

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

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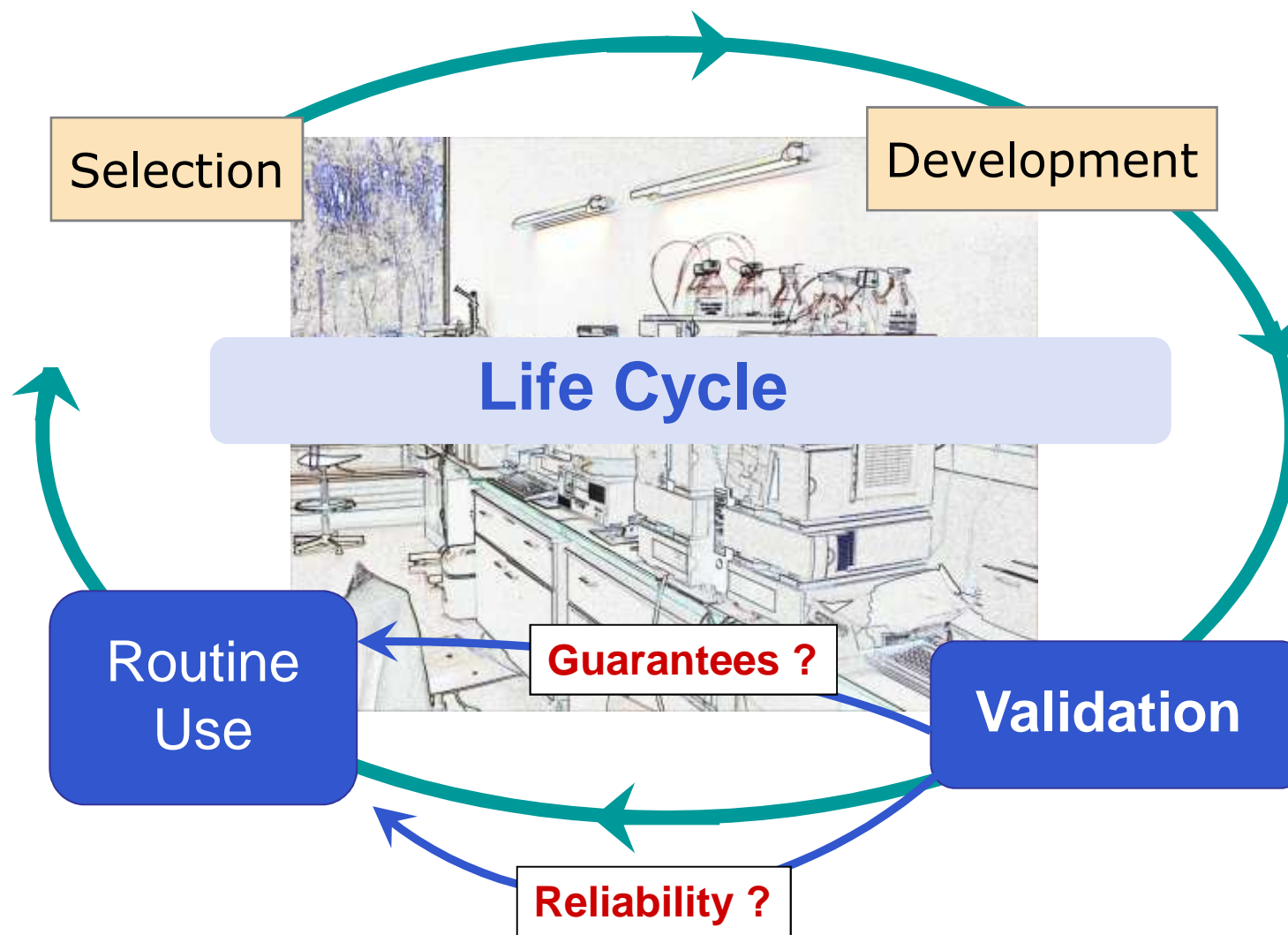
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Analytical Method Life Cycle

- 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



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 ?

Analytical Method Validation

- Traditional vision:

- The Validation Criteria Check List:

- Selectivity
- Trueness/Mean Accuracy
- Precision
- Linearity
- Range
- Limit of Quantification (LOQ)



Method Valid !

Analytical Method Validation

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

Analytical Method

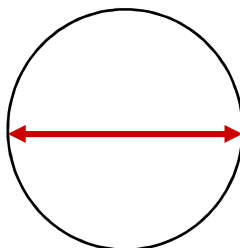
Bias



% Bias < 3%



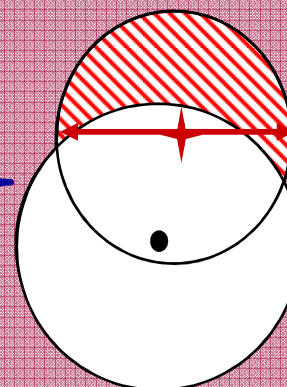
Precision



% CV < 2%



Analytical Results



Are you ready to take
this risk?

