Laboratoire de Chimie Analytique

UNIVERSITE de LIEGE

Validation of Analytical Methods

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Erasme

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Content

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

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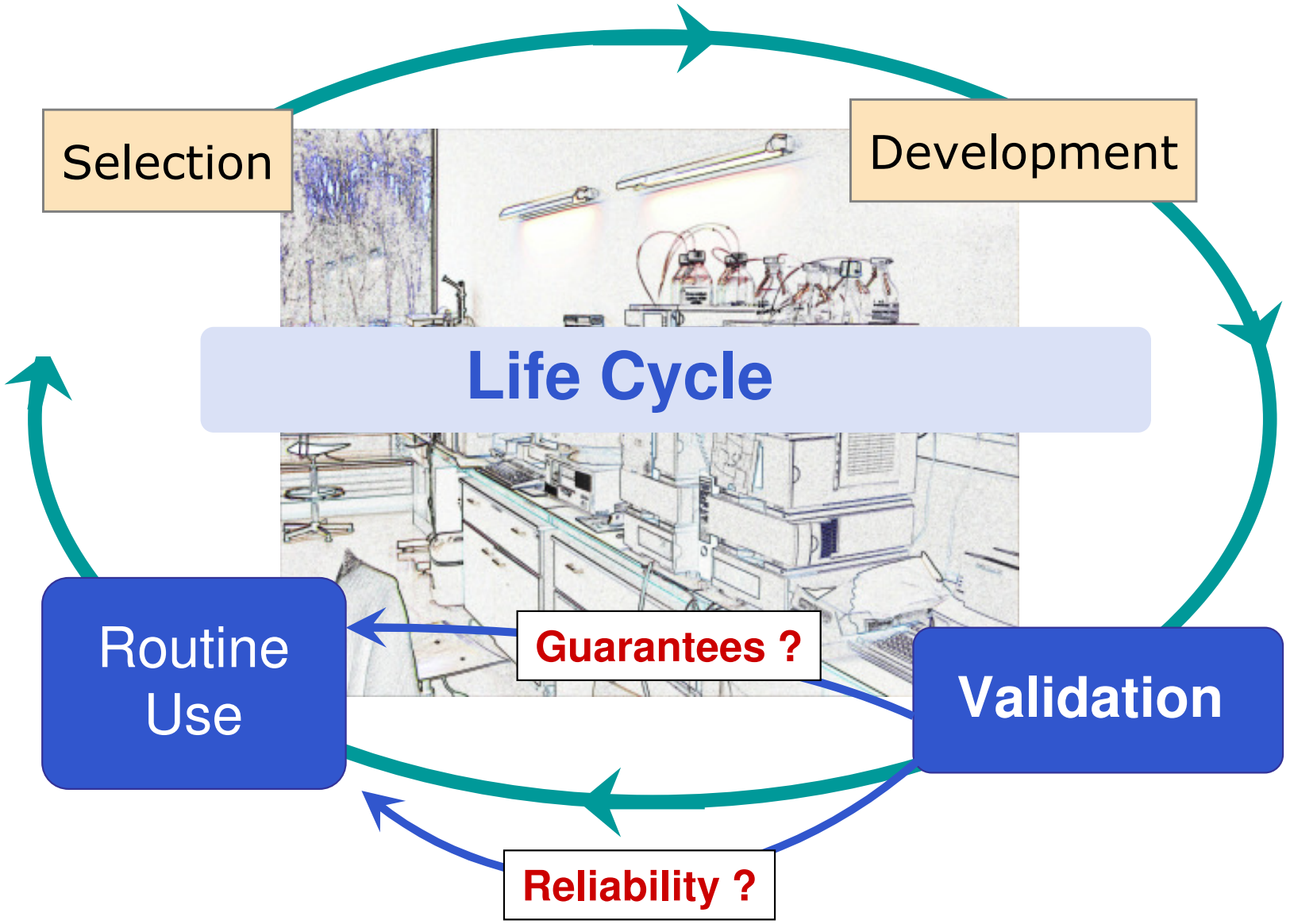
Analytical Method Life Cycle

- 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.

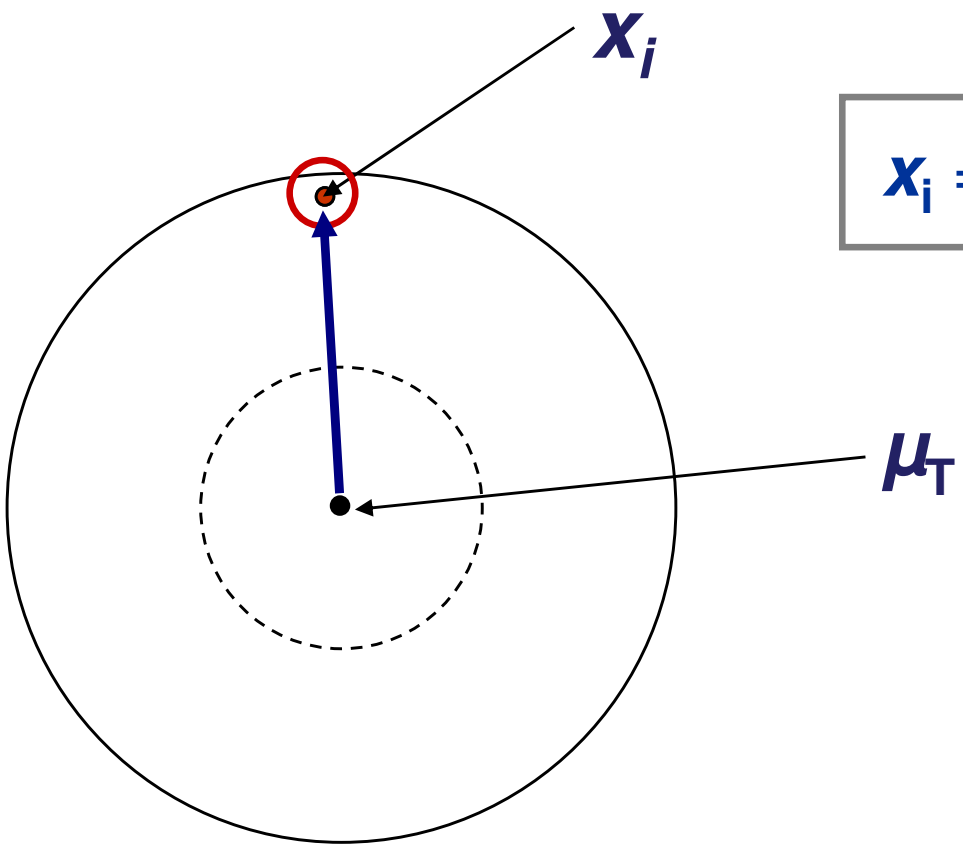
Analytical Method Life Cycle



Result in routine

X_i = observed result

μ_T = True unknown value



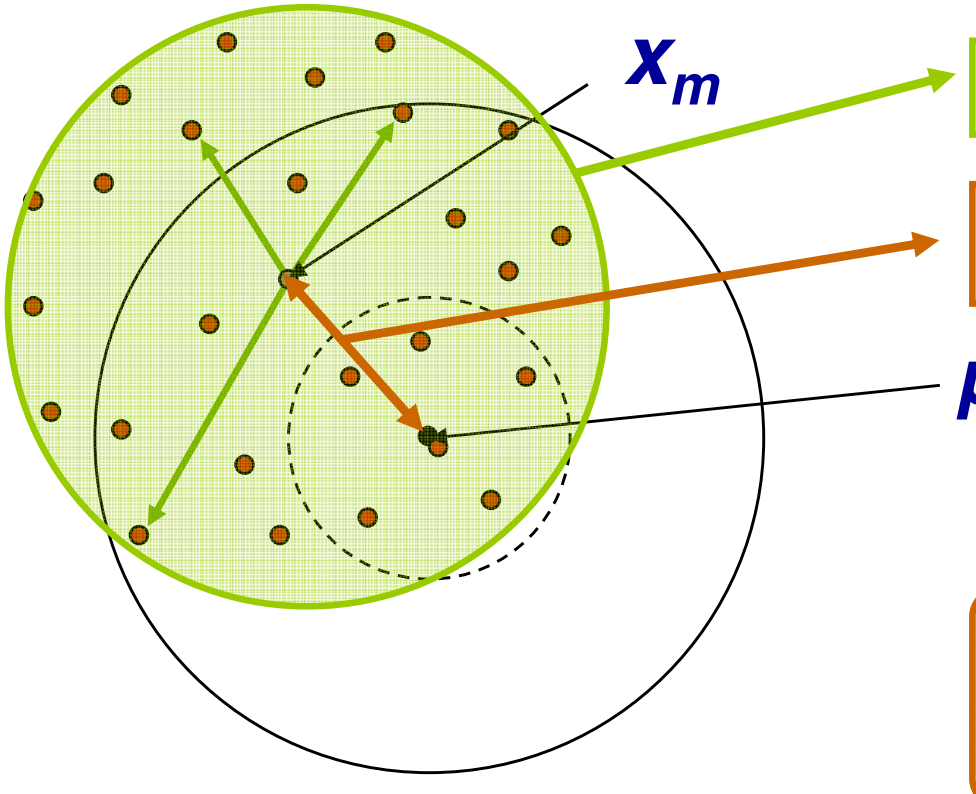
$x_i = \mu_T$ + Errors

Acceptable ?

Analytical Errors

X_m = mean of observed results

μ_T = unknown true value



Random Error

Systematic Error

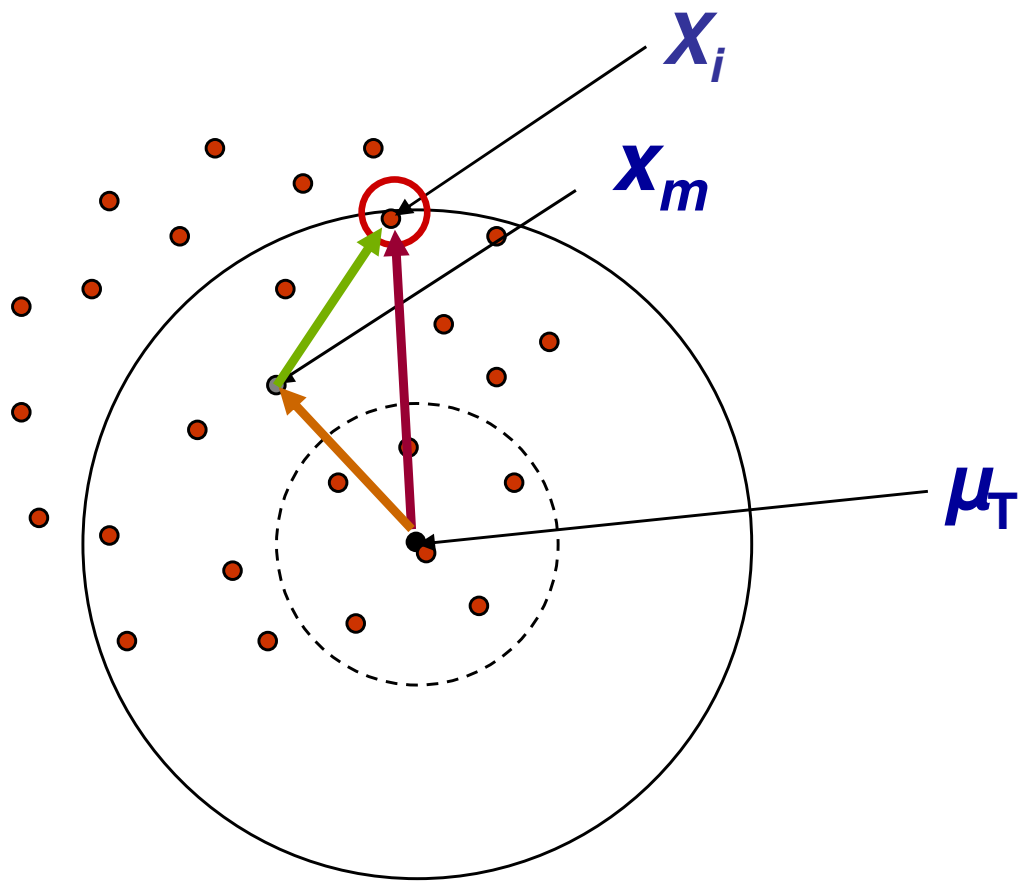
Unknown during Routine analyses

Analytical Errors

X_m = mean of observed results

μ_T = unknown true value

X_i = observed result



$x_i = \mu_T$ + **Errors**

Total Error

Systematic Error

Random Error

Total Error

Accuracy (ICH Q2R1 – ISO 5725 – SFSTP)

The accuracy of an 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**.

