Analytical Methods for the Analysis of Veterinary Drug Residues in Food Producing Animals — Guidelines

ICS 67.050

1 Scope

This Standard provides guidelines on the suitability of analytical methods used by the competent authorities and/or their officially accredited laboratories for testing programs for all residues of veterinary drugs in food producing animals.

2 Use

This Standard follows the principles provided in PNS/BAFS 380:2024 (Design and implementation of regulatory food safety assurance programs associated with the use of veterinary drugs in food producing animals — Guidelines) and should be used in conjunction with this document.

3 Normative References

The following documents are referred to in the text in such a way that some or all their contents constitute the requirements of this document. The latest edition of the referenced documents (including any amendments) applies.

Bureau of Agriculture and Fisheries Standards (BAFS)-Department of Agriculture (DA). (2022). Veterinary Drug Residues in Food — Product Standard — MRL (PNS/BAFS 48:2022)

BAFS-DA (2024). Design and Implementation of Regulatory Food Safety Assurance Programs Associated with the Use of Veterinary Drugs in Food Producing Animals — Guidelines (PNS/BAFS 380:2024)

Codex Alimentarius Commission (CAC). (2006). Guidelines for the assessment of the competence of testing laboratories involved in the import and export control of food (CAC/GL 27-1997) https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B27-1997%252FCXG_027e.pdf

CAC (2014). Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programs Associated with the Use of Veterinary Drugs in Food Producing Animals (CXG 71-2009) https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B71-2009%252FCXG_071e_2014.pdf

International Organization for Standardization (ISO). (2015). Quality Management System (QMS) — Requirements (ISO 9001:2015)

ISO. (2017). Testing and calibration laboratories — Requirements (ISO /IEC 17025:2017)

4 Terms and Definitions

For the purpose of this Standard, the following definitions shall apply:

4.1
Acceptable Daily Intake (ADI)
amount of veterinary drug, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk (CAC, 2014)

4.2
collaborative study
analysing the same sample(s) by using the same method to determine performance characteristics of the method in different laboratories, where the study allows to calculate the random measurement error and laboratory bias for the method use (European Union [EU], 2021)

4.3
competent analyst
licensed professional qualified to evaluate and interpret data to derive meaningful insights, possessing the ability to apply analytical methods effectively and communicate findings clearly to support decision-making

4.4
competent authority
government authority or official body authorized by the government that is responsible for the setting of regulatory food safety requirements and/or for the organization of official controls including enforcement (CAC, 2022)

4.5
decision limit for confirmation (CCα)
limit at and above which it can be concluded with an error probability of α that a sample is non-compliant and the value 1 – α means statistical certainty in percentage that the permitted limit has been exceeded (EU, 2021)

4.6
detection capability for screening (CCβ)
smallest content of the analyte that may be detected or quantified in a sample with an error probability of β (EU, 2021)

4.7
matrix
material or component sampled for analytical studies, excluding the analyte (CAC, 2014)

4.8
official accreditation
process wherein the DA regulatory agency having jurisdiction formally recognizes the competence of a person or an entity providing services such as testing, calibration, technical assessment or evaluation, inspection, certification, and training services to perform such services on behalf of the DA regulatory agency (DA, 2023)

4.9
quality management system
ensures that a laboratory is managed and operated in a manner that meets the requirements of an internationally recognized quality standard to produce quality data and results (e.g. ISO 17025:2017 and ISO 9001:2015) (CAC, 2014)

4.10
quantitative method
method capable of producing results, expressed as numerical values in appropriate units, with accuracy and precision which are fit for the purpose (CAC, 2014)

4.11
repeatability
precision usually expressed as RSD, obtained from the same measurement procedure or test procedure; the same operator; the same measuring or test equipment used under the same conditions; the same location and repetition over a short period of time (CAC, 2009)

4.12
residues
parent compounds and/or their metabolites in any edible portion of the animal product and include residues of associated impurities of the veterinary drug concerned (CAC, 2024)

4.13
sample processing
procedure(s) (e.g. cutting, grinding, mixing) used to make the analytical sample acceptably homogeneous with respect to the analyte distribution prior to removal of the analytical portion (CAC, 2014)

4.14
screening method
method used to detect the presence of an analyte or class of analytes at or above the minimum concentration of interest (CAC, 2014)

4.15
sensitivity
lowest concentration at which the target analyte may be reliably detected within defined statistical limits (EU, 2021)

4.16
selectivity
ability of the test to determine that samples which give a negative response are truly negative (CAC, 2014); and to distinguish between the analyte being measured and other substances (EU, 2021)

4.17
veterinary drugs
any substance applied or administered to any food-producing animal, such as meat or milk producing animals, poultry, fish or bees, whether used for therapeutic, prophylactic, or diagnostic purposes, or for modification of physiological functions or behaviour (CAC, 2024).

5 General Consideration on Analytical Methods for Residue Testing

5.1 Analytical methods used by the competent authorities for their testing programs should be fit for purpose to determine compliance for all residues of veterinary drugs in *food-producing animals.* These include residues coming from pesticides which have veterinary use.

5.2 Analytical methods may also be required in regulatory control programs for the detection of residues of substances for which ADI and MRLVD have not been established by the competent authority. In substances where an ADI or MRLVD should be established based on the toxicological evaluation, the primary concern in the method validation should be:

a) determination of the lowest concentration (LoD) at which the residue can be detected. The performance characteristics related to quantitative analyses may be less critical; and
b) *determination* of the identity of residues in a food. It is generally based on the comparison of a set of characteristics of a detected substance with those of a known standard of the suspected residue.

