

**Guidelines on method validation to be performed
in support of analytical methods for agrochemical
formulations**

1. **SCOPE**

These Guidelines refer primarily to analytical methods for formulations, but may also be applied to active ingredient content of technical material and are partially applicable to physical methods, (see 3.2.2.). They do not apply to residue or trace analysis methods.

The recommendations in these Guidelines, whilst intended specifically to apply to studies submitted to PSD should satisfy the broad requirements of method validation for formulations as laid down in the Draft EC Uniform Principles.

2. **DEFINITIONS**

2.1 **Errors**

The type of errors covered by validation in these Guidelines are:

2.1.1 **Random errors**

These are errors, usually small, which give rise to a spread of results around the average results. In other words, they define the repeatability or reproducibility of the procedures.

2.1.2 **Systematic errors**

These are errors which cause a bias in the results obtained, such that the average value observed is above or below the true value (i.e. some feature of the method which leads to inaccuracy in the results).

2.2 **Precision**

Precision is a measure of random errors, and may be expressed as repeatability and reproducibility. These terms are defined in ISO 5725-1986E:

2.2.1 Repeatability is the closeness of agreement between mutually independent test results obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time.

2.2.2 Reproducibility is the closeness of agreement between test results obtained with the same method on identical test material in different laboratories with different operators using different equipment.

2.3 Accuracy

The accuracy of a method is the degree to which the observed results correspond to the true value of the analyte in the sample.

2.4 Linearity

The linearity of a test procedure is its ability (within a given range) to obtain test results proportional to the concentration (amount) of analyte in the sample.

2.5 Specificity

The specificity of the method is a definition of the species giving rise to the signal used for quantitation.

3. METHOD VALIDATION

It is recognised that most Agrochemical manufacturers will have internal procedures for analytical method validation. It will be the manufacturer's responsibility to ensure that their procedures comply with the requirements of these Guidelines.

Method validation data submitted should address the following issues:

- Linearity of response for the analyte (and internal standard, if appropriate), in the method.
- An estimate of the precision of the procedure.
- A demonstration of the accuracy of the procedure.
- A demonstration of no interference from excipients.
- A definition of the species being determined.

Although reproducibility is important for a method proposed for wide general use, its assessment is not considered necessary as part of these Guidelines. It is

best estimated (if required) through a full collaborative study by CIPAC or AOAC.

3.1 Linearity

The linearity of response to the analyte should be demonstrated at least over the range (nominal analyte concentration $\pm 20\%$). At least 3 concentrations should be measured with duplicate measurements for each. The line generated should be submitted, together with slope, intercept and correlation co-efficient data..

The measured slope should demonstrate a clear correlation between response and analyte concentration. The results should not show a significant deviation from linearity which is taken to mean that the correlation coefficient (r) is >0.99 , over the range (nominal $\pm 20\%$). If this is not the case, the submitter must provide an explanation of how method validity is to be maintained. In cases where a non-linear response is deliberately used, an explanation must also be provided.

3.2 Precision

3.2.1. Chemical Analysis

For these Guidelines, a simple assessment of repeatability will be acceptable. A minimum of five replicate sample determinations should be made together with a simple statistical assessment of the results including the % RSD.

If considered appropriate, a suitable test for outliers (e.g. Dixons or Grubbs Test) may be applied to the results. However, it should be clearly indicated if results have been discarded and some attempt made to explain why the outlier may have occurred.

The acceptability of the results should be based on the modified Horwitz¹ equation;

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

where C = concentration of the analyte in the sample as a decimal fraction.

The derivation and worked examples of the Horwitz equation are given in Appendix I.

3.2.2. Physico-chemical measurements

For measurements of physical or physico-chemical properties, no further validation is needed where official methods (such as CIPAC or OECD) are used. This also applies to close adaptations of such methods. Where another method is used, its repeatability

must be determined, but need not comply with the Horwitz Equation.

