HBM4EU

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Validation of analytical methods

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Outline

- *Definitions*
- *Why measurement procedure must be validated*
- *Approach to validation procedure*
- *Realization of validation*

What is method validation

Method validation is the process of proving that an analytical method is acceptable for its intended purpose.

Ludwig Huber 1998, Validation and Qualification in Analytical Laboratories.

Validation

(ISO/IEC 17025)

is the **confirmation** by examination and the provision of objective evidence that the particular requirements for a specific **intended use** are fulfilled

Difference in terminology

ISO/IEC 17025

- Method: **method validation**

VIM

- Measurement procedure: **procedure validation**

GLP

- Standard operation procedure: **SOP validation**

Why validation?

Method validation is an important requirement in the practice of chemical analysis.

Importance of analytical measurement

- Provides information on procedure

The professional duty of the analytical chemist

- For analyst (the user of the procedure)
- For customer (the user of the results)

Regulatory requests

ISO 17025 requirement

Validation

Why do we need it?

ISO/IEC 17025

- Laboratories should demonstrate they operate within quality system, are technically competent and are able to generate technically valid results.

- Method (procedure) validation
- Traceability of results
- Uncertainty of results

Validation

Full validation

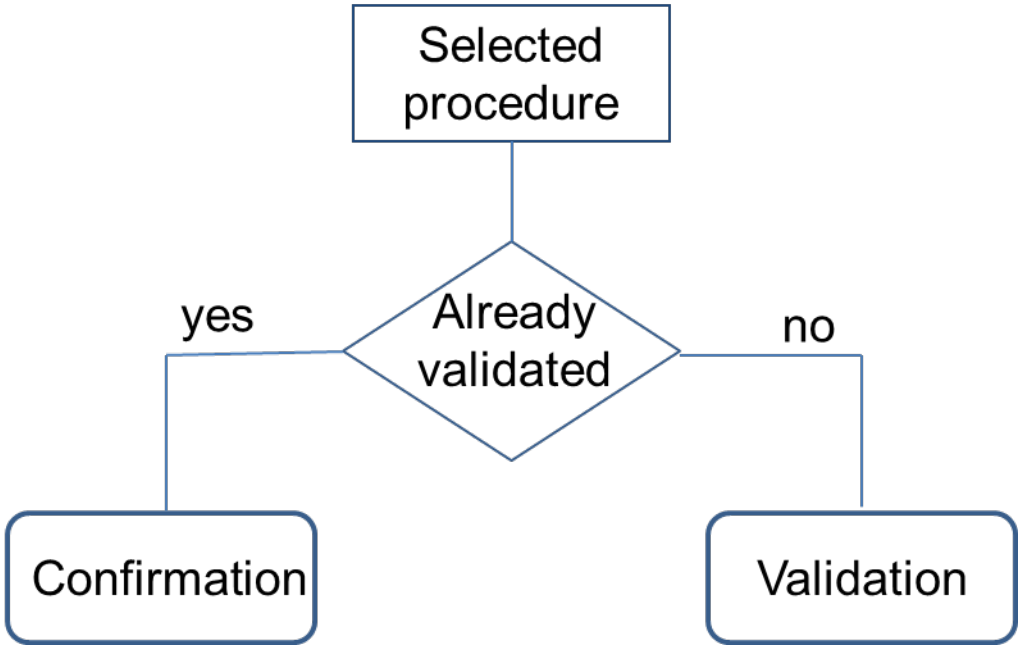
- All procedures used in the lab must be validated

Confirmation

- Procedures published as international, regional or national standards are considered to be validated