Analytical Method Validation

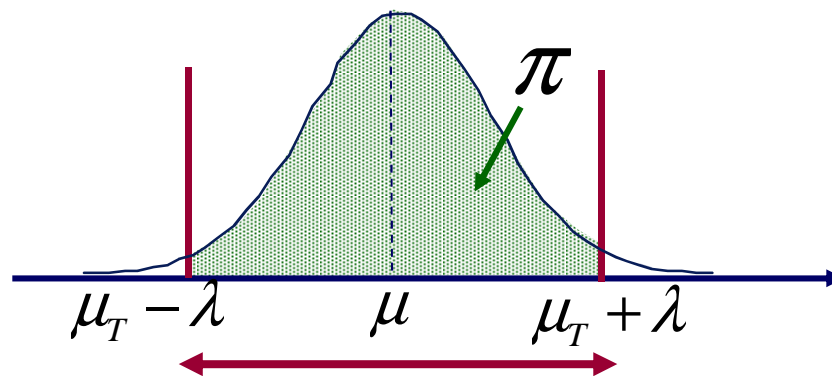
Aim of validation

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.

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

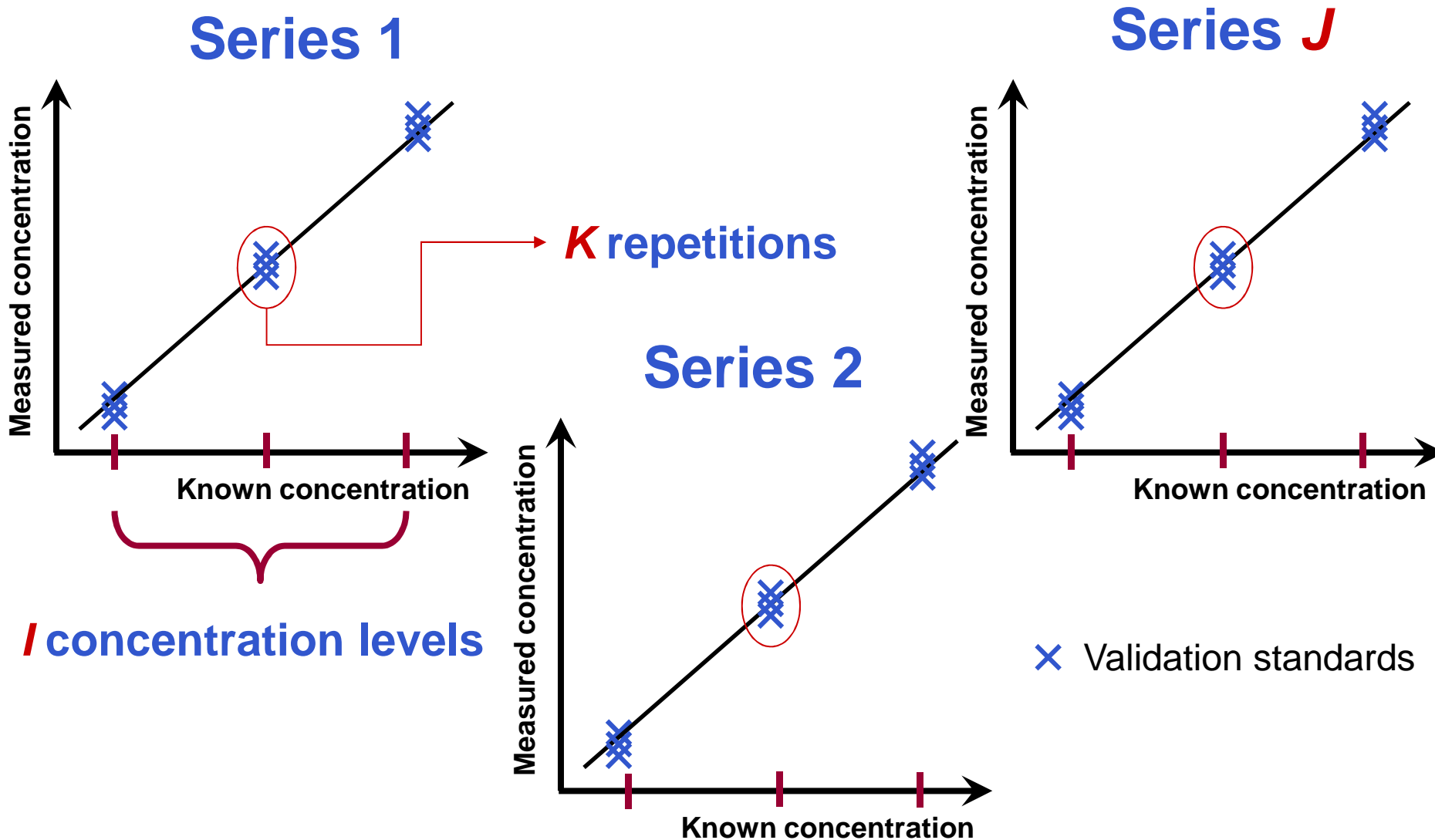
λ = predefined acceptance limits

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

$$X_{i,jk} = \mu_i + \alpha_{i,j} + \varepsilon_{i,jk}$$

$$\alpha_{i,j} \sim N(0, \sigma_{\alpha,i}^2)$$

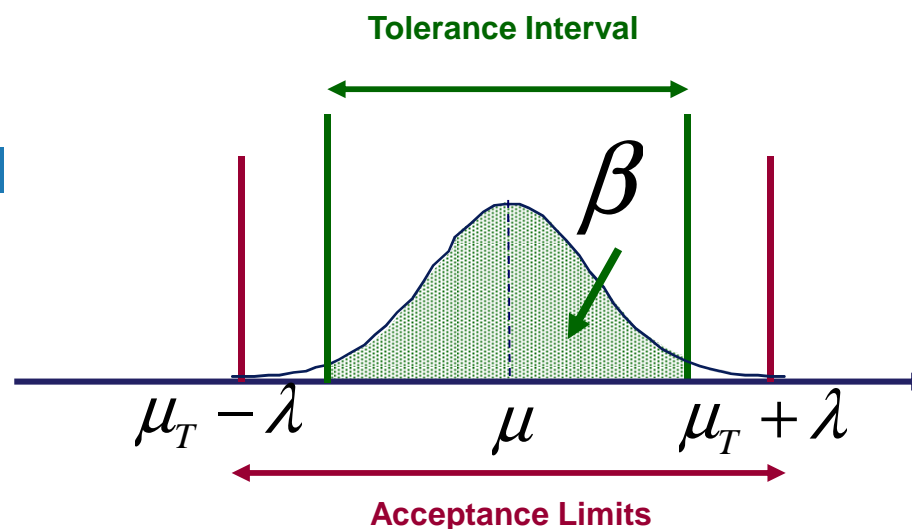
$$\varepsilon_{i,jk} \sim N(0, \sigma_{\varepsilon,i}^2)$$

