# Eurachem
## A focus for analytical chemistry in Europe

Palacký University Olomouc

**ONLINE WORKSHOP**

# TRENDS & CHALLENGES IN ENSURING QUALITY IN ANALYTICAL MEASUREMENTS
## 17th – 19th May 2021

# Book of Abstracts

**Prague**

http://www.eurachem2021.cz

Edited by Sylvie Kříženecká and David Milde

# Welcome Message

Eurachem Czech Republic is delighted to hold this year's Eurachem scientific workshop. Unfortunately, travel restrictions due to COVID-19 are still in place and we cannot welcome all of you in Prague. The scientific workshop will run as a virtual event with support from Palacky University in Olomouc.

Scientists, PhD students and professionals from laboratories and public bodies are welcome to discuss and present their findings on metrology in analytical sciences. This workshop has following aims:

- Reflect experience with the revised ISO 17025 standard after the transition period,
- Present current Eurachem activities in the field,
- Discuss future challenges in quality in analytical measurements from both research and practical perspectives.

On behalf of the organizing committee, I wish this event becomes a pleasant and rewarding opportunity for all participants who can this way contribute to Eurachem's activities for the coming years.

David Milde
Local organizing committee chair

# Committees

## Scientific committee

Miloslav Suchánek (Czech Republic) - committee chair
Vicki Barwick (UK)
Ricardo J.N. Bettencourt da Silva (Portugal)
Brian Brookman (UK)
Stephen L.R. Ellison (UK)
David Milde (Czech Republic)
Michael H. Ramsey (UK)
Lorens Sibbesen (Denmark)
Isabelle Vercruysse (Belgium)

## Local organising committee

David Milde - committee chair
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Václav Červený
Eva Klokočníková
Sylvie Kříženecká
Alena Nižnanská

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# Abstracts of invited lectures

# Eurachem – A focus for analytical chemistry in Europe

**Vicki Barwick** [1]

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**Keywords:** traceability, metrology, quality assurance, guidance

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Established in 1989, the aim of Eurachem is to provide a focus for analytical chemistry and quality-related issues in Europe. The main objectives are establishing a system for the international traceability of chemical measurement results and the promotion of good quality practices. Eurachem currently has 36 member countries and is effectively a 'network of networks'. A requirement of membership is the establishment of a national Eurachem network which supports the dissemination of Eurachem's aims and outputs.

Eurachem also has liaison arrangements with a number of European and international organisations including: Eurolab; the Technical Committee on Metrology in Chemistry (TC-MC) within Euramet; European Cooperation for Accreditation (EA); European Chemical Society-Division of Analytical Chemistry (EuChemS-DAC); European Commission-Joint Research Centre; NMKL-NordVal International; AOAC-Europe; Cooperation on International Traceability in Analytical Chemistry (CITAC); International Laboratory Accreditation Cooperation (ILAC); Consultative Committee for Amount of Substance: Metrology in Chemistry and Biology (CCQM); International Union of Pure and Applied Chemistry (IUPAC); the CODEX Alimentarius Commission (via its Committee on methods of Analysis and Sampling); Joint Committee on Traceability in Laboratory Medicine (JCTLM) and the ISO Reference Materials Committee (ISO-REMCO).

Eurachem produces authoritative guidance to support laboratories in ensuring measurement quality throughout the measurement cycle. Historically the focus was mainly on the analysis part of the cycle, with guides covering metrological traceability, method validation, measurement uncertainty and proficiency testing. However, Eurachem guides also cover other aspects of the measurement cycle, including sampling and interpretation of results against limits. All guides are available free of charge from the Eurachem website and translations of a number of guides are available [1].

In addition to the development and publication of guidance documents, a key Eurachem activity is the organization of conferences and workshops on quality assurance issues. Since 2010 Eurachem has organized **over** 20 workshops and training events, with truly international audiences.

This presentation will provide an overview of Eurachem's aims and activities.

## References

[1] https://www.eurachem.org/index.php/publications

# Experience of the Accreditation Body with the Transition to ISO/IEC 17025:2017

**Pavel Nosek** [1]

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## 1. Introduction

The introduction should briefly place the study in a broad context and define the purpose of the work and its significance.

For papers that report original research, you should use the titles "Materials and Methods", "Results", "Discussion" and "Conclusions" (optional).

In the end of 2017 we were facing a big challenge to transit more than 600 laboratories to revised standard ISO/IEC 17025. The original standard from 2005 proofed its quality as the revision was started later than usually and most of the laboratories as well as ABs was satisfied with it. We were aware of main changes as a risk based approach, the focus on impartiality or the reporting statements of conformity, but we realised some minor but important changes later on during the transition process too.

CAI's strategy was to inform laboratories in advance, do not scare laboratory managers and all the personnel and to start with transition as soon as possible. All these measures worked and the transition process worked smoothly without excessive stress on both sides.

What we did not expect is the COVID-19 pandemic all over the world which brought some difficulties into standard assessment process. Afterwards we see as the very good and important decision to do not postpone any assessment (except initial assessments) and to jump into remote assessments immediately. As the "new 17025" brought the main changes in the management systems requirements it is possible to assess those remotely well, nevertheless the on site assessment is more effective less time consuming and irreplaceable for longer time period of course. CAI welcomes the 6 months postponing of the deadline for the transition by the ILAC even finally the need to use it was not so much excessive as expected.

In my presentation I would like to inform you about some of the important changes, the big ones as well as the small ones and to share experience of more or less problematic ones. I will also touch the topics highlighted as the problematic by the other European Accreditation (EA) members. I would also show you a basic statistics of the transition in our country and review all the important milestones and deadlines.

# Revised ISO/IEC 17025 – Laboratory view

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**Keywords:** laboratory view, ISO17025:2017, implementation

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On 30 November 2017, the EN ISO/IEC 17025:2017 standard: "General requirements for the competence of testing and calibration laboratories" was published. This standard replaces the ISO/IEC 17025:2005 standard of the same name.

It was determined by ILAC that the move from the 2005 version to the 2017 version would be subject to a transition period of three years, i.e. until 30 November 2020. The revision included both new text containing new requirements and amendments of existing text into differently worded requirements, as well as the deletion of requirements from the 2005 version. The changes broadly related to:

- the structure of the standard
- terminology
- introduction of new provisions
- a strong emphasis on risk-based thinking
- new management system options
- references to new standards
- new annexes.

The revised standard is aligned with the general structure of the other standards in the ISO 17000 series and with ISO 9001:2015.

It was quite a challenge for laboratories to implement and apply the changes introduced by the revised standard.

In March 2017 Belab, the Belgian organisation of laboratories, organised a first seminar on the new standard titled "the new ISO17025 an introduction" for the accredited Belgian laboratories. This was followed in March 2018 by a seminar organised by Belac, the Belgian accreditation body, on the differences between EN ISO/IEC 17025:2017 and ISO/IEC 17025: 2005.

Since 1 January 2019, all Belac audits in Belgium have been organised according to the requirements of ISO/IEC 17025:2017. Between the publication of the standard and its implementation, the laboratories had to clear several hurdles. Not only the interpretation but also the application of the new standard in the laboratories became a real challenge.

In the spring of 2021, the accredited Belgian laboratories were questioned about, among other things, the challenges and difficulties in applying the new standard ISO/IEC 17025:2017. The results will be discussed and some specific points will be highlighted.

# Assessment of qualitative analysis performance and uncertainty

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**Keywords:** Qualitative analysis; Validation; Uncertainty

## 1. Introduction

Many chemical analyses with relevant socio-economic impact involve qualitative assessments or are only qualitative. Qualitative analysis aims to determine nominal properties that cannot be expressed numerically, such as the presence of a compound or an item's chemical identity. These analyses can be more widely described as a classification of the analysed items in classes, typically based on a specified classification criterion.

Some examples of these analyses are identifying the source of an oil spill, the presence of Covid-19 in human serum, doping substances in the urine of an athlete or pesticide residues in foodstuffs. In the first two cases and some doping analysis, no quantitative determinations after the qualitative analysis are required. In the analysis of pesticide residues in foodstuffs, the confirmed residue presence should be quantified. The qualitative analysis must be fit for its intended use and have an acceptable cost and duration. The fitness for a purpose is assessed during method validation that involves quantifying the most relevant performance characteristics and their comparison with target values. These performance characteristics should be periodically checked in the post-validation analysis of the studied items. In some cases, qualitative analysis results can be reported with a quantitative expression of their reliability, i.e., qualitative analysis uncertainty.

## 2. Qualitative analysis performance

A qualitative analysis result can be wrong when the item from class A is declared as not being from that class, defined as a false negative result, or when the item from a class different from A is wrongly declared as from that class, a false-positive result. Qualitative analysis performance can be well characterised by the false negative, *FN*, and false positive, *FP*, rates. However, for very selective classification methods where false-positive results are unlikely, the determination of *FP* is particularly demanding. The difficulties of determining *FP* can be mitigated by developing performance models or data simulation. When the qualitative analysis is supported on various independent qualitative analysis results, well-known probabilistic models can be used to quantify the reliability increase from using multiple tools. This information is handy to decide the need to confirm analysis from initial screening.

## 3. Qualitative analysis uncertainty

If sound determinations of *FN* and *FP* are possible, the qualitative analysis result can be reported with a likelihood ratio or the posterior probability of the result being correct. However, both these metrics have limitation and challenges to be faced when reporting the information.

This communication summarises the content of the guide on "Assessment of performance and uncertainty in qualitative chemical analysis" that is being finalised by the Qualitative Analysis Working Group [1].

## References

[1] QAWG, Assessment of performance and uncertainty in qualitative chemical analysis, Draft 03/2021, **2021**.

# Pros and Cons of the SARS-CoV-2 Diagnostic Tests

**Hana Zelená [1], Marketa Pomiklová [1], Ivana Vidličková [1], Jakub Mrázek [1]**

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RT-PCR testing is currently considered the most reliable technique for identification of patients infected with the new coronavirus SARS-CoV-2. However, antigen tests providing rapid response at the point-of care are also deemed suitable for screening purposes. Both of these methods usually use nasopharyngeal swabs (NPS), although nasal swabs or saliva samples are sometimes used for detection. The sensitivity of antigen tests related to RT-PCR varies, depending on the brand and setting of the evaluation experiment. The cycle threshold considered as the cut-off for SARS-CoV-2 positivity plays a major role in the sensitivity testing, with lower cycle threshold values corresponding with higher viral loads. However, few studies take into account the viability of the virus detected by PCR. We hypothesized that in a significant proportion of patients in whom RT-PCR detected SARS-CoV-2 but who were negative by antigen rapid test, only non-viable virus is present on the mucosa. We assessed virus viability test (virus cultivation) in samples the results of which differed between antigen test and RT-PCR. Only the viability test is capable of detecting really infective virus particles. Our results confirmed that there is generally very low rate of viable virus in the samples with low viral load achieved by RT-PCR and in the samples considered to be false negatives of the antigen tests. This indicates that most persons who are SARS-CoV-2 positive according to RT-PCR but missed by the good performing antigen tests are actually not infected and, hence, many patients and their relatives currently quarantined needlessly. However, the quality of antigen test differs significantly and only the best one are generally suitable for diagnostic purposes. Therefore we recommend independent evaluation of antigen tests before their implementation into the screening purposes. There is also discussed the benefit of antibody testing which is especially feasible in the differentiation between susceptible and already immune persons against repeated infection of SARS-CoV-2. Detection of neutralizing antibodies plays essential role in the screening of convalescent plasma donors used for therapeutic purposes.

## References

[1] Bullard J, Dust K, Funk D, et al. Predicting infectious SARS-CoV-2 from diagnostic samples. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2020.

[2] Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581:465-9.

[3] WHO. WHO Information Notice for IVD Users 2020/05: Nucleic acid testing (NAT) technologies that use polymerase chain reaction (PCR) for detection of SARS-CoV-2, 2021.

[4] Libster R, Pérez Marc G, Wappner D, Coviello S et al. Early High-Titer Plasma Therapy to Prevent Severe Covid-19 in Older Adults. N Engl J Med. 2021 Feb 18;384(7):610-618. doi: 10.1056/NEJMoa2033700. Epub 2021 Jan 6. PMID: 33406353; PMCID: PMC7793608.

