Validation of Analytical Methods

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Erasme

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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
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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

Selection

Development

Life Cycle

Routine Use

Guarantees ?

Validation

Reliability ?
Result in routine

$X_i = \text{observed result} \quad \mu_T = \text{True unknown value}$

$x_i = \mu_T + \text{Errors}$

Acceptable?
\( X_m = \text{mean of observed results} \)

\( \mu_T = \text{unknown true value} \)

Random Error

Systematic Error

Unknown during Routine analyses
Analytical Errors

- $X_m$ = mean of observed results
- $\mu_T$ = unknown true value
- $X_i$ = observed result

$$x_i = \mu_T + \text{Errors}$$

Total Error
- Systematic Error
- Random 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.

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

Bias

% Bias < 3%

Precision

% CV < 2%

Are you ready to take this risk?
Analytical Method Validation

- Traditional vision:
  - Preliminary Conclusion:

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

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

\(\lambda\) = predefined acceptance limits

\(\pi_{\text{min}}\) = minimum probability that a result will be included inside \(\pm \lambda\)

The aim of validation is evaluating 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.

\[ E_{\hat{\delta}, \hat{\sigma}} \{ P[|X_i - \mu_T| < \lambda] \mid \hat{\delta}, \hat{\sigma} \} \geq \pi_{\text{min}} \]
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.

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

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

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

**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

Response/Signal

LLoQ Medium High

Concentration level

J repetitions

Series 2

Response/Signal

LLoQ Medium High

Concentration level

Series I

Response/Signal

LLoQ Medium High

Concentration level

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
Traditional Approaches:

Separate evaluation of methods Trueness and Precision and comparison to predefined acceptance limits ($\lambda$).

- **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:

\[-\lambda_{\text{Tru}} \quad \hat{\delta} \quad +\lambda_{\text{Tru}}\]

Precision:

\[RSD_{I.P.} \quad +\lambda_{\text{Pre}}\]
Descriptive: performance

Valid methods but Poor results

Difference Approach

$H_0 : \delta = 0$

$H_1 : \delta \neq 0$

No rejection of $H_0 \Rightarrow$ Method valid !?

Rejection of $H_0 \Rightarrow$ 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:

- $\lambda_{Tru}$

Bias (%)

Precision:

$RSD_{I,P}$

RSD (%)

Confidence Interval (C.I.) of the bias

Upper Limit of the $RSD_{I,P}$ C.I.
Example

**Trueness**

**Paracetamol**
- 200 µg/ml
- 400 µg/ml
- 600 µg/ml

**Codeine**
- 20 µg/ml
- 25 µg/ml
- 30 µg/ml

**Precision**

- 1.8
- 1.0
- 0.3
- 0.3
- 0.8
- 1.0

-2% 0 +2% 0 +3%
Equivalence: performance

\[ \lambda_{RSD, p} = \ldots \]

- 15% risk
- 95%
- 75%
- 55%
- 35%
- 15%

Valid methods
but Poor results

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** \((\beta TI)\)

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 \(\beta\) (ex. 80%).

βTI : performance

15% risk

Accuracy Profile

### Validation experiments

**Bias**

\[ biais_j = \hat{\mu}_j - \bar{x}_j \]

**Intermediate Precision**

\[ \hat{\sigma}_{w,j}^2 + \hat{\sigma}_{B,j}^2 \]

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**Total error**

\[ E_{\mu,\sigma} \{ \Pr[|x_i - \mu| < \lambda] / \hat{\mu}, \hat{\sigma} \} \geq \beta \]

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**Predictive Interval** with a known risk

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

\( \lambda = \) acceptance limits of e.g. 5%
Example

**Paracetamol**

- 200 µg/ml
- 400 µg/ml
- 600 µg/ml

**Codéine**

- 20 µg/ml
- 25 µg/ml
- 30 µg/ml

Relative Error (%)

\[-5\% \beta = 0.95 \ +5\%\]
Example

Paracetamol

Codéine
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


Capillary Electrophoresis

NIR

Colorimetric
ELISA: Validation

LOQ=31.3 mIU/L

Accuracy Profile

Relative Error (%)

Concentration (mIU/L)

Weighted (POM) Power Regression

LOQ=62.5 mIU/L

Accuracy Profile

Relative Error (%)

Concentration (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)
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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

OK  OK  OK  OK  NOK  NOK  NOK  "shared risk"
Measurement uncertainty

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

+U OK NOK NOK NOK NOK NOK NOK "full risk"
• Use Method Validation Data:

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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 **$\beta$-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

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