GUIDELINES ON GOOD LABORATORY PRACTICE IN PESTICIDE RESIDUE ANALYSIS

CAC/GL 40-1993

Content

FOREWORD

The Guidelines are intended to assist in ensuring the reliability of analytical results in checking compliance with maximum residue limits of foods moving in international trade. Reliable analytical results are essential to protect the health of consumers and to facilitate international trade.

In addition to the present Guidelines, other relevant Codex recommendations elaborated by the Codex Committee on Pesticide Residues (CCPR) in the field of enforcement of Codex maximum limits for pesticide residues are as follows:

1   Recommended Methods of Sampling for the Determination of Pesticide Residues for compliance with MRLs (CAC/GL 33-1999).
2   Portion of Commodities to which Codex Maximum Residue Limits apply and which is analyzed (CAC/GL 41-1993).
3   List of Codex Maximum Residue Limits for Pesticides (Maximum residue limits for pesticides as adopted by the Codex Alimentarius Commission are available at: http://www.codexalimentarius.net).
4   Recommended Methods of Analysis of Pesticide Residues (CODEX STAN 229-1993).
5   Codex Classification of Foods and Animal Feeds (CAC/MISC 4-1993).

1. INTRODUCTION

It was considered that the ultimate goal in fair practice in international trade depended, among other things, on the reliability of analytical results. This in turn, particularly in pesticide residue analysis, depended not only on the availability of reliable analytical methods, but also on the experience of the analyst and on the maintenance of 'good practice in the analysis of pesticides'.

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These guidelines define such good analytical practice and may be considered in three inter-related parts:

>   The Analyst (Section 2);

>   Basic Resources (Section 3);

>   The Analysis (Section 4).

The requirements for facilities, management, personnel, quality assurance and quality control, documentation of results and raw data, and relevant subjects, which are considered as prerequisites for obtaining reliable and traceable results, are described in general in the ISO/IEC 17025 Standard (1999) and in a series of OECD GLP Guidance Documents, in the corresponding national laws and regulations. This Codex Guidelines, which are not exhaustive, outline the most essential principles and practices to be followed in the analysis of pesticide residues.

2.   THE ANALYST

2.1   Residue analysis consists of a chain of procedures, most of which are known, or readily understood, by a trained chemist, but because the analyte concentrations are in the range μg/kg to mg/kg and because the analyses can be challenging, attention to detail is essential. The analyst in charge should have an appropriate professional qualification and be experienced and competent in residue analysis. Staff must be fully trained and experienced in the correct use of apparatus and in appropriate laboratory skills. In addition, each analyst using the method for the first time should complete the tests specified in sections 4.4.5 of Table 4 to demonstrate that they can use the method within the expected performance parameters established during method validation prior to analysis of samples. They must have an understanding of the principles of pesticide residue analysis and the requirements of Analytical Quality Assurance (AQA) systems. They must understand the purpose of each stage in the method, the importance of following the methods exactly as described and of noting any unavoidable deviations. They must also be trained in the evaluation and interpretation of the data that they produce. A record of training and experience must be kept for all laboratory staff.

2.2   When a laboratory for residue analysis is set up, the staff should spend some of their training period in a well established laboratory where experienced advice and training is available. If the laboratory is to be involved in the analysis for a wide range of pesticide residues, it may be necessary for the staff to gain experience in more than one expert laboratory.

3.   BASIC RESOURCES

3.1   THE LABORATORY

3.1.1.   The laboratory and its facilities must be designed to allow tasks to be allocated to well-defined areas where maximum safety and minimum chance of contamination of samples prevail. Laboratories should be constructed of, and utilise, materials resistant to chemicals likely to be used within them. Under ideal conditions, separate rooms would be designated for sample receipt and storage, for sample preparation, for extraction and clean-up and for instrumentation used in the determinative step. The area used for extraction and clean-up must meet solvent laboratory specifications and all fume extraction facilities must be of high quality. Sample receipt, storage and preparation should be handled in areas devoted to work at residue levels. Maintenance of sample integrity and adequate provisions for personal safety are priority requirements.

3.1.2   Laboratory safety must also be considered in terms of what is essential and what is preferable, as it must be recognised that the stringent working conditions enforced in residue laboratories in some parts of the world could be totally unrealistic in others. No smoking, eating, drinking or application of cosmetics should be permitted in the working area. Only small volumes of solvents should be held in the working area and the bulk of the solvents stored separately, away from the main working area. The use of highly toxic solvents and reagents should be minimised whenever possible. All waste solvent should be stored safely and disposed of both safely and in an environmentally friendly manner taking into account specific national regulations where available.

3.1.3   The main working area should be designed and equipped for utilisation of an appropriate range of analytical solvents. All equipment such as lights, macerators and refrigerators should be "spark free" or

"explosion proof". Extraction, clean-up and concentration steps should be carried out in a well ventilated area, preferably in fume cupboards.

3.1.4   Safety screens should be used when glassware is used under vacuum or pressure. There should be an ample supply of safety glasses, gloves and other protective clothing, emergency washing facilities and a spillage treatment kit. Adequate fire fighting equipment must be available. Staff must be aware that many pesticides have acutely or chronically toxic properties and therefore, great care is necessary in the handling of standard reference compounds.

3.2   EQUIPMENT AND SUPPLIES

3.2.1   The laboratory will require adequate, reliable, supplies of electricity and water. Adequate supplies of reagents, solvents, gas, glassware, chromatographic materials, etc., of suitable quality are essential.

3.2.2   Chromatographic equipment, balances, spectrophotometers etc. must be serviced and calibrated regularly and a record of all servicing/repairs must be maintained for every such item of equipment. Calibration is essential for equipment performing measurements. Calibration curves and comparison with standards may suffice.

3.2.3   Regular calibration and re-calibration of measuring equipment must be done where the possible change in nominal value may significantly contribute to the uncertainty of the measurement. Balances and automated pipettes/ dispensers and similar equipment must be calibrated regularly. The operating temperatures of refrigerators and freezers should be continually monitored or be checked at specified intervals. All records should be kept up-to-date and retained.

3.2.4   Equipment used must be fit for purpose.

3.2.5   All laboratories require pesticide reference standards of known and acceptably high purity. Analytical standards should be available for all parent compounds for which the laboratory is monitoring samples, as well as those metabolites that are included in MRLs.

3.2.6 All analytical standards, stock solutions and reagents should be properly labelled including preparation date, analyst's identification, solvent used, storage conditions employed, and those compounds whose integrity could be influenced by degradative processes must be clearly labelled with an expiry date and stored under appropriate conditions. Reference standards must be kept under conditions that will minimise the rate of degradation, e.g. low temperature, exclusion of moisture and light. Equal care must be taken that standard solutions of pesticides are not decomposed by the effect of light or heat during storage or become concentrated by solvent evaporation.

4.   THE ANALYSIS

The methods applied for the determination of pesticide residues should generally satisfy the criteria given in Table 3.

4.1 AVOIDANCE OF CONTAMINATION

4.1.1   One of the significant areas in which pesticide residue analysis differs significantly from macro-analysis is that of contamination and interference. Trace amounts of contamination in the final samples used for the determination stage of the method can give rise to errors such as false positive or false negative results or to a loss of sensitivity that may prevent the residue from being detected. Contamination may arise from almost anything that is used for, or is associated with, sampling, sample transport and storage, and the analyses. All glassware, reagents, organic solvents and water should be checked for possible interfering contaminants before use, by analysis of a reagent blank.

4.1.2   Polishes, barrier creams, soaps containing germicides, insect sprays, perfumes and cosmetics can give rise to interference problems and are especially significant when an electron-capture detector is being used. There is no real solution to the problem other than to ban their use by staff while in the laboratory.

4.1.3   Lubricants, sealants, plastics, natural and synthetic rubbers, protective gloves, oil from ordinary compressed air lines and manufacturing impurities in thimbles, filter papers and cotton-wool can also give rise to contamination.

4.1.4   Chemical reagents, adsorbents and general laboratory solvents may contain, adsorb or absorb compounds that interfere in the analysis. It may be necessary to purify reagents and adsorbents and it is generally necessary to use re-distilled solvents. Deionised water is often suspect; re-distilled water is preferable, although in many instances tap water or well water may be satisfactory.

4.1.5   Contamination of glassware, syringes and gas chromatographic columns can arise from contact with previous samples or extracts. All glassware should be cleaned with detergent solution, rinsed thoroughly with distilled (or other clean) water and then rinsed with the solvent to be used. Glassware to be used for trace analysis must be kept separate and must not be used for any other purpose.

4.1.6   Pesticide reference standards should always be stored at a suitable temperature in a room separate from the main residue laboratory. Concentrated analytical standard solutions and extracts should not be kept in the same storage area.

