GUIDANCE DOCUMENT ON METHOD VALIDATION AND QUALITY CONTROL PROCEDURES FOR PESTICIDES RESIDUES LABORATORIES

ESYD G-PESTICIDES

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1. GENERAL COMMENTS:

The SANCO / SANTE document (e.g. 11945/2015) describes the method validation requirements to support the validity of data reported within the framework of official controls on pesticide residues. Official control laboratories perform among other checking compliance with maximum residue levels (MRLs), taking enforcement actions, and perform assessment of consumer exposure to pesticides. They have to follow all SANCO / SANTE requirements.

Laboratories not performing official controls have to comply with a number or requirements described in the present guidance document. These requirements are originated in the SANCO / SANTE document, as well as in other regulative documents, as the Decision 2002/657/EC, the EURACHEM/CITAC Guide CG 4, etc.

The extent of the validation requirements is usually related to the instrumentation used. There is a current trend for many laboratories to use gas chromatography-mass spectrometry (GC-MS or GC-MS/MS) and liquid chromatography-mass spectrometry (LC-MS/MS) based multi-residue methods, in order to greatly increase the number of pesticides of their accreditation scope. At the same time laboratories using GC or LC based methods have limited accreditation capabilities and the requirements for their accreditation differ from laboratories using MS methods.

2. OFFICIAL CONTROL LABORATORIES

Accreditation of official control laboratories is based on their proven fully compliance with the requirements of the most recent SANCO /SANTE document.

3. NON-OFFICIAL CONTROL LABORATORIES

3.1 The method must be validated to assess linearity, recovery (as a measure of trueness), RSD_R (as a measure of precision), limit of detection (LOD) and of quantitation (LOQ), and selectivity (proven by identification-confirmation procedures). Additionally, the effect of each matrix on quantitation should be evaluated and the uncertainty of results must also be estimated.

3.2 **Linearity:** The line generated for each analyte at ≥5 concentration levels should be provided, together with the slope, the intercept and correlation co-efficient data. The requirements for a calibration line to be used for quantitation are: the correlation coefficient (r) to be >0.98 over the working range and the interval ($a \pm t_{st} \times S_a$)to include zero, where a is the intercept, S_a its standard deviation and t_{st} the student test value for N-2 degrees of freedom.

If a linear relation is not supported, an alternative calibration is required, e.g. bracketing technique.

3.3 **Trueness:** A minimum of 5 replicates is required to check the recovery at the targeted LOQ or reporting level (RL) of the method, and at least one other higher level, for example, 2-10 times the targeted LOQ or the MRL. Where the residue definition includes more than one analyte, then the method should be validated for all analytes included in the residue definition if compliance with maximum residue levels (MRLs) is to be given in the report. Acceptable mean recoveries are usually those within the range 70–120%. However, in certain cases and typically with multi-residue methods, recoveries outside this range are accepted. These are cases, where recovery is low but

consistent, i.e. demonstrating acceptable precision (RSD_r values ≤20%). A correction factor for the recovery should be used in the report for these analytes. Additionally, a proficiency test should be sought for these analytes to verify the correctness of the procedure followed.

3.4 **Precision:** A minimum of 5 replicates is required to check the precision, at the targeted LOQ or reporting level (RL) of the method, and at least one other higher level, for example, 2-10 times the targeted LOQ or the MRL. Repeatability RSD_r values determined from the validation experiments should be ≤ 20%, while within-laboratory reproducibility (RSD_R), which may be determined from on-going QC-data in routine analyses, should be ≤ 25%. Contributions arising from sample heterogeneity should be excluded when evaluating precision of the method.

3.5 **LOD and LOQ:** The Limit of Quantitation (LOQ) is evaluated as the lowest spike level of the validation experiments meeting the method performance acceptability criteria, i.e. presenting acceptable trueness and precision values. The signal of a sample containing the analyte at concentration equal to the LOQ should be ≥10 compared to the signal of the noise. The signal of a sample containing the analyte at concentration equal to the limit of detection (LOD) should be ≥3 compared to the signal of the noise.

3.6 **Selectivity:** Response in reagent blank and blank control samples should be <30% of the LOQ or the reporting level response. The following Identification criteria should be met:

3.6.1 **Selectivity requirements for chromatography:**

3.6.1.a The retention time of the analyte in the extract should correspond to that of the calibration standard (may need to be matrix-matched), with a tolerance of ±0.1min, for both gas chromatography and liquid chromatography. Larger retention time deviations are acceptable, based on experimental data of the laboratory, as for example RT tolerance ≤ 2s, with s the standard deviation of the retention time over a certain time period.

3.6.1.b The peak shape of the analyte in the extract should match with that of the calibration standard, e.g. the peak width, at half of its height, must be within ±10 % of the original width of the analyte peak.

3.6.1.c The chromatographic profile of the isomers of an analyte may provide helpful evidence.

3.6.1.d Co-chromatography may be used for providing evidence of analyte identity, however it is not a fully confirmatory technique, as it is based on the retention time criterion only. According to this technique the sample extract is fortified by addition of an appropriate amount of calibration solution. The amount of analyte added must be similar to the amount of the analyte found in the sample extract.

In order not to reject the assumption that the suspect peak is the analyte peak, only the height of the analyte peak and the internal standard peak should be enhanced after taking into account both the amount added and the dilution of the extract. The peak width, at half of its height, must be within ± 10 % of the original width of the analyte peak or the internal standard peak of the unfortified sample extracts.

3.6.1.e If confirmation is not based on a MS technique, the use of a different chromatographic separation is required that will also satisfy the above mentioned criteria 3.6.1a – 3.6.1d. For example a different chromatographic separation could be the use of a LC system in the case that the initial system was a GC system. In the case that only a GC system is available, then a different chromatographic separation could be at least the use of a column of significantly different polarity.

3.6.2 **Selectivity requirements for Mass Spectrometry:** Selective ion chromatograms should have peaks exceeding S/N 3:1, of similar retention time, peak shape and response ratio to those obtained from a calibration standard analysed at comparable concentration in the same batch. Chromatographic peaks from different selective ions for the same analyte must overlap with each other. The requirements for different types of MS detectors are given in the following Table.

MS mode:	**Single MS (unit mass resolution)**	**Single MS of high mass accuracy**	**MS/MS**
Typical systems (examples)	Quadrupole, ion trap, time-of-flight (TOF)	High resolution: Q-TOF, Orbitrap, FTMS, magnetic sector	Triple quadrupole, ion trap, hybride MS (e.g. Q-TOF, Q-trap)
Acquisition:	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Full scan, Limited m/z range, Selected ion monitoring (SIM)	Selected/multiple reaction monitoring (SRM/MRM), full scan product-ion spectra
Requirements for ions:	≥ 3 ions	≥ 2 ions with mass accuracy < 5 ppm.	≥ 2 product ions
Ion ratio:	Within ±30% (relative) of average of calibration standards from same sequence.		
Other:	S/N ≥ 3, Analyte peaks in the extracted ion chromatograms must fully overlap.		

3.7 **Matrix effects:** Matrix effects are known to occur frequently in both GC and LC methods and should be assessed at the initial method validation stage. The analytes that require use of calibration standards in solvent and the analytes that require use of calibration standards in matrix should be clearly identified during this process. Comparison of response arising from solvent standards and from matrix-matched standards is used for this purpose. A maximum 20% difference is considered as acceptable for using solvent standards as calibration standards. In a different case matrix matched calibration is required.

