CIPAC Guideline

Preface

The aim of this document is to bundle the numbers of CIPAC guidelines in one document.

The reason is that in the context of this "new" guideline, the content of existing documents will be updated or deleted where appropriate. This is also necessary because of the further development of techniques since the existing guidelines have been published and in order to revise the description of procedural aspects. Furthermore, repetitions of the same or almost the same issue in different papers could be avoided.

The idea is that this CIPAC Guideline is a living document and CIPAC does not claim that it is from the beginning a document that addresses all aspects. The headings and the corresponding chapters will be filled successively and amended as appropriate.

The current version of the document will be available only as pdf-file at www.cipac.org.

Version history

Version no.	Date of adoption	Subject matter

Table of Content

1 CIPAC numbers

The CIPAC coding system has been established in order to achieve a simple and harmonized way to define the identity of pesticide active ingredients. In this system, code numbers are allocated to active ingredients of distinctive structure. The reason for the use of code numbers was to avoid misinterpretation in cases where different common names had been approved by different national standard organization, in cases that common names had been changed or caused by different spellings in different languages. The code numbers also include a subgrouping for salts and ester radicals which are listed on the internet page of CIPAC. A CIPAC number can be allocated as soon as an ISO common name for a certain compound has been accepted. In cases, where no common name was applied for at ISO an explanatory statement has to be provided.

To apply for a CIPAC number contact CIPAC by using the contact form on www.cipac.org. Concerning the principle and the purpose of CIPAC numbers see also extra document *"What are CIPAC Code Numbers?"*.

2 CIPAC methods

CIPAC methods provide the availability of standardised and internationally agreed methods for the analysis of active ingredients and relevant impurities in pesticide[1] products as well as physical, chemical and technical test methods for formulations. CIPAC methods are used by monitoring laboratories, registration authorities, for regulatory compliance, and for FAO and WHO specifications.

3 Collaborative trial to get a CIPAC method

3.1 Scope

A collaborative study is an inter-laboratory study in which each participating laboratory uses the defined method to assess the performance characteristics of the method for the given test material. It is pointed out that a collaborative study is primarily a test of the method and not of the laboratory!

3.2 How to apply

CIPAC recommends conducting first a small scale trial with a minimum of four participants to reveal problems or identify difficulties with the method before conducting a full scale trial. This is however just a recommendation and not mandatory. In case help is needed to acquire laboratories for the small scale trial, either CIPAC or one of the national Pesticides Analytical Councils (PAC) can be contacted.

[1] The term pesticide includes synthetic chemical active substances, botanicals, microorganisms, and their formulations.

a) Contact the secretary of CIPAC to clarify open issues.

b) Provide a draft information sheet including information on:

- the contact person, including e-mail address and phone number
- a method description containing the essential parameters of the method including the required equipment
- the proposed formulation types
- the time frame of the trial
- the maximum number of possible participants, if it is proposed to be limited, with a justification
- previously obtained validation data (see 5)

c) In cases where special equipment is required, expected not to be available in a pesticide quality control laboratory, the conductor of the trial should consider to provide this equipment to the participants. This information will be also included in the information sheet.

3.3 How to conduct

3.3.1 Duties of CIPAC

a) CIPAC secretary double-checks with the conductor of the trial whether all essential information is available.

b) The information sheet is circulated via CIPAC website and e-mail distribution list.

c) To facilitate the customs clearance for the distribution of the samples CIPAC will provide a corresponding confirmation letter to the collaborative trial coordinator (see 3.3.2).

3.3.2 Duties of the collaborative trial coordinator

a) Laboratories participating in the collaborative trial are chosen by the trial coordinator at random from those that are willing to take part. As the method is intended for international use, laboratories of at least five countries from two continents[2] must participate.
The trial coordinator provides feedback to the CIPAC secretary about the selection procedure, if the number was limited and the laboratories that expressed interest in taking part in the trial was higher.
It should be noted that at the end of the trial accepted results from at least eight participants which still comply with the regional criteria used for the selection must be available. Otherwise, CIPAC will not adopt the method as a full method. The participation of ten or more laboratories is therefore recommended.

