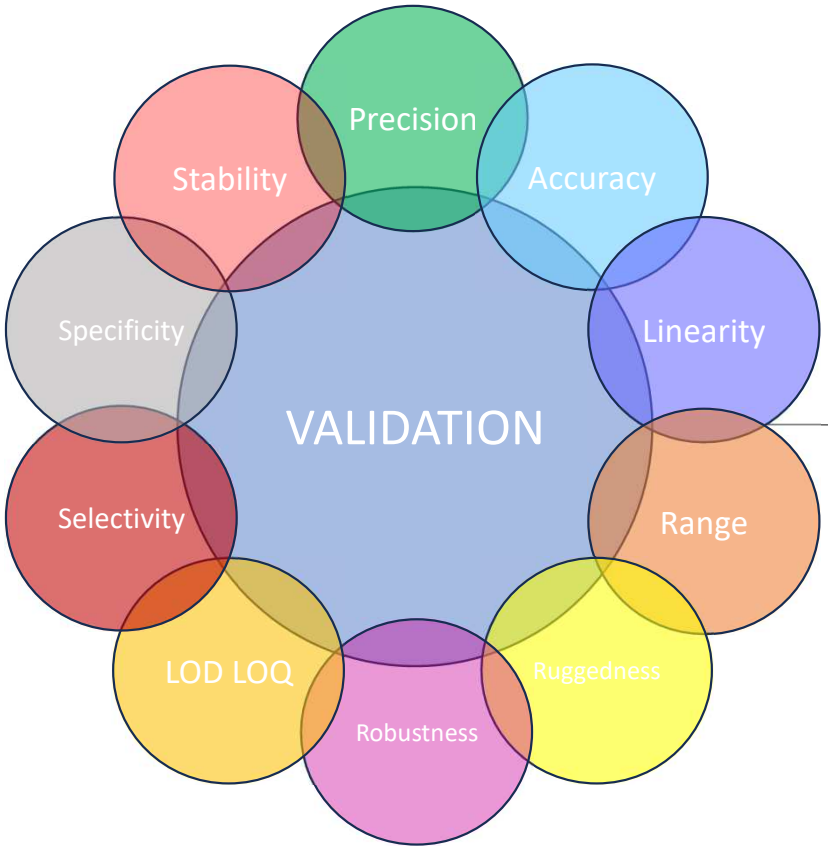


QEL, Spices Board

In-house Training – 1:
Analysis of Pesticide Residues

Dr. Ramesh BN
Spices Board

Lecture - 5 | 07-Aug-2023

Method Validation
↳ *and*
↳ Quality Control
↳ *in*
↳ Pesticide Residue Analysis

Method Validation

↦ The process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

↦ Used to judge the quality, reliability and consistency of analytical results

↦ Is an integral part of any good analytical practice

Analytical quality control (AQC)

↦ Are procedures designed to ensure that validation is maintained during routine analysis

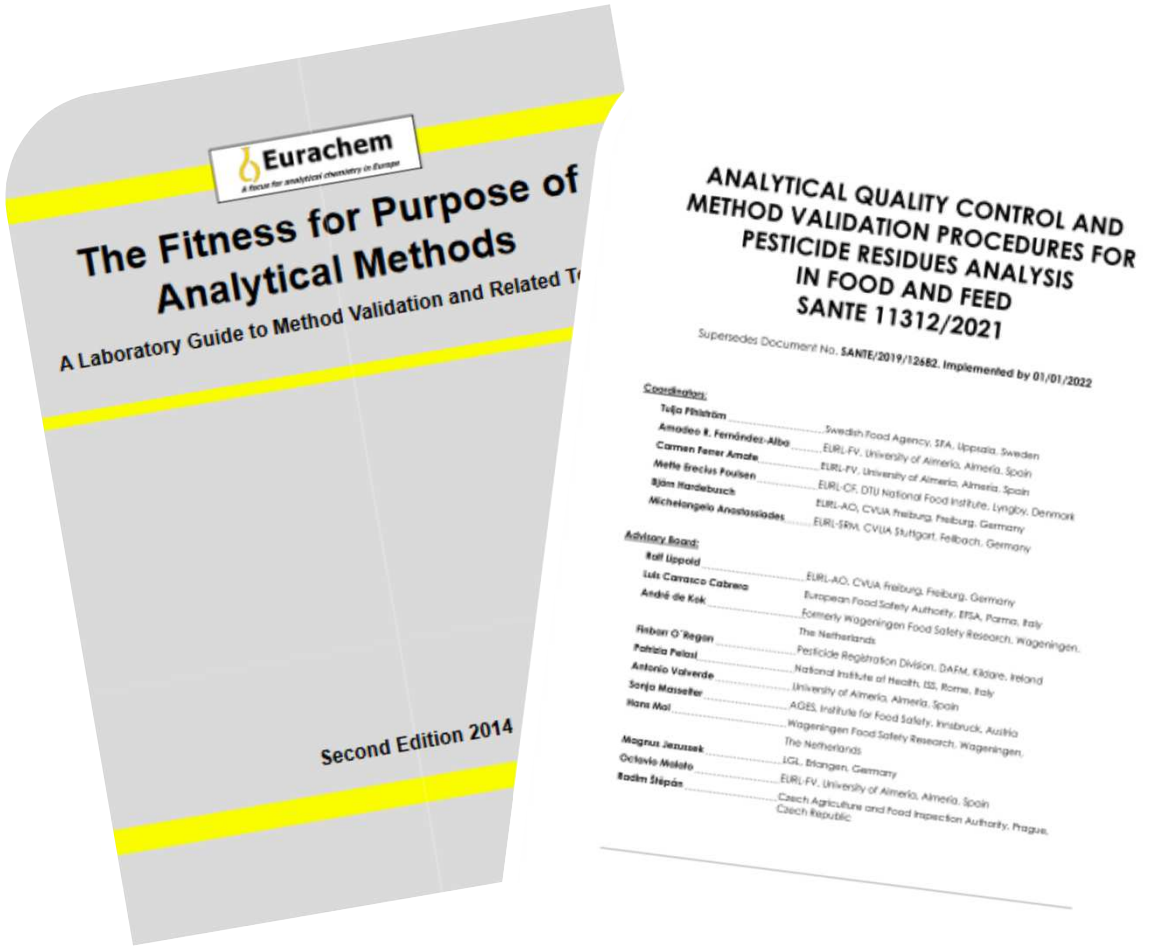
↦ That is, ensure that the results of laboratory analysis continue to be consistent, comparable, accurate and within specified limits of precision

