CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 8b **CX/RVDF 12/20/10**

December 2011

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

Twentieth Session

San Juan, Puerto Rico, 7-11 May 2012

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CHARACTERISTICS FOR MULTI-RESIDUES METHODS (APPENDIX TO CAC/GL 71-2009)

(N01-2011)

at Step 3

(Report of the CCRVDF Electronic Working Group on methods of analysis for residues of veterinary drugs in foods led by Canada and the United Kingdom with the assistance of Australia, Brazil, Costa Rica, European Union, France, Germany, The Netherlands, New Zealand, Sweden, Switzerland, United Kingdom, United States of America, Uruguay, IAEA, IDF and IFAH)

Governments and international organizations wishing to submit comments at Step 3 on the proposed draft Guidelines on performance characteristics for multi-residues methods (see Annex 1) are invited to do so **no later than 31 March 2012** as follows: U.S. Codex Office, Food safety and Inspection Service, US Department of Agriculture, Room 4861, South Building, 14th Independence Avenue, S.W., Washington DC 20250, USA (Telefax: +1 202 720 3157 ; or ***preferably*** E-mail: CCRVDF-USSEC@fsis.usda.gov , with a copy to the Secretary, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Viale delle Terme di Caracalla, 00153 Rome, Italy (Telefax: +39.06.5705.4593; E-mail: Codex@fao.org, ***preferably***).

Format for submitting comments: In order to facilitate the compilation of comments and prepare a more useful comments document, Members and Observers, which are not yet doing so, are requested to provide their comments in the format outlined in Annex 2 to this document.

Introduction

1. The 19th Session of the CCRVDF discussed the issues of methods of analysis for veterinary drug residues and the extension of existing guidance on performance criteria for single analyte methods in CAC/GL 71-2009 to include multi-residue analytical methods.

2. For this purpose and to address the issue of availability of methods, the Committee agreed to establish an electronic working group chaired by Canada and the United Kingdom, working in English and open to all member and observers with the following mandate:

- to prepare a proposed draft Appendix on performance criteria for multi-residue analytical methods for veterinary drugs residues for inclusion in the *Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs in Food Producing Animals* (CAC/GL 71-2009); and
- to consider opportunities to facilitate communication with IAEA on the development of the database on analytical methods and reference standards.

Progress

3. The electronic working group drafted an appendix on performance criteria for multi-residue analytical methods and revised this in three iterations. In addition to comments received from the drafting group, presentations were made on this topic to a number of international scientific meetings and the views of attendees sought to assist in preparing this draft appendix. The current draft of the proposed performance criteria for multi-residue analytical methods is attached for the consideration of the committee.

4. Discussions have been held with the IAEA and a database to host a collection of multi-residue analytical methods is undergoing assessment. The Committee will be updated on further progress on this action at the 20th session.

Recommendations

i. To consider the draft appendix on performance criteria for multi-residue analytical methods (see Annex 1) and provide comment on the current draft, advancing it in the step process if considered appropriate.

ii. To consider progress (to be updated at the 20th session) on the development of a database of multi-residue analytical methods by the IAEA and to request contributions of multi-residue methods to the database from member countries and others.

Annex 1

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CHARACTERISTICS FOR MULTI-RESIDUES METHODS (MRMs) OF ANALYSIS FOR VETERINARY RESIDUES

(APPENDIX TO CAC/GL 71-2009)

(N01-2011)

at Step 3

CONTENTS

Background

1. Guidelines were adopted by the Codex Alimentarius Commission (CAC) in 2008 for the design and implementation of national regulatory food safety programmes associated with the use of veterinary drugs in food producing animals (CAC/GL 71-2009). These guidelines were designed to include general guidance on the validation of analytical methods for use with single analytes under single laboratory validation conditions (as set out in CAC/GL 71-2009) and be updated as necessary to permit extension to cover additional relevant areas.

2. The 18th session of the CCRVDF recognised that current practice in analytical laboratories undertaking these analyses was to use multi-residue methods (MRMs) wherever possible to increase the efficiency of the laboratories whilst keeping analytical costs to a minimum. However, the same meeting also recognised that there was very limited guidance on the acceptable performance characteristics for MRMs. This guidance document seeks to address this need.

3. It is recognised that developing countries may need a transition period and/or technical assistance when working towards using these guidelines.

Introduction

4. Analytical methods for veterinary drug residues in foods must be capable of reliably detecting the presence or absence of a veterinary drug of interest or concern (screening methods), quantifying how much is present (quantitative methods), and providing unequivocal identification of the drug (confirmatory methods). When an analytical method has been used to determine that the defined Maximum Residue Limit (MRL) for an approved veterinary drug has been exceeded, it is imperative that the test results are confirmed before regulatory action is taken. Regulatory action could include denying the product market access, destroying the product and/or the administration of financial penalties. In cases where the detected veterinary drug is

not approved or is prohibited/banned from use in that commodity because no acceptable daily intake (ADI) and MRLs have been defined for toxicological reasons, detection of such a drug at any concentration should be confirmed using a confirmatory method which meets the criteria contained in CAC/GL 71-2009 since this finding may automatically result in regulatory action.

5. Technical Guideline documents issued by the CAC to assist countries involved in the import and export control of foods in the application of requirements for trade in foodstuffs in order to protect consumers and facilitate trade recommend that "laboratories engaged in regulatory analyses must be compliant with ISO/IEC 17025:2005 - "General requirements for the competence of calibration and testing laboratories". Laboratories should also participate in appropriate proficiency testing schemes for food analysis which conform to the requirements laid down in "The international harmonized protocol for the proficiency testing of (chemical) analytical laboratories," and, whenever possible, to use methods that have been validated according to the principles laid down by the CAC (*see* CAC/GL 27-1997). In addition, the laboratories must use internal quality control procedures that comply with such procedures as described in "The harmonized guidelines for internal quality control in applied chemistry laboratories." Section 5.4.5 ISO/IEC 17025:2005 provides general guidance for use of validated methods.