➔ **Total error** is directly linked to the definition of **accuracy.**

➔ **Total error** is the **adequate decision criterion** to accept the **validity** or the **transfer** of an analytical method **before its routine application.**

D. Kringle et al.,Pharma.Research, 24, (2007) 1157

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

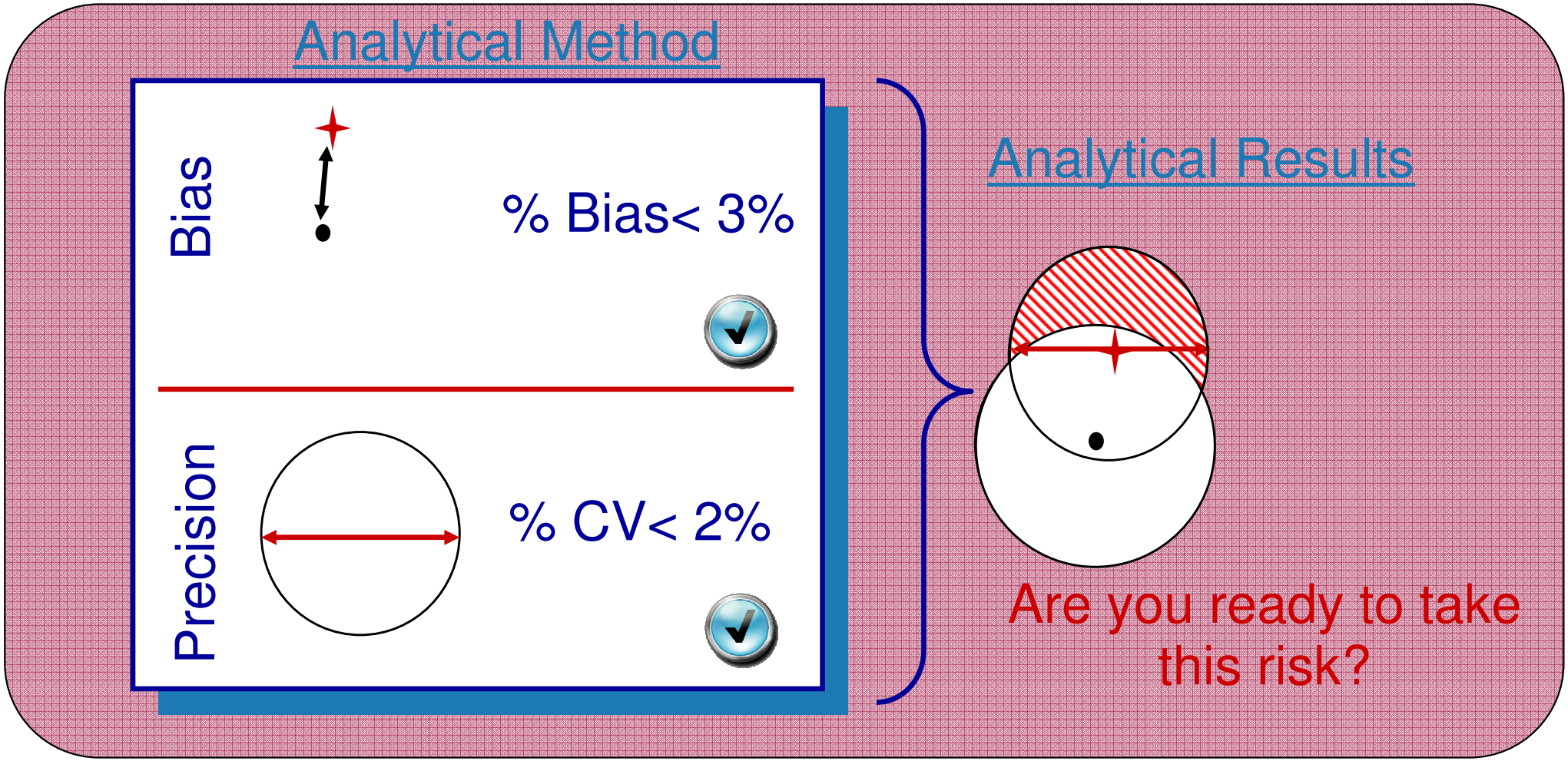
- Traditional vision:
  - The Validation Criteria Check List:

- 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 ?



Analytical Method Validation

- Traditional vision:
  - Preliminary Conclusion:

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

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Analytical Method Validation

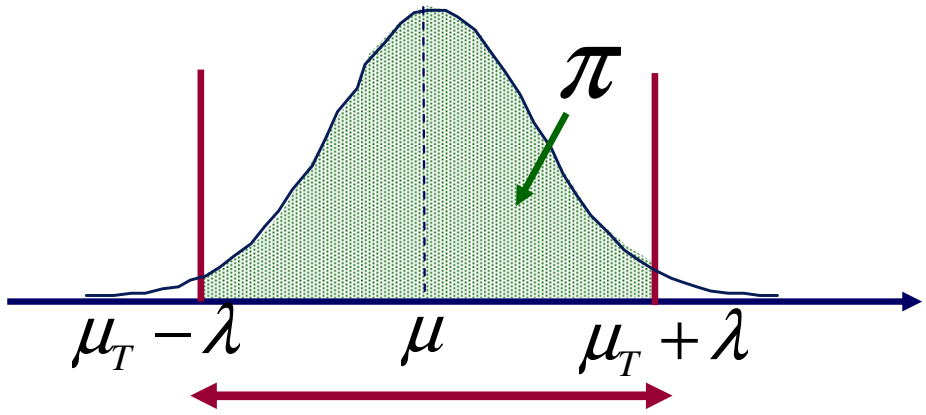
Aim of validation

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 ± λ



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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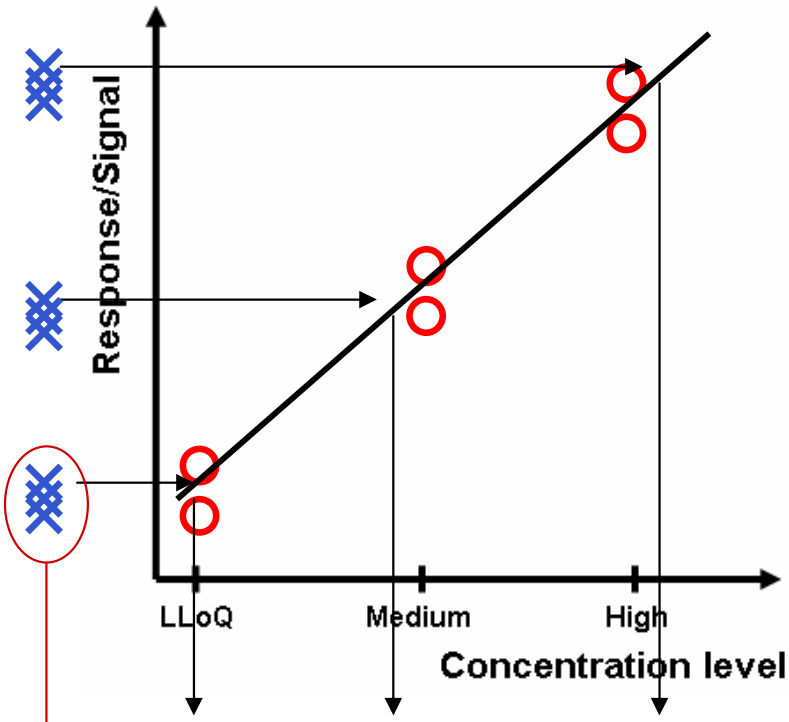
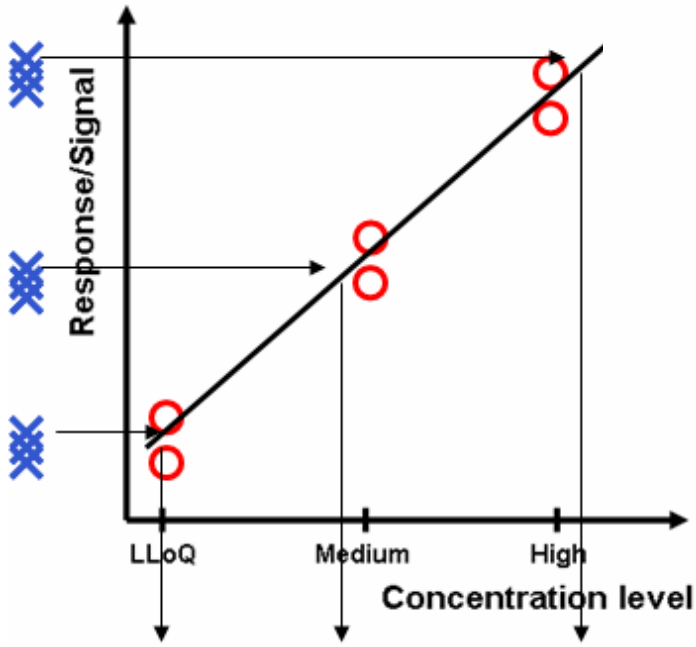
All in one Validation Design

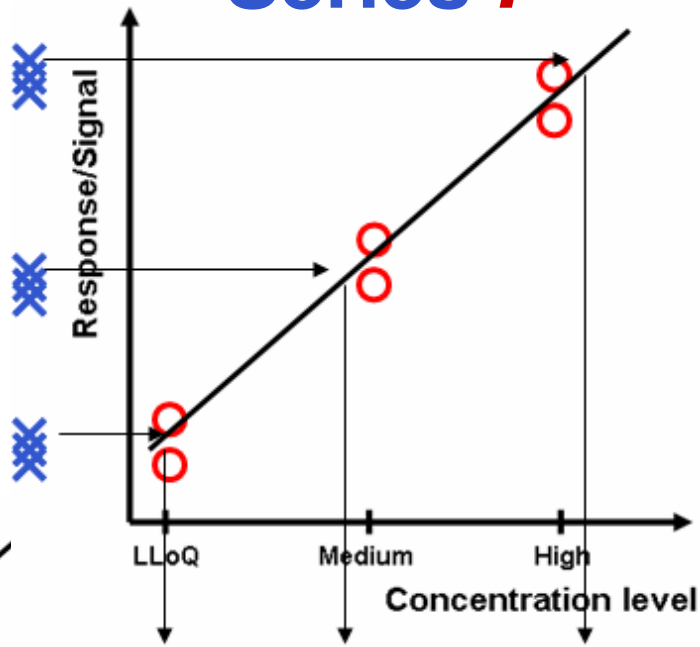
Series 1



Series 2



Series *I*



***J* repetitions**

Calibration standards

Validation standards

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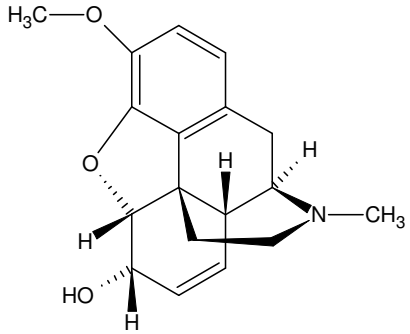
Decision Methodology

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

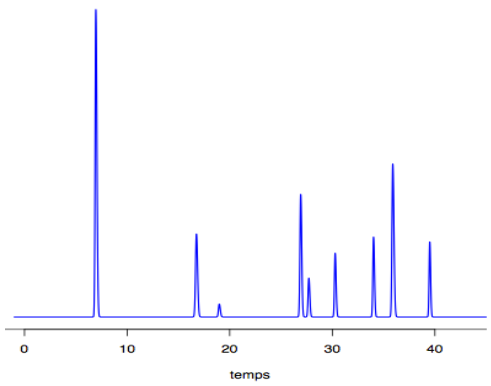
➔ Let's illustrate this through an example:

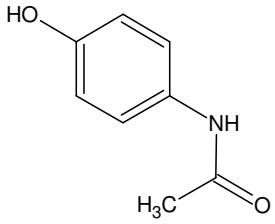
Example

- 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 ?

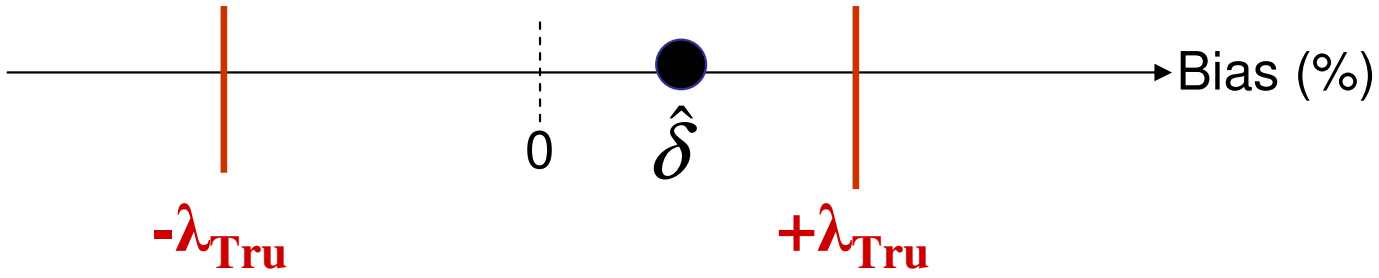
Traditional Approaches:

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

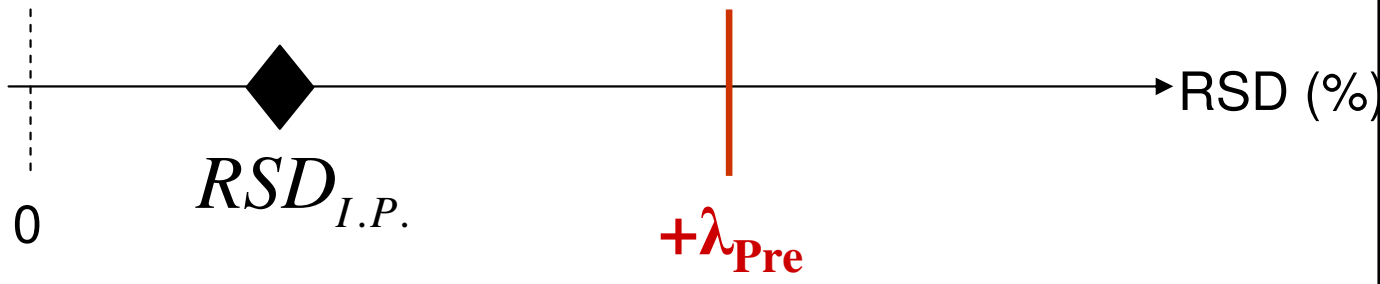
- Descriptive:
  - **trueness**: only based on estimation of method *bias*;
  - **precision**: only based on estimation of method $RSD_{I.P.}$.
- Difference:
  - **trueness**: based on bilateral Student t-test for *bias* significance.
- Equivalence:
  - **trueness**: based on confidence interval of the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