Eurachem Guide

↳ Terms and definitions, basics of procedures

EU DG-SANTE Guide

↳ Specifics on pesticide residue analysis



https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf

https://www.eurl-pesticides.eu/docs/public/tmplt_article.asp?CntID=727

Method Validation

The process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

"Intended use"

Questions arising during operations

- ↦ Is this product of required quality?
- ↦ Is this food safe for consumption?
- ↦ Will there be regulatory issues if this product is exported?
- ↦ Will this product be able to achieve good shelf life?
- ↦ ....
- ↦ ....

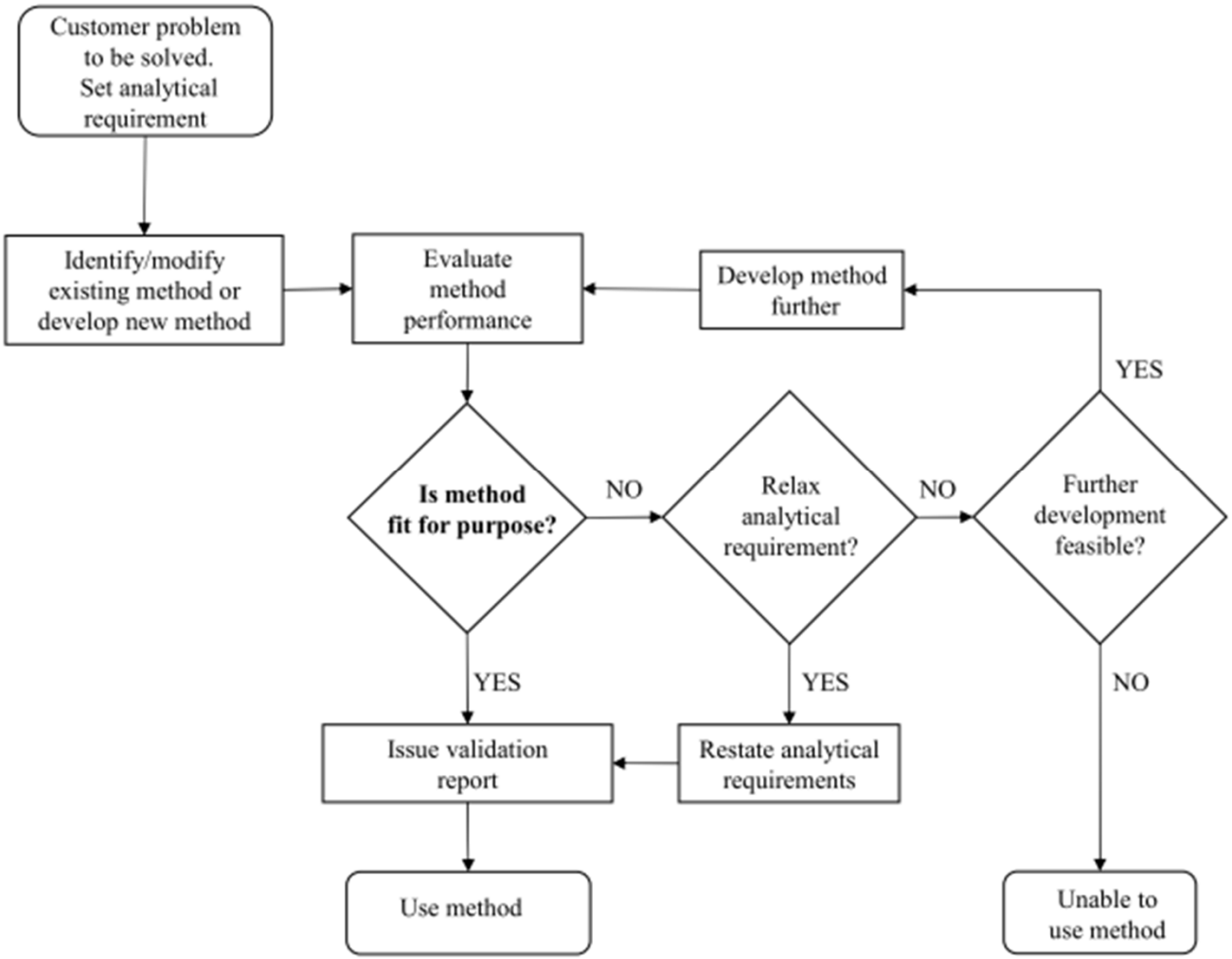
To answer these questions, we need to perform analyses

