VERIFICATION, VALIDATION AND METHOD PERFORMANCE

Dr. Marios Kostakis

National and Kapodistrian University of Athens

Member of Method validation Working group

1

Outline

- **Terminology and definitions**
- **Method's Performance Characteristics**
- **Overview about Performance characteristics**

2

Terminology

Verification: provision of objective evidence that a given item fulfills specified requirements (TAM)

Validation: verification, where the specified requirements are adequate for an intended use (TAM)

Which are the boundaries between the validation and verification?

3

Verification vs Validation

Verification:

Standard methods of ISO, and national standards.

Validation:

in-house methods/methods for literature (scientific papers):

Non-standard methods

Laboratory-designed/developed methods

Standard methods used outside their intended scope

Amplification and modification of standard methods.

Method modification: Validate or verify?

4

Method Performance Characteristics

How should methods be validated?

Extend of validation studies

Selectivity
Detectability
Limit of Detection (LOD)
Limit of Quantification (LOQ)
Working Range
Linearity
Trueness
bias, recovery
Precision
repeatability, intermediate precision, reproducibility
Ruggedness or Robustness

5

Method Performance Characteristics

How should methods be validated?

Extend of validation studies

Performance characteristic	Type of analytical application			
	Identification test	Quantitative test for impurity	Limit test for impurity	Quantification of main component
Selectivity	x	x	x	x
Limit of detection			x	
Limit of quantification		x		
Working range including linearity		x		x
Trueness (bias)		x		x
Precision (repeatability and intermediate precision)		x		x
NOTE The table is simplified and has been adapted to the structure and terminology used in this Guide.				

6

3

HISTORICAL

Decade 1990s

Introduced the term "Validation", the first horizontal guidelines for the Validation of Analytical Methods are issued. Eurachem Guide:

The Fitness for Purpose of Analytical Methods (1st ed.) (1998)

Decade 2000s

The importance of Validation is strengthened, more instructions are issued for the validation of analytical methods (EC 657/2002) as well as the performance criteria (EC 333/2007, EC 401/2006, etc.).
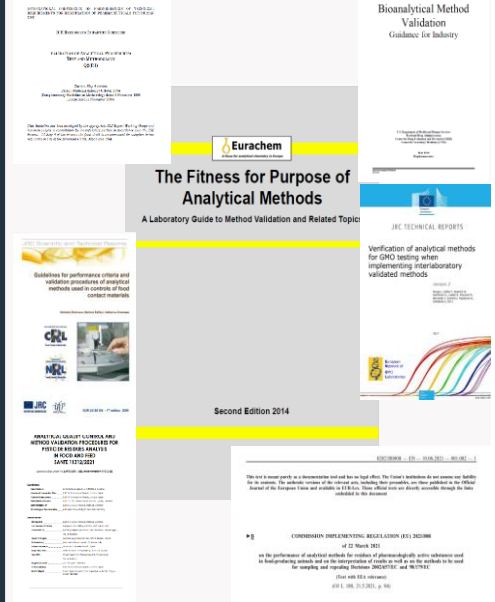
Decade 2010s

Sector specific guidelines were issued (pesticides, toxicology, food and animal feed, materials in contact with food, etc.).

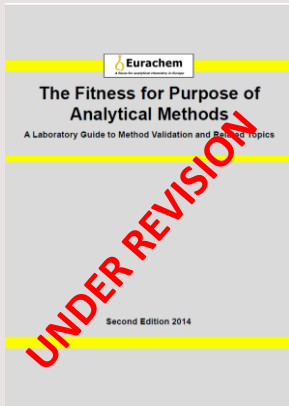Older versions are revised, more experience from laboratory.

Decade 2020s

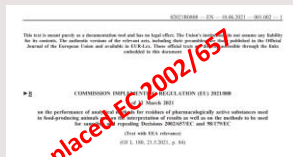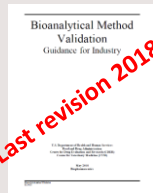Revision of EC 2002/657 from EC 2021/808.

…

7

GUIDELINES

- **Horizontal Guidelines**

- Eurachem, The fitness for purpose of Analytical Methods, 2nd edition 2014

- **Sector-specific Guidelines**

Pharmaceurical Sector

- FDA, Bioanalytical Method Validation, 2018

- ICH, Validation of Analytical Procedures, 2024

Food and Feed

- SANTE for pesticides. 2021, revision every 2 years.

- EC 2021/808, performance of analytical methods for residues of pharmacologically active substances, replace EC 2002/657.