3.8 **Uncertainty:** It is a requirement of ISO/IEC 17025 that laboratories determine and make available the uncertainty associated with analytical results. To this end, laboratories should have available sufficient data derived from method validation/verification, inter-laboratory studies (as proficiency tests) and in-house quality control tests, which are applied to estimate the uncertainties. Reproducibility RSD_R may be used as the basis, but the contribution of additional uncertainty sources (e.g. heterogeneity of the sample from which the analytical test portion is taken) should be included. The contribution of the uncertainty of a critical step (e.g. uncertainty of calibration curve) to the total uncertainty of the method should be evaluated.

4. VALIDATION FOR ACCREDITATION ON A FLEXIBLE SCOPE:

Accreditation of flexible scope has to follow all procedures described in the previews paragraphs 1-3 as well as the guidance document ΕΣΥΔ ΚΟ ΕΥΕΛ. Additionally the following should be applied:

4.1 Accreditation in "Fruits and vegetables" is performed by use of the Table:

Commodity groups	**Typical commodity categories**	**Typical representative commodities**
1. High water content	Pome fruit	Apples, pears
	Stone fruit	Apricots, cherries, peaches,
	Other fruit	Bananas
	Alliums	Bulb onion, leeks
	Fruiting vegetables/cucurbits	Tomatoes, peppers, cucumber, melon
	Brassica vegetables	Cauliflower, brussel sprouts, cabbage, broccoli
	Leafy vegetables and fresh herbs	Lettuce, spinach, basil
	Stem and stalk vegetables	Celery, asparagus
	Forage/fodder crops	Fresh alfalfa, fodder vetch, fresh sugar beets
	Fresh legume vegetables	Fresh peas with pods, petit pois, mange tout, broad beans, runner beans, French beans
	Leaves of root and tuber vegetables	Sugar beet and fodder beet tops
	Fresh Fungi	Champignons, chanterelles
	Root and tuber vegetables or feed	Sugar beet and fodder beet roots, carrots, potatoes, sweet potatoes
2. High acid content and high water content	Citrus fruit	Lemons, mandarins, tangerines, oranges
	Small fruit and berries	Strawberry, blueberry, raspberry, blackcurrant, red currant, white currant, grapes
	Other	Kiwifruit, pineapple, rhubarb

4.1.1 Validation in 1 representative commodity (e.g. apple), leads to accreditation for the whole commodity category (e.g. pome fruit) for the validated analytes.

4.1.2 Validation in 5 representative commodities, one from each 5 different commodity categories, including at least one of commodity group 2 (high acid), leads to accreditation for "Fruit and vegetables with high water content" for the validated analytes.

4.2 Accreditation to other products of plant origin or water is performed by use of the Table:

Commodity groups	**Typical representative commodities**
3. High sugar content products	Honey, raisins, dried apricots, dried plums, fruit jams
4. High fat content products of plant origin	Olive oil, rapeseed oil, sunflower oil, pumpkin seed oil Olives, avocados Nuts Oilseed rape, sunflower, cotton-seed, soybeans, peanuts, sesame Peanut butter, tahina, hazelnut paste
5. Cereals and legumes	Wheat, rye, barley, oat, maize, rice, white bread, crackers, breakfast cereals, pasta Dried bean, lentils
6. Spices, teas, coffee etc.	Hops Cocoa beans and products thereof, coffee, tea Spices
7. Waters	Potable water Surface and ground water Sea water

4.2.1 Validation in 1 representative commodity, leads to accreditation for the whole commodity group for the validated analytes.

4.2.2In the case of water, 2 different kinds of water, including sea water must be validated for accreditation for the whole commodity group "Waters".

4.3 Accreditation to products of animal origin is performed by use of the Table:

Commodity groups of animal origin food	**Typical representative commodities**
8. Meat and Seafood	Beef, pork, lamb, game, horse Chicken, duck, turkey Liver, kidney Cod, haddock, salmon, trout Shrimp, scallop, crab
9. Milk and milk products	Cow, goat and buffalo milk Cow, goat cheese Yogurt, cream
10. Eggs	Chicken, duck, quail, goose eggs
11. Fat from food of animal origin	Kidney fat, lard Butter Cod liver oil

4.3.1 Validation in 1 representative commodity, leads to accreditation for the whole commodity group for the validated analytes.

4.3.2 Validation in 2 representative commodities, one from 2 different commodity groups, including commodity group 7, leads to accreditation for "Products of animal origin" for the validated analytes.

4.4 As for the chemical class of analytes, accreditation on a flexible scope requires validation of representative analytes from each chemical class. In order to facilitate the choice of a proper number of representative analytes, the following numbers in parentheses may be considered as an estimation of the maximum number of analytes in each class: *organophosphates (70), organochlorines (15), pyrethroids (40), triazines (15), triazoles (25), antibiotics (10), dinitroanilines (10), amides (10), bendimidazoles (5), carbamates (50), aryloxy alcanoic acids (10), benzoyl ureas (10), sulfonyl ureas (30), phenyl ureas (20), dithiocarbamates(10), inorganic compounds(10), strobylurines (10), neonicotinoids (5).*

4.4.1 The selected analytes from a chemical class for a flexible validation, should be representative of the class; i.e. it should be documented that the physicochemical properties (water solubility, vapor pressure, $\log P_{ow}$) of the selected analytes cover the whole range of physicochemical properties of the chemical class.

Typical example is the class of organophosphorous compounds that contains some very polar and of high water-solubility compounds, unlike most compounds of the class.

4.4.2 For accreditation on **a fully flexible scope,** representative analytes should be selected from at least 12 different chemical classes, including carbamates, organophosphates, organochlorines, pyrethroids, and triazoles. From each one of these 12 chemical classes, at least 50% of the compounds of the class, as mentioned in paragraph 4.4, should be included in validation. This leads to accreditation scope “determination of pesticide residues”.

4.4.3 For accreditation on a **partially flexible scope**, representative analytes, at least 20% of the compounds of a class, as mentioned in paragraph 4.4, should be included in validation. This leads to accreditation of the class.

4.4.4 A procedure is required, for the determination of analytes not initially validated, including at least estimation of recovery and LOQ.

4.5 For the revision of a validated method on a fully flexible scope, the relevant validation data are sent to ESYD. Furthermore:

4.5.1 A standard operating procedure is required, describing procedures followed for revisions of methods and characterizing them according to their importance.

4.5.2 For minor revisions, a check for the accuracy is required using quality control samples.

4.5.3 For major revisions, validation in accordance with the procedures described above in respect to the categories of food and chemical analyst class is required.

5. INTERNAL QUALITY CONTROL PROCEDURES DURING ROUTINE ANALYSIS

Internal quality control should include system suitability tests, run of blank samples, routine recovery checks, checks of reporting level and of carryover. The frequency of the quality control checks should be at a rate of 5% (1 check every 20 samples) unless otherwise stated below.

5.1 **System suitability tests:** the performance of analytical instruments should periodically be tested with representative selected analytes. Parameters monitored for chromatographs should include the retention time and the detector response, and for MS systems the ratio of 3 ions. Control charts should be constructed with these data.

5.2 **Blanks:** Reagent blank and matrix blanks for different matrices should be routinely used, especially when unusual findings are detected.