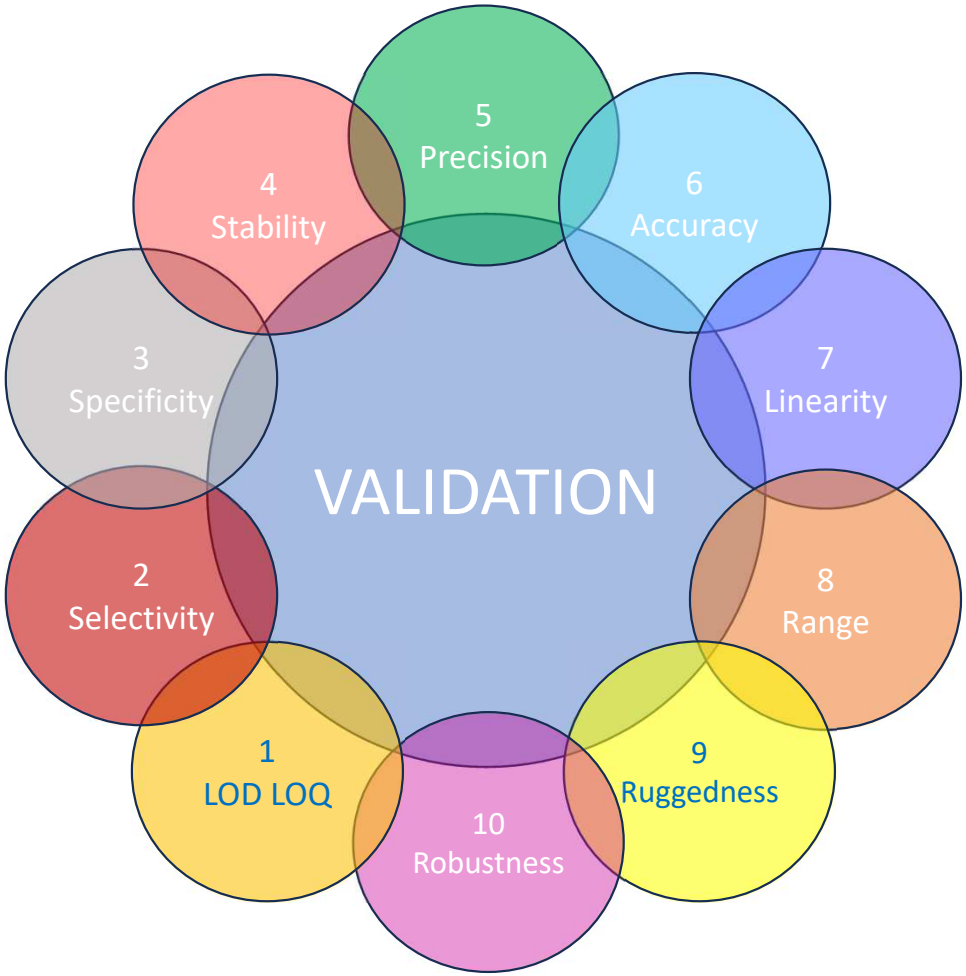
- ↦ Usually requires evaluation of compliance against specific criteria
- ↦ Will lead to decisions with economic and strategic consequences

An analytical method is considered suitable when it is established that the results produced by the method are **consistent**, **reproducible**, and **reliable**.

→ Validation parameters

The process

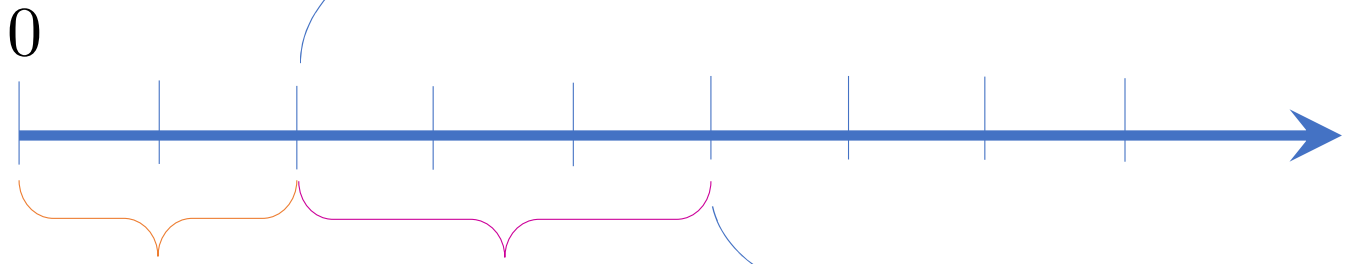

Customer problem to be solved. Set analytical requirement
Identify/modify existing method or develop new method
Evaluate method performance
Develop method further
Is method fit for purpose?
NO
Relax analytical requirement?
NO
Further development feasible?
YES
YES
YES
NO
Issue validation report
Restate analytical requirements
Use method
Unable to use method



Calculate the validation parameters

↳ Assess each parameter against acceptability criteria

LOD, LOQ, RL



LOD: the smallest amount or concentration of analyte that can be reliably distinguished from zero.

