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Reliability of Analytical Methods' Results: a Bayesian Approach to Analytical Method Validation

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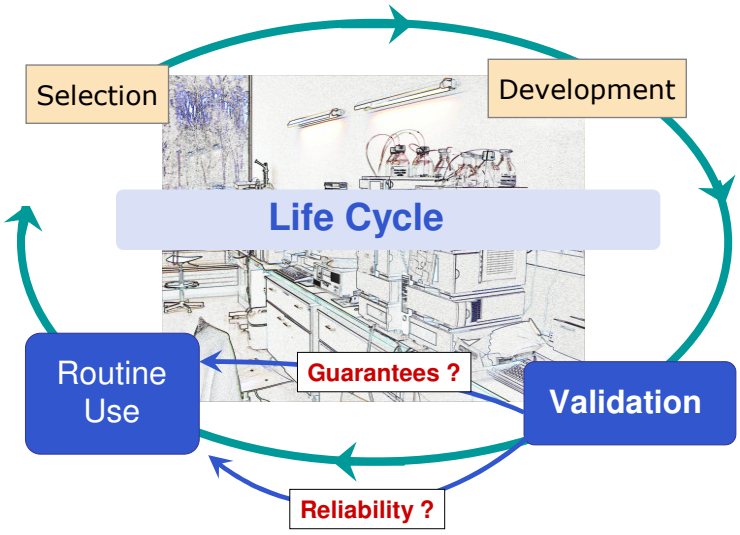
Agrostat 2012 **Paris, March 1, 2012**

Analytical Method Life Cycle

- What is the final aim of quantitative analytical methods ?
  - Start with the end !
  - Objective: provide results used to make decisions
    - Release of a batch
    - Stability/Shelf life
    - Patient health
    - PK/PD studies, …
- What matters are the results produced by the method.

Analytical Method Life Cycle



3

Analytical Method Life Cycle

- Need to demonstrate/guarantee that the analytical method will provide, in its future routine use, quality results
- This is the key aim of Analytical Method Validation !

How ?

4

Analytical Method Validation

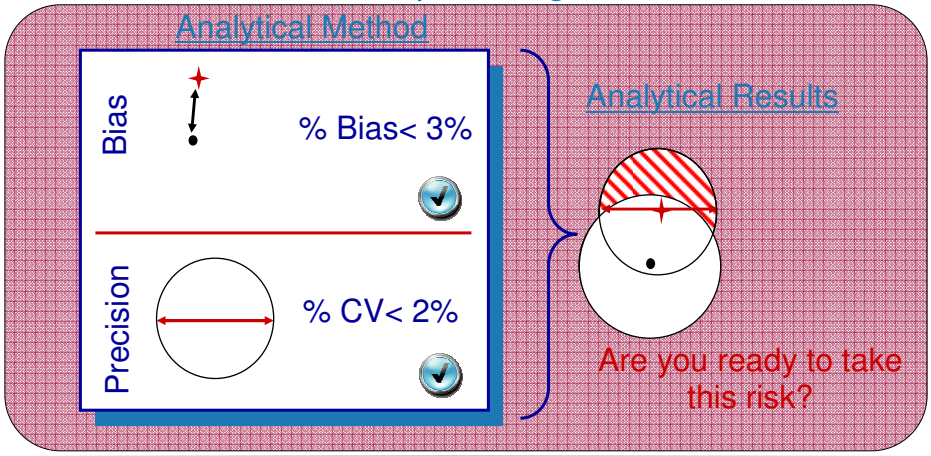
- Traditional vision:
  - The Validation Criteria Check List:
    - Selectivity
    - Trueness/Mean Accuracy
    - Precision
    - Linearity
    - Range
    - Limit of Quantification (LOQ)

Method Valid !

5

Analytical Method Validation

- Traditional vision:
  - Is a valid method providing reliable results ?



