UNIVERSITE de LIEGE

New Trends in Validation of Analytical Methods

E. Rozet, Ph. Hubert

Tbilisi **August 28-31, 2011**

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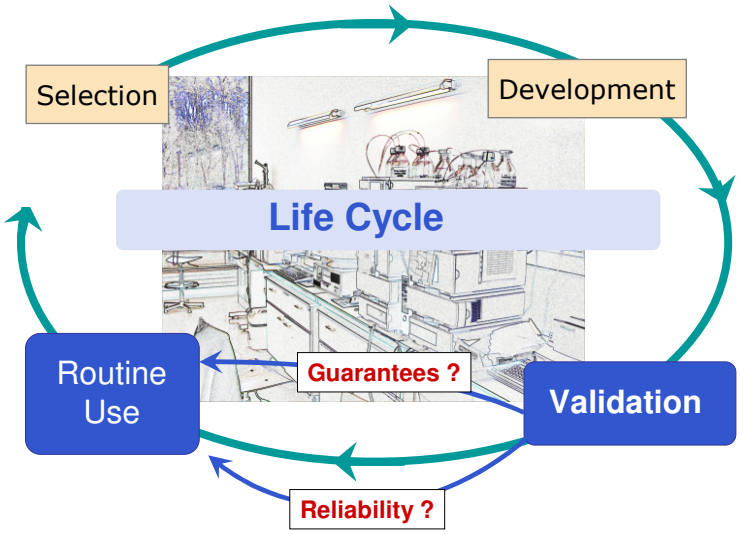
1. Aim of Analytical Method Validation
2. Traditional Analytical Method Validation
3. Rewarding Analytical Method Validation
4. Analytical Method Validation Design
5. Is my Method Valid ?
6. Applicability ?
7. Is this enough ?
8. Conclusions

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



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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 ?

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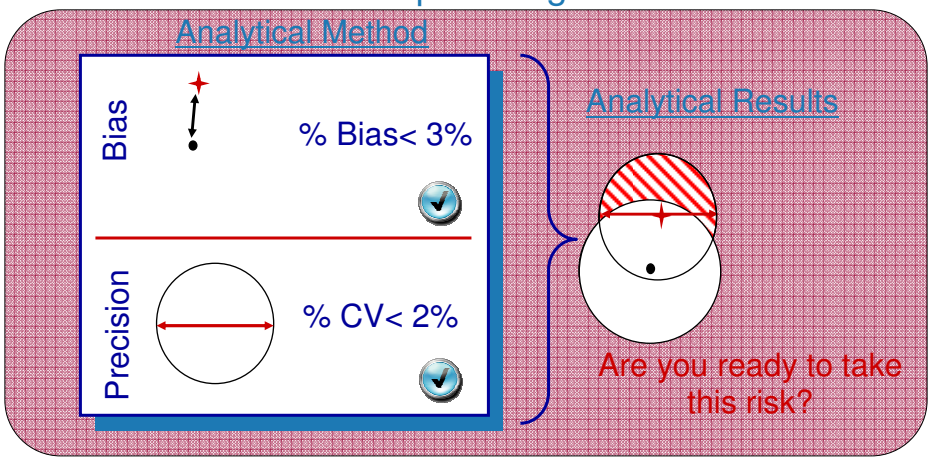
Analytical Method Validation

- 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 ?



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Analytical Method Validation

- Traditional vision:
  - Preliminary Conclusion:

"**Good**" Methods do **NOT** necessarily provide "**good**" Results !

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Analytical Method Validation

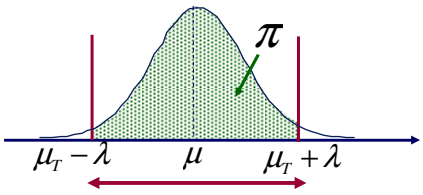
Aim of validation

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

Aim of Analytical Method Validation

The aim of **validation** is evaluting whether the probability that each future result will be included within predefined acceptance limits is acceptable.

➔ Based on the estimations of method's bias and precision.

$$E_{\hat{\delta},\hat{\sigma}}\left\{P\left[\left|X_i-\mu_T\right|<\lambda\right]\middle|\hat{\delta},\hat{\sigma}\right\}\geq\pi_{\min}$$

Aim of Analytical Method Validation

The aim of **validation** is evaluating whether the **probability that each future result** will be included within the acceptance limits.

➔ Based on the estimations of bias and precision.

$$E_{\hat{\delta},\hat{\sigma}}\left\{P\left[\left|X_i-\mu_T\right|<\lambda\right]\middle|\hat{\delta},\hat{\sigma}\right\}\geq\pi_{\min}$$

Accuracy (total error) **required** of each future result

Aim of Analytical Method Validation

The aim of **validation** is evaluating whether the **probability that each future result** will be included within the acceptance limits.