- Intermediate Precision variance

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

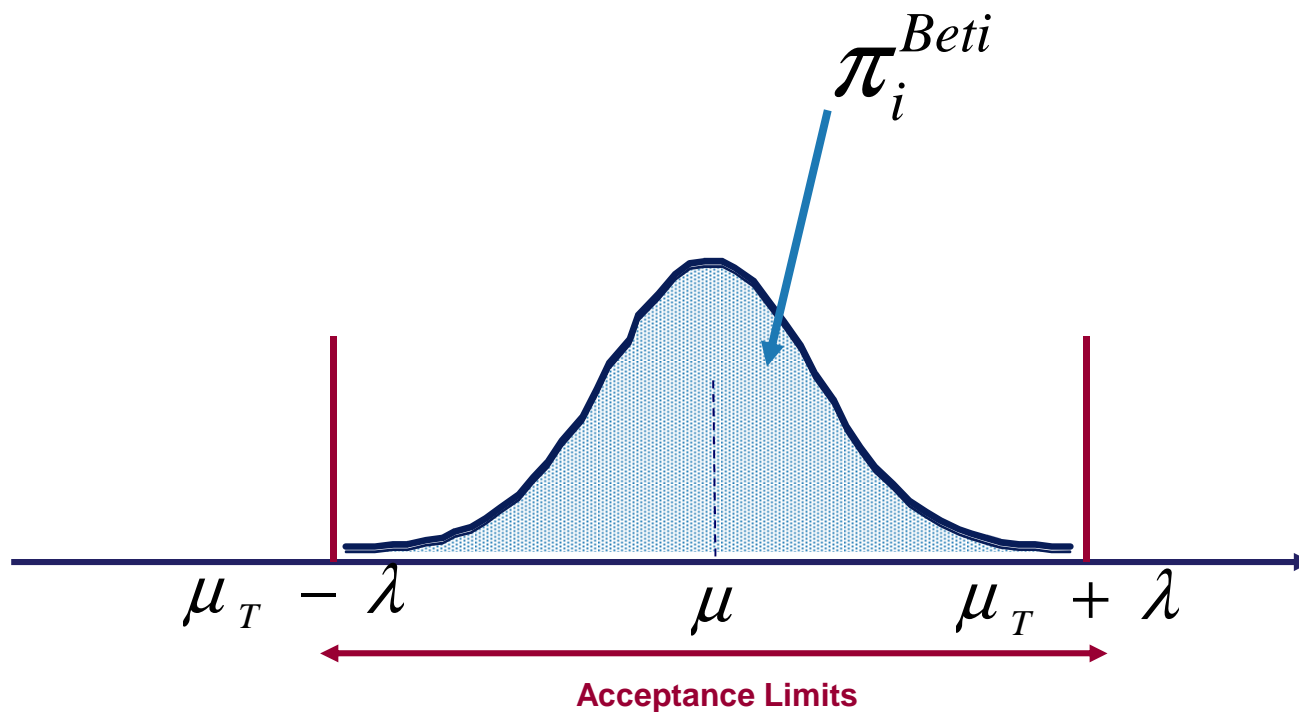
- Based on β -expectation tolerance intervals:

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%).

- Based on β -expectation tolerance intervals:



- Based on β -expectation tolerance intervals:

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

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

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

- 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.