6

Analytical Method Validation

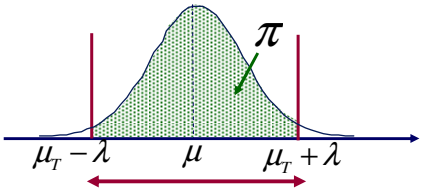
Aim of validation

Is to give to laboratories as well as to regulatory agencies the **guaranties** that each result that will be obtained in routine will be **close enough** to the unknown true value of the analyte in the sample.

$$\pi = P\left[\left|X_i - \mu_T\right| < \lambda\right] \geq \pi_{\min}$$

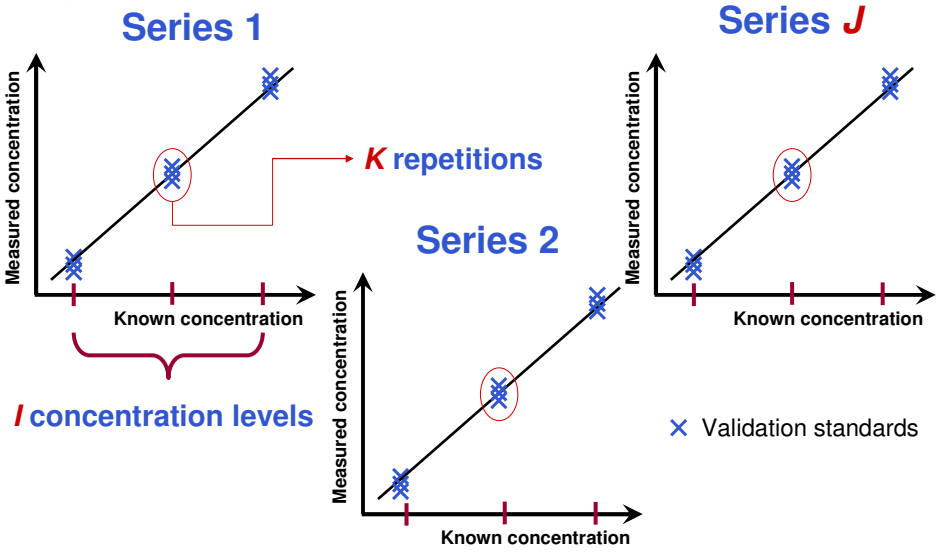
λ = predefined acceptance limits

π_{min} = minimum probability that a result will be included inside $\pm \lambda$



E. Rozet et al., J. Chromatogr.A, 1158 (2007) 126

Typical Validation Design



Typical Statistical Model

- By concentration level *i*:
  - One Way Random ANOVA model

$$X_{i,jk} = \mu_i + \alpha_{i,j} + \varepsilon_{i,jk}$$
$$\alpha_{i,j} \sim N\left(0, \sigma^2_{\alpha,i}\right)$$
$$\varepsilon_{i,jk} \sim N\left(0, \sigma^2_{\varepsilon,i}\right)$$

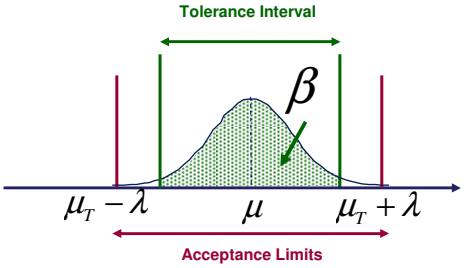
  - Intermediate Precision variance

$$\sigma^2_{I.P.,i} = \sigma^2_{\alpha,i} + \sigma^2_{\varepsilon,i}$$

Reliability Probability Estimator 1 – π^{Beti}_i

- Based on β-expectation tolerance intervals:

Allows to predict where each future result will fall (*Wald, 1942*).

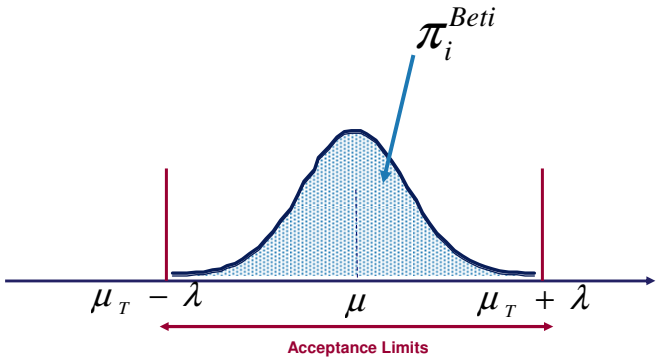


➔ If the **β-expectation tolerance interval** is included inside the acceptance limits, then the **probability that each future result will be within the acceptance limits is at least β** (ex. 80%).

