Optimization DoE for analytical method development
– how to distinguish good from bad assay conditions?

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Introduction

- In vaccine development a vaccine must be tested in clinical trials in order to prove that the vaccine is inducing an immune response in patients.
- For this reason we need an assay that can measure the level of antibodies in blood or serum.
- During development and optimization of such an assay, experimental conditions for good assay performance must be established.
- Using Optimal DoE (e.g. Jones and Nachtsheim, [1]) several combinations of influential factors are investigated systematically

Objective and question

- Ultimately, "good" assay performance requires an accurate and precise measurements
- Choice of DoE response is crucial for informative value of optimization experiment
- **Which experimental conditions correspond to best performance of assay?**

How does an ELISA work?

Step 1. Generating a standard curve

- The standard is a mixture of human sera with a known level of antibodies (reference).
- Standard is diluted on a microtiter plate in a number of serial dilution steps, in single determination or replicates.
- A signal is measured and used for fitting a standard curve. The relation between the dose of the drug substance and the response of the subject is called dose-response curve (here: four parameter logistic model, 4PL)

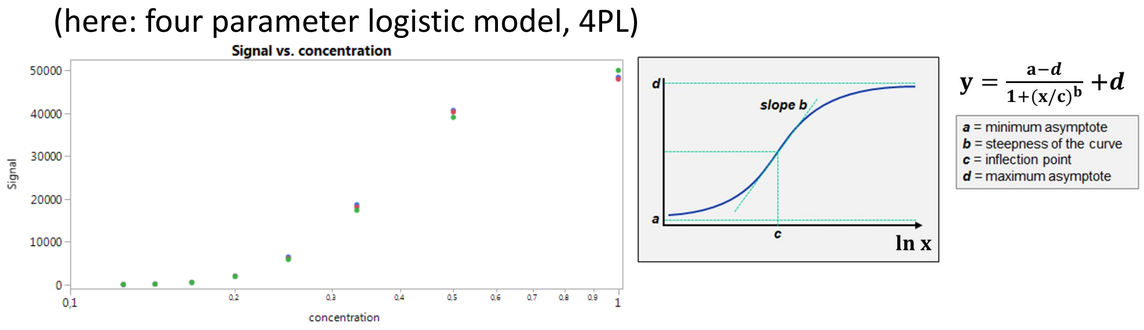


Figure 2. Measured signal (triplicate) of a Standard versus concentration (left), a four parameter logistic model visualization (right)

Step 2. Determination of potency of an unknown sample

- A sample with unknown level of antibodies (tested on the same plate) is evaluated using the standard curve.
- The standard curve is used for interpolation to relate the sample signal to its concentration.

Evaluation of an Assay

The objective of the DoE lies in finding the best experimental conditions for a precise and accurate assay ("critical quality attributes").

- Precision: Assay variability observed when testing the same sample multiple times, e.g. on one plate, on different plates or even on different days.
- Accuracy: Agreement between expected and observed concentration of a known sample.
- **Use of Precision Profile.**
  Precision profiles are functions of variability over larger range of concentrations. Thus, each profile identifies a point of maximal relative precision and yields finite-width intervals of the concentrations that can be measured within various thresholds of precision (e.g. the 15% or 25% threshold).

Precision profile: an ultimate goodness measure?

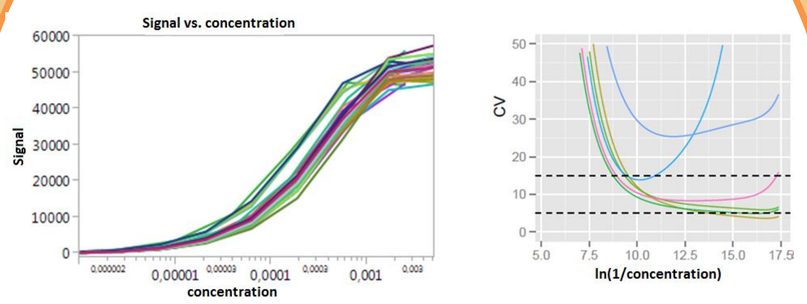


Figure 1. Signal versus dose (left) for Standard and corresponding precision profiles (right).Dashed line at 15% corresponds to the threshold of acceptable CV, the second line at 5% is a guidance for eye .

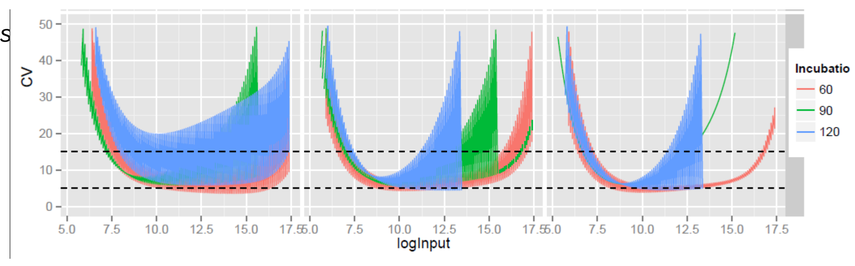
What is a "good curve"?

