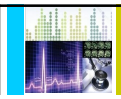



Qualification and validation of analytical and bioanalytical methods

Yossi Levy

Method life cycle




References

- ICH guidelines for validation of analytical methods:
  - Q2A: Text on validation of analytical procedures
  - Q2B: Validation of analytical procedures: methodology
- ICH draft guideline 1033: Biological assay validation
- Consensus paper: Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules (2003), DeSilva et. al. Pharmaceutical Research, Vol. 20, No. 11, November 2003

Method development

- Exploratory process
- Early development phase – check possibility of developing the method
- Optimization phase – once development of method is possible, fine tuning of method's parameters is needed for efficient implementation
- Examples of parameters: temperature, incubation time, type of equipment, etc.
- Statistical support is needed at the optimization phase
- Main statistical tool is DOE
- Usually, a series of controlled experiments is needed

Qualification and validation

- Qualification and validation are two steps in testing the performance of a (bio)analytical procedure/method and ensuring its quality
- **Qualification:** A documented testing that demonstrates with a high degree of assurance that a specific process will meet its pre-determined acceptance criteria
- **Validation:** A documented testing, performed under highly controlled conditions, which demonstrates a process consistently produces a result meeting pre-determined acceptance criteria

What is the difference?

- Key difference: whether or not the process under review operates under 'highly controlled' conditions
- Qualification can be viewed a less extensive form of validation
- Less parameters are checked
- Acceptation criteria are less strict
- In some cases, qualification is part of the method development process. Method can be modified if necessary.

The role of the statistician

- To provide, in cooperation with the development team, the experimental design for the qualification/validation.
- To develop and write the statistical methods section or a statistical analysis plan as required for the qualification/validation.
- To analyze and report the qualification/validation results according to the predefined statistical methods.
- To review and approve the qualification/validation report.

Selectivity/Specificity

- Selectivity/Specificity - the ability of an analytical method to differentiate and quantify the analyte in the presence of other components in the sample
- This includes:
  - Identification – ensuring the identity of the analyte
  - Purity – ensuring an accurate statement of the content of impurities of an analyte, i.e. related substances test, heavy metals, residual solvents content, etc.
  - Assay (content or potency) - providing an exact result which allows an accurate statement on the content or potency of the analyte in a sample

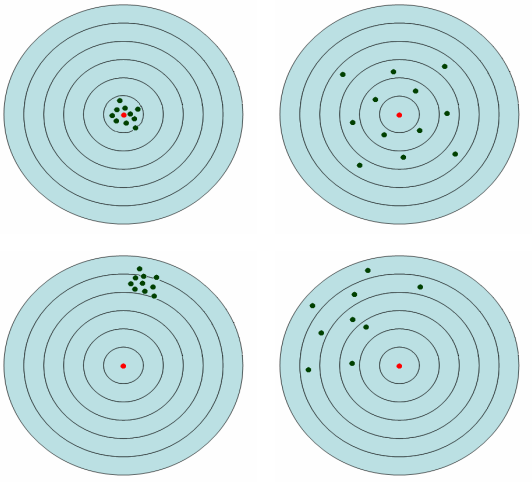
Accuracy

- The accuracy of a (bio)analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found
- This is sometimes termed as “trueness”
- Accuracy is related to systematic error or bias

Precision

- The precision of a (bio)analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions
- Precision is related to noise or variation

Accuracy vs. precision



Accuracy=Bias *Precision=Variance*

Levels of precision

- Repeatability expresses the precision under the same operating conditions over a short interval of time.
- Intermediate precision expresses within-laboratories variations: different days, different analysts, different equipment, etc.
- Reproducibility expresses the precision between laboratories.