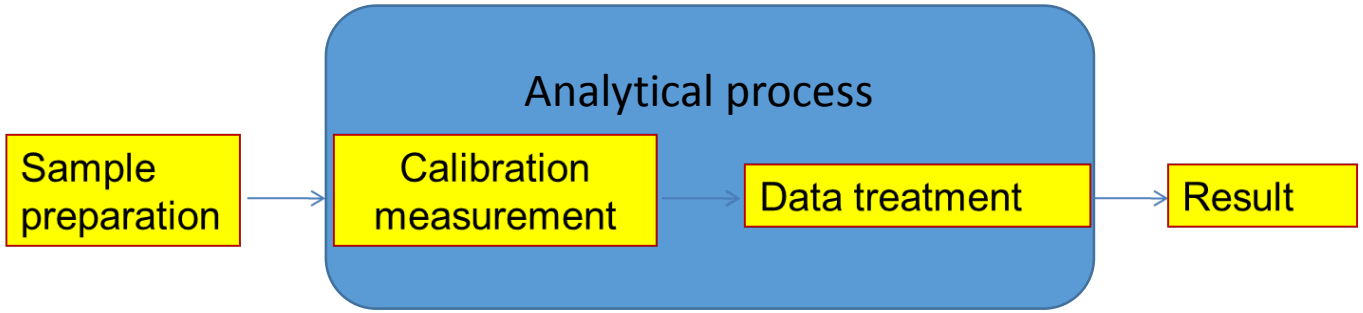
Procedures published in scientific journal are not standard procedures

Validation



- Non standard method
- New *in-house* developed
- Standard ones used outside their intended scope
- Revision of established methods
- Modify standard

Validation



Whole procedure

Full concentration range

All intended types of matrices

What must be considered in Method validation

Precision

Trueness

Accuracy

Limit of detection

Limit of quantification

Selectivity/specificity

Linearity and range

Ruggedness (or Robustness)

Sensitivity

Recovery

Validation of the method

Before validation you have to define:

- the requirements of the measurement procedure
- scope of experiments
- RM to be used
- Equipment
- Statistical tools to be used
- personnel for performing experiments and evaluating obtained results

Precision

It is the measure of the degree of repeatability of an analytical method under normal operation

Precision shows how close results are to one another

Precision

Repeatability and *reproducibility*: analysis of **routine urine samples** through the course of one year

2 replicates of each sample

Standard deviation of replicate measurements, separately for levels close to limit of quantification (LOQ), median and high levels

$$RSD_d = \frac{s_d}{\sqrt{n}} \qquad (n=2)$$

Precision

Example:

Urine samples,

concentrations close to LOQ

N=57

RSD=4.74%

Date of measurement	measurement 1, D1	measurement 2, D2	Average	D1-D2	(D1-D2)/ average
	µg/L		µg/L	µg/L	
29.1.2008	0,51	0,48	0,50	0,03	0,06
31.1.2008	0,53	0,50	0,52	0,03	0,06
6.2.2008	0,56	0,50	0,53	0,06	0,11
14.2.2008	0,42	0,43	0,43	-0,01	-0,02
21.2.2008	0,55	0,57	0,56	-0,03	-0,05
13.3.2008	0,50	0,52	0,51	-0,02	-0,04
18.3.2008	0,49	0,59	0,54	-0,11	-0,20
19.3.2008	0,60	0,55	0,58	0,05	0,09
20.3.2008	0,57	0,61	0,59	-0,04	-0,07
25.3.2008	0,61	0,62	0,61	-0,01	-0,02
27.3.2008	0,76	0,79	0,78	-0,03	-0,04
28.3.2008	0,58	0,64	0,61	-0,06	-0,10
1.4.2008	0,54	0,54	0,54	0,00	0,00
2.4.2008	0,60	0,66	0,63	-0,06	-0,09
3.4.2008	0,65	0,73	0,69	-0,08	-0,11
4.4.2008	0,56	0,65	0,61	-0,09	-0,15
24.4.2008	0,58	0,57	0,57	0,01	0,02
25.4.2008	0,65	0,67	0,66	-0,03	-0,04