4.1.7   Apparatus containing polyvinylchloride (PVC) should be regarded as suspect and, if shown to be a source of contamination, should not be allowed in the residue laboratory. Other materials containing plasticisers should also be regarded as suspect but PTFE and silicone rubbers are usually acceptable and others may be acceptable in certain circumstances. Sample storage containers can cause contamination and glass bottles with ground glass stoppers may be required. Analytical instrumentation ideally should be housed in a separate room. The nature and importance of contamination can vary according to the type of determination technique used and the level of pesticide residue to be determined. For instance contamination problems which are important with methods based on gas chromatography or high performance liquid chromatography, may well be less significant if a spectrophotometric determination is used, and vice versa. For relatively high levels of residues, the background interference from solvents and other materials may be insignificant in comparison with the amount of residue present. Many problems can be overcome by the use of alternative detectors. If the contaminant does not interfere with the residue determination, its presence may be acceptable.

4.1.8   Residues and formulation analyses must have completely separate laboratory facilities provided. Samples and sample preparation must be kept separate from the all residue laboratory operations in order to preclude cross contamination.

4.2   RECEPTION AND STORAGE OF SAMPLES

4.2.1   Every sample received into the laboratory should be accompanied by complete information on the source of the sample, on the analysis required and on potential hazards associated with the handling of that sample.

4.2.2   On receipt, a sample must immediately be assigned a unique identification code which should accompany it through all stages of the analysis to the reporting of the results. Samples should be subject to an appropriate disposal review system and all records should be kept.

4.2.3   Sample processing and sub-sampling should be carried out using procedures that have been demonstrated to provide a representative analytical portion and to have no effect on the concentration of residues present.

4.2.4   If samples cannot be analysed immediately but are to be analysed quickly, they should be stored at (1 - 5 °C), away from direct sunlight, and analysed within a few days. However, samples received deep-frozen must be kept at $\leq$ -16 °C until analysis. In some instances, samples may require storage for a longer period before analysis. In this cases, storage temperature should be approximately - 20 °C, at which temperature enzymic degradation of pesticide residues is usually extremely slow. If prolonged storage is unavoidable, the effects of storage should be checked by analysing fortified samples stored under the same conditions for a similar period. Useful information on storage stability of pesticide residues can be found in the annual publications of FAO titled: Pesticide Residues - Evaluations prepared by the FAO/WHO JMPR, and in the information submitted by the manufacturers for supporting the registration of their pesticides.

4.2.5   When samples are to be frozen it is recommended that analytical test portions be taken prior to freezing in order to minimise the possible effect of water separation as ice crystals during storage. Care must still be taken to ensure that the entire test portion is used in the analysis.

4.2.6   The containers must not leak. Neither the containers used for storage nor their caps or stoppers should allow migration of the analyte(s) into the storage compartment.

4.3   STANDARD OPERATING PROCEDURES (SOPS)

4.3.1   SOPs should be used for all operations. The SOPs should contain full working instructions as well as information on applicability, expected performance, internal quality control (performance verification) requirements and calculation of results. It should also contain information on any hazards arising from the method, from standards or from reagents.

4.3.2   Any deviations from a SOP must be recorded and authorised by the analyst in charge.

4.4   VALIDATION OF METHODS[1]

4.4.1   Guidelines have been published for validation of analytical procedures for various purposes. The principles described in this section are considered practical and suitable for validation of pesticide residue analytical methods. The guidance is not normative. The analyst should decide on the degree of validation required to demonstrate that the method is fit for the intended purpose, and should produce the necessary validation data accordingly. For instance, the requirements for testing for compliance with MRLs or providing data for intake estimation may be quite different.

4.4.2   An analytical method is the series of procedures from receipt of a sample to the production of the final result. Validation is the process of verifying that a method is fit for the intended purpose. The method may be developed in-house, taken from the literature or otherwise obtained from a third party. The method may then be adapted or modified to match the requirements and capabilities of the laboratory and/or the purpose for which the method will be used. Typically, validation follows completion of the development of a method and it is assumed that requirements such as calibration, system suitability, analyte stability, etc., have been established satisfactorily. When validating and using a method of analysis, measurements must be made within the calibrated range of the detection system used. In general, validation will precede practical application of the method to the analysis of samples but subsequent performance verification is an important continuing aspect of the process. Requirements for performance verification data are a sub-set of those required for method validation.

Proficiency testing (or other inter-laboratory testing procedures), where practicable, provides an important means for verifying the general accuracy of results generated by a method, and provides information on the between-laboratory variability of the results. However, proficiency testing generally does not address analyte stability or homogeneity and extractability of analytes in the processed sample.

Where uncertainty data are required, this information should incorporate performance verification data and not rely solely on method validation data.

4.4.3   Whenever a laboratory undertakes method development and/or method modification, the effects of analytical variables should be established, e.g. by using ruggedness tests, prior to validation. Rigorous controls must be exercised with respect to all aspects of the method that may influence the results, such as: sample size; partition volumes; variations in the performance of the clean-up systems used; the stability of reagents or of the derivatives prepared; the effects of light, temperature, solvent and storage on analytes in extracts; the effects of solvent, injector, separation column, mobile phase characteristics (composition and flow-rate), temperature, detection system, co-extractives etc. on the determination system. It is most important that the qualitative and quantitative relationship between the signal measured and the analyte sought are established unequivocally.

4.4.4   Preference should be given to methods having multi-residue and or multi-matrix applicability. The use of representative analytes or matrices is important in validating methods. For this purpose, commodities should be differentiated sufficiently but not unnecessarily. For example, some products are available in a wide range of minor manufactured variants, or cultivated varieties, or breeds, etc. Generally, though not invariably, a single variant of a particular commodity may be considered to represent others of the same commodity but, for example, a single fruit or vegetable species must not be taken to represent all fruit or

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[1]   This section is based on the recommendations elaborated by an AOAC/FAO/IAEA Consultation held in Miskolc, Hungary, in 1999. The full document is available at www.iaea.org/trc and in A. Fajgelj & A. Ambrus Principles and Practices of Method Validation, Royal Society of Chemistry, 2000

vegetables (Table 5). Each case must be considered on its merits but where particular variants within a commodity are known to differ from others in their effects on method performance, analyses of those variants are required. Considerable differences in the accuracy and precision of methods, especially with respect to the determination step, may occur from species to species.

4.4.4.1 Where experience shows similar performance of extraction and clean-up between broadly similar commodities/sample matrices, a simplified approach may be adopted for performance validation. A representative commodity may be selected from Table 5 to represent each commodity group having common properties, and used for validation of the procedure or method. In Table 5, the commodities are classified according to the Codex Classification[2].

Some examples of how far the validation data may be extended to other commodities are:

- **cereals**, validation for whole grains cannot be taken to apply to bran or bread but validation for wheat grain may apply to barley grain or wheat four;

- **animal products**, validation for muscle should not be taken to apply to fat or offal but validation for chicken fat may apply to cattle fat;

- **fruit and vegetables**, validation for a whole fresh product cannot be taken to apply to the dried product but validation for cabbages may apply to Brussels sprouts.

4.4.4.2 Similarly representative analytes may be used to assess the performance of a method. Compounds may be selected to cover physical and chemical properties of analytes that are intended to be determined by the method. The selection of representative analytes should be made based on the purpose and scope of analysis taking into account the following.

    (a) The representative analytes selected should:

        (i)   possess sufficiently wide range of physico-chemical properties to include those of represented analytes;

        (ii)   be those which are likely to be detected regularly, or for which critical decisions will be made based on the results.

    (b) As far as practicable, all analytes included in the initial validation process should be those which will have to be tested regularly and which can be determined simultaneously by the determination system used.

    (c) The concentration of the analytes used to characterise a method should be selected to cover the accepted limits (AL, see Glossary) of all analytes planned to be sought in all commodities. Therefore the selected representative analytes should include, among others, those which have high and low ALs. Consequently, the fortification levels used in performance testing with representative analytes/representative commodities may not necessarily correspond to the actual ALs.

4.4.5   Where appropriate data are already available, it may not be necessary for the analyst to perform all the tests. However, all required information must be included or referred to in the validation records. Table 1 provides an overview of parameters to be assessed for method validation according to the status of the method to be validated. Specific parameters and criteria to be assessed are listed in table 2. Parameters to be assessed should be restricted to those that are appropriate both to the method and to the purpose for which the particular method is to be applied. In many cases, performance characteristics with respect to several parameters may be obtained simultaneously using a single experiment. Test designs where different factors are changed at the same time (factorial experiment designs), may help to minimise the resources required. The performance of the analytical method should be checked, both during its development and during its subsequent use as indicated in section 4.5, according to the criteria given in Table 3.

4.4.6   Individual (single residue) methods should be fully validated with all analyte(s) and sample materials specified for the purpose, or using sample matrices representative of those to be tested by the laboratory.

