

**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.**

**Guidance document**

*Version history*

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5	For dossiers submitted on or after 1 October 2019.	General update based on comments of the EU Member States and EFSA, which were derived from experience with applications.  Main reasons: <ul style="list-style-type: none"><li>• Update to the Regulations (EU) No. 283/2013 and 284/2013</li><li>• Improvement of a common understanding on the required validation data</li></ul>

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## 1. GENERAL DEFINITIONS

For this paper the following definitions apply:

Accuracy <sup>1</sup> (comprised of ‘Trueness’ and precision)	The accuracy of an analytical procedure expresses the closeness of agreement between the value found and the value which is accepted either as a conventional true value or an accepted reference value. The accuracy is the expression of the sum of the trueness (recovery) and precision (repeatability).
Active substance (A.S.)	Correspond to pure active substance
Additive	According to Regulation 283/2013 (1.10.1), components added to the active substance, prior to manufacture of the plant protection product, to preserve stability and facilitate ease of handling
Blank formulation	A sample containing all components of the plant protection product except the active substance(s). In the case of multiple methods, each blank formulation must contain the other active substances which are not analysed to check for interferences.
Confirmation of identity	Confirmation of identity is the unequivocal establishment of the structural identity of an analyte in a particular matrix based on structural method or by comparison to standard material, which could be characterized separately.
Impurities	Any component other than the pure active substance and additives, including all the isomers not part of the active substance definition which is present in the technical active substance as manufactured originating from the manufacturing process or from degradation during storage.
Limit of quantification (LOQ)	Defined as the lowest concentration tested, at which an acceptable recovery and an acceptable precision (repeatability), is obtained.
Linearity	Ability of a method to produce an acceptable linear correlation between the measured response and the concentration of the analyte in the sample.
Precision <sup>1</sup>	The closeness of agreement between independent test results obtained under prescribed conditions. A measure of random errors, which may be expressed as repeatability.
Recovery <sup>1</sup>	The amount measured as a percentage of the amount of analyte originally added to a sample of the appropriate test item which contains either no detectable level of the analyte or a known detectable level.
Relevant impurities	All impurities of toxicological and/or ecotoxicological or environmental concern compared with the active substance, even

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<sup>1</sup> These terms are defined in ISO 5725 (6) [1]

	if present or could theoretically be formed in technical active substance at < 1 g/kg.
Relevant co-formulant	This definition of relevant is on-going at EU level (Working group on co-formulant)
Repeatability <sup>1</sup>	The closeness of agreement between 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 (one day or one analytical run).
Significant impurities	Impurities with a concentration of $\geq 1$ g/kg in the active substance as manufactured.
Specificity/selectivity	Specificity of an analytical procedure is the ability to determine the analyte in the presence of components, which may be expected to be present. The selectivity level of an assay procedure is the ability of a method to distinguish between the analyte being measured and other substances. The selectivity level of an assay procedure depends on the quality of the chromatographic separation and on the intrinsic selectivity of the detection method. The specificity/selectivity is based on absence of interference. For chromatographic techniques, the selectivity/specificity is also based on a match in the retention time of the analyte in the chromatogram of an analytical standard and in the chromatogram of the sample.
Technical active substance (T.A.S.) :	Correspond to pure active substance with its impurities which is isolated from starting materials, solvent, etc used to produce it.
Technical concentrate (T.K.)	Correspond to an active ingredient which has not been isolated from the materials, solvents, etc., used to produce it, or a minimally diluted T.A.S intended for use in preparing formulations.

## **2. INTRODUCTION**

In order to generate data for authorisation and post-registration control and monitoring purposes under Regulation (EC) N°1107/2009 [2], accurate and precise analytical methods are required.

Methods are required for the identification and quantification of the active substance (A.S.), impurities and additives in the technical active substance (T.A.S.) and, active substance, relevant impurities and relevant co-formulants (where required by the national competent authority) in the plant protection product.