➔ Based on the **estimation of bias and precision.**

$$E_{\hat{\delta},\hat{\sigma}}\left\{P\left[\left|X_i-\mu_T\right|<\lambda\right]\middle|\hat{\delta},\hat{\sigma}\right\}\geq\pi_{\min}$$

Accuracy (total error) **required** of each future result

?

Estimators of the **method performances** obtained during the **validation** phase

Missing Link

Summary of the aims

Aims

➔ Each single future result / not the past results.

➔ Futur results / not the method performances.

➔ The past performances of the method are useless to take a decision even if they provide information about the method.

➔ **Important to clarify the way the decision will be taken based on the results available.**

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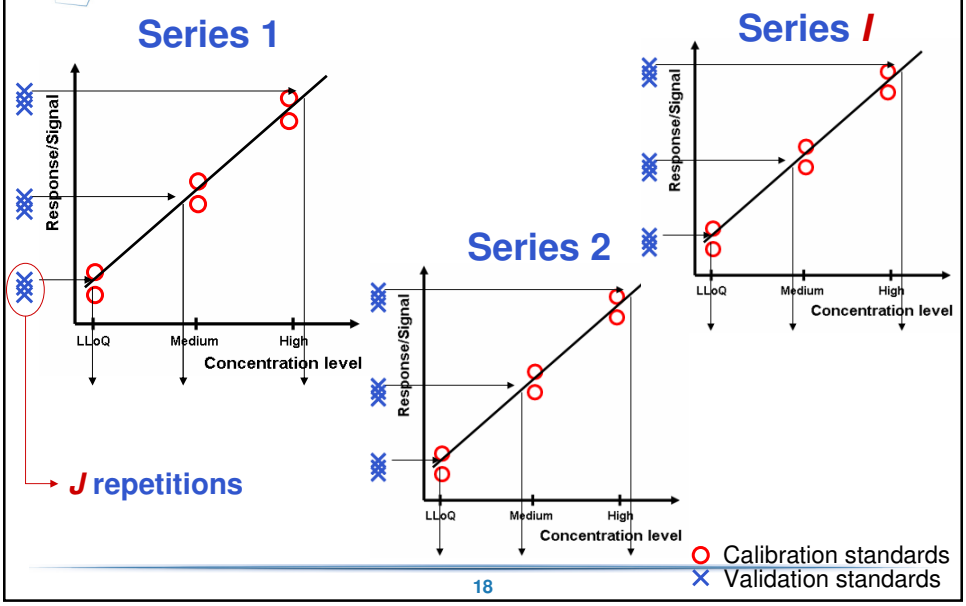
All in one Validation Design



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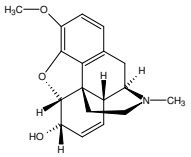
Decision Methodology

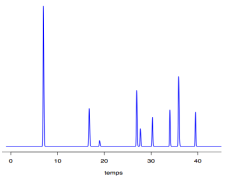
- How to decide about methods' validity ?
- Do we need statistics ?
- If yes, what statistical methodology ?

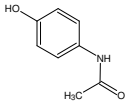
➔ Let's illustrate this through an example:

Example

- Validation of HPLC-UV method for the quantification of codeine and paracetamol in a drug product
- Design:
  - 3 series,
  - 3 repetitions per series for the validation standards
  - 3 concentration levels for the validation standards







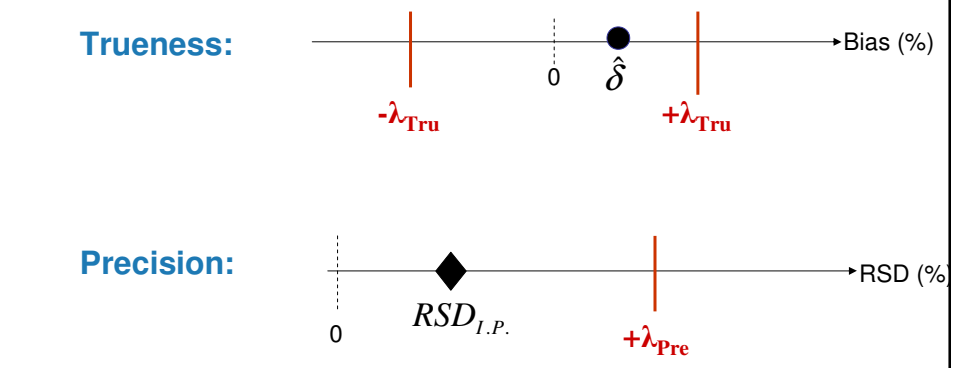
How to decide ?

Traditional Approaches:

Separate evaluation of methods **Trueness** and **Precision** and comparison to predefined acceptance limits (λ).

- Descriptive:
  - **trueness**: only based on estimation of method *bias*;
  - **precision**: only based on estimation of method $RSD_{I.P.}$.
- Difference:
  - **trueness**: based on bilateral Student t-test for *bias* significance.
- Equivalence:
  - **trueness**: based on confidence interval of the *bias (=TOST)*;
  - **precision**: based on confidence interval of the intermediate precision variance.