6. Validated analytical methods are methods with defined characteristic operational parameters which have been determined to be suitable for use in a regulatory control programme ("fit-for-purpose" in a regulatory environment). The CAC has adopted in CAC/GL 49-2003 the guidelines for the single laboratory validation of methods of analysis issued by the International Union of Pure and Applied Chemistry (IUPAC) [REFERENCE: M. Thompson, S.L.R. Ellison and R. Wood. "Harmonized guidelines for single-laboratory validation of methods of analysis" Pure Appl. Chem., 74 (5), 835-855 (2002)]. These have also been incorporated into the "General criteria for the selection of single-laboratory validated methods of analysis" contained in the CAC Procedural Manual, 20th edition. This guidance document examines the attributes of MRMs used to identify a range of analytes in the same analysis and the requirements they must satisfy before they can be considered suitable for use in regulatory control programmes for veterinary drug residues in foods.

7. Guidance has been prepared by the CCRVDF on single laboratory validation of single analyte methods (CAC/GL 71-2009). However, to increase efficiency and sample throughput, many laboratories are turning to the use of MRMs which can be used for the detection of multiple analytes of the same or different classes. For the purposes of this document, 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 are most commonly used by laboratories for screening samples for the possible presence of veterinary drugs in samples but they can also be used for both quantitative and confirmatory analyses. This guidance therefore will cover all three types of analyses/methods and forms an annex to CAC/GL 71-2009. It should be noted that a validated MRM may include some analytes for which performance requirements for quantitative analysis have been fully validated, while some other analytes may not meet precision and/or recovery criteria for quantitative analysis or the data requirements for confirmation of the residue. When the method has been validated as suitable to detect these analytes at a required accepted limit (AL), the method may be used as a screening method for such analytes, which, if present, should then be quantified using a method that has been suitably validated for the quantitative analysis of the specific analyte or confirmed using a suitably validated confirmatory method, according to the criteria provided in CAC/GL 71-2009.

8. The principles described in this section are considered practical and suitable for the determination of the performance characteristics of MRMs for use in regulatory control programmes and are based on the recommendations elaborated by an AOAC/FAO/IAEA consultation held in Miskolc, Hungary, in 1999 [REFERENCE: A. Fajgelj & A. Ambrus Principles and Practices of Method Validation, Royal Society of Chemistry, 2000]. All, or a subset of these same performance characteristics may be used during method validation to evaluate whether the method is suitable ("fit-for-purpose") for use in a regulatory environment. They are equally applicable to use with MRMs of analysis for veterinary drug residues.

9. The CAC Procedural Manual 20th edition provides "General criteria for the selection of single-laboratory validated methods of analysis". The "Working instructions for the implementation of the criteria approach in Codex, Table 1: Guidelines for establishing numeric values for the criteria" contained in this document are relevant to MRMs as discussed below, as are the "Guidelines for establishing numeric values for method criteria and/or assessing methods for compliance thereof". In the interest of harmonisation, guidance on performance criteria for analytical methods applied to veterinary drug residues should be

consistent with the general guidance already approved by the CAC. In addition, a guidance document for validation and Quality Control has recently been issued by the EU (Document No. SANCO/10684/2009) for pesticide residue analyses. The EU document covers MRMs primarily for confirmatory analyses but also addresses multi-residue screening methods using mass spectrometry. Aspects of SANCO/10684/2009 have been adopted into this document where appropriate.

Scope

10. This guidance is applicable to MRMs used to analyse veterinary drug residues, including approved veterinary drugs and pesticides. Some residue control programmes may include additional analyte groups such as agricultural pesticides and environmental contaminants. 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 pesticide residue analysis.*

Performance characteristics for MRMs

Performance characteristics of MRMs for screening analysis

11. While the following sections describe the performance characteristics for screening, quantitative and confirmatory methods in general, it must be understood that these performance characteristics must be defined and measured for every analyte listed in the scope of the fully optimised multi-residue method. This is best done after it has been determined that method development and/or modification has been completed and the method is not going to be subjected to any additional changes or modifications. In this regard, the concepts involved are very similar to those described in guidance documents for determining the performance characteristics of an analyte in a single analyte method.

12. MRMs for screening analysis are usually either qualitative or semi-quantitative in nature and often cover a range of analytes, with the objective being to discriminate samples that contain no detectable residues above a detection concentration ("negatives") from those that may contain residues above that value ("screen positives"). The validation strategy therefore focuses on establishing a detection concentration (sometimes called the cut-off level/concentration) above which results are "positive", determining a statistically based rate for both "false positive" and "false negative" results, testing for interferences and establishing appropriate conditions of use.

13. The "*selectivity*" of a qualitative (binary) screening method refers to the ability of the method to determine that samples which give a negative response are truly negative. A qualitative binary method has two possible outcomes, i.e. yes/no or positive/ negative. The method must also be able to distinguish the presence of the target analyte, or group of analytes, from other analytes that may be present in the sample material. The selectivity of a screening method may not be as great as that of a quantitative method, because screening methods often take advantage of a structural feature common to a group or class of analytes. These methods, which generally fit into the qualitative screening methods category, are often based on microbiological growth inhibition, immunoassays or chromogenic responses that may not unambiguously identify an analyte. The selectivity of a qualitative screening method may be increased when it is used as a detection system after chromatographic or other separation technique. To demonstrate a selectivity rate of at least 95 percent with 95 percent confidence (which is recommended for qualitative screening tests applied for regulating approved substances), 60 replicate analyses are conducted on representative blank sample matrix materials from a minimum of six different sources. All results should be negative. By way of comparison, to meet a minimum selectivity rate of 99 % with 95 % confidence level 299 samples would have to be tested, all of which must elicit negative results. Additional tests for potential interferences and cross-reactivity may then be conducted by testing blank matrix material spiked with a cocktail preparation of potential interfering substances (inclusivity and exclusivity), such as other drugs that might be used in animal treatment, potential environmental contaminants, drug metabolites, or chemically related compounds. Again, responses should be negative when these compounds are present at concentrations that might reasonably be expected to be present in a sample.