5.3 **Routine recovery checks:** A number of appropriately selected representative analytes (at least 5 per detection system) should be routinely checked for recovery and control charts should be constructed with these data to monitor recovery. Additionally, recovery checks for the total number of analytes should be performed at least on a yearly basis for each different method (e.g. method for high water content products, method for cereals, method for high fat content products).Analyte recovery should normally be determined by spiking within a range corresponding to 1–10 times the RL, or at the MRL, or at a level of particular relevance to the samples being analyzed. The level of addition may be changed occasionally or regularly, to provide information on analytical performance over a range of concentrations. Acceptable limits for individual recovery results should normally be within the range $r \pm 2s$, where r is the mean recovery and s the standard deviation taken from validation or QC data. However, alternatively, a range of 60-140 % may accept.

5.4 **Reporting level** should be verified with every batch of samples to avoid false negative results. This could be done by running a suitable standard solution containing the matrix.

5.5 **Carryover** should be routinely checked in order to avoid false negative results. This can be done by running a blank matrix extract following a standard solution of relatively high concentration.

7. EXTERNAL QUALITY CONTROL FOR LABORATORIES ACCREDITED ON A FLEXIBLE SCOPE

The laboratory must select proficiency testing organizers that meet the requirements of ESYD PDI and the number (N) of analytes in the sample should be the highest possible, not less than 5. The laboratory must report at least for N-1 analytes in scope if N≤10; or N-2 analytes, if N>10.

Appendix A

I. General instructions for method validation procedure

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. Possible steps for a complete method validation are listed in Table I.1.

Table I.1. Strategies and steps in analytical method validation

1. Develop a validation protocol, an operating procedure, or a validation master plan for the validation
2. For a specific validation project, define owners and responsibilities
3. Develop a validation project plan
4. Define the application, purpose, and scope of the method
5. Define the performance parameters and acceptance criteria
6. Define validation experiments
7. Verify relevant performance characteristics of equipment
8. Qualify materials, e.g. standards and reagents for purity, accurate amounts, and sufficient stability
9. Perform pre-validation experiments
10. Adjust method parameters and/or acceptance criteria if necessary
11. Perform full internal (and external) validation experiments
12. Develop standard operation procedures for executing the method in the routine
13. Define criteria for revalidation
14. Define type and frequency of system suitability tests and/or analytical quality control checks for the routine
15. Document validation experiments and results in the validation report Table

I.1. Theoretical Aspects

Validation means *"confirmation by examination and prediction of objective evidence that the particular requirements for a specified intended use are fulfilled"* (according to ISO 8402:1994).

Method validation means:

-The process of establishing the performance characteristics and limitations of a method and the identification of the influences, which may change these characteristics, and to what extent.

- Which analytes can be determined, in which matrices, in the presence of which interferences?
- Within these conditions what levels of precision and accuracy can be achieved?

-The process of verifying that a method is fit for a purpose, i.e. for solving a particular analytical problem.

Verification *means "confirmation by examination and prediction of objective evidence proving that the specified requirements have been fulfilled"* (according ISO 8402:1994).

It is necessary to make the difference between validation and verification.

Verification is applied for standardized methods and validation must be made for:

- non-standard methods;
- laboratory - designed / developed methods;
- standard methods used outside their intended purpose;
- standard methods

Validation studies for analytical methods typically determine the following parameters:

- detection limit;
- quantification limit;
- working range;
- selectivity;
- sensitivity;
- robustness;
- recovery;
- accuracy;
- precision ;
- repeatability ;
- reproducibility.

The performance parameters being tested are selected depending on the analytical requirements and based on the specifications from Table I.1.

Table I.1.1. Analytical requirements and the corresponding performance parameters

Analytical requirements	***Related performance parameters***
- Qualitative or quantitative answer?	*Confirmation of identity, selectivity/specificity, Limit of detection Limit of quantification*
For the analyte present in more than one form, is important the extractable, free or total analyte?	*Recovery*
Analyte(s) of interest and the most probable level (%, µg g^{-1}, ng g^{-1} etc.)?	*Limit of detection Limit of quantification Working range*
Level of precision and accuracy, allowed uncertainty degree.	*Recovery Accuracy Repeatability Reproducibility*
Possible interferences	*Selectivity/specificity*
Comparison of results with results from other laboratories?	*robustness Reproducibility*
Comparison of the results with external specifications?	*Accuracy Reproducibility*

Limit of Detection (LoD) means:

- *the lowest content that can be measured with reasonable statistical certainty;*
- *the lowest concentration of analyte in a sample that can be detected, but not necessarily quantified under the stated conditions of the test;*
- *the lowest analyte content, if actually present, that can be detected and can be identified.*

Where measurements are made for low concentrations of analyte (trace analysis) it is important to know what is the lowest concentration of analyte that can be confidently detected by the method. This problem must be analyzed statistically and a domain of decision criteria must be proposed.

It is normally sufficient to provide an indication of the level at which detection becomes problematic.

For **quantitative measurements**, 10 independent blank samples (a) or 10 independent blank samples fortified at lowest acceptable concentration (b) are analyzed, measured a single time each, and the **mean value** and **standard deviation (s)** of the blank sample is calculated for each set of measurements.

LoD is expressed as the analyte concentration corresponding to:

a) mean value of the blank sample + 3 s;

b) 0 + 3 s or the mean value of the blank sample + 4.65 s

For **qualitative measurements**, it is sufficient a critical concentration below which the specificity can not be identified. Thus, a series of blank samples fortified with analyte are analyzed. For each concentration level it is necessary to make 10 independent repeated measurements and a response curve of % positive or negative results versus concentration should be constructed. From this curve it can be established, by interpolation, the threshold concentration at which the test becomes unreliable.

Generally, the LoD, expressed in terms of concentration c_L, or the quantity q_L, is derived from the smallest measurement x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is calculated with the formula:

$$x_L = x_{bl} + k\, s_{bl}$$

where: x_{bl} is the mean value of the measurements for the blank sample of reagents; s_{bl} is the standard deviation of the measurements for the blank sample of reagents ; k is a numerical factor chosen according to the desired confidence level.

Table I.1.2. Limit of detection

	LIMIT OF DETECTION (LoD)
Measurements	*10 independent blank samples one time measured or* *or 10 blank samples fortified at lowest acceptable concentration , one time measured*
Determination/Estimation	*LoD = 3s + X* *in which:* *s = standard deviation for the blank or blank fortified with analyte samples* *X = measured value or mean measured value*
Optimum value	*function of tested method type*

Limit of Quantification (LoQ), known as Quantifiable Limit means:

- *the content equal to or greater than the lowest concentration point on the calibration curve;*
- *the lowest concentration of analyte in a sample that can be determined with acceptable repeatability and accuracy;*
- *performance characteristics that mark the ability of a chemical measurement process to adequately quantify an analyte.*

The ability to quantify is generally expressed in terms of the signal or analyte value that will produce estimates having a specific relative standard deviation (RSD), commonly 10%. The formula of calculation is:

$$LoQ = k_Q \sigma_Q$$

where: - σ_Q is the standard deviation at that point; k_Q is the multiple whose reciprocal equals the RSD. The IUPAC recommended value for k_Q is 10.

The following analyses will be made:

- 10 independent blank samples measured once each and the standard deviation (s) is calculated. LoQ is expressed as the concentration of the analyte corresponding to the a blank sample value + 10s;
- fortified aliquots of a blank sample at various analyte concentrations close to the LoD and the standard deviation (s) of each concentration is calculated. (s) is represented graphically against concentration and a value to the LoQ is established by interpolation.

