codex alimentarius commission

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

WORLD HEALTH ORGANIZATION

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Agenda Item 11 (a) **CX/RVDF 03/10**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS
Fourteenth Session
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REVIEW OF PERFORMANCE-BASED CRITERIA FOR METHODS OF ANALYSIS FOR VETERINARY DRUG RESIDUES IN FOODS

Governments and international organizations wishing to submit comments on the following subject matter are invited to do so in writing **no later than 3 February 2003** to: U.S. Codex Office, Food Safety and Inspection Service, US Department of Agriculture, Room 4861, South Building, 14th and Independence Avenue, S.W., Washington, DC 20250, USA (Fax No: +1.202.720.3157; e-mail: uscodex@usda.gov), with a copy to the Secretary, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy (Telefax: +39.06.5705.4593; E-mail: Codex@fao.org).

INTRODUCTION

1. The 12th Session of the Codex Committee on Residues of Veterinary Drugs in Food (CCRVDF) accepted the proposal of the *ad hoc* Working Group on Methods of Analysis and Sampling to review criteria for the validation of analytical methods with the goal of supplementing existing procedures with criteria for the validation of analytical method in a single laboratory. The 13th session of the CCRVDF held in Charleston, South Carolina in December 2001 established a drafting party to produce a document for consideration at the 14th Meeting of the Committee.

2. Two expert consultations (Vienna, 1998[1]; Miskolc, 1999[2]) have recommended that a single laboratory validation approach should be recognised as a viable means to identify methods which should be suitable for use in a regulatory program to support recommendations for Maximum Residue Limits (MRLs) specified by the Codex Alimentarius Commission.

3. These consultations not only recommended the technical specifications that should be considered in the evaluation of analytical method in a single laboratory, but also recognised that laboratories using methods must be able to demonstrate the capability to acceptably perform analytical methods. A key feature of the single laboratory evaluation procedure is the laboratory should be able to demonstrate its analytical expertise, quality assurance procedures and staff qualifications that will support the authority of their analytical conclusions. The single laboratory procedure considers that method performance is a direct result of the competence of the laboratory providing the analytical service.

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1 Validation of analytical methods for food control, FAO Food and Nutrition Paper 68, Rome, 1998.

2 Report of the Joint FAO/IAEA Expert Consultation on Practical Procedures to Validate Method Performance of Analysis of Pesticide and Veterinary Drug Residues, and Trace Organic Contaminants in Food, (http://www.iaea.org/trc).

4. A guidance document on single laboratory validation of methods, *Harmonized Guidelines for Single-Laboratory Validation of Methods of Analysis*, has recently been published as a technical report by the International Union of Pure and Applied Chemistry [3].

5. Requirements for the use of single-laboratory validation of methods for Codex purposes have been proposed for consideration by the Codex Committee on Methods of Analysis and Sampling (CX/MAS 02/11).

6. Recognizing that the main issue is mutual acceptance of laboratory data, the proposal recognizes that the method and the results obtained must be seen in the context of laboratories which operate in an accredited laboratory environment and which respects the guidelines on quality assurance, proficiency testing and single laboratory method validation. The acceptability of results obtained using single laboratory validated methods can be demonstrated through calibration using reference materials, comparison with other methods which have a well-defined performance or systematic participation in proficiency tests. The following conditions have been proposed for addition to the *Procedural Manual* to establish criteria for cases where single laboratory validated methods can be used:

i. No inter-laboratory validated method is appropriate.

ii. The single-laboratory validated methods must fulfill the following criteria

- the method is validated according to an internationally recognized protocol (for example, the IUPAC protocol referenced above);
- the use of the method is embedded in a quality assurance system under accreditation;
- external reference is given at least by systematic participation in proficiency schemes. Additional external reference can be obtained by calibration using reference materials and comparison of results with those obtained using other methods.

7. The Codex Committee on Pesticide Residues (CCPR), at its 34th meeting in April, 2002, progressed consideration of proposed amendments to the Guidelines on Good Laboratory Practice in Pesticide Residue Analysis to Step 5 of the Codex process (Alinorm 01/24, Appendix VI).These amendments incorporate concepts and recommendations elaborated by the AOAC/FAO/IAEA/IUPAC Consultation in Miskolc in 1999, published by the Training Centre of the International Atomic Energy Agency on their website (http://www.iaea.org/trc). This work will continue at the next meeting of CCPR.