### **2.1. Scope**

This document has been prepared to provide guidance to applicants on the requirements for analytical methods of chemicals supporting all submissions under Regulation (EC) No 1107/2009 and, for chemical plant protection products for pre-registration, post-registration control and monitoring purposes. Regulations (EU) No 283/2013 and 284/2013 [3,4] setting out the data requirements for active substances and plant protection products with regulation (EC) N°1107/2009 address the development of analytical methods required for pre-registration and for post-registration control and monitoring purposes. However, some of the requirements such as ‘minimum cost’ and ‘commonly available’ equipment do not apply to methods supporting pre-registration studies.

In cases where the requirements of these regulations cannot be fulfilled, full justification must be submitted. The requirements outlined in this guidance paper are applicable to a core data set for each post-registration method. Concerning pre-registration method, at least the method used for analysis of representative batches of the active substance as manufactured and for the analysis of active substance and relevant impurities in the storage stability study should be fully validated. Moreover, if the validation of the method is not performed in the laboratory where the measurements are done, the demonstration that the method can be transferred in this new laboratory should be provided (typical chromatograms and calibration lines included in the studies report).

For the analysis of plant protection products, where pre-registration and post-registration requirements differ, this is clearly stated in the regulation. The majority of validation data required is common to methods supporting both pre-registration and post-registration control and monitoring purposes.

### **2.2. Content**

The document is divided into sections addressing the requirements for methods supporting the generation data. The following topics are discussed: description of the method, validation of the method, confirmatory techniques for identity, derivatisation, and non-specific and common moiety methods. Annexes cover glossary, statistical consideration of validation results, a summary of required method validation data and a description of methods ( non-specific, specific and highly specific).

### **2.3. GLP**

Individual requirements for the validation of methods are described in each section. GLP compliance requirements for methods validation submitted in support of applications made under regulation (EC) N°1107/2009 (including minor uses GLP requirements) are detailed in the directive 2004/10/EC [5] reported in regulation (EC) 1107/2009, art 3 point 19. The development and validation of a method is not subject to GLP. However where a method is used

to generate data for safety purposes, for example the profile of batches or where the active substance degrades to (eco)toxicologically relevant product(s) (this list is not exhaustive), these studies must be conducted according to GLP, but not the development and the validation of the method itself.

## 2.4. Description and reporting of methods

Full descriptions of validated methods must be provided, including details of equipment, materials and conditions used. Where published methods are submitted, validation of the published method when applied to the relevant sample and laboratory conditions is required. However, when the published method has been validated by a collaborative study of CIPAC or AOAC for the same type of plant protection product, full validation is not required. Nevertheless, 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.

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

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. Labelling should include sample description, scale, concentration and identification of all relevant components. Statistical analyses are discussed in Appendix 2.

A summary of method validation requirements is shown in Appendix 3

This guidance document should be used in conjunction with Regulations (EU) No 283/2013 and 284/2013 [3, 4].

### 3. SCOPE AND APPLICABILITY OF METHODS SUPPORTING REGISTRATION (pre and post registration)

Validation data are required for analytical methods used for pre-registration (for example, methods used to determine the profile of batches) and analytical methods used for post-registration control and monitoring purposes. Analytical methods may be different since the purpose of these methods are different. In the dossier, there can be post and pre-registration methods different for the same substance (active substance or impurity).

When requirements are different for pre-registration method and post-registration method, this is pointed out.

Methods supporting registration may employ any suitable analytical techniques, providing the method validation criteria are adequately addressed. The use of novel/complex analytical techniques/instrumentation should be justified. Hazardous reagents shall not be used (substance classified as carc cat 1 and 2 according to directive 76/769/EEC e.g.: diazomethane, chloroform, benzene) in pre-registration methods and in monitoring and control methods.

#### 3.1. Derivatisation

For the analysis of some compounds chemical derivatisation may be necessary when the properties of the target analytes are not compatible with the analytical procedure, when detection is not sufficiently sensitive, or to improve their chromatography, thermal stability, or their identification.

Derivatives may be prepared prior to chromatographic analysis or as part of the chromatographic procedure, either pre- or post-column.