Descriptive Approach



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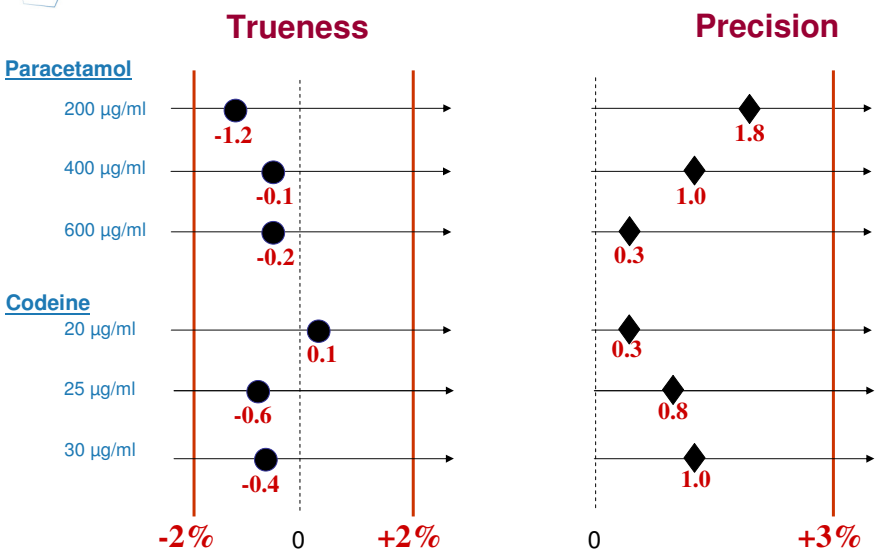
Example



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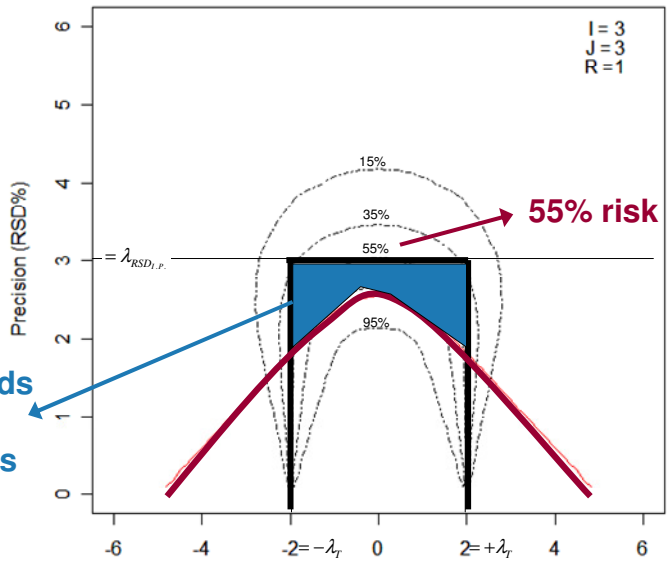
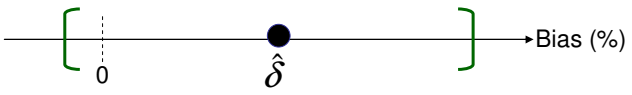
Descriptive: performance



Bouabidi *et al.*, *J. Chromatogr. A*, 1217, (2010), 3180-3192

Difference Approach

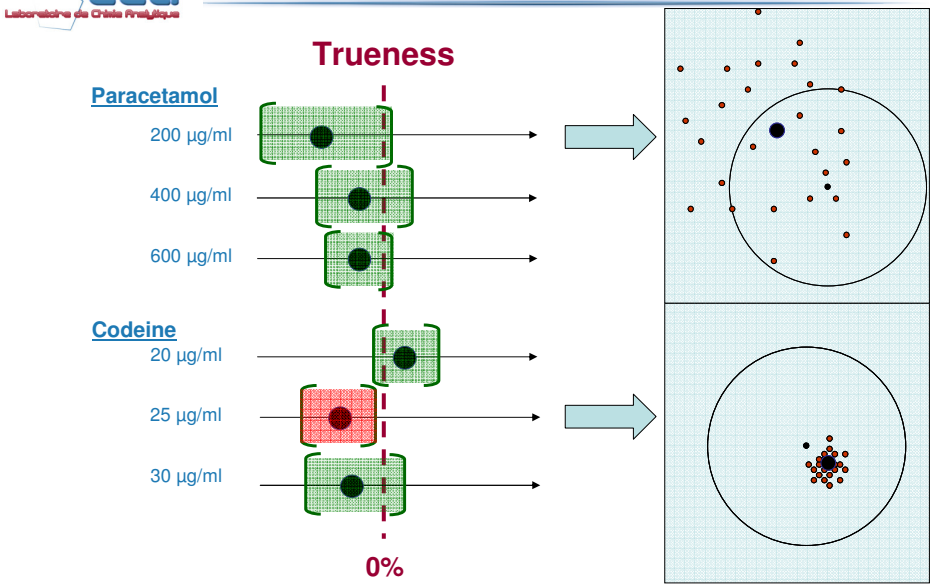
$$H_0 : \delta = 0$$
$$H_1 : \delta \neq 0$$



