case studies: Examples of outcomes of DoEs
- Using DoE to assess robustness
- Capacities: Comparison of DoE approaches (and OFaT)
Theory of OFaT vs. DoE

- **One Factor at a Time (OFaT):**
  - not possible to detect two-factor interactions
  - hard to find maximum with reasonable number of experiments

- **DoE:**
  - changing all factors at the same time
  - easy to find maximum and/or two-factor interactions (quite often observed in biological systems)
How we set up DoEs
How we set up DoE

„Classic “ Approach:
Typically 3 DoEs for assay development:

<table>
<thead>
<tr>
<th></th>
<th>Design space</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. screening (all potential factors; only linear model with low resolution)</td>
<td>wide</td>
<td>identify relevant factors</td>
</tr>
<tr>
<td>2. optimization (only relevant factors; RSM with higher resolution)</td>
<td>medium</td>
<td>two-factor-interactions and quadratic dependencies to find optimum</td>
</tr>
<tr>
<td>3. robustness (only relevant factors; only linear model with low resolution)</td>
<td>small</td>
<td>no changes of responses over design space</td>
</tr>
</tbody>
</table>

Don´t tell your technicians...
3 in 1 approach (i.e. screening, optimization and robustness in 1 DoE):

- constraining the Design Space via:
  - hands-on experience during „proof of concept“ (e.g. incubation time proliferation assay)
  - theoretical assessment of Design Space (e.g. substrate volume, cell-number)

- plan a 3 in 1 DoE i.e. with all potentially relevant factors (including hard-to-change factors)
How we set up DoEs

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Advantages vs. „Classic“ Approach:

- only single RSM DoE → far less experiments

- more replicates per factor in comparison to „classical optimization DoE“ → higher statistical power (unless not all factors turn out as relevant)

- hard-to-change factors allow identification of edge-of-failure at 1st experiment
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How we set up DoEs

Performance of experiments:
- 1 plate per run with octaplicates to assess scattering of data
- serial diltion e.g. >>1:2 steps
- swiss clock like technician
- knowing his limits in statistics
  (e.g. no rearrangement of data; no changes in design space)

Plate layout:
Choosing the right parameter
Choosing the right parameter

Which factor to assess quality of bioassay?

- dynamic range (DR) = \( \frac{asym_{high}}{asym_{low}} \)

- coefficient of correlation (R2): \( \sum_{i=1}^{n} (y_i - \bar{y})^2 \)

- mean-weighted-standard-deviation (MWSD): \( \sqrt{\frac{\sum(SD^2)}{n}} \)

- signal-to-noise (S/N): \( \frac{SD \; asym_{high}}{|asym_{high} - asym_{low}|} \)

- Z-factor * = \( 1 - \frac{(3SD \; asym_{high} + 3SD \; asym_{low})}{|asym_{high} - asym_{low}|} \)

* J. Zhang et al., (1999), Journal of Biomolecular Screening

Noise = SD asym_{high}

\[ \text{signal} \]

\[ \text{Noise} = SD \; asym_{high} \]

\[ \text{Signal} = \frac{asym_{high} - asym_{low}}{} \]

\( mAb \; [pM] \)

16/04/2018
Conclusion:
• Z-factor strongly correlates with S/N
  ➔ Focus on S/N as changes in Z-factor are small due to normalization
Choosing the right parameter

Conclusion:
• MWSD does not correlate with S/N
→ MWSD interesting to assess model quality
Choosing the right parameter

Conclusion:
- R2 correlates well but it rather describes fit-quality than noise
  → have a glance at both models
Choosing the right parameter

Conclusion:
• S/N only weakly correlates with Dynamic Range
→ assess both parameters
→ careful using Dynamic Range exclusively...
Choosing the right parameter

Conclusion:
• both parameters correlate ..... but opposite outcome!
→ careful using Dynamic Range exclusively
→ *reasonable to evaluate different responses*
Case studies
Case study #1: DoE of Reporter-Gene Assay

Conclusion:
- good model
- identification of maximum and of two-factor interactions
Case study #1: DoE of Reporter-Gene Assay

Conclusion:
- good model
- identification of maximum and of two-factor interactions
- lower substrate volume contributes to better S/N-ratio (even supported by model from MWSD)...
  → **2 in 1 works...**
  → **....and how about OFaT?!**
Conclusion:
• „numerical optimization“ allows to determine settings of all tested factors at which best assay performance is achieved
Assessment of Robustness via DoE model
Assessment of Robustness via DoE model

**Background:**
- Often assessed via Potency ...“very indirect” approach
- Rather assay quality important....

**Acceptance Criteria**
- Easy
- Difficult (only “soft“ AC)

**Significance**
- Low
- High

**Conclusion:**
Desirability plots show edge of failures and ranges of factors which do not effect assay

Approach is efficient and reasonable way to determine robustness at least for early phases