[2] here: Asia, Africa, North America, South America, Europe, Australia

b) The method has to be written up in CIPAC format.
The trial coordinator should ensure the following is provided:

- the necessary instructions to collaborators on sub-sampling and/or homogenization of samples, sub-samples or reference materials
- any particular steps which should be taken to equilibrate the equipment before use, (e.g. the number of injections of samples or standards before starting analysis, and what repeatability of duplicate injections should be attained prior to starting the analysis)
- the injection sequence of samples, calibration solutions and the number of calibration solutions required in chromatographic methods
- any questions which participating laboratories need to be asked in order to ensure the collection of full details of their operating conditions, particularly any departure from the method
- the equation and or calculations to be used to convert the data obtained into the results to be reported, including the meaning of symbols used in the equation
- special information concerning storage of reference materials samples or prepared solutions and the estimated shelf-life of solutions and reagents if necessary
- The particular instructions may deviate for physical, chemical and technical test methods. However, the trial coordinator must take care that also in this case all necessary information needed to conduct the analysis is provided.
- the number of significant digits in the required results
- the deadline for the return of results

c) The number of repetitions are specified by the trial coordinator and must be followed by the participants. ~~As a minimum requirement, duplicate analyses should be performed by each laboratory.~~ As a minimum for each sample two complete analyses (independent weighing) should be done each on two days. ~~The procedure which should be adopted is to analyse two samples from each level, with each sample weighed and analysed once, and the procedure repeated at a later date.~~ For chromatographic analyses each individual sample is injected twice, thus giving two sets of results. These duplicate injections are not required for the subsequent statistical analysis, which uses their averages, but they do give a good indication of the efficiency of the chromatography, and provide the panel or national PAC with useful additional information.
For physical, chemical and technical test methods a deviation from duplicate analyses of sub-samples might be necessary in dependence of the used method or formulation (e.g. as for MT 202 Discharge Rate of Aerosol Dispensers). In this case, an appropriate justification must be provided to CIPAC.

d) When sending out the samples the trial coordinator must assure that the declaration of the identity of the samples sent out on the label as well as in the cover letter are complete, clear and correct. Incorrect labelling can lead to the situation that samples are rejected by laboratories and their withdrawal from the collaborative trial.

To minimise problems with the customs when sending out the samples it is recommended to enclose a declaration confirming that the substance

- is intended for laboratory use only
- is not intended for further commercial distribution of the substances, and
- all residual amounts of substance not needed in the laboratory experiments will either be disposed in accordance with national regulations or sent back to the organizer.

This declaration will be made available by CIPAC (see 3.3.1).

Furthermore, the following is also helpful to minimise potential problems:

Container labelling & package labelling

Containers and the shipment packages have to be labelled correctly with the correct pictograms according to GHS and Dangerous Goods Transportation (IATA, RID, ADR, IMDG).

Material Safety Data Sheet

An up-to-date material safety data sheet for each sample and standard material has to be included in the package.

Pro forma invoice

The shipment papers should include a pro forma invoice that indicates a low commercial value for the contents of the package (e.g. stating that 10 Euro/USD or comparable low amount of money).

e) At the end of the trial, the study coordinator will notify each participating laboratory three to four weeks prior to the CIPAC meeting of the number or symbol of the own laboratory listed in the final report.

3.3.3 Duties of the participants

a) Laboratories wishing to participate in a trial have to contact the organizer by e-mailing to the contact person indicated on the information sheet with copy to CIPAC secretary.
The application must include the detailed address where the samples have to be sent to.

b) Participants must be able to perform the required analysis (e.g. availability of the equipment including the described column, except special equipment provided by the conductor) within the required timeframe.

c) The method description has to be strictly followed. The aim of the trial is to validate the method! Any deviation from the original method described has to be documented and justified. It should be noted that deviation from the original method may lead to exclusion of the results of that laboratory from the evaluation of data of the collaborative trial.

d) Fill in the result sheets and send it back to the organiser in time together with needed chromatograms, remarks on problems or proposals for changes.

3.4 How to evaluate

When all the results have been returned to the trial coordinator, they are tabulated and statistically assessed. Only results of tests without relevant deviations from the prescribed method will be taken into account.

a) Redundant data
If a laboratory carries out more than the required number of replicate analyses, all the results must be reported. Furthermore, the laboratory should provide an explanation on the reason why additional analyses have been conducted, and which results should be considered the most accurate. If the results are all valid, this may be taken into account by a statistical procedure, or the required number of results should be selected using a strictly random method.

b) Missing data
Test results may be missing, due to loss of sample or a slip in the experiment.
If only one sample could be analysed at a certain level, this result is not excluded from the subsequent statistical analysis, but it will be marked as a single value.

c) Outliers
The test for outliers is performed by using the Grubbs test. An outlier is present when the test statistic exceeds the critical value at a significance level of 1 %. If the test statistic lies between the critical values at a significance levels of 5 % and 1 %, the test result is a straggler and will be marked with an asterisk.