14. For qualitative screening tests, particularly those involving test kit technologies which provide two possible outcomes (analyte present or absent), the terms "*detection concentration or detection sensitivity*" or "*cut-off level*" refer to the lowest concentration at which the target analyte may be reliably detected within defined statistical limits. For example, in the AOAC Performance Tested Program™ for test kits, certain

conditions are necessary to meet the minimum requirements for sensitivity at 90% positive detection with 95% confidence. These conditions require that a minimum of 30 residue-free sample materials (preferably taken from no less than six different sources) spiked with the analyte(s) of interest at the target concentration(s) should all yield positive results. Three or more negative results 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. Other approaches, such as the guidance published by the EU for establishment of the cut-off level and the detection capability for screening tests may also be used. (http://ec.europa.eu/food/food/chemicalsafety/residues/Guideline_Validation_Screening_en.pdf)

15. The detection concentration for the qualitative binary test for a particular analyte is established by conducting concentration-response experiments, typically using 30 replicates (from at least six sources) spiked into known blank samples at each of a series of increasing concentrations. The same 30 blank samples are also analysed directly (i.e. without spiking) to provide control data. Once the concentrations have been established where all 30 replicates of the control samples give a negative response and all 30 spike replicates give a positive response, the experiment is repeated using the blank matrix materials spiked at four evenly spaced concentrations between the "all negative" and "all positive" concentrations. An additional set is tested at a concentration 20 percent above the "all positive" concentration. Statistical analysis of the results enables the user to establish a reliable detection concentration at the required confidence limit (usually 95 percent) [REFERENCE: Finney, D.J. 1978. *Statistical method in biological assay*. 3rd edition. New York, USA, MacMillan Publishing Co.].

16. During preparation of this Guidance document, a new concept was developed under the auspices of the AOAC International that combined the qualitative parameters for detection sensitivity, false positives, and false negatives (described above) into one single parameter called the "Probability of Detection (POD)." The POD covers all ranges of concentrations, both zero and non-zero and allows for a simple graphical representation of laboratory data as a POD curve graphed by concentration with associated error bars. Using this approach, precision and accuracy can be calculated for qualitative tests. [REFERENCE: Wehling, P., LaBude, R. A., Brunelle, S., & Nelson, T. Probability of detection (POD) as a statistical method for validation of qualitative methods. J.AOAC International 94 (1), 335-347, (2011)]. This simplified parameter merits consideration when developing validation experiments for MRM qualitative binary screening methods.

Performance characteristics of MRMs for quantitative analysis

17. Selectivity is the extent to which a method can determine particular analyte(s) in a mixture(s) or matrice(s) without interferences from other components of similar behaviour (CAC/GL 72-2009). The CAC Procedural Manual 20th edition specifies in "Table 1: Guidelines for establishing numeric values for the criteria" the limits of detection (LOD) and quantification (LOQ) expected for differing action limits and these can be used to select an appropriate detection concentration for the assay. It is the ability of an analytical method to detect and discriminate the signal response from a compound in the presence of other compounds that may be present in the sample material, which is of particular importance in defining the performance characteristics of MRMs used in regulatory control programmes for veterinary drug residues in foods. For a quantitative method, the requirement is that the signal used for quantification should relate only to the target analyte and not contain contributions from co-extracted materials or be otherwise influenced by matrix effects, unless suitably corrected. Chromatographic analyses based on peaks that are not fully resolved provide less reliable quantitative results. Use of element-specific detectors or detection wavelengths or mass-selective detectors that are more specific to a particular compound or structure, combined with chromatographic separation, improves the selectivity of quantitative methods for veterinary drug residues in foods.

18. In addition to the selectivity of a method, the ability of the method to provide a quantitative result that is reliable must be demonstrated. This consists of two factors both of which contribute to the measurement uncertainty in results generated with the method:

- the closeness of the result to the true value for the concentration of analyte present in the sample material, expressed in terms of *accuracy*, *trueness* or *bias*; and
- the ability of the method to provide consistent results on replicate determinations (or accepted value in the case of a reference material), expressed in terms of *precision* (*repeatability* and *reproducibility*).

19. It is recommended that methods used to support Codex MRLs should meet the performance standards for trueness and precision listed in Table 1. These standards are the same as the current limits applied for single analyte veterinary drug residues in CAC/GL 71-2009 and consideration of data from laboratories using MRM suggest they can be adopted for MRM, especially if confirmatory analysis are conducted using different analytical methods more specifically suited for individual analytes.

20. The *accuracy* (trueness, bias) of the results produced by a method may be determined by repeated analysis of a 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 accuracy of analyte blind-spiked into known blank sample material. The use of isotopically stable labelled analogue or internal standard wherever possible is to be encouraged and may improve the accuracy of measurements, improve the precision of analysis and permit the use of more stringent limits than those for trueness given in Table 1. The accuracy requirements of methods will vary depending upon the planned regulatory use of the results. The accuracy should be carefully characterized at concentrations around the MRL or target concentration for regulatory action (typically at concentrations from 0.5 to 2.0 times the target concentration) to ensure that regulatory action is only taken on samples containing residues that can be demonstrated to exceed the regulatory action limit with a defined statistical confidence. Where no guidance is available to provide a target concentration, it is proposed that an interim value in the range 1.0 to 10 µg/kg is adopted provided there can be reasonable confidence there will be no significant toxicological implications whilst more formal advice is sought.

21. *Recovery* is usually expressed as the percentage of analyte experimentally determined after fortification of sample material at a known concentration and should be assessed over concentrations that cover the analytical range of the method. In interpreting recoveries, it is necessary to recognize that analyte added to a sample may not behave in the same manner as the same biologically incurred analyte (veterinary drug residue). In many situations, the amount of an incurred residue that is extracted (the yield or recovered fraction) is less than the total incurred residues present. This may be due to losses during extraction, intracellular binding of residues, the presence of conjugates, or other factors that are not fully represented by recovery experiments conducted with analyte-spiked blank tissues and recoveries will vary according to the concentration of residue present. Regardless of what average recoveries are observed, recovery with low variability is desirable so that a reliable correction for recovery can be made to the final result, when required. Recovery corrections should be made consistent with the guidance provided by the Codex Alimentarius Commission (CAC/GL 37-2001).