5.3 Competent authorities responsible for designing national residue testing programs should ensure that appropriate residue methods of analysis are used to assure compliance with the established MRLVD.

5.4 A new analytical method or the extension of the validation of an existing analytical method to include a new combination of analyte and matrix may be developed and validated.

5.5 Appropriate regulatory action shall be taken against adulterated products, consistent with the reliability of the analytical data.

5.6 Integrating Analytical Methods for residue testing

5.6.1 Analytical methods for veterinary drug residues in foods shall reliably detect the presence of an analyte of interest, determine its concentration and correctly identify the analyte.

5.6.2 When residues resulting from the use of approved veterinary drugs are detected at concentrations above an established MRLVD, the results should be confirmed before regulatory enforcement actions are taken.

5.6.3 For substances which have been banned from use in food-producing animals by a competent authority, or for which an ADI and MRLVD have not been established for toxicological reasons, the confirmed presence of residues at any concentration in a food shall result in regulatory action.

5.6.4 The principal performance attributes of analytical methods used in residue testing programs should depend on whether a method is intended to simply detect, to quantify, or to confirm the presence of a target residue. Completion of a full collaborative study shall not be required for recognition of a method to be placed in one of these three categories.

5.6.5 The three categories of methods should be screening, quantitative and confirmatory and may share some performance characteristics. Each category may have other specific considerations. A balanced residue testing program should understand the relationship between these three categories of methods. These three categories of methods may be applied sequentially in a residue testing program. The performance characteristics/parameters that a multi-residue method (MRM) should have in order to provide internationally acceptable confidence in the method to produce results suitable for evaluating the residues of veterinary drugs are shown in Annex A (Performance characteristics for Multi-Residue Methods (MRM) for veterinary drug)

5.6.6 Screening methods (either qualitative or semi-quantitative in nature) should be used to identify the presence (or absence) of residues in samples.

5.6.6.1 The screening methods may be used to quickly determine which products require further testing and which can be released. However these methods

may not provide adequate information to accurately define the concentration present or, to confirm the structure of a residue.

5.6.6.3 The screening methods should have a defined and low false negative rate and should not be used alone for residue testing purposes on official samples without the availability of suitably validated quantitative and/or confirmatory methods to apply to any samples identified as potentially not in compliance with an MRLVD.

NOTE Low false negative rate may be defined by the competent authority based on the methods used.

5.6.7 Quantitative methods shall provide quantitative information which may be used to determine if residues in a particular sample exceed an MRLVD or other regulatory action limit.

5.6.7.1 Quantitative methods cannot provide unequivocal confirmation of the identity of the residue.

5.6.7.2 Quantitative methods shall perform in good statistical control within the analytical range that brackets the MRLVD or regulatory action limit.

5.6.8 Confirmatory methods shall provide unequivocal confirmation of the identity of the residue and may also confirm the quantity present and shall be the most definitive and be based on combined chromatographic and mass spectrometric techniques (e.g., such as liquid chromatography – tandem mass spectrometry [LC-MS/MS]).

5.6.8.1 When confirmatory methods are used for confirmation of residue identity, they should provide reliable structural information within established statistical limits.

5.6.8.2 When the confirmatory method does not provide quantitative information, the quantification result of the original quantitative method should be verified by analysis of replicate test portions using the original quantitative method or a suitably validated alternative quantitative method.

5.6.9 Samples which test “positive” with the screening method shall be considered as *suspect* and shall be subjected to further laboratory testing using more definitive methods. This may include repeat testing of replicate test portions with a screening method.

5.6.10 Quantitative and/or confirmatory methods should be used in the laboratory to *verify* that the sample does contain residues in excess of the regulatory limit. Such tests should be conducted on new test portions of the sample material used in the initial screening test to confirm that the analyte detected in the initial

test is definitely the suspected compound and that the MRLVD (or other regulatory action limit established by the competent authority) has indeed been exceeded.

5.6.11 The performance attributes, or characteristics, which shall be determined during method validation for each *category* of method – screening, quantitative, confirmatory – are presented in Clause 6 (Attributes of Analytical Methods for Residues of Veterinary Drugs in Foods).

5.7 Consideration for Selection and Validation of Analytical Methods

5.7.1 Identification of methods of requirements

5.7.1.1 Method scope

The following shall be considered:

a) The intended purpose of the method should be defined in a statement of scope which defines the analytes (residues), *the species (e.g., chicken),* the matrices (eg., tissues, milk, honey, etc.), and the concentration range to which the method applies.
b) The scope should also state whether the method is intended for screening, quantitative, or confirmatory use.
c) The competent authority shall establish an appropriate marker residue for each drug for which an MRLVD has been established and should also designate a preferred target tissue to be sampled for testing.

5.7.1.2 Marker residue

The following shall be considered:

a) The MRLVD should be expressed in terms of the marker residue, which may be the parent drug, a major metabolite, a sum of parent drug and/or metabolites or a reaction product formed from the drug residues during analysis.
b) In some cases, the parent drug or the metabolite may be present in the form of a bound residue which requires chemical or enzymatic treatment or incubation to be released for analysis.
c) The marker residue should, whenever possible, provide unequivocal evidence of exposure to the drug.
d) *In cases when the marker residues also result from sources other than exposure to the drug*, additional information should be required to ascertain the probable source of the residue is exposure to the drug (e.g use of semicarbazide, which may occur from other sources, as a marker residue for the drug nitrofurazone).