Table I.1.3. Limit of Quantification

LIMIT OF QUANTIFICATION (LoQ)	
Measurements	*10 independent blanks one time measured* *or* *10 blank samples fortified at lowest acceptable concentration , one time measured*
Determination /Estimation	*LoQ = 10s + X* *where:* *s = standard deviation for the blank or blank fortified with an analyte samples* *X = measured value*
Optimum value	*function of tested method type*

Working Range – the analyte concentration interval or the value for which the method can be applied is determined. Within the working interval a linear response interval can exist. Sometimes also a nonlinear response range may be used, in case of a stable situation and calculation by computer. Generally, linearity studying involves at least 10 different concentrations / property values. Anywhere, in the working range, multi - point (preferably 6+) calibration points will be necessary. It is important to retain that the working range and linearity may be different for different matrices due to the of interferences if they are not eliminated.

Table I.1.4. Working range

WORKING RANGE	
Measurements	*From the calibrating curve with 6-10 ascending and equidistant concentrations points*
Determination/Estimation	*-The lower limit corresponds with LoD or LoQ* *- The upper limit is established qualitatively by visual examination of the linearity domain of the calibrating curve or by regression coefficient determination*
Optimal value/Interpretation	*-In some cases can be used non - linear curves*

Selectivity (or specificity) means *"the ability of a testing method to determine accurately and specifically the analyte of interest in the presence of other components in a sample from a matrix, under the stated conditions of the test".*

According to IUPAC Compendium of Chemical Terminology (1987) [14], selectivity in analysis means:

- for qualitative analyses – *"the extent to which other substances interfere with the determination of a substance according to a given procedure"*
- for quantitative analyses – *"a term used in conjunction with another substantive (e.g. constant, coefficient, index, factor, number) for quantitative characterization of interferences".*

It is necessary to establish the fact that the signal produced at the measurement stage, or other measured property, which was attributed to the analyte, is only due to the analyte and not from the presence of something chemically or physically similar or arising as a coincidence. This is confirmation of identity.

Selectivity / specificity are measures which assess the reliability of measurements in the presence of interferences.

The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in test portions to which specific interferences have been deliberately introduced.

Thus, firstly:

- analysis of the samples and reference materials by the selected or other independent methods and use of the results from the confirmatory techniques to assess the ability of the method to confirm analyte identity and its ability to measure the analyte separately from other interferences. To which extent the obtained data are reasonably sufficient to provide enough reliability is then decided;

- analysis of the samples containing various suspected interferences in the presence of analytes of interest and determination of the effect of interferences - if the presence of the interference enhances or inhibits detection or quantification of the measurands. If the detection or quantification is inhibited by interferences, further method development will be required.

Specificity is generally considered to be 100% selectivity.

Sensitivity

Sensitivity is "*the slope of the response curve, i.e. the change in instrument response function of the change in analyte concentration".*

Table I.1.5. Sensitivity

SENSITIVITY	
Measurements	*From calibrating curve with 6-10 ascending and equidistant concentrations points*
Determination/Estimation	*b= calibrating curve slope* *or S = Δ Y/ Δ C* *where:* *S = sensitivity* *Δ Y= absorbance variation* *Δ C = concentration variation*
Optimal value	*-alternates on different concentration ranges*

Robustness

The robustness test is used for the analysis of the behaviour of an analytic process when slight changes in the working conditions / operating parameters are executed or by the evaluation of the effects on the results over a longer period.

Recovery

Recovery is "*the fraction of analyte added to a test sample (fortified or injected sample) before the measurement*". The percentage recovery R% is calculated with the formula:

$$R\% = [(CF-CU)/CA] \times 100$$

where: - CF is the concentration of the analyte measured in the fortified sample; CU is the concentration of the analyte measured in the unfortified sample; CA is the concentration of the analyte added in the fortified sample.

Recovery can be determined analyzing CRM and reporting the concentration found to the certified value.

Accuracy

Accuracy means *"degree of concordance between the results of a test and the accepted reference value"*. The method validation seeks to quantify the accuracy of the results by assessing systematic and random errors.

Accuracy has two components: trueness and precision. The trueness of a method is *"the degree of concordance between the mean value obtained between a large series of results for a test and the accepted reference value".*

Table I.1.6. Robustness

<table>
<tr><th colspan="2">ROBUSTNESS</th></tr>
<tr><td>Measurements</td><td>On 4 sub-samples with the same known concentration are executed measurements when three factors from the working procedure are modified. The three factors (A, B ,C) depend on the tested analysis stage.
For example, in the solvent extraction stage the three factors can be:
- stirring time for the separation funnel,
- mL of extraction agent,
-temperature of sample to be extracted.
In the GC analysis – the three factors can be:
-chromatographic column length
-carrying gas flow
-working temperature
The parameters could be modified in the range ±10% or less if major changes will occur. The amplified factors will be marked (+) and the unchanged or reduced factors with (–).</td></tr>
<tr><td>Determination /Estimation</td><td>The Youden and Steiner scheme will be applied
<table>
<tr><th rowspan="2">Experiment</th><th colspan="3">Factors</th><th rowspan="2">Result</th></tr>
<tr><th>A</th><th>B</th><th>C</th></tr>
<tr><td>1</td><td>+</td><td>+</td><td>+</td><td>Y1</td></tr>
<tr><td>2</td><td>-</td><td>+</td><td>-</td><td>Y2</td></tr>
<tr><td>3</td><td>+</td><td>-</td><td>+</td><td>Y3</td></tr>
<tr><td>4</td><td>-</td><td>-</td><td>-</td><td>Y4</td></tr>
</table>
The effect A = (∑ Y_{A+} - ∑ Y_{A-}) /2
Where : ∑ Y_{A+} is the sum of results Y_i where factor A has positive values
∑ Y_{A-} is the sum of result Y_i where factor A has negative values
The effect B = (∑ Y_{B+} - ∑ Y_{B-}) /2
The effect C = (∑ Y_{C+} - ∑ Y_{C-}) /2</td></tr>
<tr><td>Optimum value/Interpretation</td><td>The effect of modified factor will be established by applying the t-Student test or a strong modifying effect will be considered if:
The effect A (B, C) > 1.4 s_{cc}
s_{cc} = initial method standard deviation from the control chart.
The method will be considered robust if these modifications do not have an important influence on the theoretical values.</td></tr>
</table>

Table I.1.7. Accuracy

ACCURACY	
Measurements	*10 analyses repeated for a known concentration sample prepared from a reference material (standard substance)*
Determination/Estimation	***Accuracy %*** *= (* $\overline{X}$ *- μ) x 100* *where:* $\overline{X}$ *= the mean of the 10 determinations* *μ = the real value of the reference material (standard substance)* ***Bias %*** *=* $\frac{\overline{X}-\mu}{\mu}\times 100$
Optimum value/Interpretation	*-100%* *-the obtained value will be checked using the t-student test*

Trueness is normally expressed in terms of bias and can be established by using & analyzing Certified Reference Materials (with known concentration value and confidence interval) or by analyzing the same sample by the method studied and another standardized method. Than it is necessary to compare the results obtained and to check if the result obtained by the developed method belongs to the confidence interval

Precision is *"a measure of the concordance degree between the independent results of a test obtained in the provided conditions and is usually expressed as function of the standard deviation that describes the distribution of the results".*

The precision is determined after 10 repeated analyses on a sample with known concentration prepared from a reference material or from standard substance.