Descriptive Approach

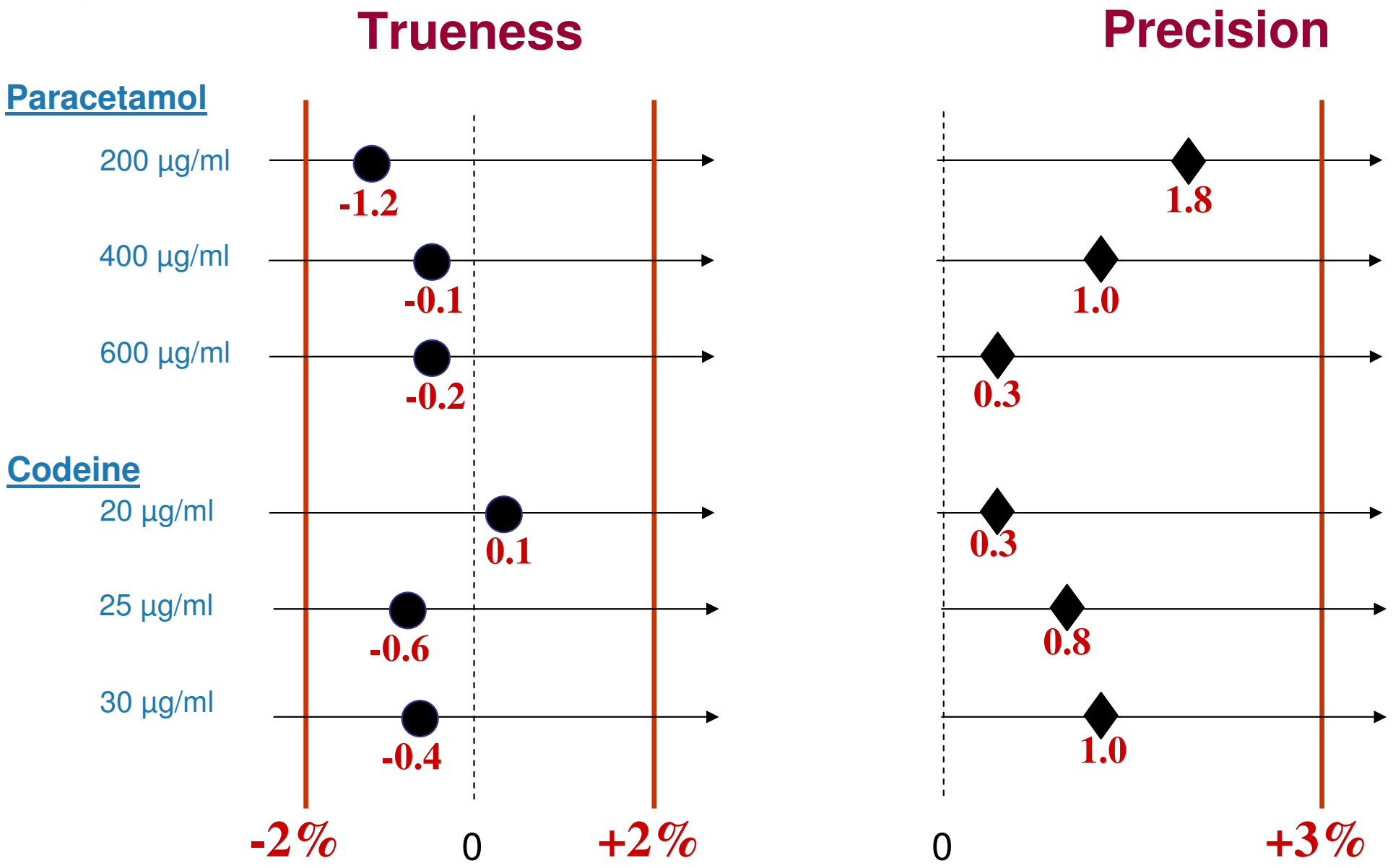
Trueness:



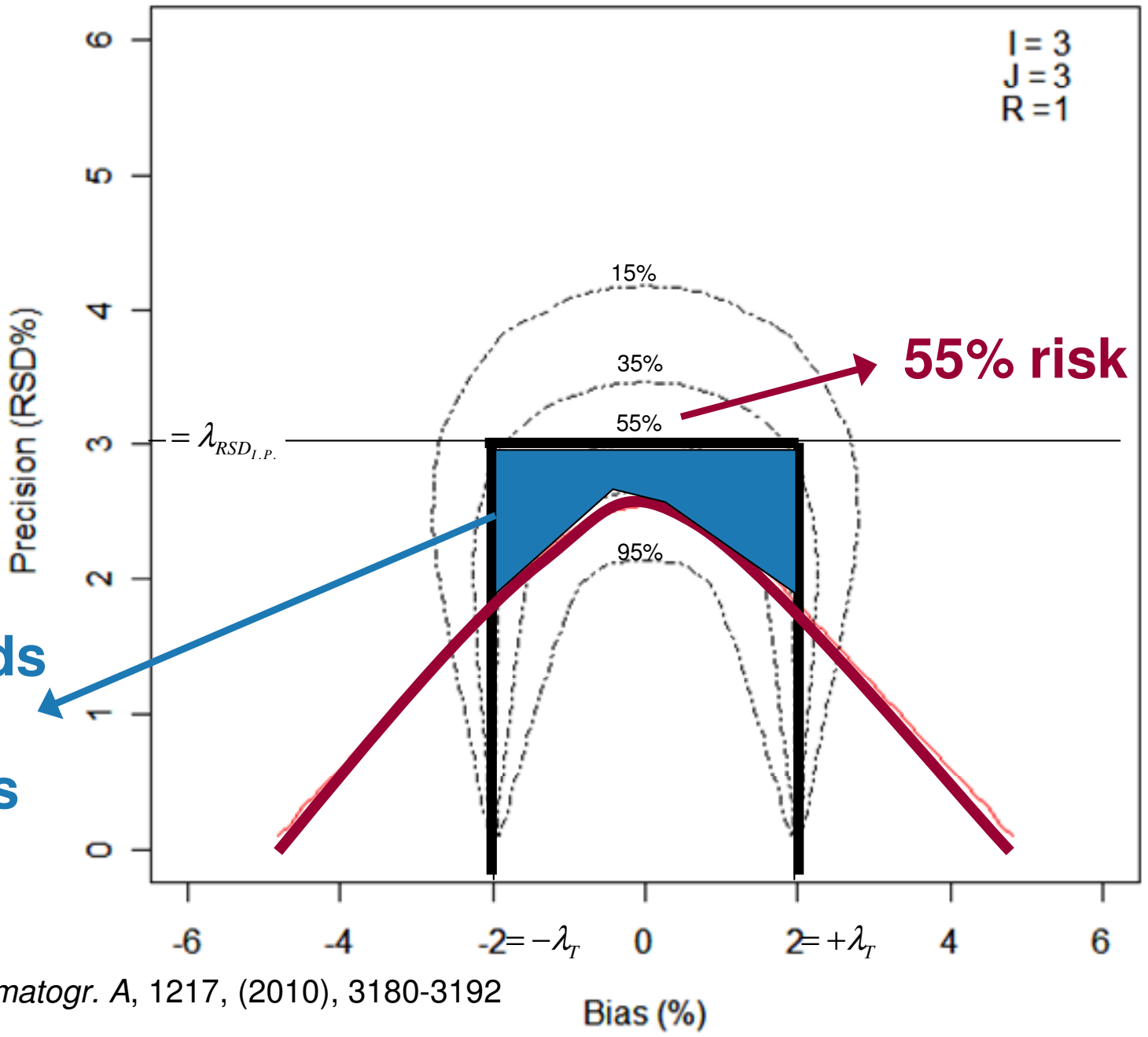
Precision:



Example


Trueness
Precision
Paracetamol
200 µg/ml
400 µg/ml
600 µg/ml
-1.2
-0.1
-0.2
1.8
1.0
0.3
Codeine
20 µg/ml
25 µg/ml
30 µg/ml
0.1
-0.6
-0.4
0.3
0.8
1.0
-2%
0
+2%
0
+3%