22. *Precision*, which quantifies the variation between replicated measurements on test portions from the same sample material, is also one of the important considerations in determining when a residue in a sample should be considered to exceed an MRL or other regulatory action limit. Precision of a method is usually expressed in terms of the within-laboratory variation (*repeatability*) and the between-laboratory variability (*reproducibility*) when the method has been subjected to a multi-laboratory trial. 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 (RSD), or coefficient of variation/variability (the standard deviation divided by the absolute value of the arithmetic mean). The RSD may be reported as a percentage by multiplying by 100.

23. Quantitative methods are usually 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 function 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. Although it is general practice to include a suitable blank with the calibration samples, this does not imply that it is acceptable to extrapolate into the region of the curve below the low standard to obtain a quantitative result. The analytical function relates the response for the analyte recovered from sample material at various concentrations throughout the range of analytical interest, with the value of all interferants remaining constant. It is, therefore, implicit that, under such circumstances, the analytical function is derived in the presence of reagents used in the method and matrix co-extractives and not from measurements using pure standard solutions alone, unless it has been adequately demonstrated that the detector signal response of the pure

standard is unaffected by the presence of method reagents and matrix components. For analytes for which a MRL or regulatory action limit has been established in a particular sample material (matrix), response is typically determined for known blank sample material and for blank sample material spiked at a range of concentrations above and below the MRL (use of a minimum of six different sources of blank materials is recommended).

24. There can be some degree of ambiguity in the scientific literature around the terms "matrix fortified" and "matrix matched". Terminology has been proposed to clarify this position (REFERENCE: Wang, J., Cheung, W., & Grant, D. (2005) Determination of pesticides in apple-based infant foods using liquid chromatography electrospray ionization tandem mass spectrometry, J. Agric. Food Chem. 53: 528-537) and the definitions below will be used in this text.

- Standard calibration curve (SCC) – a calibration curve prepared using standards in the absence of matrix, usually referred to as an "external calibration" in the literature;
- Matrix-matched standard calibration curve (MSCC) – a calibration curve prepared by addition of standards to sample extracts obtained from blank matrix after extraction; and
- Method matrix-matched standard calibration curve (MMSCC) but also known as a matrix-fortified standard calibration curve – a calibration curve prepared by addition of standards to blank matrix prior to extraction.

25. The analytical function experimental data can also be used to calculate the analytical recovery at each concentration and are of particular importance when the presence of matrix co-extractives modifies the response of the analyte as compared with analytical standards. The regression, whether it is linear or quadratic, is determined from the analytical function experiments and is the statistical expression of the curve obtained for the analysis of sample materials spiked at the target concentrations after it has been demonstrated that the fitted data meet the requirements for regression. The weighting for the regression, such as $1/x$, $1/x^2$, etc. should be considered, especially for residue analysis only after proper residual analysis testing has been conducted to determine the weighting factor based on the homoscedasticity or otherwise of the data. The weighted linear or quadratic regression is able to show a lower limit of quantification (LOQ) with a higher degree of accuracy and improved precision. It is becoming increasingly common in 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 (MMSCC) at a range of appropriate concentrations that bracket the target value (the analytical function). Use of such a method matrix-matched standard curve for calibration incorporates a recovery correction into the analytical results obtained.

26. The accepted definition for *sensitivity* (CAC/GL 72-2009) is the "quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured", a property associated with the slope of the calibration curve and the ability to discriminate changes in concentration of the analyte. It is necessary to establish the lower limits at which reliable detection, quantification or confirmation of the presence of an analyte may be performed using a particular analytical method. The *detection limit* or *limit of detection (LOD)* of a method is defined in CAC/GL 72-2009 and may be described in practical terms as the lowest concentration of the analyte in the sample that can be detected but may not be positively identified/confirmed. However, there is growing opinion that this may not be a very useful characteristic to determine due to the inherent variability associated with different detectors, etc. It is suggested that the LOD is only determined when the method performance requirements approach this limit. It may 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 (REFERENCE: Miller, J.C. & Miller, J.N. 1993. *Statistics for analytical chemistry*. 3rd Edition. Chichester, UK, Ellis Horwood Ltd.). Using this approach, the LOD is calculated using the y-intercept (assuming a positive value) of the SCC or MMSCC curve plus three times $s_{y/x}$. This approach provides a conservative estimate of the detection limit. The detection limit can also be estimated by measurements on representative test materials as the weakest relevant response of the analyte in the blank plus three times its standard deviation. It is often necessary to spike test materials at a concentration resulting in a barely detectable response to obtain an approximation of the standard deviation of the blank when using this approach.

27. The *limit of quantification* (LOQ), also referred to as quantification limit, 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 MRLs established by the Codex Alimentarius Commission, the LOQ should meet the criteria for precision and accuracy (recovery) in Table 1 and should be equal to or less than one-half the MRL. However, when the LOQ of a method is lower than the actual concentrations monitored for compliance with a MRL, the validation and subsequent application of the method should be based on a *lowest calibrated level* (LCL), which is typically 0.5× the MRL (or lower). For use in a regulatory programme, the LOD and LOQ are important parameters when the method will be applied to also estimate exposures to residues, where there may be an interest in monitoring residues at concentrations below the MRL, or when conducting residue analyses for substances that do not have ADIs or MRLs. For monitoring compliance with a MRL, it is important that a LCL be included in the analysis that adequately demonstrates that the MRL concentration may be reliably determined. The LCL of a method used to support an MRL should not be less than the LOQ. The *Procedural Manual* recommends the term *determination limit* under "Terms to be used in the criteria approach".

28. The Miskolc consultation in 1999 recognised that alternative approaches could be applied to method validation and included the terms Decision Limit (CCα) and Detection Capability (CCß) in their consideration. These terms are defined in the glossary below and have subsequently been adopted into use in some jurisdictions, e.g. in the European Union under Commission Decision 2002/657/EC and may be considered as an alternative to using LOD and LOQ.

29. *Measurement uncertainty* is defined in CAC/GL 72-2009 as the "non-negative parameter characterizing the dispersion of values being attributed to a measurand, based on the information used". There is no agreed standard approach to calculating measurement uncertainty and a number of approaches have been published on this [REFERENCE: CAC/GL 59-2006: Guidelines on estimation of uncertainty of Results (Annex, amended 2011). REFERENCE: Technical Specification ISO/TS 21748:2004: Guidance for the repeatability, reproducibility and trueness estimates in measurement uncertainty estimation. First edition 2004-03-15.]. ISO/IEC 17025:2005 requires laboratories to determine and make available the uncertainty associated with analytical results. SANCO/10684/2009 suggests a practical approach for a laboratory to estimate its measurement uncertainty and to verify its estimation based on its own within-laboratory data is by evaluating its performance during proficiency tests.