METHOD CRITERIA

8. The specifications for the evaluation of analytical methods as previously elaborated by CCRVDF in the Codex Alimentarius, Volume 3 *Residues of Veterinary Drugs in Food* (2nd Edition, 1994) are consistent with the views elaborated by these more recent consultations. For example, the set of attributes or properties which were considered to determine the usefulness of an analytical method were as follows:

i. specificity
ii. precision
iii. bias or systematic error
iv. accuracy
v. limit of detection
vi. method sensitivity
vii. practicality of use
viii. tissue/species applicability
ix. limit of quantitation
x. false positive/false negative responses

[3] Thompson, M., Ellison, S.L.R. & Wood, R. (2002) *Pure Appl. Chem*. **74**: 835-8552

9. The *Procedural Manual* (12th edition, 2001) lists criteria for methods primarily intended as international methods for the verification of provisions in Codex standards and normally derived through a formal inter-laboratory collaborative study. They are:

i. specificity
ii. accuracy
iii. precision; repeatability intra-laboratory; reproducibility inter-laboratory
iv. limit of detection
v. sensitivity
vi. practicality and applicability under normal laboratory conditions
vii. other criteria which may be selected as required.

10. This section of the *Procedural Manual* also acknowledges that other criteria, eg recovery factors, may need to be taken into account.

11. CCMAS will also be considering the inclusion of changed/additional criteria (and suggested definitions) at its 24th Session (CX/MAS 02/5). These additional criteria are:

- precision (generated through collaborative trial data)
- recovery
- selectivity
- applicability
- detection/determination limits
- linearity

12. It is proposed by CCMAS that these criteria would be used to define methods in terms of performance characteristics so that meaningful comparison can be made between alternative methodological approaches. Effectively this creates an environment suitable for acceptance of methods validated within a single laboratory.

13. Criteria identified by the AOAC/FAO/IAEA/IUPAC Consultation in Miskolc, which was considering method validation within a single laboratory, included the following:

i. specificity
ii. analytical range, including recovery through extraction, clean-up, derivatization and measurement
iii. calibration range for determination of analyte
iv. limit of detection
v. limit of quantitation
vi. reporting limit, or lowest calibrated level (LCL)
vii. analyte stability in sample extracts
viii. analyte stability during sample storage and processing
ix. analyte homogeneity in samples
x. accuracy
xi. trueness
xii. precision
xiii. selectivity
xiv. extraction efficiency
xv. purity of reagents and materials

14. In addition, the Consultation provided specific guidance on what investigations should be undertaken to determine adherence to these criteria in the two situations of pesticide residues and veterinary drug residues in foods.

15. The expanded criteria recommended for inclusion in the validation address some issues which normally would be resolved prior to a collaborative or inter-laboratory trial (ie, analyte stability, sample homogeneity, purity specifications for reagents and materials), as well as introducing the practical considerations of the specifications for calibration range required.

16. The support of an MRL requires that a method which provides the quantitative results must perform in good statistical control within the analytical range that brackets the MRL In such cases, performance of the method within that range and the inclusion of appropriate calibration points (including the lowest calibrated level, or LCL) may be more important than a characterisation of a limit of detection or limit of quantitation.

17. The issue of method selectivity (also referred to frequently as specificity) and in particular the ability of a method to provide unequivocal identification of an analyte was also seen as critical and recognised recent work to establish a minimum number of identification points which should be required to provide confidence in a confirmatory result.

CATEGORIES OF METHODS CONSIDERED BY CCRVDF

18. The Codex Alimentarius, Volume 3, *Residues of Veterinary Drugs in Foods* (2nd Edition, 1994), identifies three categories of analytical methods for use in a residue control program or to determine compliance with Codex maximum residue limits (CAC/GL 16-1993 Part III). These are Level I methods, which are intended to identify and quantify an analyte; Level II methods, which are intended to quantify, but do not unequivocally identify an analyte; and Level III methods, which determine presence or absence of a compound at or above a level of interest. Level III methods are typically referred to as screening methods, Level II methods are determinative methods and Level I methods are confirmatory methods. Level I, II and III methods roughly approximate Type II, III and IV methods as promulgated by CCMAS.