- Where a derivatisation method is used, this must be fully reported and justified. Where both the analyte and the standard are being derivatised (i.e derivative not available as standard used for calibration), the rate of derivatisation of the analyte in the sample and for the standard may be different; hence recovery and repeatability data may be unreliable. In such cases further data are required e.g. matrix matched standards should be used, or the mean yield and precision of the derivatisation addressed. In some “instances” however, for example simple solutions such as T.A.S. it may be possible to waive the need for further data if it can be confirmed that the rate of derivatisation of a standard and sample would not be expected to be significantly different.
- The derivative must be stable and must be formed reproducibly.
- Where quantification is based on the determination of a derivative, the calibration should be carried out using standards which have been derivatised using the same procedure as for the analyte or using standard solutions of that derivative, unless the derivatisation step is an on-line part of the detection system.
- In case that the derivative is not available as standard used for calibration, i.e. both the analyte and the standard are being derivatised, the mean yield and precision of the derivatisation step must be demonstrated.

The method is considered to remain specific to the analyte of interest if the derivatised species is specific to that analyte. This point should be demonstrated. However, where the derivative formed is a common derivative of two or more active substances or impurities or is classed as another active substance, the method should be considered non-specific. A consideration of non-specific methods is given in section 3.2.

### 3.2. Non-specific and common moiety methods

Common moiety methods are considered non-specific if the moiety is common to two or more analytes (active substances or significant or relevant impurities or relevant co-formulants), irrespective of how specific the determination of the common moiety is.

It is recognised that, for some analytes, a specific method may be unavailable or difficult to perform. The use of non-specific methods is discouraged. Disadvantages of using non-specific or common moiety methods are:

- Where a non-specific method has been used, the identity of the source of the analyte is likely to be called into question. For example, the method may also detect impurities or breakdown products either containing a moiety common to the intended analyte, or which have been derivatised to a common species, or which cannot be resolved from the target analyte. Such methods may also be subject to interferences from other similarly structured compounds.
- When analysing the A.S. content in a product as part of a storage stability study, degradation may be impossible to determine without a specific method. This is especially the case, when the common moiety method is only capable of detecting the sum of the active substance and the degradation product. Degradation may also cause background interferences that may make it impossible to determine the active substance accurately.
- Where the method determines a moiety common to two or more distinct analytes with different (eco)toxicity, it is important to identify the origin of the analytes, enabling an evaluation of the (eco)toxicologically significant components.

Non-specific and common moiety methods will only be acceptable in exceptional circumstances where there is no other practical means of determining the target analyte, and in these cases, full justification is required. This should include an explanation of why the compound cannot be determined by a specific analytical technique.



## 4. METHODS OF ANALYSIS FOR TECHNICAL ACTIVE SUBSTANCE AND PLANT PROTECTION PRODUCTS

(Annex, point 4.1.1 of Regulation (EU) No 283/2013 and Annex, point 5.1.1 and 5.2 of Regulation (EU) No 284/2013)

### 4.1. Technical active substance - (determination of the active substance, impurities and additives).

Methods must be appropriate to the technical specification and determine the A.S., all impurities above or equal to 1 g/kg (0.1% w/w) and all impurities of toxicological / ecotoxicological / environmental concern and additives.

Collaboratively tested standard CIPAC, CEN, ISO and AOAC methods for the analysis of the A.S. or impurities in technical active substance are regarded as validated. Therefore, no additional validation data are necessary, nevertheless the applicability of the method in terms of specificity must be demonstrated by example chromatograms.

In the other cases, validation data will be required for A.S., for impurities and for additives, as described below.

#### 4.1.1. Method validation for the active substance

- (i) *Specificity* - The degree of interference in the chromatograms for the determination of active substance in the technical active substance should be reported. Interferences from impurities should not contribute more than 3% to the total peak area measured for the target analyte. When the analytical technique used is not a chromatographic technique, a demonstration must be provided to confirm that the method detects and quantifies the analyte.

If the 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. For details of confirmatory techniques, see section 4.1.3 below.

- (ii) *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  $\pm$  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, the concentration range of the A.S. or impurity in the T.A.S. and plant protection product ((m)g/kg or % w/w) should be given to clearly demonstrate that the linearity covers the levels in the batches. 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  $r^2$  is submitted and  $\geq 0.98$ , it should be acceptable.