Performance characteristics for MRMs for confirmatory methods

30. The necessary steps to positive identification are a matter of judgement on the analyst's part and particular attention should be paid to the choice of a method that would minimise 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 [REFERENCE: Bethem, R., Boison, J. O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, J., Price, P., & Stein, S. Establishing the Fitness for Purpose of mass spectrometric methods. J. Amer. Chem. Society for Mass Spectrometry 14 (5) 528-541(2003).].

31. *Selectivity*, the ability of the method to identify unequivocally a signal response as being exclusively related to a specific analyte, is the primary consideration for confirmatory methods. Certain instrumental techniques such as Fourier transform infrared spectroscopy or mass spectrometry may be sufficiently selective to provide unambiguous identification. These are often the techniques on which confirmatory methods are based.

32. Confirmation by GC-MS is usually based on the analysis of a reference analyte contemporaneously with unknowns and require the acquisition of signals for three diagnostic GC-(SIM)/EIMS ions (i.e. three m/z values, the so-called "three ion criterion") at roughly unit mass resolution, with relative abundance matching tolerances in selected ion monitoring (SIM) measurements. [REFERENCE: Sphon, J. A. (1978) J. Assoc. Official Anal. Chemists. Use of mass spectrometry for confirmation of animal drug residues Chemists 61 (5), 1247-1252 (1978)]. In this process, it is tacitly assumed that the GC retention time also matches that of the reference standard. Sphon's approach, considered valuable and scientifically valid, uses an approach of exclusion of possibilities without claiming positive identification.

33. In 1996, Li *et al.* extended Sphon's 'three ion criterion" to LC-(ESI) MS/MS methods. Under LC-MS/MS conditions, at least, two and preferably three precursor-product ion pairs were used to replace the three-ion MS criterion that was found to be appropriate for GC-EI/MS [REFERENCE: Li, L. Y. T., Campbell, D. A., Bennett, P. K., and Henion, J. (1996) Anal Chem. 68, 3397]. According to the Li criterion, one precursor ion preferably the $[M+H]^+$ or $[M-H]^-$ ion and two structurally significant fragment ions (or product transition ions) together with matching retention time data are required to meet accepted performance criteria for regulatory methods. However, confidence in the identification will increase with the use of a greater number of structurally significant fragment (or product transition) ions or identification points and some laboratories may choose to use more than the minimum suggested.

34. Tables 2 and 3 give the identification point (IP) scheme published in the European Commission Decision 2002/657/EC and endorsed by the CCRVDF [REFERENCE: CAC/GL 71-2009] Methods based on high-resolution mass spectrometry are considered to give a higher reliability through more accurate measurement of mass than can be obtained using low-resolution mass spectrometry techniques. Method performance requirements for confirmatory methods based on low resolution gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LC-MS), as published in European Commission Decision 2002/657/EC and by an international expert body, [REFERENCE: Bethem, R., Boison, J. O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, J., Price, P., & Stein, S. Establishing the Fitness for Purpose of mass spectrometric methods. J. Amer. Chem. Society for Mass Spectrometry 14 (5) 528-541(2003).] are given in Table 4. The IP system (Table 2) is based on the arbitrary assignment of one IP to each structurally significant ion fragment detected using a low-resolution mass spectrometric method. When a low-resolution tandem instrument is used, secondary fragments are detected from a primary fragment that is isolated in the first stage of the instrument. The fact that these structurally significant fragments are produced from the fragmentation of a major fragment (precursor ion) associated with the molecule provides greater confidence, and each such product transition ion is assigned a value of 1.5 IPs. Therefore, a combination of a precursor ion and two product transition ions provides the four required IPs when low-resolution MS/MS instruments are used in a confirmatory method (Table 3).

35. Additional confidence is provided when high-resolution mass spectrometers are used in a confirmatory method, as the high resolution provides more accurate identification of the mass and may be used to predict the elemental composition of each fragment. For a single high-resolution mass spectrometer, each structurally significant fragment detected is assigned a value of two IPs, while product transition ions generated in high-resolution MS/MS experiments are assigned an IP value of 2.5 each (Table 2).

36. Regardless of the mass spectrometer resolution, at least one ion ratio must 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 high-resolution mass spectrometers. In addition, a determination of signal-to- noise should be considered.

37. As high-resolution mass spectrometers are becomingly increasingly more affordable and commonly used, it is suggested that IPs might be assigned based on mass measurement accuracy using relative mass errors rather than resolving power (Table 3). This has the advantage that the IP rating criterion is consistent across a mass range or independent of mass. Thus, for substances with established MRLs, at least two ions need to be monitored to achieve a minimum of three IPs towards satisfactory confirmation of the compound's identity with mass errors that are ≤ 5 ppm. Using in-source fragmentation or collision induced dissociation with a low and high fragmentation or collision energy, a TOF and/or Orbitrap individually or in tandem could acquire fragment rich spectra, and therefore, additional IPs can be assigned for confirmation.

38. Other techniques, when they are used in combination, may be capable of achieving a comparable degree of selectivity as confirmatory techniques (Table 5). For example, identification may be verified by combinations of methods such as:

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

Such procedures must be applicable at the designated MRL of the analyte.

39. 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. [REFERENCE: Guidelines for the implementation of Decision 2002/657/EC, SANCO/2004/2726rev2, Annex 1: *SPECLOG – the specificity log*; http://ec.europa.eu/food/food/chemicalsafety/residues/cons_2002-657ec_en.pdf] This information may be captured in a structured logging document of all the information that leads to the conclusion that 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.

General performance characteristics for MRMs for use in a regulatory control programme

40. There are some additional considerations for selection of suitable MRMs for use in a regulatory control programme for veterinary drug residues in foods. These include requirements that methods should be rugged (robust), cost-effective, relatively uncomplicated, portable, capable of simultaneously handling a set of samples in a time-effective manner, etc. The stability of analytes must also be established.

