Australian Pesticides &
Veterinary Medicines Authority

GUIDELINES FOR THE VALIDATION OF ANALYTICAL METHODS FOR ACTIVE CONSTITUENT, AGRICULTURAL AND VETERINARY CHEMICAL PRODUCTS

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1.0 INTRODUCTION

In order to generate the Part 2 Chemistry and Manufacture data for the approval of active constituents and registration of agricultural and veterinary chemical products, robust, accurate and precise analytical methods are required. Methods are required for the identification, batch analysis, and storage stability data for active constituents and agricultural and veterinary chemical products, and for post-registration compliance purposes.

1.1 SCOPE

The objective of validation of an analytical method is to demonstrate that the procedure, when correctly applied, produces results that are fit for purpose. These guidelines describe the procedures to be carried out to validate the analytical procedures included as part of an application for approval of an active constituent and registration of an agricultural and veterinary chemical product, including those used in storage stability studies. They are not intended to apply to analytical methods for residue analysis, biological and biotechnological products. Approaches other than those set forth in this guideline may be acceptable provided they are supported by adequate scientific justification.

In general, non-chromatographic analytical methods are not typically expected to comply with this guideline. However, the APVMA may require that a non-chromatographic method demonstrate some form of validation in order to satisfy itself that the method is fit for purpose [e.g. Nuclear Magnetic Resonance (NMR) methods are typically required to demonstrate certain validation parameters].

1.2 DATA REQUIREMENTS

The following is a list of information that should typically be included in support of the adequacy of the analytical procedures:

- Method description – this section should contain a full description of the analytical method. The description should include details of all-important operational parameters, such as sample preparation, including method of extraction of the active constituent from the product, details of the reference standards and reagents preparation. Documentation confirming the purity of the reference materials should also be provided.
- Validation data - all relevant data collected during validation should also be provided. Relevant data are considered to be: copies of chromatograms that are clearly labelled with peak identity and peak integration data; NMR spectra clearly showing chemical shifts and coupling constants; formulae and calculations used for calculating validation characteristics.

1.3 PARAMETERS FOR METHOD VALIDATION

To be fit for the intended purpose, the method must meet certain validation characteristics. Typical validation characteristics, which should be considered are: selectivity (specificity), linearity, range, accuracy, precision, limit of detection and quantitation.

1.3.1 Selectivity (Specificity)

Selectivity of a method refers to the extent to which it can determine particular analyte(s) in a complex mixture without interference from other components in the mixture. The terms selectivity and specificity have often been used interchangeably. The term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since very few

analytical methods respond to only one analyte, the use of the term selectivity is more appropriate than specificity. The International Union of Pure and Applied Chemistry (IUPAC) has expressed the view that "Specificity is the ultimate of Selectivity'. The IUPAC discourages use of the term specificity and instead encourages the use of the term selectivity.

The selectivity of the analytical method must be demonstrated by providing data to show the absence of interference peaks with regard to degradation products, synthetic impurities and the matrix (excipients present in the formulated product at their expected levels).

The selectivity of chromatographic methods may be assessed by examination of peak homogeneity or peak purity test (e.g., diode array, mass spectrometry) to show that the analyte chromatographic peak is not attributable to more than one component.

1.3.2 Linearity

The linearity is the ability of analytical procedure to produce test results which are proportional to the concentration (amount) of analyte in samples within a given concentration range, either directly or by means of a well-defined mathematical transformation. Linearity should be determined by using a minimum of six standards whose concentration span 80 –120% of the expected concentration range.

The linearity of a method should be established by visual inspection of a plot of analytical response as a function of analyte concentration. If there is a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of the regression line by the method of least squares. In some cases, the test data may need to be subjected to a mathematical transformation prior to regression analysis.

Reports submitted must include, the slope of the line, intercept and correlation coefficient data. The measured slope should demonstrate a clear correlation between response and analyte concentrations. The results should not show a significant deviation from linearity, which is taken to mean that the correlation coefficient, $r > 0.99$, over the working range (80 –120%). If this is not the case (i.e. r is < 0.99), the submitter must provide an explanation of how accurate calibration is to be maintained. In cases where a non-linear response is deliberately used, an explanation must be provided.

1.3.3 Range

The specified range is normally derived from the linearity studies. The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical method has suitable levels of precision, accuracy and linearity.