Method

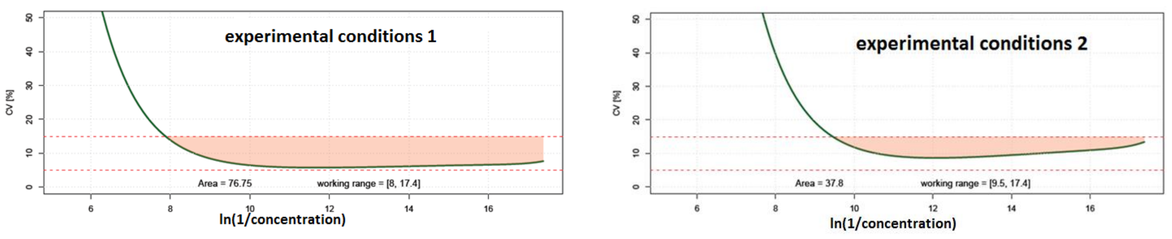
- Belanger et al. [2] consider a general non-linear heteroscedastic model: $y_{ij} = f(x_i, \boldsymbol{\beta}) + \sigma g\{f(x_i, \boldsymbol{\beta}), \boldsymbol{\theta}\}\epsilon_{ij}$ with expectation $E(y_{ij}) = f(x_i, \boldsymbol{\beta})$ and variance function $\mathrm{var}\left(y_{ij}\right) = \sigma g\{f(x_i, \boldsymbol{\beta}), \boldsymbol{\theta}\}\epsilon_{ij}$. The (x_i, y_{ij}) is data for standards ($i = 1, \dots, n$ concentrations with $j = 1, \dots, m_i$ replicates) , errors ϵ_{ij} are assumed to be iid and normally distributed.
- We applied a 4PL model to fit the standard: $f(x_i, \boldsymbol{\beta}) = \beta_2 + \frac{\beta_1 - \beta_2}{1+\left({}^{x_i}/_{\beta_3}\right)^{\beta_4}}$, the estimated concentration $\hat{x}_0 = f^{-1}(y_0, \hat{\boldsymbol{\beta}})$ is obtained using the inverse function $f^{-1}(y, \boldsymbol{\beta}) = \beta_3 \left(\frac{\beta_1 - y}{y - \beta_2}\right)^{1/\beta_4}$.
- Wald intervals for untransformed concentration scale: $\hat{x}_0 \mp c_\alpha\{\mathrm{var}(\hat{x}_0)\}^{1/2}$ where c_α is $z_{1-\alpha/2}$ or $t_{df,1-\alpha/2}$ and variability consists on variance due to variation in signal $\frac{\partial f^{-1}}{\partial y}(y_0, \hat{\beta})\frac{\hat{\sigma}^2 {y_0}^{2\hat{\theta}}}{m}$ and variability due to uncertainty in estimates of parameter $\left(\frac{\partial f^{-1}}{\partial \beta}(y_0, \hat{\beta})\right)' \sum(\hat{\boldsymbol{\beta}}) \left(\frac{\partial f^{-1}}{\partial \beta}(y_0, \hat{\beta})\right)$
- Idea: Using output from PROC NLMIXED $\hat{\boldsymbol{\beta}}, \hat{\boldsymbol{\theta}}, \hat{\boldsymbol{\sigma}}$ and $\sum(\hat{\boldsymbol{\beta}}) = \mathrm{var}(\hat{\boldsymbol{\beta}})$ to calculate %CV and build a precision profile as function of estimated concentration and %CV:

$$\%CV(x_0) = \frac{100\%}{x_0}\left[\left(\frac{\partial f^{-1}}{\partial y}(y_0, \hat{\beta})\right)\frac{\hat{\sigma}^2 {y_0}^{2\hat{\theta}}}{m} + \left(\frac{\partial f^{-1}}{\partial \beta}(y_0, \hat{\beta})\right)' \sum(\hat{\boldsymbol{\beta}}) \left(\frac{\partial f^{-1}}{\partial \beta}(y_0, \hat{\beta})\right)\right]^{1/2}$$

Figure 3. Precision profiles for different experimental conditions (left, mid and right panel) and multiple replicates of Standard. Different incubation times are depicted by red, green or blue color, the intervals cover the precision from different Standards. Dotted lines represent thresholds of %CV.



Evaluation of optimization DoE by means of Precision Profiles



Different experimental conditions could be evaluated in relation to each other:

- **Working range** is defined by intersection points between precision profile and threshold of acceptable variability: wider range corresponds to better performance of the assay
- **Upper/lower limit of precision** are the parameters of interest, strictly related to the endpoints of planned clinical studies
- **Area**, enclosed by the precision profile and threshold of acceptable variability: larger area corresponds to better performence under particular experimental conditions

References

[1] B. Jonesand, C. J. Nachtsheim (2011) "Efficient Designs with Minimal Aliasing". Technometrics, 53. 62-71
[2] B. A. Belanger, M. Davidian, D. M. Giltinian (1996). "The Effect of Variance Function Estimation on Nonlinear Calibration Inference in Immunoassay Data" . Biometrics 52:158-175.

This work was sponsored by GlaxoSmithKline Biologicals SA. Swetlana Berger, Angela Mangingelli and Walter Hoyer are employees of the GSK group of companies.

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