Analyte concentration cannot be distinguished from zero, < LOD

LOQ: The lowest concentration of analyte that can be determined with an acceptable accuracy and precision

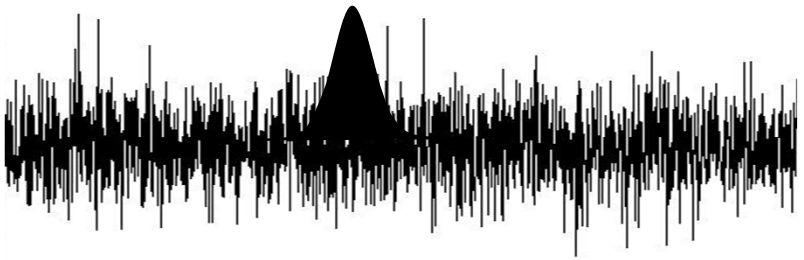
Analyte concentration cannot be determined with acceptable accuracy and precision, < LOQ

Reporting Limit (RL) The lowest level at which residues will be reported as absolute numbers. It is equal to or higher than the LOQ.

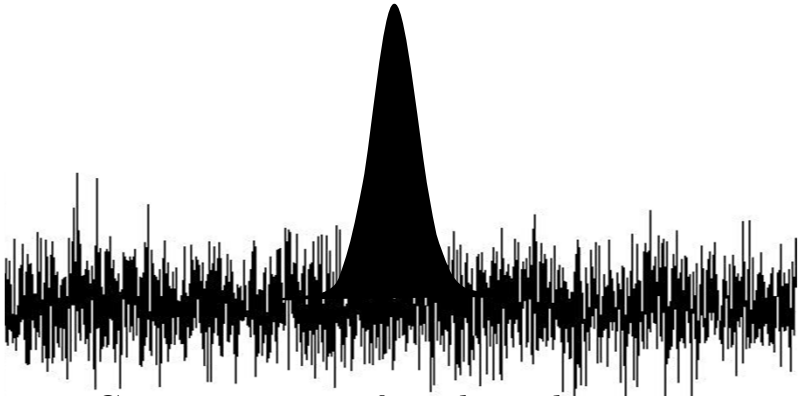
Calculation of LOD and LOQ

The ‘old’ way: Signal to noise ratio

Noise: fluctuation of the baseline



Concentration of analyte that gives response 3 times that of the noise - LOD



Concentration of analyte that gives response 5 times that of the noise - LOQ

Calculation of LOD and LOQ

The 'new' way: DG SANCO

↦ LOD is considered as deprecated (no longer useful)

↦ LOQ emerges directly from validation data

↦ Prepare validation data starting from very low concentration and increasing progressively

↦ The lowest concentration that gives acceptable values for accuracy and precision is the LOQ

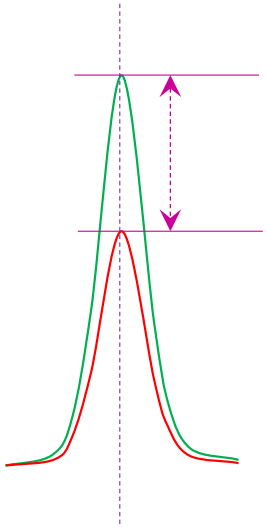
Selectivity

Analytical selectivity relates to the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behaviour.

Unambiguous Identification

Guideline for PRA

- ↦ Use 2 MRM transitions per analyte
- ↦ Analyte peaks from both product ions in the extracted ion chromatograms must fully overlap.
- ↦ Ion ratio from sample extracts should be within ±30% (relative) of average of calibration standards from same sequence



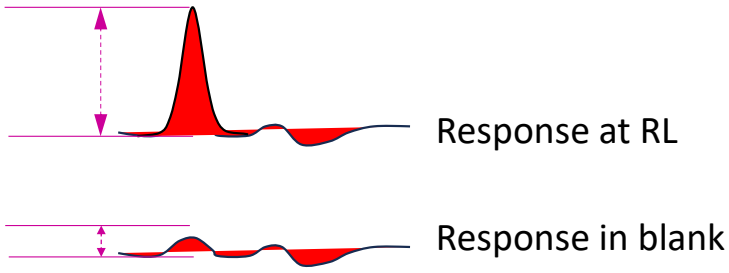
Specificity

The ability of the detector (supported by the selectivity of the extraction, clean-up, derivatisation or separation, if necessary) to provide signals that effectively identify the analyte

GC-MS with EI – low selectivity, high specificity
HRMS, MS/MS – high selectivity and high specificity

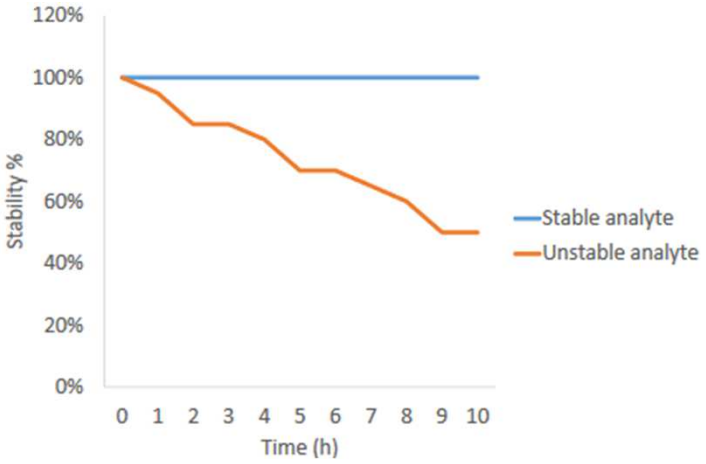
Establishing specificity

Response in reagent blank and blank control samples should be ≤ 30 % of Reporting Limit



Stability

Ensuring that the analyte remains stable (i.e. is not decomposed) during the analytical process.