Bayesian Reliability Estimator - π

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

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

$\theta = \begin{pmatrix} \beta_0 \\ \beta_1 \end{pmatrix}$ are the fixed effects

$$\theta \sim N\left(\begin{bmatrix} 0 \\ 1 \end{bmatrix}, \Gamma\right)$$

$$\Gamma^{-1} = \mathbf{0}$$

$\mathbf{U}_j = \begin{pmatrix} u_{0,j} \\ u_{1,j} \end{pmatrix}$ are the random effects of the j^{th} runs

$$\mathbf{U}_j \sim iN(\mathbf{0}, \sigma_u^2 \Sigma_{2 \times 2})$$

$$\Sigma \sim \text{Wishart}(0.0001 \mathbf{I}_2, 2)$$

$$\varepsilon_{ijk} \sim N(0, \sigma_i^2)$$

$$\sigma_i = \sigma(\mu_{T,i})^\gamma$$

$$\gamma \sim N(0, 0.0001)$$

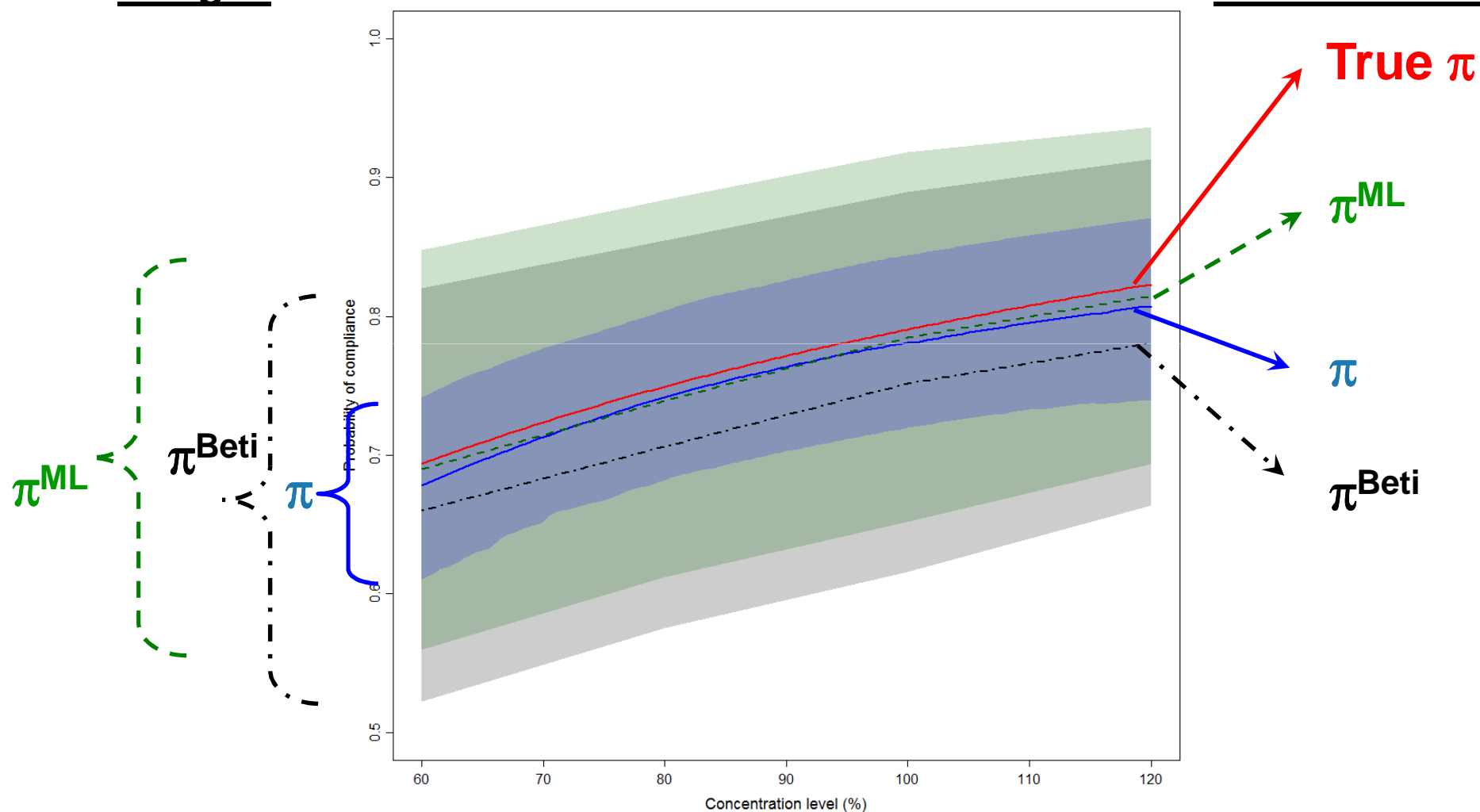
$$\tau = \frac{1}{\sigma} \sim \text{Gamma}(0.0001, 0.0001)$$

- 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: $\lambda = \pm 20\%$
 - 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 probabilities