In the first instance the mean value $\bar{x}$, standard deviation s and the relative reproducibility standard deviation $RSD_R = 100 \cdot s/\bar{x}$ (in %) are calculated with no outlying results removed, but using only valid data.

The prime decision as to whether unexplained stragglers or outliers may be retained as correct data lies with the trial coordinator.

Those laboratories or data are removed when they turn out to be outliers according to the above mentioned criteria. Removal of outliers has to be stopped before the number of concerned laboratories exceeds 22 %, e.g. only 2 outliers out of 13 laboratories are acceptable.

It should be determined whether the stragglers or outliers can be explained by some technical or computational error etc. When there is a reasonable explanation, the test item may be corrected or discarded in keeping with the explanation obtained. When several unexplained stragglers or outliers occur at different levels within the same laboratory, that laboratory may be considered an outlier.

After the outlying data has been eliminated, the mean value and precision data are then recalculated.

In case of physical, chemical and technical test methods, it may not be reasonable to identify and exclude outliers. This depends on the particular procedure and whether a defined measured value is obtained by the method. If it is not reasonable to make an outlier identification, this has to be justified.

The acceptability of the analytical quantification is based on the Horwitz Ratio

$$\text{HorRat} = \text{RSD}_R / \text{RSD}_{R\,(\text{Hor})}$$

which is the ratio of RSD_R determined from the collaborative study and the expected relative reproducibility standard deviation obtained by the Horwitz equation

$$\text{RSD}_{R\,(\text{Hor})} = 2^{(1-0.5\log C)}$$

where *C* is the content[3] of the analyte in the sample given as decimal fraction.
From the accepted results, the HorRat is calculated by the trial coordinator for each test material using all digits and without rounding. The following criteria are used for the assessment:

$0.3 \leq \text{HorRat} \leq 1$	→	acceptable
$\text{HorRat} < 0.3$ or $1 < \text{HorRat} \leq 2$	→	acceptable, but a reasonable explanation is required
$\text{HorRat} > 2$	→	not acceptable

The criterion for HorRat values < 0.3 is applied only to full scale collaborative trials.

The rationale behind these criteria is:

If HorRat is close to 1, the method precision in terms of reproducibility is close to the predicted value.

If HorRat is < 0.3, suspect comes out that the collaborative trial was not performed correctly and it gives precision values that are too optimistic (e.g. due to few participants ~~or prior knowledge of analyte content~~).

If HorRat is > 2, the analytical method is undoubtedly suspect to perform worse than expected (e.g. due to method deficiencies or due to interferences, contaminations or sample inhomogeneity).

[3] The correct term according to IUPAC is "mass fraction" (Compendium of Chemical Terminology, IUPAC Gold Book, online version, 2019). The term "content" is also used by CIPAC for multiples of the mass fraction given as g/kg or mg/kg. The term "concentration" should be used related to a volume.

3.5 How to report

to be completed later

3.6 Decision process

The conductor of the collaborative trial presents the results at the CIPAC meeting for discussion. The decision if a method is accepted and may be classified as a CIPAC method, a provisional CIPAC method, or a tentative CIPAC method is made by CIPAC members in the closed part of the technical meeting.

CIPAC methods are methods that have been investigated in accordance with internationally accepted rules and have given results falling within the accepted ranges for repeatability and reproducibility. Provisional CIPAC method are either candidate CIPAC methods, which may become full after a certain period, or methods with minor imperfections. Tentative CIPAC methods have usually not been tested in a full scale study or have provided an insufficient number of result but could be used for intended purposes.

4 Method extension

4.1 Scope

to be completed later

4.2 Procedure

to be completed later

5 Method validation for chromatographic methods

The chromatographic methods used in the collaborative trials are mainly methods for formulations, but may also be applied to active ingredient content of technical material and concentrates or for measurements of physical, chemical and technical properties (see 6).

In general, the validation of an analytical method submitted to CIPAC is performed by the collaborative trial. Only in case that the collaborative trial has shown sufficient results with respect to accuracy and precision, the method will be accepted by CIPAC.

Nevertheless, it is expected that the conductor of the trial validates the method previously with respect to specificity, linearity, recovery and repeatability according to appropriate criteria.

The obtained validation data are previously checked by the panel or national PAC and are provided to CIPAC along with the draft information sheet (see 3.2).

6 Physical, chemical and technical measurements

Besides methods for active ingredients and relevant impurities, CIPAC also provides methods to determine physical, chemical or technical properties of plant protection products, the MT (miscellaneous techniques) methods. The structure of an MT method should be the same as for other methods, but some parts may not fit for a given property (see Appendix IV). During development of an MT method, it has to be carefully assessed what kind of validation data are necessary to obtain and whether it is possible to give a measurement uncertainty. For methods which are used for plant protection products or biocides with a broad range of active ingredients, no method description regarding the chromatographic determination is needed. For MT methods for defined active ~~substances~~ ingredients, e.g. “MT 189 Free lambda-cyhalothrin in CS formulations” a detailed method description may be necessary.