Other quality parameters

- Detection limit - the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value
- Quantification limit - the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy
- Linearity – the ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample

Other quality parameters

- Range - the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity
- Robustness – measuring the method's capacity to remain unaffected by small, but deliberate variations in method parameters

Precision and accuracy estimation

"The way they do it at Chemistry ":

1. Measure accuracy and repeatability using 6 runs by the same analyst on the same day – report CV.

2. Measure reproducibility using another 6 runs by another analyst on another day – report "Reproducibility Difference "

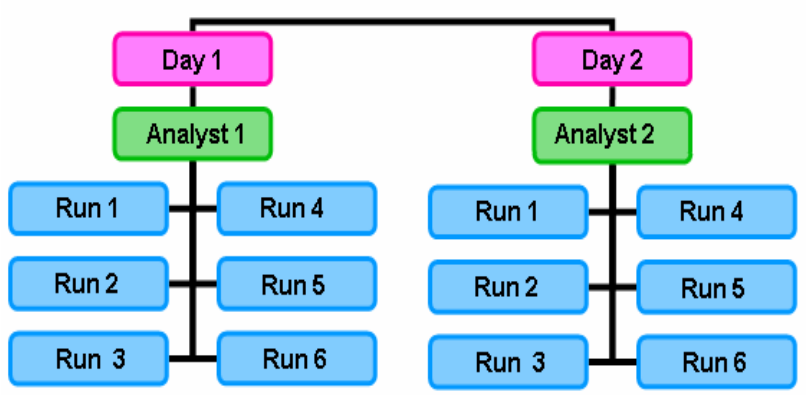
"The way they do it" advantage

- No experimental design
- No modeling
- No complex calculations
- Simple reporting

"The way they do it" problems

- Biological methods are more complicated to implement, therefore the numbers of possible runs in a single day is limited
- Variation of biological methods is generally higher compared to chemical methods
- Measuring intermediate precision is not enabled
- No statistical sense

"The way they do it" Experimental Design



Example - Biological data

Day/ Analyst	Run 1	Run2	Run 3	Mean	STD
1	0.768	0.601	0.887	0.752	0.144
2	0.460	0.398	0.519	0.459	0.061

$$\text{Accuracy} = 100 \cdot \frac{0.752}{0.7} = 107.4\%$$

$$\text{Repeatability} = 100 \cdot \frac{0.144}{0.752} = 19.1\%$$

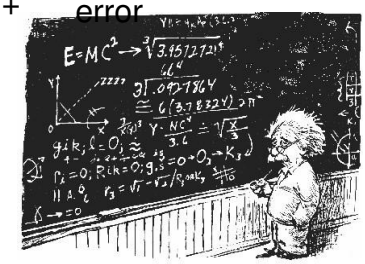
$$\text{Reproducibility Difference} = 100 \cdot \frac{|0.752 - 0.459|}{\frac{0.752 + 0.459}{2}} = 48.4\%$$

The way we would do it, at Statistics – Mixed Model!

$$Y_{ij} = \mu + b_i + c_j + \varepsilon_{ij}$$

Signal = fixed parameter + random effects + random error

Assumptions:
- Independence
- Normal distribution
- Zero mean deviations
- STDs: $\sigma_b, \sigma_c, \sigma$



Why use Mixed Models?

- Classical statistics assumes that observations are independent and identically distributed (iid)
- Often, data have a clustered structure
- When applied to clustered data, iid assumption may lead to false results
- Mixed Effects Model treats clustered data assumes two sources of variation, within cluster and between clusters
- This is the typical situation in biological data, when, observations are of the same biological category but individuals differ

Basic principles

- Two types of coefficients are distinguished in the mixed mode
  - population-averaged: same meaning as in classical statistics
  - Cluster/subject-specific: random; estimated as posteriori means

Formal modeling

$$Y = X\beta + Z\gamma + \varepsilon$$

$$\gamma \sim N(0, G)$$

$$\varepsilon \sim N(0, R)$$

$$\text{cov}(\gamma, \varepsilon) = 0$$

The matrices G and R are covariance matrices for the random effects and the random errors, respectively . As a result:

$$V(Y) = V = ZGZ' + R$$

The trick is to find a good model for G

SAS syntax

```
data example1;
  input day y @@;
cards;
1 0.768 1 0.601 1 0.887
2 0.460 2 0.398 2 0.519
;
run;
proc mixed method=reml covtest cl;
  class day;
  model y= / solution cl;
  random day;
run;
```