- EC 2006/401, mycotoxins (under revision, draft online)

8

4

Selectivity

Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques

Relative intensity (% of base peak)	GC-...	CI-GC-MS, GC-MSⁿ LC-MS, LC-MSⁿ (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± ...	± 30 %
≤ 10 %	± 50 %	± 50 %

EC 2021/808:+/- 40%
SANTE: +/- 30%

- **Selectivity:** *property of a measuring system, used with a specified measurement procedure, whereby it provides measured quantity values for one or more measurands such that the values of each measurand are independent of other measurands or other quantities in the phenomenon, body, or substance being investigated (TAM 4.2)*

- **Assessment:** From the simplest case (to prove that blank is blank) to the most complicate such as in mass spectrometry (identification criteria: Retention time, identification points, ion ratio)

- Selectivity based on the detection system.
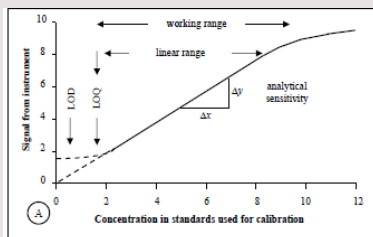
- **Specificity vs Selectivity**

- **Specificity:** *the ability to provide signals to effectively identify the analyte.*

- Biochemical methods and immunochemical methods

- It is not recommended for general purpose use. Many times, both terms are used interchangeable

9

Working Range (1)

Or **Measuring interval**: *set of values of quantities of the same kind that can be measured by a given measuring instrument or measuring system with specified instrumental measurement uncertainty, under defined conditions*

- It is not only the linear range.
- From the LOQ to the point that unacceptable change of measurement uncertainty.
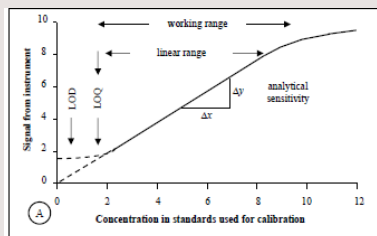
Assessment: Very often Correlation coefficient (R) is used.

Even it is the most used criterion, **it is not recommended** for linearity but only for correlation!
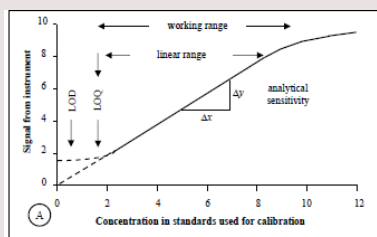
Instead:

10

Working Range (2)

- **Proposed criteria:** Residual plots, Relative back-calculated error (%RE):
- $\frac{C_{measuered} - C_{true}}{C_{true}} x100$
- **FDA, bioanalytical method:** +/- 15% RE, +/- 20% RE near to LOD
- **SANTE, EURL-POPs:** +/- 20% in all range of concentrations

More details: Raposo F., Trend in Analytical Chemistry, 77, 2016, 167-185, doi: http://dx.doi.org/10.1016/j.trac.2015.12.006

11

Working Range (3)

To weight or not weight?

Weighting calibration is useful in mass spectrometry, due to the differences in variation at low and high concentrations.

General rule: a weight $1/x^2$ is the most appropriate in mass spectrometry.

No criteria for the selection of the appropriate weight.

It not easy to perfom weighting calibration in Microsoft Excel, but all available mass spectrometer softwares used for quantification have the option for weighting calibration.
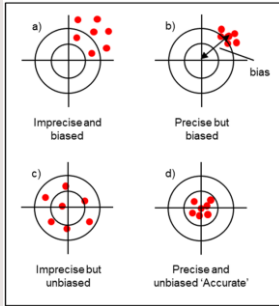
More details:

Dolan, J. W., LCGC, 27, 7, 2009,153-540

Almeida, A. M., et al, J Chromatogr. B, 774, 2, 2002, 215-222. **doi**: 10.1016/s1570-0232(02)00244-1
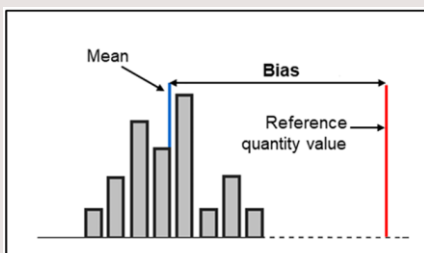
12

Accuracy

Measurement accuracy: *closeness of agreement between a measured quantity value and a true quantity value of a measurand (TAM 4.7)*

Accuracy influenced by random and systematic effects on results.

Simple words: Accuracy= Trueness and Precision.

13

Trueness

Measurement trueness: *closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value (TAM 4.5)*

Assessment: (certified) Reference Material, spiking samples, alternative well-defined method. Calculation of bias, absolute or relative (recovery).