# Ensuring Quality: A key element in analytical and clinical laboratories

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Analytical laboratories are involved in testing; however they may also be involved in sampling, associated with subsequent testing [1]. In the case of clinical laboratories, activities include examinations of materials derived from the human body [2]. What does the customer expect from a laboratory when looking for its services? In practice, this mainly refers to a report of results which need to be provided accurately, clearly and unambiguously [1,2]. In each case additional requirements may exist to fit the purpose and meet the customer's requirements [1] or any specific instructions in the examination procedures [2]. Some other requirements refer to the overall service provided for which details have to be reviewed and agreed or communicated.

A laboratory needs to ensure the quality of the services it provides; this is facilitated via the implementation of a (quality) management system which addresses both management and technical requirements, as specified by the standards ISO/IEC 17025 and ISO15189 as appropriate. These standards provide for the competence of laboratories; although they do not refer to accreditation, they represent the basis for the accreditation of laboratories. Laboratories fully complying with these requirements may declare their competence; however, the objective evidence for this lies only with their accreditation for a clearly stated scope, if this is the case.

In all cases, the main task of an analytical laboratory is the validity of the results included in the test report required by the customer; similarly, in the case of a clinical laboratory, this refers to the examination results asked for by the patient or the requester. The validity of results reflects the adequacy of policies and procedures addressing technical issues, being in place and implemented for certain disciplines. Further to this, quality refers to the whole service provided, governed by policies and procedures addressing management issues related to the operation of the laboratory. These may include time-schedules, agreements and contact reviews, communication with the customer/user of the results (see the standards), handling of complaints, management of nonconformities, consideration of risks and opportunities etc. In the case of clinical laboratories, these aspects often have even more importance.

This presentation describes all requirements with the fulfilment of which the validity of results can be ensured. Reference is made to technical aspects i.e. availability of policies and procedures, documented competence of the personnel, suitability of equipment, adequacy of the environmental conditions in the laboratory, risk-based approach, traceability of measurements, measurement uncertainty and maintenance of records. In analytical laboratories, ISO/IEC 17025 specifies that when estimating measurement uncertainty, all contributions have to be taken into account, including those arising from sampling. This is not the case with ISO 15189 which clarifies that uncertainty components are those associated with the actual measurement and this is not expected to be changed in the current revision of the standard [3]. This does not undermine the critical importance of the pre-examination phase (including sampling) for which the standard specifies very detailed requirements. Even with all these aspects being systematically and adequately addressed, the validity of results may still be under question; to this end the laboratory needs to monitor the validity of its results. This is achieved via both internal quality control, mainly with, but not limited to the use of reference materials [4,5] and external quality assurance with the participation in proficiency testing schemes or

other interlaboratory comparisons [6,7]. The presentation explains how the analytical laboratory can be supported by publications of Working Groups of Eurachem which are very active for many years on these quality issues [8,9].

The presentation also makes a comparison of requirements specified in ISO/IEC 17025 with those of ISO 15189. Some of the points discussed may change during the coming months in the perspective of the revision of the said standard, currently under way. The new standard is expected to be published next year.

## References

[1] ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories. International Organization for Standardization/International Electrotechnical Commission, Geneva

[2] ISO 15189:2012 Medical laboratories – Requirements for quality and competence (currently under revision). International Organization for Standardization, Geneva

[3] K.C.Tsimillis and S.Michael, "Uncertainty from Sampling: Could the requirements of ISO/IEC 17025:2017 be adopted in medical laboratories?" Eurachem Workshop "Uncertainty from Sampling and Analysis for Accredited Laboratories" (Berlin, November **2019**). Available from https://www.eurachem.org/events/completed

[4] ISO 17034:2016 General requirements for the competence of reference material producers. International Organization for Standardization, Geneva

[5] B. Magnusson, H. Hovind, M. Krysell, U. Lund and I. Mäkinen, Handbook - Inter Quality control, Nordtest Report TR 569 (ed. 5) **2018**. Available from www.nordtest.info

[6] ISO/IEC 17043:2010 Conformity assessment — General requirements for proficiency testing (currently under revision), International Organization for Standardization/International Electrotechnical Commission, Geneva

[7] EA-4/18 INF:2010 Guidance on the level and frequency of proficiency testing participation (currently under revision). Available from www.european-accreditation.org

[8] https://www.eurachem.org/index.php/publications

[9] https://www.eurachem.org/events/completed

# Planning method validation studies

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## 1. Introduction

Method validation should be carried out according to a documented procedure. Planning is therefore an essential step of the validation process. Although the requirements for a validation plan may be stated in sectoral guidelines, and national accreditation bodies may specify minimum requirements, it is generally left to the laboratory to devise a suitable plan to meet its particular requirements. Eurachem has published guidance on method validation planning and reporting as a supplement [1] to the guide 'The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics' [2]. The keys issues to consider when planning a validation study are outlined below.

## 2. Points to consider when planning a validation study

- The method to be validated: Before starting a validation study a detailed written procedure (such as a standard operating procedure) describing the method to be evaluated should be available.
- Critical steps in the method and instrument requirements: The analyst should be familiar with the method and aware of any critical steps that require particular attention. Any specific requirements relating to equipment/instrumentation should also be considered.
- Extent of the validation: The laboratory must decide which performance characteristics (e.g. precision, bias, limit of detection) need to be studied during the validation and the level of replication required.
- Performance criteria: The plan should include the criteria against which the chosen performance characteristics will be assessed (e.g. target values for precision, bias, limit of detection).
- Experimental design and order of evaluation of performance characteristics: Choosing suitable experimental designs is a key part of validation planning. With appropriate planning it is possible to maximise the amount of information obtained from a particular experiment. For example, it may be possible to obtain information on more than one performance characteristic. There are a number of experimental designs that can be used in a validation study, including simple replication, linear calibration and nested designs.
- Materials to be analysed: Different materials, such as certified reference materials and surplus test samples, are suitable for evaluating different performance characteristics. The validation should aim to cover a representative range of sample types in terms of matrix and analyte level. The validation study may therefore require the analysis of a number of different materials including certified reference materials (CRMs), spiked samples and test samples.
- Evaluation of the data and assessment of fitness for purpose: The plan should include details of how the data will be evaluated, including any statistical parameters that will be calculated from the data and any statistical tests that will be applied. The plan should also state how the 'fitness for purpose' of the performance characteristic will be assessed against the specified performance criteria.

## 3. Documenting the validation plan

The supplement [1] provides an example of a planning document which laboratories can use as the basis of their own plan. The plan is structured in such a way that when the experimental work has been completed, it can be easily converted into a validation report. It contains the following sections and guidance on how they can be completed:

- Title page: Method title and reference, and an overview of the method status and purpose of study.
- Analytical requirement: Information on the required scope of the method and its application, the purpose of the study, the performance characteristics to be studied, the method performance requirements, any existing performance data and the materials available for the study.
- Performance characteristics: A separate section for each performance characteristic which includes the detail of the validation study (the performance criteria, materials to be analysed, number and order of the measurements, how the data will be evaluated, and how the performance will be assessed).
- Summary: A summary of the values and/or other information obtained for each performance characteristic and a final statement on whether the aims of the study have been achieved and whether the method is fit for purpose.
- Approval: Sign off of the validation plan and the validation report.
- Learning points: Highlights any key information that has arisen from the validation, such as critical steps in the method or requirements for future quality control.

**References**

[1] V. Barwick (ed.), Planning and Reporting Method Validation Studies – Supplement to Eurachem Guide on the Fitness for Purpose of Analytical Methods (2019). Available from www.eurachem.org.

[2] B. Magnusson and U. Örnemark (eds.), Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics (2nd ed., 2014), ISBN 978-91-87461-59-0. Available from www.eurachem.org.

# Is the sampling method as valid as the analytical method?

**Lorens P. Sibbesen [1], Mike Ramsey [2]**

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**Keywords:** Method Validation; Sampling; Sample handling

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Laboratories are responsible for the validity of the methods they are applying for various tasks – through performance of either validation or verification of the methods. However, ensuring analytical results being fit for the purpose of making reliable decisions about some kind of material from which samples have been taken for testing in a laboratory, is not only depending on the validity of the test method but also (in most cases) on the validity of the sampling and sample handling preceding the testing. Uncertainty contributions from the sampling process has also come more in focus in the latest version of the ISO/IEC 17025:2017 standard. [1]

This presentation will point out some of the responsibilities of the testing laboratories in relation to ensuring that the sampling process has been valid (i.e. fit for the purpose) – and that the samples taken into work in the laboratory are valid for the testing. For the sampling process going on outside the laboratory, it depends on how much the laboratory has been involved in that process, whereas for the sample handling inside the laboratory (storing, sub-division, homogenization and various steps of preparation) it is clearly the responsibility of the laboratory. These initial steps are not always described as part of the analytical method and may therefore be overlooked in the validation or verification carried out by the laboratory. A special case is where the laboratory have been involved in field testing.

The presentation will refer to the two important Eurachem guidelines, "*The Fitness for Purpose of Analytical Methods*" [2], and "*Measurement Uncertainty arising from Sampling*" [3]

## References

[1] ISO/IEC 17025:2017 "General requirements for the competence of testing and calibration laboratories", 3rd Ed., ISO Geneva, 2017

[2]  B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2nd ed., Eurachem (2014).

[3]  M H Ramsey, S L R Ellison and P Rostron (eds.) Eurachem/EUROLAB/ CITAC/Nordtest/AMC Guide: Measurement uncertainty arising from sampling: a guide to methods and approaches. 2nd ed., Eurachem (2019)

# Ensuring validity of methods for routine use - a risk assessment approach

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**Keywords:** method validation; risk assessment

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Many laboratories are struggling with the extent of the validation – or verification - of a method, or in other words with how many experiments have to be carried out in order to ensure the validity of a specific method for routine use. Therefore, a pragmatic and risk based approach will be shown here.

This presentation aims to clarify the required extent of a method validation – or verification – that is needed in various situations to ensure that the method applied is valid (= fit for the purpose). It will focus on what and how much has to be done. The "what" is mainly a question about which of the typical performance characteristics should be investigated during the method validation study. The "how much" relates to the proper number of experiments needed in the study in order to take the appropriate decision about the fitness for purpose of the analytical method. The latter can be based on some principle for calculating the statistical power of the data resulting from the experiments, but this presentation also wants to take into account the risk management approach recommended in ISO/IEC 17025:2017 [1] and will evaluate how this approach can go beyond purely statistical considerations.

## References

[1] ISO/IEC 17025:2017 "General requirements for the competence of testing and calibration laboratories", 3rd Ed., ISO Geneva, 2017

# Measurement uncertainty and detection limits

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**Keywords:** detection capability; measurement uncertainty; detection limit

## Abstract

For many regulatory purposes, decisions about safety, health or product acceptance are based on a test for the presence of absence of a contaminant, regulated substance, or a pathogen. When quantitative analytical methods are used primarily for detection, the detection capability of the method becomes a critical characteristic.

In general, "capability of detection" – the general term used by ISO standards on this topic [0] – is the ability of an analytical method to provide reliable results corresponding to the presence or absence of a substance. To characterize a test method as acceptable, however, requires some measurable index of capability. A variety of terms and measures of detection capability are in common use; perhaps the most familiar such term is "limit of detection", essentially a true analyte level which will provide a positive test result with high confidence [0, 0]. This idea is based on the assumption that there is a separate criterion for a positive test result. In the framework of IUPAC and ISO approaches, this latter criterion is often called a "critical value" – an observed value which is unlikely if there is no analyte present. The relationship between these two concepts, and their use in interpreting analytical results, has sometimes been a source of confusion [0]; nonetheless, they form the basis of most current analytical literature on detection capability and are incorporated in some important regulations [0].

Despite the wide use of traditional concepts of detection capability, the increasing expectation that laboratories will evaluate and report measurement uncertainty raises new questions. First, measurement uncertainty is often presented as describing a symmetric distribution or a symmetric interval about the measured value; this becomes untenable when the results are so close to zero that a symmetric interval includes negative concentrations or amounts. In these circumstances, the reported measurement uncertainty may need to take account of the natural limits close to the result. Second, if it is assumed that the laboratory's measurement uncertainty is to be used in interpretation, the use of a typical expanded uncertainty – with coverage factor k=2 – does not lead to the same probabilities of acceptance and rejection as the traditional critical value.

