CODEX ALIMENTARIUS COMMISSION **E**

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 6 **CX/RVDF 10/19/6**
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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON RESIDUES OF VETERINARY DRUGS IN FOODS

Nineteenth Session

Burlington, Vermont, United States of America, 30 August – 3 September 2010

DISCUSSION PAPER ON METHODS OF ANALYSIS FOR RESIDUES OF VETERINARY DRUGS IN FOODS

(Report of the electronic working group preparing a discussion paper containing proposals for the evaluation of analytical methods provided by JECFA and guidance on the development of performance characteristics for multi-residue analysis)

Background

1. At the 18th session of the Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) in Natal Brazil (11 – 15 May 2009), the Committee agreed to establish an electronic working group under the chairmanship of the United Kingdom and Canada. The purpose of the group was to:-

(a) prepare a discussion paper containing proposals for the evaluation of analytical methods provided by JECFA; and

(b) prepare guidance on the development of performance characteristics for multi-residue analysis

for consideration by the 19th session of the CCRVDF.

Proceedings of the Electronic Working Group

2. The Working Group worked primarily by email and comment and document exchange was facilitated by an electronic forum established by the United Kingdom and the reports on the two tasks are given at Annexes I and II respectively. The first draft of each annex was prepared by a small drafting group and each annex was subsequently circulated to the wider working group for comment and revision on three separate occasions. The annexes attached reflect the views of the following countries and organisations:-

(a) United Kingdom, Canada, United States of America, Costa Rica, Brazil, Poland, the Netherlands, Germany, Sweden, Australia, France, IAEA and IFAH.

Recommendations of the evaluation of analytical methods provided by JECFA (Annex I)

3. The Working Group considered the evaluation of analytical methods by JECFA and was content to accept that evaluations were undertaken appropriately by experts. In reaching this conclusion (discussed in detail in Annex I), the Working Group made a number of recommendations for consideration by the Committee. These recommendations are set out below.

(a) It is recommended that future JECFA evaluations use the single laboratory validation (SLV) guidelines adopted at the 32nd session of the CAC.

(b) It is suggested that JECFA may wish to increase the expert representation for analytical method evaluation.

(c) It is suggested that because pharmaceutical companies do not routinely make standards of all marker residues available to analytical laboratories, JECFA may take this into consideration when selecting the marker residue especially for veterinary drugs that are no longer under patent protection and are not commercially available.

(d) *It is recommended that no further expert evaluation of analytical methods recommended by JECFA is required by the CCRVDF.*

(e) *CCRVDF should consider how analytical methods might be made available to regulatory authorities.*

(f) *CCRVDF should consider mechanisms for ensuring the availability of residue control methods for surveillance and monitoring purposes for substances for which JECFA could not establish an ADI/MRL.*

Guidance on the development of performance characteristics for multi-residue analysis

4. Preparation of this annex challenged the Working Group. It very quickly became apparent that the only international guidance on multi-residue analytical methods specifically for residues of veterinary drugs was prepared as a result of the Miskolc consultation in 1999. However, some guidance on these methods as applied to pesticides is available and was used in this paper as considered appropriate. In many areas, technology has developed since these papers were issued and more sophisticated and sensitive methods are commonly available to many laboratories.

5. Therefore, it would be appropriate to consider setting up an Expert Working Group with statisticians, academia, scientists/analysts from regulatory laboratories currently involved in developing such multi-residue methods on today's sophisticated instruments to provide data from their own research to enable appropriate limits to be defined for data generated on today's analytical instruments (instead of relying on 2-3 decade old data generated on previous instrumentation and equipment) to be used as limits for the analytical parameters discussed in this guideline.

6. The Working Group has prepared a draft paper which takes elements of good current international practice and begins to look at multi-residue analytical methods and how performance characteristics might be developed for them.

7. The attached draft at Annex II draws on elements of the following papers:-

- The Miskolc consultation
- CAC/GL 40-1993 (guidelines on good laboratory practice in residue analysis)
- CAC/GL 71-2009 (guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals)
- CAC/GL 72-2009 (guidelines on analytical terminology)
- Method validation and quality control procedures for pesticide residues analysis in food and feed (European Commission Document No. SANCO/10684/2009)

Recommendations on the preparation of guidance on performance characteristics for multi-residue analytical methods (Annex II)

8. The Working Group would like to make the following recommendations to the Committee.

- *Initial work on preparing guidance on performance characteristics should continue.*
- *Guidance should not be aimed at the highest standard available, nor should it be targeted at the standard an average performing laboratory should achieve. Rather, guidance should be developed which is "fit for purpose" and it should be recognised that different performance characteristics may be appropriate for different analytical procedures and techniques.*
- *Set up an expert group of statisticians and analysts/scientists from regulatory laboratories/academia/industry to compile and interpret data generated for multi-residue analytical methods to define and validate analytical ranges/limits as characteristic performance parameters for veterinary drugs included in such multi-residue analytical methods.*

- *The Committee should recognise the importance of linking the development of performance criteria for multi-residue analytical methods with the need to develop validation requirements for such methods, and this should build on existing guidance in CAC/GL 71-2009.*
- *Any guidance developed must not be prescriptive in nature and choices to suit local needs should be included where possible.*
- *An in-session working group should be convened to discuss this paper prior to discussion by the Committee.*

ANNEX I: Proposals for the evaluation of analytical methods provided by JECFA

Executive Summary

- *JECFA evaluates analytical methods and provides assurance that the data upon which the toxicological evaluation and the subsequent determination of an MRL were derived were based on sound science. It is recommended that future JECFA evaluations use the single laboratory validation (SLV) guidelines adopted at the 32nd session of the CAC.*
- *It is recommended that JECFA continues to increase the expert representation for analytical method evaluation.*
- *It is suggested that because pharmaceutical companies do not routinely make standards of all marker residues available to analytical laboratories, JECFA may take this into consideration when selecting the marker residue especially for veterinary drugs that are no longer under patent protection and are not commercially available.*
- *It is recommended that no further expert evaluation of analytical methods recommended by JECFA is required by the CCRVDF.*
- *CCRVDF should consider how analytical methods might be made available to regulatory authorities.*
- *CCRVDF should consider mechanisms for ensuring the availability of residue control methods for surveillance and monitoring purposes for substances for which JECFA could not establish an ADI/MRL.*

UK and Canada, co-chairs of this eWG, gratefully acknowledge the contributions of Australia, Sweden, Costa Rica, UK, Canada, USA, Brazil, France, IAEA and IFAH in preparing this draft document.

Goal 1: ***To review the recent FAO JECFA Publication Monograph 6 (70th Meeting 2008) that details the methods reviewed by JECFA at the 70th Meeting and the WHO Technical Report Series 954 "Evaluation of certain veterinary drug residues in food" 70th Report of the Joint FAO/WHO Expert Committee on Food Additives available at*** http://www.fao.org/docrep/011/i0659e/i0659e00.htm ***to consider whether it can be concluded that JECFA had done its due diligence in making sure that any methods recommended to the CCRVDF to advance the draft MRLs indeed meet the generally accepted criteria of "fit-for-purpose."***

1. At the 18th CCRVDF, it was agreed that JECFA is responsible for analytical method assessment in support of JECFA proposed MRLs. If JECFA is using the new guidelines for single laboratory validation (SLV) as set out in the recently adopted CCRVDF guidelines "Guidelines for the Design and Implementation of National Regulatory Food Safety Assurance Programmes Associated with the Use of Veterinary Drugs in Food Producing Animals" adopted at the 32nd session of the CAC, in addition to anything that is developed in response to the request detailed in paragraph 111 of the 18th CCRVDF meeting report, is there any reason for any further consideration and/or assessment by the CCRVDF? Should we not be making a strong case that one expert consideration which will have the added benefit of speeding up the MRL process is adequate? Appendix 1 below gives a detailed consideration of the assessments undertaken by JECFA in the "Evaluation of JECFA Monograph 6 & WHO Technical Report Series 954: "Evaluation of Certain Veterinary Drug Residues in Food - Seventieth Report of the JECFA."

General Comment and observation on Goal 1:

2. In general, JECFA has done its due diligence, and has determined during its most recent evaluation that, the methods evaluated are "fit-for-purpose" and suitable for routine analysis for compliance for those veterinary drugs. However, it is noted that for dexamethasone the LOQs for the analytical methods provided did not meet the general requirement that the method be capable of detecting and quantifying the analyte at 0.5 x MRL.

Recommendations arising from Goal 1:

3. JECFA Secretariat should continue to ensure that a team assigned to review a veterinary drug (s) includes at least one person with demonstrated expertise in the development and validation of analytical methods for veterinary drugs either through their publications or through their role as a scientist or manager in a residue control laboratory or residue control programme.

4. Additionally, JECFA Secretariat should ensure that any methods recommended by the JECFA process as suitable for routine surveillance/monitoring programmes must contain a section on quality assurance drafted following the SLV guidelines recommended by the CCRVDF which would require each laboratory submitting data for consideration by JECFA to demonstrate expertise and proficiency in the use of the method by generating data that meet the method specification standards.

5. JECFA is urged to work to the new SLV guidelines as agreed upon at the 18th CCRVDF and adopted at the 32nd session of the CAC.

6. If the above recommendations are adopted, then there will be no need to add another expert group to evaluate JECFA's assessment of suitable methods for compliance monitoring.

Goal 2: ***What solutions can be developed to make these validated methods evaluated by JECFA (and determined to be suitable for regulatory compliance of the drugs of interest given the confidential nature of most of the methods assessed by JECFA), easily accessible to all Codex member countries?***

7. In the USA and Canada analytical methods for regulatory control of veterinary drugs used in food animal production are considered public information. Certain considerations regarding confidentiality issues become necessary once commercial laboratories are involved. Controls are, however, carried out only in federal Government laboratories or Private laboratories under contract with the Government. Drug manufacturing companies (Sponsors) are obliged to make available any standards or unusual reagents required for the analysis of the drug that has been licensed and approved for use in food animal production.