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[2] Codex Classification of Foods and Animal Feeds (CAC/MISC 4-1993)

4.4.7   Group specific methods (GSM) should be validated initially with one or more representative commodities and a minimum of two representative analytes selected from the group.

4.4.8   MRMs may be validated with representative commodities and representative analytes.

4.5   PERFORMANCE VERIFICATION

4.5.1   The main purposes of performance verification are to:

- *monitor the performance of the method under the actual conditions prevailing during its use;*

- *take into account the effect of inevitable variations caused by, for instance, the composition of samples, performance of instruments, quality of chemicals, varying performance of analysts and laboratory environmental conditions;*

- *demonstrate that the performance characteristics of the method are broadly similar to those established at method validation, showing that the method is under "statistical control", and the accuracy and uncertainty of the results are comparable to those expected of the method. For this purpose, data obtained during method validation may be updated with data collected from performance verification during the regular use of the method.*

The results of internal quality control provide essential information on the long term reproducibility and other performance characteristics of the method including the analytes and commodities which were incorporated during the extension of the method.

The basic performance characteristics to be tested and the appropriate test procedures are described in Table 2.

For effective performance verification, analyse samples concurrently with appropriate quality control analyses (blank and recovery determinations, reference materials, etc.). Control charts may be used to check for trends in performance of the method and to ensure that statistical control is maintained.

4.5.2   *Construction and use of control charts*

4.5.2.1 Control charts may be a useful tool for demonstrating the performance of a method and the reproducibility of its selected parameter. One example for that is the control chart for recoveries. Its application depends on the tasks of the laboratory. When a large number of the same type of sample is analysed for the same active ingredients the control chart is based on the mean recovery and its standard deviation obtained during the regular use of the method. When small numbers of each of a large variety of samples are analysed for a great number of analytes with a multi-residue procedure the control charts cannot be applied in the usual way. In such cases, initially a control chart is constructed with the average recovery (Q) of representative analytes in representative matrices and the typical within-laboratory reproducibility coefficient of variation (CV_{Atyp}), obtained as described below. When the average recovery data and their coefficient of variation obtained during method validation for individual analyte/sample matrices are not statistically different, each can be considered as an estimate of the true recovery and precision of the method, and with their appropriate combination the typical recovery (Q_{typ}) and coefficient of variation (CV_{Atyp}) of the method can be established and used for constructing the initial control chart. The warning and action limits are $Q_{typ} \pm 2*CV_{Atyp}*Q$ and $Q_{typ} \pm 3*CV_{Atyp}*Q$, respectively.

4.5.2.2 When the method is applied for regular analysis of various analyte/matrix combinations represented during the validation of the method, the individual recoveries are plotted on the chart. The reproducibility of the method during its normal use may be somewhat higher than obtained at the validation of the method. Therefore, if some of the recoveries are outside the warning limits or occasionally the action limits, but they are within the ranges calculated from the CV_A values specified in Table 3, no special action is required.

4.5.2.3 Based on the additional 15-20 recovery tests performed during the regular use of the method, as part of performance verification, the mean or typical recovery and the CV_A shall be recalculated and a new control chart constructed which reflects the long term reproducibility of the application of the method. The new parameters established must be within the acceptable ranges specified in Table 3.

4.5.2.4 If this is not achievable, for example in the case of particularly problematic analytes, results from samples should be reported as having poorer accuracy or precision than is normally associated with pesticide residues determination.

4.5.2.5 During the regular use of the method, if the average of the first ≥10 recovery tests for a particular analyte/sample matrix is significantly different (P=0.05) from the average recovery obtained for the representative analyte/sample matrices, the Q_{typ} and CV_{typ} are not applicable. Calculate new warning and action limits for the particular analyte/sample matrix, applying the new average recovery and the CV values measured.

4.5.2.6 If performance verification data repeatedly fall outside the warning limits (1 in 20 measurements outside the limit is acceptable), the application conditions of the method must be checked, the sources of error(s) identified, and the necessary corrective actions taken before use of the method is continued.

4.5.2.7 If performance verification data are outside the refined action limits established according to 4.5.2.1 to 4.5.2.3 section, the analytical batch involved (or at least samples in which residues found are ≥0.7 AL or 0.5 AL, for regularly and occasionally detected analytes, respectively) should be repeated.

4.5.2.8 Re-analysis of analytical portions of positive samples is another powerful way of performance verification. Their results can be used to calculate the overall within-laboratory reproducibility of the method (CV_{Ltyp}) in general or for a particular analyte/sample matrix. In this case, the CV_{Ltyp} will also include the uncertainty of sample processing, but will not indicate if the analyte is lost during the process.

4.6 CONFIRMATORY TESTS

4.6.1   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 pesticides that are not normally associated with that commodity, or where MRLs appear to have been exceeded. Samples may contain interfering chemicals that may be misidentified as pesticides. Examples in gas chromatography include the responses of electron-capture detectors to phthalate esters and of phosphorus-selective detectors to compounds containing sulphur and nitrogen. As a first step, the analysis should be repeated using the same method, if only one portion was analyzed 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 performance verification data.

4.6.2   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 determination but, although it is difficult to quantify residues at this level, it is essential to provide adequate confirmation of both level and identity.

4.6.3   The need for confirmatory tests may depend upon the type of sample or its known history. In some crops or commodities, certain residues are frequently found. For a series of samples of similar origin, which contain residues of the same pesticide, it may be sufficient to confirm the identity of residues in a small proportion of the samples selected randomly. Similarly, when it is known that a particular pesticide has been applied to the sample material there may be little need for confirmation of identity, although a randomly selected results should be confirmed. Where "blank" samples are available, these should be used to check the occurrence of possible interfering substances.

4.6.4   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 6).

4.6.5   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 6.

4.7 MASS SPECTROMETRY

4.7.1   Residue data obtained using mass spectrometry can represent the most definitive evidence and, where suitable equipment is available, it is the confirmatory technique of choice. The technique can also be used for residue screening purposes. Mass spectrometric determination of residues is usually carried out in conjunction with a chromatographic separation technique to provide retention time, ion mass/charge ratio and ion abundance data simultaneously. The particular separation technique, the mass spectrometer, the

interface between them and the range of pesticides to be analysed are usually interdependent and no single combination is suitable for the analysis of all compounds. Quantitative transmission of labile analytes through the chromatographic system and interface is subject to problems similar to those experienced with other detectors. The most definitive confirmation of the presence of a residue is the acquisition of its "complete" electron-impact ionisation mass spectrum (in practice generally from m/z50 to beyond the molecular ion region). The relative abundances of ions in the spectrum and the absence of interfering ions are important considerations in confirming identity. This mode of analysis is one of the least selective and interference from contaminants introduced during the production or storage of extracts should be scrupulously avoided. Mass spectrometer data systems permit underlying interference (eg column bleed) signals to be removed by "background subtraction" but this technique must be used with caution. Increased sensitivity can usually be achieved by means of limited mass range scanning or by selected ion monitoring but the smaller the number of ions monitored (especially if these are of low mass), the less definitive are the data produced. Additional confirmation of identity may be obtained (i) by the use of an alternative chromatographic column; (ii) by the use of an alternative ionisation technique (eg chemical ionisation); (iii) by monitoring further reaction products of selected ions by tandem mass spectrometry (MS/MS or MS[n]); or (iv) by monitoring selected ions at increased mass resolution. For quantification, the ions monitored should be those that are the most specific to the analyte, are subject to least interference and provide good signal-to-noise ratios. Mass spectrometric determinations should satisfy similar analytical quality control criteria to those applied to other systems.

4.7.2   Confirmation of residues detected following separation by HPLC is generally more problematic than where gas chromatography is used. If detection is by UV absorption, production of a complete spectrum can provide good evidence of identity. However, UV spectra of some pesticides are poorly diagnostic, being similar to those produced by many other compounds possessing similar functional groups or structures, and co-elution of interfering compounds can create additional problems. UV absorption data produced at multiple wavelengths may support or refute identification but, in general, they are not sufficiently characteristic on their own. Fluorescence data may be used to support those obtained by UV absorption. LC-MS can provide good supporting evidence but, because the spectra generated are generally very simple, showing little characteristic fragmentation, results produced from LC-MS are unlikely to be definitive. LC-MS/MS is a more powerful technique, combining selectivity with specificity, and often provides good evidence of identity. LC-MS techniques tend to be subject to matrix effects, especially suppression, and therefore confirmation of quantity may require the use of standard addition or isotopically-labelled standards. Derivatisation may also be used for confirmation of residues detected by HPLC (paragraph 4.6.5.4).

