GUIDELINES FOR VALIDATION OF ANALYTICAL METHODS FOR NON-AGRICULTURAL PESTICIDE ACTIVE INGREDIENTS AND PRODUCTS.
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</tbody>
</table>
1. Introduction

Non-agricultural pesticides are presently regulated in Great Britain under The Control of Pesticides Regulations (COPR) 1986, as amended, and require approval before they can be sold, supplied, stored, used or advertised. The term non-agricultural pesticide covers wood preservatives, wood treatment products, surface biocides, biocidal paints, rodenticides and insecticides/acaricides for use in public hygiene situations, avicides, bird stupefying baits and antifouling products.

This guidance document is one of a series of documents that has been developed by HSE to support the approval of non-agricultural pesticides under COPR.

1.1 Background

Following agreement of the Advisory Committee of Pesticides, HSE’s Biocides and Pesticides Unit (BPU) produced a guidance document “Data requirements for non-agricultural pesticides products and their active substances” (found in Part Three/A of the Registration Handbook). The requirements published in July 1993 establish a consolidated (core) set of data requirements for all non-agricultural pesticides.

The core data requirements apply to the registration of a pesticide containing a novel active ingredient but also are used to support the review of older active ingredients and their products.

1.2 Scope

In order to generate data to support the core data requirements for COPR robust, accurate and precise analytical methods are required.

Analytical data are required for the determination of the active ingredient, impurities, degradation products and residues in the formulation, in water and in certain circumstances in other materials.

These guidelines are intended to provide information and guidance to approval holders responsible for submitting a validated analytical method to the Biocides and Pesticide Unit (BPU) of HSE to support active ingredients and formulations under the Control of Pesticides Regulations 1986 (COPR) (as amended). These guidelines highlight what is required with respect to validation of analytical methods.

In cases where the requirements of these guidelines cannot be fulfilled, a justification must be submitted. **It is not a requirement that the whole validation data set is generated every time a method is used.**
This guidance covers approvals for non-agricultural pesticide products under COPR and the document should be read in conjunction with the 1993 guidelines on core data requirements.

**With effect from 31:12:2002 these guidelines will apply to any new method of analysis submitted to BPU in fulfilment of the core data requirements for the approval of pesticidal active ingredient and products under COPR.**

This document is available on BPU’s website:

http://www.hse.gov.uk/pesticides/

1.3 Good Laboratory Practice (GLP)

Good Laboratory Practice (GLP) is concerned with the organisational process and the conditions under which studies are planned, performed, monitored, recorded and reported. The regulations are not concerned with the interpretation and evaluation of test results. The EC Directive 87/18/EEC required member States to take all measures necessary to ensure that safety studies submitted to regulatory authorities in support of notification or registration of certain classes of chemicals were in accordance with GLP. With regard to the position of COPR the following points are relevant.

COPR does not make reference to GLP since it predates Directive 87/18/EEC and the UK GLP regulations. However, with respect to the principles of GLP and its applicability to the current statutory scheme for pesticides, the following arrangements were drawn up following a consultation exercise with Approval Holders and interested parties (Pesticides Newsletter, September 1992 and the Pesticide Register Issue 3, 1992 refer)

(i) Mammalian toxicology studies started after 30th June 1988 must be carried out in compliance with the principles of GLP
(ii) Physico-chemical studies and ecotoxicology studies after 1st January 1993 must be carried out in compliance with the principles of GLP
(iii) All other safety studies started after 1st January 1993 must be carried out in compliance with the principles of GLP

Conditions (i), (ii) and (iii) apply to studies submitted either in support of an application for approval under COPR; or to satisfy a requirement for new data arising out of a review of a pesticide active ingredient or co-formulant.

In those cases where studies have not been conducted under the principles of GLP this must be justified and the quality assurance procedures used for the study must be described.

N.B. It should be noted that method validation is considered to be an intrinsic part of method development and as such does not have to be carried out to GLP. However such work should be carried out using robust scientific principles.
2. Method validation:

2.1 What is it?

In its simplest form method validation is the evaluation of a method to ensure that its performance is suitable for the analysis being carried out. ISO define validation as:

"Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled"

[ISO 8402:1994]

2.2 Why it is needed:

Validation of an analytical method will ensure that the results of an analysis are reliable, consistent and perhaps more importantly that there is a degree of confidence in the results. As either a customer of an analytical laboratory or as a performing laboratory it must be demonstrated that the parameter you determine is the right one and that the results have demonstrated “fitness for purpose”. Method validation provides the necessary proof that a method is “fit for purpose”.