Descriptive: performance



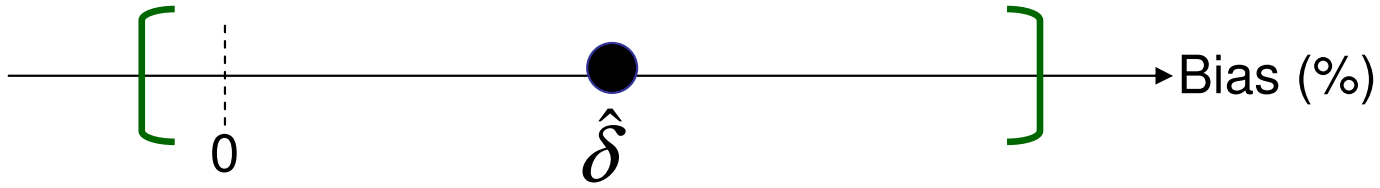
Valid methods but Poor results

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

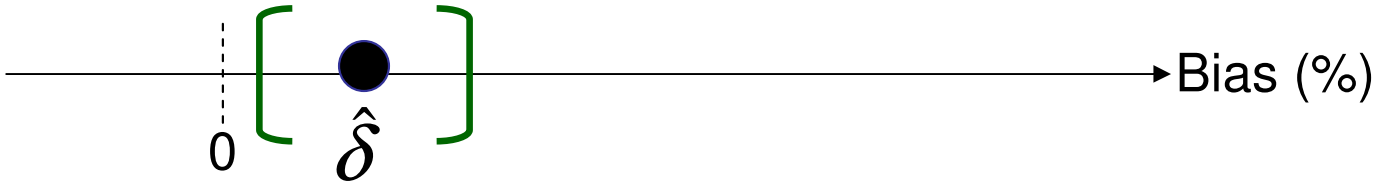
Difference Approach

$$H_0 : \delta = 0$$

$$H_1 : \delta \neq 0$$

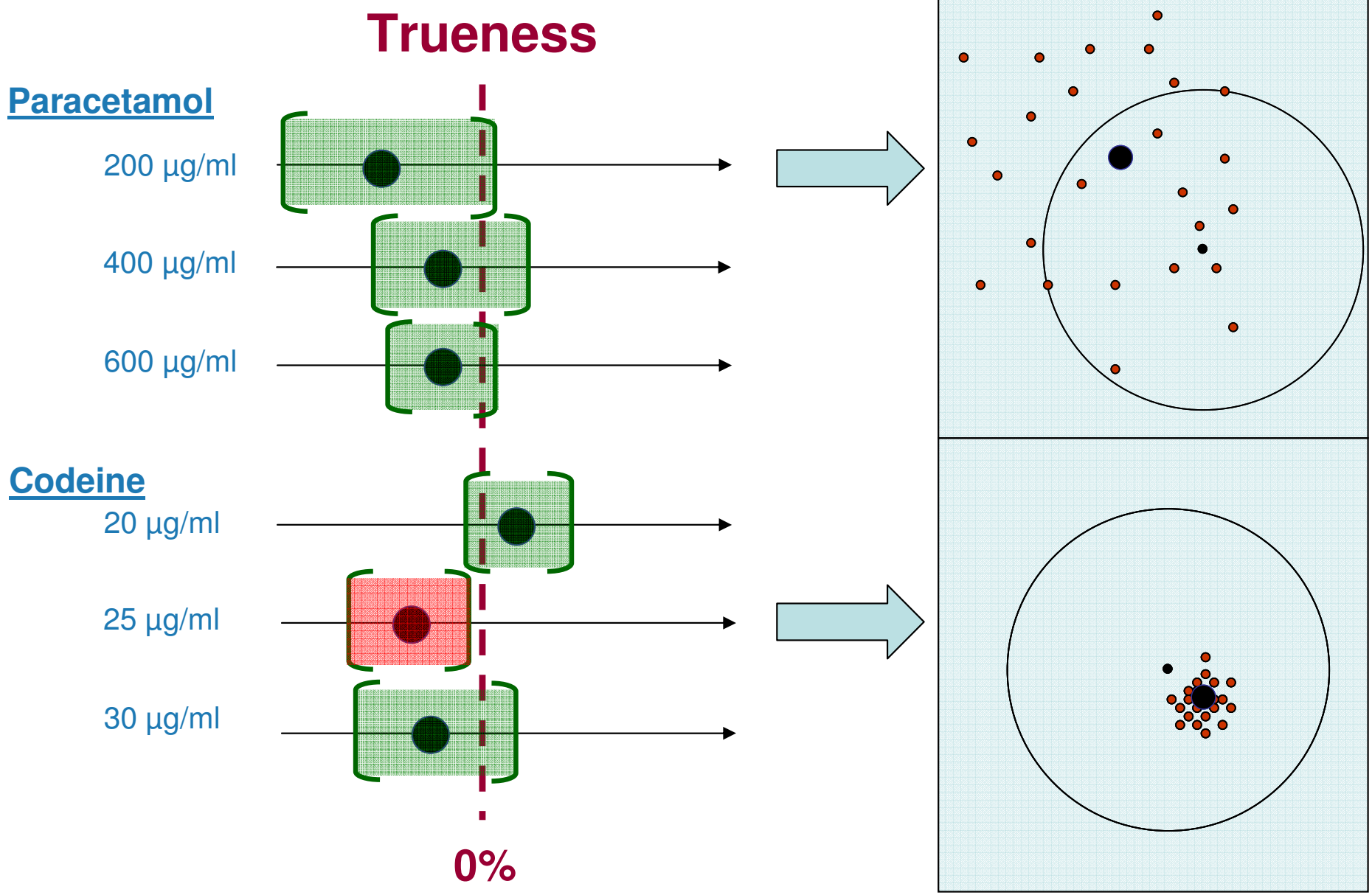


No rejection of H_0 ➔ Method **valid !?**

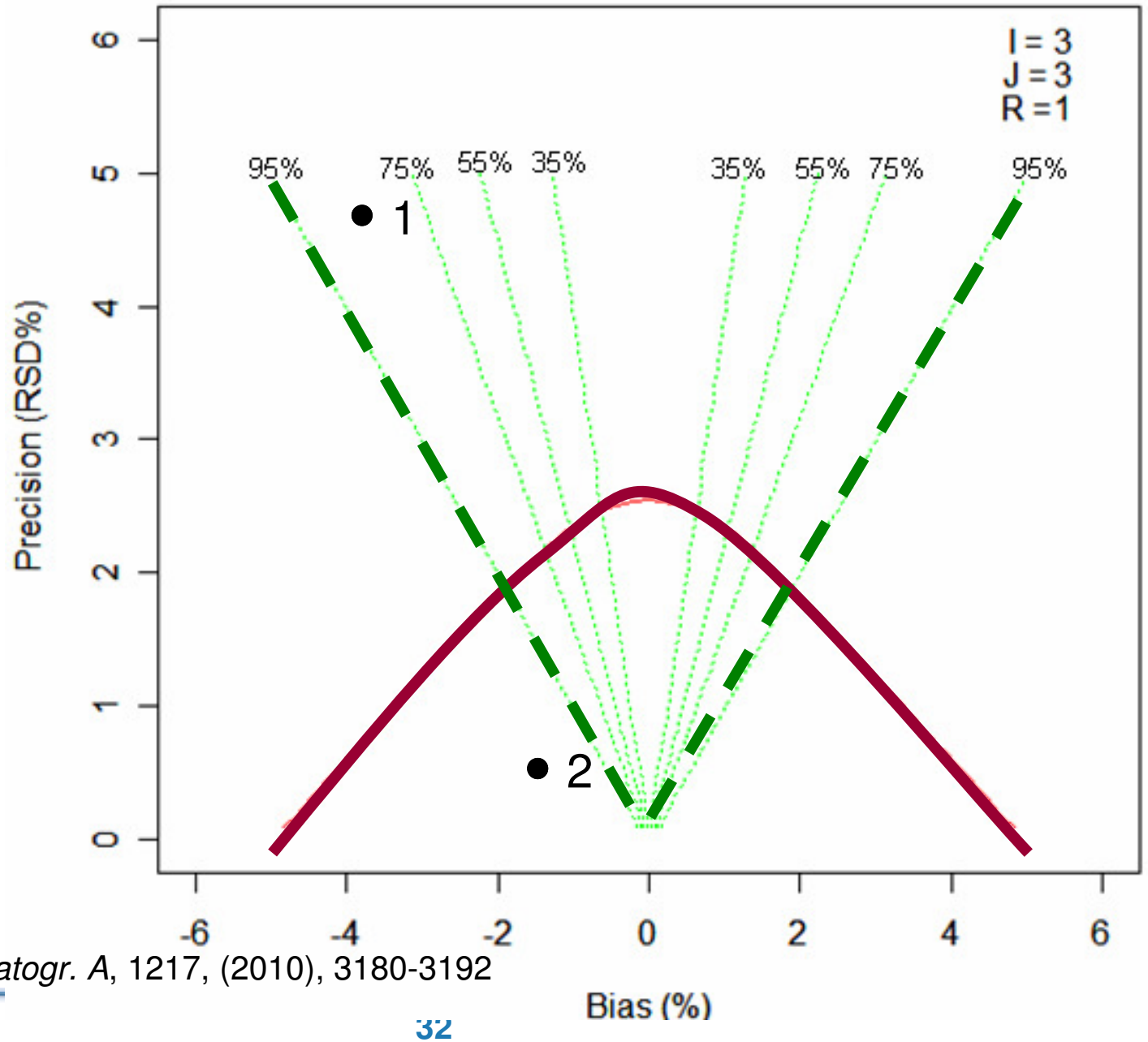


Rejection of H_0 ➔ Method **not valid !?**

Example


Trueness
Paracetamol
200 µg/ml
400 µg/ml
600 µg/ml
Codeine
20 µg/ml
25 µg/ml
30 µg/ml
0%

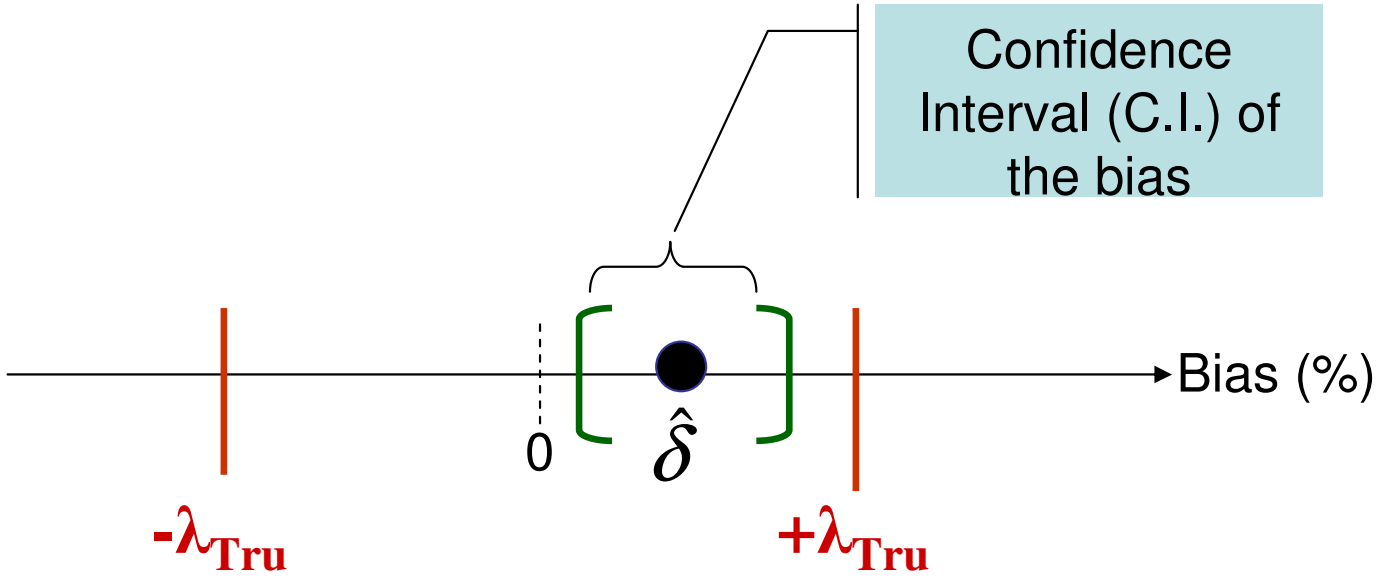
Difference: performance



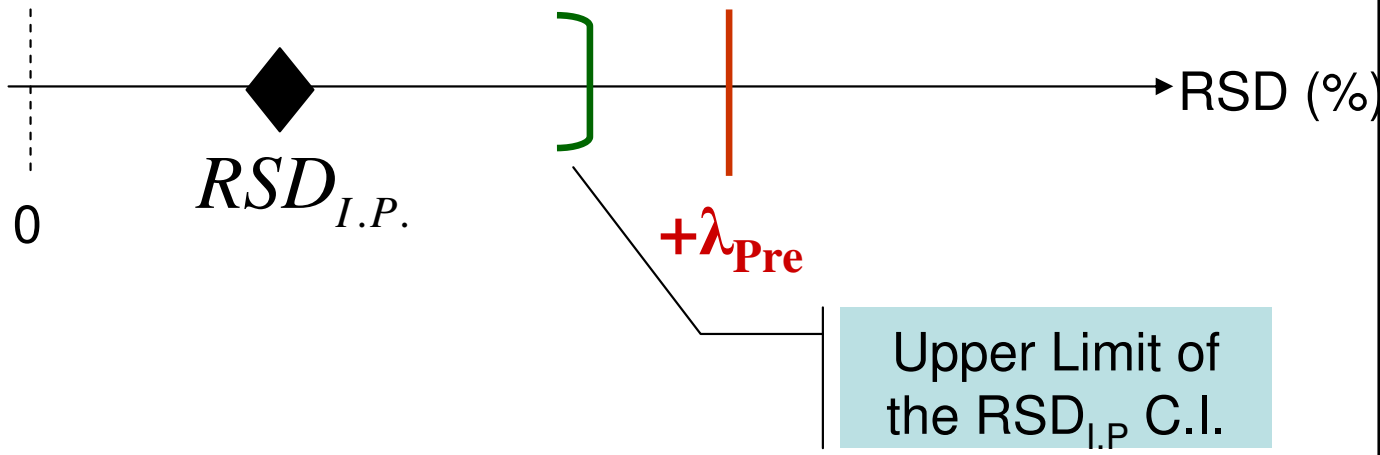
Bouabidi *et al.*, *J. Chromatogr. A*, 1217, (2010), 3180-3192

Equivalence Approach

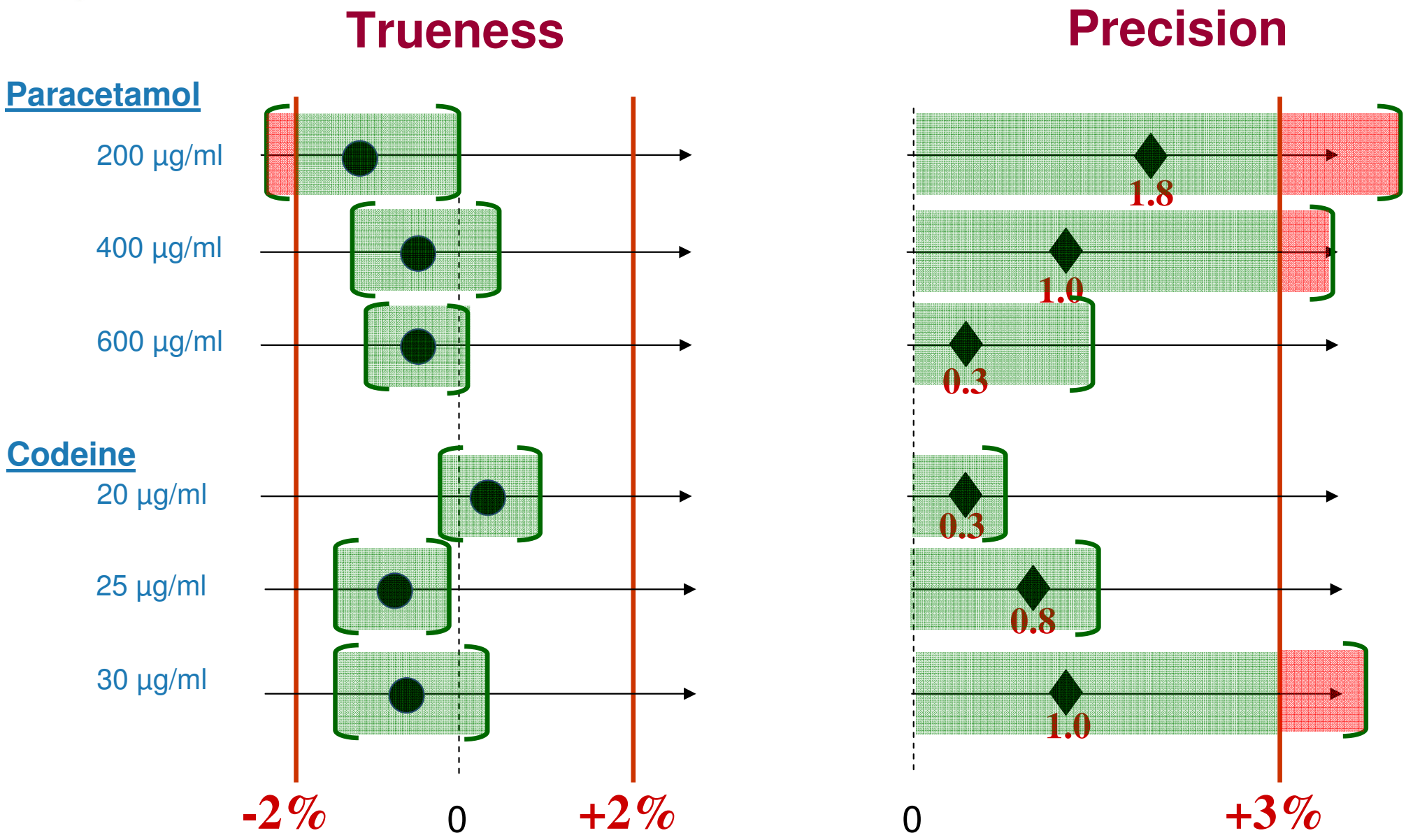
Trueness:



Precision:



Example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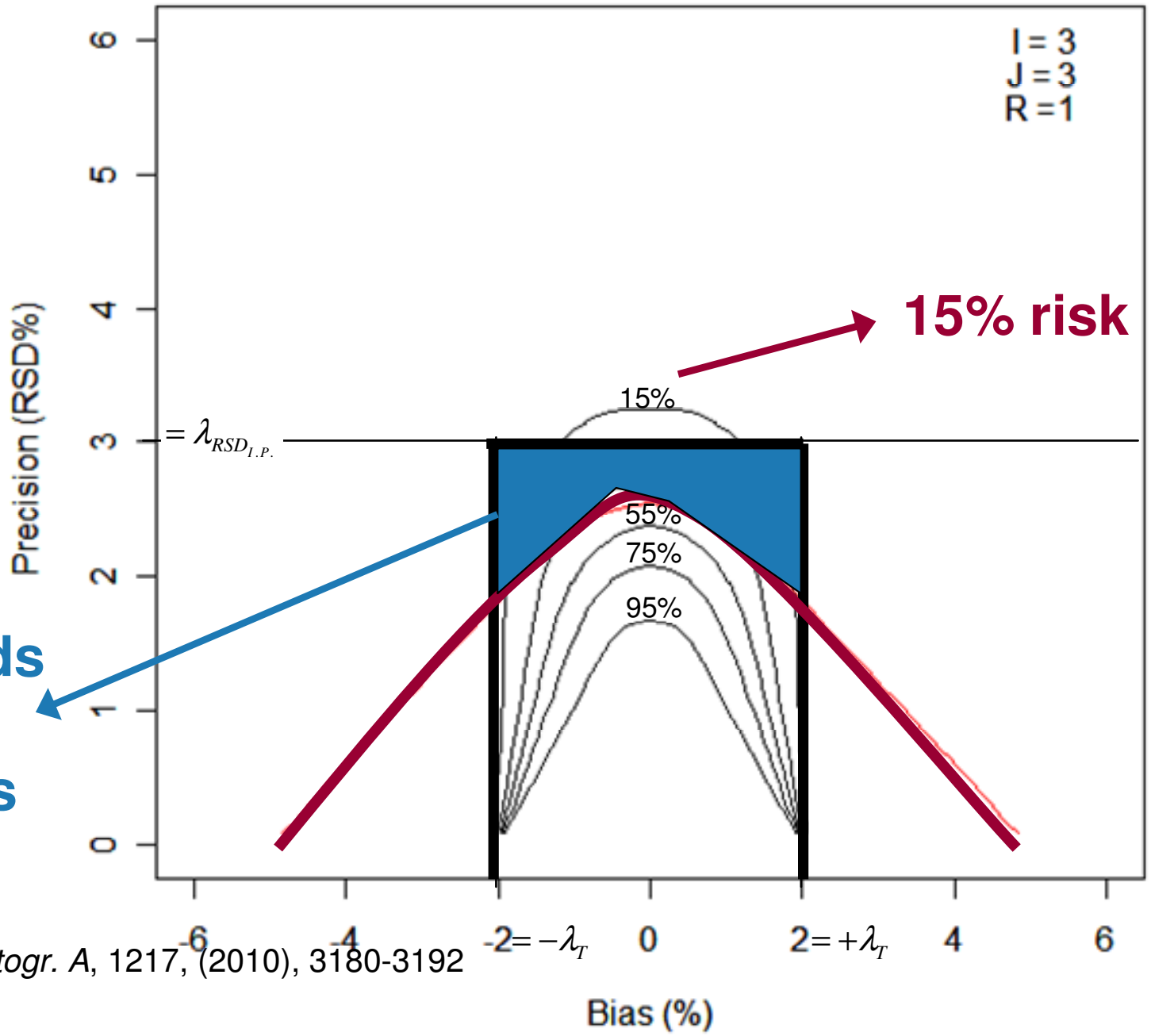
1.8
1.0
0.3
0.3
0.8
1.0
-2%
0
+2%
0
+3%