No rejection of H_0 ➔ Method **valid !?**

Rejection of H_0 ➔ Method **not valid !?**

Example



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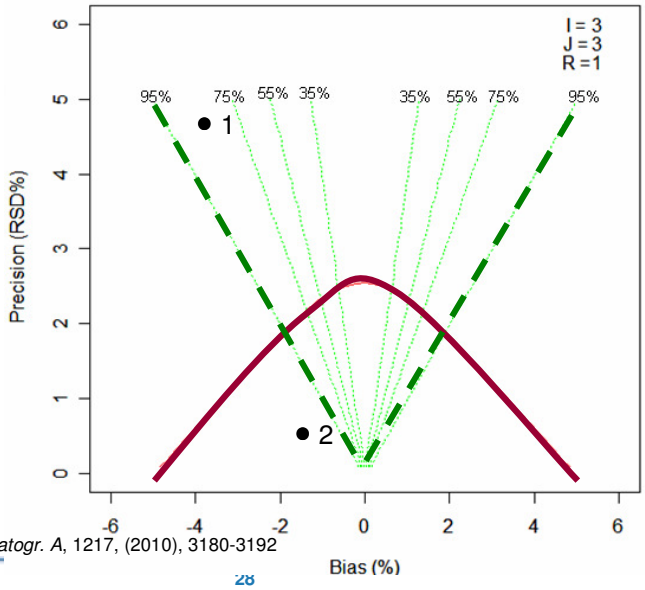
Difference: performance



Bouabidi *et al.*, *J. Chromatogr. A*, 1217, (2010), 3180-3192

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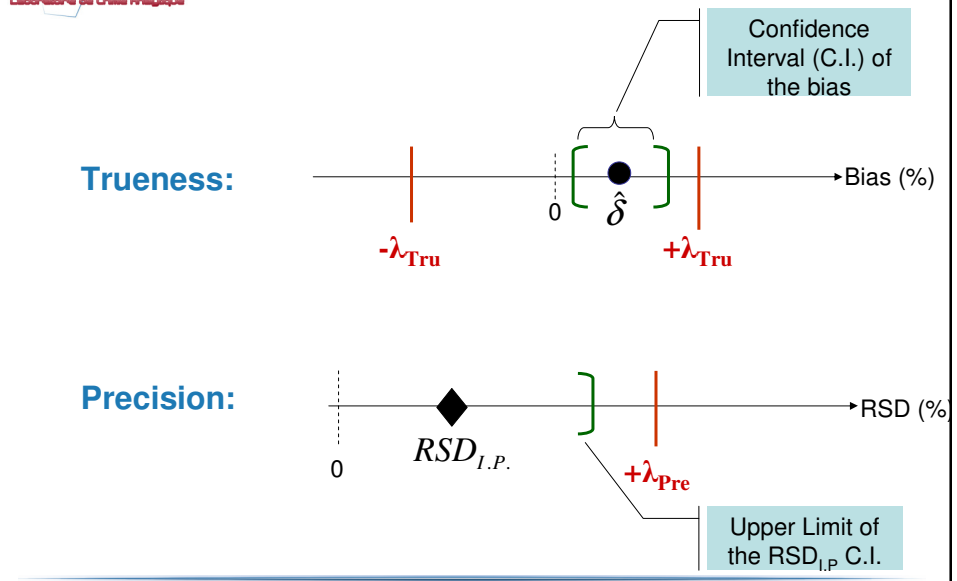
Equivalence Approach



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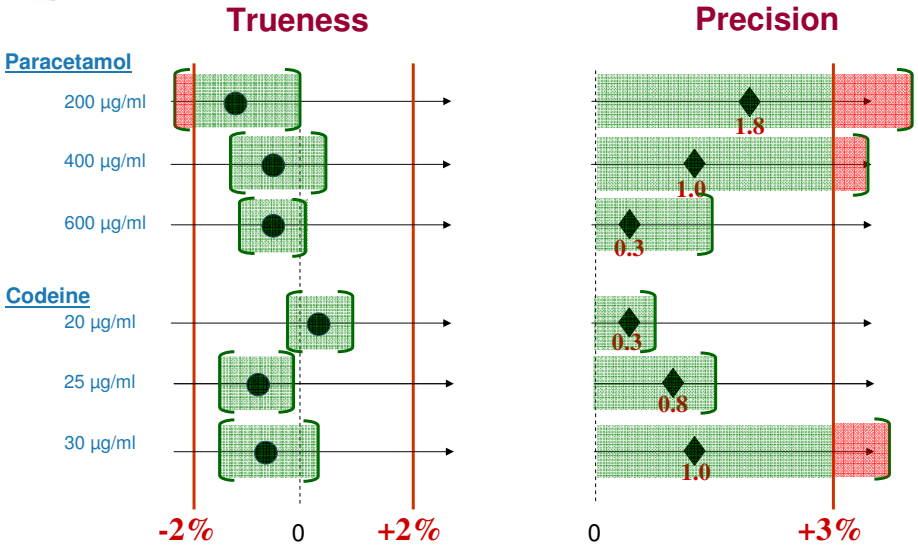
Example