Other continuous, monotonic functions (e.g. exponential/power, logarithmic) may be applied where this can be fully justified based on the detection system used.

- (iii) *Recovery* - The determination of recovery for the A.S. in the technical active substance, is not required.  
Further discussion of the measurement of recovery and statistical treatment of results is given in Appendix 2.
- (iv) *Precision (repeatability)* – Details of the precision (repeatability) of the method are required for the A.S. in the technical active substance. 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.

- (v) *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.

#### **4.1.2. Method Validation for impurities (significant and relevant) and additives**

- (i) *Specificity* - Specificity for the analysis of impurities and additives should be addressed to the extent that the technical active substance or technical concentrate is properly characterised. For details of confirmatory techniques, see section 4.1.3 below.
- (ii) *Linearity* - See section 4.1.1 (ii)
- (iii) *Recovery* - Recovery may be measured in different ways and the method should be appropriate to the matrix. The recovery of the method(s) for significant and/or relevant impurities and additives in the technical active substance and technical concentrate should be reported as mean recovery and relative standard deviation when applicable ( $n > 2$ ) (see section 4.1.1 (iv)). Recoveries should be determined at levels appropriate to the technical specification. A lower fortification level showing an acceptable recovery value would also be acceptable and can be used to determine the LOQ. At least 2 independent recovery determinations (i.e. two weighings) should be made on representative samples (T.A.S) containing a known quantity of the analyte. These 2 independent recoveries can be carried out at the same or at different fortification levels. Standard addition is an acceptable method of determining recoveries of impurities and additives in the technical active substance or technical concentrate. Further information on the measurement of recovery is given in Appendix 2.
- (iv) *Precision (repeatability)* - See section 4.1.1 (iv)
- (v) *LOQ* – According to Regulation (EU) N° 283/2013, the experimental determination of the limit of quantification (LOQ) is not required for impurities in the technical active substance. However, the method has to be validated at least at specifications level for significant impurities and at least at 20% less for relevant impurities. The recovery should be determined according to 4.1.2 (iii) by standard addition appropriate to the validation level. The precision

(repeatability) should be determined according to 4.1.1 (iv). In case that the content of the impurity is too low for quantification, samples should be fortified by standard addition appropriate to the validation level. If the specification for a relevant impurity was set at the LOQ level, the determination of the %RSD at a level 20% below might not be possible due to higher contents in the T.A.S. In this case, precision (repeatability) should be determined using unfortified T.A.S samples.

In the specific case where an impurity is identified in the technical active substance but was not isolated and there is no analytical standard available, the validation may be performed with a compound with similar structure (for example a compound with common moieties with the impurity) or with active substance as internal standard. The objective is that the quantification is done in comparison with a well characterised compound.

#### **4.1.3. Confirmation of analyte identification for pre-registration**

Regulation (EU) N°283/2013 requires information on how the structural identity of significant and relevant impurities in the technical material have been determined. Confirmation of identity needs to be addressed for each new source of the active substance.

This information must be reported as part of the identity section, as it is requested in Section 1 of the Regulation (EU) N°283/2013 and should be performed at least on one batch; however, it may be addressed as part of the validation of the method, although alternative approaches can be taken.

With regards to the preparation, confirmation of identity as part of the validation data of the method is required for relevant impurities in the plant protection product. This can either be addressed using a highly specific method or using a confirmatory method (details are outlined below).

Confirmatory techniques are required to support the identification of significant and relevant impurities, when the primary method of determination is not considered as highly specific. Highly specific methods are GC-MS with a minimum of 3 ions (ideally with an m/z ratio > 100) or HPLC-MS/MS or GC-MS/MS with at least 2 transitions or one transition with several representative fragments of the substance and must be used for identification. Full scan mass spectrum of parent or product ion spectrum should be provided and these ions or transitions should be reported and justified. When using spectra obtained from isolated compounds for comparison, GC-MS or LC-MS spectra are sufficient. Alternatively the use of high resolution/high mass accuracy MS may be applied to the confirmation too. In this case, if the determined value is within 0.003 m/z unit of the calculated value of the representative ion then this is adequate for confirmation of analyte identification.