41. *Ruggedness(Robustness)* testing may be conducted using the standard factorial design approach to determine any critical control points where minor variations in the method may result in a statistically different analytical result [REFERENCE: Youden, W.J. & Steiner, E.H. 1975. *Statistical Manual of the Association of Official Analytical Chemists*. Gaithersburg, USA, AOAC International.]. Typical factors to consider in a design include 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. Ruggedness testing may also be conducted using other designs such as the Plackett-Burman approach. Ruggedness of a confirmatory method may be required if the method differs significantly from the quantitative method previously validated (e.g. if the method uses different extraction or derivatisation procedures than are used in the quantitative method).

42. *Cost-effectiveness* is the use of reagents and supplies that 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 analysed at the same time. This reduces the analytical time requirements per sample and usually reduces the cost per sample, as there are certain fixed costs associated with the analysis of samples whether done singly or in larger sets. The ability of a method to accommodate multiple samples in a batch is important when large numbers of samples must be analysed in short or fixed time frames. *Portability* is the analytical method characteristic that enables it to be transferred from one location to another without loss of established analytical performance characteristics.

43. *Analyte stability* during analysis must 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 programme and for typical conditions of storage while a sample is awaiting analysis. The period chosen for stability during storage should cover the expected time when sample material may be stored for all required analyses, including the use of the screening, quantitative and confirmatory methods. It is prudent to conduct the storage study for a period that extends to at least 90 days beyond the expected time 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. It is also prudent to assess the effect freeze-thaw cycles would have on the stability of the analytes under frozen conditions. This will permit a decision to be made regarding whether a sample, once thawed for analysis can or cannot be returned to storage and analysed again at a later date without significant change to the previous analytical result. Improper storage or handling of samples can lead to erroneous results and in cases where analytical results are disputed, guidance given in CAC/GL 70-2009 should be followed.

Other considerations

44. Ideally, a method of analysis for veterinary drug residues should be developed and characterised for the analysis of the four major tissues generally classed as "edible tissues", which are fat, liver, kidney, and muscle. In addition, milk, eggs and honey are traded internationally and methods of analysis may also be required for these matrices. Local dietary preferences may require methods for other tissues which are normally consumed in a country or region. In addition, there may be a regulatory requirement to analyse urine or other body fluids for residues, particularly if live animal testing is part of a regulatory programme. From a practical approach, the usual minimum requirement is that an analytical method should be developed for what is normally termed as "target tissue", which is the tissue from a treated animal in which the highest and most persistent concentrations of the drug residue are expected to be found. This would usually be the tissue collected for a national residue monitoring programme. In addition, there is a requirement to test the "tissue in trade" when products are shipped between countries. This is most commonly muscle tissue, but may include other tissues and processed foods such as cheese, smoked meats and processed fish. General guidance as to the selection of suitable target tissues and the expected "tissue in trade" is provided in Table 6 and in the reports of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Knowledge of the metabolism and tissue distribution/depletion should ideally be gained for each drug residue before a final selection of appropriate tissues for validation is made.

45. The concentration of the analytes used to characterise a method should be selected to include the accepted limits of all analytes planned to be sought in all commodities.

46. Once the following parameters summarized below are experimentally determined for all the analytes listed in the scope of a multi-residue method, the method can then be considered to be ready for further assessment through a validation process to determine whether the method is suitable (i.e., "fit-for-purpose") for use in a regulatory control programme for veterinary drugs in food animal production.

47. Fajgelj et al [REFERENCE: A. Fajgelj & A. Ambrus Principles and Practices of Method Validation, Royal Society of Chemistry, 2000] provide further guidance on the relevance of the parameters below and how they can be assessed.

(a) Selectivity
    (i) Freedom from interferences – all target analytes resolved chromatographically
    (ii) Matrix effects – characterised and corrective action taken
    (iii) Qualitative, quantitative, and/or confirmatory detector response parameters determined

(b) Calibration
    (i) Sensitivity
    (ii) Calibration range
    (iii) Calibration function
    (iv) LOD and LOQ

(c) Reliability of results
    (i) Recovery
    (ii) Accuracy (trueness, bias)
    (iii) Precision and Measurement uncertainty

(d) Method portability
    (i) Identification of critical control points
    (ii) Identification of possible stopping points
    (iii) Robustness (ruggedness) testing

(e) Stability studies

(i) Analyte stability in sample extracts and standard solutions; analyte stability under sample processing and analysis

(ii) Analyte stability under frozen storage and freeze-thaw cycle conditions.

(f) Incurred residue studies (if suitable materials are available)

(i) Verify performance of steps included in method to release bound residues

(ii) Verify consistency of recovery and precision

(iii) Verify analyte stability under frozen storage and freeze-thaw cycles

Table 1: Performance criteria that should be met by MRMs suitable for use as quantitative analytical methods to support MRLs for residues of veterinary drugs in foods

Concentration	Coefficient of variability (CV)		Trueness
	Repeatability (within-laboratory)	Reproducibility (between-laboratory)	Range of mean recovery*
(µg/kg)	(%)	(%)	(%)
≤ 1	36	54	50–120
1 to < 10	32	46	60–120
10 to < 100	22	34	70–120
100 to 1 000	18	25	70–110
≥ 1 000	14	19	70–110

* If a laboratory is required to report analytical results as corrected for analytical recovery, precision for the recovery is more important than the absolute recovery. However, if analytical results are reported uncorrected for analytical recovery, absolute recovery is critical.

Table 2: The relationship between a range of classes of mass fragment and identification points earned

MS technique	**Identification points earned per ion**
Low resolution mass spectrometry	1.0
$LRMS^n$ precursor ion	1.0
$LRMS^n$ transition product ion	1.5
HRMS	2.0
$HRMS^n$ precursor ion	2.0 [a, b]
$HRMS^n$ transition product ion	2.5 [a, b]

Notes:

- Each ion may be counted only once
- GC-MS using electron ionisation is regarded as being a different technique to GC-MS using chemical ionisation.
- Different analytes can be used to increase the number of identification points only if the derivatives employ different reaction chemistries.
- Transition products include both product ion and 1st generation product ions.
- [a] Based on MS resolution ≥10,000 (10% valley over the complete mass range) or ≥ 20,000 FWHM at the mass range of interest.
- [b] Based on mass accuracy < 5ppm.