No substantial changes : *general rule for acceptable limits of recovery: 80-120% but as the detection capability of technique becoming better the criteria are changing*

Mass fraction	Criteria (EC 2021/808)
<1 µg/kg	50-120 %
>1-10 µg/kg	70-120%
≥10 µg/kg	80-120%

14

Precision
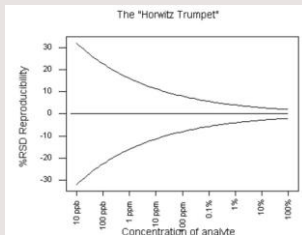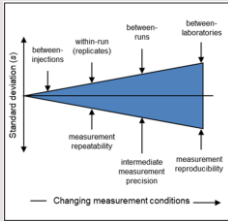
Measurement precision: *closeness of agreement between indications or measured quantity values obtained by replicate measurements on the same or similar objects under specified conditions (TAM 4.6)*

Assessment: 6-15 replicates for each material and concentration level, in different conditions: repeatability, intermediate and reproducibility conditions.
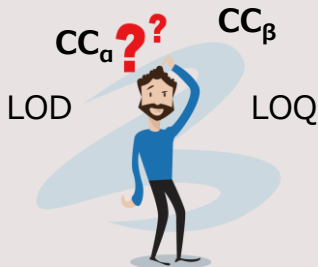
Calculating Relative standard deviation (or through ANOVA)

Criterion: Horwitz equation or HorRat

Mass fraction	Criteria (EC 2021/808)
<1 µg/kg	16 % (Horwitz)
>1-10 µg/kg	22 % (Horwitz)
≥10 µg/kg	25 % (*)
<10 µg/kg	30 % (*)

* The %RSD presented is guideline and should be as low as reasonably possible

15

LOD/LOQ, CCα/CCβ

CC_α **?** CC_β

LOD   LOQ

There are many ways to determine LOD: S/N, calibration curve, spiking at low concentrations, etc.

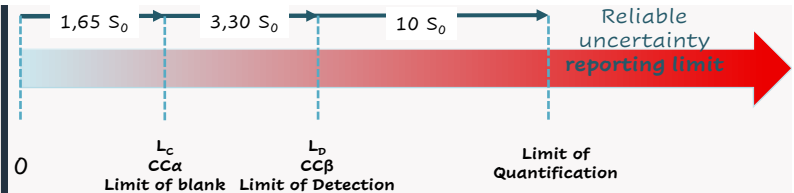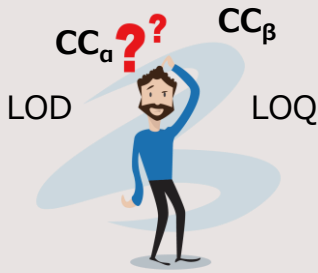**Different approaches lead to different results.**

More details: Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food, EU Technical Report.

Official control:

- comparison with the limits.

- Experimental approach: The lowest concentration level where precision and trueness are reliable (reliable uncertainty).

16

LOD/LOQ, CCα/CCβ

$1,65\ S_0$ → $3,30\ S_0$ → $10\ S_0$ →

Reliable uncertainty

reporting limit

0 L_C / $CC\alpha$ / Limit of blank L_D / $CC\beta$ / Limit of Detection   Limit of Quantification

CC_α/CC_β: another approach to find the lowest reliable concentration, especially used in veterinary drugs residues.

Many simplifications take place to calculate CC_α/CC_β

In many cases that there is MRL, are decision rule and not the limit of detection.

EC 808/2021: Changes that include the uncertainty in calculations => definitely decision rule.

e.g. $CC_\alpha = LCL + k(99\%) * u(combined)$

More details: Van Loco J. et al, Anal Chim Acta, 586, 2007, 8-12
doi:10.1016/j.aca.2006.11.058

CC_α **??** CC_β

LOD   LOQ

17

Other Parameters

Ruggedness (or Robustness) => no any change

Robustness vs Ruggedness.

Matrix Effect (ME) => included in the new EC 2021/808

- Indication of signal suppression or enhancement
- Very useful in mass spectrometry techniques, especially in LC-MS/MS
- Indication for use standard calibration curve or standard addition curve
- Same meaning as Matrix Factor but different calculation

18

Conclusions

- Validation is the important part of an analytical laboratory.
- Performance characteristics are not changing during the years
- Changes due to Technological development analytical techniques
- With increased experience, laboratories are using more sector-specific and practical ways to validate the method.

19

Thank you for your attention

Questions

HELLENIC REPUBLIC
National and Kapodistrian
University of Athens
EST. 1837

Eurachem
A Focus for Analytical Chemistry in Europe

20