8. The position is a little different in the EU. The European Medicines Agency (EMA) is responsible for the provision of analytical methods submitted in support of an MRL application to national Competent Authorities (CAs). The CAs are responsible for provision of the method to official control laboratories. Therefore, a significant number of analytical methods and information on those methods is already being exchanged, shared and distributed, and the industry is open to any system that ensures appropriate use of the

information for monitoring purposes. Where residue analyses for regulatory control are carried out by contract laboratories, provision of a contract with a government specifying the envisaged number of analyses could form the basis of access to the method and any required standards. However, it must be acknowledged that, in some cases where contract laboratories are used, it may be difficult or even impossible because of confidential reasons to make their methods available for general distribution.

9. Pharmaceutical companies may provide National Authorities with analytical standards of marker residues where these are the parent veterinary drug. However, supplies of metabolites or derivatives as marker residues may be difficult to obtain, making effective residue monitoring challenging. As risk managers, CCRVDF must consider ways for encouraging the provision of reference standards for JECFA recommended marker residues when evaluating drugs for Codex MRLs.

10. In both the USA and the EU, analytical methods provided by industry (sponsor) are those developed specifically for the tissue depletion studies as part of the requirement for drug registration and approval. In the EU, both Community Reference Laboratories (CRLs) and National Reference Laboratories (NRLs) develop routine screening, quantitative and confirmatory methods required for compliance monitoring, as necessary. These methods, which are generally multi-residue in nature and applicable to several different matrices, are developed for effective use of laboratory resources and to ensure short turn around times for reporting results. Such methods may address the needs of Codex countries better than the methods developed by industry, which by necessity, focus only on the analysis of the marker residue in an appropriate target tissue for a given MRL application.

11. Providing proprietary information on analytical methods to national laboratories is the responsibility of national governments, not the CCRVDF.

12. For substances for which JECFA could not set an ADI/MRL but for which there is a need to control residues, CCRVDF may consider establishing a mechanism to ensure the availability of suitable analytical methods.

Recommendations arising from Goal 2:

13. National Authorities should ensure that contractor laboratories providing analytical services operate to acceptable international standards.

14. Consideration should be given to the possibility of seeking the agreement of residue laboratories around the world with similar mandates to share their methods with the global or Codex country control laboratory community.

15. CCRVDF should prepare a database listing national Competent Authority contacts for authorised veterinary drugs with MRLs where routine residue control programmes are in place. This will give countries wishing to undertake residue control programmes a first “point-of-contact” for the provision of practical analytical methods used in routine residue control programmes and allow them to select an appropriate method best suited to their expertise and capabilities.

16. When selecting the marker residues for MRLs, JECFA must be aware of the potential difficulty regulatory authorities and laboratories may have in obtaining marker residues which are not the parent compound.

17. CCRVDF should urge the veterinary pharmaceutical companies to make readily available to competent authorities in member countries standards of all marker residues for veterinary drugs with Codex MRLs. Consideration must be given by the CCRVDF to making this a requirement of setting an MRL in order to better facilitate trade and ensure consumer protection.

18. For substances for which JECFA could not set an ADI/MRL but for which there is a need to control for residues, CCRVDF may consider establishing a mechanism to ensure the availability of suitable analytical methods.

APPENDIX 1

The eWG's review of the "Evaluation of JECFA Monograph 6 & WHO Technical Report Series 954: Evaluation of Certain Veterinary Drug residues in Food - Seventieth Report of the JECFA"

Avilamycin – Evaluated by Dr. Adriana Fernadez Suarez (ARG), Dr. Bruno Le Bizec (FR) & Dr. Richard Ellis (USA).

The authors acknowledged that validated LC-MS/MS methods (Eichmeir *et al*. 2006b) for the determination of avilamycin in pig fat, muscle, kidney, and liver, for chicken tissues (Eichmeir *et al*., 2006a), turkey tissues (Eichmeir *et al*., 2006c) and rabbit tissues (Eichmeir *et al*, 2006d) that measure the marker residue dichloroisoeverninic acid (DIA) were available and assessed them against the validation criteria with respect to selectivity, linearity, accuracy, recovery, repeatability, robustness, limits of quantification, limits of detection, and stability. It was concluded that even though the Limits of Quantification (LOQs) for avilamycin in the different matrices had to be re-calculated, the submitted method was satisfactory for the quantitative analysis of avilamycin in pig, turkey, rabbit and chicken tissues.

The recommended method has an LOQ of about 1/10th the recommended MRL expressed as DIA. The developer calculated LOQs were 150µg DIA/kg for liver, 100µg DIA/kg for kidney, 50µg DIA/kg for skin/fat, and 25µg DIA/kg for muscle (based on acceptable accuracy and precision) were more in line with proposed MRLs than estimates by reviewers based on signal-to-noise determinations.

The expert reviewers commented that because of the relatively complex LC-MS/MS instrumentation that may not be available in all regulatory laboratories, it may be necessary to use alternative methods in those situations where the equipment is unavailable.

	MRL (µg/kg)			
	Muscle (LOQ)	Kidney (LOQ)	Liver (LOQ)	Skin/Fat (LOQ)
Pigs	200 (24)	200 (3.3)	300 (10)	200 (22.4)
Turkeys	200 (18.4)	200 (22.4)	300 (30.4)	200 (18.7)
Chicken	200 (18.4)	200 (22.4)	300 (30.4)	200 (18.7)
Rabbits	200 (18.4)	200 (22.4)	300 (30.4)	200 (18.7)

[Ref: Eichmeir, L. S. (2006) Validation of an HPLC-MS/MS method for the determination of avilamycin in chicken liver, kidney, muscle and fat/skin. Report No. 49783, ABC Laboratories Inc. Columbia, MO USA (ABC Method 49783-MI Sponsor submitted)]

[Ref: Eichmeir, L. S. (2006) Validation of an HPLC-MS/MS method for the determination of avilamycin in chicken liver, kidney, muscle and fat/skin. Report No. 49784, ABC Laboratories Inc. Columbia, MO USA (ABC Method 49784-MI-01 Sponsor submitted)]

Dexamethasone – Evaluated by Dr. Bruno Le Bizec (FR).

Dexamethasone was evaluated at the 42nd JECFA and temporary MRLs expressed as parent drug (marker residue) based on ADI of 0 – 0.015 µg/kg bw were recommended for cattle and pigs because an adequate method to determine compliance with the MRL was not available. The same MRLs were recommended for horses at the 43rd JECFA. At the 17th CCRVDF, a request for MRL recommendations in cattle (tissues and milk) and pig tissues was raised.

Three methods were provided for assessment by JECFA:

(1) an LC-MS/MS (ESI-) for the determination of Dexamethasone residues in bovine liver (Method 1);

[Ref: National Food Administration, SLV k1-f2-v321 (2008-03-27 BGOS) Validation of the method: analysis of glucocorticosteroids Dexamethasone, Betamethasone, Flumethasone,

Prednisolone, and 6 –methylprednisolone in bovine liver using LC-MS/MS. (Submitted to FAO by the National Food Administration, Uppsala, Sweden)]

(2) an LC-MS/MS (ESI-) for the determination of Dexamethasone residues in bovine milk (Method 2);

[Ref: McDonald, M., Granelli, K., and Sjöberg, P (2007). Rapid multi-residue method for the quantitative determination and confirmation of glucocorticosteroids in bovine milk using LC-tandem mass spectrometry, Anal. Chim. Acta 588, 20-25].

(3) An LC-MS/MS (ESI+) for the determination of Dexamethasone residues in bovine muscle and kidney (Method 3).

[Ref: Boison, J., Fedeniuk, R., and Chrush, J (2008) A determinative and confirmatory method for 29 antibiotic residues in bovine muscle tissues by LC-tandem mass spectrometry. Project No. SF0103. Improved test capability for banned substances in food of animal origin. Submitted to FAO by the Canadian Food Inspection Agency, Saskatoon Laboratory, Centre for Veterinary Drug Residues, Canada]

The JECFA expert assessed the suitability of the submitted methods to measure Dexamethasone at the previously defined temporary MRL of 0.3 µg/L for milk, 0.5 µg/kg for bovine muscle and kidney and 2.5 µg/kg for bovine liver.

It was concluded after reviewing the submitted analytical parameters for selectivity, sensitivity, accuracy, precision, recovery, robustness and stability of analytes that:

(a) a suitable validated routine method is available for monitoring dexamethasone residues in bovine milk at 0.3µg/L (Method 2);

(b) a suitable validated routine method is available for monitoring dexamethasone residues in bovine liver at 2.0 µg/kg (Method 1);

(c) A suitable validated routine method is available for monitoring dexamethasone residues in bovine kidney and muscle tissues at 1.0 µg/kg instead of the temporary recommended MRL of 0.5 µg/kg (Method 3).

	Temporary MRL (CAC) (µg/kg)	Method 1 (µg/kg)	Method 2 (µg/L)	Method 3 (µg/kg)
Bovine Kidney	0.5			1.0
Bovine Liver	2.5	2.0		
Bovine Muscle	0.5			1.0
Bovine Milk	0.3		0.3	

The appropriate target tissues are liver or kidney and milk;

For Dexamethasone

- **the LOQ for the bovine liver method of 2.0 µg/kg is only 4/5th (not ½) of the Committee recommended temporary MRL of 2.5 µg/kg;**
- **the LOQ for the bovine milk method of 0.3 µg/L is equal (not ½) to the Committee recommended temporary MRL of 0.3 µg/L;**
- **the LOQ for the bovine kidney method of 1.0 µg/kg is twice (not ½) the Committee recommended temporary MRL of 0.5 µg/kg;**

NOTE: There were still no validated methods for horses and pigs but the expert noted that the method provided for cattle is adequate to be extended to pig and horse tissues.

Malachite Green (MG) – Evaluated by Dr. Bruno Le Bizec (FR), Dr. Dieter Arnold (GER) & Dr. Richard Ellis (USA).