4.7.3   In some instances, confirmation of gas chromatographic findings is most conveniently achieved by TLC. Identification is based on two criteria, Rf value and visualisation reaction. Detection methods based on bioassays (e.g. enzyme -, fungal groth or chloroplast inhibition) are especially suitable for qualitative confirmation as they are specific to certain type of compounds, sensitive and normally very little affected by the co-extracts. The scientific literature contains numerous references to the technique, the IUPAC Report on Pesticides (13) (Bátora, V., Vitorovic, S.Y., Thier, H.-P. and Klisenko, M.A.; Pure & Appl. Chem., 53, 1039-1049 (1981)) reviews the technique and serves as a convenient introduction. The quantitative aspects of thin-layer chromatography are, however, limited. A further extension of this technique involves the removal of the area on the plate corresponding to the Rf of the compound of interest followed by elution from the layer material and further chemical or physical confirmatory analysis. A solution of the standard pesticide should always be spotted on the plate alongside the sample extract to obviate any problems of non-repeatability of Rf. Over-spotting of extract with standard pesticide can also give useful information. The advantages of thin layer chromatography are speed, low cost and applicability to heat sensitive materials; disadvantages include (usually) lower sensitivity and separation power than instrumental chromatographic detection techniques and need for more efficient cleanup in case of detections based on chemicals colour reactions.

4.8   DERIVATISATION

This area of confirmation may be considered under three broad headings.

(a) Chemical reactions

Small-scale chemical reactions resulting in degradation, addition or condensation products of pesticides, 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 pesticide should be treated alongside the suspected residue so that the results from each maybe directly compared. A fortified 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. A review of chemical reactions which have been used for confirmatory purposes has been published by Cochrane, W.P. (Chemical derivatisation in pesticide analysis, Plenum Press, NY (1981)). 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 is the photochemical alteration of a pesticide residue to give one or more products with a reproducible chromatographic pattern. A sample of standard pesticide and fortified extract should always be treated in a similar manner. Samples containing more than one pesticide 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.

(c) Other methods

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

4.9 THE CONCEPT OF LOWEST CALIBRATED LEVEL (LCL)

4.9.1   When the objective of the analysis is to monitor and verify the compliance with MRLs or other ALs, the residue methods must be sufficiently sensitive to reliably determine the residues likely to be present in a crop or an environmental sample at or around the MRL or AL. However, for this purpose it is not necessary to use methods with sufficient sensitivity to determine residues at levels two or more orders of magnitude lower. Methods developed to measure residues at very low levels usually become very expensive and difficult to apply. The use of LCL (see Glossary) would have the advantage of reducing the technical difficulty of obtaining the data and would also reduce costs. The following proposals for LCLs in various samples may be useful in enabling the residue chemist to devise suitable methods.

4.9.2   For active ingredients with agreed MRLs, the LCL can be specified as a fraction of the MRL. For analytical convenience this fraction will vary and could be as follows:

MRL (mg/kg)	LCL (mg/kg)
5 or greater	0.5
0.5 up to 5	0.1 increasing to 0.5 for higher MRLs
0.05 up to 0.5	0.02 increasing to 0.1 for MRLs
less than 0.05	0.5 x MRL

When the MRL is set at the limit of determination of the analytical method, the LCL will also be at this level.

4.10 EXPRESSION OF RESULTS

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 level, rather than less than a level calculated by extrapolation. Generally results are not corrected for recovery, and they may only be corrected if the recovery is significantly different from 100%. If results are reported corrected for recovery, then both measured and corrected values should be given. The basis for correction should also be reported. Where positive results 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 value obtained should be reported. Where positive results derive from analysis of multiple test portions, the arithmetic mean of the lowest valid values 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 at lower concentrations the precision may be in the range of 50%, the residue values below 0.1 should be expressed with one significant figure only.

Figure II.1. Overview of Method Validation

ANALYTICAL

Validated for purpose — Not validated for purpose

Performance verified — No → Parameter Identification, **Table 1**

Yes → Method validation — Method modification

Yes → Currently fit for Purpose (decision to be confirmed routinely by QC)

No → Unfit for Purpose
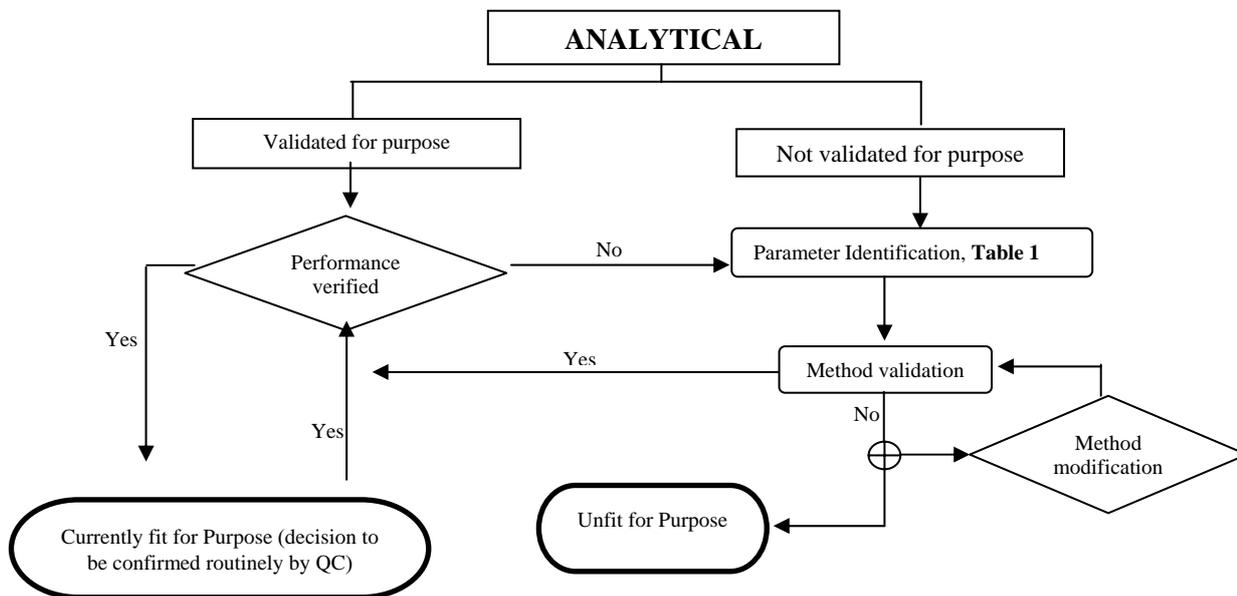
Figure II.2. Verification of Analyte Stability

ANALYTE STABILITY IN SOLVENT

Known — Unknown

Determine stability — Yes

No → Condition modification

Unfit for purpose

ANALYTE STABILITY IN EXTRACTS & SAMPLES

Known — Unknown

Yes → **Performance verification Or Full method validation**

Determine stability — Yes

No → Condition modification

Unfit for purpose
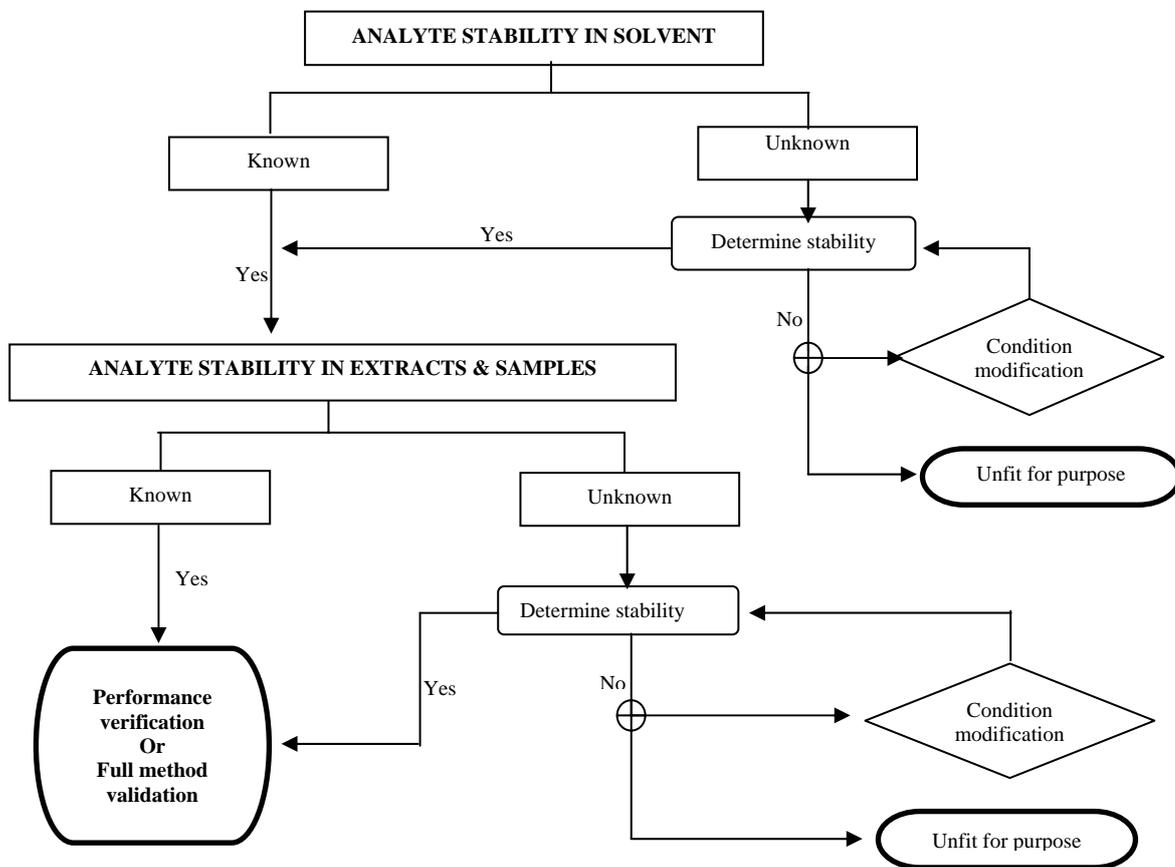
Table 1 Summary of parameters to be assessed for method validation