In this presentation, the statistical basis of limit of detection and critical value will be reviewed and their traditional use in interpretation of results discussed. Approaches to measurement uncertainty evaluation that take account of a nearby natural limit of zero concentration, including those in the current Eurachem Guide [7] – will be presented. Finally, some of the advantages and challenges of using measurement uncertainty in detection decisions will be considered.

## References

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# What is the Uncertainty factor?

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## 1. Introduction

The uncertainty of a measurement result is often as important as the measured quantity value itself, as it controls what decisions can be made using that result, such as regulatory compliance. Appropriate expression of the measurement uncertainty (MU) is crucial, and there are situations when the traditional, symmetric, expanded uncertainty interval is not sufficient. This leaflet aims to explain the concept of the uncertainty factor and how it can be used to provide a convenient and realistic uncertainty interval in particular circumstances.

## 2. Ways of expressing measurement uncertainty

Many laboratories now estimate measurement uncertainty and usually express it as either expanded uncertainty ($U$), or relative expanded uncertainty ($U'$), typically with a coverage factor ($k$) of two for approximately 95 % confidence. The measurement result is then expressed as $x \pm U$ (where $x$ is the measurement quantity value, and $\pm$ is 'plus-minus'). The range of values that contains the value of the measurand (i.e. the true value of the analyte concentration) is then between $x - U$ and $x + U$ with approximately 95 % confidence. An example of this would be for a measurement result of $50 \pm 5$ mg kg$^{-1}$, where the value of the measurand is believed to lie between 45 and 55 mg kg-*1*. This approach works well generally, unless the value of MU is high (e.g. the relative standard uncertainty $u'$ is over 20 %), or the frequency distribution of repeated measurements is positively skewed, rather than the usual Gaussian (i.e. Normal) shape. In these situations, the expanded uncertainty factor ($^{F}U$) is a more useful way to express the MU, and the measurement result is expressed as $x ^{x/} {}^{F}U$ ($k = 2$, where '$x/$' is called 'times-over'). In the previous example, but with much larger MU expressed as an uncertainty factor of $^{F}U = 2.0$, the uncertainty interval 50 $^{x/}$ 2.0 is from 25 (i.e. 50/2) to 100 (50 x 2) mg kg$^{-1}$, which is clearly an asymmetric confidence interval.

## 3. How is the uncertainty factor calculated? - A case study

One example of the calculation of $^{F}U$ is for the determination of lead at a contaminated land site, and includes the MU arising from the primary sampling of the top soil. A detailed description is given elsewhere [1], but the key issues are that 100 sampling targets were sampled in a grid across the site and sent for the determination of Pb by ICP-AES after acid digestion, in a competent laboratory. The MU was estimated using the 'duplicate method' ([1] p17-19), in which 10 randomly selected targets had duplicate samples taken, that were both analysed twice, giving 40 measurement results.
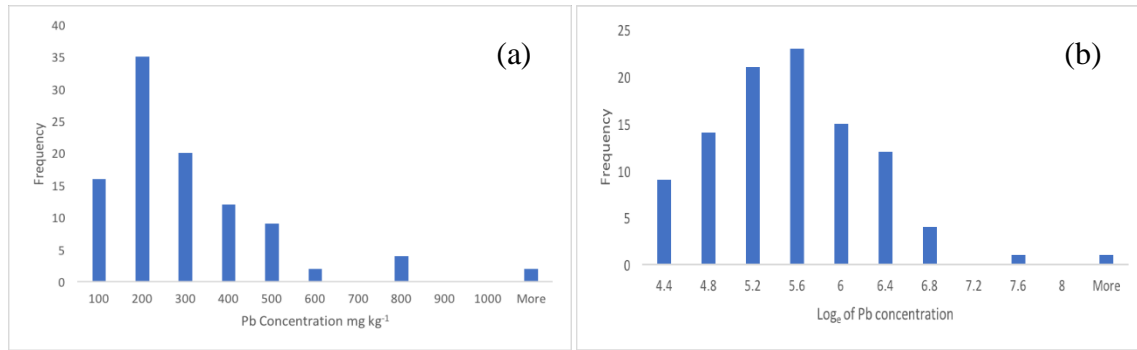
**Figure 1.** Histograms of the Pb concentration (as mass fraction in mg kg-1) measured in 100 soil samples shown on (a) the original linear scale (b) after natural logarithms were taken.

In this case the MU was estimated only as the repeatability standard deviation, which was the main source of uncertainty. The analytical bias was checked by analysing CRMs and found to be negligible.

When the MU is expressed as $U'$, it is calculated from the standard deviation ($s_{meas}$) of a measured quantity value ($x$), typically using $k = 2$ for approximately 95 % confidence, with the equation

$$U' = 100\frac{2s_{meas}}{x}\ \%\qquad\qquad(1)$$

The validity of this equation assumes that the frequency distribution of the replicated measurement results is Gaussian. However, if this distribution is shown to be positively skewed (Fig 1a - *In this example the distribution is from 100 different sampling targets. The positive skew is caused by the heterogeneous distribution of the analyte at that scale. This heterogeneity is also likely to apply at the smaller scale within each sampling target, which is reflected in the estimate of MU*), it may well be lognormal. This can be confirmed by taking natural logarithms of all of the measurement results, ln($x$) or log$_e$($x$), and determining if this then gives a near-normal distribution (Fig 1b).

The uncertainty factor FU can be calculated from the standard deviation ($s_{L,meas}$) of these 40 log-transformed measurement results, produced by applying the 'duplicate method', using

$$^FU = \exp(2s_{L,meas}) = e^{2s_{L,meas}}\qquad\qquad(2)$$

For practical purposes, $^FU$ can be calculated by inputting the original 40 measurement results into a software package that applies Analysis of Variance (ANOVA), e.g. [2]. For this example, the value of $^FU$ was calculated as 2.62, and it is applicable over the range of concentration represented by the duplicates. For a typical single measurement result of 300 mg kg$^{-1}$, the value of the measurand would lie, therefore, between 115 (300/2.62) and 784 (300 x 2.62) mg kg$^{-1}$. This wide and asymmetric confidence interval is mainly caused by uncertainty from the sampling process, due to the high level of heterogeneity of the Pb distribution in the soils within each sampling target.

## 4. Broader implications

High levels, and asymmetric distributions, of uncertainty can also arise in the analytical part of the measurement process. For example, in one study on the determination of genetically modified organisms (GMO) in soya [3] (Fig 2), the distribution suggests that FU could be the most applicable way to express MU in some purely analytical systems, as well as for those dominated by uncertainty from sampling. In such situations, FU can be calculated using Equation (2), without the need for ANOVA.
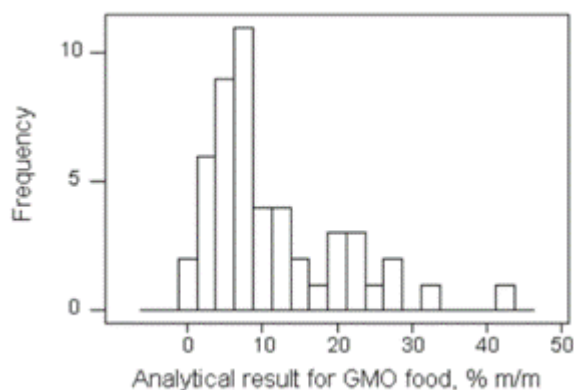
**Figure 2** Mass fraction (cg/g) of GMO in soya

## 5. Communication of MU

One challenge in using $^F U$ to express MU is to communicate its meaning clearly to the user of the measurement results. The statement of a measurement result can take the form $x^{x/F}U$. Hopefully, this leaflet will be one way to assist in communicating the meaning of a result expressed in this form.

## References

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# Demonstrating the validity of measurement results through reliable proficiency testing

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**Keywords:** proficiency, validity, assurance

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A regular independent assessment of the technical performance of a laboratory is necessary to assure the validity of measurement results and should form part of an overall quality strategy. A well-established approach to achieve this independent assessment is for a laboratory to participate in independently provided proficiency testing (PT) schemes or external quality assessment (EQA) schemes, the term often used within the medical sector. The important role of PT is well recognised in the international laboratory competency standards, ISO/IEC 17025 [1] and ISO 15189 [2].

The primary aim of PT is to provide the infrastructure for a laboratory to monitor and improve the quality of its routine analytical measurements. A PT scheme provides laboratories with a framework for obtaining a regular external and independent assessment of their performance. PT not only addresses the measurement phase in the measurement cycle, it also plays an important role in addressing the pre-analytical and post-analytical phases such as sampling and the interpretation of the measurement results. Globally, laboratories undertake millions of measurements every year, with the measurement results underpinning important decisions to support, for example, compliance, trade, health and security. Thus, valid measurements underpinned by PT participation helps to ensure the reliability of these important decisions.

To ensure that laboratories maximise the benefits of PT participation it is essential that they select the most appropriate PT scheme, that those selected are used appropriately, and that they understand how their performance has been evaluated by the PT provider to enable them to correctly interpret their PT results. Equally, it is important that they ensure the validity of the PT schemes chosen and that they are managed and operated competently by the PT provider. To support laboratories in these important aspects of their PT participation, Eurachem has recently updated its popular guide on the 'Selection, Use and Interpretation of Proficiency Testing (PT) Schemes' [3]. This presentation will provide an overview of the guide, highlighting some of the key aspects to support laboratories in establishing their PT participation plan.

The Eurachem PT guide addresses key principles that help to ensure the appropriateness of participation in PT schemes, and need to be considered and understood by interested parties:

- the PT scheme selected should resemble as closely as possible the laboratory's routine work;
- laboratories should treat PT items as routine samples;
- the PT scheme documentation, such as scheme protocols, must provide clear information in order for all parties to understand how the PT scheme operates;
- all unsatisfactory or repeated questionable results must be thoroughly investigated so that the laboratory can understand the reasons for poor performance and correct as necessary;
- the evaluation and interpretation of the performance in a PT scheme should take into account the risk associated with the measurement;
- the performance of a laboratory over several rounds of a PT scheme and analysis of trends is paramount to determining the successfulness of participation;
- the PT provider should be open to discussion amongst interested parties in order to gain a more accurate understanding of the PT scheme and its operation;
- laboratories should view PT participation as an educational tool, using the PT scheme results in the improvement process and to give feedback to staff.

The aims of the Eurachem PT guide is to provide laboratories with guidance on:

- aims and benefits of participation in PT schemes;
- selecting the most appropriate PT scheme;

- understanding the basic statistics and performance scoring used by the PT providers;
- using and interpreting the PT results in order to improve the overall performance of the laboratory.

The guidance is relevant to all organizations that are performing sampling, testing, calibrations and examinations, for example, testing laboratories, calibration laboratories, inspection bodies, biobanks, etc. It covers measurements, examinations and interpretations.

It is key that a laboratory, before selecting a PT scheme, should evaluate the level and frequency of their participation and establish their PT participation strategy. Once undertaken the laboratory can select an appropriate PT scheme to fulfil the needs of their participation strategy. There are a number of key aspects to consider when selecting the most appropriate scheme including:

- the PT items being offered;
- the participant base of the scheme;
- the distribution of the PT items;
- how the results are reported by the participant and handled by the PT provider;
- the PT reports provided back to the participants;
- the competency of the PT provider.

The Eurachem PT guide provides valuable advice and assistance to the laboratory on these aspects.

By participating appropriately in carefully selected PT schemes, a laboratory can gain many benefits; the use of PT should be much wider than the basic statement of whether the laboratory is competent or not. The Eurachem PT guide explores how laboratories can benefit from participation in PT schemes in various ways. A good overview on how a PT provider evaluates the performance of the laboratory participating is given along with guidance on how the laboratory should interpret their PT results, both in terms of performance in a particular PT round and in terms of reviewing longer term performance over multiple PT rounds. Importantly, guidance includes investigating and addressing poor performance, looking at the various causes of poor performance.

One of the key selection criteria for the laboratory to consider when choosing the most appropriate PT scheme in which to participate is the competency of the PT provider, and as such, if they comply with the international standard ISO/IEC 17043 [4]. This international standard is currently under revision, so an update on the direction of this revision will be presented.