Usually not calculated as a separate parameter in PRA

if the analyte is unstable, its decomposition influences the trueness and precision of the procedure and is, thus, accounted for by these two parameters.

Very important in bioanalytical chemistry, is calculated as an independent parameter

Many variables can affect analyte stability:

↳ sample processing,

↳ temperature,

↳ pH,

↳ Time...

Should be dealt with during method development.

Reference standards also can degrade on keeping – part of AQC checks

Accuracy

Closeness of agreement between an analytical result and the true or accepted reference value.

Calculated as the Recovery (%)

A known quantity of the analyte(s) in the form of reference standard solution is added into the sample, and analysed with replicates, average of results is calculated.

$$R(\%) = \frac{\bar{X}}{X_{Ref}} \times 100$$

Where $\bar{X}$ is the average result from experiments and X_{Ref} is the reference value

"Spiking" or "Fortification"

Ideally, blank sample is required

Consider purity of reference standard

Average recovery for each spike level tested: 70- 120%

Types of recovery calculations

Pre-extraction spikes

- ↦ Analyte is spiked into dry matrix prior to extraction step
- ↦ Attempt is made to mimic natural incidence of residues, and to ensure uniformity
- ↦ Used for recovery calculations

Post-extraction spikes

- ↦ After completion of extraction step in a blank samples, analyte is spiked into blank extract: 'matrix matching'
- ↦ Gives estimate of matrix effects

Recovery checks can be a diagnostic tool

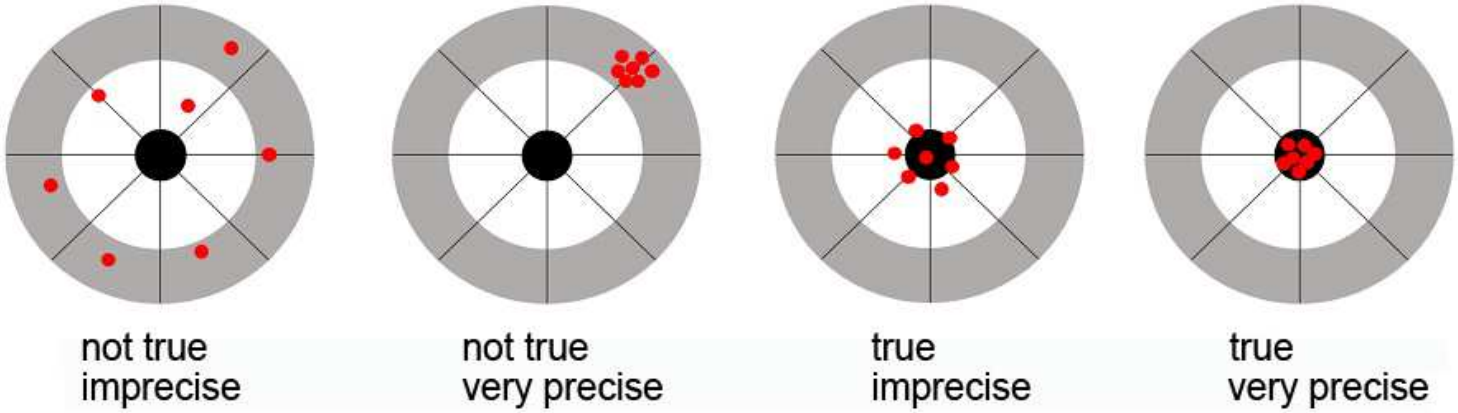
Recovery can be used to isolate problematic steps in the procedure

Precision

The closeness of agreement between independent analytical results obtained by method.

Precision is calculated in terms of the relative standard deviation (RSD), also called the coefficient of variation.

$$RSD = \left| \frac{\sigma}{\mu} \right| \times 100\%$$



Two Types of Precision

Repeatability

The precision (RSD_r) of measurement of an analyte (usually from accuracy calculations), obtained using the same method on the same sample in a single laboratory over a short period of time, during which differences in the materials and equipment will not occur.

.

Reproducibility

The precision (standard deviation RSD_{wR}) of measurement of an analyte (usually from accuracy calculations), obtained using the same method in a number of laboratories, by different analysts, or over a longer period in which differences in the materials and equipment will occur

RSD_r
RSD_{wR} } 20%

When this is done within the laboratory, it is called within-laboratory precision or intermediate precision

Linearity

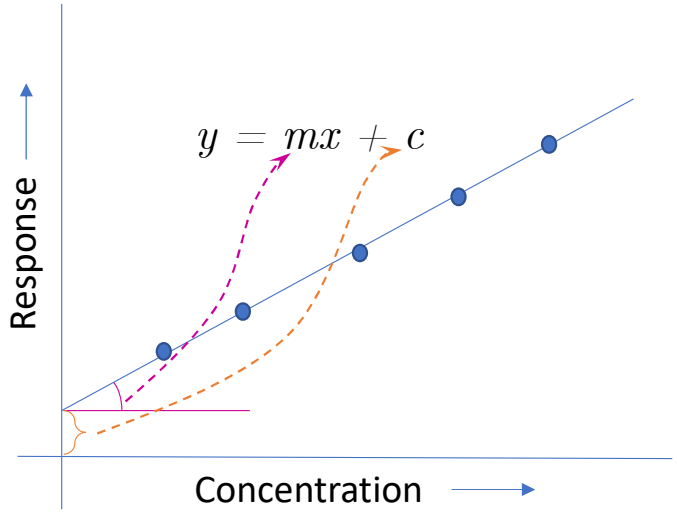
The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample.