Parameters to be tested	Existing analytical method, for which previous tests of the parameter have shown that it is valid for one or more analyte/matrix combinations					Modification of an existing method	New method, not yet validated	Experiment types which may be combined
	Performance verification*	Additional matrix	Additional analyte	Much lower concentration of analyte	Another laboratory			
Specificity (show that the detected signal is due to the analyte, not another compound)	No (provided criteria for matrix blanks and confirmation of analyte are met)	Yes, if interference from matrix is apparent in QC	Yes	Yes, if interference from matrix is apparent in QC	Rigorous checks not necessary if the performance of the determination system is similar or better	Yes or No. Rigorous checks may be necessary if the determination system is fundamentally different or where the extent of interferences from the matrix is uncertain	Yes. Rigorous checks may be necessary if the determination system is different or where the extent of interferences from the matrices are uncertain, compared with existing methods	
Analytical Range, Recovery through extraction, clean-up, derivatisation and measurement	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Calibration range Analytical range LOD/LOQ Matrix effect
Calibration range for determination of analyte	No	No	Yes	Yes	Yes, for representative analytes	Yes, for representative analytes	Yes, for representative analytes	Linearity, reproducibility and signal/noise
LOD and LOQ	No	Yes, (partial if matrix is from a represented class)	Yes, partial for represented analytes	Yes	Yes	Yes	Yes	Lowest calibrated level, and low level spike recovery data
Reporting Limit, LCL	Yes	No	No	No	No	No	No	
Analyte stability in	No	Yes, unless	Yes, unless	Yes	No	No, unless	Yes, if extraction/final	

| Parameters to be tested | Existing analytical method, for which previous tests of the parameter have shown that it is valid for one or more analyte/matrix combinations | | | | | Modification of an existing method | New method, not yet validated | Experiment types which may be combined |
	Performance verification*	Additional matrix	Additional analyte	Much lower concentration of analyte	Another laboratory			
sample extracts$^{\Xi\ =}$		matrix is from a represented class	the analyte is represented			extraction/final solvent is different, or the clean-up is less stringent	solvent is different from that used in an existing method, or the clean-up is less stringent, compared with existing methods used.	
Analyte stability during sample storage$^{\Xi 9}$	Yes	Yes	Yes,	Ideally	No	No	No	
Extraction efficiency$^{\Xi\upsilon}$	No	Ideally	Ideally	Ideally	No	No, unless different extraction conditions employed	Yes, unless previously tested extraction procedure is used.	
Homogeneity$^{\Xi}$ of analytical samples	Yes$\cong$	No, unless the matrix is substantially different	No	No	No, unless the equipment is changed	No, unless the equipment is changed	Yes, unless a previously tested sample processing procedure is used	See below
Analyte stability in sample processing$^{\Xi}$	No	Yes, unless a represented matrix	Yes, unless a represented analyte	Ideally	No	No, unless procedure involves higher temperature, longer time, coarser comminution, etc.	No, unless procedure involves higher temperature, longer time, finer comminution, etc. than validated procedures.	Repeatability, reproducibility

* On-going quality control

$^{\Xi}$ If relevant information is not available

$^{=}$ Representative analytes may be chosen on the basis of hydrolysis, oxidation and photolysis characteristics

[9] Stability data in/on representative commodities should provide sufficient information. Additional tests are required, for example, where:

 a samples are stored beyond the time period tested (eg. stability tested up to 4 weeks and measurable analyte loss occurs during this period, samples not analyzed until 6 weeks),

 b stability tests were performed at $\leq$ -18 $^{\circ}$C, but the samples are stored in the laboratory at $\leq$ 5 $^{\circ}$C;

 c samples are normally stored at $\leq$–15°C, but storage temperature rises to +5 $^{\circ}$C).

[υ] Information on efficiency of extraction may be available from the manufacturer or company that is registering the compound.

≅ Occasionally with repeated analysis of test portions of positive samples.

Table 2 Parameters to be assessed for method validation in various circumstances

Parameter	Level(s)	No. of analyses or type of test required	Quantitative method	Criteria Screening method	Comments
1. Within-Laboratory (single laboratory) performance of optimised method					
1.1 Analyte stability in extracts and standard solutions	At ≤AL, or with well detectable residues	≥5 replicates at each appropriate point in time (including zero) and for each representative analyte/commodity. Fortify blank sample extracts to test stability of residues. Compare analyte concentration in stored and freshly made standard solutions.	No significant change in analyte concentration in stored extracts and analytical standards (P = 0.05)	At the end of the storage period, residues added at LCL are detectable	The test of stability in extracts is required if the analytical method is suspended during the determination process, and the material will likely be stored longer than during deter-mination of precision, or if low recoveries were obtained during optimisation of the method. During method optimisation, recovery should be measured against both "old" and "freshly prepared" calibration standards, if the recovery extracts are stored. Storage time should encompass the longest period likely to be required to complete the analysis.
1.2 Calibration function Matrix effect	LCL to 2 (3) times AL	Test the response functions of all analytes included in the method with ≥2 replicates at ≥3 analyte levels plus blank sample. For non-linear response, determine response curve at ≥7 levels and ≥3 replicates. Test the matrix effect with all representative analytes and matrices. Apply the standards prepared in solvent and sample extracts randomly.	For linear calibration: regression coefficient for analytical standard solutions (r) ≥ 0.99, the SD of residuals ($S_{y/x}$) ≤ 0.1 For polynomial function (r) ≥ 0.98. The matrix effect is confirmed if the difference is significant at P = 0.05.	For linear calibration: regression coefficient (r) ≥ 0.98. SD of residuals ≤ 0.2 For polynomial function (r) ≥ 0.95	Calibration parameters may be established during optimisation of the procedure, determination of precision or detection capability. Prepare calibration solutions of different concentrations For MRM perform calibration with mixtures of analytes ("standard mixture"), which can be properly separated by the chromatographic system. Use matrix matched analytical standards for further tests if matrix effect is significant. The method validation may not give definite information for the matrix effect, because ma-trix effects change with time, with sample (sometimes), with column, etc.
1.3 Analytical range, accuracy,	LCL to 2 (3) times AL*	Analyse representative analyte matrix combinations: ≥ 5 analytical portions spiked at zero, LCL, AL and ≥3 replicates at 2-3	LOQ should be fit for purpose. Mean recovery and CV_A see Table 3.	All recoveries are detectable at LCL	The analysts should demonstrate that the method is suitable for determining the presence of the analyte at the appropriate AL with the maximum (false negative and false positive)

Parameter	Level(s)	No. of analyses or type of test required		Criteria		Comments
			Quantitative method	Screening method		
trueness precision, limit of detection (LD), limit of quantitation (LOQ)		AL level. The recovery tests should be divided among the analysts, who will use the method, and instruments that will be involved in the analysis.	Mean residue* measured in reference material is not significantly different from the consensus value (P = 0.05).			errors specified. For MRM, the fortification level of blank samples should cover the ALs of analytes represented. Consequently they may not correspond with the actual AL for the representative analytes. Fortify analytical portions with standard mixtures. The accuracy and precision ranges determined for representative analyte/matrix combinations can be considered typical for the method, and will be used as applicability criteria for extension to new analytes and commodities, as well as initial guidance for internal quality control of the method. Report uncorrected results, mean recovery and CV_A of replicates. CV_A is equivalent to the within laboratory reproducibility of analysis of samples. * Correct the results for mean recovery if it is significantly different from 100 %. Where the method does not permit recovery to be estimated, accuracy and precision are those of calibration.
1.4 Specificity and selectivity of analyte detection	At lowest calibration level (LCL)	Identify by mass spectrometry, by a similarly specific technique, 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.	Measured response is solely due to the analyte. Residues measured on two different columns should be within the critical range of replicate chro-matographic determinations.	The rate of false negative samples (β error) at AL should typically be < 5%.		Applies only to a specific combination of separation and detection technique. Samples of known treatment history may be used instead of untreated samples, for analytes other than that applied during treatment. Maturity of sample matrices may significantly affect the blank sample response. Blank values shall also be regularly checked during performance verification (see Section 4 below). Report typical peaks present in the extracts of

