QA/QC measurements for the implementation of analytical methods

Joint FAO, IAEA and Latin American and Caribbean Analytical Network Workshop on Quality Assurance and Quality Control Measures in Food Testing Laboratories

May_05 _2019

Msc. Miriam Loewy
rmloewy@gmail.com
Ralaca Board
Neuquen-Argentina

Reference Document

The guidance in this document is intended for laboratories involved in the official control of pesticide residues in food and feed across the European Union. This document describes the method validation and analytical quality control (AQC) requirements to support the validity of data reported within the framework of official controls on pesticide residues.

Implemented by 01/01/2018

EUROPEAN COMMISSION
DIRECTORATE GENERAL FOR HEALTH AND FOOD SAFETY
Safety of the Food Chain
Pesticides and Biocides

SANTE/11813/2017
21 – 22 November 2017 rev.0

Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed.

SANTE/11813/2017

Supercedes

SANTE/11945/2015
Implemented by 01/01/2018

KEY Objectives

- To provide a harmonized, cost-effective quality assurance and quality control system across the EU
- To ensure:
  - quality and comparability of analytical results
  - acceptable accuracy
  - false positives or false negatives avoided
- To support compliance with, and specific implementation of ISO/IEC 17025

Sampling, transport, traceability and storage of laboratory samples

4

Sampling

- Food samples should be taken in accordance with Directive 2002/63/EC or superseding legislation. For feed, the regulations are laid down in Appendix I of Regulation (EC) No. 152/2009 and amendments.

- Where it is impractical to take primary samples randomly within a lot, the method of sampling must be recorded.

Transport

- Appropriate conditions to the laboratory : clean containers and robust packaging
- Very fragile or perishable products (e.g. ripe raspberries) may have to be frozen to avoid spoilage
- Samples that may be damaged by chilling (e.g. bananas) must be protected from both high and low temperatures.
- Rapid transport to the laboratory, preferably within one day, is essential for samples of most fresh products.

The condition of samples delivered to the laboratory should approximate to that which would be acceptable to a discerning purchaser, otherwise samples should be unfit for analysis

Traceability

- Samples must be identified clearly and indelibly
- Avoid the use of marker pens containing organic solvents for labelling bags containing samples to be analyzed for fumigant residues, especially if an electron capture detector is to be used

On receipt, each laboratory sample must be allocated a unique code by the laboratory.

Storage

- Laboratory samples which are not analyzed immediately should be stored under conditions that minimize decay.
- Fresh products should be stored in the refrigerator, but typically **no longer than 5 days**
- Dried products may be stored at room temperature, but if storage time is expected to exceed **two weeks**, they should be sub-sampled and stored in the freezer

Sample analysis: Preparation and processing procedures

The Shortest time possible

- In order to minimize sample decay and pesticide losses
- Residues of very labile or volatile pesticides should be started as soon as possible
- Specially the procedures which could lead to loss of analyte should be completed, preferably on the day of sample receipt

Sample preparation and processing

- It must be demonstrated that:
  - Sample processing and storage procedures have no significant effects on the residues present in the sample
    - Avoid prolonged heating
    - Dry ice

  - Sample comminution is homogeneous enough to ensure that sub sampling variability is acceptable
    - eg . save the proportions of pulp and shell
    - Use bigger sub-samples or more replicas

Pooling of subsamples

Low frequency of pesticide residues

Pooling of individual samples or simple extracts

When pooling 5 samples,

LOQ = RL/5 (at least)

- Providing that the detection system is sensitive enough
- The original samples or the extracts must be re-analyzed in cases of pesticide residue findings at relevant levels

Extraction conditions and efficiency

- A number of parameters can affect extraction efficiency and analyte stability

  (T, pH, shaking time impact, etc.)

- To improve the extraction efficiency of **low moisture commodities**, addition of water prior to extraction is recommended (cereals, dried fruits)

- The recovery of **incurred residues** can be lower tan the percentage recovery obtained from the analysis of spiked samples.