… n=57

Trueness

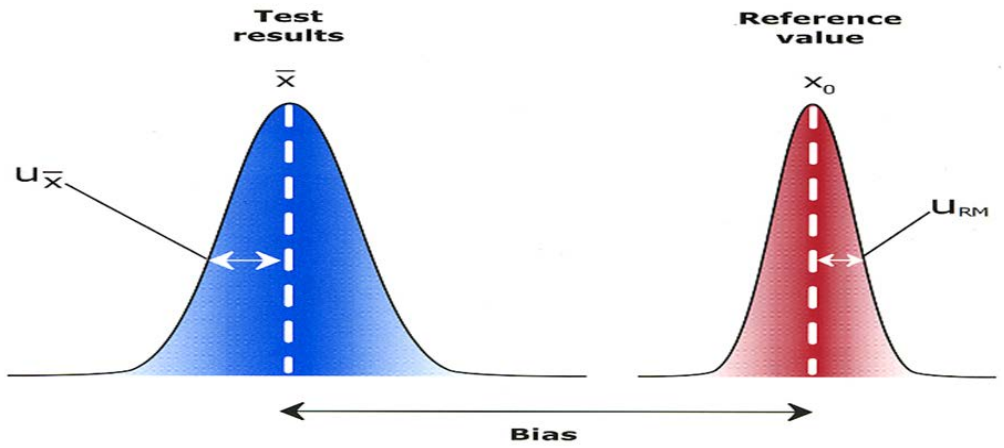
Closeness of agreement between the average of an infinite number of replicate **measured quantity values** and a **reference quantity value.**

Estimation of trueness by:

- Using Certified Reference Materials
- Using RM or *in-house* materials
- Using Reference methods
- Results from proficiency testing
- Spiked samples

Bias

Bias is a quantitative expression of trueness.



Picture outline from: In House Method Validation, LGC

The trueness of result improves when bias decreases.

Recovery

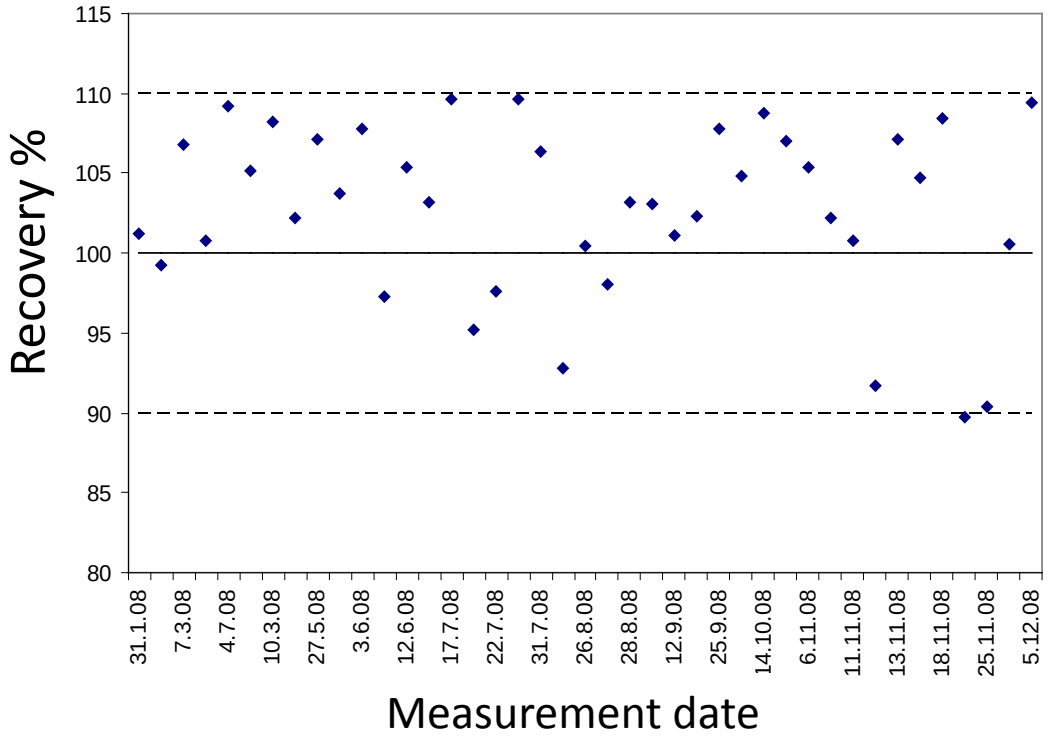
A measure of the trueness of a measurement procedure