Example - SAS output

Covariance Parameter Estimates							
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
day	0.03887	0.06077	0.64	0.2612	0.05	0.007062	175.52
Residual	0.01215	0.008593	1.41	0.0786	0.05	0.004362	0.1003

Repeatability

Between Day precision

Solution for Fixed Effects								
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
Intercept	0.6055	0.1465	1	4.13	0.1511	0.05	-1.2560	2.4670

Accuracy

Results that make biological sense

$$\text{Accuracy} = 100 \cdot \frac{0.6055}{0.7} = 86.4\%$$

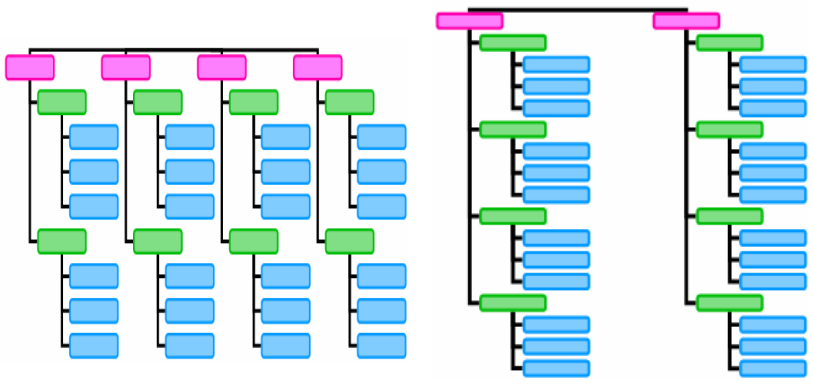
$$\text{Repeatability} = 100 \cdot \frac{\sqrt{0.01215}}{0.6055} = 18.2\%$$

$$\text{Reproducibility} = 100 \cdot \frac{\sqrt{0.03887 + 0.01215}}{0.6055} = 37.3\%$$

Results that make statistical sense

	Parameter	Estimate	95% confidence interval
Accuracy	μ	0.6055	-1.2560 - 2.4670
Repeatability	σ	0.0122	0.004362 - 0.1003
Between Days precision	$\sqrt{\sigma_D^2+\sigma^2}$	0.2259	????

DOE to measure intermediate precisions



4 Days, 2 Analysts or 2 Days, 4 Analysts

Reporting intermediate precisions

$$\text{Accuracy} = 100 \cdot \frac{\mu}{\mu_0}$$

$$\text{BetweenDay Precision} = 100 \cdot \frac{\sqrt{\sigma^2_{\text{Day}} + \sigma^2}}{\mu}$$

$$\text{BetweenAnalyst Precision} = 100 \cdot \frac{\sqrt{\sigma^2_{\text{Analyst}} + \sigma^2}}{\mu}$$

$$\text{Repeatabilty} = 100 \cdot \frac{\sigma}{\mu}$$

CI for μ, σ, $\sqrt{\sigma^2_{\text{Day}} + \sigma^2}$, $\sqrt{\sigma^2_{\text{Analyst}} + \sigma^2}$

Example 2

Covariance Parameter Estimates							
Cov Parm	Estimate	Standard Error	Z Value	Pr Z	Alpha	Lower	Upper
Analyst	0.000548	0.000796	0.69	0.2455	0.05	0.000106	0.8027
Day	0.002582	0.002132	1.21	0.1130	0.05	0.000821	0.03765
Residual	0.000177	0.000057	3.08	0.0010	0.05	0.000102	0.000377

Repeatability

Between Day precision

Between Analyst precision

Solution for Fixed Effects								
Effect	Estimate	Standard Error	DF	t Value	Pr > |t|	Alpha	Lower	Upper
Intercept	0.5621	0.03045	1	18.46	0.0344	0.05	0.1753	0.9490