Confirmation can be achieved by an independent analytical method using for example a chromatographic technique different from the original, a different stationary phase and/or mobile phase with significant different selectivity or alternative detector (see appendix 4). In that case, a fully validation of independent analytical method is not required, however the specificity of the method should be demonstrated.

Where the primary method is not specific or highly specific, for example titration, the technical limitations for not applying a more specific method should be reported.

Confirmation may also be possible by use of chromatographic peak (fraction) collection (retention time of standard/analyte) followed by spectroscopic/spectrometric analysis or to use spectroscopic/spectrometric method only (e.g. IR, NMR, MS). In that case representative spectrum (test and reference if available) should be enclosed.

## 4.2. Plant protection product - (determination of the active substance, relevant impurities and relevant co-formulants)

Where collaboratively tested CIPAC or AOAC methods are available, additional validation data are not required providing the method was collaboratively tested on the plant protection product type under consideration. However, the applicability of the method in terms of specificity must be demonstrated by example chromatograms (standard, blank : formulation without active substance, and formulation with active substance). Moreover, 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)

Methods should be validated 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.

Methods must be provided for the determination of all relevant impurities identified in the technical active substance or formed during manufacture of the plant protection product or during storage

However, in certain cases the content of the active substance in the plant protection product can be too low in order to determine a relevant impurity at the level derived from the maximum content in the technical active substance. In this case, the validation must be performed at the lowest possible concentration. However, for relevant impurity it is necessary to demonstrate that it is not technically possible to reach the theoretically required LOQ (with chromatograms or some experiment data) and to provide a (eco) toxicological argumentation demonstrating that the reached LOQ is acceptable.

### 4.2.1. Method validation for the active substance

- (i) *Specificity* - 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.

The degree of interference should be reported. Interferences from other substances present in the plant protection product, including other active substances, should not contribute more than 3% to the total peak area measured for each A.S. or the sum of the components of that A.S.. Example chromatograms of formulation blank, standard and sample should be provided.

For pre and post-registration , 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.

- (ii) *Linearity* - as detailed in section 4.1.1 (ii).

- (iii) *Recovery* - 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 ( $n > 2$ ) (see section 4.1.1 iv). At least 2 independent recovery determinations (i.e. two weighings) should be made on representative product samples (formulation of the dossier or same type of formulation containing the active substance) 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. Further information on the measurement of recovery is given in Appendix 2.
- (iv) *Precision (repeatability)* - Details of the precision of the method are required for the active substance in the plant protection product, as detailed in section 4.1.1 (iv). This must be performed on representative plant protection product samples.

#### **4.2.2. Method validation for relevant impurities and relevant co-formulants**

- (i) *Specificity* - The method(s) must be capable of determining the relevant impurities and relevant co-formulants in the presence of the other compounds (active substance and formulants(s)). In case that the respective impurity is an enantiomer, the method should be capable of determining the individual components present by for example an enantioselective method. In the case, where the relevant impurity content is very low in plant protection product and thus the detection of (minor) enantiomer is very difficult, a non-enantioselective method could be sufficient. However, it should be demonstrated that the determination is not possible.

When a relevant impurity is unstable in the plant protection product (e.g. dimethylsulfate, isocyanate in presence of water or water traces from other co-formulants), an analytical method for their determination is not possible and can be waived. However, it should be demonstrated that the determination is not possible.

- (ii) *Linearity* - as detailed in section 4.1.1 (ii).
- (iii) *Recovery* - The recovery of the method should be reported as mean recovery for relevant impurities in the plant protection product. The recovery and precision (repeatability) can be determined by fortification of blank formulations. See section 4.2.1 (iii).
- (iv) *Precision (repeatability)* - Details of the precision of the method are required for relevant impurities and relevant co-formulants in the plant protection product, as detailed in section 4.1.1 (iv).
- (v) *LOQ* - The LOQ must be reported and determined for relevant impurities and relevant co-formulants. It should be at least at the anticipated concentration of the impurity in plant protection product taking into consideration the maximum limit of the relevant impurity in the T.A.S. (as specified in the approval regulation of the active substance) and the content of the T.A.S. in the plant protection product or at the concentration which is formed during storage of the product, whichever is more appropriate. In the case where the content of active substance is too low in order to determine the relevant impurity at the level derived from the maximum content in the T.A.S., the validation must be performed at the lowest possible concentration. However, it should be demonstrated that the desired LOQ cannot be reached.

