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ANALYTICAL QUALITY CONTROL FOR PESTICIDE RESIDUES AND MYCOTOXINS ANALYSIS IN PLANT PROTECTION AND FOOD SAFETY

Analytical Chemistry Laboratory

Lesotho Plant Protection and Quarantine

The primary aim of this Laboratory Analytical Qaulity Control Guideline is to detail the quality system procedures that ensures the consistent validation of the data generated by Plant Protection and Quarantine – Analytical Chemistry Laboraotry (PPQ – ACL). It is meant to be used in conjunction with system procedures including Laboratory Standard Operating Procedures (SOPs) which contain method specific details to ensure accuracy, precision and completeness of the individual results and the supporting quality control (QC) measurements, resulting in a scientifically defensible curriculum.

PPQ – ACL provides analytical survices to support regulatory and non-regulatory programs requiring data quality objectives that meet a variety of clients requairements. The manual was developed and approved in June 2019.

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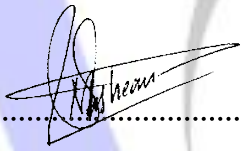

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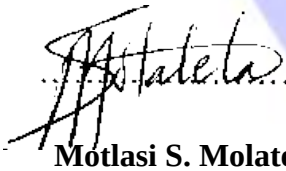

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Foreword

The control of plant health and hence the food safety and quality is an integral part of national programmes for the development. National plant control systems are devised to protect the plants, food and the health of consumers in an attempt to advance the development of trade in plants and plant products as well as food products. The emphasis is mainly on the prevention of chemical and biological hazards which may result from contamination of plants and food.

A significant part of the national plant and food control system is the ability for the analytical laboratory service to detect and quantify plants and food pollutants including residual pesticides and mycotoxins. The main objective of this guideline is to assure that the plant protection and food safety laboratory produces high-quality analytical results using an analytical methodology which has been proven to be reliable and reproducible for selected residual pesticides and mycotoxins analysis. This includes a brief discussion of sampling and sample preparation techniques as well as method development, validation and results reporting.

The following precautions apply to all the procedures in this guideline:

a) Use only deionized water (the worst case distilled water).
b) Use of the high-grade reagents with the best purity.
c) Use all laboratory safety procedures and safety equipment.
d) Follow the method instructions.

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1. Introduction

It is considered that the ultimate goal in fair practice in international trade depended, among other things, on the reliability of analytical results. This in turn, particularly in pesticide residue and mycotoxins analysis, depended not only on the availability of reliable analytical methods but also on the maintenance of good practice in the analysis of pesticides and mycotoxins.

The guidelines in this document are intended for the monitoring of pesticides and mycotoxins in Lesotho. The guidelines define the analytical quality control (AQC) requirements to support the validity of the data used for checking compliance with minimum residue limits (MRLs), enforcement actions or assessment of consumer exposure to pesticides and mycotoxins. The quality requirements detailed in this document are meant for as guidance for accreditation purposes.

The primary objectives of the guideline are:

a) to ensure that false positive or negative results are not reported.
b) to ensure that acceptable accuracy is attained.
c) to accord cost effective AQC in Lesotho.
d) to support compliance with ISO 17025, the accreditation standard.

2. Sample preparation

Sample preparation is considered as the bottleneck in the analytical chemistry laboratories. It is key for effective and accurate analysis of trace pesticide residues and mycotoxins. The primary aim of samples preparation is to isolate the trace amounts of analytes from a large number of complex matrices and eliminate interference from the sample matrix as much as possible. Typical sample preparation steps include sampling/homogenization, extraction, and cleanup.

2.1. Sampling

2.1.1. Representative samples

Each primary samples that make up the field sample should be sampled randomly, systematically or selectively in the lot. Representative samples of the crop in each plot must be taken considering the following point:

a) when taking a sample at harvest, avoid taking diseased or under-sized crop parts or commodities at a stage when they would not normally be harvested.
b) sample the parts of the crop that normally constitute the commercial commodity.
c) take samples in such a way as to be reasonably representative of typical harvesting practice.
d) take care not to remove surface residues during handling, packing or preparation.
e) take and bag the required weight of samples in the field and do not sub-sample.

2.1.2. Control samples

Always take control samples. These are as important as samples from test plots. Control samples should be of similar quality to that of the test samples. Control samples should be taken before the treated samples, so as to avoid the possibility of contamination from handling. The control sample is normally needed for residual pesticides analysis.

2.1.3. Sampling procedures for field crop

The number of different commodities required to constitute a satisfactory sample obviously vary according to the commodity itself. The recommended size of the field samples may differ from those recommended for the enforcement of maximum residue limit because field samples are often required to satisfy other needs including research programmes.