19. Each category of method requires the determination of conformance to a set of performance criteria which normally would be elaborated through a multi-laboratory trial . Currently, a validated method is defined by CCRVDF as “an analytical method which has been subjected to a multi-laboratory study fo accuracy, precision, reproducibility performance and ruggedness” (CAC/MISC 5 – 1993). In the absence of such information, the contemporary view is that a single laboratory validation conducted according to the principles discussed above, with appropriate peer-review, would be acceptable (Vienna. 1998; Miskolc, 1999).

20. One element, sample stability under normal conditions of storage, is common to all categories of methods and must be determined in all cases. This property should be determined using samples collected, transported and stored prior to analysis under conditions which are representative of the situation for samples which will normally be processed by the laboratory. Once this information has been obtained, it is not necessary to repeat the investigations for different methods or categories of methods unless there is a significant change in conditions associated with the use of the method.

DEFINITIONS

Minimum validation criteria requirements to address for Level III methods

21. For Level III or “screening” methods, it is necessary to demonstrate the statistical reliability of the method performance at the concentration considered critical for analyte detection, usually a MRL. The performance parameters which must be assessed are the following, for each type of sample material (for example, beef liver, beef muscle, sheep muscle, cow’s milk) to be tested for the target analyte:

i. **Selectivity**, defined in CX/MAS 02/5 as the "extent to which a method can determine particular analyte(s) in mixtures or matrices without interferences from other components. Selectivity is the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence of interferences from other components. Selectivity can be graded. The use of the term specificity for the same concept is to be discouraged as this often leads to confusion."

The AOAC Performance Tested Program ™ for test kits requires demonstration of at least 90% selectivity with 95% confidence on known residue-free sample tested for the target analyte**.[** This is determined experimentally by testing a minimum of 30 residue-free sample materials which are from at least six different sources (that is, at least 5 replicates from each of at least 6 sources), all of which should yield a negative result. Three or more positive results constitute a failure of the selectivity test. If one or two of the results are positive, the experiment should be repeated and two positive results would then constitute failure.

Other organizations or authorities have elaborated other requirements. For example, the OIE recommends 1000 negative samples (see http://www.oie.int/eng/normes/mmanual/A_00013.htm), while screening methods used in conformity with EU Directive 96/23/EC are required to be "validated and have a false compliant rate of <5% (∃-error) at the level of interest (see Commission Decision 2002/657/EC).

ii. **Sensitivity** may be defined for a screening test as lowest concentration at which the target analyte may be reliably detected. In the AOAC Performance Tested Program ™ for test kits, this is determined experimentally by testing a minimum of 30 residue-free sample materials fortified with the analyte at the target concentration. The sample materials should be from at least six different sources (that is, 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. 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.

iii. The **dose-response** curve for the test should be determined in conjunction with the sensitivity test. This requires selecting a range of concentrations which should include the target concentration for detection of non-compliance. In the AOAC Performance Tested Program ™ for test kits, for each concentration, a minimum of 30 residue-free sample materials (six sources) are fortified with the analyte at the required concentration and the percentage of positive results is determined. The response at the target concentration for monitoring non-compliance and at higher concentrations should meet the sensitivity requirement stated above. An accurate dose-response determinations should include fortification at a minimum of four evenly spaced concentrations between "all negative" and "all positive", plus a concentration at least 20% higher than the "all positive" concentration, which should be the target concentration for detection of non-compliance.

iv. The **ruggedness** is defined in the Procedural Manual as the "ability of a chemical measurement process to resist changes in results when subjected to minor changes in environmental and procedural variables, laboratories, personnel, etc.". A screening test should be assessed by varying appropriate factors such as amount of reagent added, incubation time or temperature and visual accuity of tester in determining end-point, to determine any critical control points which should be identified.

v. In the case of "test kit" assays, the **conditions of storage** and **shelflife** should be clearly stated.

vi. **Cross-reactivity,** which is the ability of the test to generate a positive response with other compounds than the target analyte, including metabolites or other substances which may be present in samples, should be determined.

vii. **Interferences** which mask, obscure or enhance the signal from sample materials should be determined. This information can be derived from the analysis of the blank sample materials obtained from six or more sources, used in the selectivity and sensitivity experiments. For tests used in the determination of residues in milk, potential interferences from typical bacteria and somatic cells should be assessed.

22. In addition, the stability of the analyte both in standards and in samples under the normal conditions of analysis required by the test should be assessed.