Equivalence: performance



15% risk

Valid methods but Poor results

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

Summary

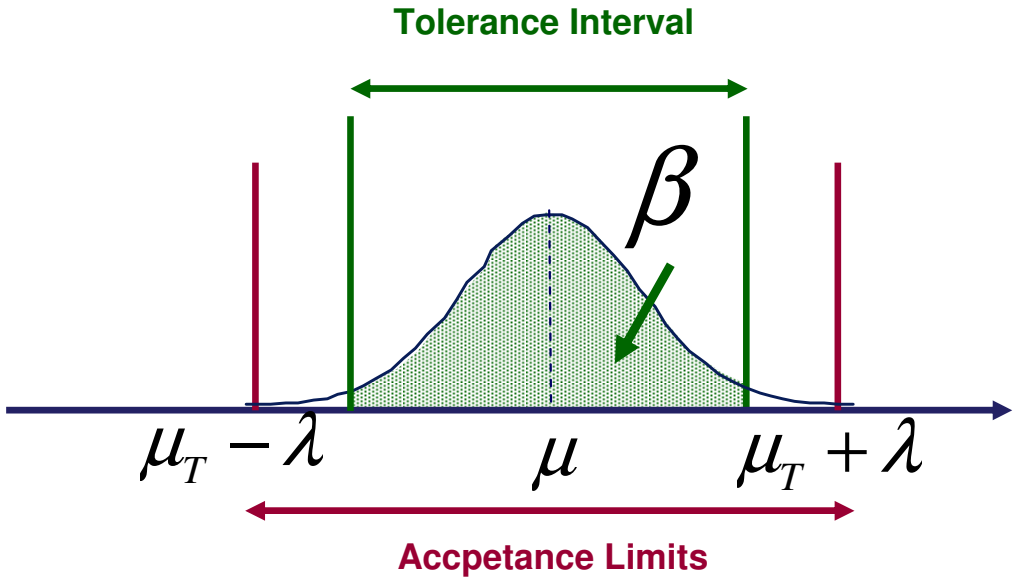
- Descriptive approach:
  - no risk management
  - Up to 50% risk to take wrong decision
- Difference approach:
  - Useless for Method Validation purpose: Avoid it !
- Equivalence approach
  - Patient risk controlled
  - Nonetheless do not fully answer method validation aim: the method is “good” but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

- Is there any better decision methodology ?

Tolerance Intervals

β-Expectation Tolerance Interval (βTI)

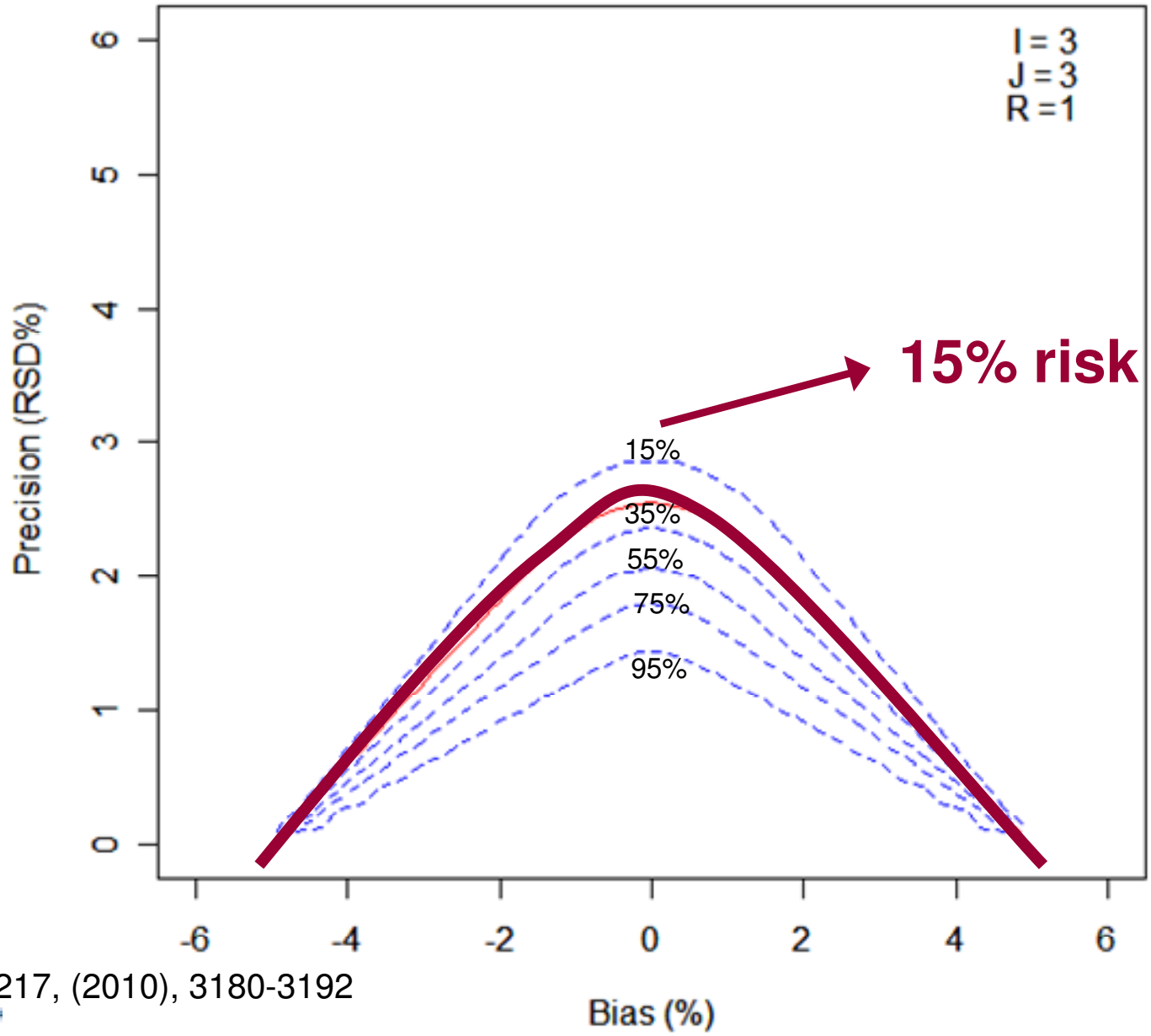
Allows to predict where each future result will fall (*Wald, 1942*).



➔ 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

Accuracy Profile

Validation experiments

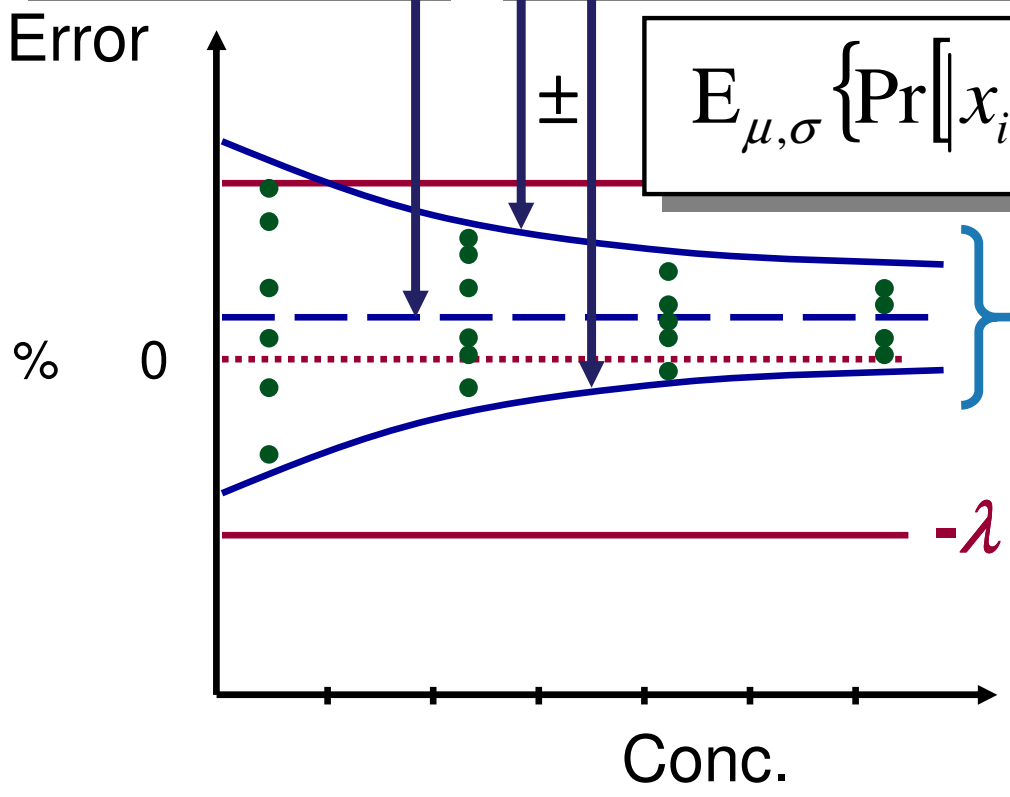
Bias

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

Intermediate Precision

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

→ Total error



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

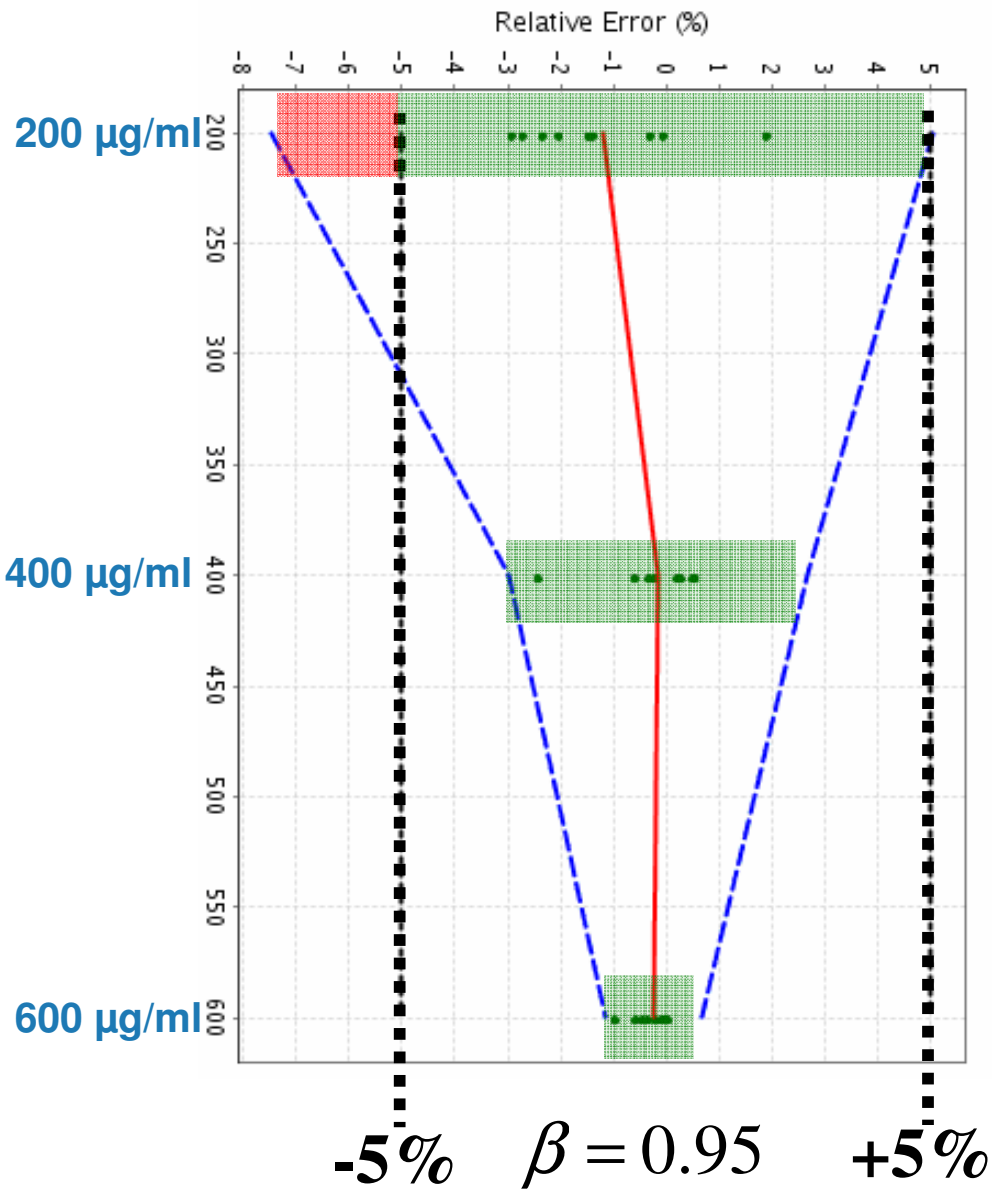
→ **Predictive Interval** with a known risk