Accuracy

Relative Standard Deviation

- Let $X_1,\ldots X_n \sim N(\mu,\sigma^2)$ iid. Define $CV = \sigma / \mu$

  $RSD = s / \bar{X}$

- McKay derived the approximate distribution of RSD in 1932:

$$f_b(t) = \left(\frac{n}{\sigma^2}\right)^{n/2} \frac{t^{n-2}}{2^{\frac{n-1}{2}}\sqrt{\pi}\,\Gamma\left(\frac{n-1}{2}\right)} \int_{-\infty}^{\infty} |x|^{n-1} e^{-n[t^2x^2+(x-\mu)^2]/2\sigma^2} dx.$$

- This can be used to obtain CI for CV, but would one extend that to Mixed Models?

Jackknife

- Idea: systematically re-computing the statistic estimate leaving out one or more observations at a time from the sample set
- From this new set of replicates of the statistic, an estimate for the bias and an estimate for the variance of the statistic can be calculated
- If we delete one observation at a time we get n sub-samples
- Then we calculate estimate CV out of the n subsamples, and obtain an estimate for it variation
- This estimate can be used to obtain a CI

Fieller's theorem application

- Same trick as in Fieller's theorem – look at

$$U = s - CV \cdot \overline{X}$$

- Then

$$V(U) = V(s) + CV^2 \cdot \frac{\sigma^2}{n} = \frac{\sigma^2}{2(n-1)} + CV^2 \cdot \frac{\sigma^2}{n}$$

- The obtained CI is

$$(0{,}100 \times (\overline{x^2} - t_{0.05}^2 s^2/n)(\overline{xs} + \sqrt{\overline{x^2}s^2 - (\overline{x^2} - t_{0.05}^2 s^2/n)(s^2 - t_{0.05}^2 s^2/2(n-1))}))$$

Delta method

- Let T_n be a MLE of a (multidimensional) parameter θ.
- It is known that T_n is asymptotically Normally distributed:

$$\sqrt{n}(T_n - \theta) \xrightarrow{D} N(0, \Sigma)$$

- Consider a function h(θ). We can expand its according to Taylor :

$$h(T_n) \approx h(\theta) + \nabla h(\theta)' \cdot (T_n - \theta)$$

Delta method

$$V\left[h(T_n)\right] \approx V\left[h(\theta) + \nabla h(\theta)' \cdot (T_n - \theta)\right] =$$

$$= V\left[h(\theta) + \nabla h(\theta)' \cdot T_n - \nabla h(\theta)' \cdot \theta)\right] =$$

$$= V\left[\nabla h(\theta)' \cdot T_n\right] = \nabla h(\theta)' \cdot V\left[T_n\right] \cdot \nabla h(\theta) =$$

$$= \frac{1}{n} \nabla h(\theta)' \cdot \Sigma \cdot \nabla h(\theta)$$

Application of Delta method

- In our framework:

$$\theta = \left(\mu, \sigma^2\right)$$

$$T_n = \left(\bar{X}_n, S_n^2\right) \quad \text{where} \quad S_n^2 = \frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{X}_n)^2$$

$$h(x, y) = \sqrt{y} / x$$

- This leads to the following CI:

$$\left(0{,}100 \times \left[\frac{s_n}{\bar{x}} + z_{0.95}\sqrt{\frac{s_n^4}{\bar{x}^4 \mathrm{n}} + \frac{s_n^2}{2\bar{x}^2 n}}\right]\right)$$

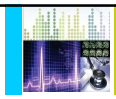
Parametric bootstrap

Parametric bootstrap algorithm

- Estimate model parameters
- Simulate N new datasets based on estimated parameters
- Estimate parameter under interests for each of the simulated datasets to get a sample of N simulated estimates
- Use 2.5% and 97.5% sample quartiles as a CI

Note: for RSD, we use the 95% quartile as an upper confidence limit, since the lower confidence limit is zero.

Which method should we use?



- We should consider
  - Distributional assumptions – are they correct? Are they needed?
  - Robustness
  - Ease of computation
  - “back calculation” – Can we calculate sample sizes?