Repeatability

Repeatability and reproducibility represent the two measures of precision.

Repeatability (*the smallest expected precision*) *will give information on the variability of the method when replicates of the same sample are performed, by a single analyst, on the same equipment, over a short period of time*.

Usually repeatability and reproducibility depend on analyte concentration and should be determined on a number of relevant concentrations levels.

To determine the repeatability, the same analyst must analyze the same samples or Reference Materials making 10 determinations, on the same equipment, in a short timescale. Then the mean and standard deviation at each concentration must be calculated.

Table I.1.8. Precision

PRECISION	
Measurements	10 analyses repeated for a known concentration sample prepared from a reference material (standard substance)
Determination/Estimation	CV (RSD) % = $\frac{s}{\overline{X}} \times 100$ where: $\overline{X}$ = mean value of the 10 determinations s = standard deviation
Optimal value	- depends on the tested method

Table III.1.9. Repeatability

REPEATABILITY	
Measurements	*10 analyses repeated for a known concentration sample prepared from a reference material (standard substance). The analyses will be achieved in the same laboratory, by the same analyst, with the same equipment, with the same method within close time intervals*
Determination/Estimation	*r = 2.8 x s_r* *where:* *s_r = repeatability standard deviation*
Optimal value	*-depends on the methods and the laboratory's level of proficiency*

Reproducibility *(the largest expected precision) will give information on the variability of the method when the same sample is analyzed in different laboratories, by different analysts, on different equipment, over a long period of time*.

To determine the **intra-laboratory reproducibility**, different analysts of the same laboratory must analyze the same samples or Reference Materials making 10 determinations, on different equipment, in an extended timescale. Then the mean and standard deviation at each concentration must be calculated.

To determine the **inter-laboratory reproducibility**, different analysts of different laboratories must analyze the same samples or Reference Materials making 10 replicates, on different equipment, in an extended timescale. For the inter-laboratory reproducibility is necessary to organize a collaborative study.

Table I.1.10. Internal reproducibility

INTERNAL REPRODUCIBILITY	
Measurements	*10 analyses repeated for a known concentration sample prepared from a reference material (standard substance). The analyzes will be conducted in the same laboratory by different analysts, different equipments, the same procedure at larger time intervals.*
Determination/Estimation	$R_L = 2.8 \times 1.6 \times s_r = 1.6 \times r$ *where:* s_r *= repeatability standard deviation* *r = repeatability*
Optimal value	*- depends of the methods and the laboratory's level of proficiency*

Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented. The Method Documentation Protocol must contain:

- updates and review;
- title;
- scope;
- definitions;
- principle;
- normative references;
- reagents and materials;
- apparatus and equipment;
- sampling and samples;
- drawing of the calibration curve;
- procedure;
- calculation and expressing of the results including final units, ± uncertainty, confidence interval.

Appendix B. General Instructions for Quality Control

II.1. Theoretical Aspects

Quality Control (QC) is a *"part of Quality Management focused on fulfilling quality requirements"*

The ISO/REMCO document 271 introduces the concept of Quality Control of analytical data, described as *"the complete set of procedures undertaken by the laboratory for continuous monitoring of operations and results in order to decide whether the results are reliable enough to be used".*

The techniques and activities involved in Quality Control can be divided into three levels of control, as shown below:

- **first line of control:** Instrument check/calibration;
- **second line of control:** Batch control (control sample);
- **third line of control:** Inter-laboratory proficiency tests.

The first and the second lines of control represent the internal quality control, while the third line of control is the external quality control.

The level and type of quality control will depend on the nature and frequency of analysis, batch size, degree of automation, and test difficulty.

The obtaining of quality data is a bold action for the laboratories requiring a continuous human and financial effort. A fundamental rule is that 10-20% of the total costs of analysis should be spent on quality control. Therefore, for quality work at least four conditions should be fulfilled:

- availability of means (adequate personnel and facilities);
- efficient use of time and means (cost aspects);
- availability of expertise (answering questions);
- maintaining and improvement level of output (process continuity).

Internal quality control checks

As part of the quality systems and to monitor day-to-day and batch-to-batch analytical performance, the laboratories must apply an appropriate level of internal quality control checks and participate wherever possible in inter-laboratory comparison schemes as external quality control checks.

The level and type of quality control will depend on the nature and frequency of analyses, batch size and degree of automation, test difficulty and confidence level for the test.

A. *Internal QC* is realized on two different levels of control:

The first level of control may take a variety of forms, including the use of reference samples, spiked samples, blank samples, replicate analysis and QC samples, as shown in Table II.1. .

Table II.1 Checks on the first level of control.

Level of control	***Type of checks***	***What is checked?***	***Who checks?***
Internal QC Level 1	*-Reagent reference*	*-Reagent contamination*	*Analyst*
	-Blank spiked with analyte	*-The analytical performance of the equipment*	*Analyst*
	-Spiked samples	*-Effect of matrix; recovery*	*Analyst*
	-Replicate analysis	*-Check of repeatability*	*Analyst*
	-Control samples (control charts)	*-Drift of the system*	*Analyst*
	-Checks and correlations of the results	*-Calculation* *-Results for different characteristics of the tested sample*	*Head of laboratory*

A special check is represented by the use of control charts, particularly for monitoring QC control samples.

The control samples are typical samples, which are sufficiently stable and available in sufficient quantities to be available for analysis over an extended period of time. Over this period the random variation in the performance of the analytical process can be monitored by monitoring the analyzed value of the QC sample, usually by plotting it on a control chart.

The control chart is perhaps the most useful and most commonly used analytical tool available to the laboratory. The control chart provides a graphic method for observing of the manner in which aberrations or deviations from the allowed variation are produced. This implies the fact that the systematic checks, e.g. per day or per batch, must show that the test result remains reproducible and that the methodology of measuring the analyte is respected. Several types of control charts can be applied, but the most usual types are:

- Control chart of the Mean ($\bar{x}$-chart) for the control of bias
- Control chart of the recovery rate (R-chart) for the control of precision.

- A control chart is obtained by plotting the analytical data in specific units on the vertical scale, against time or sequence of tests on the horizontal scale.

1. An $\bar{x}$ **-chart** can be started when a sufficient number of measured values for the control sample are available. For this, it is recommended to start with at least 10 replicates collected in each bath. The mean, $\bar{x}$, and standard deviation, s, of a set of result are calculated and then the warning limits ($\pm$2s) and the action limits ($\pm$3s) are drawn on one and the other side of the mean value (Figure II.1).

Each time that a result for the control sample is obtained in a batch of test samples, this result is recorded on the control chart. When a chart is full, a new chart must be started. Quality control rules have been developed to detect excess bias and imprecision as well as shift and tendencies in the analysis.

Warning rule - (if occurring), then data requires further inspection:

-one control result beyond warning limit

Rejection rules - (if occurring), then the data is rejected:

-one control result over the Action Limit;

-two successive control results over the Warning Limit;

-ten successive control results on the same side of the mean;

-four out of five successive points in decreasing or increasing order.

If any of the rejection rules is violated, specific action must be taken.