5.7.1.3 Target *matrix*

The following shall be considered:

a) Edible tissue and animal food products should be the target *matrix* selected by competent authorities to be tested for veterinary drug residues in a residue testing program as the residues of the marker residue occur at the highest concentrations and are most persistent.
b) In cases where drugs are normally administered as injectable formulations, testing of muscle tissue from suspected injection sites may be required.
c) The competent authorities and laboratories shall clearly identify the testing objectives and the analytical requirements required in terms of target *matrix*, marker residues and concentration ranges to ensure suitable methods are used in the regulatory control program.
d) When applicable, competent authorities may also use biological fluids such as urine or serum to indicate the presence or absence of residues of interest.

5.7.2 Implementing other guidelines

5.7.2.1 Laboratories involved in the import/export testing of food products should conform with CAC/GL 27-1997 (Guidelines for the assessment of the competence of testing laboratories involved in the import and export control of food) or other relevant existing guidelines.

5.7.2.2 Methods used for analyses of veterinary drug residues in foods should be capable of detecting the compounds included in the residue testing program.

5.7.2.3 The analytical recovery and precision for the target *matrices* should meet the criteria stated in 6.3.2.5 and 6.3.2.6

5.7.2.4 The methods should be used within an established laboratory Quality Management System (QMS) which is consistent with the principles in the document on internal quality control referenced above. When methods which have not been subjected to a multi-laboratory performance trial are used in a regulatory program for control of veterinary drug residues in foods, the quality control and quality assurance procedures applied with these methods shall require careful definition, implementation, and monitoring.

5.7.2.5 In the case of methods which have been through multi-laboratory trials, performance characteristics, such as *trueness* and precision, shall be defined through the results obtained during the study.

5.7.2.6 For a method validated within a single laboratory, data shall be generated to define the performance characteristics expected of the method when used by

analysts within that laboratory. The ongoing performance shall be monitored through the QMS in place in the laboratory.

5.7.3 Method validation and fitness for purpose

5.7.3.1 The process of method validation shall demonstrate that a method is fit-for-purpose.

5.7.3.2 The validation should address the issues of marker residue, target *matrix* and concentration range identified by the laboratory in consultation with the *competent authority*.

5.7.3.3 When the protocol of the validated method is followed using suitable calibration standards, the results within the established performance limits obtained on the same or equivalent sample material by any *competent* analyst shall be comparable.

5.7.4 Multi-laboratory validation or collaborative approach

5.7.4.1 Multi-laboratory method performance studies shall satisfy the analytical requirements for use in a regulatory program. These methods should be subjected to a properly designed inter-laboratory study *so that variabilities in method performance characteristics that includes the analysts, standards and reagents, other materials, and equipment are considered*

5.7.4.2 Quantitative methods should be studied collaboratively according to the revised harmonized protocol prescribed by the relevant standards and have been evaluated in a minimum of 8 laboratories.

5.7.4.3 When applicable, multi-laboratory studies may be conducted which do not have the minimum number of laboratories required to qualify as a collaborative study. Such studies can provide useful information on method performance in the hands of multiple analysts in different laboratories but do not provide the same degree of statistical confidence obtained from the results of a collaborative study.

5.7.4.4 In the absence of methods validated through inter-laboratory method trials, *competent residue testing* laboratories shall frequently use *single laboratory validation* which have been subjected to studies conducted within their own laboratory to characterize the method performance.

5.7.5 Single laboratory validation – The criteria approach

In the case that inter-laboratory validated methods are not available or applicable, particularly for multi-analyte/ multi-substrate methods and new

analytes, methods may be validated in a single laboratory to meet the General Criteria for the Selection of Methods of Analysis, as well as the additional criteria:

a) The method is validated according to an internationally recognized protocol, such as the IUPAC guidelines;
b) The method is part of a QMS compliant with ISO/IEC 17025:2017 or Good Laboratory Practice principles;
c) Accuracy is demonstrated through:
   i) regular participation in proficiency schemes, if available;
   ii) calibration using certified reference materials, if applicable;
   iii) recovery studies at the expected concentration of the analytes; and
   iv) verification of results with other validated methods, if available.

6 Attributes of Analytical Methods for Veterinary Drug Residues in Food-Producing Animals

6.1 The performance characteristics of analytical methods used to determine compliance with MRLVD shall be defined and proposed methods evaluated accordingly. This will assure reliable analytical results and provide a secure basis for determining veterinary drug residues in food-producing animals in international trade. The Clause 5 (General Considerations of Analytical Methods for Residue Testing) above, presents a discussion of general types or categories of regulatory methods, and provides a scheme for using these analytical methods based upon their intended purpose in a regulatory framework.

6.2 Method development consideration

6.2.1 The development of an analytical method shall require *competent* analysts experienced in the analytical techniques to be used, as well as *adequate* laboratory space, equipment, and financial support.

6.2.2 The intended use and need for a method in a residue testing program should *first* be established, including the required performance parameters. Other considerations should include the following:

a) required scope of the method (compound or class of compounds of interest and sample *matrix*);
b) potential interfering substances;
c) the required performance characteristic of the measurements system;
d) the pertinent physical and chemical properties that may influence method performance;

e) the specificity of the desired testing system and how it will be determined, analyte and reagent stability data and purity of reagents;
f) the acceptable operating conditions for meeting method performance factors;
g) sample preparation guidelines;
h) environmental factors that may influence method performance, safety considerations; and
i) and any other specific information pertinent to program needs.