Parameter	Level(s)	No. of analyses or type of test required		Criteria		Comments
			Quantitative method	Screening method		
		Determine and report selectivity (δ) of detector and relative response factors (RRF) of representative analytes with specific detectors used..				blank samples. The LCL should preferably be $\leq 0.3AL$, except when the AL is set at or about the limit of quantitation. The test may be performed in combination with the determination of decision limit and detection capability and will also provide information for the relative RRts and RRFs of compounds. Alter chromatographic conditions if blank sample response interfere with the analyte or use an alternative detection system. Suitable combination of selective detectors increases specificity, because the amount of information about the analyte is increased.
1.5 Selectivity of separation	At AL	Determine 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 RRt-s on columns of different polarity. Determine and report resolution (R_S) and tailing factors (T_f) of critical peaks.	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.	Tentative identification of all analytes tested. (Not all analytes need to be separated)		Unless the chromatographic separation and spectrometric detection is used in combination, report RRt values on columns of different polarity, which enable the separation (minimum $R \geq 1.2$) of all analytes tested. The test may be combined with the determination of calibration function and matrix effect (see. 1.7)
1.6 Homogeneity of analyte in analytical sample	At about AL or well detectable residues	Analyse ≥ 5 replicate test sample portions of one representative commodity from each group (Table 5), post-processing. Determine CV_{Sp} with analysis of variance. The analyte homogeneity should be checked with analytes known to be stable.	$CV_{Sp} \leq 10\%$.	$CV_{Sp} \leq 15\%$ For screening methods it may be desirable to take a portion in which residues can be expected to be highest (e.g. citrus peel) and achievement of homogeneity may be unnecessary.		Use preferably commodities with incurred stable surface residues or treat the surface of a small portion of the natural units ($<20\%$) of laboratory sample before cutting or chopping to represent worst scenario of sample processing. Processing validated for use with any subsequent procedure. Validation applicable to other commodities with similar physical properties, and it is independent of the analyte. The test may be combined with testing stability

Parameter	Level(s)	No. of analyses or type of test required		Criteria		Comments
			Quantitative method	Screening method		
						of analyte (see Section 1.7 of this Table) Determine the sampling constant[3,4] to calculate the size of analytical portion required to satisfy quality criteria of $CV_{Sp} \le 10\%$ specified. The CV_{Sp} may not need to be determined separately if the CV_L of the incurred residues are within the limits specified in Table 2.
1.7 Analyte stability during sample processing	About AL	Fortify commodities with known amounts of analytes before processing the sample. Analyse ≥5 replicates of each commodity, post-processing, Apply a stable marker compound together with the analytes tested For MRM and group specific methods, GSM, several analytes, which can be well separated, can be tested together.	The stability of the analyte need not be specified if the average overall recovery of analyte added before sample processing (including procedural recovery) and CV_A are within the ranges specified in Table 3. Quantify stability if the overall recovery and the procedural recovery is significantly different (P=0.05).	Analyte added at LCL remains detectable after processing		The temperature of the sample during processing 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_L of labile and stable marker compounds. Use these compounds for internal QA tests (see section 4). Express the ratio of average concentration of labile and stable compounds to indicate stability of residues. CV's of stable compounds will indicate the within laboratory repeatability as well.
1.8 Extraction efficiency	About AL or readily measurable residues	Analyse ≥5 replicate portions of samples or reference material with incurred residues. Compare the reference (or different) procedure with that under test. For MRM the analytes tested should preferably have a wide range of Pow values. Only to be determined using incurred residues.	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. Or, the consensus value of reference material and the mean residue should not differ significantly at	The mean incurred residues, known to be present at or about the LOQ or LCL, are actually detectable in the samples.		Temperature of the extract, speed of blender or Ultra Turrax, time of extraction and solvent/water/matrix ratio may significantly affect the efficiency of extraction. The effect of these parameters can be checked with ruggedness test. The optimised conditions should be kept constant as far as possible. Validation is generally applicable for commodities within one group and represented analytes of similar physical and chemical properties. Validation is independent from

[3] Wallace, D. and Kratochvil, B., Analytical Chemistry, **59**, 1987, 226.

[4] Ambrus, A., Solymosné, E.M. and Korsós, I., J. Environ. Sci. and Health, **B31**, 1996, 443.

Parameter	Level(s)	No. of analyses or type of test required		Criteria		Comments
			Quantitative method	Screening method		
			P=0.05 level when calculated with CV_A of the method tested. 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 following the extraction).			subsequent procedures in the method. The average recovery of each method shall be determined from spiked analytical portions. Correct results with average recovery of analysis if it is significantly different from 100%. According to some regulations the ability of screening kits should be tested to detect a positive at 95% confidence.
1.9 Analyte stability during sample storage	About AL	Analyse freshly homogenised samples containing incurred residues, or homogenise and spike blank samples (time 0), and then analyse samples stored according to normal procedures of the laboratory (usually at ≤ -18 °C). The storage time should be ≥ than the longest interval foreseen between sampling and analysis. ≥5 replicates at each time point. When the stored portions are analysed ≥ 4 occasions, test ≥2 spiked portions, and ≥ 1 blank portion spiked at the time of analysis. Analytical portions should be thawed only	No significant loss of analyte during storage (P = 0.05)	Analyte added at lowest calibration level, LCL, remains detectable after storage		Storage is validated for use with any subsequent procedure. Validation is 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 (e.g. hydrolysis) of the analyte and the intended use of the substance. Useful information can be obtained on stability during storage from the JMPR evaluations[5] or from dossiers submitted for registration. Report the initial residue concentration, the remaining residue concentration and the procedural recovery of the analyte. Unnecessary sample storage can be avoided by a careful planning for sampling and consequent

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[5] FAO, Pesticide Residues in Food – Evaluations; published annually in the series of FAO Plant Production and protection Papers

Parameter	Level(s)	No. of analyses or type of test required		Criteria		Comments
			Quantitative method		Screening method	
		immediately before or during extraction.				analysis through administrative arrangement, which is not a part of analytical method.
2. Extension of the validated method						
2.1 Analyte stability during sample storage, processing, and in extracts and standard solutions.	See 1.1, 1.2 & 1.9					Only if information on stability under the processing conditions and on the representative matrix is not already available
2.2 Calibration function, matrix effect	LCL to 2 (3) AL:	Three point calibration embracing AL with and without matrix matched analytical standards	For linear calibration: regression coefficient for analytical standard solutions (r) $\geq$ 0.99. SD of relative residuals ($S_{y/x}$) $\leq$ 0.1 For polynomial function (r) $\geq$ 0.98.		For linear calibration: regression coefficient (r) $\geq$ 0.98. SD of relative residuals $\leq$ 0.2 For polynomial function (r) $\geq$ 0.95.	The method validation may not give definite information for the matrix effect, because matrix effects change with time, with sample (sometimes), with column, etc.
2.3 Accuracy, precision, LD, LOQ	at AL	Planned in advance: (a) Analyse 3 analytical portions of representative sample matrices of interest fortified at AL Unexpectedly found: Fortify 2 preferably 3 additional portions of analytical sample approximately at the level of the new analyte. Calculate the recovery of added analyte. Use similar sample matrix for recovery test if appropriate amount of analytical sample is not available..	The residues recovered should be within the repeatability limits of the method: Three portions: $C_{max} - C_{min} \leq 3.3 CV_{Atyp}Q$ Two portions: $C_{max} - C_{min} \leq 2.8 * CV_{Atyp}Q$ CV_{Atyp} is the typical repeatability coefficient of variation of the method to be adapted. Q =average recovery of the new analyte, and it shall comply with Table 3.		Analytes added to blank samples at target reporting level should be measurable in all tests.	Use CV_{Atyp} established during method validation. The method should only be tested with commodities representing the intended use (possible misuse) of the analyte.