A comprehensive analysis of approaches to the analysis of MG and leucomalachite green (LMG) are presented by the authors but there are no indications that any particular methods were reviewed for suitability/appropriateness for compliance with the exception of a statement made under a discussion of the kinetics of the depletion of MG in fish that a validated method by Andersen et al., (2006) was used to measure the sum of the concentrations of MG and LMG in MG-treated salmon. The authors identified several screening, quantitative and confirmatory methods with defined performance characteristics, but except in cases where it was noted that the sensitivity requirements exceeded the EU MRPL, there were no definite conclusions reached as to the suitability of any of the methods for the regulatory control of MG and LMG.

[*Ref: Andersen, Et. al., (2006). Quantitative and confirmatory analyses of malachite green and leucomalachite green residues in fish and shrimp. J. Agric Food Chem., 54, 4517-4523]*

Monensin – Evaluated by Dr. Lynn Friedlander (USA) & Dr. Pascal Sanders (FR).

The authors indicated in a comprehensive review that several methods had been used to generate depletion data, but summarized the method performance characteristics for 2 newly developed and validated methods which were used to estimate the MRLs for monensin in foods of animal origin one using post-column derivatization and the other mass spectrometric detection as suitable for routine monitoring:

(a) For cattle, the MRL was estimated from unlabeled drug depletion studies conducted with a validated HPLC method with post-column derivatization;

[*Ref: Anon (undated) Analytical method AM-AA-CR-R152-AA-791 Determination of monensin in chicken tissues by HPLC using post-column derivatization. Sponsor submitted*]

(b) For chicken and turkey, the MRL was estimated from unlabeled drug depletion studies conducted with a validated HPLC-MS/MS method;

[*Ref: Cordroc'h, S. (2007). Non-clinical laboratory study (GLP): Validation of an HPLC-MS/MS method for the assay of monensin A in bovine muscle, liver, kidney, fat and milk. Unpublished study No A061485 from Avogadro, Fontnenilles, France, Elanco Animal Health, a division of Eli Lily and Company. Greenfield, IN. Sponsor submitted*]

(c) For cow's milk, the MRL was estimated from unlabeled drug depletion studies conducted using the validated HPLC-MS/MS method.

[*Ref: Cordroc'h, S. (2007). Non-clinical laboratory study (GLP): Validation of an HPLC-MS/MS method for the assay of monensin A in bovine muscle, liver, kidney, fat and milk. Unpublished study No A061485 from Avogadro, Fontnenilles, France, Elanco Animal Health, a division of Eli Lily and Company. Greenfield, IN. Sponsor submitted*]

The LOQ (based on the lowest standard of standard curve) for the validated method for cow's milk was 1/8th the recommended MRL.

Narasin – Evaluated by Dr. Betty San Martin (CHILE) & Dr. Lynn Friedlander (USA).

The authors noted that several methods had been used in both non-GLP and GLP compliant studies to generate residue depletion data for narasin. They cited specifically 5 validated methods - two HPLC/UV methods with post column derivatization and three HPLC-MS/MS methods that they claim can be considered suitable for routine monitoring of narasin drug residues in chicken and pig tissues. Even though the methods had not been validated for cattle tissues, the Committee recommended the same MRLs as temporary for cattle. In their assessment, liver or fat (where available) is suggested as the target tissue and the parent drug as the marker residue.

The validated HPLC/UV method had an LOQ of 25 µg/kg which was ½ the recommended MRL for chicken or pig liver (or fat) of 50 µg/kg.

[*Ref: Method 1. Lacoste, E and Larvor, A (2003). Residue study in edible tissues of broiler chickens fed with narasin at 80 ppm for five consecutive days. European Animal Science Research. Elanco Animal Health, Division of Eli Lily and Company, Report No T2NAFR0103. Sponsor submitted*].

The validated HPLC/UV method had an LOQ of 7 µg/kg which was ½ (0.5 times) the recommended MRL for chicken of pig muscle and kidney of 15 µg/kg

[*Ref: Method 2. Ward, TL., Moran, JW., Turner, JM., and Coleman, MR. (2005). Validation of a method for the determination of narasin in edible tissues of chickens by liquid chromatography. J. AOAC Intl. 88, 95-101.*

It is surprising to note that while both HPLC/UV methods were validated for skin/fat, muscle, liver and kidney, the authors chose to define the MRL for liver on one method and that for kidney and muscle on another method without any justification for the choice of method. In addition, even though the authors noted that mass spectrometric methods of detection and confirmation provided good specificity and sensitivity, their LOQs (reported at 1 µg/kg for liver and eggs for one of them) were not considered at all in the determination of the MRLs.

Note: A validated regulatory method with all its performance characteristics will be required to be submitted to JECFA before the end of 2010 before re-evaluation to propose permanent MRLs for narasin residue in cattle can be undertaken by JECFA.

Tilmicosin – Evaluated by Dr. Shixin Xu (PRC) & Dr. Dieter Arnold (GER).

A very short section on methods of analysis which identified a validated HPLC/UV method for chicken and turkey tissues with an LOQ of 60 µg/kg for liver and kidney and 25 µg/kg for muscle and fat.

[*Ref: Lily Method B04228 rev 7. Lily Laboratory Procedure for Method B04228 revision 7. Determination of tilmicosin residues in chicken, swine, cattle, and sheep edible tissues by HPLC. Sponsor submitted]*

[Ref: Hawthorne, P (1999). Validation of an analytical method for the determination of tilmicosin residues in turkey liver, kidney, muscle and skin/fat samples. Unpublished Study No CEMS-1035 CEM Analytical Services, Berkshire, England for Elanco Animal Science Research, Lily Industries limited, Basingstoke, UK., Sponsor submitted]

A validated LC-MS/MS method with an LOQ of 25 µg/kg was provided for evaluation.

[*Ref: McCracken, B. (2007). Validation of the analytical method, "Method of analysis for the determination of tilmicosin in whole chicken by LC-MS/MS", Study P0002796 includes Appendix I Analytical Method V003516, Method of analysis for the determination of tilmicosin in whole chicken eggs by LC-MS/MS from MPI Research Inc., State College, PA, USA. Sponsor submitted*].

For tilmicosin, the data from the marker residue study enabled the MRLs for chicken and turkey to be calculated on the basis of the upper one-sided 95% confidence limits over the 95th percentile of residue concentrations.

	MRLs (µg/kg) for Tilmicosin			
	Muscle (LOQ)	Kidney (LOQ)	Liver (LOQ)	Skin/Fat (LOQ)
Chicken	150 (25)	600 (60)	2400 (60)	250 (25)
Turkey	100 (25)	1200 (60)	1400 (60)	250 (25)
Chicken egg	Validated LC-MS/MS method with LOQ of 25 µg/kg			
Bovine and Sheep milk	Validated LC/UV method with an LOQ of 10 µg/kg			

Triclabendazole – Evaluated by Dr. Philip Reeves (AUS) & Dr. Gerald Swan (SA).

The 17th CCRVDF requested JECFA to re-evaluate the MRLs for triclabendazole in cattle and sheep. While no new PK or metabolism studies were submitted for evaluation, 3 new residue studies in cattle using a pour-on-formulation were submitted. The Committee recommended that:

(a) the marker residue is the sum of all residues extracted and converted to keto-triclabendazole;

(b) liver and muscle are suitable target tissue;

(c) MRLs for liver, kidney and muscle from cattle and sheep were calculated from the upper one-sided 95% confidence limit over the 95th percentile of the residues of keto-triclabendazole on day 28;

(d) **The LOQ for the validated method was ½ the recommended MRL for fat**;

(e) The following analytical methods were considered to be suitable for the routine analysis of triclabendazole residues in cattle and sheep:

[*Ref: Adams, S (2004b) Validation of analytical procedure no 193.F00. Tissue residue of triclabendazole, measured as CGA 110754, in cattle following repeated oral dosing with Fasinex 10%, Novartis Animal Health Australasia Pty ltd., Report No 04/02/1875, Study Y03/49*

Adams, S (2004c). Validation of analytical procedure no 193.F00. Determination of residues of triclabendazole in animal tissues by HPLC. Novartis Animal Health Australasia Pty ltd., Report No TR 04/05/1886, Study V03/57.

Adams, S (2005). Extended validation of analytical procedure no 193.F00. for sheep and cattle tissues. Novartis Animal Health Australasia Pty ltd., Report No TR 05/06/1945.

Dieterle, R., Kissling, M (1995). Validation of method REM 15/83: Determination of common moiety CGA 110754 in muscle, liver, kidney and fat of cattle as well as in muscle and liver of sheep after administration of 14C-CGA 89317 by HPLC. Ciba-Geigy Report on special study 132/94.

Giannone, C. (1983). Determination of total residues in tissues and fat of sheep and cattle. REM 3-38. Ciba-Geigy Limited.

Giannone, C., and Formica, G (1983). Determination of total residues in tissues and fat. REM 15/83. Ciba-Geigy Limited.

Study No AA031 (2001), Determination of tissue residues following treatment of cattle with an abamectin/triclabendazole pour-on formulation. Sponsor: M. Forster, Ancare NZ Limited. Study Director, B. Chick, Veterinary Health Research Pty Ltd., West Armidale, Australia, Laboratory Amdel New Zealand Ltd., Auckland, NZ.

Study No ANT1274 (2002). Determination of tissue residues in beef cattle following administration of an abamectin and triclabendazole pour-on formulation. Sponsor: M. McArthur, Ancare NZ Limited. Study Director, M. Chambers, Veterinary Health Research Pty Ltd., West Armidale, Australia, Laboratory: D. Hennessy, VHR Analytical Laboratory, North Ryde, Australia.

	MRL (µg/kg)			
	Liver (LOQ)	Kidney (LOQ)	Muscle (LOQ)	Fat (LOQ)
Cattle	850 (74)	400 (58)	250 (36)	100 (20)
Sheep	300 (24)	200 (34)	200 (41)	100 (42)

Tylosin – Evaluated by Dr. Jack Lewicki (POL), Dr. Philip Reeves (AUS) & Dr. Gerald Swan (SA).