6.2.3 Stability of standards, both under normal conditions of storage and use and during processing of samples, should be assessed. Analyte stability in samples during typical conditions of sample storage prior to analysis should also be determined, including any period for which a sample may be held pending a potential re-analysis for confirmatory purposes.

6.2.4 Method performance attributes shall be established, as these provide the necessary information for *competent authorities* to develop and manage their *residue testing* programs.

6.2.5 Method performance requirements may vary, depending on whether the method is used for the screening, quantification, or confirmation of a residue for which MRLVD has been established, or for residues of a drug for which an ADI and MRLVD have not been recommended.

6.2.6 If there are no established MRLVD, the competent authority may set a minimum performance standard for analytical methods used for regulatory control purposes. When no safe concentrations of these compounds in foods have been established, the competent authority shall review such limits periodically to ensure they reflect improvements in technology and analytical capability. When such limits have not been formally established by the competent authority, they should be established as *de facto* by the detection capabilities of the methods deemed *acceptable to the competent residue testing* laboratories.

6.3 Analytical performance characteristics

6.3.1 Performance characteristics of screening methods

6.3.1.1 Screening methods may either be qualitative or semi-quantitative, with the objective to discriminate samples which contain no detectable residues above a threshold value (“negatives”) from those which may contain residues above that value (“*suspect*”).

6.3.1.2 The validation strategy should focus on the following:
a) establishing a threshold concentration above which results are “*suspect*”;

b) determining a statistically based rate for both “false positive” and “false negative” results;
c) testing for interferences; and
d) establishing appropriate conditions of use.

6.3.1.3 The “cut-off” or threshold for the test for a particular compound should be established by conducting concentration response experiments, typically using 10 replicates (from at least *one* source) fortified at each of a series of increasing concentrations. Once the concentrations have been established where all 10 replicates give a negative response and all 10 replicates give a positive response, the experiment should be repeated using the blank matrix materials fortified at four evenly spaced concentrations between the “all negative” and “all positive” concentrations *typically using 30 replicates from at least six sources*. An additional set *(10 replicates)* should be tested at a concentration 20% above the “all positive” concentration. Statistical analysis of the results enables the user to establish a reliable detection concentration at the required confidence level (usually 95%).

6.3.1.4 For a screening test, particularly those involving test kit technologies, sensitivity may be determined experimentally by testing a minimum of 30 residue-free sample materials fortified with the analyte at the target concentration (AOAC Performance Tested Program for test kits).

6.3.1.5 The sample materials should come from at least six different sources (at least 5 replicates from each of at least 6 sources), all of which should yield a positive result when fortified at the target concentration.

6.3.1.6 Three or more negative results should constitute a failure of the sensitivity test. If one or two of the results are negative, the experiment should be repeated and two negative results would then constitute failure. The experiment should be repeated with known incurred material at the target concentration, if such material is available.

6.3.1.7 The “selectivity” of a screening method shall be able to distinguish the presence of the target compound, or group of compounds, from other substances which may be present in the sample *matrix*.

6.3.1.8 The selectivity of a screening method may be increased when it is used as a detection system after chromatographic or other separation techniques. To demonstrate a selectivity rate of at least 90% with 95% confidence (recommended for screening tests) the following should be observed:
a) 30 replicate analyses shall be conducted on representative blank sample matrix from a minimum of six different sources and shall all have negative results;
b) additional tests for potential interferences and cross-reactivity may then be conducted by testing blank matrix fortified with potential interfering

substances, such as other drugs which might be used in animal treatment, potential environmental contaminants, drug metabolites, or chemically related compounds; and

c) responses should be negative when these compounds are present at concentrations which might reasonably be expected to be present in a sample.

6.3.2 Performance characteristics for quantitative methods

6.3.2.1 Selectivity, the ability of an analytical method to detect and discriminate the signal response from a compound in the presence of other compounds which may be present in the sample *matrix*, is of particular importance in defining the performance characteristics of methods used in regulatory control programs for veterinary drug residues in foods.

6.3.2.2 Selectivity of quantitative methods should consider the following aspects:

a) the ability of the method to provide a signal response which is free from interferences from other compounds which may be present in a sample; or sample extract; and

b) the ability of the method to unequivocally identify a signal response as being exclusively related to a specific compound.

6.3.2.3 For a quantitative method, the signal used for quantification should relate only to the target analyte and should not contain contributions for co-extracted materials.

6.3.2.4 Quantitative methods should be based on a comparison of the response from an analyte in a sample with the response from standards of the analyte in solution at known concentrations. In method development and validation, the calibration curve should first be determined to assess the detector response to standards over a range of concentrations. These concentrations (a minimum of five, plus blank) should cover the full range of analytical interest and the resultant curve should be statistically expressed.

6.3.2.5 In addition to the selectivity of a method, the ability of the method to provide a reliable quantitative result shall be demonstrated with the following factors:

a) the closeness of the result to the true or accepted value for the concentration of analyte present in the sample *matrix*, expressed in terms of trueness or bias; and

b) the ability of the method to provide consistent results on replicate determinations, expressed in terms of precision (repeatability and *within laboratory* reproducibility).

6.3.2.6 Methods used to support Codex MRLVD and PNS/BAFS 48:2022 (PNS on Veterinary Drug Residues in Food — Product Standard — MRL) should meet the performance standards for trueness and precision listed in Table 1.