In conclusion, participating in PT schemes is an essential requirement for any laboratory wishing to ensure and demonstrate the validity of their analytical measurements. Key to this is establishing a participation strategy and selecting the most appropriate PT schemes in which to participate. The recently revised Eurachem PT guide provides valuable advice to assist the laboratory in doing this, and the international standard ISO/IEC 17043, currently under revision, provides the framework for assessing the competency of the providers of such PT schemes.

**References**

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# Guides, leaflets and more – online calculation/validation tools

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**Keywords:** educational material, web application, calculation validation

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As stated on its website "Eurachem promotes best practice in analytical measurement by producing authoritative guidance on quality of analytical measurement". A wealth of useful information is freely available from the many Eurachem guides and information leaflets related to quality assurance, measurement uncertainty, method validation and proficiency testing (https://eurachem.org/index.php/publications). These valuable documents are greatly appreciated by many laboratory practitioners that download them and implement the recommended metrological practices in their laboratories. Not to mention the successful Eurachem workshops and dedicated training seminars. What could Eurachem do more?

The Eurachem guides provide all the relevant mathematical formulas in the detailed annexes. This is certainly useful to practitioners fluent in XLS or programming. However, the training seminars show that this is not sufficient. Eurachem could upload some "calculations modules" on their website. Users would be able to use them for checking their calculations or for validating the modules they have developed in-house. Some examples will be presented and discussed.

# Use of in-house validation data in measurement uncertainty evaluation

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**Keywords:** Top-down evaluations; Measurement uncertainty; Method validation

## 1. Introduction

The last stage of a complete measurement method validation is checking if produced measurements can have adequately low uncertainty for the intended use of the analysis [1]. The adequacy of a method for an intended use also depends on the analysis's cost and duration. Method validation is a relevant part of analytical costs that only becomes negligible after many unknown samples' characterisation. If method validation is not adequately thorough and the subsequent analysis quality control is not well designed, unnecessary expenses in repeating analysis and managing other bad performance will have a relevant impact on analysis costs.

Top-down uncertainty evaluations are very popular due to the low cost of algorithm development and the resources required to quantify the uncertainty components. Although bottom-up uncertainty evaluations are more expensive regarding model design, they can save some experimental resources by modelling the uncertainty components.

## 2. Trueness and precision models

Top-down uncertainty evaluations are challenging regarding the development of uncertainty models for a wide concentration interval and in using all available reference materials to assess measurement trueness [2]. For cases where results should be corrected for recovery, the mean recovery observed in various matrices and days should be used in results correction instead of the daily recovery test.

## 3. Correlation of method validation data

In some highly regulated analytical fields, guidelines for method validation are established that produce correlated information challenging for being used in measurement uncertainty evaluation. However, uncertainty evaluation algorithms can be adapted to the experimental reality to overcome this difficulty [3]. Uncertainty evaluation should adapt to available experimental data and not the other way around!

This communication discusses the most challenging, controversial, and relevant aspects of top-down measurement uncertainty evaluation while suggesting solutions to overcome difficulties.

## References

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# Eurachem Guidance on compliance assessment

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## 1. Introduction

The measurement cycle, Figure 1, starts with a client's problem or issue which leads to a request for a measurement to be undertaken, e.g.:

1.  Consumer products – can this toy be used by children below 3 years of age?

2.  Industry – in the batch of stainless steel delivered, is the mass fraction of nickel between 16 and 18 %?

3.  Forensic – the driver has a measured alcohol level of 0.052 mg/100 ml; can one be confident that the alcohol content in the blood is over the limit of 0.05 mg/100 ml?

4.  Food – are there are any pesticides present above the maximum residue limit in this batch of shrimps?

5.  Manufacturing – do the QC results obtained today show that the production process is under control?

The measurement cycle ends with the client taking a decision on the results – a statement on compliance or non-compliance. The Eurachem Guide gives guidance on compliance assessment.
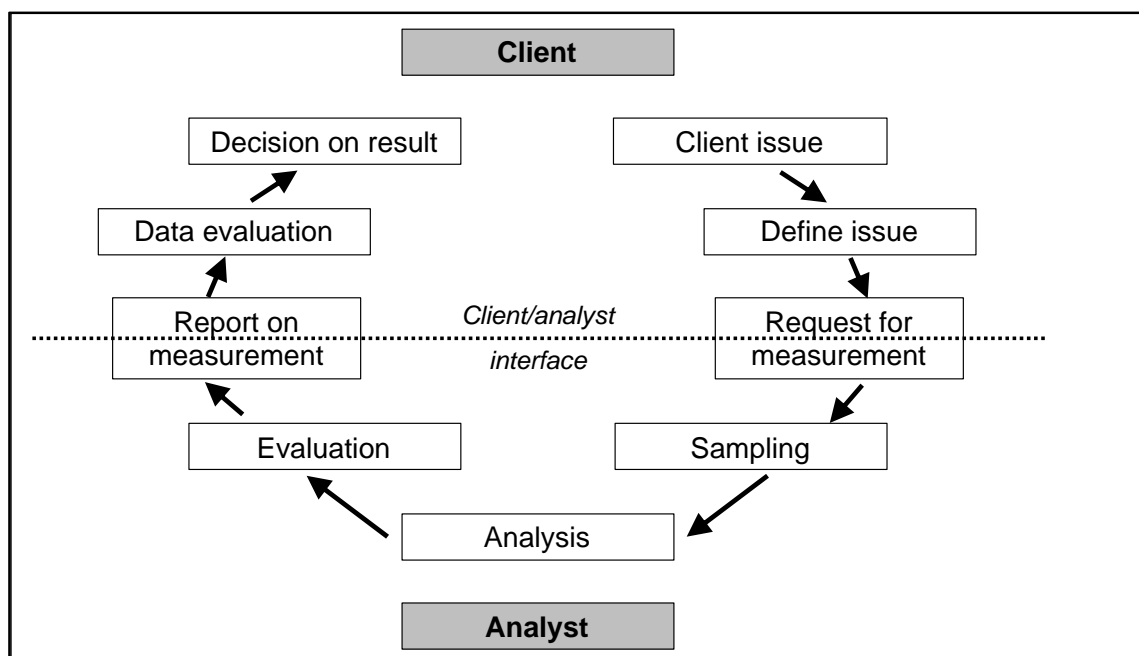


**Figure 1** The measurement cycle

## 2. Information needed for compliance assessment

The following information is needed to make an assessment of compliance:

- measurand clearly specified;
- specification stating upper or lower limit or both;
- decision rule;
- measured value:
- measurement uncertainty for a measured value at the limit(s).

In cases 1-4 above the measurement uncertainty is needed but in case 5 instead, the intermediate precision is required. Case 5 is not treated in the Guide but similar principles can be applied. The key concept is the use of a decision rule to take a decision on whether the results indicate compliance or non-compliance.

## 3. Decision rule

A decision rule should have a well-documented method of determining the location of acceptance and rejection zones, including the probability, P, that the value of the measurand 1) lies within the specification limit, high confidence of correct acceptance (low probability of false acceptance) or 2) lies outside the specification limit, high confidence of correct rejection (low probability of false rejection).

## 4. Summary

The Eurachem Guide on compliance assessment [1] presented here describes the information needed and how to use this information to state compliance or non-compliance.

## References

[1] A. Williams and B. Magnusson (eds.) Eurachem/CITAC Guide: Use of uncertainty information in compliance assessment (2nd ed. 2021). ISBN 978-0-948926-38-9. Available from www.eurachem.org.

# Poster abstracts

# POSTERS

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| 2 | **Study of reproducibility in scientific publications based on the statistical evaluation of validation results and uncertainty estimation in analytical chemistry**<br>Bruna Ferreira, Vitor Pacces, Igor Olivares, Emanuel Carrilho<br>Contact email: brunadrielen@iqsc.usp.br |
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| 11 | **Traceable reference gas mixtures to calibrate analyzers used to measure atmospheric VOCs**<br><br>Maitane Iturrate-Garcia, Celine Pascale, Tobias Bühlmann, Stefan Reimann<br><br>Contact emails: maitane.iturrate@metas.ch (presenter);<br><br>celine.pascale@metas.ch (project coordinator) |
| 12 | **Evaluation of measurement uncertainty of environmental matrices indicators including sampling uncertainty**<br><br>David Milde, Alena Nižnanská<br><br>Contact email: david.milde@upol.cz |
| 13 | **Bottom-up evaluation of the uncertainty of a titration with visual end-point detection: Determination of dissolved oxygen by the Winkler Method**<br><br>Ariely Carvalho, Ruben Miguel, Sara Neves, Cristina M. Oliveira, Ricardo J. N. Bettencourt da Silva<br><br>Contact email: ariely_cardoso_100@hotmail.com |

# Evaluation of the uncertainty of complex sample preparation - Monte Carlo bottom-up approach

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## 1. Introduction

Many chemical analyses involve a complex sample preparation and some, based on an instrumental method of analysis such as spectrometric and chromatographic methods, are affected by matrix effects. The objective interpretation of the results of these analyses, performed in the framework of a research or of a conformity assessment, requires the quantification of the measurement uncertainty. Top-down assessments of the measurement uncertainty are known to involve the oversimplification of the measurement process and a pessimist quantification of the uncertainty [1]. This work presents a novel methodology for the bottom-up modelling of the performance of complex analytical operations, such as sample digestion or extraction, by the Monte Carlo simulation of their performance independently of the performance of the other analytical steps [2].

### 1.1. Methodology

The developed modelling requires the replicate analysis of items, where at least some should have a known reference value, in n different days (e.g. n = 10). The simulation of m measured values (e.g. m = 16000), considering all analytical steps except the sample preparation, of the analysis of each sample in the n days allows the determination of (n × m) differences between n sample means with m simulations of between-days means. These differences allow simulating the between-days precision component of the measurement error, Eb, and the mean analyte recovery, $\bar{R}$, from the analysis of reference materials. The Eb and $\bar{R}$ from the analysis of various items are pooled simulating the complex probability density of these uncertainty components. For the post-validation analysis of unknown samples, the simulated uncertainty of all analytical steps except sample preparation is merged with the simulated Eb and $\bar{R}$, and results corrected for the $\bar{R}$ reported as relevant percentile intervals or the distribution of simulated measured values.

### 1.2. Results and Discussion

The developed methodology was successfully applied to the determination of total or acid-extractable As (following OSPAR or EPA 3051A methods, respectively) [3], [4] in sediments where Eb was simulated from the analysis of one Certified Reference Material, CRM, and three sediment samples and $\bar{R}$ simulated from the analysis of the CRM and two spiked samples. The evaluated uncertainty is fit for environmental monitoring considering performance criteria defined for Quasimeme proficiency tests [5]. The developed measurement models were successfully cross-validated by randomly extracting data from the validation set subsequently used to check the compatibility between estimated and reference values for 95% or 99% confidence level. The observed success rate of these assessments is compatible with the confidence level of the tests.

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# Study of reproducibility in scientific publications based on the statistical evaluation of validation results and uncertainty estimation in analytical chemistry

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**Keywords:** reproducibility, validation, software, uncertainty, statistics.

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## 1. Introduction

The data presented in 2016 by the article "1500 scientists lift the lid on reproducibility" by Monya Baker, brought up a discussion about the potential and significant crisis of reproducibility in scientific research. In the same article, the potential causes were raised according to the experience of the consulted researchers, and the most prominent points refer to the low power and application of statistical tools and a better application of intra-laboratory validation. Within this context, in this work, the research was developed based on the search for verification of this information in Chemistry (area whose researchers reported the most difficulty in reproducing results).

First, the objective was to assess whether the perception of the existence of a reproducibility crisis was capable of being confirmed and quantified. Then, the second objective was to evaluate the relationship between this potential existence of the reproducibility crisis with the application of the performance criteria for the validation of analytical methods (presented in scientific documents), as mentioned in that article. Based on the criteria for application and acceptance of results in validations of methods contained in current protocols and legislation, conformity assessments and checks of statistical calculations of published analytical data were carried out.