- Where practicable, samples containing incurred residues can be analyzed using varying extraction conditions to **obtain further information on extraction efficiency**

Clean-up, concentration/reconstitution and storage of extracts

A clean-up, or dilution step may be necessary to:

- reduce matrix interferences
- reduce contamination of the instrument system.

Clean-up techniques take advantage of the difference in physicochemical properties (e.g. polarity, solubility, molecular size) between the pesticides and the matrix components. However, the use of a clean-up step in a multi-residue method can cause losses of some pesticides.

Concentration of sample extracts can cause precipitation of matrix-components and in some cases losses of pesticides. Similarly, **dilution** of the extract with a solvent of a different polarity can also result in pesticide losses because of decreased solubility.

Extracts evaporation and storage

- Temperature should be kept as low as is practicable.
- A small volume of a "keeper".
- Foaming, vigorous boiling of extracts, dispersion of droplets, must be avoided.

- A stream of dry nitrogen or vacuum centrifugal evaporation for small-scale evaporation.
- Analyte stability in extracts should be evaluated during method validation.

Avoid losses of pesticides in extracts at room temperature

On-going method performance verification during routine analysis

- Where practicable, recoveries of all target analytes should be measured within each batch of analyses.

  If this requires a disproportionately large number of recovery determinations, the number of analytes may be reduced. However, it should be in compliance with the minimum number specified in the next Table

- At least 10% of the representative analytes (with a minimum of 5) should be included per detection system.

- Reporting level

Minimum frequency of recovery checks

	Representative analytes	All other analytes
Minimum frequency of recovery checks	10% of representative analytes (at least 5) per detection system, in each batch of analyses	Within a rolling programme to include all other analytes at least every 12 months, but preferably every 6 months
	Within a rolling programme covering all representative analytes as well as representative commodities from different commodity groups, at least at the level corresponding to the reporting Limit	At least at the level corresponding to the reporting limit

Recovery Concentration

- The recovery of an analyte should normally be determined by spiking within a range corresponding to the

  RL and 2-10 x the RL

  or at the MRL

  or at a level of particular relevance to the samples being analyzed.

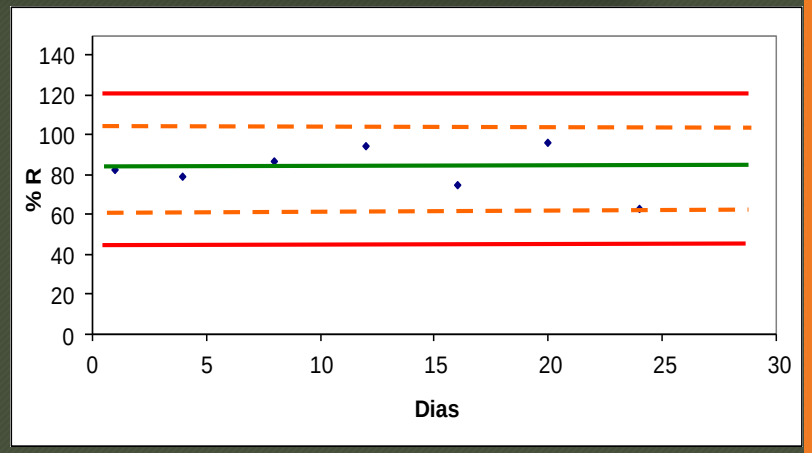
- The spiking level may be changed to provide information on analytical performance over a range of concentrations.
- Recovery at levels corresponding to **MRL and RL** are particularly important

Blank Matrix Extract

- The analyte (or apparent analyte) concentration in such a blank matrix extract should be determined from multiple test portions
  - the blank values should not be higher than **30% of the residue level corresponding to the RL**
- Where blank material is not available or where the only available blank material contains an interfering compound, the spiking level for recovery should be
  - **≥3 times the level present in the blank material**

Acceptance criteria for routine recoveries

Criteria	
Mean rec ± 2S	From initial validation
Practical default range 60-140% (IndRec)*	Outside de range Re-analysis **
RSD > 20%	Investigate
CRM	Seldom available
In house reference materials	Exchange between Laboratories



Mr +2SD
Mr
Mr -2SD

For each commodity group the mean recovery results and RSDs may be taken from initial method validation or from on-going recovery results (within laboratory reproducibility, RSDwR).