2.3 What does it entail?

There are a number of criteria for validating an analytical method and different performance characteristics will require different validation criteria. For example low-level analysis may require different assessments of validation to those required for routine analysis. These guidelines are intended to provide a “rule of thumb” for various analyses commonly used to support active ingredients under COPR and the validation deemed appropriate for that method.

Method validation data submitted to BPU should, as appropriate, address the following parameters:

- A demonstration of the accuracy and precision of the procedure
- A demonstration of the specificity of the procedure
- Linearity of response for the analyte (and internal standard, if appropriate)
- Limit of detection
- Limit of quantitation
- Sensitivity
- Ruggedness/Robustness

Where appropriate all methods using standard materials should use Certified Reference Materials (or traceable as such). It is important to note that it is the method and not the results that are validated.

N.B. Further descriptions of these parameters are given in Section 3 and a summary of the regulatory requirements for the different analytical methods is given in Section 5.
3. Parameters for Method Validation

3.1 Accuracy:

Accuracy expresses the closeness of a result to a true value and is often described using two components, trueness and precision.

Trueness:

“The closeness of agreement between the arithmetic mean of a large number of test results and the true or accepted reference value”

[BS ISO 5725-1:1994]

For some methods the true value cannot be determined exactly and it may be possible to use an accepted reference value to determine this value. For example if suitable reference materials are available or if the reference value can be determined by comparison with another method.

Analysing a sample with known concentrations can assess accuracy, for example a Certified Reference Material (CRM) and comparing the measured value with the true value as supplied with the CRM.

An alternative method for assessing accuracy is the recovery of known amounts of analyte spiked into a sample matrix by either:

i) Standard additions:

This method is used if a blank sample cannot be prepared without the analyte being present. Mean recovery should be appropriate to the concentration tested, as specified in Table 1, encompassing a range 20 % below the lowest expected concentration and 20 % above the highest expected concentration. For an impurity method the mean recovery should be within 0.1 % absolute of the theoretical concentration or 10 % relative, whichever figure is greater, for impurities in the range 0.1 - 2.5 % w/w.

ii) Assay methods:

This method involves spiking analyte in blank matrices. Spiked samples are generally prepared at 3 levels in the range 50 - 150 % of the target concentration. The matrix is constructed to mimic representative samples in all respects where possible. For impurities, spiked samples are prepared over a range that covers the impurity content, for example 0.1 - 2.5 % w/w. The analyte determination should be done using the same quantitation procedure as will be used in the final method. Recoveries should be appropriate to the concentration tested as specified in Table 1.

Recoveries depend largely on the sample matrix, sample-processing procedure and on the analyte concentration. EC document SANCO/3030/99 rev.4 11/07/00 includes a table relating expected recovery with analyte concentration.
Table 1: Expected recovery as a function of analyte concentration.

<table>
<thead>
<tr>
<th>% Active (nominal)</th>
<th>Analyte ratio</th>
<th>Unit</th>
<th>Mean Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;10</td>
<td>&gt;10⁻¹</td>
<td>&gt;10%</td>
<td>98 - 102</td>
</tr>
<tr>
<td>1-10</td>
<td>10⁻²-10⁻¹</td>
<td>1-10%</td>
<td>97 - 103</td>
</tr>
<tr>
<td>&lt;1</td>
<td>&lt;10⁻²</td>
<td>&lt;1%</td>
<td>95 - 105</td>
</tr>
<tr>
<td>0.01-0.1</td>
<td>10⁻³-10⁻²</td>
<td>0.01-0.1%</td>
<td>90 - 110</td>
</tr>
<tr>
<td>&lt;0.01</td>
<td>&lt;10⁻⁴</td>
<td>100 ppm</td>
<td>80 - 120</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>% Impurity (nominal)</th>
<th>Analyte ratio</th>
<th>Unit</th>
<th>Mean Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1</td>
<td>&gt;10⁻²</td>
<td>&gt;1%</td>
<td>90 - 110</td>
</tr>
<tr>
<td>0.1-1</td>
<td>10⁻³-10⁻²</td>
<td>0.1-1%</td>
<td>80 - 120</td>
</tr>
<tr>
<td>&lt;0.1</td>
<td>&lt;10⁻³</td>
<td>&lt;0.1%</td>
<td>75 - 125</td>
</tr>
</tbody>
</table>

3.1.1 Precision:

In contrast with accuracy being the closeness of results to a measured or true value, precision is the closeness of results of multiple analyses to each other. Figure 1 below is a schematic representation of accuracy and precision.