The following minimum specified ranges should be considered:

- For the assay of the active constituent or an agricultural/veterinary chemical product: normally from 80 –120% of the test concentration/label concentration; and
- For the determination of an impurity: from the specification level of the impurity to 120% of the specification.

1.3.4 Accuracy

The accuracy of an analytical method is defined as the degree to which the determined value of analyte in a sample corresponds to the true value. Accuracy may be measured in different ways and the method should be appropriate to the matrix. The accuracy of an analytical method may be determined by any of the following ways:

- Analysing a sample of known concentration and comparing the measured value to the 'true' value. However, a well characterised sample (e.g., reference standard) must be used.
- Spiked – placebo (product matrix) recovery method. In the spiked – placebo recovery method, a known amount of pure active constituent is added to formulation blank [sample that contains all other ingredients except the active(s)], the resulting mixture is assayed, and the results obtained are compared with the expected result.
- Standard addition method. In the standard addition method, a sample is assayed, a known amount of pure active constituent is added, and the sample is again assayed. The difference between the results of the two assays is compared with the expected answer.

In both methods (spiked – placebo recovery and standard addition method), recovery is defined as the ratio of the observed result to the expected result expressed as a percentage.

The accuracy of a method may vary across the range of possible assay values and therefore must be determined at several different fortification levels. The accuracy should cover at least 3 concentrations (80, 100 and 120%) in the expected range.

Accuracy may also be determined by comparing test results with those obtained using another validated test method.

Acceptance criteria: the expected recovery depends on the sample matrix, the sample processing procedure and on the analyte concentration. The mean % recovery should be within the following ranges:

% Active/impurity content	Acceptable mean recovery
≥ 10	98 –102%
≥ 1	90 –110%
0.1 – 1	80 – 120%
< 0.1	75 – 125%

1.3.5 Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. For these guidelines, a simple assessment of repeatability will be acceptable. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. A minimum of 5 replicate sample determinations should be made together with a simple statistical assessment of the results, including the percent relative standard deviation. If considered appropriate, a suitable test for outliers (Dixon's or Grubbs Test) may be applied to the results. Where outliers have been discarded, that fact must be clearly indicated. An explanation as to the reason for the occurrence of individual outliers must be attempted.

The following levels of precision are recommended.

Component measured in sample	Precision
≥ 10.0%	≤ 2%
1.0 up to 10.0%	≤ 5%
0.1 up to 1.0%	≤ 10%
< 0.1%	≤ 20%

1.3.6 Limit of Detection (LOD)

The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value.

The LOD may be determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level (lowest calibration standard) at which the analyte can be reliably detected. The lowest calibration standard which produces a peak response corresponding to the analyte should be measured n times (normally 6-10). The average response (X) and the standard deviation (SD) calculated. The LOD is X + (3 x SD).

1.3.7 Limit of Quantitation (LOQ)

The limit of quantitation is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions. The limit of quantitation is a parameter of quantitative assays for low levels of compounds in sample matrices and is used particularly for the determination of impurities and/or degradation products or low levels of active constituent in a product.

The LOQ may be determined by preparing standard solutions at estimated LOQ concentration (based on preliminary studies). The solution should be injected and analysed n times (normally 6-10). The average response and the standard deviation (SD) of the n results should be calculated and the SD should be less than 20%. If the SD exceeds 20%, a new standard solution of higher concentration should be prepared and the above procedure repeated. The LOQ is X + (10 x SD).

1.4 VALIDATION CHARACTERISTICS AND REQUIREMENTS

The extent to which a method needs to be validated depends on its application.
The following characteristics are recommended for consideration for each of the categories of analytical test methods described in the guidelines:

Type of test/characteristics	Assay of active constituent in bulk active constituent	Quantitative test for toxicologically significant impurities in bulk active constituent and/or ag/vet chemical product	Assay of active constituent in an ag/vet chemical product
Specificity	Yes	Yes	Yes
Linearity	Yes	Yes	Yes
Accuracy	No	Yes	Yes
Precision	Yes	Yes	Yes
Range	*	Yes	Yes
Limit of detection	No	Yes	No
Limit of quantitation	No	Yes	*