Case 1: 0% bias – 16.0% RSD

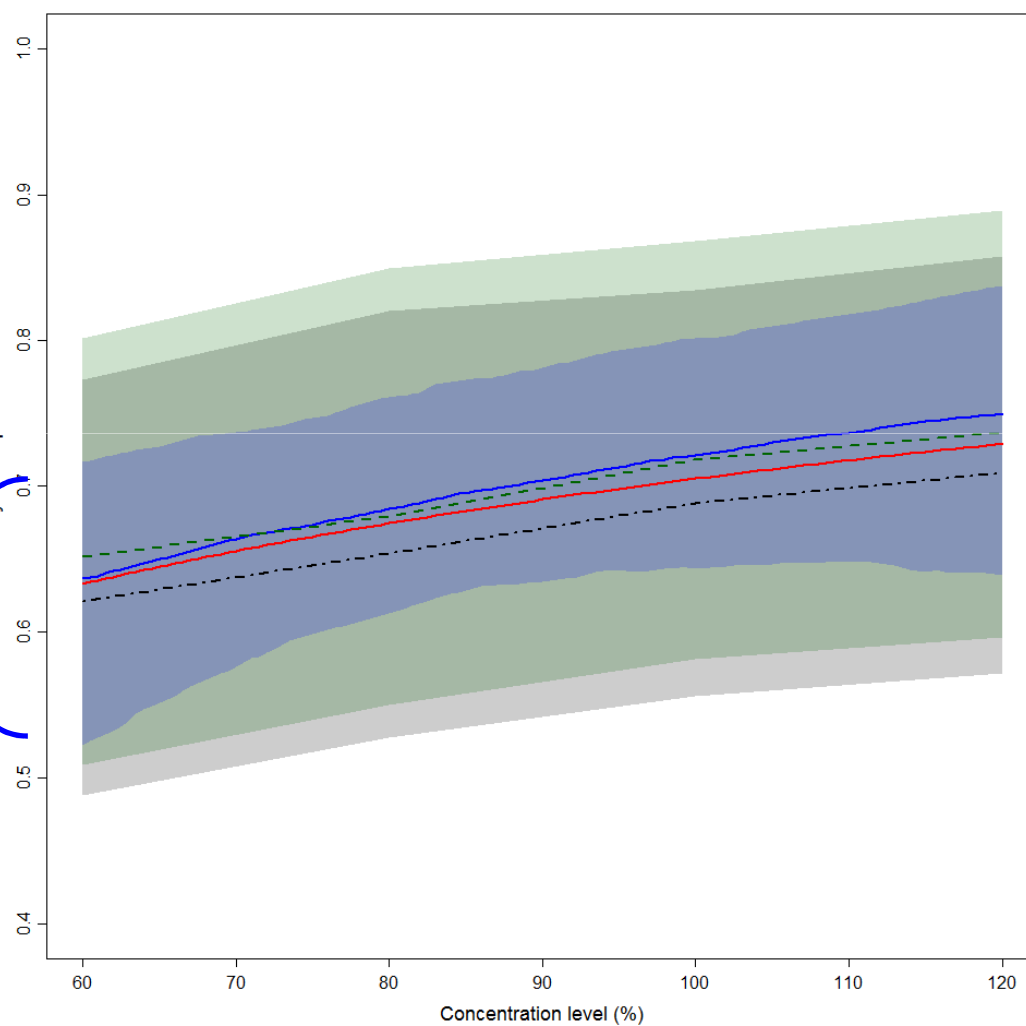
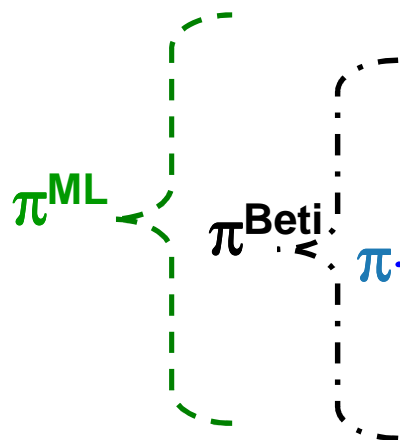
Ranges