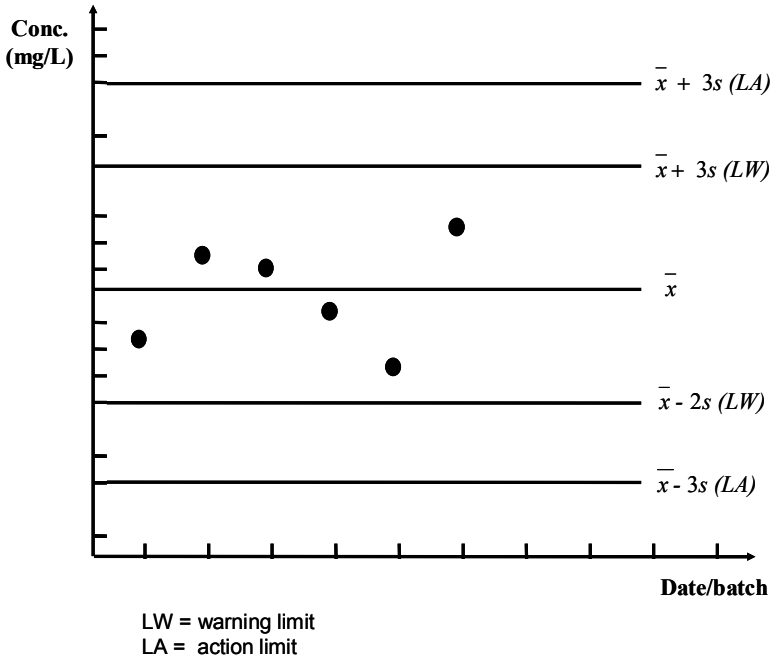


Fig. II.1. Control chart of the Mean ($\bar{x}$ -chart).

2. The **R-chart** can be obtained by running duplicate analysis in the same batch on control samples or test samples.

The differences between the results allow the calculation of the $\overline{R}$-mean difference between duplicate samples and the S_R –standard deviation of the range of all pairs of duplicates. The parameters $\overline{R}$ and S_R are determined for at least 10 initial pairs of duplicates. The warning and action limits can be drawn at 2s and 3s distance from the mean of differences. The graph is single–sided so that the lowest observable value of the difference is zero (Figure II.2).

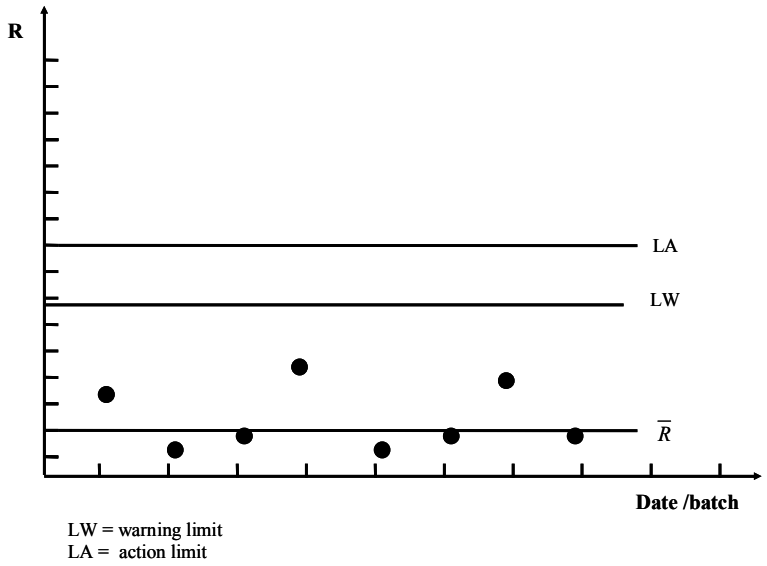


Fig. II.2. Control chart of the type recovery rate ($\overline{R}$ -chart).

Running duplicates of a control sample in each batch is the simplest way of controlling the precision.

A limitation of the use of duplicates of a control sample to verify precision is that this may not fully reflect the precision of the analysis of the test sample both in matrix composition and in concentration. The most convenient way to deal with this problem is to use more than one control sample with different concentrations of the attribute or to use test samples instead of control samples.

Quality control rules are similar to those of the Mean Chart. The response to violation of the rejection rules is also similar: the analysis is repeated and the problem is investigated if the repeat is not satisfactory.

It is widely accepted that for routine analysis, an internal QC level of 5% is reasonable, i.e. 1 in every 20 samples analyzed should be a QC sample. However, for robust routine methods with high sample throughput, a lower QC level may be reasonable. For more complex procedures a 20%

level is not unusual, and on occasion even 50% may be required. For analyses performed infrequently, it is necessary to use a reference material containing a certified or known concentration of analyte, followed by repeated analyses of the sample and spiked samples. Analyses undertaken more frequently should be the subject of systematic QC procedures incorporating the use of control charts and check samples.

The second level of internal QC is represented by *"intra-laboratory testing"* or in-house proficiency testing of the analysts. The purpose of such activities is to identify the sources of measurement method error and to estimate their bias (accuracy), repeatability and reproducibility. Some of the potential error sources during the test are the operator, the analyst, the equipment, the calibration and the operating conditions. The results may be analyzed by making comparisons against each other or against reference standards. The major problems associated with designing a program of intra- laboratory testing concern the following questions:

- what kind of samples to use;
- how to prepare and introduce the samples into the run without the recipient's knowledge;
- how often to check the analyst's proficiency.

The solutions or criteria for decision-making are listed in Table V.4.3

Table V.II.2. Problems in assessing intra-laboratory tests

No.	Problem	Solutions and decision criteria
1	*Kind of samples*	*-Replicate samples of unknowns of reference materials* *-An aliquot from one day can be introduced for analysis by another analyst* *-Supervisor can place known samples or replicates in the system occasionally*
2	*Introducing the sample*	*-Samples should have the same labels and appearance as unknowns* *-Samples must be exposed by the analyst to the same preparatory steps as normal unknown samples*
3	*Frequency of checking performance*	*-Function of the method precision* *-Function of the degree of automation* *-Function of the analyst's training, attitude and performances recorded*

Such intra-laboratory tests are organized periodically by the supervisor of the laboratory and by the quality manager, in accordance with a scheduled program.

B. ***External QC***

The third line of control is constituted by external quality control. It consists in the participation of laboratories in different types of inter-laboratory programs organized locally, regionally, nationally or internationally, as well as within the organization. Before a scheme is joined, the purpose of participation must be clear, so that a rational choice can be made.

The following operational types can be distinguished:

- **Evaluation of the performances of an analytical method** by participating to:
  - Collaborative study – establishing the performance, characteristics of an analytical method;
  - Comparative study – comparing analytical methods by comparing the results.
- **Testing of the performances of the laboratory** by participating to :
  - Proficiency test (one method) – comparing the performance of the laboratories on the basis of the same analytical method;
  - Proficiency test (different methods) – comparing the performance of laboratories by comparing the results obtained with their own methods.
- **Tests for the certification of the reference materials:**
  - Certification study – establishing benchmark values for components or properties of a material;
  - Consensus study – establishing characteristic values for components or properties of a material, for quality control.