For obtain data and reference values, softwares validated aimed at testing laboratories, called ConfLab Validation and ConfLab Uncertainty, were used, which were designed to attended the main Quality Management Systems related to these requirements. The discussions took place by comparing the results obtained as they were presented in the original documents with the results obtained by the software. The results presented provide evidence that justifies the possible existence of problems related to the lack of knowledge about the correct use of tests and statistical tools, which promotes the misapplication of performance parameters and, consequently, an inappropriate analysis of the data.

In the next step, discussions based on calculations of estimation of measurement uncertainty will be added to assess their potential contribution to the effects of the reproducibility of analytical methods.

## 2. Results about application criteria

The results about the use of the protocol, tests of the performance parameters and establishment of acceptance criteria showed that, although 100% of the documents mention the use of protocols to perform the method validation, there are many inconsistencies about the ability of authors to conduct the process of validating the methods in their entirety. See Figure 1.
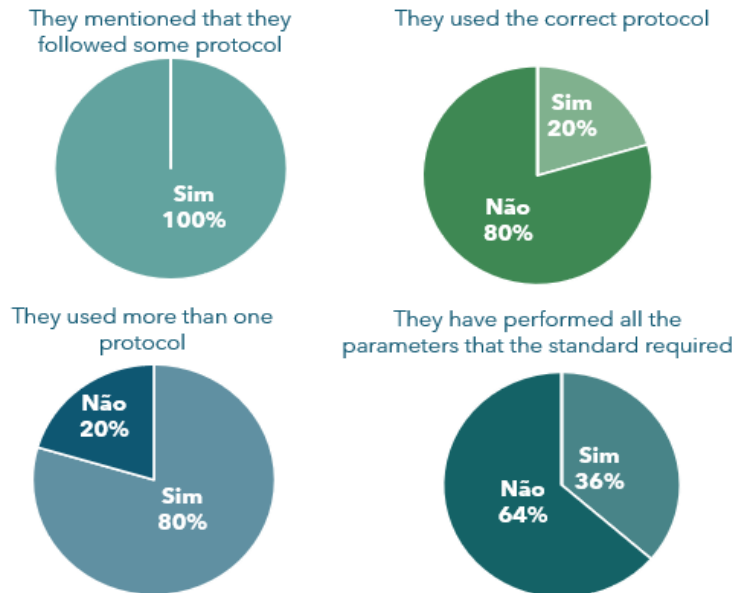
**Figure 1:** Characterization about the application of the protocols a) frequency that the authors understood the contexts of application of their method b) of the performance parameters that were performed, the author correctly understood the form of application of the test in all of them.

The linearity with 75% was the parameter that got the most mistakes, and the accuracy test, of the times that was performed in 56.8% of them, was through a correct application about the protocol, being the performance parameter with the highest percentage of correct applications. See Figure 2.
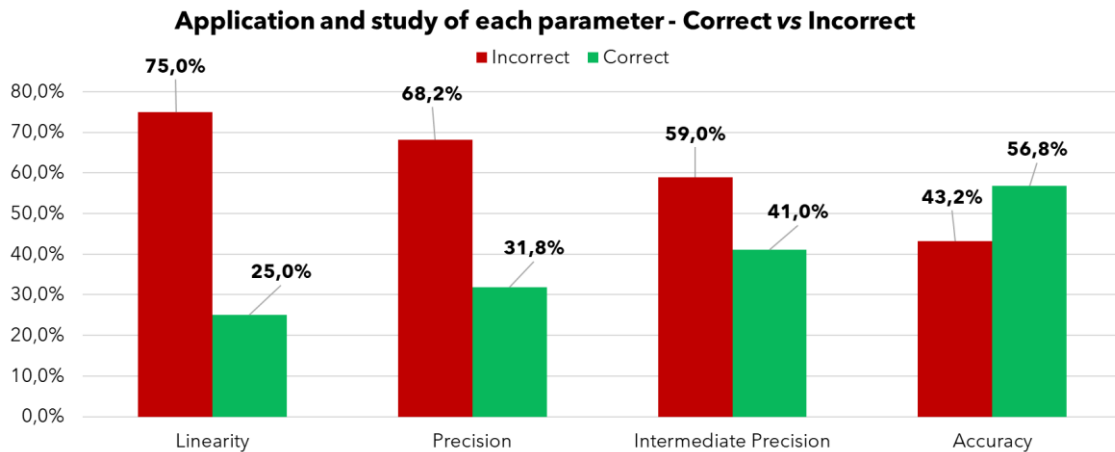


**Figure 2 -** Comparison between the frequencies that the test and the study of the performance parameter were performed correctly with incorrect ones.

## 3. Conclusion

With the data obtained so far, it is possible to be close to establishing a confirmation of the lack of knowledge about the correct application of method validation and uncertainty calculations as statistical tools, which can result in the misuse of performance parameters. This information increases the likelihood of admitting the significant negative influence of this confirmation on the reproduction of published methods, as stated in that article (BAKER, 2016). However, for a more precise

conclusion, studies based on uncertainty estimation calculations will still be carried out to relate these conclusions to the existence of a crisis of reproducibility of analytical methods.

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# Metrological tools applied in test methods for the detection of SARS-CoV-2

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**Keywords:** SARS-CoV-2; qualitative analysis; diagnostic tests; metrological tools.

## 1. Introduction

Qualitative analysis is defined by the EU as: *analytical method that identifies a substance based on its biological or physical properties.* Where a binary response can come through a measuring instrument, such as test kits. A 'qualitative method' effectively gives a 'Yes' / 'No' answer at a given cut-off concentration of an analyte. This type of analysis is recommended mainly for screening purposes using low-cost methods or at analyte concentrations close to the limit of detection (LOD). [1]–[4]

Emerging and reemerging infectious diseases are considered constant challenges for public health worldwide, as well as the current global pandemic caused by Severe Acute Respiratory Syndrome 2 (SARS-CoV-2). In order to detect and diagnose the contagion of the virus, diagnostic methods have been developed. These in vitro methods or tests act for a quick and effective response in this crisis, as they contribute to the screening, diagnosis, monitoring/treatment of patients, as well as epidemiological recovery/surveillance. Moreover, to assess the method's compliance with legislation, its performance must be qualified and reported. However, according to the database currently available, there is a mismatch between an existing or reported results from applied metrological tools method/test/device information, and performance criteria. This results in the need to ensure that the performance characteristics of the method are defined, ensuring that the method is scientifically consistent in the conditions in which it is used. [4]–[8]

Despite the great importance of applying these methods for the detection of SARS-CoV-2, there is no standardization for this. This reveals the importance of applying a set of qualitative analysis checks to the method using different metrological tools to ensure that the tests must comply and suitable for their application. Among these tools, the following stand out: validation, uncertainty estimation, and proficiency testing (Table 1). Figure 1 shows the complete set of measures that the laboratory must carry out to guarantee the obtaining of high-quality data. In addition to the use of validation and/or standardized methods, these measures are: effective internal quality control (IQC) procedures (use of reference materials (MRs), control charts, etc.); participation in proficiency tests; accreditation to an international standard, usually ISO / IEC 17025 and registration of the test with regulatory agencies. [9], [10]

Considering the significant importance of the correct application of metrological tools to ensure the quality and reliability of results from methods for the detection of SARS-CoV-2, the purpose of this work is to review and evaluate these tools highlighting the best practices.

**Table 1.** Terms used and their respective definitions: validation, uncertainty, and proficiency testing.

| Term | Definition |
|---|---|
| **Validation** | *It is the verification, where the specified requirements are suitable for an intended use.* [11] |
| **Uncertainty** | *Non-negative parameter that characterizes the dispersion of the values attributed to a measurand, based on the information used.* [11] |

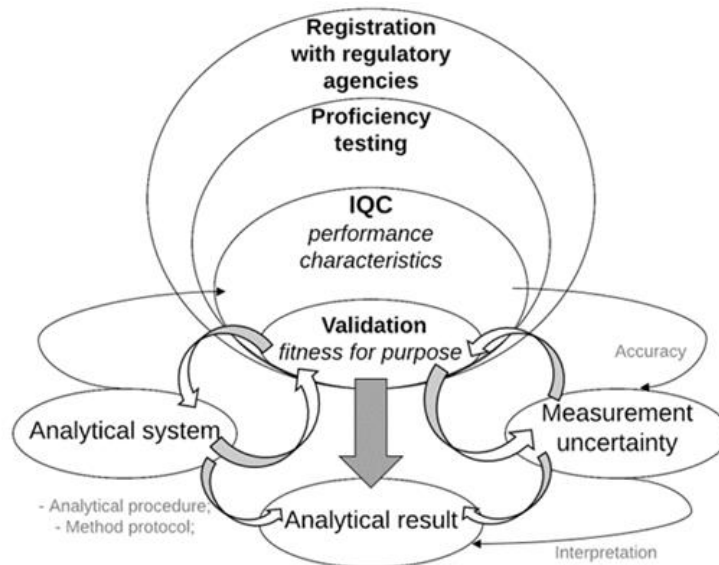| | |
|---|---|
| **Proficiency testing** | *"Proficiency testing activities" refer to monitoring the performance of the laboratory by comparing it with results from other laboratories (external mechanisms).*[12] |



**Figure 1.** Different levels of quality validation categories for the analysis of diagnostic methods for SARS-CoV-2. Adapted [9]

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# Application of statistical tools for laboratory quality management aiming the ethyl carbamate (EC) monitoring in unsweetened cachaça

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## 1. Introduction

Certified reference materials (CRMs) are essential to quality control of the routine analytical data evaluation. The development of an CRM entails quite a research investment to solve problems as original matrices adequation to the analytical method, homogeneity assays, and stability studies of the CRMs regarding proper storage, packing, and transportation conditions.

In distilled beverages, the formation of ethyl carbamate (EC), a potentially carcinogenic organic compound, is common. For this reason, the Ministry of Agriculture, Livestock and Supply (MAPA) is interested in ethyl carbamate monitoring in distilled beverages produced and commercialized in Brazil, following a maximum residue limit (LMR) of 210 µg.L$^{-1}$ established by the Normative Instruction N. 28, of August 8th, 2014 [1]. However, this monitoring was temporarily interrupted by some analytical and governmental problems and was not carried out in our country.

Therefore, at the request of MAPA, this work aimed to develop and validate the chromatographic method for analysis of ethyl carbamate. To assist during the analyzes, a certified reference material (CRM) of ethyl carbamate was also developed in the unsweetened cachaça matrix, with adequate homogeneity and stability. Additionally, a proficiency test (PT) with this CRM was carried out, and the diagnosis essay was carried out to determine ethyl carbamate concentration in unsweetened sugar spirits. The developed CRM lot was donated to MAPA for routine analysis usage, collaborating to this compound monitoring.

## 2. Materials and Methods

The method validated for MAPA used a gas chromatograph Shimadzu model GC 2010, equipped with an automatic sampler model AOC-5000, coupled to a mass spectrometer model GCMS-QP2010 Plus; developed with a stationary phase column of polyethylene glycol in dimensions 30m x 0.25mm x 0.25µm. The MAPA notified that in the analysis, there were many doubts about the identification of the EC only by ion 62. The methods developed were performed with ions 44, 62, and 74 to ensure the identification and quantification continuing with ion 62, which is the most used for the determination of EC [2,3].

For the development of the certified reference material, a sample of unsweetened cachaça was fortified with ethyl carbamate standard up to the threshold of the legislation, 210 µg.L$^{-1}$. The homogeneity study was carried out with a sample of 30 bottles analyzed in quintuplicate, chosen in a stratified random way. For the storage stability study, each month, two vials were removed from the conditioned batch between -1 ° C and -4 ° C and placed at room temperature. Each vial was analyzed in triplicate. It was done in the first four months and then again in the ninth month, at the end of the study. For the short-term stability study, to reproduce the transport conditions of the CRM candidates to the laboratories participating in the promoted PT, two vials were stored at 40 ° C in a thermostated oven for 72 hours, analyzed in triplicate immediately afterward.

The PT was carried out with four laboratories accredited by the Brazilian Network of Testing Laboratories (RBLE). For the diagnosis of ethyl carbamate contents, MAPA provided 18 samples from the inspection program.

## 3. Results and Discussion

A CRM for ethyl carbamate was developed in an unsweetened sugar spirit matrix, presenting a reference value of (236,500 ± 105,006) µg/L (k=2, normal) with adequate homogeneity and stability. This CRM uncertainty is close compared to the uncertainty of other contaminants in whisky CRM developed by Fapas.