Table 3: Examples of the number of identification points earned for a range of techniques and combinations thereof (n = an integer)

Technique	Source of Identification	Number of Identification Points
GC-MS (EI or CI)	N	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	N
GC-MS/MS	1 precursor ion + 2 product ions	4
LC-MS/MS	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 2 2nd 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/MS and GC-HRMS/MS	1 precursor ion + 2 product ions	6

Table 4 Performance requirements for relative ion intensities (sample compared to standard) using various mass spectrometric analytical techniques

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
20–50	≤ 15	≤ 25
> 10– < 20	≤ 20	≤ 30
≤10	≤ 50	≤ 50

Table 5: Examples of detection methods suitable for the confirmatory analysis of substances, as recommended by the Miskolc Consultation

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
GC/ECD, NPD, FPD	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

[a] Other chromatographic systems (applying stationary and/or mobile phases of different selectivity) or other techniques.

Table 6: Practical guidance on selection of appropriate test matrix for examination for residues of veterinary drugs in foods

Species/Commodity	Usual target tissue or matrix for method development	
	Water-soluble	Fat-soluble
Non ruminant (e.g. pig)*	Liver or kidney, muscle**	Fat, muscle
Poultry (e.g. chicken, turkey)*	Liver, muscle	Fat ,or muscle with adhering skin in normal proportions**
Shellfish/Crustacean (e.g. prawn)	Muscle	Muscle
Milk (usually cows' milk)	Whole milk	Whole milk
Honey	Honey	Honey
Egg	Whole –without shell	Whole-without shell

* Method development and characterization of analytical parameters should be conducted for all major species from which samples will be collected for routine testing. For minor use applications, it may be acceptable to demonstrate method applicability for the new species if the method has been previously demonstrated to be applicable to another species from the group (e.g., ruminant) and meets the previously verified performance standards for the "equivalent" major species.

** Residues of water-soluble compounds are usually found at highest concentrations in either liver or kidney, with the choice of tissue being made based on distribution studies provided by the drug sponsor at the time of registration by a national or regional authority. Fat-soluble compounds are usually present as residues at highest concentrations in fat, so in such instances the selection of test matrices is typically fat and muscle. However, in the case of poultry and fin-fish, where food preparation and consumption frequently include both the muscle and skin with fat, a suitable guideline may be "muscle with adhering skin in normal proportions", reflecting the combined muscle tissue, fat and skin which may be consumed. Such requirements should be clearly established with the client (the purchaser or user of results) before beginning method development. National or regional authorities or purpose of testing may require method applicability for different or additional matrices.

*** Fin fish can have high lipid concentrations (e.g. salmonids) or low lipid concentrations (e.g. tilapia, perch) and this can affect the choice of analytical method.

GLOSSARY OF TERMS*

This glossary includes only terms not defined in "Guidelines on Analytical Terminology", CAC/GL 72-2009.

Accepted Limit (AL)	Concentration value for an analyte corresponding to a regulatory limit or guideline value which forms the purpose for the analysis, e.g. MRL, trading standard, target concentration limit (dietary exposure assessment), acceptance level (environment), etc. for a substance without an MRL or for a banned/prohibited substance there may be no AL (effectively it may be zero or there may be no limit) or it may be the target concentration above which detected residues should be confirmed (action limit or administrative limit).
Alpha *(α)* Error	Probability that the true concentration of analyte in the laboratory sample is less than a particular value (e.g. the AL) when measurements made on one or more analytical/test portions indicate that the concentration exceeds that value (false positive). Accepted values for this probability are usually in the range 1 to 5%.
Beta (β) Error	Probability that the true concentration of analyte in the laboratory sample is greater than a particular value (e.g. the AL) when measurements made on one or more analytical portions indicate that the concentration does not exceed that value (false negative). Accepted values for this probability are usually in the range 1 to 5%.
Confirmatory Method	Methods that provide complete or complementary information enabling the analyte to be identified with an acceptable degree of certainty [at the Accepted Limit or concentration of interest]. As far as possible, confirmatory methods provide information on the chemical character of the analyte, preferably using spectrometric techniques. If a single technique lacks sufficient specificity, then confirmation may be achieved by additional procedures consisting of suitable combinations of clean-up, chromatographic separation(s) and selective detection. Bioassays can also provide some confirmatory data. In addition to the confirmation of the identity of an analyte, its concentration shall also be confirmed. This may be accomplished by analysis of a second test portion and/or reanalysis of the initial test portion with an appropriate alternative method (e.g. different column and/or detector). The qualitative and quantitative confirmation may also be carried out by the same method, when appropriate.
Decision Limit (CCα)	Limit at which it can be decided that the concentration of the analyte present in a sample truly exceeds that limit with an error probability of a (false positive). In the case of substances with zero AL, the CCα is the lowest concentration, at which a method can discriminate with a statistical probability of 1 -α whether the identified analyte is present. The CCα is equivalent to the limit of detection (LOD) under some definitions (usually for α = 1%). In the case of substances with an established AL, the CCα is the measured concentration, above which it can be decided with a statistical probability of 1 -α that the identified analyte content is truly above the AL.
Detection Capability (CCβ)	Smallest true concentration of the analyte that may be detected identified and quantified in a sample with a beta error (false negative). In the case of banned/prohibited substances the CCß is the lowest concentration at which a method is able to determine the analyte in contaminated samples with a statistical probability of 1 -β. In the case of substances with an established MRL, CCß is the concentration at which the method is able to detect samples that exceed this MRL with a statistical probability of 1 - ß. When it is applied at the lowest detectable concentration, this parameter is intended to provide equivalent information to the Limit of Quantification (LOQ), but CCß is always associated with a specified statistical probability of detection, and therefore it is preferred over LOQ.
False negative result	See beta error
False positive result	See alpha error
Incurred Residue	Residues of an analyte in a matrix arising by the route through which the trace concentrations would normally be expected by treatment or dosing according to intended use, as opposed to residues from laboratory fortification of samples.
Individual Method	Method which is suitable for determination of one or more specified compounds. A separate individual method may be needed, for instance to determine some metabolite included in the residue definition of an individual pesticide or veterinary drug.
Lowest Calibrated Level (LCL)	Lowest concentration of analyte detected and measured in calibration of the detection system. It may be expressed as a solution concentration in the test sample or as a mass and must not include the contribution from the blank