3.3 Accuracy

The accuracy of the procedure should be assessed by the preparation and analysis of at least four samples of laboratory-prepared 'synthetic' formulation containing known weights of analyte. The results may be assessed using the Students t-statistic or other acceptable approaches, given in Appendix II.

3.4 Non-analyte interference

This is covered to some extent by the assessment of accuracy, since any interference from excipients will confer a systematic error on the method. However, an analysis should be carried out using an excipient blank, either to demonstrate lack of interference or quantify any which is occurring. Sample chromatograms or other results should be submitted.

Where specific impurities are known to occur in the technical active ingredient, it must be demonstrated that these do not contribute more than 3% to the total peak area measured for the analyte or internal standard under the conditions used for the analysis. If there is such a known bias, it must be indicated whether or not submitted results are corrected.

3.5 Specificity

The specificity of the method should be defined in terms of the species analysed. This should normally be done through a spectrometric examination of the species, eg. using GC/MS, LC/MS diode array detection or peak collection followed by spectroscopic examination.

This will normally be done, either for the active ingredient characterisation method or for the method for the validation of the analytical standard. These will almost invariably be chromatographic methods. Where the formulation method is based on one of these methods, it is unnecessary to repeat this work.

If the chromatographic method is entirely original, then the specificity of the method should be established. If a spectroscopic method is used, the identity of the species can be inferred from the spectra.

The species actually being determined should be stated in the submission. If this cannot be done, a reasoned explanation must be provided for consideration.

4. GENERAL NOTES

- 4.1** 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-checked.
- 4.2** 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.
- 4.3** Any further data considered useful in support of method validity should be submitted.

This might include an assessment of reproducibility (eg. from a collaborative study) or some information on the robustness of the procedure to minor changes.

- 4.4** 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:

- a) The formulations should contain the same (or very similar) co-formulants. Any qualitative change in co-formulants should be checked for potential interference.
- b) The formulations should not differ markedly in physico-chemical properties (e.g. pH).
- c) The concentrations of active ingredients in the analytical solutions must remain within the demonstrated linearity ranges.
- d) Any changes in relative co-formulant concentrations should not yield significant interference.

Any methods submitted under this cross-applicability of validation should be accompanied by a consideration of the above points.

- 4.5** Any method validation presented should use only certified reference materials (or traceable to such) as standards. This requirement does not apply to internal standards.

5. REFERENCE AND USEFUL DOCUMENTS

1. Boyer, K.W. Horwitz, W and Albert, R Analytical Chemistry 57, 454-9 (1985).

Miller J.C. and Miller J.N. Statistics for Analytical Chemistry, Ellis Horwood, 1988 (2nd edition).

Guidelines for CIPAC Collaborative Study Procedures for Assessment of Performance of Analytical Methods (published through GIFAP).

International Standard ISO 5725. Precision of Test Methods - Repeatability and reproducibility. Reference number: ISO 5725 - 1986 (E).

APPENDIX I - The Horwitz equation for acceptable repeatability

This equation was defined by Horwitz et al¹ from a practical consideration of a number of collaborative studies done by AOAC over many years.

For the purposes of this document, examples are given over the range of ~ 0.25% up to 100%.

The equation is: $\% \text{RSD}_R = 2(1-0.5\log C)$

where $\% \text{RSD}_R$ is the inter-laboratory CV and
C is the concentration of analyte in the sample
as a decimal fraction

So, for a 100% pure sample, , C = 1, LogC = 0

$$\text{so, } \text{RSD}_R = 2 (1-(0.5 \times 0)) = 2^1 = 2$$

For a 50% sample (e.g. a 500g/kg WP), C = 0.5 Log C = -0.3010

$$\text{so, } \text{RSD}_R = 2 (1-(0.5 \times -0.3010)) = 2^{1.1505} = 2.22$$

Other values are:

| | | | | |
|-------|---|----------------|---|------|
| 20% | , | RSD_R | = | 2.55 |
| 10% | , | RSD_R | = | 2.83 |
| 5% | , | RSD_R | = | 3.14 |
| 2% | , | RSD_R | = | 3.60 |
| 1% | , | RSD_R | = | 4.00 |
| 0.25% | , | RSD_R | = | 4.93 |

Horwitz noted that values for RSD_r (the repeatability CV) were usually between half and two-thirds that of RSD_R. For this reason, repeatability acceptabilities are proposed as the Horwitz values for RSD_R x 0.67.