The PT was carried out with a small group of participants, and it was necessary to study a new way of evaluating the laboratories. ISO 13528: 2015 [4] recommends using the z score as a performance parameter. However, for PTs with less than 20 participants, robust Algorithm A statistics for calculating robust means and standard deviations are compromised and the z score cannot be calculated based on the participants' standard deviation value. They must resort to using the model Horwitz adapted by Thompson for the calculation of the standard deviation based on the concentration of the designated value. However, the Horwitz's mathematical model for calculating the standard deviation of analytical methods is an empirical model carried out in the 80's. In 40 years, instrumental analysis has evolved a lot. The analytical power that we currently have available compared to when the Horwitz model was developed is unmatched. This way, the PT evaluation compared the z score results (using Horwitz) with zeta score and normalized error that considers the uncertainties from the CRM and the laboratory results.

## 4. Conclusions

A chromatographic method was developed and validated, and a batch of CRM for MAPA was produced, contributing to the inspection of EC in distilled beverages in the country.

A proficiency test was carried out with ISO/IEC 17025 accredited laboratories, and with this study, it was verified the importance that the estimates of uncertainties assume in proficiency tests of few participants, which can assume greater relevance as a performance parameter of the laboratories; as some authors have already suggested, which may become a global evaluation parameter [5]. More and more, we tend towards more diversified products and with more specific niches. It is a tendency that few participants' proficiency tests are more requested, and it is necessary to be adequately prepared for this demand.

Applying for all the CRM production work, developing and validating the method for the analysis of unsweetened cachaça, samples from the MAPA inspection program were analyzed and it was revealed that many cachaças are still being marketed with high levels of EC. Producers must maintain greater control of this compound's content in their production since the country is a significant producer and exporter of this product.

With this work, it was possible to provide tools for correct monitoring of the EC in cachaça, since in partnership with the MAPA, it was possible to validate a method for routine analyzes, in addition to producing an CRM that can be used in the control of the quality of these analyzes. PT demonstrated that, despite few participants, most of them had adequate results. However, although the laboratories analyzed correctly, the diagnosis regarding EC in cachaça reinforced the importance of this monitoring since a significant number of failed results were found. These results reinforce that the tools developed in this work will be useful and applied in this monitoring.

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# Pioneering development of automatic identification of microplastics by micro-ATR-FTIR spectra

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## 1. Statement

Environment contaminated by micro(plastics) issue is very trending nowadays.

Plastics are ubiquitous and have a wide range of applications due to their versatile properties. Its production reached over 368 million tonnes worldwide and 57.9 million tonnes in Europe in 2019 [1,2]. However, plastic materials are somehow discarded into aquatic environments, becoming responsible for over 62% of the marine litter's composition worldwide [3].

The scientific community has become interested in monitoring the presence of these contaminants in different matrices, namely, surface water, column water, seafloor sediment, and beaches. The impact of microplastics in open ocean, rivers, estuarine areas, and coastal regions compartments is only possible to understand if this contamination is characterized adequately and objectively regarding the physical and chemical properties (i.e., polymer type) of particles.

### 1.1. Microplastics ID

The type of polymer is largely identified by Fourier-Transformed-Infrared spectroscopy, FTIR, where the acquired infrared, IR, spectrum works as a molecular fingerprint of the plastic. Identifying microplastics from the IR spectrum can be a challenge in some cases, especially when a biofilm covers the particles or whenever spectral inconsistencies appear due to differences in plastic additives and copolymers, ageing, or other coating types.

This work aimed at developing and validating a methodology towards the automatic identification of microplastics by micro-ATR-FTIR, overcoming the complexity and time consuming of a manual interpretation of characteristic spectral bands. The automatic identification of the IR spectra can be supported on a fast mathematical comparison between the unknown microparticle and reference spectra using an agreement index such as correlation.

This work describes the development and validation of the use of different correlation algorithms for spectra correlation based on the modelling of their distribution by the Bootstrap method. A previous evaluation of the presence of spectral contamination by biofilm and of the level of attenuation of characteristic spectral bands of the polymer was performed. The methodology was implemented in a user-friendly *MS-Excel* spreadsheet used to define and validate statistically sound criteria for accurate and automatic identification of microplastics with a true positive result rate, *TP*, not lower than 95%, and a false positive result rate, *FP*, not greater than 5%. The quality of the identification was expressed by the Likelihood Ratio, *LR*(+), of the identification. Considering the defined criteria for the *TP* and *FP*, the analysis fitness for purpose can be controlled by assessing if the *LR*(+) is greater than 19 [4].

The methodology for identifying microplastics with adequate uncertainty was successfully applied to the identification of Polyethylene, PE, Polyethylene terephthalate, PET, Polypropylene, PP, and Polystyrene, PS, microparticles from sediments collected in Portuguese rivers, improving the preliminary results on the polymer type identification already reported [5].

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# Particular and total risks in the conformity assessment of paracetamol oral solutions

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## 1. Introduction

Paracetamol (acetaminophen) is one of the most commonly prescribed painkillers and it's widely available and used as an over-the-counter medicine worldwide [1]. As a multiparameter product, this medicine has multiple quality parameters to attend to guarantee its safety and efficiency.

The measurement uncertainty associated with all measure values must be considered to provide traceability and reliable results. It enables the risk evaluation in conformity assessment, that plays an important role in the decision making process, reducing the probability of making an erroneous decision such as accept an out-of-specification medicine/batch or reject an within-the-specification medicine/batch [2].

When more than one quality parameter is considered for evaluation, all of them need to be in compliance regarding the quality specifications. The particular risks of the parameters can't solely influence the conformity decision, being necessary to consider the total risk. However, the evaluation of multiple quality parameters can generate metrological correlation due to the sharing of analytical steps. This correlation affects the total risk value, and therefore should be consider in the determination of the total risk [3].

Given the above, the aim of this work was to determine the measurement uncertainties and the particular and total risks regarding the quality parameters of relative density, active pharmaceutical ingredient (API) content, and dose per drop of paracetamol in oral solutions, considering the metrological correlation.

## 2. Materials and Methods

Three brands of paracetamol oral solution 200 mg/mL were evaluated regarding the relative density, API content (by ultraviolet absorption spectrophotometry), and dose per drop, according to the Brazilian Pharmacopeia.

The individual uncertainty values were estimated by the analytical balance calibration, by repeatability studies of the glassware, and by ANOVA analysis of the relative density, absorbance and drop mass. The standard uncertainty of the drop volume, API content, and dose per drop were obtained by Monte Carlo Method (standard deviations of 50,000 simulations for each parameter) in a Microsoft Excel worksheet. The particular and total risks for each brand were also estimated by Monte Carlo Method, considering whether the 50,000 simulation values are within or out-of-specification.

## 3. Results and Discussion

The total risk takes into account all quality parameters. Among the three brands evaluated, only brand C presented all its parameters within specification (Table 1). Brands A and B, on the other hand, presented out-of-specification dose/drop values (Table 1).

**Table 1.** Mean values and standard uncertainties of the evaluated quality parameters of paracetamol oral solution brands and the risks of false decision in their conformity assessment.

| Quality parameters evaluated | Specification limits | Brand A Mean value ± u (R%) | Brand B Mean value ± u (R%) | Brand C Mean value ± u (R%) |
|---|---|---|---|---|
| relative density (g/mL) | 1.10-1.20 | 1.13 ± 0.03 (0.11[1]) | 1.15 ± 0.00 (0.00[1]) | 1.13 ± 0.03 (0.00[1]) |
| API content (mg/mL) | 180-220 | 189.34 ± 2.21 (7.50[1]) | 203.80 ± 2.27 (0.00[1]) | 204.89 ± 2.33 (2.95[1]) |
| dose/drop (mg/drop) | 11.3-15.3 | 9.49 ± 0.70 (2.03[2]) | 10.30 ± 0.28 (0.57[2]) | 12.00 ± 1.10 (31.56[1]) |
| Conformity assessment | | not comply (1.98[4]) | not comply (0.57[4]) | comply (32.84[3]) |

Mean values of 50,000 Monte Carlo simulations for each parameter; u: standard uncertainty; R: risk of false decision; [1] Particular consumer risk; [2] Particular producer risk; [3] Total consumer risk; [4] Total producer risk.

When one or more quality parameters are out-of-specification, the product cannot be considered adequate for use. Thus, brands A and B do not meet the specifications regarding conformity assessment, and should be rejected; therefore the particular risks for dose/drop and the total risk correspond to the producer risk, which is the probability of rejecting a batch within specification, considering the 50,000 Monte Carlo simulations (Table 1). On the other hand, the risks regarding brand C refer to consumer risk, which is the probability of approving an out-of-specification batch, considering the 50,000 Monte Carlo simulations (Table 1).

Although brands A and B are considered not-conforming, the risks of false decision related to them are relatively low. With the exception of the consumer risk regarding the API content of brand A, which was above the maximum permissible risk of 5% (7.5%), the producer's risks were below 5% (total risk 1.98% and 0.57% for brands A and B, respectively). The decision to accept brand C is inconclusive, since the consumer's total risk is considerably high (32.84%), much higher than 5%.

## 4. Conclusions

The three brands of paracetamol oral solutions were evaluated regarding the risks of false conformity decisions, considering 50,000 Monte Carlo simulations. The risks were estimated using a frequentist approach, which takes into account the measured value, its measurement uncertainty, and the specification limits. Also, using the Monte Carlo method, the metrological correlation between the quality parameters measured values were considered in the risk estimations.

Regarding the evaluated quality parameters, brands A and B were considered non-conforming, yet presented low risk of rejecting a batch within specification. Brand C was considered conforming; however, it presented high risk of approving an out-of-specification batch. These results reinforce the importance of considering the total risk of false decisions in the conformity assessment along with the quality parameters evaluation.

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# First assessment of total risks in oil spill source identification

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## 1. Introduction

Oil spills have been a concern over the years due to the growth of marine traffic and oil extraction and processing to answer present society demands. The main sources of oil pollution are marine transportation or land-based sources resulting from accidental or intentional discharges [1]. The well-known socio-economic, human health and environmental impacts reveal the importance of identifying the origin of this type of pollution and holding the offender liable under the law.

Chemical analyses of samples taken from oil spills have been used as evidence in legal proceedings. The chemical composition of samples collected in the spill (spill sample) and in the suspected sources of the incident (suspected source sample) are compared to identify the spill's source. This is possible because oils and refined products have distinct relative content of hydrocarbons that confer unique characteristics, namely fingerprint, due to the refining processes and the different organic materials and geochemical conditions of oil formation [2]. Using chromatographic techniques, *e.g.*, Gas Chromatography-Mass Spectrometry (GC-MS), each sample collected are characterized by identifying and quantifying a wide range of hydrocarbons. The data from these instruments can be subject to either multivariate or univariate statistical assessments, allowing identifying the type, geographical origin, or oil source. Multivariate statistical methods provide relevant information about the origin of a spill. However, the oil identification experts have preferred the direct assessments of the compositional equivalence of the spill and suspected sources' samples. The ratio of the abundance of hydrocarbons present in the samples, *i.e.* diagnostic ratios (*DR*), is widely used to assess oil fingerprints' equivalence on two samples. One of the methods used to compare *DR* obtained from each sample is the Student's t statistics, which, from triplicate determinations of one sample, define the limits for the *DR* comparison of the second sample [3-5]. The statistical equivalence of *DR* is concluded if the *DR* estimated from the second sample is within the confidence interval defined. However, to support the identification of the spill source is necessary the agreement of a set of characteristic *DR* observed for the samples compared [1,3]. The Student's t method (S-t) for *DR* comparison assumes the *DR* probability distribution normality. This approach can lead to a higher risk of false decisions of compositional equivalence between two samples if the *DR* distributions deviate significantly from normality. Therefore, the estimation of the risks associated with the compositional equivalence decision between two samples is relevant, leading the chemical analyses to more valuable evidence.

This work intends to describe a tool developed to estimate the risks of true acceptance and false rejection of the compositional equivalence of an oil present in two samples by simulation of correlated chromatographic signals using Monte Carlo Method (MCM), and the comparison with the risks estimated for S-t method. In addition, it is presented an alternative methodology for chemical composition comparison, which leads to a lower risk of false rejection of the compositional equivalence between two samples.