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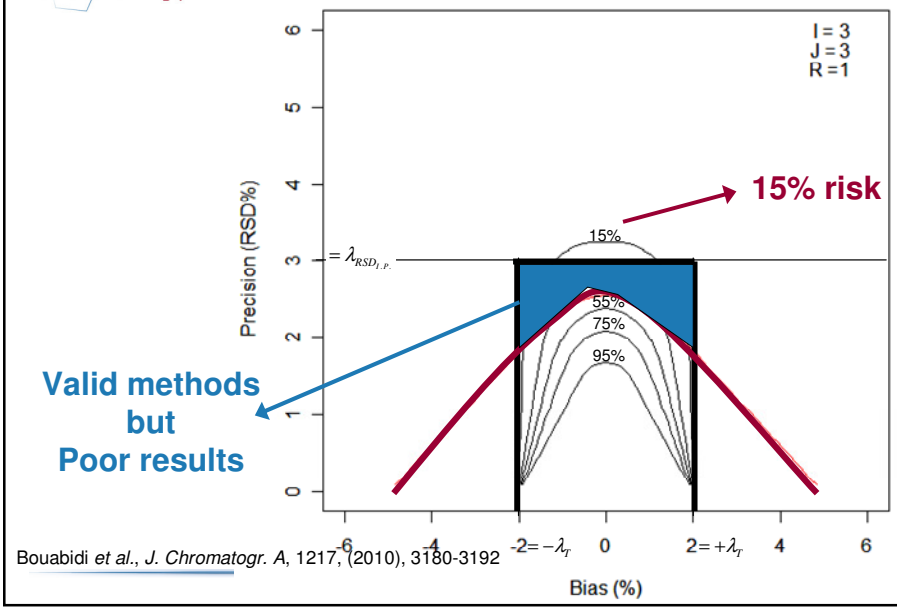
Equivalence: performance



Bouabidi *et al.*, *J. Chromatogr. A*, 1217, (2010), 3180-3192

Summary

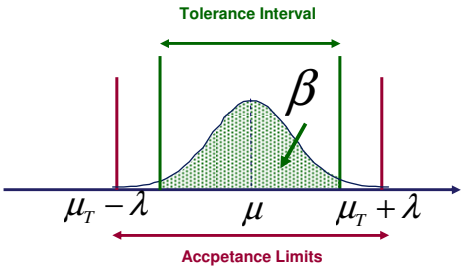
- Descriptive approach:
  - no risk management
  - Up to 50% risk to take wrong decision
- Difference approach:
  - Useless for Method Validation purpose: Avoid it !
- Equivalence approach
  - Patient risk controlled
  - Nonetheless do not fully answer method validation aim: the method is “good” but not necessarily the results !

- Is there any better decision methodology ?

Tolerance Intervals

β-Expectation Tolerance Interval (**βTI**)

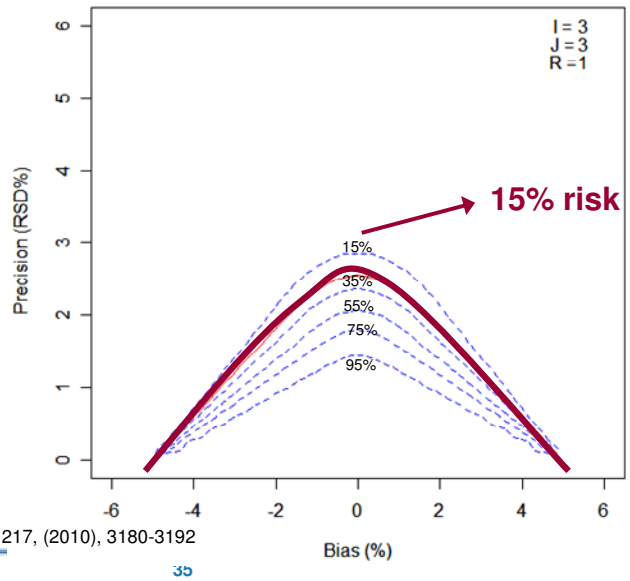
Allows to predict where each future result will fall (*Wald, 1942*).



➔ 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

βTI : performance



Bouabidi *et al.*, *J. Chromatogr. A*, 1217, (2010), 3180-3192

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Accuracy Profile

Validation experiments

Bias

$biais_j = \hat{\mu}_j - \bar{x}_{.j.}$

Intermediate Precision

$\hat{\sigma}^2_{W,j} + \hat{\sigma}^2_{B,j}$

Total error

Error

±

$\mathrm{E}_{\mu,\sigma}\{\Pr[|x_i - \mu| < \lambda] / \hat{\mu}, \hat{\sigma}\} \geq \beta$

% 0

Predictive Interval with a known risk

-λ

$TL_B = \frac{(\bar{x}_c - \bar{x}_i) \pm k\, s_{IP}}{\bar{x}_i} \cdot 100$

Conc.