Instrument Linearity

The calibration curve

Sensitivity is defined as the slope of the calibration curve

The regression coefficient for good linearity is > 0.9



Linearity in practice

Criteria: For each calibration point, the deviation of back-calculated concentration from true concentration should be $\leq \pm$ 20 %

Calculating from the calibration curve

For each calibration point x_c, the back-calculated concentration x_b can be calculated as

$$y = mx_b + c$$

$$x_b = \frac{y - c}{m}$$

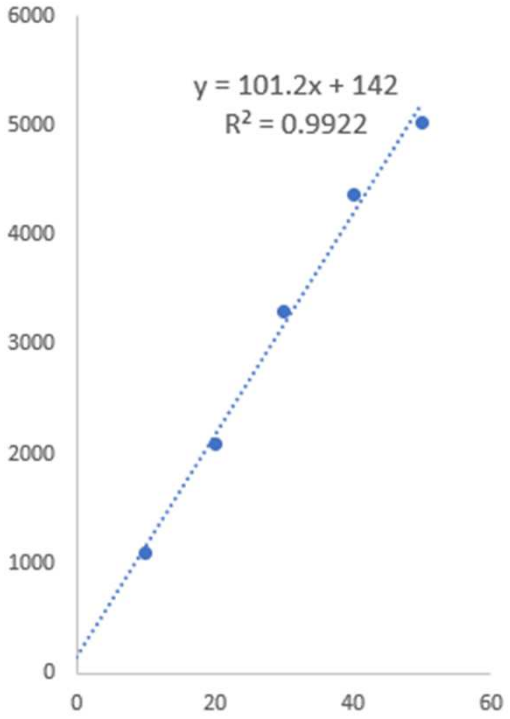
$$\frac{x_c - x_b}{x_c} \times 100 \quad \text{should be} \leq \pm 20\ \%$$

Linearity in practice (2)

Criteria: For each calibration point, the deviation of back-calculated concentration from true concentration should be $\leq \pm$ 20 %

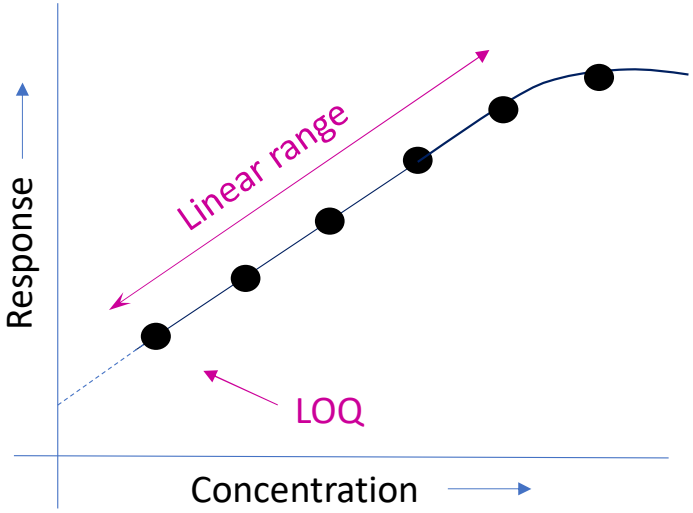
Worked example

Xc (true conc)	Response	m	c	xb (back calc)	(xc-xb).xc*100
10	1100	101.2	142	9.5	5
20	2100			19.3	3.5
30	3300			31.2	-4
40	4360			41.7	-4.25
50	5030			48.3	3.4



Range

The range is the interval of analyte concentrations where the method provides reliable and consistent results



Calibration should cover ± 10 % of the expected analyte concentration

Where necessary extracts containing high-level residues above the calibrated range must be diluted and re-injected

Ruggedness and Robustness

Ruggedness

Ruggedness is a measure of the reproducibility of test results under normal, expected operational conditions from laboratory to laboratory and from analyst to analyst.