**

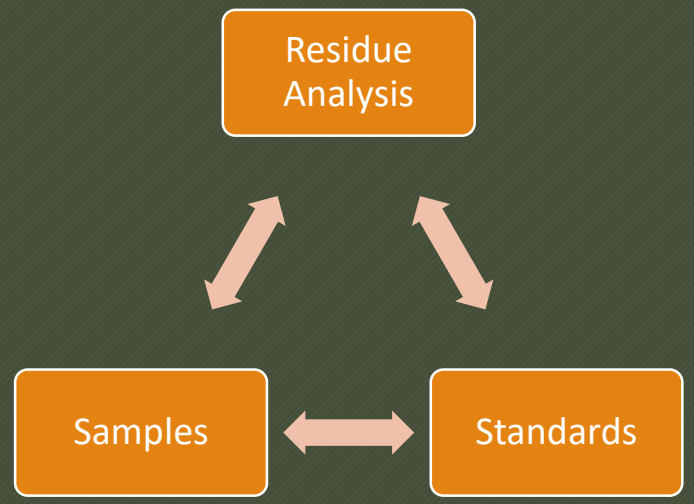
If at some point during the rolling programme the recovery of an analyte is outside of the acceptable range, then all of the results produced since the last satisfactory recovery must be considered to be potentially erroneous.

Proficiency testing

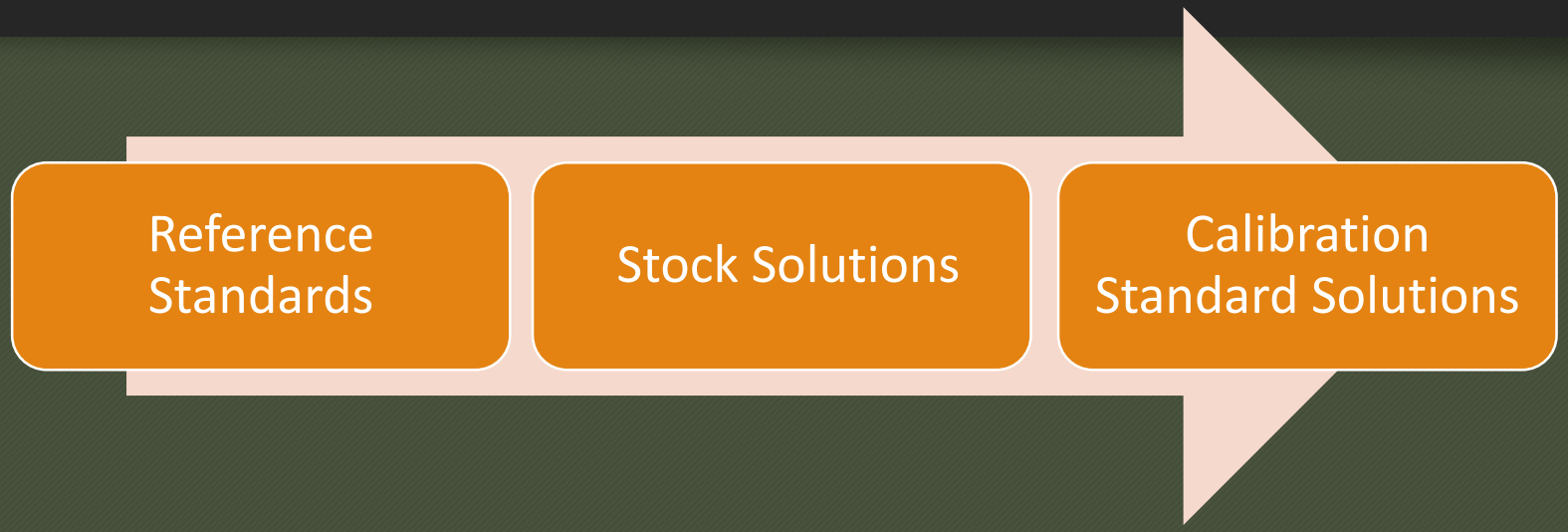
- For all official control laboratories **it is mandatory** to participate regularly in proficiency test schemes