λ = acceptance limits of e.g. 5%

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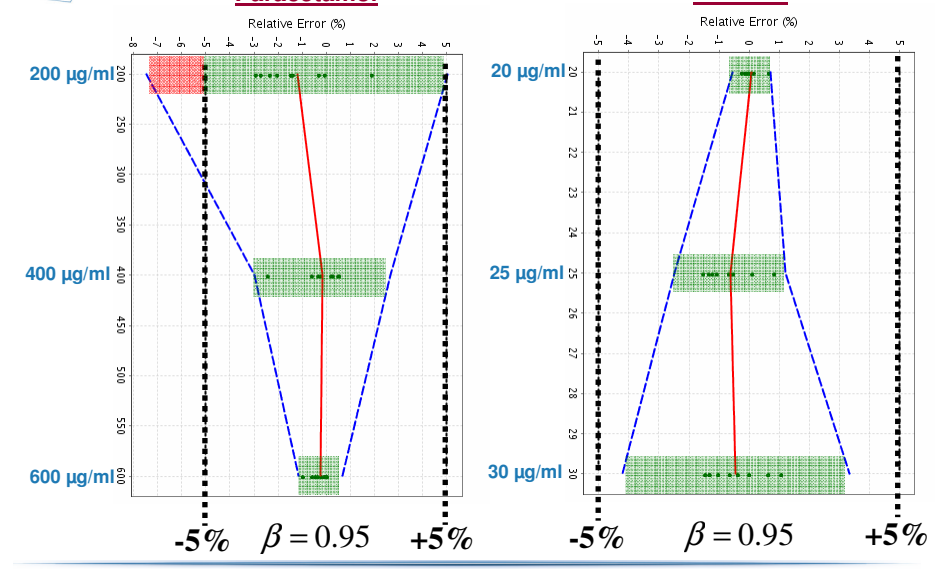
Example



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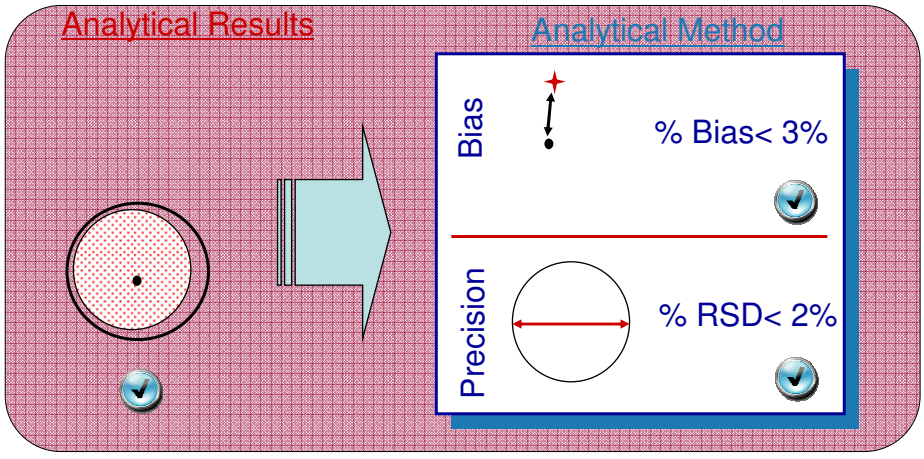
Analytical Method Validation



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Analytical Method Validation

- Accuracy Profile Approach:
  - Preliminary Conclusion:

    "**Good**" Results can only be obtained by "**good**" Methods !

  - Make a decision on the results, the very reason of an analytical quantitative method.
  - This way, it will guarantee your method is valid