The most common type of inter-laboratory comparison scheme in which laboratories participate consists of proficiency tests, where sub-samples of a large sample are sent to participating laboratories at regular intervals. Depending on the material to be analyzed, the laboratories can follow their own analytical procedures or can perform analyses according to a detailed method proposed by the organizer of the test. When sub-samples have been analyzed for one or more attributes by the participants, the results are sent to the scheme's coordinator, where they will be processed. The "normal" way of data treatment would be to calculate the mean and the standard deviation. However, in proficiency tests and consensus studies there is a preference for using the median value rather than the mean, in order to reduce the influence of extreme data. Individual rating of the proficiency of a laboratory is done with the so-called "Z-score",

The Z – score is based on the estimated deviation and the target value for the standard deviation of the results:

$$Z = \frac{x - \bar{x}}{s}$$

where: x = individual result

$\bar{x}$ = mean of all results

s = standard deviation of x

Before the mean is calculated, outliers are removed. A direct estimation of the laboratory performance is done by comparing the following quality limits:

/Z/ $\leq$2: the laboratory performance is considered satisfactory

2< /Z/ < 3: the laboratory performance is considered questionable

/Z/ $\geq$3: the laboratory performance is considered unsatisfactory

After the data are processed, the report on each round is sent to participants. Usually, after a number of rounds, a more extensive report is made, since more data allow more and better statistical conclusions. The participants can inspect their results, and when significant and/or systematic deviations are noticed, they may take corrective action in the laboratory.

Regular participation in proficiency testing schemes is one of the best ways for an analytical laboratory to monitor its performance against both its own requirements and the performances of other laboratories.

Proficiency testing helps to highlight not only repeatability and reproducibility performance between laboratories but also systematic errors, e.g. bias.

Appendix C. Glossary [SANTE/11945/2015]

Accuracy	Closeness of agreement between an analytical result and the true, or accepted reference value. When applied to a set of results, it involves a combination of random error (estimated as precision) and a common systematic error (trueness or bias) (ISO 5725-1).
Analyte	The chemical species for which the concentration (or mass) is to be determined. For the purposes of these procedures: a pesticide or a metabolite, breakdown product or derivative of a pesticide or an internal standard.
AQC	Analytical quality control. Measurement and recording requirements intended to demonstrate the performance of the analytical method in routine practice. The data supplement those generated at method validation. AQC data may be used to validate the extension of methods to new analytes, new matrices and new levels. Synonymous with the terms internal quality control (IQC) and performance verification. Concurrent AQC data are those generated during analysis of the batch in which the particular sample is included.
Batch (analysis)	For extraction, clean-up and similar processes, a batch is a series of samples dealt with by an analyst (or team of analysts) in parallel, usually in one day, and should incorporate at least one recovery determination. For the determination system, a batch is a series undertaken without a significant time break and which incorporates all relevant calibration determinations (also referred to as an "analysis sequence", a "chromatography sequence", etc.). A determination batch may incorporate more than one extraction batch.
Bias	The difference between the mean measured value and the true value.
Blank	(i) Material (a sample, or a portion or extract of a sample) known not to contain detectable levels of the analyte(s) sought. Also known as a matrix blank. (ii) A complete analysis conducted using the solvents and reagents only; in the absence of any sample (water may be substituted for the sample, to make the analysis realistic). Also known as a reagent blank or procedural blank.
Calibration	Determination of the relationship between the observed signal (response produced by the detection system) from the target analyte in the sample extract and known quantities of the analyte prepared as standard solutions. In the present document, calibration does not refer to calibration of weighing and volumetric equipment, mass calibration of mass spectrometers, and so on.
Calibration standard	A solution (or other dilution) of the analyte (and internal standard, if used) used for calibration of the determination system. May be prepared from a working standard and may be matrix-matched.
Certified reference material (CRM)	See reference material.

Confirmation	Confirmation is the combination of two or more analyses that are in agreement with each other (ideally, using methods of orthogonal selectivity), at least one of which meets identification criteria. It is impossible to confirm the complete absence of residues. Adoption of an "RL" at the LCL avoids the unjustifiably high cost of confirming the presence, or absence, of residues at unnecessarily low levels. The nature and extent of confirmation required for a positive result depends upon importance of the result and the frequency with which similar residues are found. Assays based on an ECD tend to demand confirmation, because of their lack of specificity. Mass spectrometric techniques are often the most practical and the least equivocal approach to confirmation. AQC procedures for confirmation should be rigorous.
Contamination	Unintended introduction of a target analyte into a sample, extract, internal standard solution etc., by any route and at any stage during sampling or analysis.
Fragment ion	Product ion that results from the dissociation of a precursor ion
GC	Gas chromatography (gas-liquid chromatography).
Identification	Is a qualitative result from a method capable of providing structural information (e.g., using mass spectrometric (MS) detection) that meets acceptable criteria for the purpose of the analysis. The process of generating of sufficient evidence to ensure that a result for a specific sample is valid. Analytes must be identified correctly in order to be quantified. AQC procedures for identification should be rigorous.
Interference	A positive or negative response produced by a compound(s) other than the analyte, contributing to the response measured for the analyte, or making integration of the analyte response less certain or accurate. Interference is also loosely referred to as "chemical noise" (as distinct from electronic noise, "flame noise", etc.). Matrix effects are a subtle form of interference. Some forms of interference may be minimised by greater selectivity of the detector. If interference cannot be eliminated or compensated, its effects may be acceptable if there is no significant impact on accuracy.
Internal quality control	See AQC.
Within-laboratory	See reproducibility.
Internal standards	Definitions are given in the main body of text.
Laboratory sample	The sample sent to and received by the laboratory.
LC	Liquid chromatography (primarily high performance liquid chromatography, HPLC and Ultra high performance liquid chromatography, UPLC).
LCL	Lowest calibrated level. The lowest concentration (or mass) of analyte with which the determination system is successfully calibrated, throughout the analysis batch. See also "reporting limit".

LC-MS/MS	Liquid chromatographic separation coupled with tandem mass spectrometric detection.
Level	In this document, refers to concentration (e.g. mg/kg, µg/ml) or quantity (e.g. ng, pg).
LOD	Limit of determination (LOD) means the validated lowest residue concentration which can be quantified and reported by routine monitoring with validated control methods; In this respect it can be regarded as the LOQ (see below)
LOQ	Limit of quantitation (quantification). The lowest concentration or mass of the analyte that has been validated with acceptable accuracy by applying the complete analytical method. LOQ is preferable to LOD because it avoids possible confusion with "limit of detection". However, In Reg.396/2005 MRLs that are set at the limit of quantification/determination are referred to as "LOD MRLs", not "LOQ MRLs".
Mass accuracy:	Mass accuracy is the deviation of the measured *accurate* mass from the calculated *exact* mass of an ion. It can be expressed as an absolute value in milliDaltons (mDa) or as a relative value in parts-per-million (ppm) error and is calculated as follows: (accurate mass – exact mass) Example: the experimentally measured mass = 239.15098, the theoretical exact mass of the ion m/z = 239.15028. The mass accuracy = (239.15098 – 239.15028) = 0.7 mDa or (accurate mass – exact mass) / exact mass * 10^6 Example: the experimentally measured mass = 239.15098, the theoretical exact mass of the ion m/z = 239.15028 The mass accuracy=(239.15098–239.15028)/239.15028 * 10^6=2.9 ppm
Mass resolution	The resolution of a mass spectrometry instrument is the ability to distinguish between two ions with similar m/z values (IUPAC definition[20]: the smallest mass difference between two equal magnitude peaks so that the valley between them is a specified fraction of the peak height).
Mass resolving power	The resolving power, defined at full-width half maximum (FWHM), is m/Δm, where m is the m/z being measured and Δm the width of the mass peak at half peak height. Note 1: for magnetic sector instruments another definition is used ("10% valley"). Roughly the difference between the two definitions is a factor of 2 (i.e. 10,000 resolving power by the 10% valley method equals 20,000 resolving power by FWHM). Note 2: mass resolving power is often confused or interchangeably used with mass resolution (see definition above).
Matrix blank	See blank.
Matrix effect	An influence of one or more co extracted compounds from the sample on the measurement of the analyte concentration or mass. It may be observed as increased or decreased detector response, compared with that produced by solvent solutions of the analyte. The presence, or absence, of such effects may be demonstrated by comparing the response produced from the analyte in a solvent solution with that obtained from the same quantity of analyte in the sample extract.