$$R = \frac{observed\ value}{reference\ value}$$

- CRM
- Spike of pure substance

Recovery

Example: Standard addition method



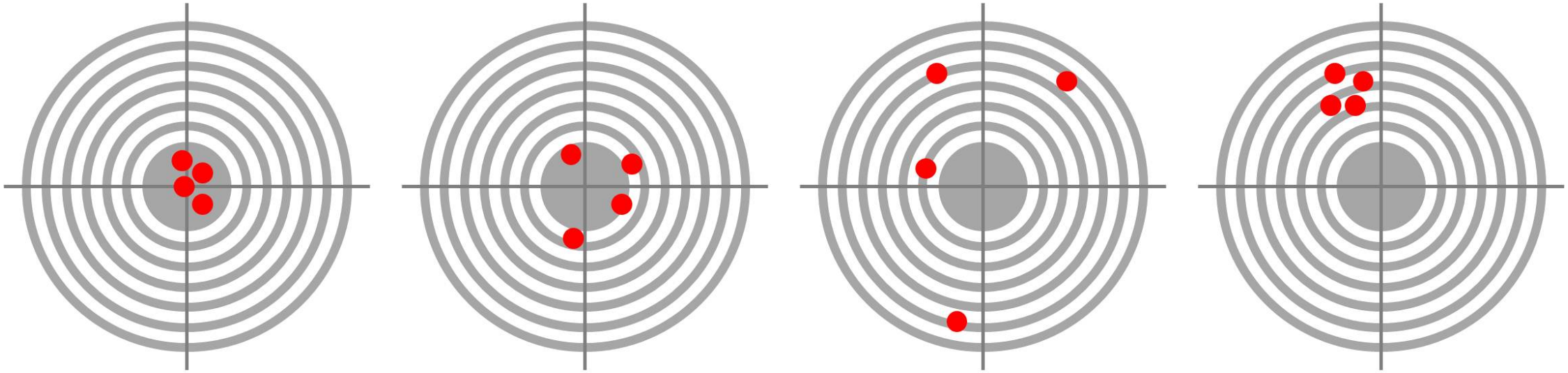
$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{spike}} = 0.98$$

Accuracy

- Closeness of agreement between a measured quantity value and a “true” quantity value of a measurand.

- Describes the measure of exactness of an analytical method.

Precision and trueness = accuracy



High accuracy, good precision, good trueness

Low accuracy, poor precision, good trueness

Low accuracy, low precision, poor trueness

Low accuracy, high precision, poor trueness

Limit of detection

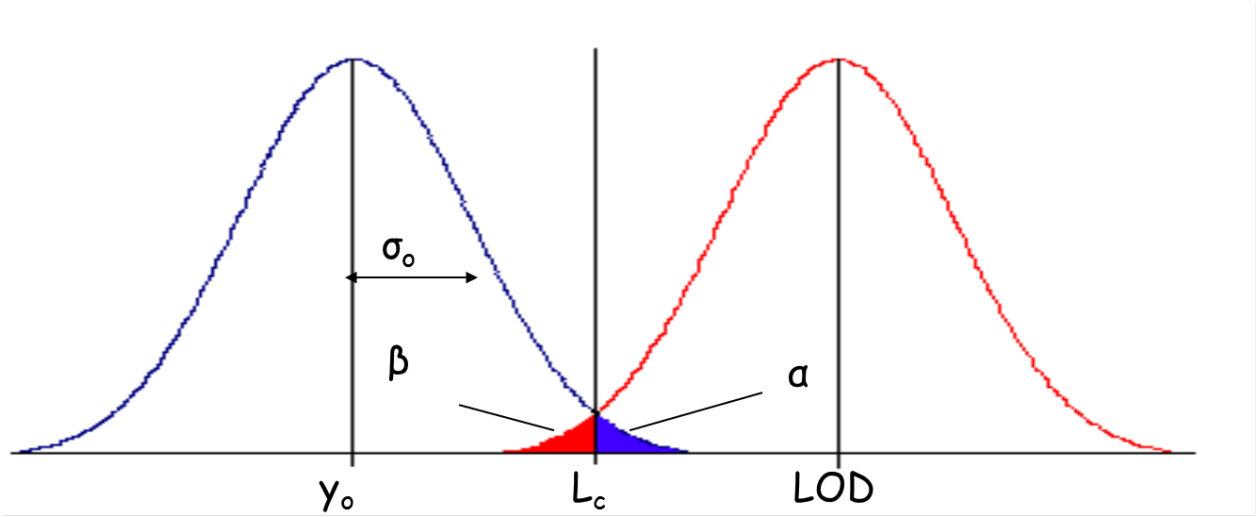
LOD is defined as the lowest concentration of an analyte in a sample that can be distinguished from a blank