2.1.4. Sampling of processed commodities

Where a commodity is normally processed between harvest and marketing, such as by milling, pressing, fermentation, drying or extraction, data may be required on the processed crop or its

products. Details of the processing method should be supplied with the samples along with storage and handling histories. In such cases, the trials should be planned to provide samples with appropriate residue levels so that the fate of residues can be studied during the processing.

2.1.5. Sampling of stored commodities

Trials with stored products/post-harvest treatments should be carried out over a wide range of storage facilities and the sampling technique must be carefully chosen if a valid sample from most commodities in storage units is well established. Such procedures are acceptable in sampling for pesticide residue and mycotoxins analysis and may be used if adequate references are given.

2.1.6. Sampling record

The sampling personnel must record the nature and origin of the lot; the owner, supplier or carrier of it; the date and place of sampling; and any other relevant information. Any deviation from the recommended method of sampling must be recorded. A signed copy of the record must accompany each replicate laboratory sample and a copy should be retained by the sampling personnel. A copy of the sampling record should be given to the owner of the lot, or a representative of the owner, whether or not they are to be provided with a laboratory sample.

2.1.7. Sample transportation

The laboratory samples must be in a clean, inert container which provides secure protection from contamination, damage, and leakage. The container should be sealed, securely labeled and sampling record must be attached. Samples of commodities prepacked for retail sale should not be removed from the packaging before transportation. Very fragile or perishable products such as ripe raspberries may have to be frozen to avoid spoilage and then transported in dry ice to avoid thawing in transit. Samples that are frozen at the time of collection must be transported without thawing. Samples that may be damaged by chilling including bananas must be protected from both high and low temperatures. Transportation to the laboratory within one day is crucial for samples of most fresh products.

2.2. Preparation and storage the analytical sample

On receipt, each of the samples must be given a unique identification which together with the date of receipt and the sample size should be added to the sample record. The analytical sample should be comminuted and mixed well to enable representative portions to be withdrawn. The size of the analytical portion will be determined by the analytical method and efficiency of mixing. The methods for comminution should be recorded and should not affect the residues and mycotoxins present in the sample. If the analytical samples are to be stored before analysis, the method and length of time of storage should be such that they do not affect the level of residues and mycotoxins present. An additional portion must be withdrawn for replicate and confirmatory analyses, as required.

2.2.1. Extraction

It involves the separation of analyte from the sample matrix using a solvent. The extraction procedure should be such that it quantitatively removes the analyte from matrix efficiently but does not change the analyte chemically. The extraction method and solvent type determine the extraction efficiency. Common extraction preparation techniques for pesticide residue and mycotoxins analysis include liquid-liquid extraction (LLE). LLE is a classic method for routine sample preparation due to its simplicity, robustness, and efficiency. In this procedure the homogenized solid or liquid samples are repeatedly extracted with an immiscible organic solvent and extract are then centrifuged, concentrated and/or purified before analysis.

2.2.2. Clean up of the extract

During extraction, the solvent comes in contact with the sample matrix, in order to enable extraction of the pesticides and mycotoxins along with some other constituents of the matrix along get solubilized. The extract not only contains pesticide residues or mycotoxins but also other constituents called co-extractives. The removal of interfering co-extractives from the extract is called clean up. The co-extractive typically extracted along with pesticides or mycotoxins from the matrix are moisture, pigments including chlorophyll, xanthophyll, and anthocyanins, colorless compounds like oil fat and wax. The most common clean up method

is liquid-liquid partitioning. In this technique, co-extractives from the extract are removed by partitioning the residues between two immiscible solvents.

2.2.3. Storage of the extract

The extracts must be stored in a refrigerator or freezer to minimize degradation. At times the loss of pesticides and/or mycotoxins in the extracts at room temperature may occur. Analyte stability in extracts should be evaluated during method validation.

3. Standard and calibration solutions

3.1. Identity, purity, and storage of standards

Standards of analytes and internal standards should be of known purity and each must have a unique identity and the date of receipt recorded. They should be stored under the conditions that minimize the rate of degradation such as in the freezer where there is no light and moisture. The identity of the standards should be checked and verified.

3.2. Preparation and storage of stock standards

The identity and mass or volume of pure standards and the identity and amount of solvent must be recorded when preparing stock standards. The solvent must be appropriate to the analyte (solubility, no site reactions) and the method of analysis. Moisture must be avoided during equilibration of the pure standards to room temperature before use.