Table 1. Performance criteria which should be met by methods suitable for use as quantitative analytical methods to support MRLVDs for residues of veterinary drugs in foods (CAC, 2001; Thompson et al., 1999)

Concentration µg/kg	Coefficient of Variability (CV)				Trueness
	Repeatability (Within-Laboratory, CV_A) %	Repeatability (Within-Laboratory, CV_L) %	Reproducibility (Between-Laboratory, CV_A) %	Reproducibility (Between-Laboratory, CV_L) %	Range of Mean % Recovery
≤ 1	35	36	53	54	50-120
1 to 10	30	32	45	46	60-120
10 to 100	20	22	32	34	70-120
100 to 1000	15	18	23	25	70-110
≥1000	10	14	16	19	70-110

NOTE CV_A refers to the coefficient of variation determined by test portions of blank matrix fortified prior to extraction

CV_L is the overall laboratory variability which includes a 10% estimate for variability of sample processing

6.3.2.7 The *trueness* of a method may be determined by analysis of a *matrix* certified reference material, by comparison of results with those obtained using another method for which the performance parameters have previously been rigorously established (typically, a collaboratively studied method) or, in the absence of reference materials or methods validated by inter-laboratory trial, by determination of the recovery of analyte fortified into known blank sample matrix.

6.3.2.8 Recovery should be expressed as the percentage of analyte experimentally determined after *spiking* of sample matrix at a known concentration and should be assessed over concentrations which cover the analytical range of the method.

6.3.2.9 Precision, which quantifies the variation between replicated measurements on test portions from the same sample *matrix*, is also an important consideration in determining when a residue in a sample should be considered to exceed an

MRLVD or other regulatory action limit. Precision of a method is usually expressed in terms of the repeatability and the between-laboratory variability (reproducibility) when the method has been subjected to a multi-laboratory trial.

6.3.2.10 For a single laboratory method validation, precision should be determined from experiments conducted on different days, using a minimum of six different tissue pools, different reagent batches, preferably different equipment, etc., and preferably by different analysts. Precision of a method is usually expressed as the standard deviation. Another useful term is relative standard deviation, or coefficient of variation (the standard deviation, divided by the absolute value of the arithmetic mean). It may be reported as a percentage by multiplying by one hundred.

6.3.2.11 The analytical function experiment data may also be used to calculate the analytical recovery at each concentration and is of particular importance when the presence of matrix co-extractives modifies the response of the analyte as compared to analytical standards. The linearity should be determined from the analytical function experiments and is the statistical expression of the curve obtained for the analysis of sample *matrices spiked* at the target concentrations. It should be determined from a linear regression analysis of the data, assuming there is a linear response.

6.3.2.12 Lower limits should be established which reliable detection, quantification, or confirmation of the presence of an analyte may be performed using a particular analytical method. The detection limit may be described in practical terms as the lowest concentration where the analyte can be identified in a sample. It can be estimated using the standard deviation ($S_{y/x}$) from the linear regression analysis of the standard curve generated in the analytical function experiment described above.

6.3.2.13 The limit of quantification (LOQ), may be established from the same experiments using the y-intercept of the curve plus ten times $S_{y/x}$. For methods used to support MRLVD established by the *competent authority,* the limit of quantification should meet the criteria for precision and *trueness* (recovery) in Table 1 and should be equal to or less than one-half the MRLVD. However, when the limit of quantification of a method is lower than the actual concentrations monitored for compliance with a MRLVD, the validation and subsequent application of the method should be based on a lowest calibrated level (LCL), which is typically 0.5x the MRLVD.

6.3.3 Performance characteristics for confirmatory methods

6.3.3.1 Selectivity, the ability of the method to unequivocally identify a signal response as being exclusively related to a specific compound, should be the primary consideration for confirmatory methods. Minimum of four identification points

shall be required to meet accepted performance criteria for regulatory methods. Method performance requirements for confirmatory methods based on low resolution GC/MS and LC/MS are shown in Table 2.

Table 2. Performance requirements for relative ion intensities (sample compared to standard) using various mass spectrometric analytical techniques (CAC, 2014)

Relative ion intensity (% of base peak)	GC-MS (EI) (relative)	GC-MS (CI), GC-MS/MS LC-MS, LC-MS/MS (relative)
>50%	≤10%	≤20%
20% to 50%	≤15%	≤25%
10% to 20%	≤20%	≤30%

6.3.3.2 Other techniques, when they are used in combination, may be capable of achieving a comparable degree of selectivity as confirmatory techniques. For example, identification may be verified by combinations of methods such as:

a) thin layer chromatography;
b) element-specific gas-liquid chromatography and accompanying detection systems;
c) formation of characteristic derivatives followed by additional chromatography; or
d) determining compound specific relative retention times using several chromatographic systems of differing polarity.

6.3.3.3 Such procedures shall be applicable at the designated MRLVD of the analyte. When a confirmatory method such as mass spectrometry is not available, information on the selectivity associated with the analysis of a particular veterinary drug residue in a sample may be developed from various sources. This information may be captured in a structured logging document of all the information that leads to the conclusion a method has detected a particular compound in a sample, at a measured concentration as reported. While no single measurement or analysis may provide the unequivocal proof of compound identity and/or quantity present that is desired, the combined information that has been compiled provides evidence that the analyst has made a conscientious effort to arrive at a logical result consistent with the data and other information available. Examples of analytical techniques which may be suitable to meet criteria for confirmatory analytical methods are summarized in Table 3.