$LOD = B+3S_0$ or $0+3S_0$

B=Blank

S_0=standard deviation of 10 blank measurements

Limit of detection



Y_o -Signal equal to the blank signal
L_c- decision level
LOD- limit of detection

Limit of quantification

LOQ is defined as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy under the stated operational conditions of the method

$$LOQ = B+10S_0$$

Limit of detection, limit of quantification

Example: Measurement of 10 blank samples

$$c_{blk} = \frac{h_{blk} - h_{BB}}{h_{STD} - h_{BB}} \cdot \frac{1}{V_{blk}} \cdot c_{STD} \cdot V_{STD} \cdot 1000$$

h_{blk} – blank signal (height in mm)
h_{BB} – measurement procedure blank signal
h_{STD} – standard solution signal
c_{STD} – concentration of standard solution
V_{blk} – volume of blank sample
V_{STD} – volume of standard solution

$$LOD[\mu g/L] = 3s(c_{blk}) = 0.17\ \mu g/L$$

$$LOQ[\mu g/L] = 10s(c_{blk}) = 0.58\ \mu g/L$$

Sample	THg [µg/L]
blk 1	0.53
blk 2	0.45
blk 3	0.52
blk 4	0.45
blk 5	0.39
blk 6	0.43
blk 7	0.52
blk 8	0.47
blk 9	0.35
blk 10	0.45
Average	**0.46**
Standard dev. (s)	**0.06**

Selectivity / specificity

Selectivity refers to the extent to which method can be used to determine particular analytes in mixtures of matrices without interferences from other components of similar behaviour.