Median values



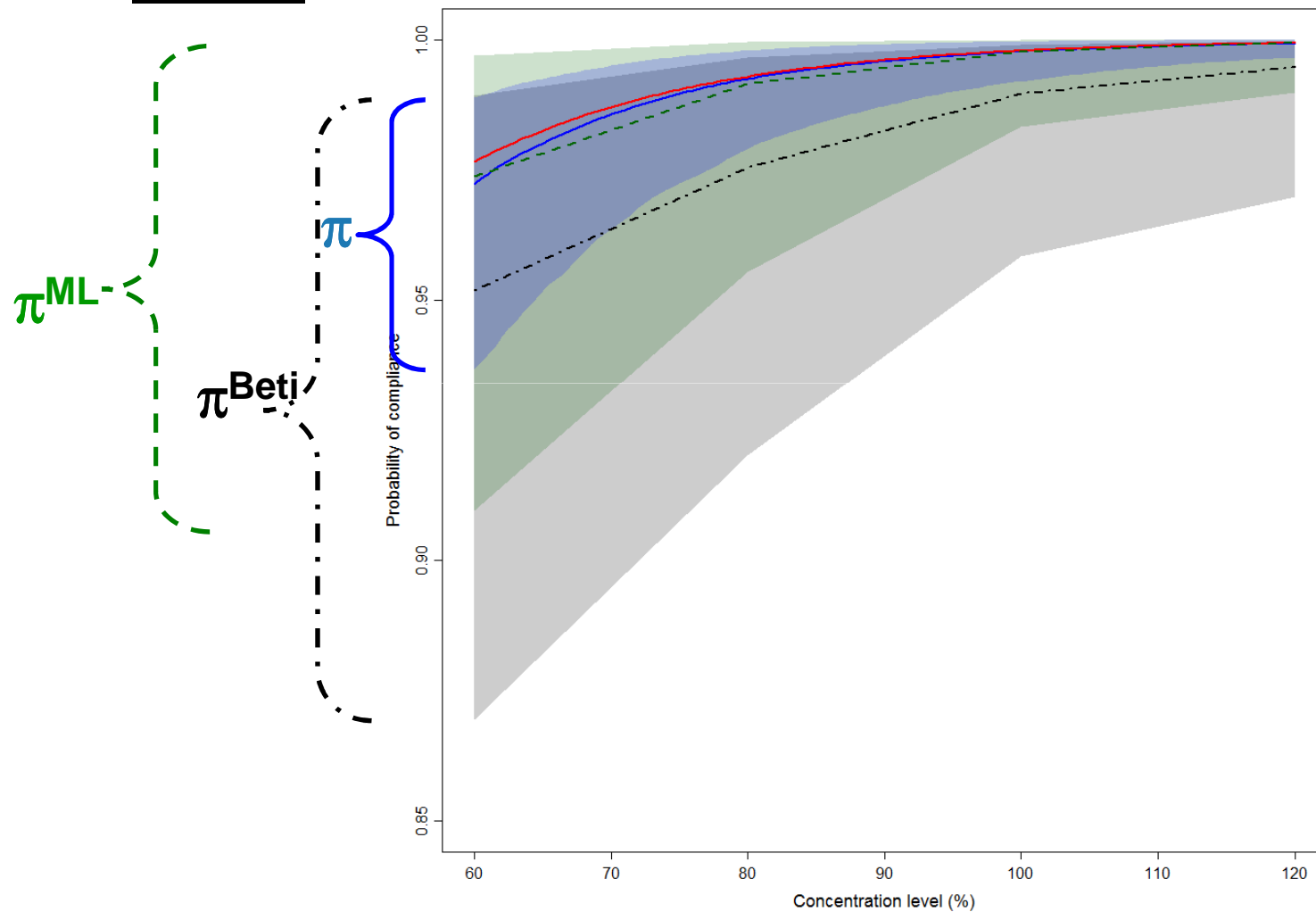
Case 2: 10% bias – 16.0% RSD

Ranges