Same as intermediate precision RSD_{wR}

Robustness

The robustness of an analytical procedure as a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters

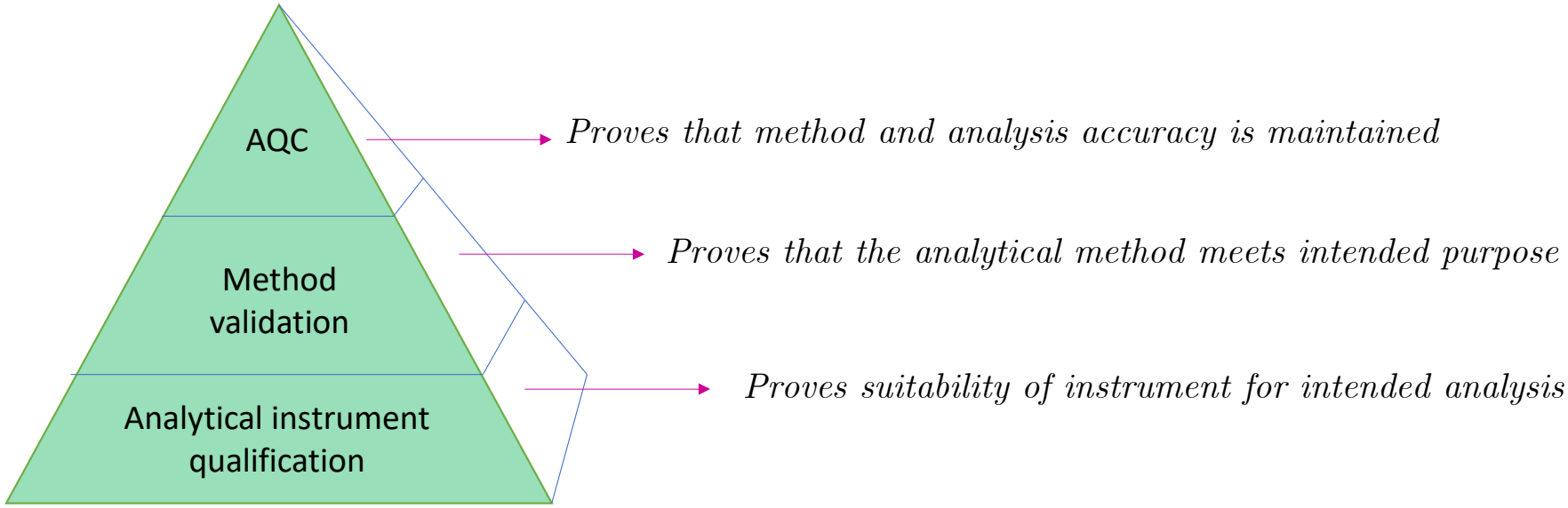
Robustness in practice

- ↦ Identify different parameters for making deliberate variations in the method
  - ↳ e.g. *moisture content, extraction time, amounts of QuEChERS reagents, oven temperature, injection volume...*
- ↦ Make 5 sets of combinations of such parameters
- ↦ Analyse a fortified sample (e.g. at 2 x LOQ) and analyse using the five set of parameters
- ↦ Acceptance at RSD $\leq 20\%$

Analytical quality control (AQC)

Are procedures designed to ensure that validation is maintained during routine analysis

Routine AQC


AQC
Method validation
Analytical instrument qualification
Proves that method and analysis accuracy is maintained
Proves that the analytical method meets intended purpose
Proves suitability of instrument for intended analysis

ISO 17025:2017

AQC checks including, but not limited to:

① use of reference materials or quality control materials;

② use of alternative instrumentation that has been calibrated to provide traceable results;

③ functional check(s) of measuring and testing equipment;

④ use of check or working standards with control charts, where applicable;

⑤ intermediate checks on measuring equipment;

⑥ replicate tests or calibrations using the same or different methods;

⑦ retesting or recalibration of retained items;

⑧ correlation of results for different characteristics of an item;

⑨ review of reported results;

⑩ intralaboratory comparisons;

⑪ testing of blind sample(s)

