Validation of analytical MT method

Introduction
This document has been prepared to provide guidance on how to validate the MT analytical methods for the determination of active ingredient in plant protection product by GC or HPLC technique.

Content
The topics are:
- description of the method
- validation parameter
- acceptability criteria

Description of methods
Full descriptions of validated methods must be provided, including details of equipment, materials and conditions used.
The method description must include the following:
- principle of the method (including scope and technique of the method used)
- method summary; equipment/reagents (including details of any hazards or precautions required and reagent stability information)
- full details of standard compound purity where relevant
- Standards; purified active substance and reference substances for relevant impurities used in an analytical method
- storage of validation samples prior to analysis (where appropriate, details of conditions and period of storage)
- general sample preparation techniques (include extraction procedure)
- analytical procedure (including analytical instrumentation)
- details of calibration (concentrations, number of samples,)
- Range of tested concentrations in relation to the expected concentration of the analyte
- where chromatographic technique used, representative chromatograms, including peak assignments (e.g. control blank(s), analytical standard/ standard(s), lowest fortification(s), extract preparation/test item, formulation with and without active substance (blank formulation)) and it is recommended, for better comparison, to provide an overlap of the representative chromatograms of the blank(s), analytical standard(s) and the sample(s))
- calculations
- references

It has to be demonstrated that there is no interference > 3% with example chromatograms of blank samples, test samples and fortified samples. In cases where the method deviates from the standard method, additional validation data will be required.

Quantification procedures should be described, including detection system, calibration, calculation of analyte concentration and any compliance with statistical parameters required. Supporting chromatograms/spectra or non-chromatographic data should be clearly labelled.

Validation parameter

The methods should be validate for individual formulations. Nevertheless, some validation data such as linearity, recovery and precision can be extrapolated from one plant protection product to another plant protection product of the same type, but of a different composition, on a case by case basis. The applicant should submit a robust argument as to why the validation data can be extrapolated. However, in all cases chromatograms (of the blank plant protection product, of the plant protection product and of the standard) should be provided for the specific plant protection product being assessed and recovery data could be requested if the differences of composition between the two plant protection products are considered as significant

(i) Specificity - The degree of interference in the chromatograms for the determination of active substance in the technical active substance and in the plant protection product should be reported. Interferences from impurities should not contribute more than 3% to the total peak area measured for the target analyte.

If the active substance (a.s.) is specified as being optically pure or ratio of isomers, the method must support this. Where an a.s. contains more than one non-active isomer, the method should be capable of determining the individual components present. In case of manufacturing processes that yield racemic mixtures, it should be demonstrated with argument that the synthetic steps do not promote the formation of a specific enantiomer; in such a case the submission of an enantiomer-selective method is not requested. Otherwise, an enantiomer-selective method must be submitted and used to support the specified ratio of enantiomers that define the active substance.

If the a.s. is specified as being optically pure or a specific isomer ratio is set, the method must support this. Where more than one inactive isomer of an active substance is known to occur, the method(s) should distinguish between individual isomers where this is relevant, with the exception that this requirement does not include determination of optical isomers in racemic mixtures. In this case, an enantioselective method is not necessary.

Where the plant protection product contains more than one active substance the method(s) must be capable of determining each in the presence of the other. When the analytical technique used is not a chromatographic technique, it must be demonstrated that the method is suitable to detect and quantify the analyte.

a)Linearity

The analytical calibration should extend over the lowest and highest nominal concentration of the analyte in relevant analytical solutions with an appropriate range of± at least 20%. Duplicate determinations (independently weighed samples) at either three or more concentrations or single determinations at five or more concentrations must be made. A typical calibration plot, the equation of the calibration curve and the corresponding correlation coefficient (r) must be reported. The concentration of the solutions (mg/L) used and the concentration range of the a.s. must be reported.

b) *Recovery (accuracy)* - The recovery of the method should be reported as mean recovery of the pure active substance in the plant protection product and relative standard deviation when applicable ($n > 2$). At least 2 independent recovery determinations (i.e. two weighings) should be made on representative product samples containing a known quantity of the analyte (e.g. between 90 and 110 % of the target concentration). Ideally, samples should be laboratory-prepared co-

formulant mixes to which a known quantity of analyte is added and the whole sample analysed to reduce sampling error. However, where it is not possible to prepare a sample test item without the presence of the analyte, or there are difficulties in replicating the sample to be analysed (for example with pellet formulations), the standard addition method may be used.