Parameter	Level(s)	No. of analyses or type of test required		Criteria		Comments
			Quantitative method	Screening method		
2.4 Specificity and selectivity of analyte detection	At LCL	Identify by mass spectrometry, or by the appropriate combination of separation and detection techniques available. Planned in advance: (a) Analyse one representative blank sample from each commodity group of interest (in which the new analyte is likely to be present). Analyse new matrix with representative compounds. Unexpectedly found: (b) Check response of blank sample (if available), or demonstrate that the response measured corresponds solely to the analyte, using the best technique available in the laboratory. Check δ and RRF of detection and RRts of representative analytes. Compare RRt and response of new analyte with other analytes tested during method validation and with blank responses obtained during extension of the method and the prior validation of the method.	Measured response is solely due to the analyte. The detection system used should have equal or better detector performance than those applied during method validation. Residues measured on two different columns should be within the critical range of replicate chromatographic determinations. Relative retentions of representative analytes obtained during method validation and measured should be within 2 % for GLC and 5 % for HPLC determinations.	The rate of false negative samples (β error) at AL should be < 5%.		When the extension for a new analyte is planned, the applicability of the method shall be checked for all representative sample matrices in which the analyte may occur. When an analyte is unexpectedly detected, the performance check may be carried out for the actual matrix alone See also 1.4. The responses of blank sample(s) should not interfere with the analytes, which are likely to be measured in the sample. Report typical peaks present in blank extracts. The background noise of a new matrix extract should be within the range obtained for representative commodities/sample matrices. If the selectivity of detection does not eliminate the matrix response, use appropriate combination of chromatographic columns that enable the separation of analytes from the matrix peaks. See other options in Table 6.
2.5 Selectivity of separation	See 1.5	See 1.5	See 1.5	See 1.5		See 1.5 Only if information is not available
2.6 Extraction efficiency	See 1.8	See 1.8	See 1.8	See 1.8		See 1.8 Only if information is not available

3. Adaptation of the validated 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 derivatization without and with sample.	No interfering response above 0.3 LCL.	No interfering response above 0.5 AL	Some of the most common problems in method transfer involve differences in selection of reagents, solvents and chromatographic media, or in equipment capabilities. Whenever possible, try to confirm actual materials and equipment used by the method developer, if that information is not provided with the method or publication, as received. Substitutions can be tried after the method is working within your laboratory.
3.2 Analyte stability in extracts and standard solutions	See 1.10	See 1.1	See 1.1	See 1.1	This testing may be omitted if full information on analyte stability is provided with the method or if the method is replacing a previously used method for the analyte and the stability information has been previously generated for the previous method.
3.3 Calibration function Matrix effect	LCL to 2 (3) times AL	Test the response functions of representative analytes included in the method at $\geq$3 analyte levels plus blank. For non-linear response, determine response curve at$\geq$7 levels and $\geq$3 replicates. Test the matrix effect with representative analytes and matrices.	For linear calibration: regression coefficient for analytical standard solutions (r) $\geq$ 0.99. The SD of relative residuals $(S_{y/x}) \leq 0.1$ For polynomial function (r) $\geq$ 0.98.	For linear calibration: regression coefficient (r) $\geq$ 0.98. The SD of relative residuals $\leq$ 0.2 For polynomial function (r) $\geq$ 0.95.	Sees: 1.2
3.4 Analytical range accuracy and precision, limit of detection, limit of quantitation	Blank extract and or AL	Analyse representative analyte/matrix combinations: $\geq$ 5 analytical portions each of blank samples spiked at 0 and AL, and 3 portions spiked at 2 AL. The recovery tests should be divided among the analysts, who will use the method, and instruments that will be involved in the analysis.	Average recovery and CV_A should be within the ranges given in Table 3.	All recoveries detectable at LCL. Reference materials at AL: analyte detected.	See comments in 1.3.

3.5 Specificity and selectivity of analyte detection	At 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 1.4.	Measured response is solely due to the analyte. The detector performance (sensitivity and selectivity) should be equal or better than specified in the method. See section 1.4	The rate of false negative samples (β error) at AL should typically be < 5%.	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 section 1.4.
3.6 Analyte "homogeneity"	At about AL or well detectable residues	Test two representative commodities of different nature	$CV_{Sp}<10\%$	$CV_{Sp}<15\%$ For screening methods it may be desirable to take a portion in which residues can be expected to be highest (e.g. citrus peel) and achievement of homogeneity may be unnecessary.	The tests are performed to confirm similarity of application conditions and applicability of parameters obtained by the laboratory validating the method. When the test results in similar CV_{Sp} as reported, the conditions of sample processing may be considered similar and further tests are not required for the validation of the method.
3.7 Analyte stability in extracts and standard solutions	See 1.1	See 1.1	See 1.1	See 1.1	This testing may be omitted if full information on analyte stability is provided with the method or if the method is replacing a previously used method for the analyte and the stability information has been previously generated for the previous method.

Table 3. Within Laboratory Method Validation Criteria for Analysis of pesticide residues

Concentration	Repeatability		Reproducibility		Trueness[2,]
	$CV_A\%$ [3]	$CV_L\%$ [4]	$CV_A\%$ [3]	$CV_L\%$ [4]	Range of mean % recovery
≤1 µg/kg	35	36	53	54	50–120
> 1 µg/kg ≤ 0.01 mg/kg	30	32	45	46	60–120
> 0.01 mg/kg ≤ 0.1 mg/kg	20	22	32	34	70–120
> 0.1 mg/kg ≤ 1 mg/kg	15	18	23	25	70–110
> 1 mg/kg	10	14	16	19	70–110

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

2. These recovery ranges are appropriate for multi-residue methods. Stricter criteria may be necessary for some purposes e.g. methods for single analytes or veterinary drug residues (see Codex V3, 1996).

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

4. CV_L: Overall coefficient of variation of a laboratory results, including up to 10% variability of residues between analytical portions (CV_{Sp}). Note: the variability of residues in between analytical portions can be calculated from the uncertainty of the measurement of replicate portions of samples (CV_L) containing residues; $CV_L^2 = CV_{Sp}^2 + CV_A^2$.

Table 4 Requirements for performance verification

Parameter	Level(s)	No. of analyses or type of test required		Criteria	Comments
			Quantitative method	Screening method	
4. Quality control (performance verification)					
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 derivatization without sample.	No interfering response ≥0.3 LCL.	No interfering response ≥ 0.5AL.	Alternately, if the sample blank, calibration and the recovery are satisfactory then the suitability of reagents etc. are confirmed.
4.1.2 Calibration and analytical range		Single point calibration may be used with standard mixtures, if the intercept of calibration function is close to 0. Apply multi point calibration (3x2) for quantitative confirmation.	The analytical batch may be considered to be under statistical control if the analytical standards and sample extracts are injected alternately, and the calculated SD of relative residuals is ≤0.1.	Analyte is detected at LCL.	Standard solution and samples should be injected alternately. Bracketing with appropriate standard injections may provide a time saving alternative to multi point calibration especially if auto sampler is not available. As system response often changes multi point calibration shall be performed regularly to confirm that the intercept is close to zero. Multi point calibration is not necessary for quantitative confirmation if the calibrant is very close in concentration to that of the sample.
4.1.3 Accuracy and precision	Within analytical range	Include in each analytical batch ≥1 sample either fortified with standard mixture, or the reanalysis of a replicate portion of a positive sample.	The performance of detector and chromatographic column shall be equal or better than specified in the method. Preferably all recoveries should be within the warning limit of control chart constructed according to section 4.5.2. On a long run one of every 20 or 100 samples may be outside the warning and action limits, respectively. 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.		Fortify analytical portion with standard mixture(s). Alter standard mixtures in different batches to obtain recoveries for all analytes of interest at regular intervals. Perform alternately recovery studies at AL as well as at LCL and 2 times AL, as appropriate, to confirm applicability of the method within the analytical range. The frequency of recovery studies at AL should be 2 to 3 times higher then those at other levels. Repeated analysis of positive samples may

			$C_{max} - C_{min} > 2.8 * CV_{Ltyp} Q$		replace the recovery test in a particular batch.
			Q is the average residue obtained from the replicate measurements, the CV_{Ltyp} is the measure of within laboratory reproducibility, which includes the combined uncertainty of sample processing and analysis.		For MRM prepare commodity/sample specific standard mixtures from the analytes which may occur in a particular sample. The selection of analytes for one mixture should assure selective separation/detection without any problem.
					For tentative identification: prepare analytical batches containing the appropriate detection test mixture, and samples.
					For quantitative determination/confirmation include in the analytical batch the detection test mixture, appropriate number of calibration mixtures, fortified blank sample(s), or one repeated positive sample and the new positive samples
					Inject standards and samples alternately.
4.1.4 Selectivity of separation, Specificity of detection Performance of detectors		Include appropriate detection test mixture in each chromatography batch. Include untreated commodity (if available) 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 ≥0.7 AL level.	R_s, T_f of test compounds, and RRF and δ of the detection should be within the specified range. Relative retention should be within 2 % for GLC and 5 % for HPLC determinations. Detector performance should be within specified range. Sample co-extractives interfering with the analyte should not be present ≥ 0.3 LCL. The recovery of added standard should be within the acceptable recovery range of the analyte.	Detector performance should be within specified range. Analyte should be seen above LCL or CCα for banned compounds.	This is also sometimes referred to as a "system suitability" test. Prepare detection test mixture for each method of detection. Select the components of the mixture in order to indicate the characteristic parameters of chromatographic separation and detection. Adjust RRt database for the compounds of detection test mixture and analytes used for calibration. Define the RRF specific for the detection system. Perform quantitative confirmation with analytical standards prepared in blank matrix extract if matrix effect is significant.