Minimum validation criteria requirements to address for Level II methods

23. For Level II or determinative tests, a more comprehensive set of parameters relating to the statistical reliability of the quantitative result obtained must be assessed. These include the following:

i. **Accuracy** (also sometimes referred to as **trueness** or **bias**) is defined in the Procedural Manual as the "closeness of agreement between the reported result and the accepted reference value". **[**The Procedural Manual defines **trueness** as the "closeness of agreement between the average value obtained from a series of test results and an accepted reference value" and **bias** as the **"**difference between the expectation of the test results and an accepted reference value". **Accuracy** is therefore the ability of a method to provide a result consistent with the true concentration of the analyte present in the test material. The accuracy of a method may be determined by 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 (that is, a recognized reference method) or, in the absence of reference materials or methods, by determination of the recovery of analyte fortified into known blank sample material.

ii. **Recovery** is defined in CX/MAS 02/5 as the proportion of the amount of analyte present or added to the test material which is extracted and presented for measurement . It is typically expressed as the percentage of analyte experimentally determined after fortification of sample material at a known concentration and should be assessed at concentrations which cover the analytical range of the method.

iii. The **calibration curve** should be determined to assess the detector response to standards. The concentrations (a minimum of five, plus blank) should cover the full range of analytical interest and the resultant curve should be statistically expressed. Typically, a linear response is desirable and the curve is statistically expressed in terms of linear correlation. For a determinative method, the related term **sensitivity** is defined in the Procedural Manual as "the change in the response divided by the corresponding change in the concentration of a standard (calibration curve); i.e. the slope, s_i, of the analytical calibration curve". It has also been defined as "the gradient of the calibration function"[3].

iv. The **analytical function** relates the response for the analyte recovered from sample material at various concentrations throughout the range of analytical interest. For analytes for which an MRL has been established in a particular sample material (matrix), response is typically determined for known blank sample material and for blank sample material fortified at each f 0.5x, 1.0x and 2.0x the MRL (use 6 different source of blank materials). The analytical function experiment can be combined with the recovery experiment described above and is of particular importance when the presence of matrix co-extractives modifies the response of the analyte as compared to analytical standards. It is 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 at a range of appropriate concentrations which bracket the target value. Use of such a "tissue standard curve" for calibration incorporates a recovery correction into the analytical results obtained.

v. **Linearity** is defined in CX/MAS 02/4 **as "**the ability of a method of analysis, within a certain range, to provide an instrumental response or results proportional to the quality of analyte to be determined in the laboratory sample." This proportionality is expressed by an *a priori* defined mathematical expression. The linearity limits are the experimental limits of concentrations between which a linear calibration model can be applied with a known confidence level (generally taken to be 1%). For a method in which fortified blank matrix material is used for quantitation, the linearity is determined from the analytical

function experiments as described and is the statistical expression of the curve obtained for the analysis of sample materials fortified at the target concentrations bracketing the Maximum Residue Limit. It is typically determined from a linear regression analysis of the data, assuming there is a linear response

vi. The **limit of detection** or detection limit (CX/MAS 02/4) is conventionally defined as field blank + 3s, where s is the standard deviation of the field blank signal (IUPAC definition) –It is also defined as "the smallest amount or concentration of analyte in the test sample that can be reliably be distinguished from zero"[3]. Typically, the calculation of the mean signal for the field blanks and the standard deviation is made from 20 or more determinations . This approach can yield an optimistic estimate of the limit of detection. An alternative approach involves the calculation of the limit of detection from the standard deviation $s_{y/x}$ from the linear regression analysis of the standard curve generated in the analytical function experiment described above[4]. The limit of detection is then calculated using the y-intercept of the curve plus three times $s_{y/x}$. This approach provides a more conservative estimate of the limit of detection.

vii. The **limit of quantitation,** as defined by IUPAC**,** is the lowest concentration of analyte in a defined matrix which can be determined with the required precision and accuracy[5]. It may be expressed as 10 times the standard deviation of the mean value for 20 or more determinations of the response from known blank matrix material. For methods used to support Maximum Residue Limits established by the Codex Alimentarius Commission, the limit of quantitation should meet the criteria for precision and accuracy (recovery) in Table 1. However, given that the limit of quantitation of a method may be considerably lower than the actual concentrations monitored for compliance with a Maximum Residue Limit, the validation and subsequent application of the method may be based on a **lowest calibrated level**, which is typically 0.5x the MRL. For use in a regulatory program, the limits of detection and quantitation are important parameters when the method will be applied to estimate exposures to residues, where there may be an interest in monitoring residues at concentrations below the MRL. For monitoring compliance with an MRL, it is important that a lowest calibrated level be included in the analysis which adequately demonstrates that the MRL concentration may be reliably determined.