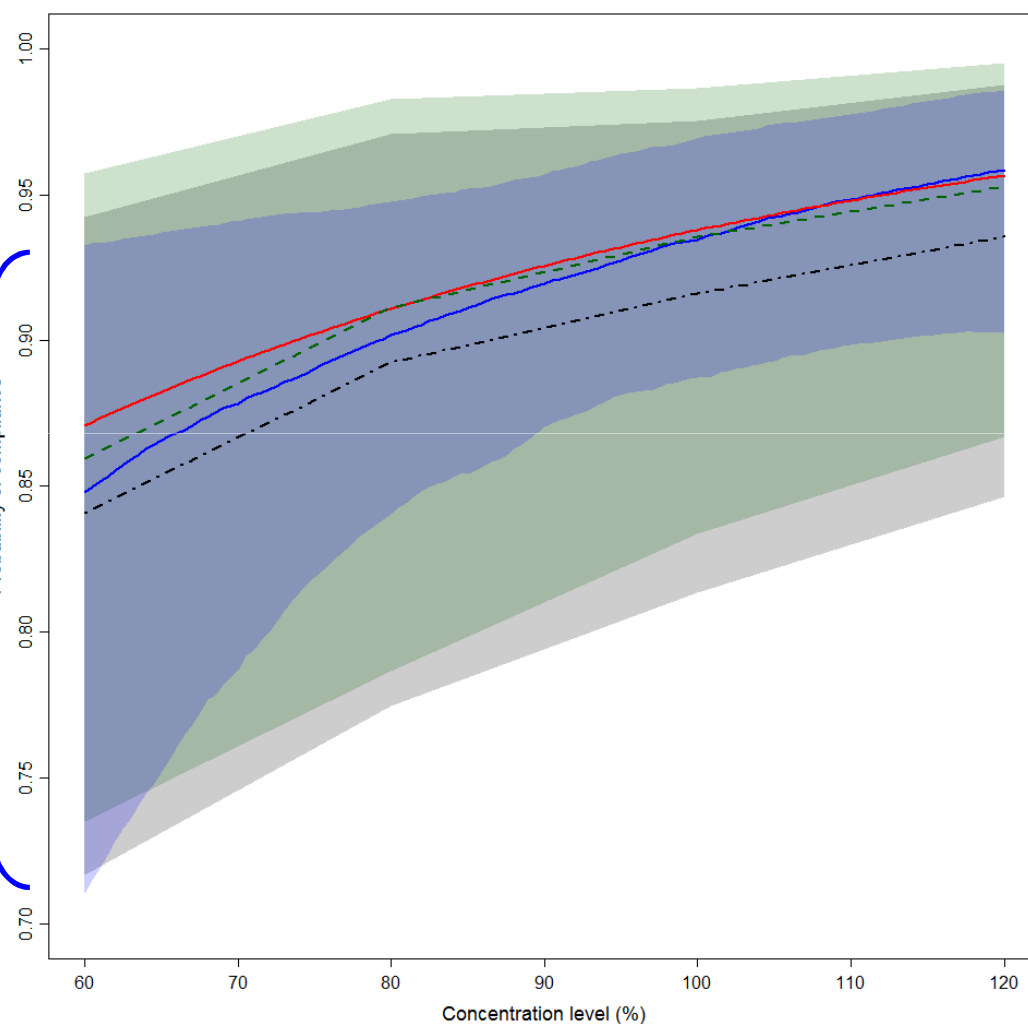
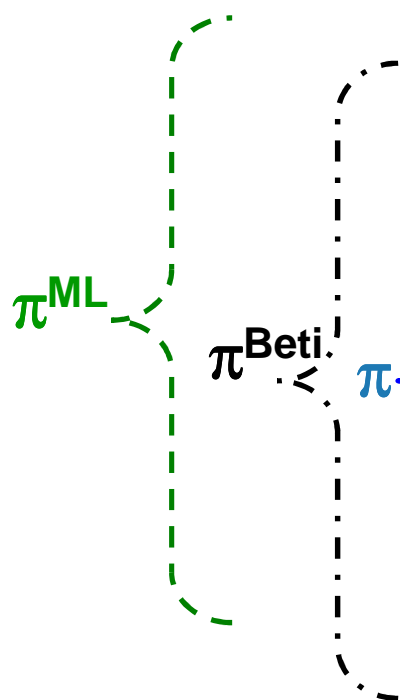
Case 3: 0% bias – 6.5% RSD