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

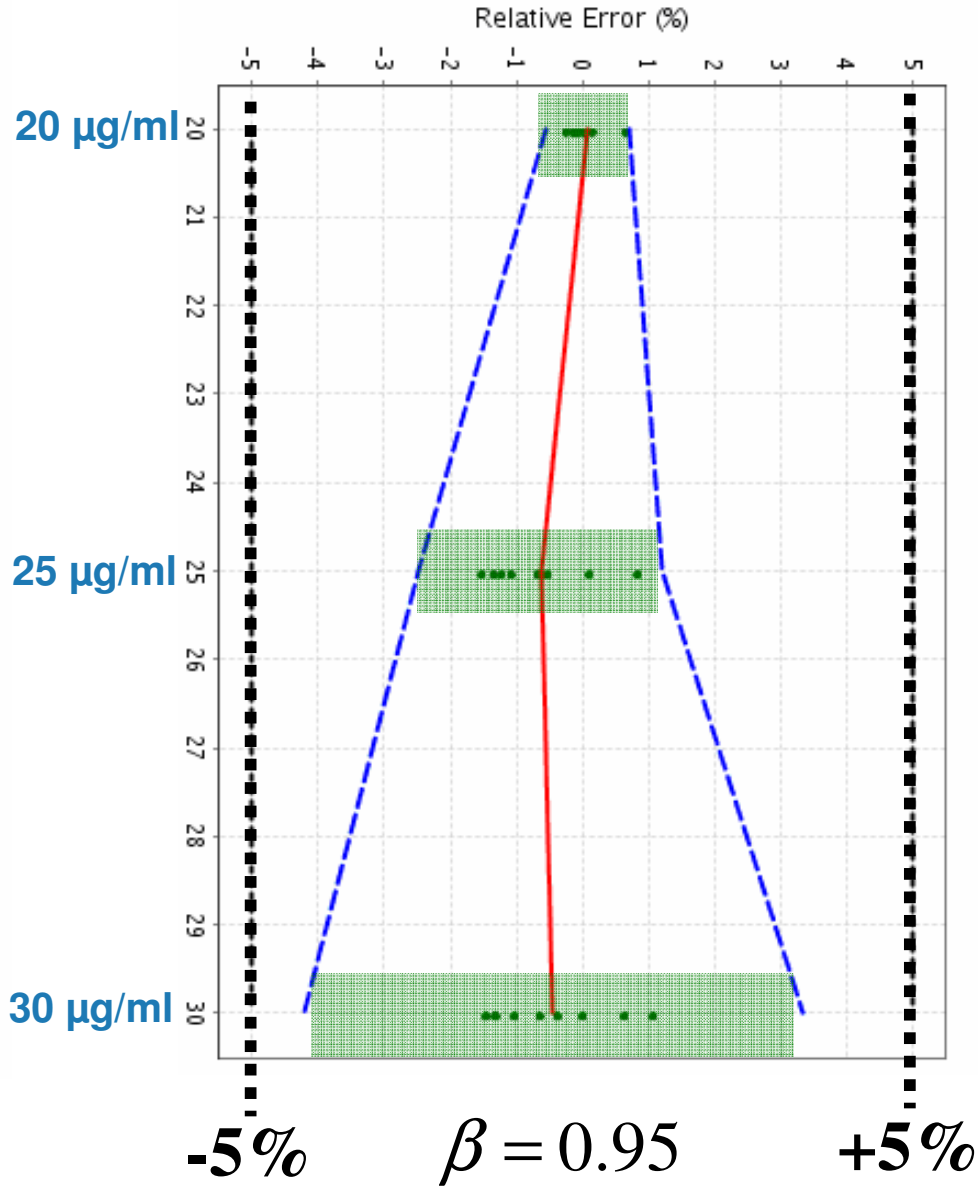
λ = acceptance limits of e.g. 5%

Example

Paracetamol

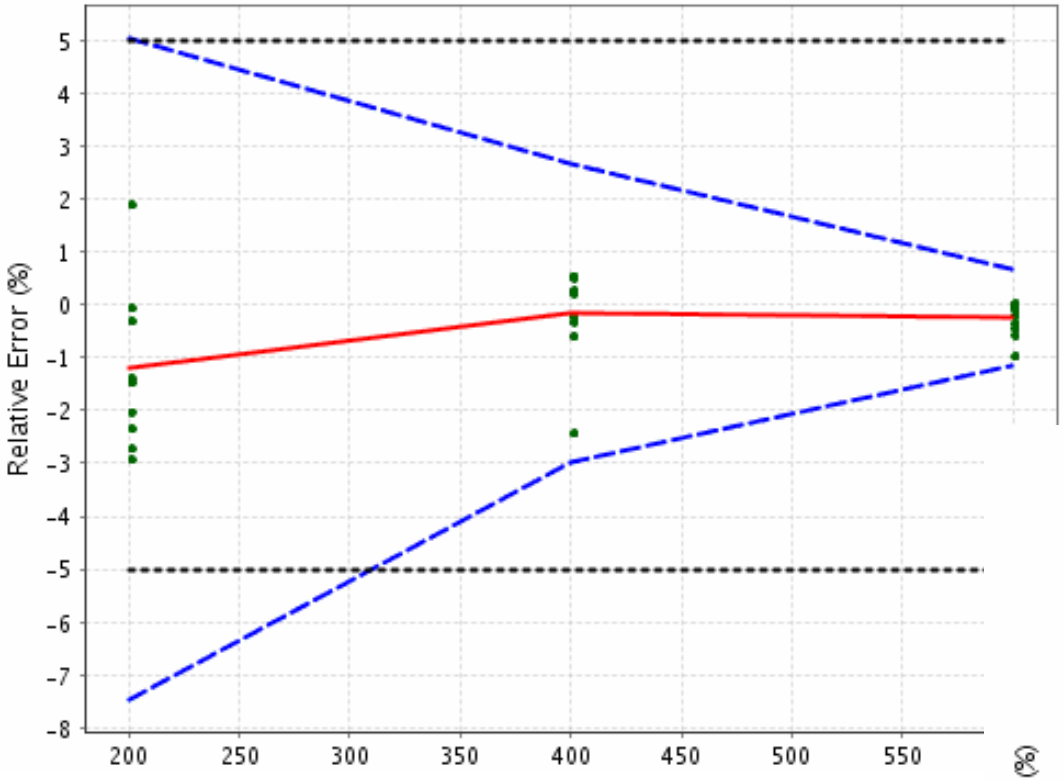


Codéine

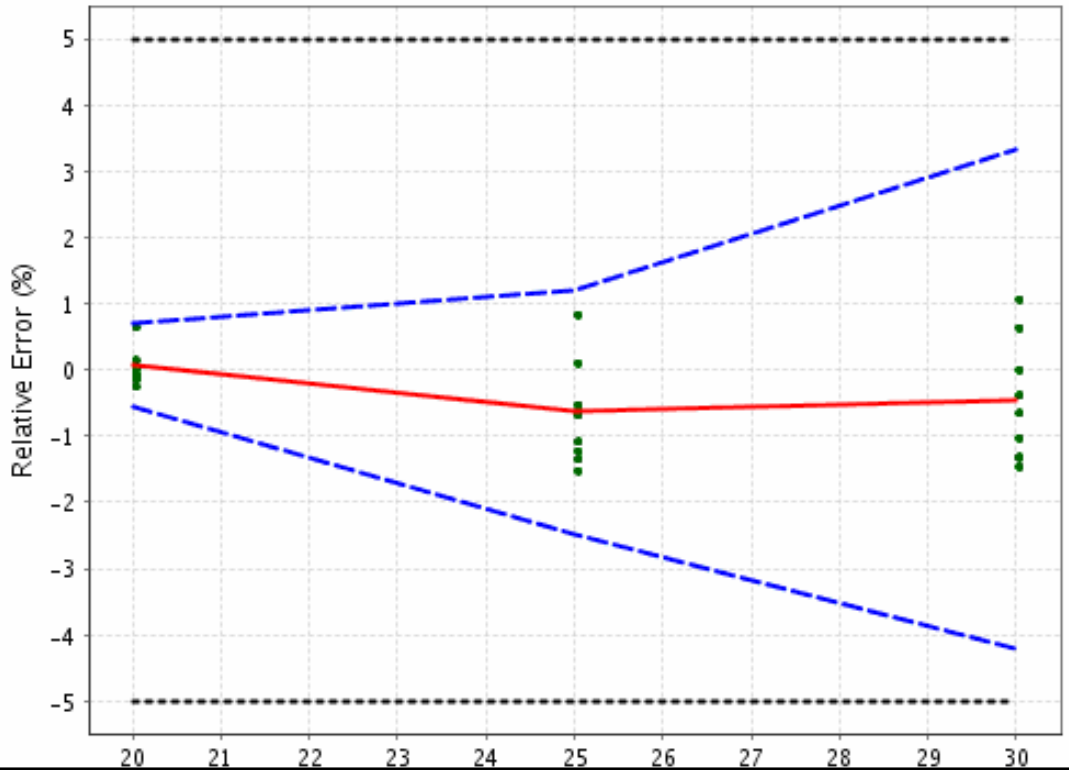


Example

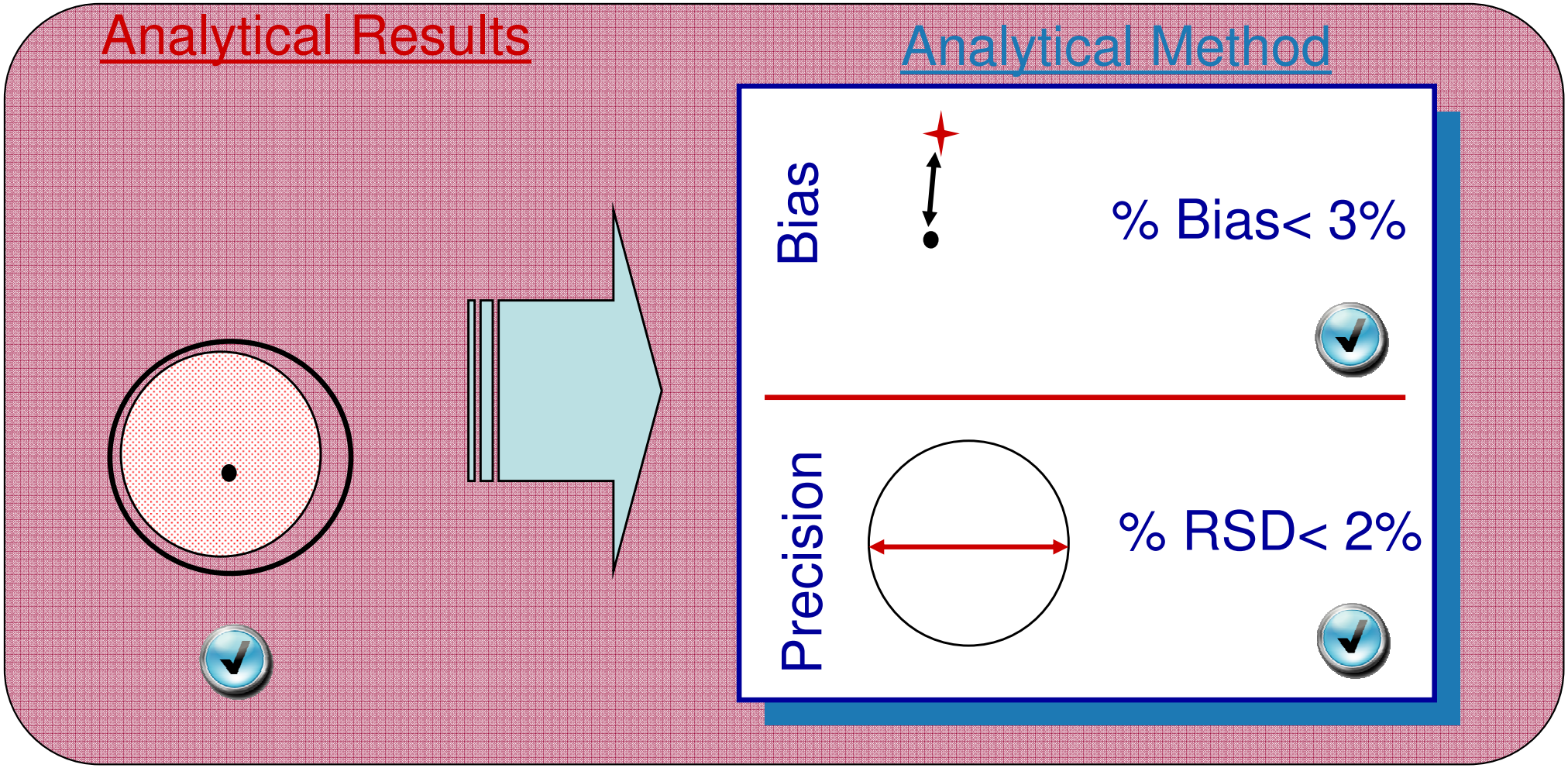
Paracetamol



Codéine



Analytical Method Validation



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

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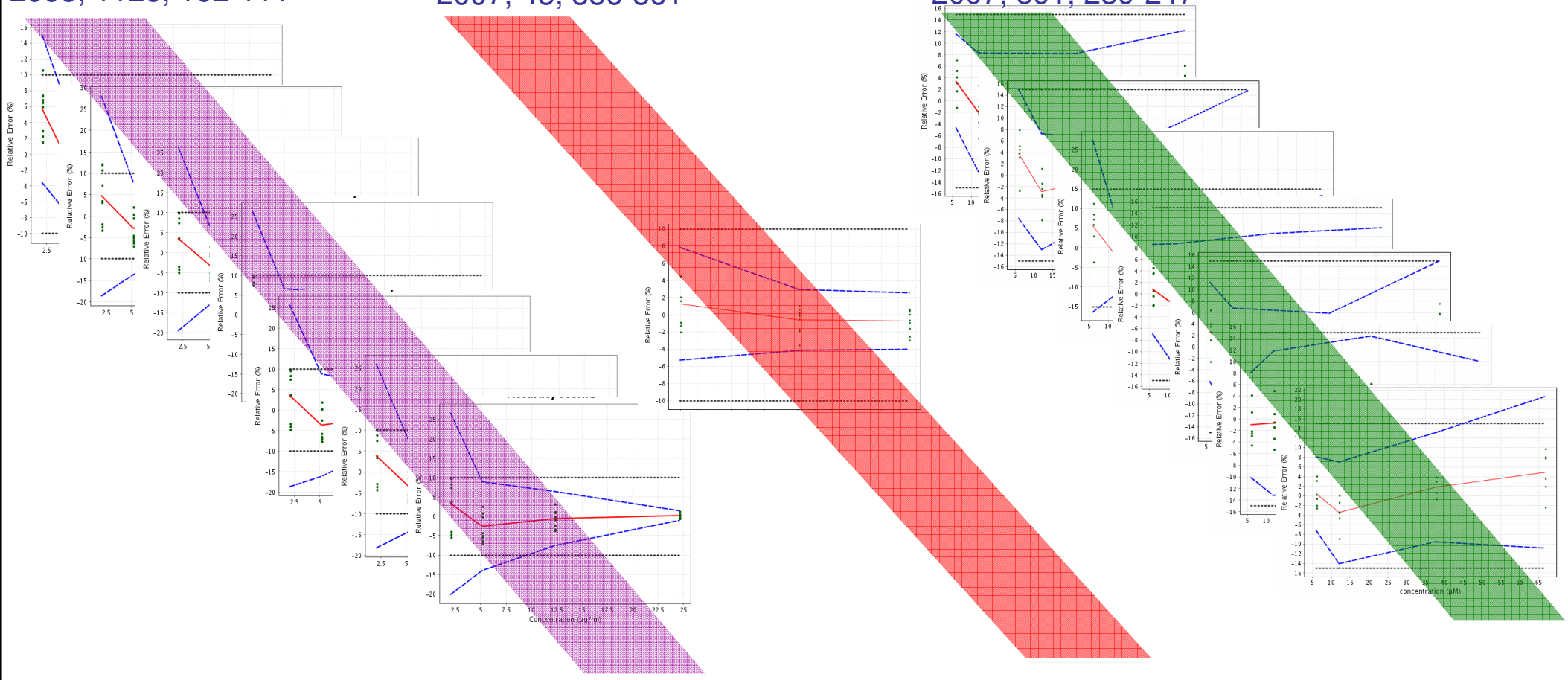