4.1.5 Analyte homogeneity in processed sample	At well detectable analyte concentration.	Select a positive sample randomly. Repeat analysis of another one or two analytical portions.	The residues measured on two different days should be within the reproducibility limit of replicate analytical portions: $C_{max} - C_{min} \leq 2.8 * CV_{Ltyp}Q$ Q is the average residue obtained from the replicate measurements, the CV_{Ltyp} is the combined uncertainty of sample processing and analysis obtained during method validation.	Perform test alternately to cover each commodity analysed. Test homogeneity at the beginning of growing season, or 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.
4.1.6 Extraction efficiency				The efficiency of the extraction cannot be controlled during the analysis. To ensure appropriate efficiency, the validated extraction procedure should be carried out without any change.
4.1.7 Duration of analysis			The samples, extracts etc. should not be stored longer than the period for which the storage stability was tested during method validation. Storage conditions should be regularly monitored and recorded.	Examples for the need of additional storage stability tests are given under Table 1.
4.2 Analyte detected occasionally				
FOLLOW TESTS DESCRIBED IN 4.1 WITH THE FOLLOWING EXCEPTIONS				
4.2.1Accuracy and precision	At around AL	Reanalyse another analytical portion; Use standard addition at the measured level of analyte.	The residues measured on two different days should be within the critical range: $C_{max} - C_{min} \leq 2.8 * CV_{Ltyp}Q$ Q is the average residue obtained from the replicate measurements, the CV_{Ltyp} is obtained during method validation. The recovery following standard addition shall be within action limits.	Check accuracy if residue found at $\geq 0.5AL$.
4.3 Methods used at irregular intervals				
Follow tests described in 4.1 with the following exceptions				
4.3.1 Accuracy and precision	At AL and LCL	Include one fortified sample at LCL and two samples at AL in each analytical batch. Use standard addition if untreated	Minimum two recoveries shall be within warning limit, one may be within action limit. The residues measured in replicate portions should be	The acceptable results also prove the suitability of chemicals, adsorbents and reagents used. Confirm residues above 0.5AL.

(repeatability)		sample (similar to those analysed in the batch) is not available. Perform analysis with ≥2 analytical portions.	within the critical range: $C_{max} - C_{min} \leq 2.8 \cdot CV_{Ltyp}Q$ or $C_{max} - C_{min} \leq f_{(n)} \cdot CV_{Ltyp}Q$ Q is the average residue obtained from the replicate measurements, the CV_{Ltyp} is obtained during method validation, $f_{(n)}$ is the factor for calculation of extreme range depending on the number of replicate samples.		If performance criteria were not satisfied, the method shall be practised and its performance characteristics (Q, CV_{Atyp}, CV_{Ltyp}) 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 1**.		
4.4.1 Chromatographic column	Test selectivity of separation, resolution, inertness, RRt values.		Performance characteristics should not be affected		Apply appropriate test mixtures to obtain information on the performance of the column.
4.4.2 Equipment for sample processing	Homogeneity of processed sample; Stability of analytes.		Test described in 1.6 and 1.7 shall be performed and they should give results conforming to the relevant criteria..		Homogeneity test is only necessary if the degree of comminution and/or mixing is inferior to that of the original equipment. The stability of analytes needs to be tested if the processing time and temperature are significantly increased.
4.4.3 Equipment for extraction	Compare field incurred residue levels detected with the old and new equipment in ≥ 5 replicates		The mean residues should not be significantly different at p=0.05 level.		Test is necessary if a new type of equipment is used
4.4.4 Detection	Test selectivity of separation and selectivity and sensitivity of detection		Performance characteristics should be the same or better specified in the description of the method.		Test also detectability separately with new detection reagents.
4.4.5 Analyst	≥5 recovery tests at each level (LCL, AL and 2 (3) AL), re-analysis of one blank sample and two positive samples (unknown to 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.		This is a minimum requirement. Laboratories in some areas of residue work 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 analytes fortified by the analyst at a minimum of 3 levels in duplicate; (3) minimum of 1 analytical run containing fortified or incurred samples, 3 levels in duplicate, provided as unknowns to the analyst. All results must meet acceptability criteria, or be repeated.

4.4.6 Laboratory	Accuracy and precision ≥3 recovery tests at each level (LCL, AL and 2 (3) AL) by (different) analyst(s) on different days.	All results should be within the warning limits specified for the method in the laboratory.	The reproducibility of the method under the new conditions must be established and it has to be done by more than one analyst if available.

Table 5. Representative commodities/samples for validation of analytical procedures for pesticide residues

Commodity Group	Common properties	Commodity class[6]	Representative species
Plant products			
I.	High water and chlorophyll content	Leafy vegetables Brassica leafy vegetables Legume vegetables	spinach or lettuce broccoli, cabbage, kale green beans
II.	High water and low or no chlorophyll content	Pome fruits Stone fruits Berries Small fruits Fruiting vegetables Root vegetables	apple, pear peach, cherry Strawberry grape, tomato, bell pepper, melon mushroom potato, carrot, parsley
III.	High acid content	Citrus fruits	orange, lemon
IV.	High sugar content		raisins, dates
V.	High oil or fat	Oil seeds Nuts	avocado, sunflower seed walnut, pecan nut, pistachios
VI.	Dry materials	Cereals	wheat, rice or maize grains
		Cereal products	wheat bran, wheat floor
	Commodities requiring individual test		e.g. garlic, hops, tea, spices, cranberry
Products of animal origin			
		Meats	Cattle meat, chicken meat
		Edible offals	Liver, kidney
		Fat	Fat of meat
		Milk	Cow milk
		Eggs	Chicken egg

Note: The method should be validated with representative pesticides for each commodity group. Commodities which are difficult to analyse require individual tests.

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[6] Codex Classification of Foods and Animal Feeds (CAC/MISC 4-1993)

Table 6. Examples of detection methods suitable for the confirmatory analysis of substances

Detection method	Criterion
LC or GC and Mass spectrometry	if sufficient number of diagnostic ions are monitored
LC-DAD or scanning UV	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[1]
Derivatisation	if it was not the first choice method
LC-immunogram	in combination with other techniques
LC-UV/VIS (single wavelength)	in combination with other techniques

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

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, MPL; 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[7] describes the relationship between analytical portion size and the expected variation in a well mixed analytical sample: $K_S = w\,(CV_{Sp})^8$, 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.

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[7] Wallace, D. and Kratochvil, B., Analytical Chemistry, 59, 226-232, 1987

[8] Ambrus, A., Solymosné, E.. and Korsós, I. J. Environ. Sci. Health, B31, (3) 1996

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 level 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 re-analysis 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 α (false positive). In the case of substances with zero AL, the CCα is the lowest concentration level, at which a method can discriminate with a statistical probability of $1 - \alpha$ whether the identified analyte is present. The CCα is equivalent to the limit of detection (LOD) under some definitions (usually for $\alpha = 1\%$).
	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 $1 - \alpha$ that the identified analyte content is truly above the AL.
Detection Capability (CCß)	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 CCβ is the lowest concentration at which a method is able to determine the analyte in contaminated samples with a statistical probability of $1 - ß$. In the case of substances with an established MRL, CCβ is the concentration at which the method is able to detect samples that exceed this MRL with a statistical probability of $1 - ß$.
	When it is applied at the lowest detectable concentration, this parameter is intended to provide equivalent information to the Limit of Quantitation (LOQ), but CCβ 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 factors 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 values. 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. E.g. phenoxy acetic acids, dithiocarbamates, methyl carbamates.
Incurred Residue	Residues of an analyte in a matrix arising by the route through which the trace levels would normally be expected, as opposed to residues from laboratory fortification of samples. Also weathered residue.

Individual Method	Method, which is suitable for determination of one or more specified compounds. A separate individual method may be needed, for instance to determine some metabolite included in the residue definition of an individual pesticide or veterinary drug.
Laboratory Sample	The sample as received at the laboratory (not including the packaging).
Limit of Detection (LD)	Smallest concentration where the analyte can be identified. Commonly defined as the minimum 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. IUPAC and ISO have recommended the abbreviation LD. See also Decision Limit.
Limit of Quantitation (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 level 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 system. 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 quantitation 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 level. (see also Limit of Detection)
Performance Verification	Sets of quality control data generated during the analysis 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 level.
Precision	Closeness of agreement between independent test results obtained under stipulated conditions.
Quantitative Method	A method capable of producing results, expressed as numerical values in appropriate units, with accuracy and precision which fit for the purpose. The degree of precision and trueness must comply with the criteria specified in Table 3.
Recovery	Fraction or percentage of an analyte recovered following extraction 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 physico-chemical 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 analytes.
Reproducibili-ty	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 $\beta = 5\%$). 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.
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		