→ even 3 in 1 works
Case study #2: Apoptosis-Assay
Case study #2: Apoptosis-Assay

Design-Expert® Software
Factor Coding: Actual
S/N

Actual Factors
a: Trypsination = 6
B: Incubation time = 24
C: Cell Number = 7378.38
d: Pre-Culture = 1

Conclusion:
- No relevant effects on S/N over whole design space => assay seems very robust

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Case study #2: Apoptosis-Assay

Design-Expert® Software
Factor Coding: Actual
Original Scale
MWSD
- Design Points

Actual Factors
- a: Trypsination = 3
- B: Incubation time = 24
- C: Cell Number = 3000
- d: Pre-Culture = 0

Conclusion:
- No relevant effects on S/N over whole design space => assay seems very robust
- ...but not clear why 2 factor interaction of incubation time and cell number highly relevant for MWSD
Case study #2: Apoptosis-Assay

Conclusion:
- certain settings caused curves too steep to be fitted
Case study #2: Apoptosis-Assay

Conclusion:
- using „Hill slope“ as a response clearly showed that Pre-Culture is crucial to get nice curves
Case study #2: Apoptosis-Assay

Conclusion:
- low „Hill slope“ was ranked as highest desirability, followed by MWSD and S/N to obtain
- desirability clearly showed that Pre-Culture is crucial to get nice curves
...further procedure...

• confirmation of results during fine-tuning i.e.:
  o if necessary, check wider design space
  o adaptation of starting-concentration and serial dilution and plate-layout at set-point
  o plate-uniformity at set-point
  o pre-testing of accuracy/precision before assay-validation/qualification at set-point
# Capacities

<table>
<thead>
<tr>
<th></th>
<th>3 in 1 approach</th>
<th>DoE „classic“ approach</th>
<th>OFaT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planing of experiments</td>
<td>~3h expert</td>
<td>2 DoEs</td>
<td>Interpretation of every set of experiments</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~6h expert</td>
<td>=&gt; &gt;&gt;3h expert</td>
</tr>
<tr>
<td>Capacitiy for experiments (screening &amp; optimization)</td>
<td>40-50 assays =&gt; ≤10 days</td>
<td>&gt;60 assays =&gt; &gt;15 days</td>
<td>Preparation and performance of different experiments</td>
</tr>
<tr>
<td></td>
<td>~50h technician</td>
<td>&gt;75h technician</td>
<td>=&gt; &gt;50h technician</td>
</tr>
<tr>
<td>Evaluation</td>
<td>~3h expert</td>
<td>~9h expert</td>
<td>Interpretation of every set of experiments</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>=&gt; &gt;&gt;3h expert</td>
</tr>
<tr>
<td>Report</td>
<td>Template ~3h expert</td>
<td>~8h expert</td>
<td>Individual report</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>=&gt; &gt;3h expert</td>
</tr>
<tr>
<td>Robustness</td>
<td>Evaluation of existing data ~1h expert</td>
<td>Separate DoE ~30h technician</td>
<td>Experiments performed separately</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~6h expert (design &amp; evaluation)</td>
<td>=&gt; &gt;50h technician</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>=&gt; &gt;3h expert (planing and report)</td>
</tr>
<tr>
<td>Sum</td>
<td>~50h technician ~10h expert</td>
<td>&gt;100h technician</td>
<td>&gt;100h technician</td>
</tr>
<tr>
<td></td>
<td></td>
<td>~29h expert</td>
<td>=&gt; &gt;&gt;12h expert</td>
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</table>

16/04/2018
Summary

• 2 in 1 approach less labor-intensive than good OFaT or „classical“ DoE-approach
• robustness via DoE at least for early phase reasonable => 3 in 1
• considering different parameters for assay-quality (S/N often of avail)
• some front-loading required to get statistical know-how
• provides much more insight into critical assay parameters => fast break even because better assays during later routine
Backup (Effect of Substrate Temperature on Substrate Volume)

Design-Expert® Software
Factor Coding: Actual
Original Scale
S/N-ratio
- Design Points

Actual Factors
A: Substrate volume = 100
B: Substrate Temperature = 37
C: Cell-Number = 2
D: Plate Type = Flat-bottom

Warning! Factor involved in AB interaction.

Warning! Factor not in model.
Backup (Effect of Substrate Temperature on Substrate Volume)

Design-Expert® Software
Factor Coding: Actual
Original Scale
S/N-ratio
- Design Points

Actual Factors
A: Substrate volume = 100
B: Substrate Temperature = 4
C: Cell-Number = 2
D: Plate Type = Flat-bottom

Warning! Factor involved in AB interaction.

Warning! Factor not in model.
Case study: DoE of ADCC assay

Conclusion:
- rather poor model for S/N shows „only“ maximum
- finding supported by good model from dynamic range
- for ADCC S/N and dynamic range correlate