Table 3. Examples of detection methods suitable for the confirmatory analysis of substances, as recommended by the Miskolc Consultation (CAC, 2014)

Detection method	***Criterion***
LC or GC and Mass Spectrometry	If sufficient number of fragment ions are monitored
LC-DAD	If the UV spectrum is characteristic
LC – fluorescence	In combination with other techniques
2-D TLC – (spectrophotometry)	In combination with other techniques
Gas Chromatography with Electron Capture Detector (GC-ECD),)	Only if combined with two or more separation techniques[a]
Derivatization	If it was not the first-choice method
LC-immunogram	In combination with other techniques
LC-UV/VIS (single wavelength)	In combination with other techniques
NOTE [a] Other chromatographic systems (applying stationary and/or mobile phases of different selectivity) or other techniques.	

6.3.4 General performance characteristics for methods for use in a regulatory control program.

6.3.4.1 There are some additional considerations for selection of suitable methods for use in a regulatory control program for veterinary drug residues in foods. Methods should be rugged (robust), cost effective, relatively uncomplicated, portable, and capable of simultaneously handling a set of samples in a time effective manner. The stability of analytes shall also be established.

6.3.4.2 Ruggedness testing includes variations in reagent volumes or concentrations, pH, incubation or reaction time and temperature, reagent quality, and different batch or source of a reagent or chromatographic material.

6.3.4.3 Cost-effectiveness is the use of reagents and supplies which are readily available in the required purity from local suppliers and equipment for which parts and service are also readily available. The method efficiency is increased when multiple samples can be analyzed at the same time.

6.3.4.4 Analyte stability during analysis shall be established for both standards and analyte in the presence of sample material, during processing through the complete analysis for all methods used in a regulatory control program and for typical conditions of storage while a sample is awaiting analysis.

6.3.4.5 Storage study should be conducted *as recommended by the competent authority* for all screening, quantitative, and confirmatory analyses to be completed and the results reported in case there is a challenge and a request for re-analysis.

6.4 Method development and validation considerations for residue testing methods

6.4.1 Selection of appropriate test *matrix* for validation

6.4.1.1 In developing and validating a residue testing method, data should be derived from three types of sample *matrix*:

a) Control test *matrix* from non-treated animals provides information about analytical background and matrix interferences;
b) *Spiked* test *matrix*, containing known amounts of the analyte added to the control *matrix*, yields information about the method's ability to recover the analyte of interest under controlled conditions; and
c) Analysis of biologically incurred *matrices* from food producing animals that have been treated with the drug provides information about biological or other interactions that may occur when analyzing residue testing samples.

Matrices should be obtained from multiple sources to cover the variations resulting from factors such as different diets, husbandry practices, sex, and breed of animals. A minimum of six different sources of matrix is recommended.

6.4.1.2 In some instances, known drug free sample *matrices* may not be available for use in residue testing laboratories. In these instances an equivalent sample *matrix* may be used.

6.4.1.3 When a *matrix* is used from an unknown source for quantitative or screening methods, a second method should be used to demonstrate that the matrix does not contain residues of the drug. Residue testing laboratory *should* demonstrate fitness for the purpose of the equivalent sample *matrix* .

6.4.1.4 Laboratories shall demonstrate that the methods in use for analysis of regulatory samples have been suitably validated. The multi-laboratory method validation study should be the preferred approach to provide analytical data to define method performance characteristics.

6.4.1.5 Other models may be considered which include multi-laboratory trials with smaller numbers of laboratories than are required to conduct a full collaborative study and single laboratory validation based on rigorous in-house evaluation of method performance, supported by a QMS, independent audits and analysis of proficiency or reference materials, when available.

6.4.2 Measurement uncertainty

Laboratories should provide their customers on request with information on the measurement uncertainty or statement of confidence associated with the quantitative results produced by each quantitative method. Guidance on

estimation of measurement uncertainty should be followed in accordance with guidelines developed by relevant scientific bodies (e.g., IUPAC and ISO).

6.4.3 Use of internal standards

Some residue methods are designed using internal standards for analytical control. A properly used internal standard will compensate for some of the analytical variability of an analysis, improving precision. However, an improperly used internal standard may obscure variables that are an important part of the analytical measurement. If an internal standard is used, it should be added to a sample as early as possible in the procedure, preferably to the test *matrix* before analysis begins.

6.4.4 Environmental considerations

If residue testing methods may be subjected to widely variable physical test environments, this should be taken into account in the development and validation of these methods. Addressing these issues may help improve method ruggedness.

6.4.5 Animal welfare considerations

Sample collection involving live animals, animal welfare shall be taken into consideration in accordance with Republic Act No. 8485 (Animal Welfare Act) as amended by Republic Act No. 10631 (Philippine Animal Welfare Act of 2013) and its future amendment.

6.4.6 Choice of Validation Model

6.4.6.1 An analytical method developed and used in only one laboratory may have limited use in a residue testing program unless care is taken to meet the rigorous expectations for single laboratory method validation associated with accreditation under ISO/IEC 17025:2017 (Testing and calibration of laboratories).

6.4.6.2 The principles for conducting a single laboratory method validation, a multi-laboratory method trial or a collaborative study of a residue testing method are the same. Samples for evaluating method performance should be unknown to the analyst, in randomized replicates, containing the residue near the MRLVD or other target concentration, as well as samples with the analyte above and below the concentration of interest, and test material blanks. A minimum of three individual datasets should be generated over three analysis periods, on at least three separate occasions (at least one day apart), preferably with replicate analysis, to improve statistical evaluation of method performance and provide an estimate of inter-day variability.