For the values above, this gives the following:

| % Analyte | Horwitz RSD _R | Proposed acceptable RSD _r |
|-----------|--------------------------|--------------------------------------|
| 100 | 2 | 1.34 |
| 50 | 2.22 | 1.49 |
| 20 | 2.55 | 1.71 |
| 10 | 2.83 | 1.90 |
| 5 | 3.14 | 2.10 |
| 2 | 3.60 | 2.41 |
| 1 | 4.0 | 2.68 |
| 0.25 | 4.93 | 3.30 |

The unmodified Horwitz equation is currently used as a criterion of acceptability for methods collaboratively tested by CIPAC.

APPENDIX II - Estimation of Accuracy

The following procedures illustrate various approaches to the estimation of accuracy of a procedure.

- 1) The accuracy of a procedure may be determined by the examination of a number of 'samples' containing a known quantity of the analyte. These should be laboratory-prepared co-formulant mixes to which a known quantity of analyte (corresponding to the quantity demanded by the method) is added. The analyte added should be technical active ingredient of known purity. The whole sample should be analysed to eliminate sampling error. At least 4 recoveries should be done, following exactly the proposed procedure. The results should be treated as follows:-
 - (a) Calculate mean recovery and relative standard deviation of the recoveries.
 - (b) Apply an F-test to the RSD's of these results and the results from the estimation of repeatability to establish that the recovery results do not show a significantly different RSD to the estimated precision (as the samples have been prepared slightly differently).
 - (c) If (b) is satisfactory, apply Student's t-test to the recovery results, i.e. attempt to prove that the observed recovery (mean) differs from the concentration added only by random errors (no evidence of systematic error).

$$|t| = \left| (\bar{x} - m) \frac{\sqrt{n}}{s} \right|$$

\bar{x} = sample mean

m = true value

n = no. of samples

s = standard deviation

Critical values of t are given in statistical tables for various degrees of freedom, (i.e. no. of samples - 1). If the value obtained for t does not exceed the critical value, then there is no proven evidence of systematic error at the given confidence interval (95% will usually be satisfactory).

- 2) The above expression may be re-arranged to yield a confidence interval for the mean. This is defined as the range within which the true value may be said to lie (to a given degree of confidence)

$$m = \bar{x} \pm t \left(\frac{s}{\sqrt{n}} \right)$$

Calculate the mean % recovery for the synthetic formulation as follows:

$$\text{Mean \% recovery:} = \frac{\text{Mean \% content determined} \times 100}{\text{Theoretical \% content}}$$

This mean % recovery should be within the following ranges:

| <u>% active (nominal)</u> | <u>Mean % recovery</u> |
|---------------------------|------------------------|
| >10 | 98.0 - 102.0 |
| 1 - 10 | 97.0 - 103.0 |
| <1 | 95.0 - 105.0 |

- 3) For both the above cases, a complete synthetic mixture is analysed to eliminate sampling error. The same procedures may also be applied to sub-samples of a mixture of known composition, but it must be noted that this will tend to give an artificially large value to the confidence interval of the mean for formulations which are not homogeneous at the time of sampling.
- 4) Where it is very difficult to replicate the formulation to be analysed (e.g. for a pellet or block bait type) the accuracy may be estimated through a standard additions procedure. In this case, full details of how the standard additions were done should be submitted.