#### **4.2.3. Confirmation of analyte identification**

This is not required for the analysis of the active substance in the plant protection product. However, the identity of relevant impurities, e.g. N-nitrosamine compounds, in the plant protection product should be confirmed when possible. Otherwise, an argument demonstrating the impossibility to identify the impurities should be provided. For a discussion of confirmatory techniques, see section 4.1.3.

## 5. REFERENCES

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- [2] Regulation (EC) No 1107/2009 published in the Official Journal on 24.11.2009
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Validation of analytical procedures : text and methodology Q2(R1). ICH Harmonised Tripartite Guideline

## 6. Appendix 1

### GLOSSARY OF TERMS

AOAC	AOAC International (formerly the Association of Official Analytical Chemists)
C and c	concentration
CIPAC	Collaborative International Pesticides Analytical Council
FAO/WHO	Food and Agricultural Organization of United Nation/ World Health organization
GC	gas chromatography
GLP	good laboratory practice
HPLC	high performance liquid chromatography
IR	infra-red (spectroscopy)
kg	kilogram
L	litre
LC	liquid chromatography
µg	microgram
m	metre
mg	milligram
MS	mass spectrometry
NMR	nuclear magnetic resonance (spectroscopy)
RSD	relative standard deviation
RSD <sub>R</sub>	reproducibility relative standard deviation
RSD <sub>r</sub>	repeatability relative standard deviation
UV	ultra-violet (spectroscopy or detection)



## 7. Appendix 2

### 7.1. Recovery

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 [6]:

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 ( $C_U$ )  
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 ( $C_A$ )  
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 ( $C_F$ )  
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:

$$\text{Total \% recovery} = 100 \times (C_F)/(C_U + C_A)$$

#### 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:

$$\text{Marginal \% recovery} = 100 \times (C_F - C_U) / C_A$$

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 %
$\geq 10$	97 - 103
$\geq 1 - < 10$	90 - 110
$\geq 0.1 - < 1$	80 - 120
$\geq 0.01 - < 0.1$	75 - 125
$< 0.01$	70 - 130

A consideration of the measured recovery data in relation to these values should be provided. The details of any statistical approach used must be reported.

## 7.2 Precision

A suitable test for outliers may be applied to the precision data, for example the Grubbs or Dixons Tests [7, 8]. 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 ( $RSD_R$ ) and concentration (c) :

$$\%RSD_R = 2^{(1-0,5*\log(c))}$$

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

$$\%RSD_r = 0,67 * 2^{(1-0,5*\log(c))}$$

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  $H_r$  [11]

$$H_r = \%RSD/\%RSD_r$$

$\%RSD$ : obtained repeatability (see section 4.1.1(iv))

$\%RSD_r$ : 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:

$H_r \leq 1$ , acceptable

$1 < H_r \leq 2$ , acceptable in case of a suggested explanation

$H_r > 2$ , not acceptable

For concentrations  $< 10 \mu\text{g}/\text{kg}$ , the RSD obtained should be  $< 30\%$