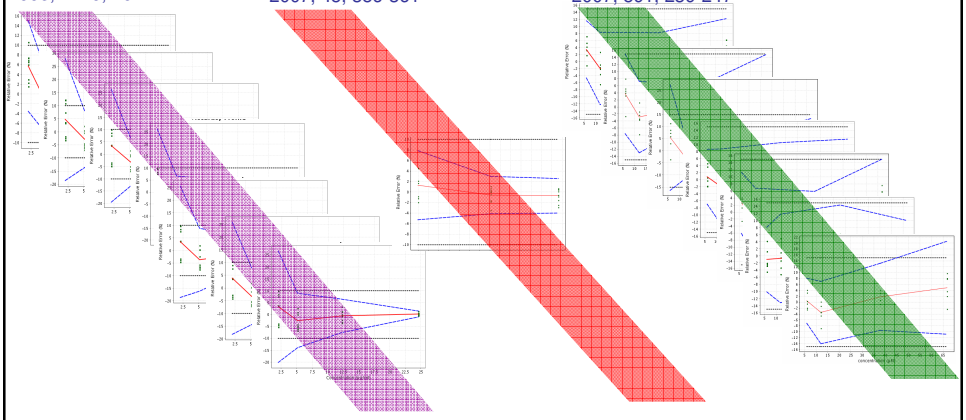
Content

Other examples

Marini et al.,
J. Chromatogr. A,
2006, 1120, 102-111

Bodson *et al.*,
J. Pharm. Biomed. Anal.,
2007, 45, 356-361

Rozet *et al.*,
Anal. Chim. Acta,
2007, 591, 239-247



Capillary Electrophoresis **NIR** **Colorimetric**

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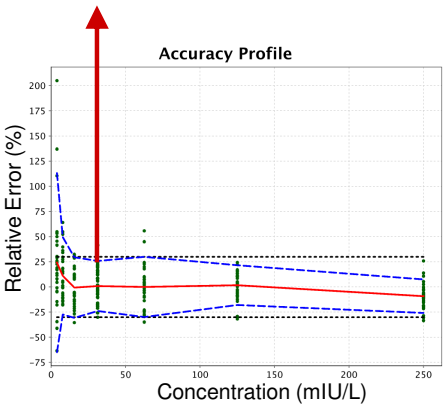
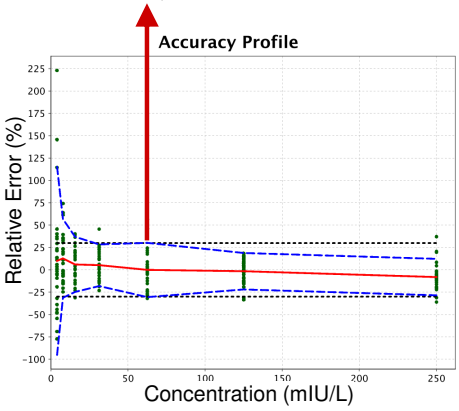
ELISA: Validation

LOQ=31.3 mIU/L



Weighted (POM) Power Regression

LOQ=62.5 mIU/L



4 parameters Logistic Regression

Boemer *et al.*, *J. Chromatogr. B*, 877, (2009), 2412-2417

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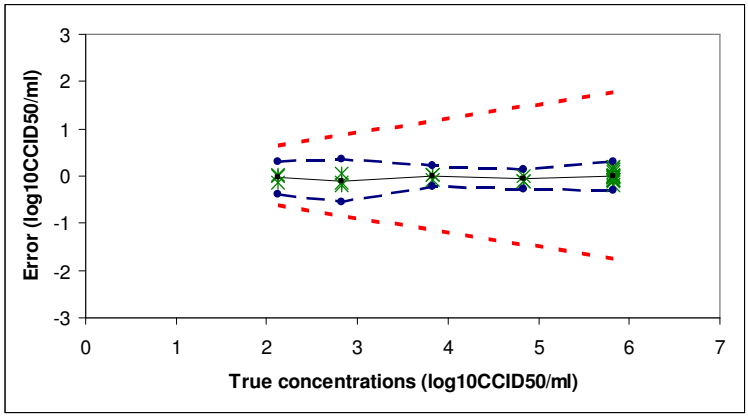
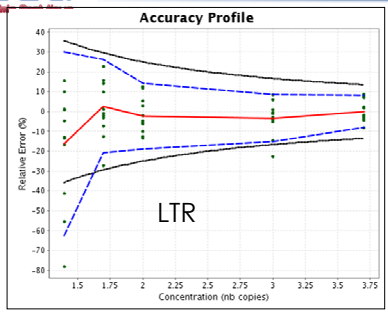
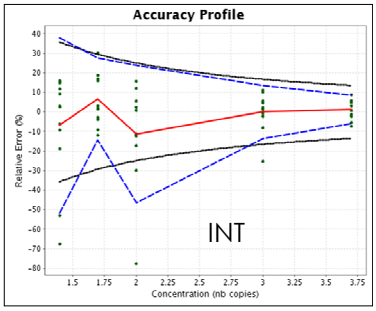
Viral activity : Validation

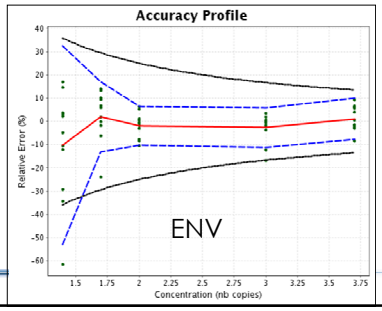


Gibelin *et al.*, *J. Chromatogr. B*, 877, (2009), 2407-2411

Q-PCR of 3 HIV genes: validation







In collaboration with Dr. C. Devaux (CRP-santé - Luxembourg)