The authors acknowledged that besides the data generated from radiolabeled studies, several HPLC/UV or HPLC-MS/MS methods for Tylosin A were submitted for review by JECFA. JECFA only evaluated methods which were fully validated for tylosin. This method described by Roberts "LC-MS/MS method for the determination of Tylosin A residues in edible tissues of chickens and in eggs with an LOQ of 50 g/kg for

liver, kidney, muscle and skin/fat" and 100 µg/kg for eggs was the only one considered to be suitable for the routine analysis of tylosin A residues in edible tissues of chickens and in eggs.

[Ref: *Roberts, S (2007). Validation of an analytical method for the determination of tylosin in chicken liver, kidney, muscle, skin with fat and in eggs. Analytical method No 1610. Study No 211608. Charles River Laboratories, Tranent, Edinburgh, UK., Sponsor submitted*]

The authors commented that this method could easily be extended to other matrices and is a suitable method for regulatory analysis of tylosin A in edible tissues of cattle, pigs, chicken, milk and in eggs.

The authors then also considered several validated methods published in the open literature for the analysis of tylosin residues in:-

animal feed [*Ref: Peng, Z., & Bang-Ce, Y (2006). Small molecule micro arrays for drug residue detection in feedstuffs. J. Agric Food Chem., 54, 6978-6983. Gonzalez de la Huebra et al (2007). Sample preparation strategy for the simultaneous determination of macrolides antibiotics in animal feeding stuffs by liquid chromatography with electrochemical detection (HPLC-ELCD). J. Pharm. Biomed. Anal., 43, 1628-1637.; Vincent et. al., (2007). Validation of an analytical method for the determination of spiramycin, virginiamycin and tylosin in feeding stuffs by TLC and bioautography. J Food Add. Contam. , 24, 351-359*],

biological fluids and animal tissues [*Ref: Garcia-Mayor et. al., (2006). Liquid chromatography UV diode array detection method for multi-residue determination of macrolides antibiotics in sheep's milk. J. Chromatogr. A., 1122, 76-83.; Tang et. al., (2006). High throughput screening for multi-class veterinary drug residues in animal muscle using LC-tandem mass spectrometry with on-line solid phase extraction. Rapid Commun. Mass Spectrometry 20, 2565-2572.; Wang et. al., (2006). Determination of five macrolides antibiotic residues in raw milk using LC-electrospray ionization tandem mass spectrometry. J. Agric Food Chem., 54, 2873-2880*],

honey [*Ref: Wang, J. (2004). Determination of five macrolides antibiotic residues in honey by LC-ESI-MS and LC-ESI-MS/MS. J. Agric Food Chem., 52, 171-181; Benetti, C., et. al., (2004). Unauthorised antibiotic treatments in beekeeping. Development and validation of a method to quantify and confirm tylosin residues in honey using LC-tandem mass spectrometric detection. Anal. Chim. Acta, 520, 87-92.; Caldow, M., et. al., (2005). Development and validation of an optical SPR biosensor assay for tylosin residues in honey. J. Agric Food Chem., 53, 7367-7370.; Thompson, TS., et. al., (2005). Determination of lincomycin and tylosin residues in honey by LC-tandem mass spectrometry. Rapid Commun. Mass Spectrometry., 19, 309-316.; Nalda, MJN., et. al., (2006). Trace analysis of antibacterial tylosin A, B, C and D in honey by LC-electrospray ionization-mass spectrometry. J. Sep. Sci. 29, 405-413.; Thompson, TS., et. al., (2007). Degradation of incurred tylosin to desmycosin – implications for residue analysis for honey. Anal. Chim. Acta, 586, 304-311.; Hammel, YA., et. al., (2008). Multi-screening approach to monitor and quantify 42 antibiotic residues in honey by LC-tandem mass spectrometry. J. Chromatogr. A., 1177, 58-76.*] but made no comments as to their suitability or otherwise for compliance monitoring.

The validated methods had an LOQ of 50 g/kg which is ½ the recommended MRLs for milk and animal tissues.

	MRLs (µg/kg)					
	Kidney (LOQ)	Liver (LOQ)	Muscle (LOQ)	Skin/Fat (LOQ)	Milk (LOQ)	Eggs (LOQ)
Cattle	100 (50)	100 (50)	100 (50)	100 (50)	100 (50)	
Pig	100 (50)	100 (50)	100 (50)	100 (50)		
Chicken	100 (50)	100 (50)	100 (50)	100 (50)		300 (100)

ANNEX II: DRAFT CCRVDF GUIDELINES FOR THE DEVELOPMENT OF PERFORMANCE CHARACTERISTICS FOR MULTI-RESIDUE ANALYSIS.

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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 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 performance characteristics for multi-residue analytical methods. 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 of a veterinary drug of interest or concern (screening methods), quantifying how much is present (quantitative methods), and providing unequivocal identification (confirmatory methods) of the drug. 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 permitted, banned or prohibited 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 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, use methods of analysis 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 validation methods.

6. Validated analytical methods are methods with defined characteristic operational parameters which have been determined (subjected to independent assessment) to be "fit-for-purpose" in a regulatory environment. This guidance document examines the attributes of analytical methods used for a range of substances 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.

Scope

7. Whilst some residue control programmes may include additional analyte groups such as pesticides and environmental contaminants, this guidance has been prepared specifically to cover only veterinary drug residues.

8. 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 multi-residue analytical methods. Multi-residue analytical methods are methods which can be used for the detection of multiple analytes of the same or different classes. For the purposes of this document, a multi-residue analytical method 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 methods 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.

9. The principles described in this section are considered practical and suitable for the determination of the performance characteristics of multi-residue analytical methods 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 (http://www.iaea.org/programmes/rifa/trc/pest-qa_val_guide.pdf and in 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 to evaluate during method validation whether the method is suitable ("fit-for-purpose") for use in a regulatory environment. For the reasons above, the information in Table 5 below (which resulted from the Miskolc consultation in 1999 http://www.iaea.org/trc/pest-qa_val_annex2.pdf) has been retained intact as it is the most recent comprehensive guidance relevant to the work of this group on residues of veterinary drug residues, being important for both analytical method validation and derivation of performance characteristics.

10. Guidance 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 multi-residue analytical methods primarily for confirmatory analyses but also addresses multi-residue screening analytical methods using mass spectrometry. Aspects of SANCO/10684/2009 have been adopted into this document where appropriate.

Performance characteristics for analytical methods

Performance characteristics of screening methods

[*NOTE: 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*]

11. Screening methods 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 below a detection concentration or sensitivity ("negatives") from those that may contain residues above that value ("positives"). The validation strategy therefore focuses on establishing a detection 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.

12. The detection concentration for the test for a particular compound is established by conducting concentration-response experiments, typically using 30 replicates (from at least six sources) spiked at each of a series of increasing concentrations. Once the concentrations have been established where all 30 replicates give a negative response and all 30 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).[1]

13. For screening tests, particularly those involving test kit technologies, the term "*detection concentration or detection sensitivity*" refers 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, it is required that in order to meet the minimum requirement for sensitivity at 90% incidence with 95% confidence, a minimum of 30 residue-free sample materials (preferably taken from no less than six

[1] Finney, D.J. 1978. *Statistical method in biological assay*. 3rd edition. New York, USA, MacMillan Publishing Co.

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 on screening tests (http://ec.europa.eu/food/food/chemicalsafety/residues/Guideline_Validation_Screening_en.pdf) may also be used.

14. The "*selectivity*" of a screening method refers to the ability of the test to determine that samples that give a negative response are truly negative. The test must also be able to distinguish the presence of the target compound, or group of compounds, from other substances that may be present in the sample material. It is normally not 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 compounds. These methods, which generally fit into the screening methods category, are often based on microbiological growth inhibition, immunoassays or chromogenic responses that may not unambiguously identify a compound. The selectivity of a 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 screening tests), 60 replicate analyses are conducted on representative blank sample matrix materials from a minimum of six different sources. All results should be negative. Additional tests for potential interferences and cross-reactivity may then be conducted by testing blank matrix material spiked with potential interfering substances, 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.

Performance characteristics for quantitative methods

15. *Selectivity*, 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, is of particular importance in defining the performance characteristics of methods used in regulatory control programmes for veterinary drug residues in foods. There are two aspects that must be considered – the ability of the method to provide a signal response that is free from interferences from other compounds that may be present in a sample or sample extract, and the ability of the method to identify unequivocally a signal response as being exclusively related to a specific compound. For a quantitative method, the requirement is that the signal used for quantification should relate only to the target analyte and not contain contributions for co-extracted materials. 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.

[NOTE: Request data to be compiled and reviewed to determine whether the 5 % (False Positive/False Negative criteria) for single analyte methods can safely be extended to multi-residue methods].

16. 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:

- 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*). The precision data can be used for calculation of propagation of experimental error (i.e., measurement uncertainty, MU) for the method.

17. It is recommended that methods used to support Codex MRLs should meet the performance standards for trueness and precision listed in Table 1 *[subject to verification after compiling and reviewing a large database of current data that the limits are still useful]*, where CV_A refers to the coefficient of variation

determined by test portions of blank matrix spiked prior to extraction and CV_L is the overall laboratory variability, which includes a 10 percent estimate for variability of sample processing.[2]

18. 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 (typically, a collaboratively studied method) or, in the absence of reference materials or methods validated by inter-laboratory trial, by determination of the *recovery* of analyte spiked into known blank sample material. The determination of accuracy as recovery is frequently used in validation of methods for veterinary drug residues in foods, as both certified reference materials and methods validated by inter-laboratory trial are often not available. The accuracy of a measurement is closely related to *systematic error* (analytical method bias) and analyte recovery (measured as percent recovery). The accuracy requirements of methods will vary depending upon the planned regulatory use of the results. The accuracy should be carefully characterized at concentrations near 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.

19. *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. At relatively high concentrations, analytical recoveries are expected to approach 100 percent. At lower concentrations, particularly with methods involving extensive extraction, isolation and concentration steps, recoveries may be lower. 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).

20. *Precision*, which quantifies the variation between replicated measurements on test portions from the same sample material, is also an important consideration 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, or coefficient of variation (the standard deviation divided by the absolute value of the arithmetic mean). It may be reported as a percentage by multiplying by 100.