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827 **6.4.6.3** Expanding the validation *should* include other laboratories, preferably to the
828 number required for a collaborative study. Analysis of blind duplicates, as
829 required in the collaborative study protocol. The validation of a collaboratively
830 studied method can be extended to include additional tissues and species in
831 a subsequent study conducted by a single expert laboratory, as required.
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833 **6.4.7 Quality Management Systems**
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835 The testing laboratory conducting residue analysis shall have a QMS
836 conforming to ISO 9001:2015 (Quality Management System) requirements,
837 and the testing methods shall be accredited to ISO/IEC 17025:2017 (Testing
838 and calibration laboratory).
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Annex A
(Informative)

Performance Characteristics for Multi-Residue Methods (MRM) for Veterinary Drugs

A.1 The purpose of this Annex is to describe the performance characteristics/parameters that a multi-residue method (MRM) should have in order to provide internationally acceptable confidence in the method to produce results suitable for evaluating the residues of veterinary drugs for either domestic programs or in international trade. The uses may include screening, quantification, and/or confirmation, each having different performance requirements.

A.2 This Annex is applicable to MRM used to analyze all residues of veterinary drugs and substances which may be used as veterinary drugs. These MRMs include certain pesticides which have veterinary uses and which may be present as residues in commodities. Guidance on the validation of multi-residue methods for non-veterinary use of pesticides is contained in CAC/GL 40-1993 (Guidelines on good laboratory practice in residue analysis).

A.3 In this Annex, a MRM is considered to be a method which includes three or more analytes in the same class or more than one class of veterinary drugs in its scope. These MRMs may be used for screening samples for the possible presence of veterinary drugs or quantitative and/or confirmatory analyses. This guidance covers all three types of situations. It should be noted that a validated MRM may include some analytes where performance characteristics for quantitative analysis have been fully validated and other analytes where precision and/or recovery criteria for quantitative analysis or the data requirements for confirmation of the residue are not available. However, those analytes should be clearly identified in the method and shall not be used for those purposes until they have been validated and/or demonstrated to be fit for purpose.

A.4 Summary of performance parameters to be characterized and defined for multi-residue analytical methods

The following characteristic parameters need to be measured for every analyte and for each matrix under study, as applicable:

1. **Selectivity**
   a) freedom from interferences;
   b) matrix effects – characterized and controlled by the method if they occur; and

c) qualitative, quantitative, and/or confirmatory detector response parameters determined (and CCβ for screening analyses where this is included below to cover cut-off or threshold limits)

2. **Calibration**
   a) sensitivity;
   b) calibration range;
   c) calibration function; and
   d) LOD and LOQ, and/or CCα and CCβ

3. **Reliability of results**
   a) recovery;
   b) accuracy (trueness and precision);
   c) measurement uncertainty; and
   d) robustness (ruggedness) testing

4. **Stability of Analytes**
   a) stability in sample extracts and standard solutions;
   b) stability under sample processing and analysis; and
   c) stability under frozen storage and freeze-thaw cycle conditions.

5. **Incurred residue studies** (if suitable materials are available)
   a) verify that incurred residues are as effectively extracted as fortified analytes;
   b) verify performance of any steps included in method to release chemically bound residues where required; and
   c) verify consistency of recovery and precision.

A.5 Performance characteristics for MRM

A.5.1 It should be understood that the performance characteristics listed in A.4 (Summary of performance parameters to be characterized and defined for multi-residue analytical methods) should be defined and measured for every analyte listed in the scope of the fully optimized multi-residue method. This is best done after it has been determined that method development and/or modification has been completed and the analytical method is not to be subjected to any additional changes or modifications. In this regard, the concepts involved are very similar to those for determining the performance characteristics of an analyte in a single analyte method elaborated in 6.3 (Analytical performance characteristics). To avoid repetition, only differences from single analyte consideration will be highlighted in this Annex.

A.5.2 The requirement on MRMs to successfully detect residues of a variety of different veterinary drugs in a complex food matrix can be expected to result in an increased risk of interference by other material from the sample matrix compared to single analyte methods. If the MRM is required to analyse different

matrices or a matrix from different species the risk is increased. This necessitates particular emphasis on performance characteristics related to detection capability and selectivity when considering the performance of MRMs

A.6 Performance characteristics of MRM for screening analysis

A.6.1 MRMs for screening analysis are usually qualitative in nature and often cover a range of analytes, species and matrices, with the objective being to differentiate samples that contain no detectable residues above a threshold or cut-off value (”negatives/compliant”) from those that may contain residues above that value (”positives/presumptive positives/suspect positives”).

A.6.2 Screening methods for approved veterinary drugs should demonstrate a selectivity of 90% with 95% confidence and sensitivity at the lowest concentration at which the target analyte may be reliably detected within defined statistical limits, usually 95% confidence limit. For regulatory purposes, these screening methods can tolerate a small number of “false positive” results, as any screen “positive/presumptive positive/suspect positive” sample should be carried forward for additional confirmatory and/or quantitative analysis to identify, confirm and/or quantify the presence of the “suspect” residue. For all other veterinary drugs which are NOT approved for use, this Annex may be used to inform decisions on the performance criteria which may need to be developed.

A.6.3 Criteria for identifying cut-off or threshold limits for screening methods are indicated in 6.3.1.4.