B. Boulanger et al., J. Chromatogr. B, 877 (2009) 2235

Reliability Probability Estimator 1 – π^{Beti}_i

- Based on β-expectation tolerance intervals:



11

Reliability Probability Estimator 1 – π^{Beti}_i

- Based on β-expectation tolerance intervals:

$$\pi_i^{Beti} = P\left[X_i > \mu_{T,i} - \lambda\right] + P\left[X_i < \mu_{T,i} + \lambda\right]$$

$$= P\left[t(f) > \frac{\left(\mu_{T,i} - \lambda\right) - \bar{X}_i}{\hat{\sigma}_{I.P.,i}\sqrt{1 + \frac{K\hat{R}_i + 1}{N\left(\hat{R}_i + 1\right)}}}\right] + P\left[t(f) < \frac{\left(\mu_{T,i} + \lambda\right) - \bar{X}_i}{\hat{\sigma}_{I.P.,i}\sqrt{1 + \frac{K\hat{R}_i + 1}{N\left(\hat{R}_i + 1\right)}}}\right]$$

- N=JK.
- $\bar{X}_i$ is the mean results
- $t(f)$: Student distribution with f degrees of freedom using Satterthwaite approximation
- $\hat{R}_i = \frac{\hat{\sigma}_\alpha^2}{\hat{\sigma}_\varepsilon^2}$

W. Dewé et al., Chemometr. Intell. Lab. Syst. 85 (2007) 262-268.

12

Reliability Probability Estimator 2 – π^{ML}_i

- Maximum likelihood estimator

$$\pi_i^{ML} = P\left[Z > \frac{(\mu_{T,i} - \lambda) - \bar{X}_i}{\hat{\sigma}_{I.P.,i}}\right] + P\left[Z < \frac{(\mu_{T,i} + \lambda) - \bar{X}_i}{\hat{\sigma}_{I.P.,i}}\right]$$

where Z is a standard normal variable.

B. Govaerts et al., Qual. Reliab. Engng. Int. 24 (2008) 667-680.

13

Bayesian Reliability Estimator - π

- Aims: modeling the reliability probability over the whole concentration range
- Model: Linear model with random slopes and intercepts

$$X_{ijk} = \beta_0 + \beta_1 \mu_{T,i} + u_{0,j} + u_{1,j}\mu_{T,i} + \varepsilon_{ijk}$$

$\boldsymbol{\theta} = \begin{pmatrix} \beta_0 \\ \beta_1 \end{pmatrix}$ are the fixed effects $\quad \boldsymbol{\theta} \sim N\left(\begin{bmatrix} 0 \\ 1 \end{bmatrix}, \boldsymbol{\Gamma}\right) \quad \boldsymbol{\Gamma}^{-1} = \mathbf{0}$

$\mathbf{U}_j = \begin{pmatrix} u_{0,j} \\ u_{1,j} \end{pmatrix}$ are the random effects of the j^{th} runs $\quad \mathbf{U}_j \sim iN(\mathbf{0}, \sigma_u^2 \underset{2x2}{\boldsymbol{\Sigma}}) \quad \boldsymbol{\Sigma} \sim \text{Wishart}(0.0001\mathbf{I}_2, 2)$

$\varepsilon_{ijk} \sim N(0, \sigma_i^2) \quad \sigma_i = \sigma(\mu_{T,i})^{\gamma} \quad \gamma \sim N(0, 0.0001)$