* May be required, depending on the nature of the specific test

1.5 GENERAL NOTES

1.5.1 Regulatory Analytical Methods

Analytical methods described in Collaborative International Pesticide Analytical Council (CIPAC) handbooks and Association of Official Analytical Chemists (AOAC International) Manual for agricultural active constituents and agricultural chemical products, and in British Pharmacopoeia (BP), British Pharmacopoeia (Veterinary) [BP (Vet)], European Pharmacopoeia (Ph Eur) and United States

Pharmacopoeia (USP) for veterinary active constituents and veterinary chemical products are legally recognised as the regulatory methods, and these procedures (if one is available) are used by the APVMA for determining compliance with the Agricultural and Veterinary Chemicals Code Act. It is recommended that analytical methods described in BP, BP (Vet), Ph Eur, USP, CIPAC handbooks and AOAC for a particular active constituent or formulated product be used, where available.

Note: Analytical methods described in CIPAC handbooks and AOAC International Manual, and in recognized pharmacopoeias [BP, BP (Vet), Ph Eur and USP] for a particular active constituent or formulation are regarded as validated and do not require revalidation. However, the suitability of these methods must be verified under actual conditions of use i.e., the selectivity and accuracy of the method should be demonstrated for the published method when applied to the relevant sample matrix and laboratory conditions.

1.5.2 Alternative Analytical Methods

An alternative analytical method is an analytical method proposed by the registrant for use instead of the regulatory analytical method. Validated alternative analytical methods may be proposed by the registrants in place of regulatory methods.

1.5.3 Typical Characteristics for Nuclear Magnetic Resonance (NMR) Data

Analytical data generated using NMR methods should include demonstration of a suitable relaxation time, selectivity, accuracy and LOQ validation parameters using properly characterised internal and/or external reference standards.

1.5.4 Analytical Reference Standards

Well characterised analytical reference standards with documented purity should be used throughout the validation study. If analytical reference standards are not available for a given analyte then this should be reported.

1.5.5 Good Laboratory Practice

It is not a requirement that analytical validation studies carried out for generation of batch analysis data required for approval of an active constituent and generation of stability data for agricultural and veterinary chemical products be carried out in compliance with the principles of Good Laboratory Practice.

1.6 REVALIDATION

Analytical methods require validation whenever the conditions for which the methods have been developed change. Revalidation of the analytical method is required in the following circumstances:

- An existing method is modified to meet special requirements;
- Changes in the route of synthesis of the active constituent which may lead to different impurity profile; and
- Changes to the formulation composition of an agricultural and veterinary chemical product.

Revalidation should be performed to ensure that the analytical method maintains its characteristics. The degree of revalidation depends on the nature of the change i.e. a new dosage strength in a product may require validation of the method in terms of recovery and linearity at the new dosage strength; a new formulation would require revalidation in terms of selectivity, recovery, etc.

1.7 DEFINITIONS AND ABBREVIATIONS

Acceptance criteria – Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures.

Active constituent – The substance(s) in an agricultural or veterinary chemical product that is primarily responsible for the biological or other effects that make the product a pesticide or veterinary medicine.

AOAC – Association of Official Analytical Chemists.

Biological compound - An agricultural or veterinary chemical product where the active constituent, whether living or not, are derived from plants, animals, viruses or other microorganisms, whether living or not, formulated either singly or in combination and where some essential characteristics of the source are retained in the product.

BP – British Pharmacopoeia.

BP (vet) – British Pharmacopoeia (Veterinary).

CIPAC – Collaborative International Pesticide Analytical Council.

EURACHEM – 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.

Excipient – All other intentionally added components of an agricultural or veterinary chemical product except the active constituent(s).

FAO – Food and agricultural Organisation of the United Nations.

Pharmacopoeia An authoritative work containing descriptions of active drugs listing specifications, their formulae and dosage forms and directions for determining purity and strength.

Ph. Eur. – European Pharmacopoeia.

Placebo (or blank) - Dosage form that contains all other ingredients except the active(s).

Spiking – The addition of a small known amounts of a known compound to a standard, sample, or placebo, typically for the purpose of confirming the performance of an analytical method.

USP – United States Pharmacopoeia/National Formulary.

Validation – the procedures involved in checking data or programs for correctness, compliance with standards and conformance with the requirement specifications.

1.8 REFERENCES

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