Figure 1: schematic representation of accuracy and precision. The circle represents the ‘target’ value.

3.1.2. Repeatability:

Repeatability aims to ensure that contributing factors to the variability of results, such as the operator, equipment, calibration and environmental considerations, remain constant and have little or no contribution to the final results.

Repeatability consists of 2 factors:

1) Intra-laboratory assay; repeated analysis (a minimum of 5 replicate determinations must be made and the mean, % Relative Standard Deviation (RSD) and number of
determinations reported) of an independently prepared sample on the same day by the same operator in the same laboratory.

2) Intermediate precision; repeated analysis (a minimum of 5 replicate determinations must be made and the mean, % RSD and number of determinations reported) of an independently prepared sample by different operators on different days in the same laboratory.

EC document SANCO/3030/99 rev.4 11/07/00 includes a table relating expected RSD values with analyte concentration.

Table 2: Suggested maximum RSD as a function of analyte concentration.

<table>
<thead>
<tr>
<th>Analyte (%)</th>
<th>Analyte ratio</th>
<th>Unit</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>1</td>
<td>100%</td>
<td>1.34</td>
</tr>
<tr>
<td>10</td>
<td>10⁻¹</td>
<td>10%</td>
<td>1.89</td>
</tr>
<tr>
<td>1</td>
<td>10⁻²</td>
<td>1%</td>
<td>2.68</td>
</tr>
<tr>
<td>0.1</td>
<td>10⁻³</td>
<td>0.1%</td>
<td>3.79</td>
</tr>
<tr>
<td>0.01</td>
<td>10⁻⁴</td>
<td>100 ppm</td>
<td>5.36</td>
</tr>
<tr>
<td>0.001</td>
<td>10⁻⁵</td>
<td>10 ppm</td>
<td>7.58</td>
</tr>
<tr>
<td>0.0001</td>
<td>10⁻⁶</td>
<td>1 ppm</td>
<td>10.72</td>
</tr>
<tr>
<td>0.00001</td>
<td>10⁻⁷</td>
<td>10 ppb</td>
<td>15.16</td>
</tr>
<tr>
<td>0.000001</td>
<td>10⁻⁸</td>
<td>1 ppb</td>
<td>21.44</td>
</tr>
<tr>
<td>0.0000001</td>
<td>10⁻⁹</td>
<td>1 ppb</td>
<td>30.32</td>
</tr>
</tbody>
</table>

These RSD are based on the modified Horwitz equation which suggests that:

$$\text{RSD} < 2 \left(1 - 0.5 \log C\right) \times 0.67$$

Where C is the concentration of the analyte expressed as a decimal fraction (i.e. 0.1, 1x10⁻⁶ etc.)

3.1.3 Reproducibility:

This is a measure of a methods ability to perform a routine analysis and deliver the same results using a particular method irrespective of laboratory, equipment and operator changes.

Confirmation of reproducibility is important if the method is to be used in different laboratories for routine analysis. Reproducibility is expressed in terms of relative standard deviation. The unmodified Horwitz equation is used as a criterion of acceptability for measured reproducibility.

Validation of the reproducibility is particularly important if the method is to be used for analysis in other laboratories. Collaborative methods do not require validation of reproducibility since by their nature they are validated in this way (providing that the analysis falls within the validated range of that method).
3.2 Specificity (and selectivity):

The role of an analytical method is to determine some predetermined parameter with a degree of confidence that the measured parameter is solely due to one particular species. It is necessary to determine that the signal assigned to an analyte is only due to that particular analyte and not a chemically or physically similar co-eluent.

Specificity and selectivity are measures that assess the reliability of measurements in the presence of interferences. Interferences may inhibit confirmation of an analyte by distortion of the signal or may affect the slope of the calibration curve. The co-eluent may not be distinct from the analyte of interest on a chromatographic trace but spectral analysis may reveal the presence of an impurity.

For chromatographic methods developing a separation involves demonstrating specificity. The response of the analyte in test solutions containing the analyte of interest and all potential sample components is compared with the response of a solution containing only the analyte.

Selectivity: The USP monograph defines selectivity as a method's ability to ‘measure accurately an analyte in the presence of interferences such as synthetic precursors, excipients, enantiomers and known (or likely) degradation products that may be expected to be present in the sample matrix’.