> *[NOTE: Data should be requested, compiled and interpreted to determine whether the current recovery ranges as well as ranges for trueness acceptability represented in Table 1 are still acceptable or need to be revised significantly.]*

21. 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 curve should first be determined to assess the detector response to standards over a range of concentrations. These concentrations (a minimum of five, plus blank) should cover the full range of analytical interest and the resultant curve should be statistically expressed. However, although it is recommended 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

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[2] Alder, L., Holland, P.T., Lantos, J., Lee, M., MacNeil, J.D., O'Rangers, J., van Zoonen, P. & Ambrus, A. 2000. *Guidelines for single-laboratory validation of analytical methods for trace-level concentrations of organic chemicals* (available at http://www.iaea.org/trc/pest-qa_val2.htm).

concentrations throughout the range of analytical interest. For analytes for which an 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 concentration above and below the MRL (use of six different sources of blank materials is recommended).

22. 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 *linearity* 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. It is typically determined from a linear regression analysis of the data if the data has been tested and found to meet the requirements for linear regression. 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 prior to analyte extraction at a range of appropriate concentrations that bracket the target value (the analytical function). Use of such a "tissue standard curve" for calibration incorporates a recovery correction into the analytical results obtained.

23. It is also 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)* may be described in practical terms as the lowest concentration where the analyte can be detected (but may not be identified/confirmed) in a sample. It can be estimated using the standard deviation ($s_{y/x}$) from the linear regression analysis of the standard curve generated in the analytical function experiment described above.[3] Using this approach, the limit of detection is calculated using the y-intercept (assuming a positive value) of the 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.

24. 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 (subject to revision) 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 an 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. For use in a regulatory programme, the limits of detection and quantification 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, or when conducting residue analyses for substances that do not have ADIs or MRLs. For monitoring compliance with an MRL, it is important that an 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".

25. 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 should be accepted as an alternative to using LOD and LOQ.

Performance characteristics for confirmatory methods

26. When analyses are performed for monitoring or enforcement purposes, it is especially important that confirmatory data are generated before reporting on samples containing residues of veterinary drugs that are not normally associated with that commodity, or where MRLs appear to have been exceeded. Samples may

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[3] Miller, J.C. & Miller, J.N. 1993. *Statistics for analytical chemistry*. 3rd Edition. Chichester, UK, Ellis Horwood Ltd.

contain interfering chemicals that may be misidentified as veterinary drugs. As a first step, the analysis should be repeated using the same method, if only one portion was analysed initially. This will provide evidence of the repeatability of the result, if the residue is confirmed. It should be noted that the only evidence supporting the absence of detectable residues is provided by the system performance suitability data run concurrently with the sample of interest.

27. Confirmatory tests may be quantitative and/or qualitative but, in most cases, both types of information will be required. Particular problems occur when residues must be confirmed at or about the limit of quantification but, although it is difficult to quantify residues at this concentration, it is essential to provide adequate confirmation of both concentration and identity.

28. The need for confirmatory tests may depend upon the type of sample or its known history. In some commodities, certain residues are frequently found. For a series of samples of similar origin, which contain residues of the same veterinary drug, it may be sufficient to confirm the identity of residues in a small proportion of the samples selected randomly. Where "blank" samples are available, these should be used to check the occurrence of possible interfering substances in the species/matrix of interest.

29. Depending upon the initial technique of determination, an alternative procedure which may be a different detection technique, may be necessary for verification of quantity. For qualitative confirmation (identity) the use of mass-spectral data, or a combination of techniques based on different physico-chemical properties, is desirable (see Table 3).

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 compounds. The technique(s) chosen depend(s) upon the availability of suitable apparatus and expertise within the testing laboratory. Some alternative procedures for confirmation are given in Table 3.

31. *Selectivity*, the ability of the method to identify unequivocally a signal response as being exclusively related to a specific compound, 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. Typically, a minimum of four identification points is required to meet accepted performance criteria for regulatory methods. However, confidence in the identification will increase with a greater number of identification points and some laboratories may choose to use more than the minimum of four. Table 1a and 1b give the identification point scheme published in European Commission Decision 2002/657/EC. 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 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,[4] are given in Table 2 [subject to review]

[Data may be requested, compiled and considered to determine whether the limits defined in Table 3 are still acceptable and appropriate.]

33. It is considered that one identification point should be assigned to each structurally significant ion fragment detected using a low-resolution mass spectrometric method. When a tandem low-resolution instrument, such as a "triple quadrupole" mass spectrometer 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 identification points. A combination of a precursor ion and two product transition ions provides the four required identification points when low-resolution MS/MS instruments are used in a confirmatory method.

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[4] Bethem, R., Boison, J.O., Gale, J., Heller, D., Lehotay, S., Loo, J., Musser, S., Price, P. & Stein, S. 2003. Establishing the fitness for purpose of mass spectrometric methods. *Journal of the American Society for Mass Spectrometry*, 14(5): 528–541.

34. Additional confidence is provided when high-resolution mass spectrometers are used in a confirmatory method, as the high resolution provides more precise 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 2 identification points, while product transition ions generated in high-resolution MS/MS experiments are assigned an identification point value of 2.5 each. In addition, 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.

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

- 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.

36. 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.[5] 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. Examples of analytical techniques that may be suitable to meet criteria for confirmatory analytical methods are summarized in Table 3.

37. Derivatisation can also be used for confirmation of veterinary drug residues and may be considered under three broad headings.

(a) Chemical reactions

Small-scale chemical reactions resulting in degradation, addition or condensation products of veterinary drugs, followed by re-examination of the products by chromatographic techniques, have frequently been used. The reactions result in products possessing different retention times and/or detector response from those of the parent compound. A sample of standard veterinary drug should be treated alongside the suspected residue so that the results from each maybe directly compared. A spiked extract should also be included to prove that the reaction has proceeded in the presence of sample material. Interference may occur where derivatives are detected by means of properties of the derivatising reagent. Chemical reactions have the advantages of being fast and easy to carry out, but specialised reagents may need to be purchased and/or purified.

(b) Physical reactions

A useful technique for a limited number of veterinary drugs is the photochemical alteration of a residue to give one or more products with a reproducible chromatographic pattern. A sample of standard veterinary drug and spiked extract should always be treated in a similar manner. Samples containing more than one veterinary drug residue may give problems in the interpretation of results. In such cases pre-separation of specific residues may be carried out using TLC, HPLC or column fractionation prior to reaction.

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[5] Stephany, R.W. 2003. *SPECLOG – the specificity log*. CRD-9, Codex Committee on Residues of Veterinary Drugs in Foods, 14th Session, Arlington, USA, 4–7 March.

(c) Other methods

Many veterinary drugs are susceptible to degradation/transformation by enzymes. In contrast to normal chemical reactions, these processes are very specific and generally consist of conjugation, oxidation, hydrolysis or de-alkylation. The conversion products possess different chromatographic characteristics from the parent veterinary drug and may be used for confirmatory purposes if compared with reaction products using standard veterinary drugs.

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

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

39. *Ruggedness(Robustness)* 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. Ruggedness testing of a confirmatory method may be required if the method differs significantly from the quantitative method previously validated (if the method uses different extraction or derivatisation procedures than are used in the quantitative method).

40. *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.

41. *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 cycle 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.

Other considerations

42. 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

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[6] Youden, W.J. & Steiner, E.H. 1975. *Statistical Manual of the Association of Official Analytical Chemists*. Gaithersburg, USA, AOAC International.

may include other tissues. General guidance as to the selection of suitable target tissues and the expected "tissue in trade" is provided in Table 4 and in the reports of the Joint FAO/WHO Expert Committee on Food Additives. 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.

43. The concentration of the analytes used to characterise a method should be selected to cover the Accepted Limits (ALs) of all analytes planned to be sought in all commodities.

44. 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.

45. Table 5 provides further guidance on the relevance of the parameters below and how they can be assessed.

(a) Selectivity

  (i) Matrix effects - direct interference effects (false negative rates), masking/enhancement effects, cross reactivity effects

(b) Sensitivity

  (i) Calibration range

  (ii) Calibration function, LOD. LOQ, precision, accuracy (bias), MU, recovery

  (iii) Chromatographic separation retention parameters,

  (iv) Qualitative, quantitative, and/or confirmatory detector response parameters

(c) Ruggedness (Robustness)

  (i) Identification of critical control points

  (ii) Identification of possible stopping points

(d) Stability studies

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

  (ii) Conditions; analyte stability under frozen storage and freeze-thaw cycle conditions.

(e) Incurred residue studies

Expression of results

46. For regulatory purposes, only confirmed data should be reported, expressed as defined by the MRL. Null values should be reported as being less than lowest calibrated value, rather than less than a concentration calculated by extrapolation. For veterinary drug residue analyses, results are generally corrected for recovery. If results are reported corrected for recovery, then both measured or corrected concentrations should be given together with the correction factor. The basis for correction should also be reported. Where positive results are obtained by replicate determinations (e.g., on different GC columns, with different detectors or based on different ions of mass spectra) of a single test portion (sub-sample), the lowest valid concentration obtained should be reported. Where positive results derive from analysis of multiple test portions, the arithmetic mean of the lowest valid concentrations obtained from each test portion should be reported. Taking into account, in general, a 20-30% relative precision, the results should be expressed only with 2 significant figures (e.g.: 0.11, 1.1, 11 and 1.1×10^2). Since precision decreases more rapidly at lower concentrations, residue values below 100 µg/kg should be expressed with one significant figure only.

Table 1 **Performance criteria that should be met by methods suitable for use as quantitative analytical methods to support MRLs for residues of veterinary drugs in foods**[7][*Subject to review and revision if required*]

Concentration	Coefficient of variability (CV)				Trueness
	Repeatability (within-laboratory, CV_A)	Repeatability (within-laboratory, CV_L)	Reproducibility (between-laboratory, CV_A)	Reproducibility (between-laboratory, CV_L)	Range of mean % recovery*
(μg/kg)	(%)	(%)	(%)	(%)	(%)
≤ 1	35	36	53	54	50–120
1 to 10	30	32	45	46	60–120
10 to 100	20	22	32	34	70–110
100 to 1 000	15	18	23	25	70–110
≥ 1 000	10	14	16	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.