Discrimination between the analyte and closely related substances

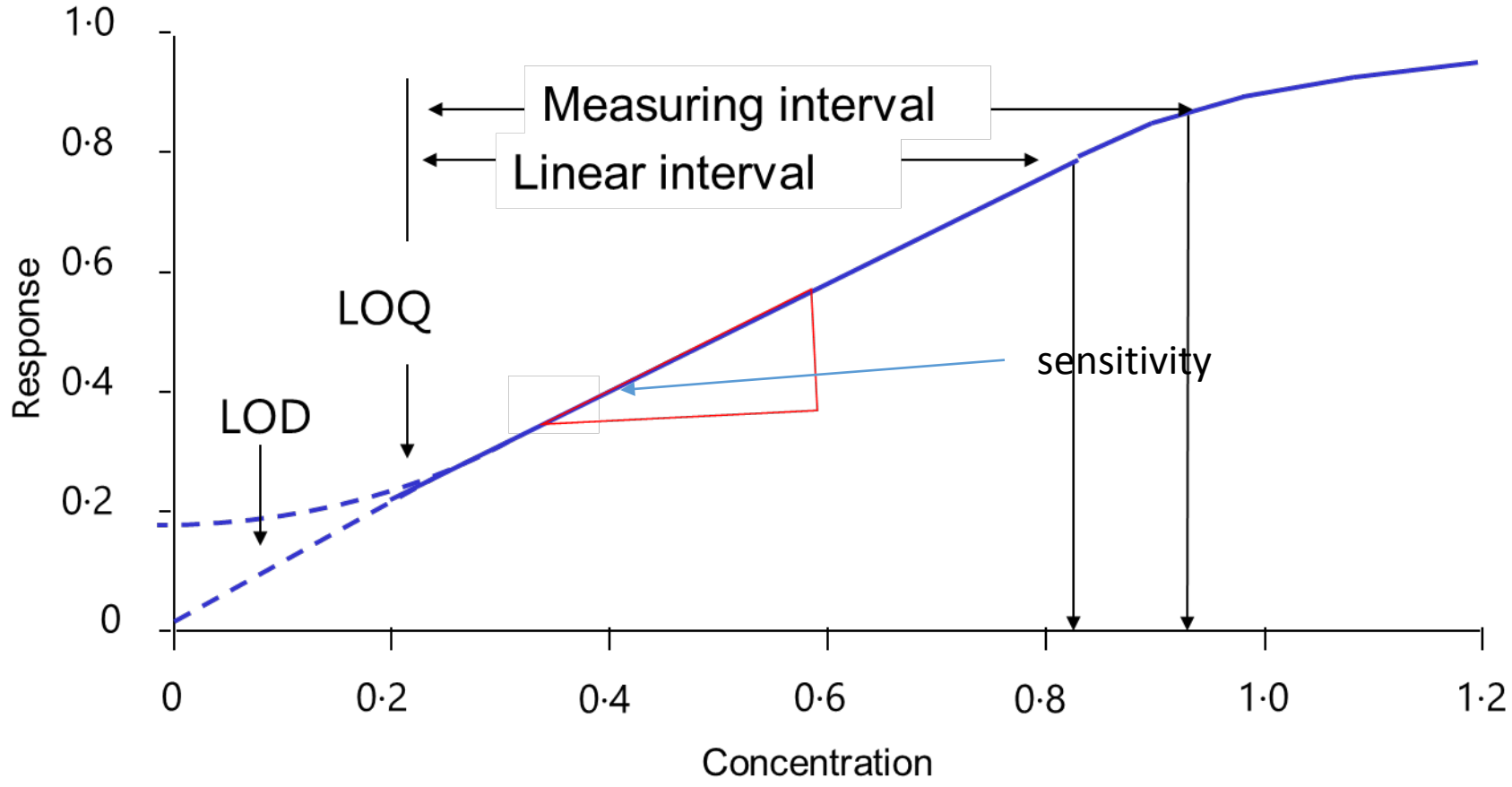
- The potentially interfering substances must be chosen
- The relevant blank sample must be analysed
- Presence of possible interferences must be detected
- Estimation of the effect of interferences must be done

Sensitivity

The sensitivity of method is the rate of change of the measured response with change in the concentration of analyte

Linearity and range

The ability of the method to obtain test results which are proportional to the concentration of the analyte