Matrix-matched /matrix- based calibration	Calibration using standards prepared from extracts of the same (matrix-matched) or any other (matrix-based) blank matrix.
Method	A sequence of procedures or steps, from receipt of a sample through to the calculation and reporting of results.
Method validation	The process of characterizing the performance to be expected of a method in terms of its scope, specificity, accuracy sensitivity, repeatability and within laboratory reproducibility. Some information on all characteristics, except within laboratory reproducibility, should be established prior to the analysis of samples, whereas data on reproducibility and extensions of scope may be produced from AQC, during the analysis of samples. Wherever possible, the assessment of accuracy should involve analysis of certified reference materials, participation in proficiency tests, or other inter-laboratory comparisons.
MRL	Maximum residue level. In Regulation 396/2005 list MRLs for pesticide/commodity combinations, an asterisk indicates that the MRL* is set at or about the LOQ, with the LOQ being here a consensus figure rather than a measured value.
MRM	In pesticide residue analysis: multi-residue method
MRM	In mass spectrometry: Application of selected reaction monitoring (SRM) to multiple product ions from one or more precursor ions
MS	Mass spectrometry.
MS/MS	Tandem mass spectrometry, here taken to include MS. An MS^n procedure in which ions of a selected mass to charge ratio (*m/z*) from the primary ionisation process are isolated, fragmented usually by collision, and the product ions separated (MS/MS or MS^2). In ion-trap mass spectrometers, the procedure may be carried out repetitively on a sequence of product ions (MS^n), although this is not usually practical with low-level residues.
Performance verification	see analytical quality control (AQC).
Precision	The closeness of agreement between independent analytical results obtained by applying the experimental procedure under stipulated conditions. The smaller the random part of the experimental errors which affect the results, the more precise the procedure. A measure of precision (or imprecision) is the standard deviation[21].
Precursor ion	Ion that reacts to form particular product ions or undergoes specified neutral losses. The reaction can be of different types including unimolecular dissociation, ion/molecule reaction, change in charge state, possibly preceded by isomerization.
Procedural blank	See blank.
Product ion	Ion formed as the product of a reaction involving a particular precursor ion

"Reference" standard	A solid, liquid or gaseous compound that has been prepared in a largely purified form and packed appropriately to ensure stability and allow transportation and storage. The storage conditions, expiry date, purity must be indicated as well as the hydratation water content and the isomer composition where this is relevant. Where standards are bought in solution they should be treated as secondary standards (i.e. as stock or working solutions).
Reagent blank	See blank.
Recovery (of analyte through an analytical method)	The proportion of analyte remaining at the point of the final determination, following its addition (usually to a blank sample) immediately prior to extraction. Usually expressed as a percentage. Routine recovery refers to the determination(s) performed with the analysis of each batch of samples.
Reference material	Material characterized with respect to its notionally homogeneous content of analyte. Certified reference materials (CRMs) are normally characterized in a number of laboratories, for concentration and homogeneity of distribution of analyte. In-house reference materials are characterized in the owner's laboratory and the accuracy may be unknown.
Repeatability (r)	The precision (standard deviation) of measurement of an analyte (usually obtained from recovery or analysis of reference materials), obtained using the same method on the same sample(s) in a single laboratory over a short period of time, during which differences in the materials and equipment used and/or the analysts involved will not occur. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result. May also be defined as the value below which the absolute difference between two single test results on identical material, obtained under the above conditions, may be expected to lie with a specified probability (e.g. 95%).
Reporting limit (RL)	The lowest level at which residues will be reported as absolute numbers. It is equal to, or higher than the LOQ. For EU monitoring purposes where samples for surveys are analysed over a 12-month period, the same RL should be achievable throughout the whole year.
Representative analyte	An analyte used to assess probable analytical performance in respect of other analytes notionally sought in the analysis. Acceptable data for a representative analyte are assumed to show that performance is satisfactory for the represented analytes. Representative analytes must include those for which the worst performance is expected.
Residuals	The residuals are the deviations of the measurement values from their values predicted by the regression line.

Reproducibility (R)	The precision (standard deviation) of measurement of an analyte (usually by means of recovery or analysis of reference materials), obtained using the same method in a number of laboratories, by different analysts, or over a period in which differences in the materials and equipment will occur. The measure of precision usually is expressed in terms of imprecision and computed as standard deviation of the test result. Within-lab-reproducibility (RSDwR) is that produced in a single laboratory under these conditions. May also be defined as the value below which the absolute difference between two single test results on identical material, obtained under the above conditions, may be expected to lie with a specified probability (e.g. 95%).
Response	The absolute or relative signal output from the detector when presented with the analyte.
RSD	Relative standard deviation (coefficient of variation).
Sample	A general term with many meanings but, in these guidelines, refers to laboratory sample, test sample, test portion, or an aliquot of extract.
Sample preparation	The first of two processes which may be required to convert the laboratory sample into the test sample. The removal of parts that are not to be analysed, if required.
Selectivity	The ability of the extraction, the clean-up, the derivatisation, the separation system and (especially) the detector to discriminate between the analyte and other compounds. GC-ECD is a selective determination system providing no specificity.
SIM	Selected ion monitoring. Operation of a mass spectrometer in which the abundance of several ions of specific m/z values are recorded rather than the entire mass spectrum
SRM	Selected reaction monitoring. Measurement of specific product ions corresponding to m/z selected precursor ions recorded via two or more stages of mass spectrometry (MS^n).
S/N	Signal-to-noise ratio.
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 is a fairly non- selective determination system capable of high specificity. High resolution mass MS and MS^n can be both highly selective and highly specific.
Spike or spiking	Addition of analyte for the purposes of recovery determination or standard addition.
Standard	A general term which may refer to a “pure” standard, stock standard, working standard, or calibration standard.
Stock standard solution	The most concentrated solution (or solid dilution, etc.) of the “pure” standard or internal standard, from which aliquots are used to prepare working standard solutions or calibration standard solutions

Trueness	The measure of trueness is normally expressed as 'bias'. The closeness of agreement between the average value obtained from a series of test results (i.e. the mean recovery) an accepted reference or true value (ISO 5725-1).
Uncertainty (of measurement)	A range around the reported result within which the true value can be expected to lie with a specified probability (confidence level, usually 95%). Uncertainty data should encompass trueness (bias) and reproducibility
Unit (sample)	A single fruit, vegetable, animal, cereal grain, can, etc. For example, an apple, a T-bone steak, a grain of wheat, a can of tomato soup.
Unit mass resolution	Mass resolution such that it is possible to clearly distinguish a peak corresponding to a singly charged ion from its neighbours 1 Dalton away, usually with no more than 5–10 % overlap
Validation	See method validation.
Working standard solution	A general term used to describe dilutions produced from the stock standard, which are used, for example, to spike for recovery determination or to prepare calibration standard solutions.

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