[7] *Harmonized IUPAC Guidelines for the use of recovery information in analytical measurement* (CAC/GL 37-2001); see also Thompson, M., Ellison, S.L.R., Fajgelj, A., Willetts, P. & Wood, R. 1999. Harmonized guidelines for the use of recovery information in analytical measurement. *Pure Applied Chemistry*, 71(2): 337–348.

Table 1a: 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
$HRMS^n$ transition product ion	2.5

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 .

Table 1b: 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 + 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 1st generation product ions	5.5
HRMS	N	2n
GC-MS and LC-MS	2 + 2	4
GC-MS and HRMS	2 + 1	4

Table 2 Performance requirements for relative ion intensities (sample compared to standard) using various mass spectrometric analytical techniques[4]

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 3. Examples of detection methods suitable for the confirmatory analysis of substances, as recommended by the Miskolc Consultation (http://www.iaea.org/programmes/rifa/trc/pest-qa_val_guide.pdf)

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 4. 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**
Ruminant (e.g. cattle, sheep)*	Liver or kidney, muscle**	Fat, muscle
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**
Fish	Muscle with adhering skin in normal proportions	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	Whole

* 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).

** 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.

Table 5. Summary of parameters and criteria for development adaptation and validation of single analyte, group specific and multi-residue analytical procedures for veterinary drug residues, as prepared by the Miskolc consultation. (http://www.iaea.org/trc/pest-qa_val_annex2.pdf)

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
Within laboratory performance of optimised method						
1.1 Analyte stability during sample storage	About Accepted Limit (AL)	Analyse representative samples (time 0) and samples stored according to normal procedures of the laboratory (e.g. at ≤ -18°C). The storage time should be ≥ than the longest interval foreseen between sampling and sample disposal. Repeat at -70°C if analyte stability does not meet criteria at ≤-18°C. ≥5 replicates at each time point.	No significant loss of analyte during storage (P = 0.05)	No significant loss of analyte during storage (P = 0.05)	No false negatives at after storage.	Storage stability should be assessed using incurred tissues, when available. Otherwise, prepare spiked test materials using different pools of blank tissue to reflect the expected variability of the samples to which the method is to be applied. Storage is validated for use with any subsequent procedure. Validation may be specific to analyte. However, generally storage stability data obtained with representative sample matrices can be considered valid for similar matrices. The matrices shall be selected taking into account the chemical stability of the analyte. Useful information can be obtained on stability during storage from the JECFA evaluations, or from dossiers submitted for registration.
1.2 Analyte stability during sample processing	About AL	Treat representative tissue matrices with known amount of analyte(s). Analyse ≥5 replicates of each representative commodity, post-processing,	No significant loss of analyte during processing (P = 0.05)	No significant loss of analyte during processing (P = 0.05).	No false negatives at AL after processing.	Factors such as exposure to light, the temperature of the sample during processing and the extent of sample processing (e.g. homogenisation time) may be critical. Processing validated for use with any subsequent procedure. Validation may be specific to analyte and/or sample matrix. For testing stability determine the mean recovery and CV of representative marker compounds. Use these compounds for internal QA tests (see section 5). CV of each compound will indicate the within laboratory repeatability as well.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
1.3 Analyte stability in extracts and standard solutions	At AL, with well detectable residues	≥5 replicates at each appropriate point in time (including zero) and for each representative analyte/matrix. Spike blank extracts to test stability of residues. Compare analyte concentration in stored and freshly made standard solutions.	At the end of the storage period, the recoveries should be within the range specified in Table 1. No significant change in analyte concentration in stored analytical standards ($P = 0.05$)	At the end of the storage period, the recoveries should be within the range specified in Table 1. No significant change in analyte concentration in stored analytical standards ($P = 0.05$)	At the end of the storage period, all recoveries detectable at AL.	The test of stability in extracts is required if the semi-processed material will likely be stored longer than during determination of precision, or low recoveries were obtained during optimisation of the method. Storage time should encompass the longest period likely to be required to complete the analysis, including any subsequent confirmation using the extract.
1.4 Extraction efficiency	About AL	Analyse ≥5 replicate portions of samples or reference material with incurred residues. Compare the reference (or different) procedure with that under test.	For samples with incurred residues, the mean result obtained with the reference procedure and the tested procedure should not differ significantly at $P=0.05$ level applying CV_L in the calculation. If using a reference material, the mean concentration of the residue should not differ significantly at $P=0.05$ level, calculated with CV_A of the method tested, from the consensus value for the residue in the reference material. When the CV_A of the method is larger than 10%, the number of replicate analyses has to be increased to keep the relative standard error of the mean < 5%. Otherwise quantify and report the efficiency of extraction (excluding the recovery of analytical phase).	For samples the mean residues obtained with the reference procedure and the tested procedure should not differ significantly at $P=0.01$ level applying CV_L in the calculation. Or, the consensus value of reference material and the mean residue, calculated with CV_A of the method tested, should not differ significantly at $P=0.01$ level. Otherwise quantify and report the efficiency of extraction (average recovery of extraction excluding the recovery of analytical phase).	No false negatives at AL	Some residues may be conjugated or otherwise bound to the tissue matrix and sample pre-treatment (e.g. glucuronidase) may be required to release such residues and thereby improve analyte recovery. Temperature of the extract, speed and duration of blending or homogenizing, time of extraction and volumes and ratios of extracting solvents may significantly affect the efficiency of extraction. The effect of these parameters can be checked with a ruggedness test. The optimised conditions should be kept constant as far as possible and may be generally applicable for similar matrices and analytes of similar physical and chemical properties.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
1.5 Selectivity of separation	About AL	Determine Relative Retention time (RRt) values for all analytes to be tested by the method (not only the reference compounds). When chromatographic techniques are used without spectrometric detection, apply different separation principles and/or determine RRts on columns of different polarity. Determine and report resolution (RS) and tailing factors (Tf) of critical peaks.	Peaks should be baseline resolved or sufficiently separated to permit accurate identification and quantification. The nearest peak maximum should be separated from the designated analyte peak by at least one full width at 10% of the peak height, or more selective detection of all analytes is required.	Peaks should be baseline resolved or sufficiently separated to permit accurate quantification. The nearest peak maximum should be separated from the designated analyte peak by at least one full width at 10% of the peak height, or more selective detection of all analytes is required.	For chromatographic methods, peaks should be sufficiently resolved to permit tentative identification of all analytes tested at AL. Other types of screening methods, such as ELISA, should detect analytes at the AL.	Use information obtained from these experiments in establishing system suitability criteria for the analysis. System suitability involves injection of analytes to demonstrate adequate performance of the chromatographic system (i.e. peak resolution as specified by method or client requirement).
1.6 Specificity and selectivity of analyte detection	About AL	Identify by mass spectrometry, or by the appropriate combination of separation and detection techniques available. Analyse ≥5 blanks of each representative commodity obtained preferably from different sources. Report analyte equivalent of blank response. Determine and report selectivity (δ) of detector and relative response factors of representative analytes (RRF) with specific detectors used.	Analyte may be identified and, if necessary, quantified, by mass spectrometry or other suitable technique. Analyse ≥5 blanks of each representative commodity obtained preferably from different sources. Report analyte equivalent of blank response. Determine and report selectivity (δ) of detector and relative response factors of representative analytes (RRF) with specific detectors used.	Analyte peak sufficiently resolved from other peaks in chromatogram for quantitative determination. Evidence of no co-eluting compounds should be provided.	False negatives (β-error) ≤5%; false positives (α-error) ≤10%. (see CAC/GL 40-1993 Rev. 1-2003	Applies only to a specific combination of separation and detection technique. Samples of known treatment history may be used instead of untreated samples. Maturity of sample matrices may significantly affect the blank response and consequently the selectivity of detection. Blank values shall also be regularly checked during performance validation. Report typical peaks present in blank extracts. The LCL should preferably be ≤ 0.5AL. Alter chromatographic conditions if blank response interferes with the analyte. The targets for false positive and false negative rates for screening tests are based on CAC/GL 71-2009.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
1.7 Calibration function Matrix effect	About AL	Test the response functions of all analytes included in the method on a minimum of 2 occasions with ≥2 replicates at ≥3 analyte concentrations plus blank.	For linear calibration: regression coefficient for analytical standard solutions (r) ≥ 0.99. SD of residuals (Sy/x) ≤ 0.1	For linear calibration: regression coefficient (r) ≥ 0.99. SD of residuals (Sy/x) ≤ 0.1	Not applicable.	Establish calibration parameters during optimisation of the procedure, determination of precision or detection capability. Prepare calibration solutions of different concentrations independently from stock solution. For MRM perform calibration with mixtures of analytes ("standard mixture"), which can be properly separated by the chromatographic system to take into account the "multi component effect".
1.8 Analytical range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ).	About AL	Analyse ≥5 blank samples and analytical portions spiked at LCL, plus ≥3 analytical portions spiked at each of ≥ .5, 1 and 2 times AL. Where practical, method performance tests should be divided among the analysts, who will use the method, and instruments which will be used in the analysis.	Method must positively confirm presence of analyte at AL and, used quantitatively, must meet performance criteria in Table 1. LOQ must be fit for purpose.	Method must meet performance criteria in Table 1.	False negatives (β-error) ≤5%; false positives (α-error) ≤10%. (see CAC/GL 40-1993 Rev. 1-2003).	The analysts should demonstrate that the method is suitable for determining the presence of the analyte at the appropriate AL with the maximum errors specified. The confidence interval around the calculated mean depends on the number of data points used for the calculation. The decision limit and detection capability for specified analyte/matrix combinations can be determined by analysing ≥ 5 blank samples and analytical portions spiked at AL and 0.5 and 2 times the AL, or by applying ISO Standard 11843. Estimates of method accuracy, precision and recovery should be available to users of data generated with the method.
2. Extension of the method to new analyte and matrices having similar properties to those of representative analytes and matrices						
2.1 Analyte stability during sample storage, processing, and in extracts and standard solutions	See. 1.1, 1.2, and 1.3	See. 1.1, 1.2, 1.3	See. 1.1, 1.2, 1.3	See. 1.1, 1.2, 1.3	See. 1.1, 1.2, 1.3	See. 1.1, 1.2, 1.3
2.2 Extraction Efficiency	About AL	See 1.4	See 1.4	See 1.4	See 1.4	See 1.4
2.3 Selectivity of separation	About AL	See 1.5	See 1.5	See 1.5	See 1.5	See 1.5
2.4 Specificity and selectivity of analyte	About AL	Check response of ≥ 3 different (if available) blank samples.	See 1.6.	See 1.6.	See 1.6	Some authorities recommend that 6 or more representative blanks be used for each new matrix. If the selectivity of detection does not eliminate the matrix response, use appropriate combination of