Replicate testing and retesting

Replicate testing

↦ Testing a given sample in replicates : Repeatability precision

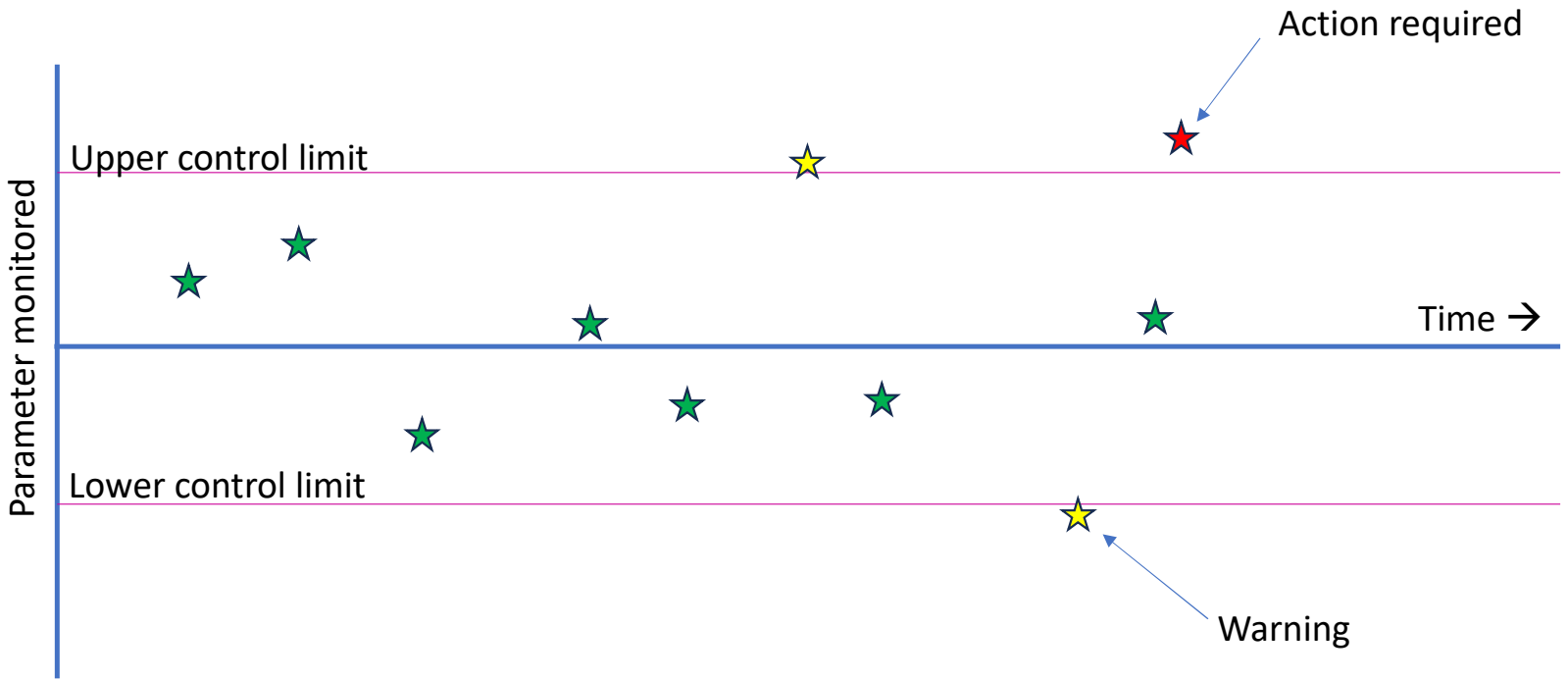
Retesting of retained samples

↦ A previously tested sample is reissued to analyst as a blind sample

↦ Variation of result as compared to past analysis is verified

↦ In this case, repeatability will be defined as the value below which the absolute difference between the two single test results on identical samples, obtained under the above conditions, may be expected to lie with a specified probability (e.g. 95%).

Control charts



Interlaboratory comparison

A homogenous sample is prepared and analysed for a parameter by many different laboratories

The Z-score is calculated for each lab:

$$Z = \frac{x - \mu}{\sigma}$$

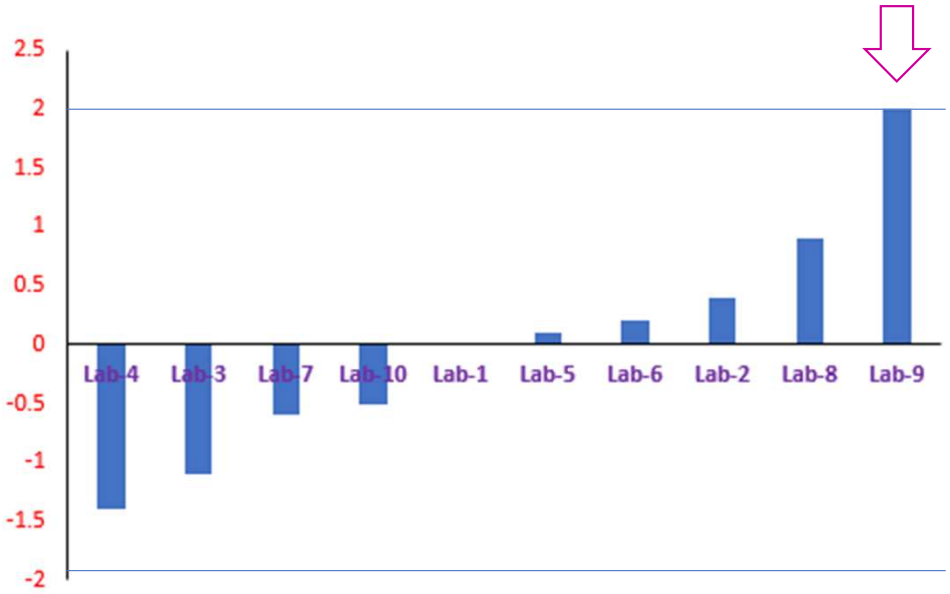
Where
x *is the average result from a laboratory,*
μ *is the average result from all labs,*
σ *is the standard deviation of results*

Interpretation of Z scores:

$|Z| \leq 2$ Acceptable results
$2 < |Z| \leq 3$ Questionable results
$|Z| \geq 3$ Unacceptable results

ILC – worked example

Lab Number	Results	Mean	Stdev	Z Score
Lab-4	3.1	10.92	5.51	-1.4
Lab-3	4.7			-1.1
Lab-7	7.6			-0.6
Lab-10	8.3			-0.5
Lab-1	10.8			0
Lab-5	11.5			0.1
Lab-6	12.1			0.2
Lab-2	13.1			0.4
Lab-8	16			0.9
Lab-9	22			2



More to learn..

Next session 13th Aug 2023

- ☑ (1) Introduction to instrumentation: LC-MS/MS, 03-Jul-23
- ☑ (2) Introduction to instrumentation: GC-MS/MS, 10-Jul-23
- ☑ (3) Modern pesticide residue analysis - Introduction, 17-Jul-23
- ☑ (4) Advanced pesticide residue analysis, 24-Jul-23
- ☑ (5) Method validation: requirements and practice, 7-Aug-23
- 🕒 (6) Introduction to measurement uncertainty calculation, 13-Aug-23 ⟸

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Thank you!

§ Questions?