Some measurements of physical, chemical or technical properties, such as suspensibility or spontaneity of dispersion, may also require a chromatographic analytical determination. The decision, whether the chromatographic method is considered as fit for purpose, is made in the context of the measurement of the corresponding property.

7 Special matrices

Further guidance concerning CIPAC methods that are related to special matrices is provided in extra documents. In this regard, reference is made to the document

Work flow for integration of analytical and physical-chemical methods for LN into related CIPAC methods for corresponding active ingredients, CIPAC 4567/R (May 2009)

8 Relevant impurities

CIPAC provides analytical methods for the determination of certain toxicologically and/or ecotoxicologically relevant impurities. These methods are needed for the quality control of the technical material and formulations and for regulatory monitoring purpose.

For further information, see extra document *CIPAC Guideline for analytical methods for the determination of relevant impurities (June 2009)*.

9 Instructions for writing CIPAC methods

CIPAC methods are written in English, which is often not the native language of the people for whom they are intended. Writers of methods should keep this in mind and should use straightforward wording and give clear and concise, but sufficiently detailed and unambiguous description of the procedures to be carried out.

9.1 General instruction

CIPAC methods are written in the imperative mode except for the section OUTLINE OF METHOD, which is written in the passive voice.

Use no abbreviations unless they are internationally accepted, or are fully explained when used for the first time.

Use of notes

Avoid the use of notes; they will distract the attention of the reader. Incorporate their content in the text or use a special section or sub-section.

Footnotes should not be used; the only exception is the footnote giving the status of the CIPAC method, with the year of acceptance and the committee that prepared the method.

Units, symbols and nomenclature

Use SI units. Express quantity, concentrations in the following way (IUPAC rules[4]):

c (NaOH) = 1 mol/l or γ (NaOH) = 1 g/l .

Use the ISO common names of active ingredients ~~pesticides~~. Chemicals should be named according to the IUPAC rules for nomenclature. In the descriptive section of the active ingredient, the name according to Chemical Abstracts should be added. Do not use molecular formulae in the text of the method. Long names may be abbreviated or substituted by trivial names. In that case the abbreviation or the trivial name has to be explained in the reagent section.

9.2 Format

The best way to get familiar with the CIPAC format is to take an example from one of the latest volumes of the CIPAC Handbook. Further, you find four Appendixes on the CIPAC website with formatted examples of the common methods (GC-FID, HPLC-UV, method extension and MT-Method as Word.docx).

Coding of methods and cross reference

The letter **"M"** stands for "analytical methods"

The letter **"MT"** for "miscellaneous techniques"

[4] Quantities, Units, and Symbols in Physical Chemistry, IUPAC Green Book, 3rd ed., 2007

Methods are identified by the CIPAC coding system (CIPAC Code Number) and make use of the two-letter codes for formulations (FAO and WHO: Manual on the development and use of FAO and WHO specifications for chemical pesticides, current edition).

"M" stands for *"full CIPAC method"*

"(M)" stands for *"provisional CIPAC method"*

"m" stands for *"tentative CIPAC method"*

The last position stands for the current section in the method.

So, the code for ampropylofos technical is: **500**/TC/M/2.1.

Remark: If CIPAC had already published a method with the same ~~a.i.~~ active ingredient and formulation, the new method gets in our example the following code: **500**/TC/M2/-.

In principle, each formulation type should have its own method. If there are no differences between the methods for the respective formulation, it is sufficient to refer to the basic method. In other cases where the differences are partial or small, shorten the text by using sentences like:

As for **500**/TC/3 except: .....

In this example, the number "3" refers to the corresponding chapter 3 in the method.

The order of the methods for the different formulations is: technical, technical concentrates, solid formulations, liquid formulations, and within these groups in alphabetical order.

Layout and arrangement of the sections

The following description provides information on the content of the corresponding sections used for analytical methods. Examples are given in the Appendices I - III. It is pointed out that the layout for MT methods differs from that of analytical methods. Concerning the format of MT methods, reference is made to Appendix IV.

A short descriptive section that gives information about the active ingredient concerned should precede new compounds for which no method has been published before.

1 Sampling

In this section the amount of material is specified. If special precautions should be taken e.g. to obtain homogeneity of the sample, they are mentioned in this section.