Ranges



Case 4: 10% bias – 16.0% RSD

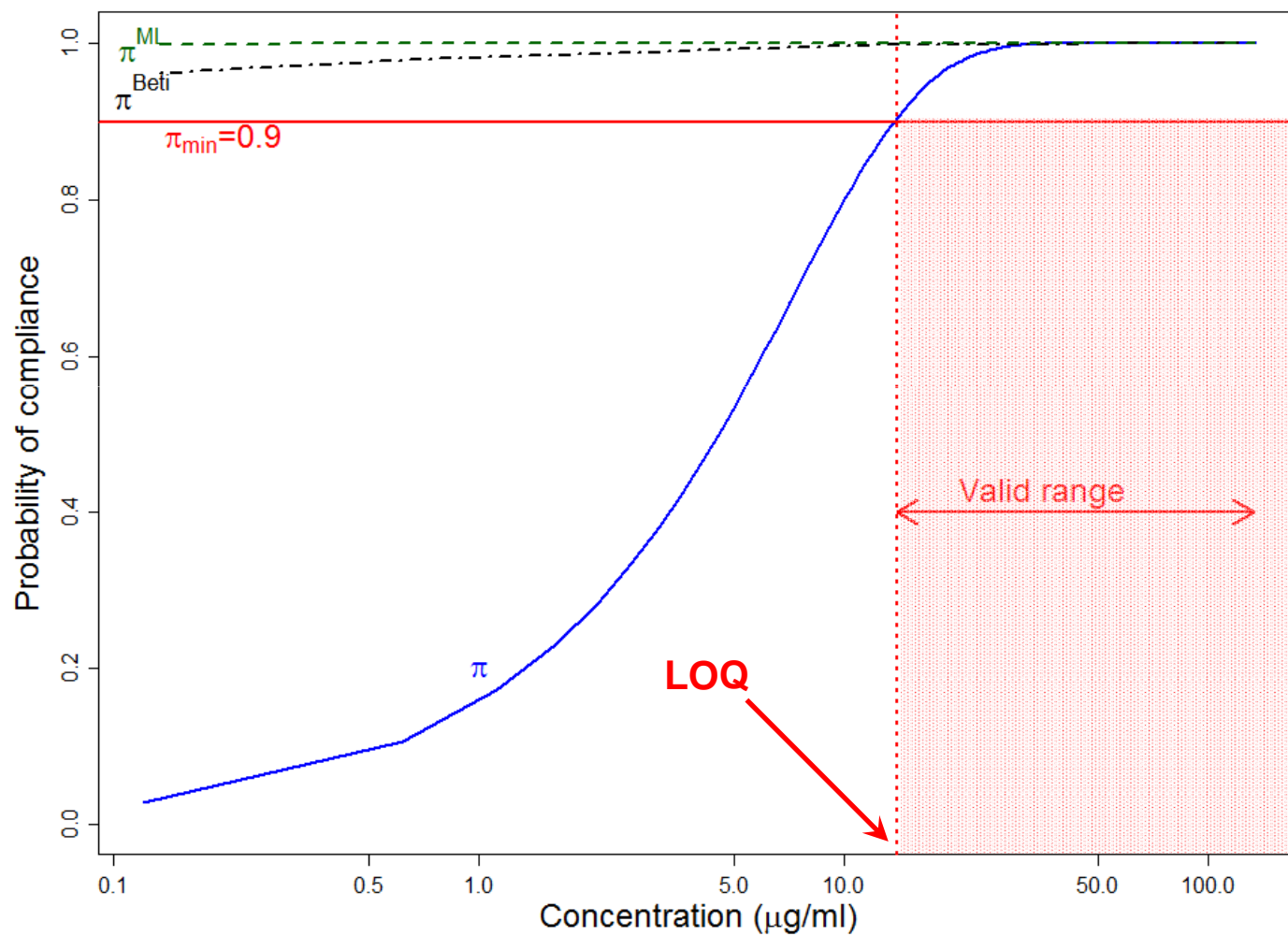
Ranges



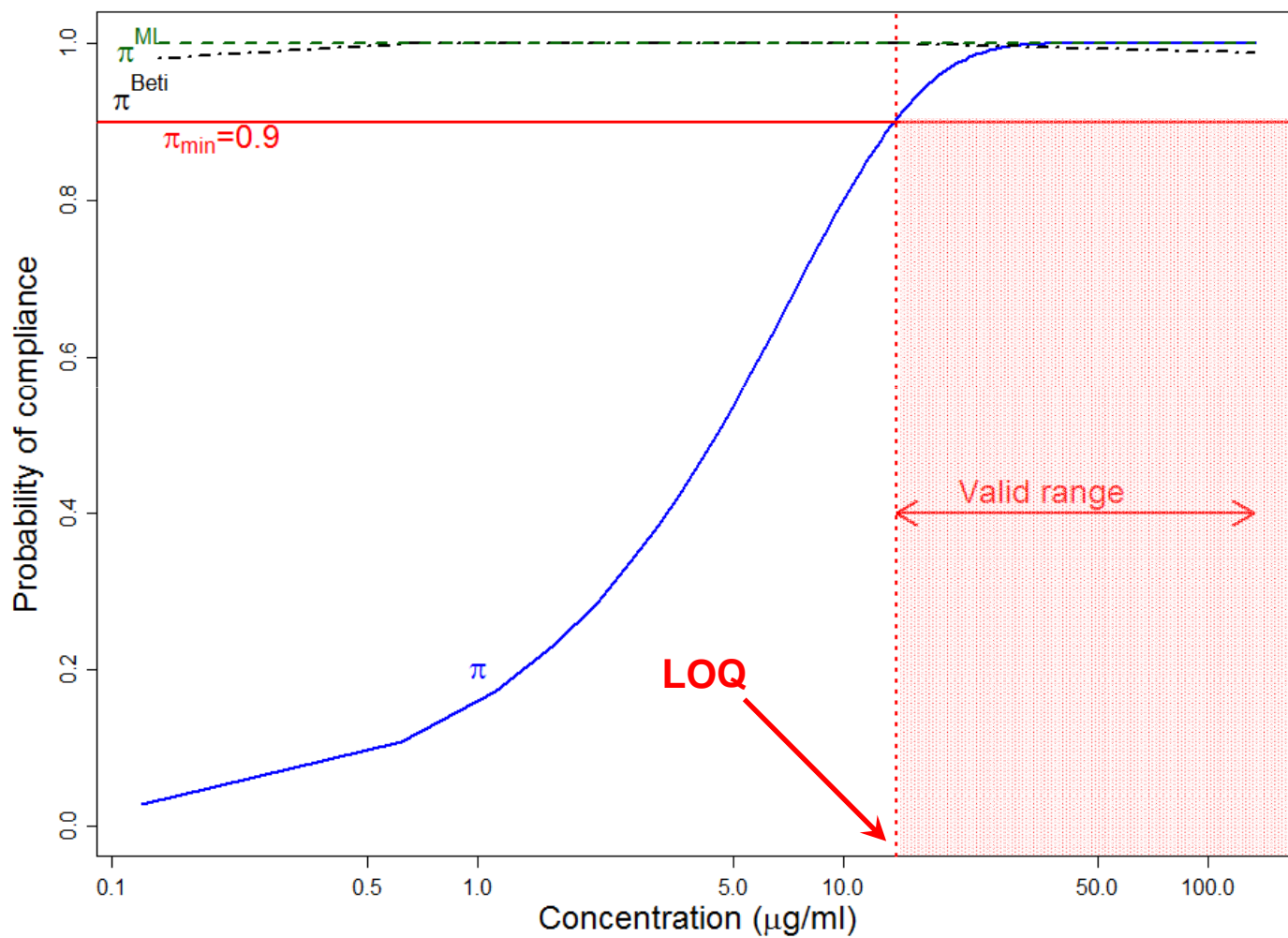
Example of application

- 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 $\mu\text{g/ml}$
 - Nb Series: $J=3$
 - Nb Repetitions: $K=4$
 - Acceptance limits: $\lambda=\pm 20\%$
 - Minimum reliability probability: $\pi_{\min}=0.90$

Ketoglutaric acid



Hydroxymethylfurfural



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:

<http://orbi.ulg.ac.be/>



- Contact:

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