- When:
  - false positive(s)
  - or negative(s) are reported
  - or the accuracy (z scores) achieved is questionable or unacceptable

the problem(s) should be investigated and rectified before proceeding with further determinations of the analyte/matrix combinations involved



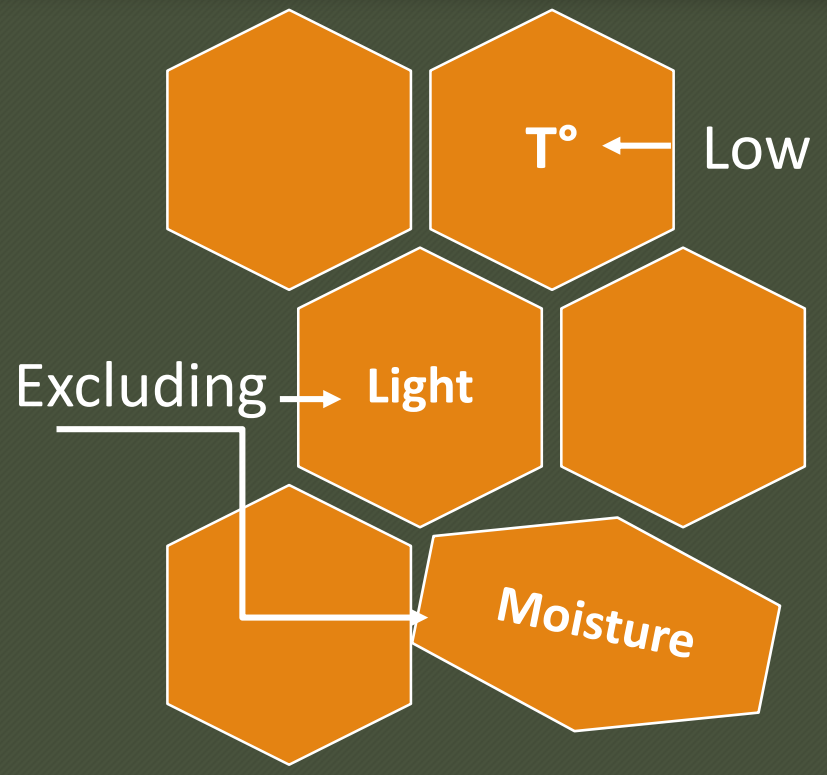
Pesticide standards, stock solutions and calibration standard solutions



Reference standards: Identity & Purity

- Reference standards of analytes should be of **known purity** and must be assigned with a **unique identification code** and recorded in a way that **ensures full traceability**
  - Source of supply
  - Badge number
  - Date of receipt
  - Place of storage
  - Expiring date

Storage of reference standards



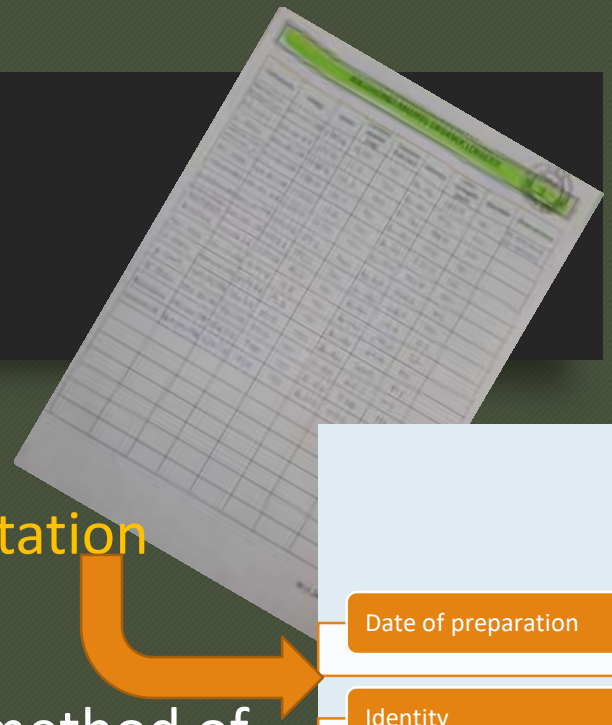
Under conditions that minimize the rate of degradation