Matrix	Material or component sampled for analytical studies, excluding the analyte.
Matrix Blank	Sample material containing no detectable concentration of the analytes of interest.
Matrix-matched standard calibration curve (MSCC)	Matrix-matched standard calibration curve (MSCC) – a calibration curve prepared by addition of standards to sample extracts obtained from blank matrix after extraction.
Method matrix-matched standard calibration curve (MMSCC)	Method matrix-matched standard calibration curve (MMSCC) but also known as a matrix-fortified standard calibration curve – a calibration curve prepared by addition of standards to blank matrix prior to extraction.
Method	The series of procedures from receipt of a sample for analysis through to the production of the final result.
Method Validation	Process of verifying that a method is fit for purpose.
Multi-residue Method, MRM	Method which is suitable for the identification and quantification of a range of analytes, usually in a number of different matrices.
Negative Result	A result indicating that the analyte is not present at or above the lowest calibrated concentration. (see also Limit of Detection)
Performance Verification	Sets of quality control data generated during the analyses of batches of samples to support the validity of on-going analyses. The data can be used to refine the performance parameters of the method.
Positive Result	A result indicating the presence of the analyte with a concentration at or above the lowest calibrated concentration.
Quantitative Method	A method capable of producing results, expressed as numerical values in appropriate units, with accuracy and precision which are fit for the purpose. The degree of precision and trueness must comply with the criteria specified in Table 1.
Reagent Blank	A sample consisting of all reagents that have been run through the method excluding the sample matrix.
Reference Method	Quantitative analytical method of proven reliability characterised by well-established trueness, selectivity, precision and sensitivity. These methods will generally have been collaboratively studied and are usually based on molecular spectrometry. The reference method status is only valid if the method is implemented under an appropriate QA regime.
Reference Procedure	Procedure of established efficiency. Where this is not available, a reference procedure may be one that in theory should be highly efficient and is fundamentally different from that under test.
Representative Analyte	Analyte chosen to represent a group of analytes which are likely to be similar in their behaviour through a multi-residue analytical method, as judged by their physicochemical properties e.g. structure, water solubility. K_{ow}, polarity, volatility. hydrolytic stability, pKa, etc.
Represented Analyte	Analyte having physico-chemical properties which are within the range of properties of representative analytics.
Representative Commodity	Single food or feed used to represent a commodity group for method validation purposes. A commodity may be considered representative on the basis of proximate sample composition such as water. fat/oil. acid. sugar and chlorophyll contents. or biological similarities of tissues, etc.
Sample Preparation	The procedure used, if required, to convert the laboratory sample into the analytical sample by removal of parts not to be included in the analysis.

Sample Processing	The 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. The processing element of preparation must be designed to avoid inducing changes in the concentration of the analyte.
Screening Method	A method used to detect the presence of an analyte or class of analytes at or above the minimum concentration of interest. It should be designed to avoid false negative results at a specified probability (generally ß = 5%). Qualitative positive results may be required to be confirmed by confirmatory, quantitative or reference methods. See Decision Limit and Detection Capability.
Standard Addition	A procedure in which known amounts analyte are added to aliquots of a sample extract containing the analyte (its initially measured concentration being X), to produce new notional concentrations (for example, 1.5X and 2X). The analyte responses produced by the spiked aliquots and the original extract are measured, and the analyte concentration in the original extract (zero addition of analyte) is determined from the slope and intercept of the response curve. Where the response curve obtained is not linear, the value for X must be interpreted cautiously.
Standard calibration curve (SCC)	A calibration curve prepared using standards in the absence of matrix, usually referred to as an "external calibration."

ABBREVIATIONS

CCRVDF	Codex Committee on Residues of Veterinary Drugs in Foods	LC-MS/MS	Liquid chromatography-tandem mass spectrometry
CI	Chemical ionisation	LRMS	Low resolution mass spectrometry
CIMS	Chemical ionisation mass spectrometry	MMSCC	Method matrix-matched standard calibration curve
DAD	Diode array detection	MRL	Maximum Residue Limit
ECD	Electron capture detector	MRM	Multi-residue method
EI	Electron ionisation	MS	Mass spectrometry
EIMS	Electron ionisation mass spectrometry	MSCC	Matrix-matched standard calibration curve
FAO	Food and Agriculture Organisation	NPD	Nitrogen phosphorus detector
FPD	Flame photometric detector	$S_{y/x}$	Standard deviation of the residuals calculated from the linear calibration function
GC	Gas chromatography	SCC	Standard calibration curve
GC-MS	Gas chromatography-mass spectrometry	TLC	Thin layer chromatography
GC-MS/MS	Gas chromatography-tandem mass spectrometry	UV	Ultraviolet light detection
HRMS	High resolution mass spectrometry	WHO	World Health Organization
IP	Identification point	VIS	Visible light detection
JECFA	Joint FAO/WHO Expert Committee on Food Additives		
LC-MS	Liquid chromatography-mass spectrometry		

Annex 2

GENERAL GUIDANCE FOR THE PROVISION OF COMMENTS

In order to facilitate the compilation and prepare a more useful comments' document, Members and Observers, which are not yet doing so, are requested to provide their comments under the following headings:

(i) General Comments

(ii) Specific Comments

Specific comments should include a reference to the relevant section and/or paragraph of the document that the comments refer to.

When changes are proposed to specific paragraphs, Members and Observers are requested to provide their proposal for amendments accompanied by the related rationale. New texts should be presented in **underlined/bold font** and deletion in ~~strikethrough font~~.

In order to facilitate the work of the Secretariats to compile comments, Members and Observers are requested to refrain from using colour font/shading as documents are printed in black and white and from using track change mode, which might be lost when comments are copied / pasted into a consolidated document.

In order to reduce the translation work and save paper, Members and Observers are requested not to reproduce the complete document but only those parts of the texts for which any change and/or amendments is proposed.