$\tau = \frac{1}{\sigma} \sim Gamma\,(0.0001,\ 0.0001)$

14

Simulations

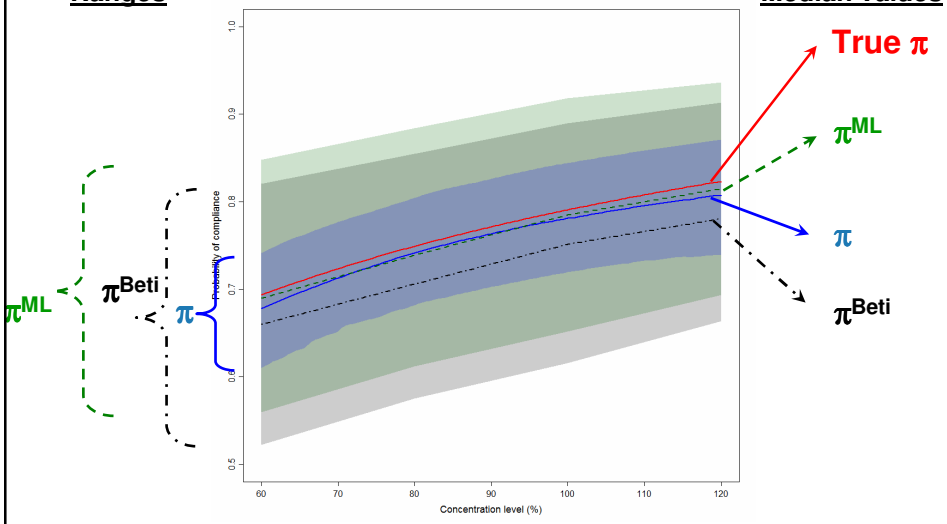
- 4 scenarios:
  - Conditions
    - Analytical Method relative bias: 0% and 10%
    - Analytical Method I.P. RSD: 6.5% and 16%
    - Known concentrations ($\mu_{T,i}$):60%, 80%, 100% and 120%
    - Acceptance limits: λ=±20%
    - Nb Series: J=4
    - Nb Repetitions: K=4
  - Criteria
    - Compare median estimated reliability probabilities to true probability
    - Compare ranges (min to max) of estimated reliability probabilites