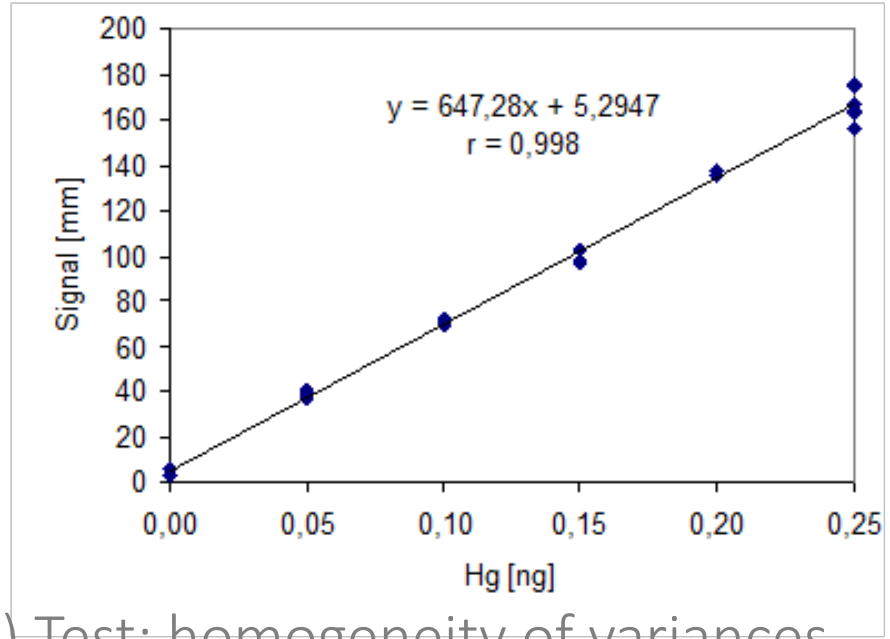
Linearity and range

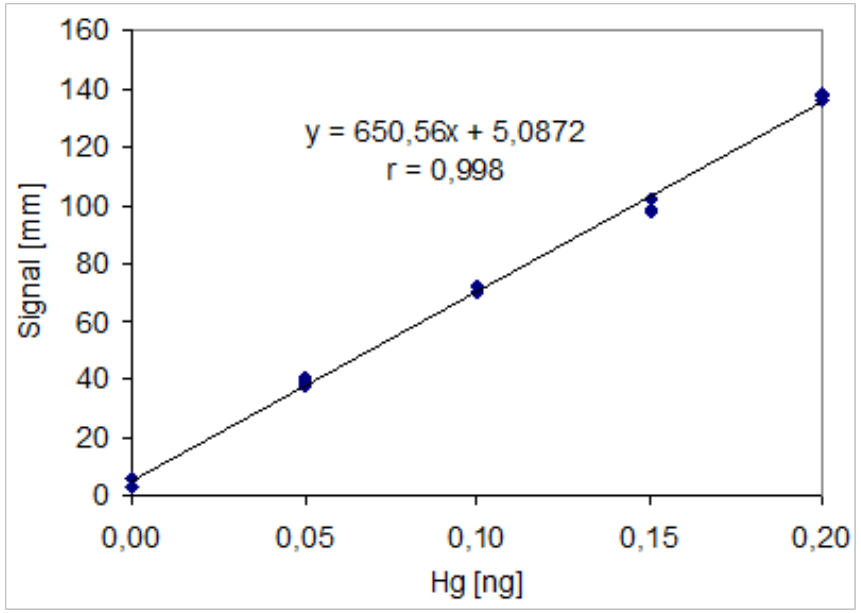
Example:

1) Blank

Added Hg (ng): 0, 0.05, 0.10, 0.15, 0.20, 0.25

2) Linear regression





3) Test: homogeneity of variances

Ruggedness (robustness)

The ruggedness (robustness) of an analytical method is the resistance to change in the result produced by an analytical method when minor deviations are made from the experimental conditions described in the procedure.

Example:

Precision estimated at different concentration levels

RSD_{LOQ}=4.74%

RSD_{median}=3.31%

RSD_{high}=3.27%

$$RSD_{pool} = \sqrt{\left(\frac{(n_1 - 1)\cdot RSD_1^2 + (n_2 - 1)\cdot RSD_2^2 + \ldots}{(n_1 - 1) + (n_2 - 1) + \ldots}\right)}$$

F-test → no significant difference found

RSD_{pool}=4.4%

Measurement uncertainty

Based on model equation, GUM approach & validation data

Details about estimation: *to be presented on Friday, A04*

References

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Speaker's information

Janja Snoj Tratnik works at the Department of Environmental Sciences, Jožef Stefan Institute, Ljubljana, Slovenia. Her backround is in biology. She has 10 years experience in trace element analysis in biological samples, HBM recruitment, database management and statistical evaluation of the results. In HBM4EU she is part of the Cd chemical group.

Prof. Milena Horvat is the Head of the Department of Environmental Sciences at the Jožef Stefan Institute and a Dean of the International Postgraduate School Jožef Stefan. By basic training she is an analytical chemist. She coordinated the implementation of the Slovenian HBM and several research project in the domain of environment and health studies. Within the HBM4EU she is chemical group leader for Cd and involved in Training activities.

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