- Identity of a freshly acquired reference standard should be checked if the analyte is new to the laboratory.
- For screening purposes only, the reference standards and derived solutions may be used after the expiry date
- Certified reference standard and calibration standard solution made has to be used for quantification.

The supplier's expiry date, often based on less stringent storage conditions, may be replaced, as appropriate for each standard, by a date allowing for storage up to 10 years.

Preparation of stock solutions

- When preparing stock of reference standards documentation should be such as to ensure full traceability.
- The solvent(s) must be appropriate to the analyte and method of analysis.
- Moisture must be excluded during equilibration of the reference standard to room temperature before use.
- Concentrations must be corrected for the purity of the reference standard.



- Date of preparation
- Identity
- Mass or Volume measured
- Identity and Volume of solvent

Preparation and storage of stock solutions

- ✓ Not less than 10 mg of the "reference" standard should be weighed using a 5 decimal place balance.

- ✓ The ambient temperature : glassware has been calibrated

- ✓ Volatile liquid analytes should be dispensed by volume or weight (if the density is known) directly into solvent.

- ✓ Gaseous (fumigant) analytes may be dispensed by bubbling into solvent and weighing the mass transferred.

Stock standards must be labelled indelibly, allocated an expiry date and stored at low temperature in the dark in containers that prevent any loss of solvent and entry of water

Use of stock Solutions

- Equilibration to room temperature, solutions re-mixed and ensure that the analyte remains completely dissolved.
- Use of a different solvent, different storage conditions or solutions with lower concentration can help to overcome the problem of limited solubility.

Currently available data show that:

stock solutions of the large **majority of pesticides**

when stored in tightly closed glass containers in the freezer,

are sufficiently stable for at least **5 years in toluene or acetone**

and for **at least 3 years in acetonitrile, methanol or ethyl acetate.**

Working solutions

0,05 ppm

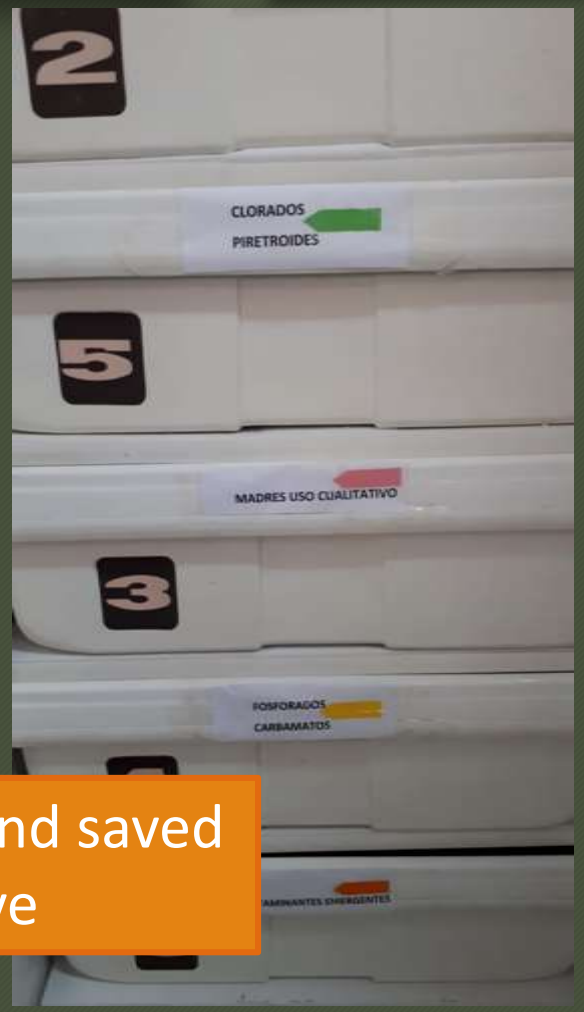
0,10 ppm

0,20 ppm

0,30 ppm

0,50 ppm

5 viales of 0,05 ppm



The last dilution can be divided into vials and saved for direct use in the calibration curve

Testing and replacement of standards

The stability of an existing and possibly expired “reference” standard may be checked against a new one

vs

using appropriate dilutions of individual standards or mixtures of standards.