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
detection						chromatographic columns to enable the separation of analytes from the matrix peaks. Report typical peaks present in blank extracts. See 1.6
2.5 Calibration function, matrix effect	About AL	See 1.7	See 1.7	See 1.7	See 1.7	See 1.7
2.6 Analytical range, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ).	About AL	Spike blank analytical portions with relevant representative analytes at 3 concentrations, in duplicate. See 1.8	Method must positively confirm presence of analyte at AL and, used quantitatively, must meet performance criteria in Table 1. See 1.8	Meets performance specifications in Table 1. See 1.8	Analytes added to blank samples at AL should be detectable in all tests. See 1.8	Relevant representative analyte: analyte which may occur in a particular sample. See 1.8
2.7 Analyte homogeneity	See 1.3.	See 1.3.	See 1.3.	See 1.3.	See 1.3.	Biological variability may result in differences in analyte homogeneity in, for example, liver from different species.
2.8 Matrix effect	About AL	Test the matrix effect using blanks in combination with 3.4.	Method must positively confirm presence of analyte at AL and, used quantitatively, must meet performance criteria in Table 1.	Meets performance specifications in Table 1. No matrix effect observed.	Analytes added to blank samples at AL should be detectable in all tests.	If method performance criteria are not met due to matrix effects, method requires revision to be applied to the new matrix.
3. Adaptation of the method in another laboratory						
3.1 Purity and suitability of chemicals, reagents and ad(ab)sorbents		Test reagent blank, applicability of ad(ab)sorbents and reagents. Perform derivatisation without and with sample.	No interfering response.	No interfering response.	Verify screening test performs within manufacturer's specifications.	
3.2 Analyte "homogeneity"						No test required unless evidence of heterogeneity is found through quality control procedures during method application.
3.3 Selectivity of separation	About AL	Verify system suitability.	Specified separation achieved.	Specified separation achieved.	Analytes added to blank samples at AL should be detectable in all tests.	System suitability samples are usually prepared by dissolving the analyte(s) in the solvent used in the final extract of the method. They are injected prior to running samples to ensure that the chromatographic separation achieved is within the requirements of the method.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	**Codex Level II method (Quantitative)**	**Codex Level III method (Screening – qualitative or semi-quantitative)**	
3.4 Calibration function , matrix effect	About AL	Test the response functions of representative analytes included in the method on a minimum of 2 occasions at ≥3 analyte levels plus blank, in duplicate on each occasion. Test the matrix effect with representative analytes and matrices.	Method must positively confirm presence of analyte at AL and, used quantitatively, must meet performance criteria in Table 1. No matrix effects observed.	Meet requirements of Table 1. No matrix effects observed.	Analytes added to blank samples at AL should be detectable in all tests.	Calibration parameters may be established during optimisation of the procedure, determination of precision or detection capability. Prepare calibration solutions independently from the stock solution. For MRM perform calibration with mixtures of analytes ("standard mixture"), which can be properly separated by the chromatographic system to take into account the "multi component effect". Use matrix matched analytical standards for quantitative tests if matrix effect is significant.
3.5 Specificity of analyte detection	About AL	Check performance characteristics of detectors used and compare them with those specified in the method. Check response of one blank of each representative commodity, otherwise perform test as described in section 1.6.	Measured response is solely due to the analyte. The detector performance (sensitivity and selectivity) should be equal or better than specified in the method. Response of blank sample should not interfere with those of the analytes.	False negatives (β-error) ≤ 5%; false positives (α-error) ≤10% (see CAC/GL 40-1993 Rev. 1-2003).	The relative response of specific detectors can substantially vary from model to model. Proper checking of specificity of detection is critical for obtaining reliable results. Compare blank response observed with typical peaks reported in blank extracts. See other comments under 1.6.	
3.6 Analytical range, accuracy, precision, decision limit, detection capability.	About AL	See 1.8	See 1.8	See 1.8	Establish that original performance characteristics of method are met or exceeded, or document performance achieved. If method is fit for purpose, establish QC criteria based on the within-laboratory performance achieved during validation	See comments in 1.8.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
3.7 Analyte stability in extracts and standard solutions.		No test, unless problems arise during evaluation of performance.				See 1.9 if problems arise
4. Quality control (performance validation)						
4.1 Methods used regularly						
4.1.1 Suitability of chemicals, adsorbents and reagents		For each new batch: test reagent blank, applicability of ad(ab)sorbents and reagents. Perform derivatisation without sample.	No interfering response ≥LCL.	No interfering response ≥ LCL.	No interfering response at minimum concentration specified.	
4.1.2 Analyte stability during sample processing and analysis	About AL	Spike blank sample matrix of known origin with appropriate test compounds (See 1.2) and analyse them together with other samples in the analytical batch.	Recoveries of the test compounds should be within the action limits of control chart, if method used for quantification; otherwise, analyte should be confirmed at lowest concentration specified by requirement.	Recoveries of the test compounds should be within the specified limits (usually 2σ) of control chart.	Analyte added at lowest concentration specified by requirement remains detectable after storage.	Test stability during period when seasonal changes may result in fluctuations in laboratory environment (temperature, humidity, etc.).
4.1.3 Analyte homogeneity in processed sample	About AL	Select a positive sample randomly. Repeat analysis of another one or more analytical portions.	The replicates should be within the reproducibility limit of Table 1, if method includes quantification. For confirmation only, results should confirm within method criteria (e.g. ion ratios).	The replicates should be within the reproducibility limit of Table 1.	All results should be positive at or above the minimum detection requirements specified.	Perform test alternately to cover each commodity analysed. Test homogeneity at the start of the analysis of the given type of samples. The acceptable results of the test also confirm that the reproducibility of the analyses (CV_A) was appropriate.
4.1.4 Extraction efficiency						The efficiency of the extraction cannot be controlled during the analysis. To ensure appropriate efficiency, the extraction should be carried out without any change.
4.1.5 Selectivity of separation, performance of detectors	About AL	Include appropriate detection test mixture (system suitability) in each chromatography batch.	System suitability demonstrated.	System suitability demonstrated.	Not applicable for most screening tests (test kits).	Prepare detection test mixture for each method of detection. Select the components of the mixture to indicate the characteristic parameters of chromatographic separation and detection. Adjust chromatographic conditions to obtain required separation, if required.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	**Codex Level II method (Quantitative)**	**Codex Level III method (Screening – qualitative or semi-quantitative)**	
4.1.6 Specificity of analyte detection	About AL	Include blank matrix in analytical batch. Use standard addition if no untreated sample (similar to those analysed in the batch) is available. Confirm identity and quantity of each analyte present AL.	Sample co-extracts interfering with the analyte should not be present.	Sample co-extracts interfering with the analyte should not be present.	False negatives (β-error) ≤5%; false positives (α-error) ≤10%. (see CAC/GL 40-1993 Rev. 1-2003).	Include appropriate detection test mixture in each chromatography batch (system suitability). Perform quantitative analysis with analytical standards prepared in blank matrix extract if matrix effect is significant.
4.1.7 Calibration and analytical range	About AL	Usually prepared at a minimum of 0.5, 1 and 2 times the AL of each analytical run.	Ion ratios for peaks used for mass spectral confirmation must be within limits specified in method. Usually r = 0.98 or better for each calibration curve used in quantification.	Usually r = 0.98 or better for each calibration curve.	Usually not applicable. Include appropriate standards to verify test performance at minimum concentration specified by requirement.	
4.1.8 Accuracy and precision	Within analytical range	Include in each analytical batch ≥ 1 blank sample either: spiked with standard mixture, replicate portion of a positive sample, or a re-analysis of a positive sample. Certified reference materials, if available, may also be used.	The performance of detector and chromatographic column shall be equal or better than specified in the method. For quantitative methods, preferably all recoveries should be within the warning limit of control chart constructed with the specific or typical CVA of analytes. On a long run one of 20 or 100 samples may be outside the specified limits for the control chart. The analytical batch should be repeated if any of the recoveries falls outside the action limits, or the results of the replicate analyses of the positive sample exceeds the critical range.	The performance of detector and chromatographic column shall be equal or better than specified in the method. For quantitative methods, preferably all recoveries should be within the warning limit of control chart constructed with the specific or typical CV_A of analytes. Occasionally one sample may be outside the specified limits for the control chart. The analytical batch should be repeated if any of the recoveries fall outside the action limits, or the results of the replicate analyses of the positive sample exceeds the critical range.	Usually not applicable. Apply criteria for false positives and false negatives.	Spike analytical portion with standard mixture(s) within the analytical range of interest, particularly at concentrations near an AL to be detected.

