CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 7 **CX/RVDF 13/21/7**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

Twenty-first Session

Minneapolis, Minnesota, United States of America, 26 – 30 August 2013

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CHARACTERISTICS FOR MULTI-RESIDUES METHODS (APPENDIX TO CAC/GL 71-2009) (N01-2011)

(Report of the CCRVDF Electronic Working Group on Multi-Residue Analytical Methods)

Governments and international organizations wishing to submit comments on the proposed draft Guidelines on Performance Characteristics for Multi-Residues Methods (*see* Annex 1) are invited to do so **no later than 30 June 2013** 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; 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; E-mail: Codex@fao.org).

Please note that only comments submitted by the above deadline will be compiled, translated and made well in advance to the 21st CCRVDF.

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. At the 20th session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) in San Juan, Puerto Rico, (7 – 11 May 2012), the Committee agreed to establish an electronic working group. The purpose of the group is:

- To revise the draft report on performance criteria for multi-residue analytical methods that was submitted to the 20th Session for inclusion as an Appendix to 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 develop a generic validation protocol for multi-residue methods.

Proceedings of the Electronic Working Group

2. The electronic Working Group (eWG) worked primarily by email and comment and document exchange was facilitated by an electronic forum established by the United Kingdom. This document reflects the input and views of the following countries: Australia, Brazil, Canada, France, Germany, the Netherlands, Switzerland, United Kingdom, Uruguay and the United States of America.

Discussion

3. The eWG agreed at an early stage that the Appendix to CAC/GL 71-2009 should be developed and simplified from earlier documents considered by the CCRVDF. It was considered that it would be too difficult at this stage to develop a generic validation protocol for multi-residue analytical methods and that it was more appropriate to reference a number of national or regional guidelines which would serve to inform those wishing to validate such methods for their own purposes.

Recommendations

4. The Committee is invited to consider the draft Appendix for CAC/GL 71-2009 at Annex 1 for amendment and advancement as appropriate.

ANNEX 1

PROPOSED DRAFT GUIDELINES ON PERFORMANCE CHARACTERISTICS FOR MULTI-RESIDUES METHODS (APPENDIX TO CAC/GL 71-2009)
(N01-2011)

(At Step 3 of the Procedure)

APPENDIX C: PERFORMANCE CHARACTERISTICS FOR MULTI-RESIDUE METHODS (MRMs) FOR VETERINARY DRUGS

Purpose

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

Scope

2. It is applicable to multi-residue methods (MRMs) used to analyse all residues of veterinary drugs and substances which may be used as veterinary drugs. This includes certain pesticides which have veterinary uses and that 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 pesticide residue analysis*.

3. In this Appendix 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 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 must not be used for those purposes until they have been validated and/or demonstrated to be fit-for-purpose (1).

Summary of performance parameters to be characterised and defined for multi-residue analytical methods

4. The following characteristic parameters (2) need to be measured for every analyte and for each matrix under study:-

(a) Selectivity

(i) Freedom from interferences – all target analytes resolved chromatographically

(ii) Matrix effects – characterised and controlled by the method if they occur

(iii) 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)

(b) Calibration

(i) Sensitivity

(ii) Calibration range

(iii) Calibration function

(iv) LOD and LOQ, and/or CCα and CCβ

(c) Reliability of results

(i) Recovery

(ii) Accuracy (trueness, bias)

(iii) Precision and Measurement uncertainty

(iv) Robustness (ruggedness) testing including identification of critical control points and possible stopping points

(d) Stability of Analytes

(i) Stability in sample extracts and standard solutions

(ii) Stability under sample processing and analysis

(iii) Stability under frozen storage and freeze-thaw cycle conditions

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

(i) Verify that incurred residues are as effectively extracted as fortified analytes

(ii) Verify performance of any steps included in method to release chemically bound residues where required (3)

(iii) Verify consistency of recovery and precision

Performance characteristics for MRMs

5. It should be understood that the performance characteristics listed in paragraph 4 should 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 analytical method is not going 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 CAC/GL 71-2009, paragraphs 160 – 181. To avoid repetition, only differences from single analyte consideration will be highlighted in this Appendix.

6. 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.

Performance characteristics of MRMs for screening analysis

7. 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 ("negatives/compliant") from those that may contain residues ("positives/presumptive positives/suspect positives") above a threshold or cut-off value.

8. Screening methods for approved veterinary drugs should demonstrate a selectivity rate of 95% with 95% confidence and a sensitivity rate of 90% with 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 verify the presence of the "suspect" residue. For all other veterinary drugs which are NOT approved for use, this appendix may be used to inform decisions on the performance criteria which may need to be developed.

9. Criteria for identifying cut-off or threshold limits for screening methods are given in CAC/GL 71-2009 (paragraph 163) and in documents such as the EU CRL guidelines on screening method validation for veterinary drugs (4).

Performance characteristics of MRMs for quantitative analysis

10. The requirement to recover a range of different veterinary drug residues in one extraction increases the potential for compromised selectivity in MRMs compared to single analyte methods. The need to use 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 MRMs to ensure that quantification will not be affected by interference from other compounds present in the sample matrix. It is recommended that MRMs used to support Codex MRLs should meet the performance standards for trueness and precision listed in Table 1 of CAC/GL 71-2009. 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 be based on measurements on a minimum of six different sources of blank sample material. The intermediate precision for recovery of analytes fortified into these different samples should be used for comparison to the criteria in Table 1 of CAC/GL 71-2009 rather than the repeatability precision.