c) *Precision (repeatability)* –The precision (repeatability) of the method is required for the a.s. in the technical active substance and in the plant protection product. A minimum of 5 independently, weighed sample determinations at the same concentration must be made and the mean, % RSD and number of determinations must be reported for repeatability.
The acceptability of the % RSD (from repeatability) should be assessed using the Horwitz ratio (Horrat, Appendix 2), however it must be noted that this is empirically derived. Further details are given in Appendix 2. Where outliers have been identified and discarded using appropriate statistical methods (Grubbs or Dixons test, Appendix 2) this should be made clear and justified, if possible. A maximum of 1 outlier by fortification level may be discarded. Where more than one outlier has been identified, additional determinations must be included.

d) LOQ – According to Regulation (EU) No 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for the technical active substance.

Acceptability criteria

Linearity – 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 ® is > 0.99 over the range (nominal ± 20%). Where a linear correlation coefficient (r) is < 0.99, an explanation on how accurate linearity is to be maintained should be submitted. However, if the coefficient of determination r2 is submitted and ≥ 0.98, it should be acceptable.

Recoveries – Recovery is the fraction or percentage of the analyte that is recovered when the test sample is analysed using the entire method. There are two types of recoveries, according to [2]:

1. Total recovery based on recovery of the native plus added analyte, and
2. Marginal recovery based only on the added analyte.

The following information is needed for calculation of the recovery:

(1) The initial concentration of the analyte in the unfortified sample (CU)
The initial concentration is the mean of at least two determinations. It should be stated as (m)g/kg in technical active substance/formulation and as mg/mL in the measuring solution. In case a blank formulation is used as initial sample no mean concentration has to be determined.

(2) The concentration of the analyte added (CA)
The concentration added should be stated as (m)g/kg in technical active substance/formulation and as mg/mL in the measuring solution.

(3) The measured concentration of the fortified sample (CF)
The measured concentration should be stated as (m)g/kg in technical active substance/formulation.

Total recovery
If the initial concentration in the unfortified sample is less than about 10% of the concentration added then the total recovery is used. It is calculated in the following way:

Total % recovery = 100 x (CF)/(CU + CA)

Marginal recovery
If the initial concentration in the unfortified sample is more than about 10% of the concentration added then the marginal recovery is used. It is calculated in the following way:

Marginal % recovery = 100 x (CF – CU) /CA
Confidence intervals for % mean recovery from active substance and impurities in technical active substance and in plant protection product, are as follows:

% (w /w) substance (active substance or impurity)	**Mean recovery %**
≥ 10	97 - 103
≥ 1 - < 10	90 - 110
≥ 0.1 - < 1	80 - 120
≥ 0.01 - < 0.1	75 - 125
< 0.01	70 - 130

Precision – a suitable test for outliers may be applied to the precision data, for example the Grubbs or
Dixons Tests [3, 4].
If an outlier is identified with Grubbs or Dixons tests, an explanation must be provided to discard the value (e.g. analytical problem).
The Horwitz equation [9, 10] correspond to an exponential relationship between the among-laboratory relative standard deviation (RSDR) and concentration (c) :

%RSDR = 2(1-0,5*log(c))

For an estimation of the intra-laboratory repeatability (RSDr), the Horwitz equation is modified to:

%RSDr = 0,67 * 2(1-0,5*log(c))
The Horwitz equation is used as a benchmark for the performance of single analytical method (intra-laboratory) via the so called Horrat (Horwitz ratio) value Hr [5]

Hr = %RSD/%RSDr
%RSD: obtained repeatability (see section 4.1.1(iv))
%RSDr: expected repeatability obtained with modified Horwitz equation

Therefore, the acceptability of the %RSD results for precisions should be based on the Horrat value with these criteria:
Hr ≤ 1, acceptable 1 < Hr ≤ 2, acceptable in case of a suggested explanation
Hr > 2, not acceptable

For concentrations < 10 µg/kg, the RSD obtained should be < 30%

References

1) Document SANCO 3030/99/rev.5 "Technical Active Substance and Plant protection products: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex (Section 4) of Regulation (EU) No 283/2013 and Annex (Section 5) of Regulation (EU) No 284/2013".

2) Appendix F: Guidelines for standard method performance requirements, AOAC official methods of analysis, 2012

3) F. E. Grubbs, G. Beck. *Technometrics*. 1972. 14 847.

4) W. J. Dixon. *Ann. Math. Stat*. 1951. 22 68.

5) I Taverniers, M De Loose and E Van Bockstaele, trends in analytical chemistry, 23, n°8, 2004