Individual stock standard solutions typically are prepared in a concentration of 1 mg/mL. The required amount of pure pesticide or mycotoxin reference standard, typically 10 – 50 mg, should be weighed out using a 4 - or 5 - decimal place balance or scale, depending on the method. Transfer to an appropriate volumetric flask, dissolve in a suitable solvent and makeup to the required volume to achieve the desired concentration. Label the flask with all relevant information, allocate an expiry date and store in the freezer. Liquid reference standards can be dispensed, by weight, directly into the solvent.

3.3. Preparation and storage of working standard solutions

Working standard solutions typically have very low concentrations in a range of 0.005 – 0.01 µg/mL. To achieve such low concentrations, it may be necessary to create intermediate solutions and dilute further to achieve the required concentration. For a multi-residue method, several pesticides or mycotoxin standards are mixed. Combine appropriate quantities of the individual stock standard solutions to create the required concentrations to compare against the sample under investigation.

4. Analytical method verification

4.1. Calibration of method

Standard curves are developed by the operator using calibration standards and are used to quantify the concentration of the analyte in the sample. Calibration standards prepared for each analyte shall include at least not less than two low-end concentration, two different mid-range concentration, and high-end concentration. This range of concentration value must bracket the expected range of concentrations in the samples. Typically, the concentration should be from 70% of the lowest to 130% of the highest expected in the sample. Use the literature values from multiple sources to establish the bracketing concentrations with multiple points around the typical concentrations reported. The calibration is valid only within the bracketing concentrations.

Combining the multiple analytes into a single standard vial is acceptable, provided that there is a clear separation between peaks. All calibration standards shall be run at the beginning and the unknown samples, provide a blank, a spiked sample, and duplicate unknown sample for each run or for every 15 unknown samples, the three extra samples are used for QC. These 18 samples represent a group of samples. The blank will be a sample that includes the background matrix, that is, any reagent used in sample preparation but does not contain the analyte of interest. The spiked sample should be prepared by splitting an existing unknown sample and then adding a known quantity of each analyte to the sample. The duplicate sample is a split unknown sample that can come from an unknown, it is often taken from the same sample that

is used in the spiking process. The QC sample shall be eventually spaced among the unknown samples so that the QC check occurs throughout the grouping.

There must be at least one calibration verification sample per group or two per run. The sample must be designated as a verification sample in the system during method development. Run at least one standard from each analyte group several times during the run in order to determine if the peak is shifting in time to provide additional calibration points or calibration verification points

4.2. Validation of methods

Validation is the process of verifying that a method is suitable for the intended purpose. The method may be developed in-laboratory, taken from the literature or obtained from the third party. The method may then be adapted or modified to match the requirements and capacities of the purpose for which the method will be used. Generally, validation follows completion of the development of the method and it is assumed that the requirements such as calibration, system suitability, and analyte stability have been established satisfactory. Typically, validation will precede the practical application of the method to the analysis of samples. Proficiency testing provides an important means for verifying the accuracy of the results generated by the method.

4.3. Confirmatory test

When analyses are performed for monitoring or enforcement purpose, it is important that confirmatory data are generated before reporting on samples containing residues of pesticides or mycotoxins that are not normally associated with that particular commodity. The need for confirmatory tests may depend upon the type of sample or its known history. For a series of samples of similar origin which contain residue of the sample pesticides or mycotoxins, it may be enough to confirm the identity of the residues in a small proportion of the samples selected randomly. Similarly, when it is known that a particular pesticide has been applied to the sample material there may be a little need for confirmation of identity, although randomly selected

results should be confirmed. The occurrence of interfering substances should be checked against the blank.

5. Reporting results

For regulatory purposes, only confirmed data should be reported. Null values should be reported as being less than the lowest calibration level, rather than a level calculated by extrapolation. Where positive results are obtained by replicate determination of a single test portion (sub-sample), the lowest valued value obtained should be reported. Where positive results derived from the analysis of multiple test portions, the arithmetic mean of the lowest valid values obtained from each test portion should be reported. Typically, for a 20 – 30% relative precision the results should be expressed only in two significant figures. Since at lower concentrations the precision may be in the range of 50%, the residue values below 0.1 may be expressed with one significant figure.

6. References

1. Joint FAO-WHO Food Standard Programme. Codex Alimentarius Commission. Report on the thirty-fifth session of the Codex Committee on pesticide residues, Rotterdom, The Netherlands. 31 March – 5 April 2003.
2. European Commission – Directorate General for Health and Food Safety. Guidance document on analytical quality control and method validation procedures for pesticide residues and analysis in food and feed. SANTE/11813/2017, 21 – 22 November 2017.
3. National Food Administration- Sweden. Quality control procedures for pesticide residues analysis. SANCO/10232/2006, 28 September 2007.