viii. **Precision** is defined in the Procedural Manual as the "closeness of agreement between independent test results obtained under stipulated conditions". It may be expressed in terms of **repeatability** (intra-laboratory) and **reproducibility** (inter-laboratory). For a single laboratory method validation, precision as repeatability should be determined from experiments conducted on different days, using different reagent batches and preferably by different analysts. An initial estimate of repeatability may be made from the data generated during the analytical function experiments described above. Additional data may be obtained from subsequent method familiarizations conducted by other analysts in the laboratory. Precision expressed as reproducibility requires a multi-laboratory trial of the method**.** CCMAS have proposed that precision should be generated from collaborative trials data rather than measurement uncertainty considerations(CX/MAS 02/5).

ix. **Specificity** is defined in the Procedural Manual as "the property of a method to respond exclusively to the characteristic or analyte defined in the Codex standard. However, CCMAS have proposed (CX/MAS 02/5) that the term **selectivity,** defined as "the extent to which a method can determine particula analyte(s) in mixtures or matrices without interferences from other components", is "the recommended term in analytical chemistry to express the extent to which a particular method can determine analyte(s) in the presence of interferences from other components". This property should be determined by the analysis of known blank sample materials. No interfering substances should be detected when the method is applied to typical sample materials representative of those which would be submitted for analysis. The method should be able to discriminate the analyte in the presence of potential interfering substances (**selectivity**), such as other drugs which might be expected to be present as residues in typical field samples.

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4 Miller, J.C., & Miller, J.N. (1993) *Statistics for Analytical Chemistry, 3rd Edition*, Ellis Horwood Ltd., Chichester.

5 *Pure & Appl. Chem*., **68**: 1167-1193, 1996

x. The Procedural Manual defines **ruggedness as "the ability of a chemical measurement process to resist changes in results when subjected to minor changes in environmental and procedural variables, laboratories, personnel, etc."] Ruggedness** testing should be conducted using the standard factorial design approach to determine any critical control points[6]. Typical factors to include 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.

xi. **Analyte stability** of standards and during processing of samples should be assessed. Analyte stability 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.

Table 1. Current Performance Criteria for Methods for the Analysis of Residues of Veterinary Drugs in Foods(CAC/GL 16 - Codex Alimentarius, Volume 3)

Concentration	CV(%) **Repeatability** (within laboratory)	CV(%) **Reproducibility** (between laboratories)	Acceptable Range for Accuracy, expressed as % Recovery
≤1 μg/kg	#30	35	50–120
> 1 μg/kg ≤ 0.01 mg/kg	#30	30	60–120
> 0.01 mg/kg ≤ 0.1 mg/kg	#20	20	70–110
> 0.1 mg/kg	#15	15	80–110

24. A revised version of these performance requirements has been suggested in the report of the Miskolc Consultation[2]. These recommendations are summarized in Table 2.

Table 2. Within Laboratory Method Validation Criteria[a] for Analysis of Residues of of Pesticide and Veterinary Drug Residues in Foods, as recommended by Miskolc Consultation[2]

Concentration	**Repeatability**		**Reproducibility**		**Trueness[b,]**
	CV_A% [c]	CV_L%[d]	CV_A% [c]	CV_L%[d]	Range of mean % recovery
≤1 μg/kg	35	36	53	54	50–120
> 1 μg/kg ≤ 0.01 mg/kg	30	32	45	46	60–120
> 0.01 mg/kg ≤ 0.1 mg/kg	20	22	32	34	70–120
> 0.1 mg/kg ≤ 1 mg/kg	15	18	23	25	70–110
> 1 mg/kg	10	14	16	19	70–110

a) With multi-residue methods, there may be certain analytes where these quantitative performance criteria cannot be strictly met. The acceptability of data produced under these conditions will depend on the purpose of the analyses e.g. when checking for MRL compliance the indicated criteria should be fulfilled as far as technically possible, while any data well below the MRL may be acceptable with the higher uncertainty.

b) These recovery ranges are appropriate for multi-residue methods. Stricter criteria may be necessary for some purposes e.g. methods for single analytes or veterinary drug residues (see Table 1).