Discrepancies between the concentrations of new and old solutions maybe due to a number of factors other than simply analyte degradation

- analyte precipitation
- solvent evaporation
- differences in the purities
- errors in weighing
- errors in the instrumental analysis

Testing and replacement of standards

Means should not differ more tan 10%

- When the difference exceeds ± 10%, storage time or conditions may have to be adjusted. Both old and new solution should be checked against another new solution that is prepared independently from the first two.
- The variability of (at least 5) replicate injections(expressed as repeatability-RSDr) should also be taken into account. Low variability should be pursued to minimize the uncertainty of the calculated concentration.
- An internal standard may be used to reduce measurement variation. It is recommended to inject the old and new standards in alternating order to reduce any bias caused by signal drift.

Testing and replacement of standards

Where sufficient evidence exists (data from ≥2 other labs) that a certain pesticide is stable using specified storage conditions (time, solvent, temperature etc.) then other laboratories reproducing these storage conditions can reduce their own stability checks accordingly.

Additional recommendations

Contamination and Interferences

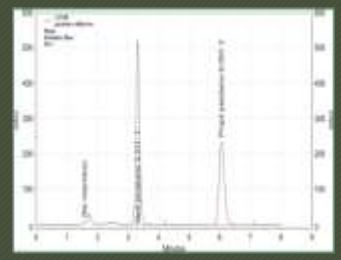
Contamination

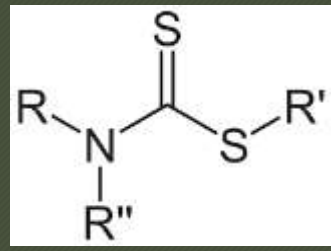
- Samples separated:
  - from each other
  - from other sources of potential contamination
- Volumetric equipment must be cleaned scrupulously
  - Separated for samples and standards
  - The use of excessively scratched or etched glassware should be avoided

- Solvents used for fumigant residues analysis should be checked to ensure that they do not contain target analyte(s)

Contamination

- Where the analyte occurs naturally, or is produced during the analysis (e.g. carbon disulfide from the Brassicaceae), low-level residues from pesticide use cannot be distinguished from background levels.
- Natural occurrence of these analytes must be considered in the interpretation of results.
- Dithiocarbamates, precursors of carbon disulfide, ethylenethiourea or diphenylamine can occur in certain types of rubber articles and this source of contamination must be avoided.





Interferences

- Sources of possible interferences:
  - Equipment, containers, solvents (including water), reagents, filter aids, etc.
  - Rubber and plastic items, polishes and lubricants

- Vial seals
  - should be PTFE-lined
  - extracts should be kept out of contact with seals, especially after piercing (by keeping vials upright)
  - may have to be replaced quickly after piercing, if re-analysis of the extracts is necessary.

Analysis of reagent blanks should identify sources of interference: equipment or materials used

Interferences

- Matrix effects or matrix interferences from natural constituents of samples are frequent

The interference may be peculiar to the determination system used, variable in occurrence and intensity, and may be subtle in nature.

- If the interference takes the form of a response overlapping that of the analyte, a different clean-up or determination system may be required.
- Matrix effects in terms of suppression or enhancement of the detection system response is dealt with:

matrix matched standards
analytical protectants
standard addition methods

If it is not practicable to eliminate or compensate for matrix effects the overall accuracy of analysis should nonetheless comply with the established criteria

Muchas Gracias !

Obrigado !

Thank you !