Other examples

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



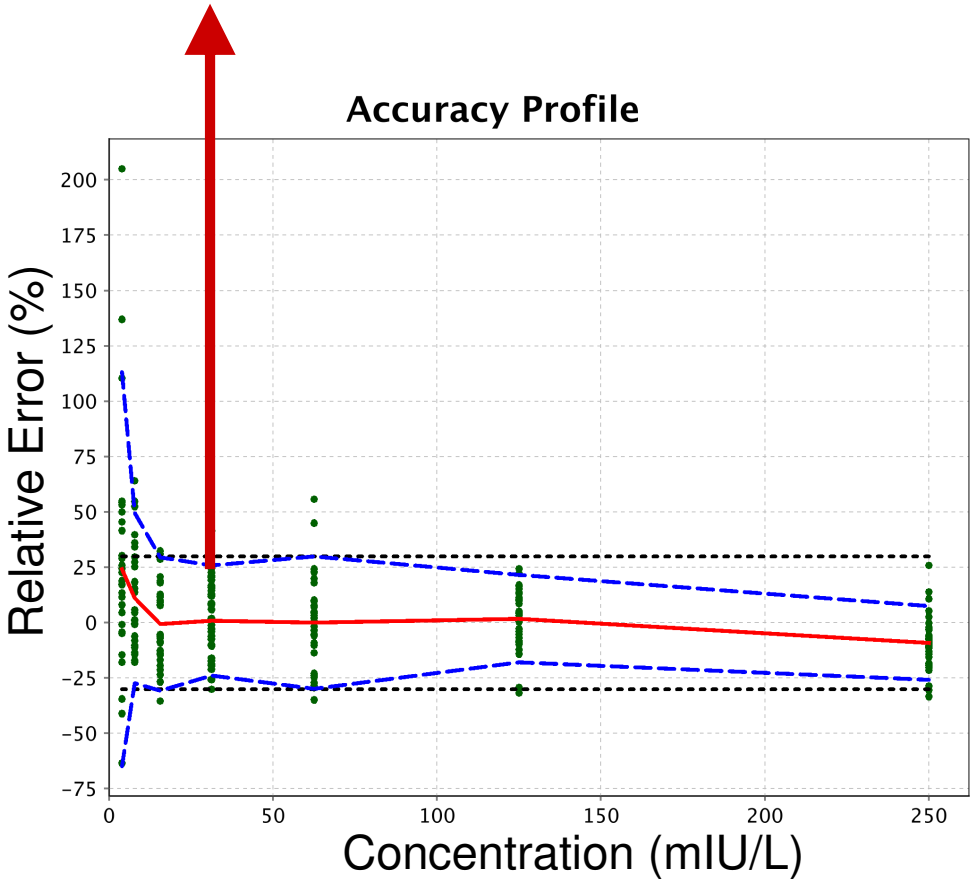
Capillary Electrophoresis

NIR

Colorimetric

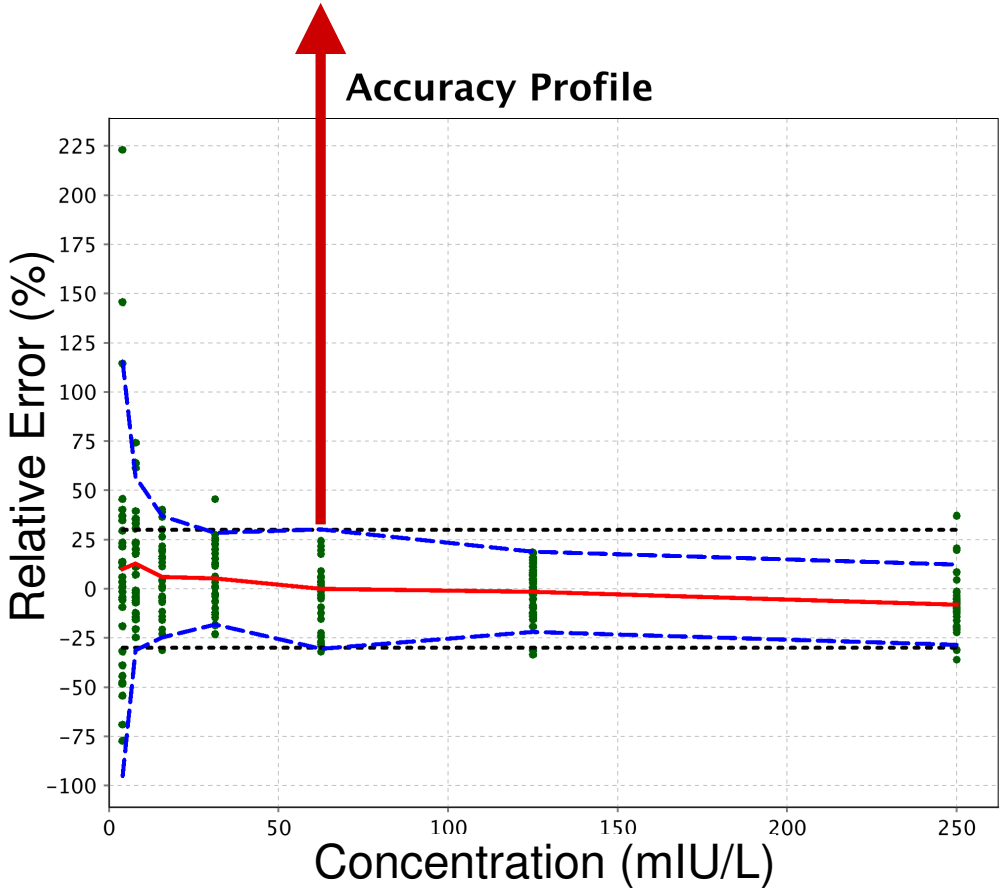
ELISA: Validation

LOQ=31.3 mIU/L



Weighted (POM) Power Regression

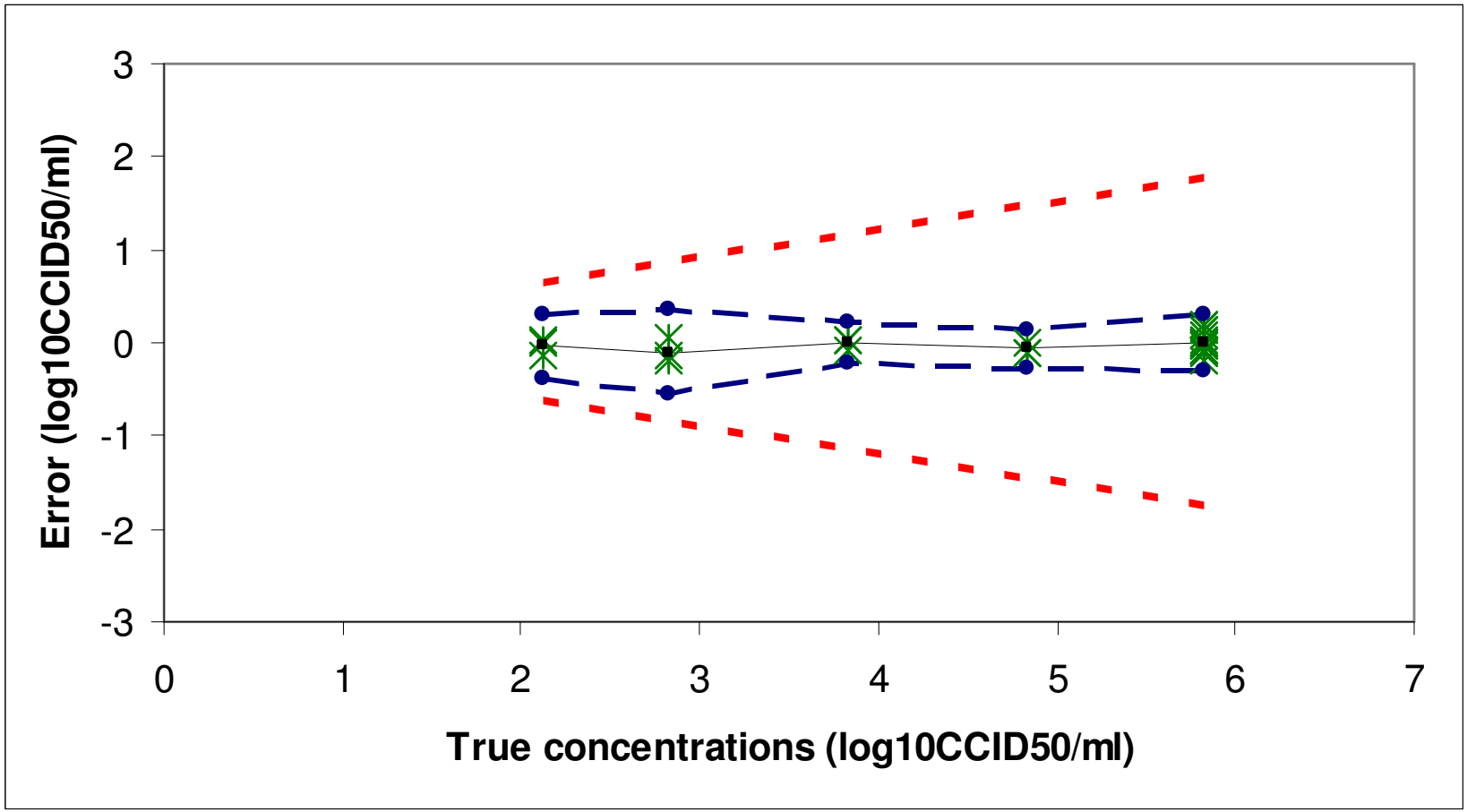
LOQ=62.5 mIU/L



4 parameters Logistic Regression

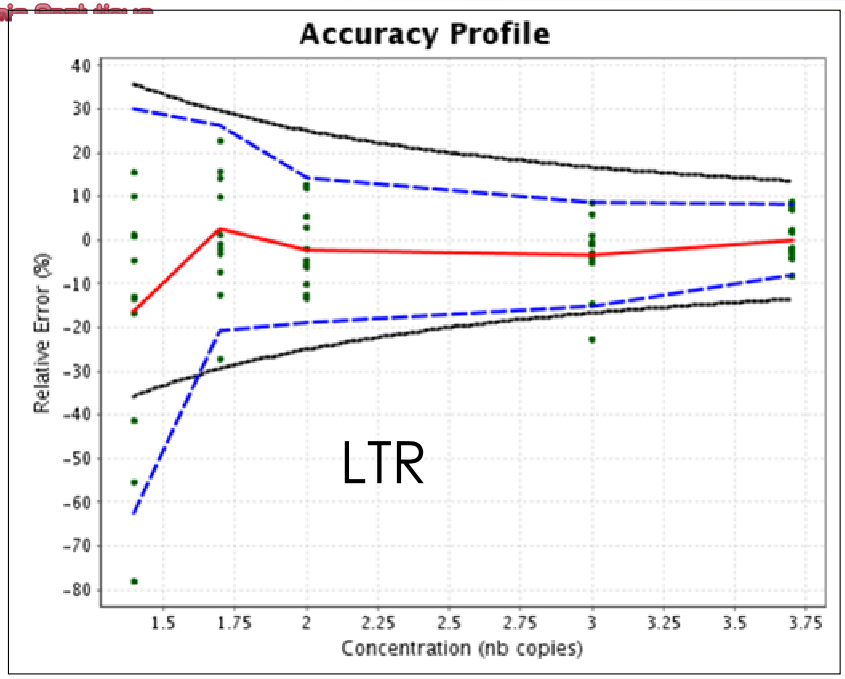
Boemer *et al.*, *J. Chromatogr. B*, 877, (2009), 2412-2417