## 2. Methodology

The developed tool estimates the confidence limits used for DR comparison between samples, as well as the risks of a true acceptance and false rejection of the compositional equivalence of two samples. The MCM estimates the confidence limits for DR comparison and the risks above referred. Fifty-seven chromatographic signals used for the 69 DR determination are simulated simultaneously. Experimental data from GC-MS analysis of oil samples are used to estimate the correlation and dispersion of data since the chromatographic signals of the compounds used in the DR determination are correlated.

To define the confidence limits for the DR comparison, 100 000 simulations are performed for each chromatographic signal to generate 100 000 values of the studied DR. An additional set of 10 000 results of the 69 DR is obtained to perform DR significance tests using the confidence limits estimated. This set of 10 000 results $\times$ 69 DR is used to estimate the probability of all the DR set ratios being statistically equivalent, i.e., the risk of the true acceptance of the compositional equivalence between two samples. The risk of false rejection of the compositional equivalence between two samples is obtained by subtracting the risk of the true acceptance of the compositional equivalence between two samples to 100%.

## 3. Conclusions

The developed tool was successfully applied to assess the compositional equivalence of two oil samples from the comparison of 69 DR defined from 57 correlated chromatographic signals. The DR distributions have shown deviations from normality, leading to MCM confidence intervals wider than S-t ones. As expected, the probability of two samples with the same oil producing 69 statistically equivalent DR was lower than the confidence level studied since the 69 DR studied are not perfectly correlated. Higher probabilities were found when the MCM method was applied in comparison to the S-t method.

A developed alternative methodology to conclude about the compositional equivalence between two samples reduced the risk of false rejection of compositional equivalence.

This work is the first statistically sound assessment of the probability of correctly concluding about the compositional equivalence of two oil samples from the comparison of multiple DR. The detailed understating of the success rate of the tests allowed defining a methodology for increasing analysis reliability.

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# Assessment of the application of quality management systems requirements related to computer systems in laboratories by assessors and their application by accredited laboratories

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## 1. Introduction

Considering the fact that nowadays it is practically impossible to perform laboratory tests without using some kind of computer, the standards that orient laboratory activities needed to incorporate guidelines on the use of computerized systems in quality management systems [1, 2].

However, it is not uncommon for laboratories to be unable or unwilling to apply these rules fully, either due to lack of clarity of the standards or negligence in enforcing them. In addition, the assessment of compliance with requirements related to computerized systems depends on the level of knowledge of the auditors themselves [3].

Thus, the final objective of this study is to compare the laboratories' perception of the assessors' collection of the requirements of the standards and the laboratories' execution of them, as well as to analyze the auditors' level of knowledge about computerized systems applied to Quality Management Systems using an online questionnaire.

## 2. Methods

Data was collected using electronic forms created on the Google Forms platform, given its simplicity of use with which internet users are familiar, making it more intuitive to fill out and less prone to errors caused by misunderstandings about its use. Two forms were created to collect responses from auditors and laboratories. The auditor form had three sections (Characterization of the participant, Computer skills, State of implementation of computerized systems in the laboratories) and the laboratories form had two sections (Characterization of the company/institution, About the items evaluated and compliance with the requirements). Initially, the link to the forms was sent to the participants via e-mail, along with a presentation text of the researchers and their objectives, as well as the address of the website created in the Wix platform to disclose the research, containing more detailed information about it. The e-mail addresses to contact the laboratories were obtained from Inmetro's website, in its catalog of laboratories belonging to the Brazilian Network of Test Laboratories (RBLE). Additionally, the survey was disseminated on the IQSC-USP news website and in social networking groups related to auditors and laboratories with implemented management systems.

## 3. Results

There were 111 laboratories participating, located in thirteen different Brazilian states. Most of the participating institutions - 69.37% - declared to be private. In this group, only 3.90% are not accredited to ISO/IEC 17025, and 2.60% are in the process of accreditation. As for the classes of tests, 36.94% of laboratories work with chemical tests, another group in which accreditation in ISO/IEC 17025 prevails: 87.80% are accredited in this standard. The purpose of evaluating the non-conformities received by the laboratories during their last external audit was not only to identify the items related to computer systems with the highest incidence of non-conformities, but also to

ascertain the rate of verification of the raters of each item. Questions were asked involving the following topics:

- validation of software used in the laboratory;
- formal testing for verification and suitability;
- computer operating systems;
- antimalware use;
- data backup;
- user control for computer access;
- preventive and corrective maintenance;
- confidentiality contract;
- software manuals;
- spreadsheets used in the lab.

## 4. Conclusions

Even with a quick observation of the data obtained, it can be seen that a high number of auditors fail to evaluate several items related to computerized systems, even when there is the possibility of data security being affected. The four most neglected items were the periodicity of anti-malware updates, the corrective maintenance of computers and software, the existence of antimalware, and the preventive maintenance of computers and software. In a hypothetical case in which there were nonconformities in such items, it would not be difficult for malicious software to infiltrate the computers and generate damage that would force the pause of laboratory activities, for example.

With the exception of the items related to backups, even those with higher values of verification performed had responses corresponding to "the rater did not check this item" in the 20 to 25% range. Together with the possibility that the auditors' knowledge of computerized systems is not consistent, this opens gaps for failures that can lead from the loss of documents of little or no importance (which can be redone, such as form templates) to the generation of erroneous test results or the loss of single-observation documents that cannot be redone due to lack of sample, or for some other impeding reason. When considering that 54.95% of the participating laboratories perform chemical, biological, and clinical testing, the impact of these consequences becomes clearer.

The percentage of non-compliance detected is low - the highest of all was the blocking of cells, with 8.11% - and the five items with most non-compliances are simple to be corrected by the laboratories, because they consist in blocking cells of the spreadsheets used, confidentiality agreement when data are kept by third parties, validation of spreadsheets used, writing validation reports of software and frequency of performing backups. However, it is still not possible to know if this is really due to their absence or the evaluators' inability to detect them, reinforcing the need to examine their knowledge.

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# Metrological aspects of the certification of reference standards of the State Pharmacopoeia of Ukraine

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## 1. Introduction

The concept of measurement uncertainty is a globally recognized scientific basis for assuring consistency and comparability of measurement results, the ISO 17025 requirements for application of which have recently become mandatory for the members of the network of official medicines control laboratories (OMCLs) in the European Union [1]. However, applying the uncertainty concept and implementing the principle of metrological traceability is virtually impossible when using reference standards (RSs) established by leading pharmacopoeias since they do not provide the user with the uncertainty of the value assigned to a property of an RS and the homogeneity for the RS property.

Consequently, the development of the approach to the certification of pharmacopeial RSs that would satisfy the ISO requirements is crucial.

## 2. Results and Discussion

The State Pharmacopoeia of Ukraine (SPhU) adopted the following approaches to the SPhU RS certification. Hereinafter, we assume that a level of confidence is 95 % unless otherwise specified; under uncertainty we understand the expanded uncertainty.

Combining the uncertainty components in quadrature was proposed to evaluate the overall uncertainty, based on which the principle of insignificance was formulated: any uncertainty component is considered insignificant relative to the overall uncertainty if their quotient is not more than 0.32. Relying on the insignificance principle and the standardization rules accepted in the pharmaceutical sector, recommendations for the target measurement uncertainty ($U_{Target}$) for basic pharmacopeial quantitative applications were developed and introduced in the SPhU (Table 1).

**Table 1.** Recommendations for the target measurement uncertainty.

| Use | Recommendation |
|---|---|
| Assay of some pharmaceutical substances (two-sided specification limits) | $U_{Target} = B_{Upper} - 100\ \%$[1] |
| Assay of medicinal products and some pharmaceutical substances (two-sided symmetric specification limits) | $U_{Target} = 0.32 \times (B_{Upper} - B_{Lower})/2$ |
| Assay of medicinal products and pharmaceutical substances (one-sided upper specification limit) | $U_{Target} = 6.4\ \%$ |
| Uniformity of dosage units, Dissolution | $U_{Target} = 3.0\ \%$ |
| Related substances | |
| Limit tests | $U_{Target} = 16\ \%$ |
| Quantitative tests | $U_{Target} = 5.0\ \%$ |

[1] proposed by the Ph. Eur. [2]; other recommendations are developed by the SPhU;

$B_{Upper}$ is the upper specification limit; $B_{Lower}$ is the lower specification limit.

The maximum permissible uncertainty of the value assigned to a property of an RS ($\max U_{RS}$) shall be negligible compared with the target measurement uncertainty:

$$\max U_{RS} = 0.32 \times U_{Target}.$$

Consequently, for SPhU RSs intended for assays of medicinal products with specification limits of 100 % ± 5 % (the most stringent requirements), the maximum permissible uncertainty of the assigned value shall not exceed 0.51 %.

As uncertainty lower than 0.51 % may not be reached in the RS establishment, the strictest requirement for any uncertainty source contributing to the uncertainty of the assigned value, namely the uncertainty associated with the RS characterization, homogeneity, and stability, shall not exceed 0.51 %; if fulfilled, the uncertainty budget is not established.

Typically, the SPhU RS assigned value is determined by a mass balance method and verified by an independent method. It may be additionally verified by comparison to the previous batch or another material. The characterization uncertainty aroused from any method should not exceed the $\max U_{RS}$, and any difference between the results of the property value determination should not exceed $\sqrt{2} \times \max U_{RS}$. The user is informed of the maximum permissible uncertainty of the assigned value, which remains unchanged when replacing the SPhU RS batch.

The SPhU RS homogeneity is studied on data obtained from at least ten determinations, using the test portion mass indicated in the analytical procedure. An RS is considered sufficiently homogeneous if both following criteria are met:

1. A two-sided confidence interval should not exceed the maximum permissible uncertainty of the assigned value.

2. The difference between any result and the assigned value should not exceed the maximum permissible uncertainty of the assigned value at a 99 % confidence level ($\max U_{RS(99)}$):

$$\max U_{RS(99)} = (t_{95} / t_{99}) \times \max U_{RS},$$

where $t_{95}$ and $t_{99}$ are the two-tailed critical values for Student's $t$ with 9 degrees of freedom for the confidence levels of 95 % and 99 %, respectively.

If the maximum permissible uncertainty of the assigned value at a 95 % confidence level is equal to 0.51 %, that at a 99 % confidence level is: $(2.59 / 1.96) \times 0.51 = 0.67$ %. The approach is similar to the one used in pharmacy for standardization of the uniformity of dosage units. If the first criterion is used alone, critical individual deviations from the assigned value may be erroneously considered acceptable.

The SPhU RS is accompanied by a certificate, which states the following information:

– intended use (tests and/or assays, and analytical methods),

– value assigned to the RS property and its maximum permissible uncertainty (for quantitative RSs),

– minimum test portion that provides sufficient homogeneity of the RS (if necessary, for quantitative RSs),

– expiration date under prescribed storage conditions (by default, 1 year from the dispatch date).

The approach adopted to the SPhU RS certification was called the "principle of transparency".

At present, the nomenclature of the SPhU RSs certified according to the established principles comprises about 900 items.

## 3. Conclusion

Being in line with the ISO recommendations, the approach to the certification of reference standards employed by the State Pharmacopoeia of Ukraine allows using the uncertainty concept and ensuring the metrological traceability of measurement results, which makes it possible to take a scientifically sound decision about the suitability of the SPhU RS for the user's intended purpose.

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# The use of Monte Carlo Simulations of georeferenced information to evaluate composition trends in oceanic waters

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## 1. Introduction

The assessment of the environmental status of a large oceanic area and evaluation of temporal trends is demanding due to its seasonality, heterogeneity and size. Until recently, the uncertainty associated with sampling was not considered in oceanic monitoring [1].

This work describes a novel tool based on Monte Carlo Simulations of georeferenced information to assess if temporal silicate concentration variations in a large ocean area cannot be justified by system heterogeneity or analytical uncertainty and is, therefore, meaningful.