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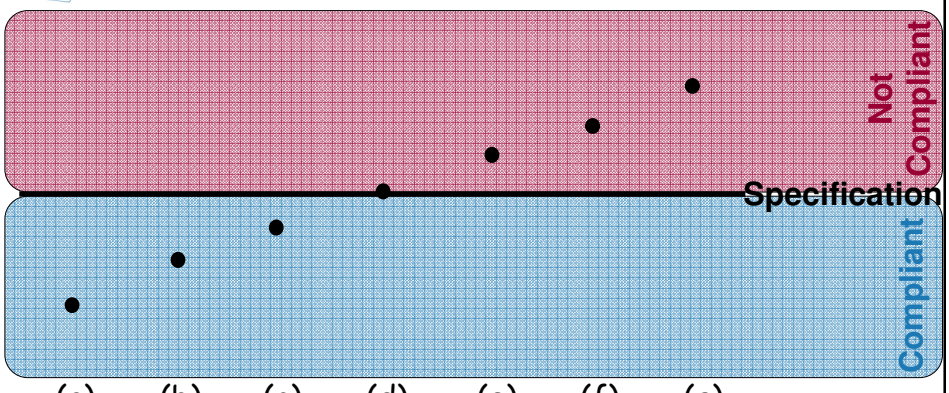
Measurement uncertainty

- The method is valid, is this enough ?
- Need measurement uncertainty:

$$\text{Results} \pm U$$

- to:
  - **Interpret** adequately results
  - **Compare** results between them

Measurement uncertainty



(a) (b) (c) (d) (e) (f) (g)

OK OK OK OK NOK NOK NOK "shared risk"

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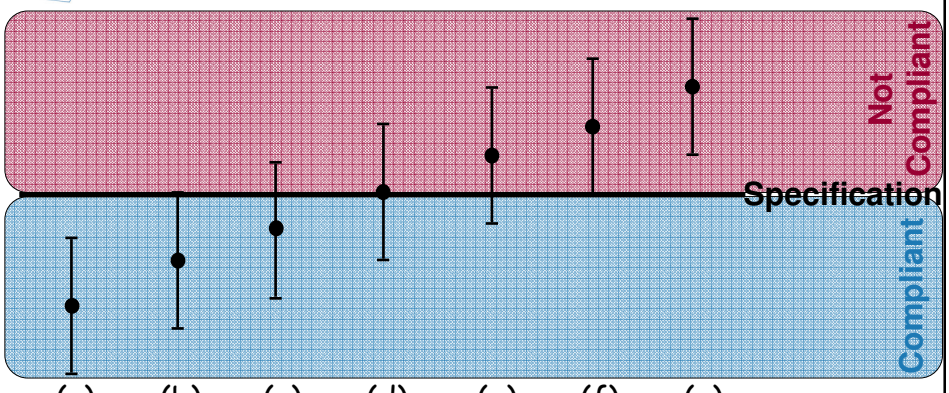
Measurement uncertainty



(a) (b) (c) (d) (e) (f) (g)

+U OK NOK NOK NOK NOK NOK NOK "full risk"

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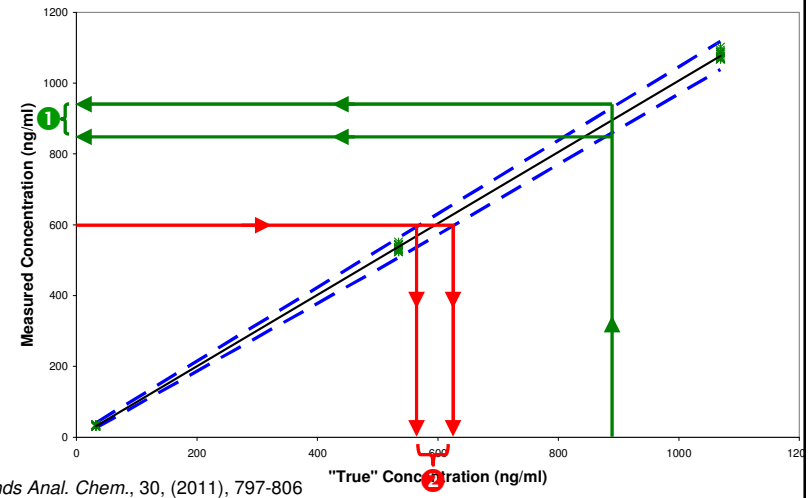
Measurement uncertainty

- Use Method Validation Data:



Rozet *et al.*, *Trends Anal. Chem.*, 30, (2011), 797-806

Content

Conclusions

- **Switch** from the traditional check list validation to a rewarding, useful and predictive method validation
- The **quality of future results** ($\approx \pi$) must be the objective and not the past performances of the method.
- The **β-expectation tolerance interval/Accuracy profile** fulfils this objective.
- In such a way, the **risks** are known at the end of the validation.

Conclusions

- Use method validation to obtain estimates of **measurement uncertainty** for routine real/incurred samples.
- **Universal** methodology applicable to **any** quantitative assay.

Thanks for your attention

- Check our publications at:

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

- Contact:

  Eric.Rozet@ulg.ac.be

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