A.7 Performance characteristics of MRM for quantitative analysis

A.7.1 The requirement to recover a range of different veterinary drug residues in one extraction increases the potential for compromised selectivity in MRM compared to single analyte methods. Using less selective extraction and clean-up procedures is likely to result in greater co-extracted matrix material in the final extract. The nature and quantities of such co-extracted material can vary markedly depending on the history of the individual sample. Particular care is therefore required when setting criteria for the precision and trueness of MRM to ensure that quantification will not be affected by interference from other compounds present in the sample matrix. It is recommended that MRM used to support Codex MRL should meet the performance standards for trueness and precision listed in Table 1 of 6.3.2.3. To ensure that the effects of different samples are taken into account when assessing performance against these criteria, it is recommended that determinations of these parameters follow the guidance in 6.3.2 (Performance characteristics for quantitative methods). The intermediate precision for recovery of analytes fortified into these different samples should be used for comparison to the criteria in Table 1 of 6.3.2.3 rather than the repeatability precision.

A.7.2 However, where no guidance is available to provide a target concentration for a specific analyte, a value based on an assessment of public health risk, and not based on the detection limits of the available analytical instrumentation may be considered.

A.7.3 It is becoming increasingly common in analytical methods for veterinary drug residues in foods to base the quantitative determination on a standard curve prepared by addition of standard to known blank representative matrix material prior to analyte extraction at a range of appropriate concentrations that bracket the target concentration. Use of such a method matrix-matched standard curve for calibration inherently incorporates a recovery correction into the analytical results obtained but may introduce a new bias related to the behavior of the particular blank matrix used to construct the standard curve. It is recommended that the trueness of methods that employ matrix-matched calibration curves follow the guidelines provided in 6.3.2 (Performance characteristics for quantitative methods).

A.7.4 Alternative approaches may be applied to method validation that use the parameters Decision Limit (CCα) and Detection Capability (CCß). These two parameters incorporate a consideration of measurement uncertainty.

A.8 Performance characteristics for MRM for confirmatory methods

A.8.1 The necessary steps to positive identification are for the expert judgement of the analyst and particular attention should be paid to the choice of a method that would minimize the effect of interfering analytes. Ultimately, it is the responsibility of the analyst to make choices, provide supporting data, and interpret results according to scientific principles and qualified judgement as outlined in 6.3.3 (Performance characteristics for confirmatory methods).

A.8.2 Method performance requirements for confirmatory methods based on low resolution gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS) listed in Table 2 of 6.3.3.1 have been extended to include situations where the relative ion intensity may be less than 10%. Under these conditions, a 50% relative ion intensity between standard and sample is acceptable.

A.8.3 Table A.1 lists the number of identification points (IPs) earned for a combination of mass spectrometry based analytical techniques and provides necessary and sufficient criteria for confirmatory analysis. Typically, a minimum of four identification points is required to meet accepted performance criteria for regulatory methods. Therefore, a combination of a precursor ion and two product ions will provide the four IPs required when low resolution MS/MS instruments are used in a confirmatory method. Examples of non-MS based detection methods are listed in Table 3 in 6.3.3.5.

Table A.1 Examples of the number of identification points (IPs) earned for a range of mass spectrometric detection techniques and combinations thereof (n = an integer)

Technique	Source of Identification	Number of Identification Points (IPs)
GC-MS (EI[a] or CI[b])	n characteristic ions	N
GC-MS (EI +CI)	2 (EI) + 2 (CI)	4
GC-EIMS or GC-CIMS (2 derivatives)	2 (Derivative A) + 2 (Derivative B)	4
LC-MS	n characteristic ions	N
GC-MS/MS[c]	1 precursor ion + 2 product ions	4
LC-MS/MS[d]	1 precursor ion + 2 product ions	4
GC-MS/MS	2 precursor ions, each with 1 product ion	5
LC-MS/MS	2 precursor ions, each with 1 product ion	5
LC-MS/MS/MS	1 precursor, 1 product ion and 22nd generation product ions	5.5
HRMS	N	2n
GC-MS and LC-MS	2+2	4
GC-MS and HRMS	2+1	4
LC-HRMS/M	1 precursor ion + 2 product ions	6

[a] Electron ionisation (EI)
[b] Chemical ionisation (CI)
[c] Gas chromatography tandem mass spectrometry (GC-MS/MS)
[c] Liquid chromatography tandem mass spectrometry (LC-MS/MS)

A.8.4 Regardless of the mass spectrometer resolution, at least one ion ratio shall also be measured to eliminate the potential for fragments of the same mass arising from isobaric compounds of similar structure. Retention times, or better still relative retention times, should also be determined to avoid the potential for false identifications when using mass spectrometers for detection.

A.8.5 Non-magnetic sector type high-resolution mass spectrometers (HRMS) are becoming increasingly more affordable and commonly used. If using this equipment, it is suggested that confirmation of a compound be based on the high mass accuracy and the resolving power of the mass spectrometer.

A.9 Validation of the fully characterized MRM

A.9.1 Determination of the parameters in A.4 for all the analytes and matrices listed in the scope of a MRM will allow an objective assessment to be made of the fitness-for-purpose of the analytical method for use in a regulatory control program. For screening methods, analytes whose measured performance parameters in a set of validation experiments are achieved in ≥ 90% of the measurements taken at each analyte/matrix/concentration combination could be considered acceptable for inclusion in the method.

A.9.2 The 6.4.1 (Selection of appropriate test material for validation) recommends the use of biologically incurred material in the characterization and validation of analytical methods where possible, but the cost of generating such incurred material for the validation of each analyte in a MRM could be prohibitive. However, where it is economically feasible and possible to administer several different veterinary drugs to a food animal, incurred material may be generated for a few carefully selected analytes representative of drug classes and/or groups based on their prevalence of use and potential for causing residues that exceed established MRL. The target incurred concentration should be close to the MRL or expected concentration.

A.9.3 Alternative protocols may be used for validation of MRM, adapted as necessary for individual circumstances.

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