6 Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the Association of Official Analytical Chemists*, AOAC International, Gaithersburg, VA.

c) CV_A: Coefficient of variation for analysis excluding sample processing. The parameter can be estimated from tests performed with reference materials or analytical portions spiked before extraction. A reference material prepared in the laboratory may be used in the absence of a certified reference material.

d) CVL: Overall coefficient of variation of a laboratory result, allowing up to 10% variability of sample processing.

Minimum validation criteria requirements to address for Level I methods

25. For Level I or confirmatory methods, the performance criteria for Level II methods apply when the Level III method is used as a determinative method. In addition, the following criteria should be considered in establishing the performance of the method for analyte identification:

i. The **specificity** , or **selectivity,** of the method must be established. As noted above, although both the *Procedural Manual* and *Codex Alimentarius Volume 3* currently refer to method **specificity**, CCMAS has recommended **selectivity** as the preferred term in analytical chemistry for this property of a method (CX/MAS 02/5). The method must be able to discriminate unequivocally between true field blanks and sample materials containing the analyte at concentrations within the range of analytical interest. For methods based on spectral determinations, this is typically expressed as the presence of a minimum number of characteristic features in the spectra obtained for an analyte in a sample material as compared with the spectrum obtained for an authentic standard of the analyte. The most commonly used confirmatory methodologies in organic residue analysis are based on low resolution mass spectrometry in combination with gas chromatography or liquid chromatography (GC/MS, LC/MS). Other less commonly used spectral techniques include infrared spectrometry and nuclear magnetic spectroscopy. Typically, a minimum of four identification points are required to meet accepted performance criteria for regulatory methods. Methods based on high resolution mass spectrometry are considered to give a higher reliability through more precise measurement of mass than can be obtained using low resolution mass spectrometry techniques. Method performance requirements for confirmatory methods based on low resolution GC/MS and LC/MS, as recently published by the European Commission[7] are given in Table 3.

Table 3: Performance requirements for relative ion intensities (sample compared to standard) using various mass spectrometric analytical techniques[7].

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 %
< 10%	∀ 50 %	∀ 50 %

ii. **Analyte stability** during analysis must be established for both standards and analyte in the presence of sample material, during processing through the complete analysis. Analyte stability in stored samples should be determined if the conditions of storage studied for the determinative or screening methods are not sufficient to include the conditions under which samples may be stored while awaiting analysis by the confirmatory method.

7 Commission Decision 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, *Official Journal of the European Communities, L221/8*, August 17, 2002.

iii. **Ruggedness testing** may be required if the method differs significantly from the determinative method previously validated (if the method uses different extraction or derivatization procedures than are used in the determinative method).

26. Examples of analytical techniques which may be suitable to meet criteria for confirmatory analytical methods is summarized in Table 4.

Table 4. Examples of detection methods suitable for the confirmatory analysis of substances, as recommended by the Miskolc Consultation[2]

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]
Derivatisation	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.

Statistical considerations in method validation

27. The reliability of a method at the target concentration in sample material, particularly when testing for compliance with a Maximum Residue Limit, is a significant aspect of method validation and may be statistically expressed.

a) The **alpha (∀) error** expresses the probability that the true concentration of analyte in a sample material is less than the defined action level (for example, a Maximum Residue Limit) when results on one or more test portions indicate that the concentration exceed that value (false positive). Accepted values for this probability are usually from 1 to 5%. The **decision limit, CC∀,** is defined as the limit at which the concentration of the analyte truly present in a sample material exceeds that concentration with an error probability of ∀; that is with a statistical probability of 1-∀. For determination of compliance with an MRL, the decision limit would usually be determined with a statistical probability of 95%.

b) The **beta (∃) error** expresses the probability that the true concentration of analyte in a sample material is greater than the action level when measurements made on one or more test portions indicate that the concentration does not exceed that value (false negative). Accepted values for this probability are 1 to 5%. The **detection capability, CC∃,** is defined as the smallest true concentration of the analyte that may be detected, identified and quantified in a sample with an error probability of ∃; that is, with a statistical probability of 1 - ∃. For determination of compliance with an MRL, the detection capability would usually be determined with a statistical probability of 95%.