15

Case 1: 0% bias – 16.0% RSD

Ranges

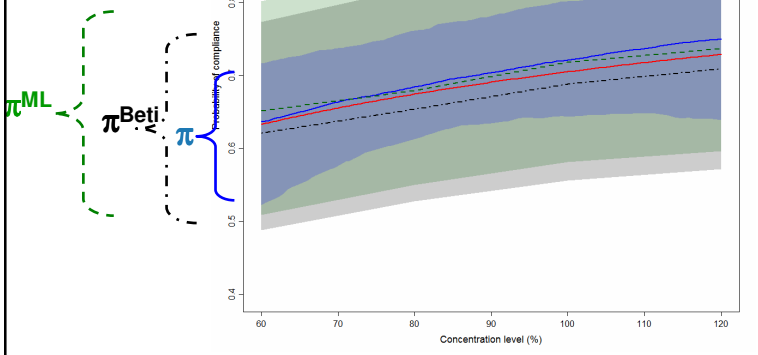
Median values



16

Case 2: 10% bias – 16.0% RSD

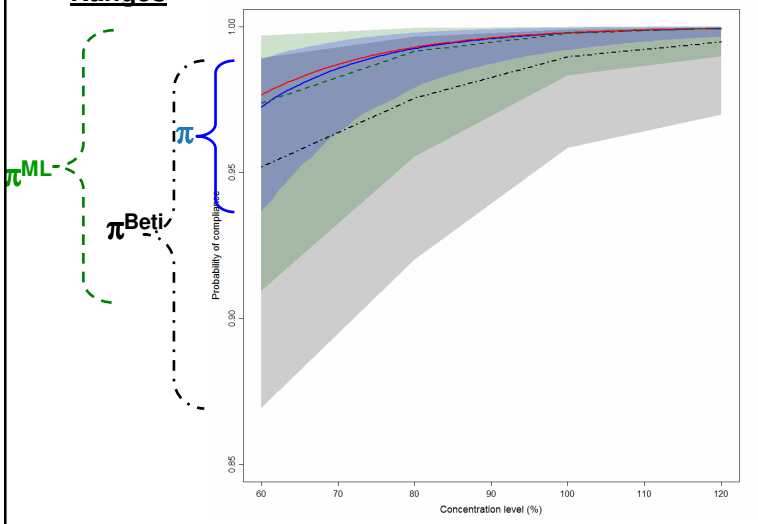
Ranges



17

Case 3: 0% bias – 6.5% RSD

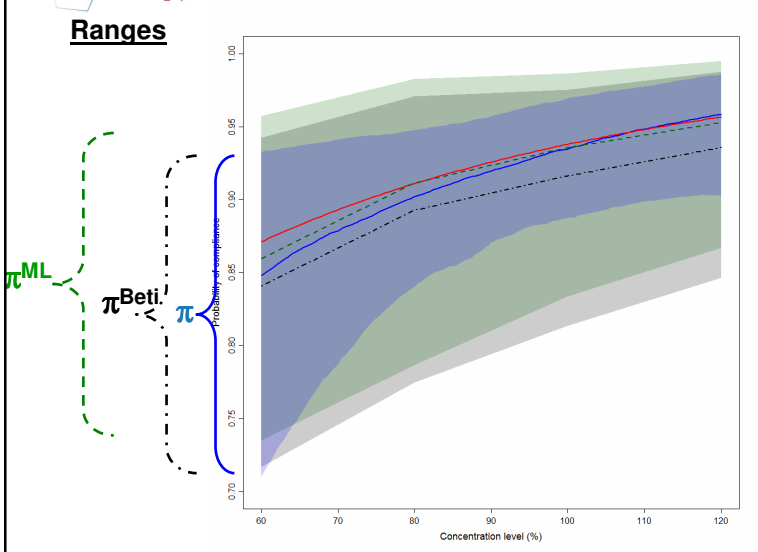
Ranges



18

Case 4: 10% bias – 16.0% RSD

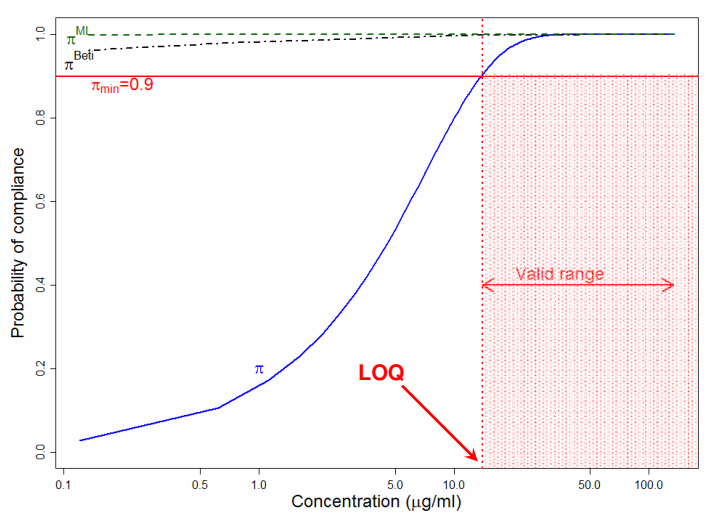
Ranges



Example of application

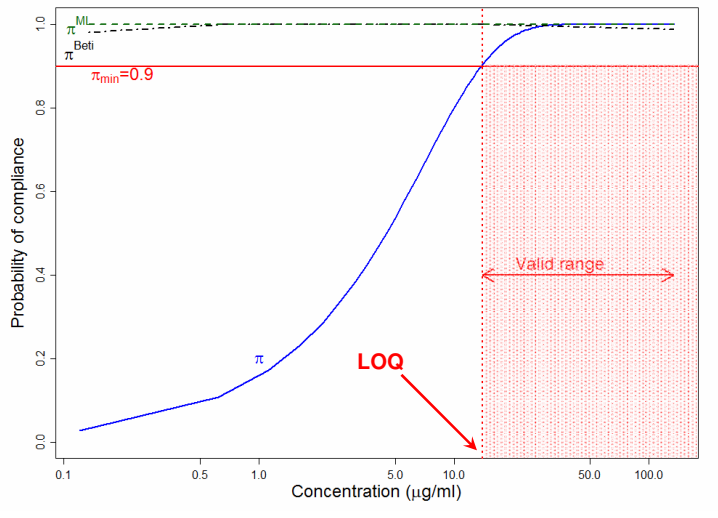
- Validation of a bioanalytical method:
  - SPE-HPLC-UV method for the quantification of ketoglutaric acid (KG) and hydroxymethylfurfural (HMF) in human plasma
    - Known concentrations ($\mu_{T,i}$): 0.13, 0.67, 3.33, 66.67 and 133.33 µg/ml
    - Nb Series: J=3
    - Nb Repetitions: K=4
    - Acceptance limits: λ=±20%
    - Minimum reliability probability: π_{min}=0.90

Ketoglutaric acid



21

Hydroxymethylfurfural



22

Conclusions

- **Switch** from the traditional check list validation to a rewarding, useful and predictive method validation
- The **quality of future results** (π) must be the objective of method validation and not the past performances of the method.
- The Bayesian reliability probability estimator is **less biased** and **more precise**.
- In such a way, the **risks** are known at the end of the validation.
- This decision methodology is **fully compliant** with actual regulatory requirements

Thanks for your attention

- Check our publications at:

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