Specific: a method that produces a response for a single analyte.

Selective: a method that produces a response for a number chemical entities that may or may not be distinguished from each other.

3.3 Linearity:

For any quantitative analytical method it is essential to determine the range (where ‘range’ is defined as the ‘interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the method as written’.) of analyte concentrations over which the method may be used.

Within this range may exist a ‘linear response range’ i.e. a range over which the signal response has been determined to be linear with respect to the analyte concentration. (figure 2).
Fig 2: plot of concentration vs. detector response to show linearity over a given range.

Linearity is the ability of a method to elicit test results that are directly proportional to the concentration of analytes in samples within a given range.

Linear regression of a plot of concentration of analyte vs. detector response should have an intercept of (or close to) zero and a coefficient of correlation of >0.99. The range should extend 20 % below the lowest expected concentration and 20 % above the highest expected concentration.

3.4 Limit of Detection:

Where analysis is made at low analyte concentrations, for example in trace analysis or ensuring compliance with the Drinking Water Directive, it is important to determine the lowest level of analyte that can be confidently detected by the method in question. For validation it is usually sufficient to indicate the level at which detection becomes problematic.

Limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. In chromatography, limit of detection is an amount that produces a peak with a height at least 3 times that of the baseline noise level.

3.5 Limit of Quantitation:

The limit of quantitation is described as the minimum injected amount that gives precise, (that is an acceptable level of repeatability and trueness) measurements, i.e. a peak height 10 - 20 times that of the baseline noise. The LOQ can then be expressed as the lowest validated concentration (in terms of accuracy and precision).

The EURACHEM approach is to inject 6 samples of decreasing concentrations of analyte. The calculated RSD is plotted against concentration and the amount that corresponds to a predetermined RSD (see table 2) is defined as the limit of
quantitation (it should be noted that this particular approach uses calculated data as opposed to “real” data).

Fig 3: the limit of quantitation expressed as a function of analyte concentration.

3.6 Sensitivity:

Sensitivity is effectively the gradient of the response curve, i.e. the change in instrument response that corresponds to a change in analyte concentration. Where the response has established as linear with regard to concentration, i.e. within the linear range, sensitivity is a useful parameter to use in formulae for quantitation.

3.7 Ruggedness/Robustness:

The measure of an analytical method is how well it stands up to less than perfect implementation. At any given point in a method there may be a step in which an error may have a large effect on the performance of the method. These steps should be identified and their potential influence on the method evaluated through deliberate variations to the method and subsequent analysis of the results. This identifies the parts of the method most susceptible to significant variation. It also identifies the steps that may be improved to further improve the method. This should be evaluated during method development.
4. General Notes

4.1 Where collaboratively tested standard CIPAC and AOAC methods for analysis are available and relevant to the active ingredient or formulation under consideration such methods are regarded as validated and no additional information will be required, providing that the sample being analysed falls within the concentration range of that method.

4.2 The range of linearity response for a detection system is frequently very instrument-dependent. If a method is used with a different system, linearity should be re-evaluated.

4.3 If methods are submitted with performances below the minimum stipulated in these guidelines a detailed argument as to why the method is considered acceptable should be provided.

4.4 Any further data considered useful in support of method validity should be submitted.

4.5 Applicability of validation data to more than one formulation:

In general, validation data should be considered formulation specific. However, it is recognised that manufacturers may produce a number of very similar formulations and it may be possible to use a single method for these. The criteria for cross-applicability are:

- The formulation should contain the same (or very similar) co-formulants. Any qualitative change in co-formulants should be checked for potential interference.

- The formulations should not differ markedly in physico-chemical properties (e.g. pH)

- The concentrations of active ingredients in the analytical solutions must remain within the demonstrated linearity ranges.

- Any changes in relative co-formulant should not yield significant interference

Any methods submitted under this cross-applicability of validation should be accompanied by a consideration of the above points. If you have any questions regarding the cross applicability of methods between formulations please contact BPU at the address given in the reference section of this document.

4.6 Any method validation presented should use only certified reference materials (or traceable to such) as standards. This requirement does not apply to internal standards. If certified reference materials are not available for a given analyte then this should be reported.