OTHER CONSIDERATIONS

28. In selecting an analytical method for use in a regulatory program to ensure compliance with MRLs as established by the Codex Alimentarius Commission, laboratories should ensure that the performance of the methods selected meet the performance criteria in Table 1 and the requirements of their clients. It is also expected under ISO-17025 that laboratories will make available to their clients information which provides an

estimate of the uncertainty of the measurement and the estimated result obtained with an analytical method. International guidance on measurement uncertainty is being developed by the International Union of Pure and Applied Chemistry. However, until such a guidance document is finalized, conducting the experiments to assess the criteria described above will provide a laboratory with statistically derived information to provide to clients on method performance and reliability of results.

29. In determining requirements for method validation within a laboratory, the following should be considered:

i. When methods are selected for implementation which have been previously validated through an inter-laboratory collaborative study, a full validation of the method within the laboratory is not required. Rather, the laboratory must demonstrate that the analysts using the method can meet the performance criteria for the method as defined through the collaborative study. Any data on specific issues which are not available from the collaborative study report, such as sample and analyte stability under typical conditions of storage and analysis in the laboratory and regulatory program that it supports, may require additional investigation. Any modifications, substitutions or other variances from the method as collaboratively studied must be fully validated.

ii. Methods which have been validated in one or more other laboratories prior to transfer to a new user laboratory may not require additional validation if a complete validation report is available to the new user. Again, as for implementation of a collaboratively studied method, the laboratory must demonstrate that the analysts using the method can meet the performance criteria for the method established by the original users, as documented in the validation report. Any data on specific issues which are not available from the validation report, such as sample and analyte stability under typical conditions of storage and analysis in the laboratory and regulatory program that it supports, may require additional investigation. Any modifications, substitutions or other variances from the method as originally validated must be fully validated.

iii. Extension of a validated method to additional matrices or analytes should be suitably validated. Issues to address will include analyte stability in the new matrix (or stability of the new analyte), freedom from interferences, plus recovery, precision and linearity of response for determinative methods.

iv. Any modifications or changes to a method after the initial validation should be validated and the results of the re-validation should be documented.

30. Method validation is the beginning of a process, not an end in itself. Laboratories must have in place a quality assurance system which demonstrates that methods and the analysts using them continue to meet the performance criteria established during method validation. Evidence of this may be provided through internal quality assurance activities, including re-analysis of samples, analysis of internal check samples and participation in proficiency programs.

RECOMMENDATIONS:

31. In consideration of the deliberations of the continuing discussions within CCMAS and CCPR regarding single laboratory method validation, the following recommendations be considered by the *Ad hoc* Working Group on Analytical Methods and Sampling:

i. That the new work recommended by the 13th Session of the CCRVDF to review CAC/GL 16-1993, and to prepare revised Draft "Guidelines for the Establishment of a Regulatory Programme for Control of Veterinary Drug Residues in Foods", include a review and revision of "Part II, General Considerations on Analytical Methods for Residue Control", which deal with criteria for selection of suitable analytical methods for use in a regulatory program, to reflect the single laboratory method validation approach. Such an amendment would be based on the contents of this working paper and the on-going work in CCMAS, CCPR and IUPAC.

ii. That the Working Group continue to harmonise procedures for the identification of suitable methods of analysis to support Codex MRLs with those procedures developed by CCMAS and CCPR.

iii. That the Working Group recommend to the Committee (CCRVDF) the basic principle that additional guidance details associated with analytical methods (incorporated in CAC/GL 16-1993 and any successor guidelines) should be, whenever possible, in the form of references to available standards and guidelines elaborated under the auspices of relevant international scientific organisations, such as IUPAC, Iso and AOAC International.

iv. Should the Committee consider that more detailed instructions should be provided to members for implementation of appropriate method validation procedures, the Committee may wish to consider the adoption of a guidance document developed from the recommendations of FAO/IAEA Consultation in Miskolc with respect to the criteria and practical guidance for the validation of analytical methods for residues of veterinary drugs in foods. (A draft document was available to members as a room document at the 13th Session and comments will be considered by the Working Group when it meets prior to the 14th Session. This document is the product of a drafting group assignment (Australia, Canada, Costa Rica, France, Netherlands, United States, COMISA) by the 12th Session of the CCRVDF and can be further developed and finalized by the Working Group if such a document is required, in the opinion of the Committee.)