11. 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. It is suggested that an interim value of 10 µg/kg is temporarily adopted provided there can be reasonable confidence there will be no significant toxicological implications whilst more formal advice is sought.

12. 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 behaviour 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 are determined using a minimum of six different sources of blank material for each matrix for which the method is validated.

13. The Miskolc consultation in 1999 (5) 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 two parameters incorporate a consideration of measurement uncertainty.

Performance characteristics for MRMs for confirmatory methods

14. The necessary steps to positive identification are a matter of expert 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 (6).

15. 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 CAC/GL 71-2009 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 (7).

16. Table 1 in this Appendix lists the number of identification points (IPs) earned for a combination of 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.

17. 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 mass spectrometers for detection.

18. Non-magnetic sector type high-resolution mass spectrometers are becomingly 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**.**

Validation of the fully characterized MRM

19. Determination of the parameters in paragraph 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 programme. For screening methods, those analytes whose measured performance parameters are achieved in a combination of validation experiments in which ≥90% of the measurements taken at each analyte/matrix/concentration combination of interest pass could be considered acceptable for inclusion in the method.

20. Paragraph 189 of CAC/GL 71-2009 recommends the use of biologically incurred material in the characterisation 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 MRLs. The target incurred concentration should be close to the MRL or expected concentration.

21. Alternative protocols may be used for validation of MRMs, adapted as necessary for individual circumstances. For example and for guidance only, the EU Community Reference Laboratories (CRL) have published a guideline (4) on screening method validation for veterinary drugs, and the SANCO Document (SANCO 12495/2011) describes a method validation and quality control procedures for pesticide residues analysis in food and feed (8). Similar documents have been published in the USA by the FSIS (9) and in Canada by the CFIA (10).

Table 1: Examples of the number of identification points (IPs) earned for a range of techniques and combinations thereof (n = an integer)

Technique	Source of Identification	Number of Identification Points (IPs)
GC-MS (EI or CI)	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	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 2^{nd} 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

GLOSSARY OF TERMS

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

Compliant or Negative Result	A result indicating that the analyte is not present at or above the lowest calibrated concentration. (see also Limit of Detection in CAC/GL 72-2009)
Confirmatory Method	A method that provides complete or complementary information enabling the analyte to be identified with an acceptable degree of certainty at the concentration of interest.
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 **α** (false positive).
Detection Capability (CCβ)	Smallest true concentration of the analyte that may be detected, identified and quantified in a sample with an error probability of ß-(false negative).
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.
Matrix	Material or component sampled for analytical studies, excluding the analyte.
Matrix Blank	Sample material containing no detectable concentration of the analytes of interest.
Method	The series of procedures from receipt of a sample for analysis through to the production of the final result.
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 and includes three or more analytes in the same class or more than one class of veterinary drugs in its scope.
Presumptive positive or suspect Result	A result suggesting the presence of the analyte with a concentration at or above the lowest calibrated concentration.

Positive Result	A result indicating that the analyte has been confirmed to be present 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 of CAC/GL 71-2009.
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.
Screening Method	A method used to detect the presence of an analyte or class of analytes at or above the minimum concentration of interest.

ABBREVIATIONS

CI	Chemical ionisation
CIMS	Chemical ionisation mass spectrometry
EI	Electron ionisation
EIMS	Electron ionisation mass spectrometry
GC	Gas chromatography
GC-MS	Gas chromatography-mass spectrometry
GC-MS/MS	Gas chromatography-tandem mass spectrometry
HRMS	High resolution mass spectrometry
IP	Identification point
LC-MS	Liquid chromatography-mass spectrometry
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LRMS	Low resolution mass spectrometry
MRL	Maximum Residue Limit
MRM	Multi-residue method
MS	Mass spectrometry

REFERENCES

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3. Glossary of Terms. Appendix to 35th Session Joint FAO/WHO Food Standards Programme Codex Alimentarius Commission, Rome Italy 2-7 July 2012. http://www.codexalimentarius.net/vetdrugs/data/reference/glossary.html

4. The EU CRL guidelines on screening method validation for veterinary drugs. http://ec.europa.eu/food/food/chemicalsafety/residues/Guideline_Validation_Screening_en.pdf

5. Harmonized IUPAC Guidelines for single laboratory validation of methods of analysis CAC/GL 49-2003 [Pure Applied Chemistry 74 (5), 835-855 (2002)]

6. 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. Society for Mass Spectrometry 14 (5), 528-541(2003).

7. Milman, B.L. Identification of compounds. Trends in Analytical Chemistry 24 (6), 493-508 (2005).

8. Method validation and quality control procedures for pesticide residues analysis in food and feed. SANCO 12495/2011.

9. Type I Validations of Chemistry Methods. FSIS Laboratory-wide SOP LW-0050.00

10. Validation of CVDR Test Methods CVDR-S-0027.08 (2011/06)

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.