2 Identity tests

An identity test is not required in case that certificated reference standards are use. Checking the (relative) retention time(s) of the chromatographic method used for quantification is then sufficient.

In other cases, at least two identity tests, based on different analytical techniques, are required, one of which may be the check of the (relative) retention time(s) of the chromatographic method used for quantification. If necessary, refer to example chromatograms or spectra. For spectroscopic techniques, it is necessary to separate the active ingredient from other components of the formulation. Use standard wording according to the templates wherever possible.

3 Active ingredient

In this section an accurate description of the analytical method must be given together with a list of the materials and apparatus needed. Use the following sub-headings:

OUTLINE OF METHOD

Give the analytical principles of the method, important conditions (e.g. the wavelength at which the absorption is determined, the use of internal or external standardisation) and a short description of the most important manipulations. Mention chemicals that play a key-role in the procedure, avoiding at the same time to present too many details. Use the passive voice.

SCOPE

This subsection can usually be omitted, because the scope is sufficiently clear from the code above each method. Use this section only if there are other limitations to the applicability of the method.

REAGENTS

Give a list of the reagents and their quality required in the method. Use IUPAC names for all chemicals. Give here the abbreviations to be used in the rest of the method.

Include the preparation of internal standard solutions and calibration solutions.

APPARATUS

Give a list of important apparatus with sufficient definition.

As far as possible use less toxic reagents.

PROCEDURE

Write this section in the imperative mode. Use standard wording for the weighing procedure and indicate the required accuracy (see Appendices). Express quantities in the following way:

"Dissolve in acetonitrile (40 ml), add by pipette internal standard solution (25.0 ml), and dilute to volume"

Examples of headings for sub-sections are:

(a) Operating conditions

(b) System equilibration

(c) Sample preparation

(d) Determination

(e) Calculation

For the sake of uniformity use, where possible, standard symbols in the formulae to calculate the content of the active ingredient. Keep the final calculation formula as simple as possible.

Close this section with the repeatability and reproducibility[5] figures that were calculated based on the ring trial results of the method.

Repeatability r = ...g/kg at ...g/kg active ingredient content

Reproducibility R = ...g/kg at ...g/kg active ingredient content

4 Impurities

Add methods for impurities if requested, e.g. by FAO specifications.

5 Determination of the active ingredient after a physical, chemical or technical test

If after the application of a physical, chemical or technical test (e.g. suspensibility) the concentration or the amount of the active ingredient has to be determined in the processed formulation or in a fraction thereof, the method to be used must be mentioned, either by referring to the method for that particular formulation, or by giving a modified method.

Example:

As for **500**/TC/3 except…….

[5] The terms "repeatability" and "reproducibility" refer to the same “repeatability limit” and “reproducibility limit”, respectively, as defined in ISO 5725-1:2023.

9.3 Typing

This section gives instructions for the final typescript only.

- Type the text in 14 pt Times New Roman font.
- Use single spacing between the lines.
- Paper size: A4.
- Margin: up 2.5 cm, down 2.5 cm, left 1.5 cm, right 1.5 cm, header 1 cm, footer 1 cm.

File format: preferable as Word.docx, alternative unprotected pdf file.

9.4 Graphs, line drawings, formulas

- Graphs: Embedded in the word.docx as PNG or JPG graph (important high-resolution quality). Alternatively, the graphs can be submitted as separate files.
- Line drawings: Embedded in the word.docx as GIF or PNG graph (important high-resolution quality). Alternatively, the line drawings can be submitted as separate GIF or PNG files.
- Formula: Embedded with the formula editor (word) in the document. Alternatively, as an embedded picture e.g. PNG or JPG file).

9.5 Standard symbols to be used typically in the calculation formulae

t	ml required for the sample determination
b	ml required for the blank determination
a	ml required for the back titration
f_i	single response factor, i = 1,...
f	~~average~~ mean response factor
H_s	peak area of the....(*a.i.*)....peak in the calibration solution
H_w	peak area of the....(*a.i.*)....peak in the sample solution
I_r	peak area of internal standard peak in the calibration solution
I_q	peak area of internal standard peak in the sample solution
s	~~mass~~ weight of ....(*a.i*).... in the calibration solution (mg)
w	~~mass~~ weight of sample taken (mg)
r	~~mass~~ weight of internal standard in the calibration solution (mg)
q	~~mass~~ weight of internal standard in the sample solution (mg)
P	purity of ...(*a.i.*).... reference substance (g/kg)

R	...(*a.i.*).... to internal standard peak area (height) ratio for the sample solution
R'	...(*a.i.*).... to internal standard peak area (height) ratio for the calibration solution