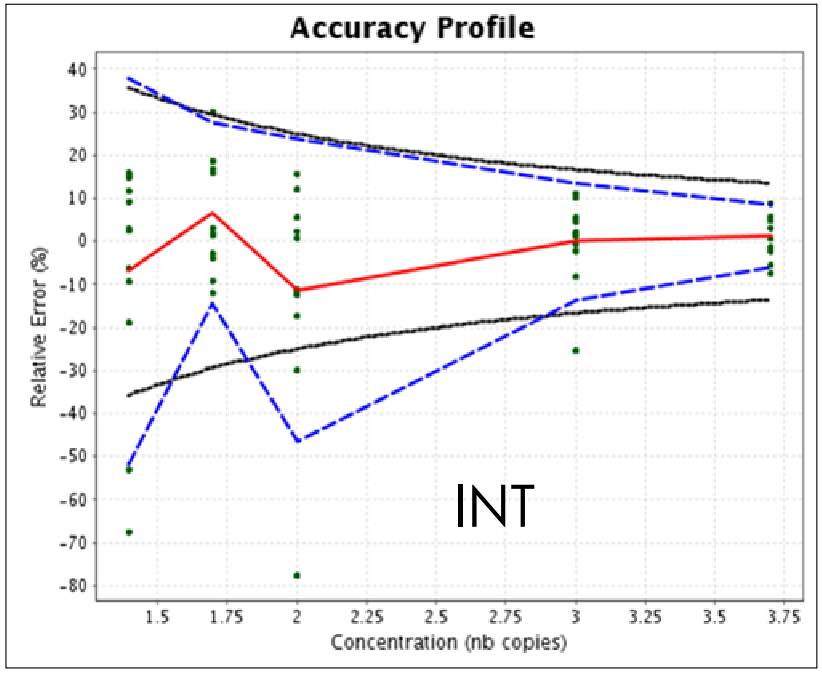
Viral activity : Validation

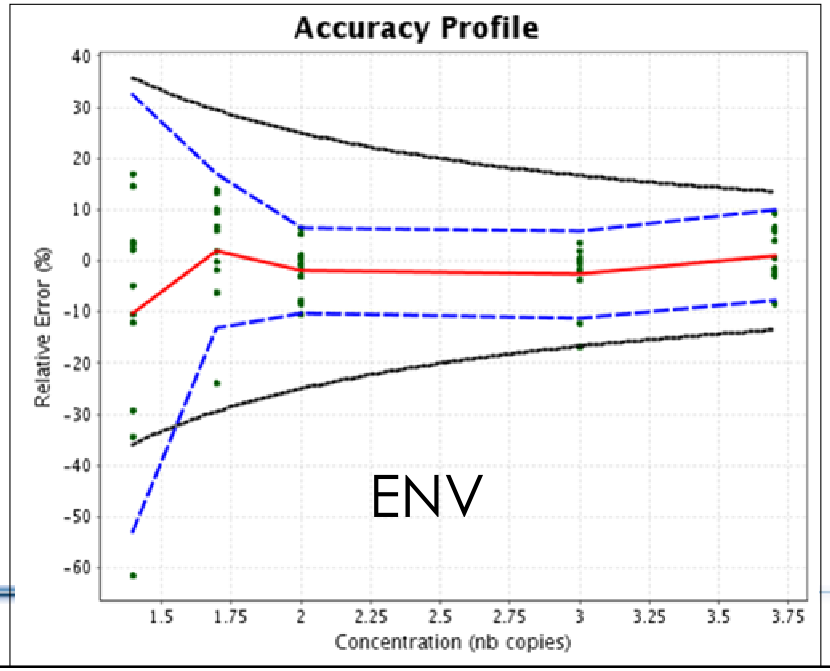


Gibelin *et al.*, *J. Chromatogr. B*, 877, (2009), 2407-2411

Q-PCR of 3 HIV genes: validation







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

Content

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

Measurement uncertainty

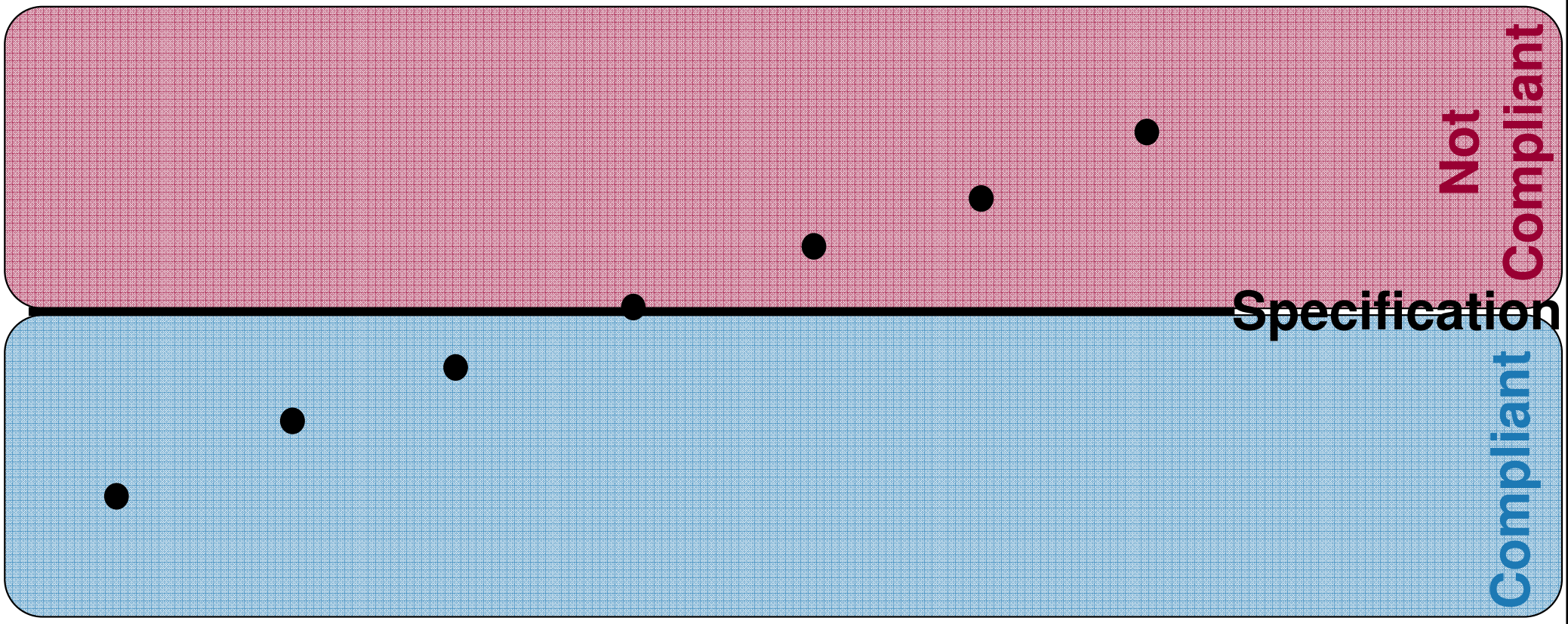
- The method is valid, is this enough ?

- Need measurement uncertainty:

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

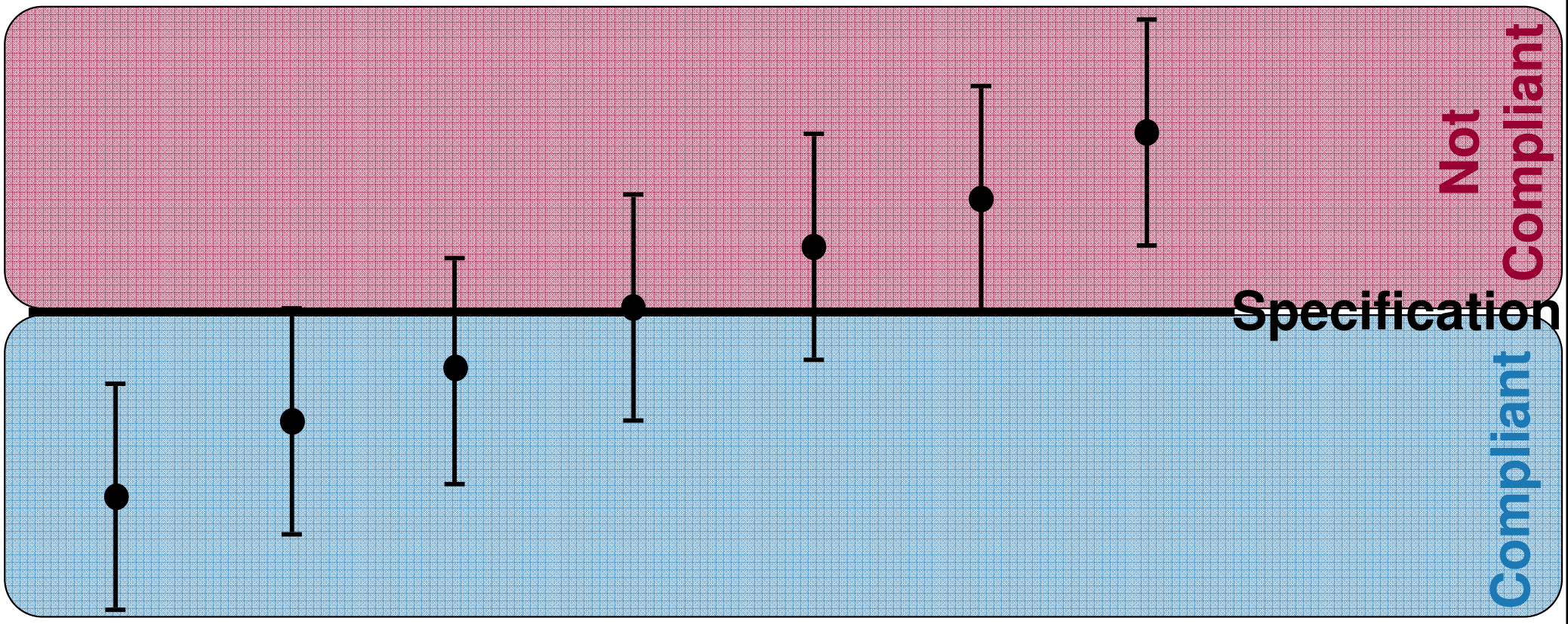
- to:
  - **Interpret** adequately results
  - **Compare** results between them

Measurement uncertainty



(a)	(b)	(c)	(d)	(e)	(f)	(g)	
OK	OK	OK	OK	NOK	NOK	NOK	"shared risk"

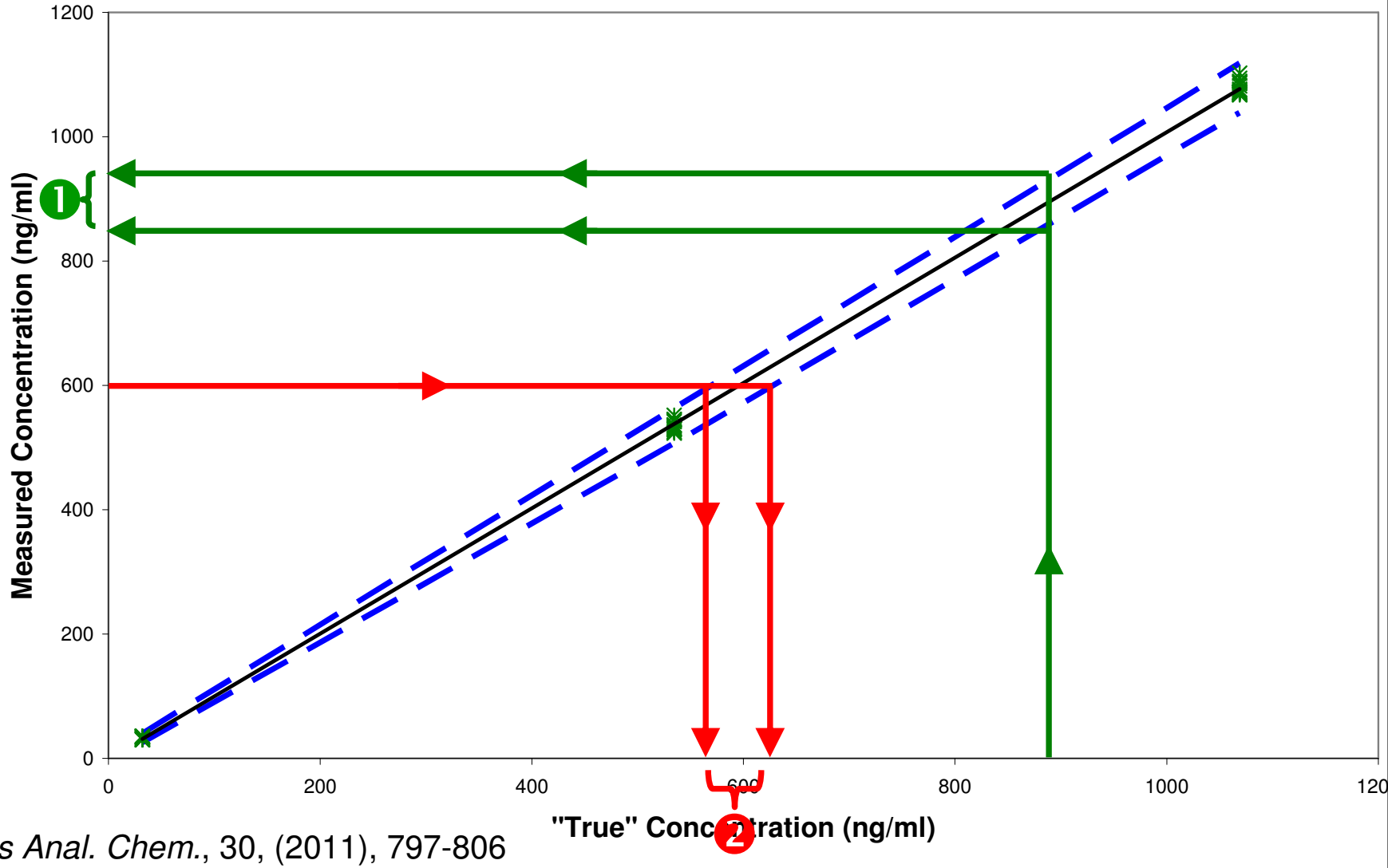
Measurement uncertainty



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

Measurement uncertainty

- Use Method Validation Data:



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

Content

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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:

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