## 8. Appendix 3

### SUMMARY OF REQUIRED VALIDATION

Sample/purpose	Analyte consideration	Recovery	Precision	LOQ	Linearity	Interference	Specificity	Confirmatory of identity
Technical active substance	Active substance	Not required	Minimum 5 independently weighed samples determination at the same conc.	Not required	Range appropriate to the lowest and highest nominal concentration of the analyte $\pm 20\%$ . Range in % w/v and w/w Duplicate (independently weighed samples) determination at 3 conc or single determination at 5 conc $r > 0.99$	Interferences from impurities in a.s. < 3% of the total peak measured for the target analyte	For a.s. specified as being optically pure or ratio of isomer set, the method must support this	not required
	Significant impurities/relevant impurities and additives	Recoveries determined at levels appropriate to the material specification 2 independent recoveries (i.e. 2 weighings). Standard addition is an acceptable method of determining recoveries of impurities and additives	Minimum 5 independently weighed samples determination at the same conc.	Not required. However, the method has to be validated at least at specifications level for significant impurities and at least at 20% less for relevant impurities.	Range appropriate to the lowest and highest nominal concentration of the analyte $\pm 20\%$ . Range in % w/v and w/w Duplicate (independently weighed samples) determination at 3 conc or single determination at 5 conc $r > 0.99$	Interferences from impurities in a.s. < 3% of the total peak measured for the target analyte	Addressed to the extent that the technical active substance or technical concentrate is properly characterised	Must be addressed for impurities if the primary method is not highly specific and no identity data provided (i.e. mass spectrum)
Section references	4.2	4.1.1(iii) 4.1.2 (iii)	4.1.1(iv) 4.1.2(iv)	4.1.2(v)	4.1.1(ii) 4.1.2(ii)	4.1.1(i) 4.1.2(i)	4.1.1(i) 4.1.2(i)	4.1.3
plant protection product	Active substance	2 independent recovery determinations on representative samples containing known quantity of	Minimum 5 independently weighed samples determination at the same conc	Not required	Range appropriate to the lowest and highest nominal concentration of the analyte $\pm 20\%$ . Range in % w/v and w/w	Interferences from impurities in a.s. < 3% of the total peak measured for	The method must be capable of determining each active substance in presence of each other	Not required

		analyte (between 90-110% of the target conc)			Duplicate (independently weighed samples) determination at 3 conc or single determination at 5 conc r>0.99	the target analyte	For a.s. specified as being optically pure or ratio of isomer set , the method must support this	
	Relevant impurities	2 independent recoveries determinations (i.e. 2 weighings) on representative samples containing known quantity of analyte at the level of (eco)toxicological significance in the plant protection product taken into account the ratio of A.S. in the product or at the concentration which is formed during storage of the product, where relevant. Standard addition is an acceptable method of determining recoveries of impurities and additives	Minimum 5 independently weighed samples determination at the same conc	The recovery should be determined according to 4.1.2 (iii) by standard addition appropriate to the validation level	Range appropriate to the lowest and highest nominal concentration of the analyte $\pm 20\%$ . Range in % w/v and w/w Duplicate (independently weighed samples) determination at 3 conc or single determination at 5 conc r>0.99	Interferences from impurities in a.s. < 3% of the total peak measured for the target analyte	the method must be capable of determining the relevant impurities and relevant co-formulants in the presence of the other compounds (active substance(s) and co-formulant(s)). In case that the respective impurity is an enantiomer, the method should be capable of determining the individual components present for example by an enantioselective method except in the case where the relevant impurity content is very low, however it should be demonstrated that the determination is not possible	must be addressed for relevant impurities. If the method used is regarded as highly specific and is fully validated further data are not required.
Section references		4.2.1(iii) 4.2.2(iii)	4.2.1(iv) 4.2.2(iv)	4.2.2(v)	4.2.1(ii) 4.2.2(ii)	4.2.1(i) 4.2.2(i)	4.2.1(i) 4.2.2(i)	4.2.3

## 9. Appendix 4

Description of the non-specific, specific and highly specific methods

Non-specific method	Any analytical method in which quantification is based on a functional group (moiety) within the analyte rather than for the specific analyte (ex: titration)
Specific method:	HPLC or GC method with a retention match with a reference standard of the analyte. HPLC-DAD: Increased specificity based on the UV spectrum match of the chromatographic peak (peak purity) but not considered highly specific.
Highly specific method	<ul style="list-style-type: none"><li>- LC-MS/MS with two ion transitions validated and a retention time match with a reference standard of the analyte or</li><li>- GC-MS or LC-MS with three ion transitions validated and a retention time match with a reference standard of the analyte</li><li>- Chromatographic peak (fraction) collection (retention time of standard/analyte) followed by spectroscopic/spectrometric analysis or to use spectroscopic/spectrometric method only (e.g. NMR, MS)</li><li>-</li><li>- HPLC-UV with a changing of the stationary phase or mobile phase in order to change the selectivity</li><li>- HRMS (2 ions)</li></ul>