4.7 Methods should be revalidated following, for example, changes in active ingredient/formulation synthesis, changes of the technical specification of the active
ingredient/formulation or changes in the analytical procedures used to analyse the active ingredient/formulation.
5. Summary of regulatory requirements:

It is not practical to specify a precise set of regulatory requirements that correspond exactly to every method submitted to a regulatory body. It is recognised that laboratories have their own validation procedures. Based on other regulatory bodies, expert groups and organisations the following is a suggested set of minimum validation requirements for individual methods commonly submitted in support of an active ingredient/product under COPR.

<table>
<thead>
<tr>
<th>Quantification and analysis of a.i. in technical material</th>
<th>Quantification and analysis of significant impurities (&gt;0.1 % and substances of toxicological concern below this level) in technical material</th>
<th>Quantification and analysis of a.i. in a matrix (formulation)</th>
<th>Quantification and analysis of a.i. in drinking water (0.1 μg l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Repeatability</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Where the method is to be used in other laboratories reproducibility should be addressed</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>LOD</td>
<td>×</td>
<td>×</td>
<td>✓</td>
</tr>
<tr>
<td>LOQ</td>
<td>×</td>
<td>✓</td>
<td>×</td>
</tr>
<tr>
<td>Linearity</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Range</td>
<td>×</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Robustness</td>
<td>Robustness should be addressed as part of method development</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key:
✓ - required.
× - not required.
**GLOSSARY**

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Accuracy</strong></td>
<td>The accuracy of a method is defined as the degree to which the determined value of analyte in a sample corresponds to the accepted reference value (for example ISO 5725)</td>
</tr>
<tr>
<td><strong>Active ingredient</strong></td>
<td>The component of a product which fits it for use as a pesticide</td>
</tr>
<tr>
<td><strong>Additives</strong></td>
<td>Any component added to a formulation or active ingredient</td>
</tr>
<tr>
<td><strong>AOAC</strong></td>
<td>AOAC International (formerly the Association of Official Analytical Chemists)</td>
</tr>
<tr>
<td><strong>CIPAC</strong></td>
<td>Collaborative International Pesticides Analytical Council</td>
</tr>
<tr>
<td><strong>EURACHEM</strong></td>
<td>A collaborative European working group with the objective of establishing a system for the traceability of chemical measurements and the promotion of good quality practices.</td>
</tr>
<tr>
<td><strong>Formulation</strong></td>
<td>A pesticide preparation containing technical active ingredient(s) and formulant(s) in a form suitable for use</td>
</tr>
<tr>
<td><strong>Impurities</strong></td>
<td>Material other than the active ingredient present as a result of the manufacturing process.</td>
</tr>
<tr>
<td><strong>ISO</strong></td>
<td>The International Standards Organisation</td>
</tr>
<tr>
<td><strong>Limit of Detection</strong></td>
<td>The lowest concentration of a pesticide that can be detected</td>
</tr>
<tr>
<td><strong>Limit of Quantitation</strong></td>
<td>The lowest concentration of a pesticide that can be identified and quantitatively measured by the method of analysis</td>
</tr>
<tr>
<td><strong>Linearity</strong></td>
<td>Linearity is defined as the ability of the method, within a given range, to obtain an acceptable linear correlation between the results and the concentration of analyte in samples</td>
</tr>
<tr>
<td><strong>Pesticide</strong></td>
<td>As defined in the Food and Environment Protection Act 1985 (FEPA) (Part III., Section 16 (15) + (16)) and COPR (Section 3, (1)).</td>
</tr>
<tr>
<td><strong>Precision</strong></td>
<td>Precision is defined as the closeness of agreement between independent test results obtained under prescribed conditions</td>
</tr>
<tr>
<td><strong>Recovery</strong></td>
<td>The percentage of the amount of active substance or relative metabolite originally added to a sample of the appropriate matrix, which contains no detectable level of the analyte</td>
</tr>
<tr>
<td><strong>Residue</strong></td>
<td>Any pesticide, including specified derivatives such as degradation products, metabolites or impurities considered to be of toxicological significance, found in a sample</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>Specificity is the ability of a method to distinguish between the analyte being measured and other substances</td>
</tr>
<tr>
<td><strong>Technical active</strong></td>
<td>unformulated active ingredient as manufactured</td>
</tr>
</tbody>
</table>
References and other sources of information:


2) Method Validation. Guidance at WWW.labcompliance.com


10) Accuracy (trueness and precision) of measurement methods and results Part 1: General principles and definitions. BS 5725-1:1994


BPU can be contacted at the following address:

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Room 123, Magdalen House
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Merseyside L20 3QZ

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Fax: 0151 951 3317