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
4.1.9 Duration of analysis			The samples, extracts etc. should not be stored longer than the period for which the storage stability was tasted during method validation. Storage conditions should be regularly monitored and recorded.			
4.2 Analyte detected occasionally						
Follow tests described in 4.1 with the following exceptions						
4.2.1 Accuracy and precision	About AL	Reanalyse another analytical portion; or use standard addition at the measured concentration of analyte.	Replicate analyses should agree within confirmatory criteria of method. For quantitative purposes, replicates should agree within the specifications in Table 1.	Replicates should agree within the specifications in Table 1.	Replicates should be in agreement.	Check accuracy if residue found at ≥0.5 AL.
4.3 Methods used at irregular intervals						
Follow tests described in 4.1 with the following exceptions						
4.3.1 Accuracy and precision (repeatability)	About AL	Include spiked samples at 0.5, 1 and 2 times AL in each analytical batch. Use standard addition if untreated sample (similar to those analysed in the batch) is not available. Perform analysis with ≥2 analytical portions.	Replicate analyses should agree within confirmatory criteria of method. For quantitative purposes, replicates should agree within the specifications in Table 1.	Replicates should agree within the specifications in Table 1.	Replicates should be in agreement.	The acceptable results also prove the suitability of chemicals, adsorbents and reagents used. If performance criteria were not satisfied, the method shall be practised and it performance characteristics re-established during partial revalidation of the method.
4.4 Changes in implementation of the method						
Change	**Parameters to be tested**		**For test methods and acceptability criteria see the appropriate sections of Appendix I**			
4.4.1 Reagent/ new materials: different supplier or quality	Test blank value and perform derivatisation without sample. Test recoveries in two replicates at 0.5, 1 and 2 times AL.					Method performance characteristics should not be changed. Modify method protocol to include acceptable change in specified quality or supplier.
4.4.2 Chromato-graphic column	Test selectivity of separation, resolution, inertness, RRt values (system suitability).					Method performance characteristics should not be changed significantly. Some adjustment or modification of chromatographic conditions may be required and should be documented accordingly. Modify method

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	Codex Level II method (Quantitative)	Codex Level III method (Screening – qualitative or semi-quantitative)	
						protocol to include acceptable change in specified item or supplier.
4.4.3 Equipment for sample processing	Homogeneity of processed sample; Stability of analytes					Method performance characteristics should not be changed. Modify method protocol to include acceptable change in item or supplier.
4.4.4 Equipment for extraction	Compare results using incurred samples or suitable surrogates (spiked matrix blanks when these have been shown previously to reflect extraction for incurred samples) detected after extraction with the old and new equipment in ≥ 5 replicates		The mean residues should not be significantly different at p=0.05.	Method performance characteristics should not be changed. Modify method protocol to include acceptable change in item or supplier.		
4.4.5 Detection	Test selectivity of separation and selectivity and sensitivity of detection					Test also detectability separately with new detection reagents. Method performance characteristics should not be changed. Modify method protocol to include acceptable change in item or supplier.
4.4.6 Analyst	≥5 recovery tests at each concentration (LCL, AL and 2 (3) AL), re-analysis of one blank sample and two positive samples (unknown for the analyst).		All results should be within the warning limits specified for the method in the laboratory. Replicate sample analysis shall be within the critical range.	Document analyst familiarization with method (i.e. analyst is “ready to perform” tests on samples). This is a minimum requirement. Some veterinary drug residue laboratories use a more detailed protocol which includes (1) generation of standard curve within acceptability criteria; (2) minimum of 2 analytical runs for each matrix, containing representative samples spiked by the analyst at a minimum of 3 concentrations, in duplicate; and (3) minimum of 1 analytical		

Parameter	Concentration(s)	No. of analyses or type of test required	Criteria			Comments
			Codex Level I method (Confirmatory/ Quantitative)	**Codex Level II method (Quantitative)**	**Codex Level III method (Screening – qualitative or semi-quantitative)**	
				run containing spiked or incurred residues, at 3 concentrations in duplicate, provided as unknowns to the analyst. All results at each stage must meet acceptability criteria, or be repeated.		
4.4.7 Laboratory	Accuracy and precision ≥3 recovery tests at each concentration, 0.5, 1 and 2 times AL, preferably by (different) analyst(s) on different days.		All results should be within the warning limits specified for the method in the laboratory.			

GLOSSARY OF TERMS

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 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).
Accuracy	Closeness of agreement between a test result and the accepted reference value.
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%.
Analyte	The chemical substance sought or determined in a sample.
Analyte Homogeneity (in sample)	Uniformity of dispersion of the analyte in matrix. The variability in analytical results arising from sample processing depends on the size of analytical portion. The sampling constant describes the relationship between analytical portion size and the expected variation in a well mixed analytical sample: $Ks = w (CV_{Sp})^8$, where w is the mass of analytical portion and CV_{Sp} is the coefficient of variation of the analyte concentration in replicate analytical portions of w [g] which are withdrawn from the analytical sample
Analytical portion	A representative quantity of material removed from the analytical sample, of proper size for measurement of the residue concentration.
Analytical sample	The material prepared for analysis from the laboratory sample, by separation of the portion of the product to be analysed and then by mixing, grinding, fine chopping, etc., for the removal of analytical portions with minimal sampling error.
Applicability	The analytes, matrices and concentrations for which a method of analysis has been shown to be satisfactory.
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%.
Bias	Difference between the mean value measured for an analyte and an accepted reference value for the sample. Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias. A larger systematic difference from the accepted reference value is reflected by a larger bias value.
Commodity Group	Group of foods or animal feeds sharing sufficient chemical characteristics as to make them similar for the purposes of analysis by a method. The characteristics may be based on major constituents (e.g. water, fat, sugar, and acid content) or biological relationships, and may be defined by regulations.
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 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 -13. 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 I - 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.
Detection Test Mixture	Mixture of analytical standards which are suitable to check the conditions of chromatographic separation and detection. The detection test mixture should contain analytes which provide information for the selectivity and response factor s for the detectors, and the inertness (e.g. characterised by the tailing factor Tf) and separation power (e.g. resolution Rs) of column, and the reproducibility of RRt value s. The detection test mixture may have to be column and detector specific.
False negative result	See beta error
False positive result	See alpha error
Group specific method	Method designed to detect substances having either a common moiety or similar chemical structure.
Incurred Residue	Residues of an analyte in a matrix arising by the route through which the trace concentrations would normally be expected, as opposed to residues from laboratory fortification of samples.
Individual Method	Method which is suitable for determination of one or more specified com pounds. 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.
Laboratory Sample	The sample as received at the laboratory (not including the packaging).
Limit of Detection (LOD)	Smallest concentration where the analyte can be identified. Commonly defined as the mini mum concentration of analyte in the test sample that can be measured with a stated probability that the analyte is present at a concentration above that in the blank sample. See also Decision Limit.
Limit of Quantification (LOQ)	Smallest concentration of the analyte that can be quantified. Commonly defined as the minimum concentration of analyte in the test sample that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test. See also Detection Capability.
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 Calibration	Calibration using standards prepared in an extract of the commodity analysed (or of a representative commodity). The objective is to compensate for the effects of co-extractives on the determination sys tem. Such effects are often unpredictable, but matrix-matching may be unnecessary where co-extractives prove to be of insignificant effect.
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.
Precision	Closeness of agreement between independent test result s obtained under stipulated conditions.
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.
Recovery	Fraction or percentage of an analyte recovered following ex traction and analysis of a blank sample to which the analyte has been added at a known concentration (spiked sample or reference material).
Reagent Blank	Complete analysis made without the inclusion of sample materials for QC purpose.
Reference Material	Material one or more of whose analyte concentrations are sufficiently homogeneous and well established to be used for the assessment of a measurement method, or for assigning values to other materials. In the context of this document the term "reference material" does not refer to materials used for the calibration of apparatus.
Reference Method	Quantitative analytical method of proven reliability characterised by well-established trueness. specificity, precision and detection power. 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.
Repeatability	Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on replicate analytical portions in the same laboratory by the same operator using the same equipment within short intervals of time. (ISO 3534-1)
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.
Reproducibility	Closeness of agreement between results obtained with the same method on replicate analytical portions with different operators and using different equipment (within laboratory reproducibility). Similarly, when the tests are performed in different laboratories the inter-laboratory reproducibility is obtained.
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.
Ruggedness	Ability of a chemical measurement process to resist changes in test results when subjected to minor changes in environmental and method procedural variables, laboratories, personnel, etc.
Sample Preparation	The procedure used, if required, to convert the laboratory sample into the analytical sample by removal of parts (soil. stones, bones. etc.) 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 level (generally ß = 5%). Qualitative positive results may be required to be confirmed by confirmatory or reference methods. See Decision Limit and Detection Capability.
Selectivity	Measure of the degree to which the analyte is likely to be distinguished from other sample components. either by separation (e.g., chromatography) or by the relative response of the detection system.
Specificity	Extent to which a method provides responses from the detection system which can be considered exclusively characteristic of the analyte. A procedure in which known amounts analyte are added to aliquots of a sample extract
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.
Tailing Factor	Measure of chromatographic peak asymmetry; at 10% peak height maximum, the ratio of the front and tail segments of peak width, when separated by a vertical line drawn through the peak maximum.
Test Portion	See "Analytical Portion "
Test Sample	See "Analytical Sample"
Trueness	Closeness of agreement between the average value obtained from a large series of test results and an accepted reference value.
Uncertainty of measurement	Single parameter (usually a standard deviation or confidence interval) expressing the possible range of values around the measured result, within which the true value is expected to be with a stated degree of probability. It should take into account all recognised effects operating on the result, including: overall long-term precision (within laboratory reproducibility) of the complete method; the method bias; sub-sampling and calibration uncertainties; and any other known sources of variation in results.

ABBREVIATIONS

C_{max}	Highest residue detected in replicate analytical portions	**MRM**	Multi-Residue Method
C_{min}	Lowest residue detected in replicate analytical portions	**RRF**	Relative response factor
CV_{Atyp}	Typical coefficient of variation of residues determined in one analytical portion.	**RRt**	Relative retention value for a peak
CV_{Ltyp}	Typical coefficient of variation of analyses of portions of a laboratory sample.	**Rs**	Resolution of two chromatographic peaks
CV_{Sp}	Coefficient of variation of residues in analytical portions.	**SD**	Standard Deviation
GLP	Good Laboratory Practice	**$S_{y/x}$**	Standard deviation of the residuals calculated from the linear calibration function
GSM	Group Specific Method	**WHO**	World Health Organization
MRL	Maximum Residue Limit		