## 2. Materials and Methods

Water from an area of the Portuguese Continental Platform was sampled during two field surveys of the AQUIMAR project (Marine Knowledge supporting Aquaculture): October 2018 and May 2019. The sampling stations were located on a grid of approximately 15 by 20 nautical miles, between 40.12 and 40.46 degrees N and 8.96 and 9.30 degrees W at a distance of 5 nautical miles between them. In each sampling occasion, samples of 4 L to 5 L were collected at 25 m depth from the Portuguese R/V NRP Almirante Gago Coutinho, using 8 L Niskin bottles assembled on a Rosette sampler.

Immediately upon collection, at the field laboratory, samples were homogenized, filtered using 0.45 mm pore size cartridges and then preserved below -20 ºC, in high density polyethylene bottles until analysis.

The determinations were performed by Segmented Flow Analysis using a Skalar SANplus Segmented Flow Auto-Analyzer designed for saline water analysis. The analyses were performed using previously validated procedures and checked using metrologically sound quality control. Details regarding the determinations can be found elsewhere [2].

The developed methodology involves transforming the known coordinates and concentration of samples collected in the studied area in a spatial model of concentration variation represented by a 3D ($x, y, z$) surface, where $x$ and $y$ are the coordinates, and $z$ the sample concentration. This surface is subsequently randomized, given coordinates and samples analysis uncertainty, and the simulated concentrations of the analyzed component used to estimate concentration distribution and mean value with uncertainty (Single Sampling, SS). The simulated surface is also used to predict the uncertainty of two types of composite samplings, namely: random (RS) or linear composite sampling (LS), where samples are collected randomly or in a radial line that starts in the center of the sampled area [2]. This methodology was implemented in a user-friendly MS-Excel spreadsheet.

The total uncertainty associated with the measurement is, then, calculated by combining the pertaining sampling uncertainty with sample analysis uncertainty, according to Eq. (1), where $s_S$ is the uncertainty arising from sampling that depends on the sampling strategy used, and $s_r$, $s_I$ and $u_T$ are the standard deviation of measurement repeatability, the standard deviation of measurement intermediate precision and the standard uncertainty of measurement trueness, respectively.

$$U = 2 \times u = 2 \times \sqrt{s_S^2 + s_r^2 + s_I^2 + u_T^2} \qquad (1)$$

## 3. Results

Table 2 presents the results of Monte Carlo simulations of the mean concentration of silicate in the two studied occasions and of the respective uncertainty when different types of samplings are considered, namely: 1) "Single Sampling" (SS) where only one sample is taken from the sampling circle (the actual case), 2) "Random Composite Sampling" (RS (m)) where m samples from randomly selected positions of the sampling circle are collected mixed in a single solution and analyzed, and 3) "Linear Composite Sampling" (LS(m; d)) where m samples are collected in a line that starts at the center of the sampling circle and has any radial direction with samples being collected at d m distances and mixed before one analysis. RS and LS were studied from 1 to 7 samples, with LS being studied in a radial distance of 15000 m (d = 15000 m to 2500 m), but only results for m = 2, 4 and 7 are presented. Analytical components of uncertainty are $s'_r = 2.95\%$, $s'_I = 2.51\%$ and $u'_T = 3.09\%$.

**Table 2.** Simulated variability of mass concentrations of silicate in an area of the Portuguese Continental shelf on two different sampling periods, and estimated sampling and combined expanded uncertainties using different sampling strategies. (§ - Value obtained by the Monte Carlo Method; Analytical components of uncertainty are $s'_r = 2.95\%$, $s'_I = 2.51\%$ and $u'_T = 3.09\%$)

| Sampling | October 2018 | | | May 2019 | | |
|---|---|---|---|---|---|---|
| | Mean § | $s'_S$ (%) § | $U'$ (%) | Mean § | $s'_S$ (%) § | $U'$ (%) |
| SS | 1.97 | 27.03 | 54.96 | 1.77 | 52.11 | 104.7 |
| RS(2) | - | 19.11 | 39.48 | - | 36.85 | 74.4 |
| RS(4) | - | 13.52 | 28.80 | - | 26.06 | 53.0 |
| RS(7) | - | 10.22 | 22.72 | - | 19.70 | 40.6 |
| LS(2; 15000) | 1.91 | 6.61 | 16.52 | 2.76 | 5.90 | 15.4 |
| LS(4; 5000) | 1.91 | 13.89 | 29.49 | 2.60 | 10.80 | 23.8 |
| LS(7; 2500) | 1.92 | 16.71 | 34.86 | 2.55 | 12.88 | 27.6 |

## 4. Discussion and Conclusions

Analyzing the main features of Table 1, the mean uncertainty decreases with the increase of m for RS strategy, but the opposite behavior is observed for the LS strategy, which is mainly due to the system's heterogeneity. The observed behaviors indicate that the LS strategy is not adequate for heterogeneous systems.

When comparing data from the two sampling occasions and compare the estimated silicate mean concentrations we obtain (1.97 ± 0.29) µmol L$^{-1}$ and (1.77 ± 0.44) µmol L$^{-1}$. Since the two intervals overlap, it can be stated directly that for silicate no trend can be observed for the studied area. This is confirmed by the application of the statistical t test, that allows the estimation of differences with the associated uncertainty and returns the value (-0.20 ± 0.78) µmol L$^{-1}$.

This observed behavior can be related to the low concentrations and high heterogeneity observed.

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# Traceable reference gas mixtures to calibrate analyzers used to measure atmospheric VOCs

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## 1. Introduction

Volatile organic compounds (VOCs) play an important role in the atmospheric chemistry, especially in the oxidative capacity of the lower atmosphere [1]. Besides their role as ozone and aerosol precursors, VOCs contribute directly and indirectly to the radiative forcing and in turn to climate change [2, 3]. In order to identify climate trends, comparable datasets at regional and temporal scales are essential. For that purpose, long-term, accurate and traceable measurements of VOCs are needed. However, the lack of stable and traceable standards to the international system of unit for some VOCs, together with effects linked to reactivity with surfaces (i.e. memory effects, decomposition artefacts) and to ozone and humidity interferences, are common issues for sites monitoring VOCs in the atmosphere.

Within the framework of the European Metrology Programme for Innovation and Research (EMPIR) of the European Association of Metrology Institutes (EURAMET), the project "Metrology for Climate Relevant Volatile Organic Compounds" (MetClimVOC, 2020-2023) [4] pursues to minimize these limitations by generating reference gas mixtures (RGMs) of relevant VOCs. These novel SI-traceable RGMs will be produced at atmospheric amount fraction level with a well-defined uncertainty (amount fraction between 1 nmol/mol and 1 µmol/mol with expanded uncertainty < 5% for oxy-VOCs and terpenes and < 1 nmol/mol with expanded uncertainty < 3% for halogenated VOCs). Moreover, the project aims to optimize sampling and analytical methods used in monitoring stations along with the development of fit-for-purpose working standards allowing full uncertainty estimations on real air measurements.

## 2. Current calibration strategies for volatile organic compound used in monitoring station

Because of their complex chemical speciation (thousands of species) [eg. 5, 6], analysis of atmospheric VOC is not straightforward. Gas chromatography coupled to either flame ionization detection (GC-FID) or mass spectrometry (GC-MS) is the most common technique used for measuring VOC gas mixtures. However, in recent years, the use of proton-transfer reaction mass spectrometry (PTR-MS) has increased.

Different strategies are applied to calibrate the analytical techniques used for measuring VOCs. We present an overview of calibration strategies and working standards in the field. In addition, gaps identified for achieving SI-traceability are included.

## 3. SI-traceable reference gas mixtures realization and its uncertainty estimation

One of the aims of the project MetClimVOC is the realization of accurate, stable and traceable static and dynamic RGMs for VOCs relevant for climate change at low amount fractions (atmospheric levels). Thus, this project will contribute to cover current calibration strategy gaps for achieving SI-traceability.

Within the project, one of the methods to generate dynamically SI-traceable VOC RGMs is permeation [6]. The first step to generate these RGMs is to estimate the permeation rate of a permeation unit containing the pure liquid form of the VOC of interest. The permeation rate is calculated as the mass loss of the permeation unit with time. To determine the mass loss, the permeation unit is placed in a magnetic suspension balance (MSB). Pressure, temperature and flow in the MSB permeation chamber are regulated precisely. Once the permeation rate is stable (ca. 5 – 7 days), RGMs are generated using a dynamic dilution system coupled to the MSB. Dilution flows are set to obtain RGMs of different amount fractions. The uncertainty of each step is considered in the overall uncertainty budget. The impurity assessment of the permeation units using GC-FID or GC-MS is crucial to estimate the full uncertainty of the RGMs and to ensure SI-traceability.

Here, the generation of SI-traceable VOC RGMs using permeation is described in more detail. Furthermore, the analytical method to measure and validate the generated RGM is presented along with a full uncertainty budget according to the Guide to the Expression of Uncertainty in Measurement [7]. Main uncertainty contributions are highlighted.

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# Evaluation of measurement uncertainty of environmental matrices indicators including sampling uncertainty

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## 1. Introduction

Testing laboratories are interested in estimating uncertainties of indicators of environmental matrices, which may include raw and surface water, sewage sludge, waste and sediments. The main reasons for that are requirements of accreditation bodies, legal regulations and customers. Uncertainties reported by the laboratory quite often do not include sampling uncertainties and do not respect the effect of the concentration level of the analyte of interest. A validation study suitable for the so-called empirical approach to the evaluation of measurement uncertainty, which would include sampling and analysis, would entail significant financial and time costs for each laboratory and would include only an in-house experiment. Obtaining the estimation of real values of uncertainties achieved in the analysis of environmental samples is a challenging task and the methodology of a targeted interlaboratory experiment is an effective way to obtain such estimates of measurement uncertainty values, including sampling.

## 2. Statistical model for measurement uncertainty evaluation

An empirical approach for estimating the uncertainty and statistical procedures given in the Eurachem/CITAC Guide [1] was chosen. To design test methods for the empirical estimation of uncertainty, it is necessary to have a statistical model describing the relationship between the measured and the true value of the analyte concentration. This random effects model considers a single measurement of analyte concentration ($x$), on one sample (composite or single), from one particular sampling target:

$$x = X_{true} + \varepsilon_{sampling} + \varepsilon_{analytical}$$

where $X_{true}$ is the true value of the analyte concentration, the total error due to sampling is $\varepsilon_{sampling}$ and the total analytical error is $\varepsilon_{analytical}$. If statistical estimates of variance ($s^2$) are used to approximate measurement and sampling variances, we get:

$$s_{meas}^2 = s_{sampling}^2 + s_{analytical}^2$$

The standard uncertainty ($u$) can be estimated using $s_{meas}$, which is therefore given by

$$u = s_{meas} = \sqrt{s_{sampling}^2 + s_{analytical}^2}$$

To obtain the expanded uncertainties (e.g., 95% confidence level), this value must be obtained by an expansion factor of 2. The expanded uncertainty is then calculated as $U = 2 \cdot s_{meas}$.

## 3. Results and conclusion

Selected results of raw and surface water, sediment, waste and sewage sludge indicators obtained in 2016–2019 during interlaboratory comparisons organized by an accredited proficiency testing provider, CSlab Ltd. Will be presented as tables. A brief summary of findings for individual matrices and interlaboratory comparisons has shown:

• A comparison of the results processed by ANOVA and RANOVA confirmed that robust methods should be used when clear outliers appear as part of a typical set of sampled cases.
• Laboratories with a better technical equipment and well-established internal quality control system report lower measurement uncertainties, which puts them at a disadvantage compared to laboratories with a higher uncertainty, often estimated by a "qualified estimate".
• Laboratories usually do not have evaluated uncertainties for different concentration levels of analytes, the uncertainty reported by laboratories is a constant value, it is not concentration dependent.
• A sampling plan is very important for performing sampling, which includes the purpose for which sampling is performed.

## Acknowledgment

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# Bottom-up evaluation of the uncertainty of a titration with visual end-point detection: Determination of dissolved oxygen by the Winkler Method

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## 1. Introduction

Dissolved oxygen concentration in water is a crucial parameter to assess the condition or evolution of aquatic ecosystem health. This determination can be performed using an electrochemical sensor or the reference Winkler method that allows a more reliable measurement of this parameter. The comparison of dissolved oxygen values determined on two occasions or two samples requires calculating the measurement uncertainty. This uncertainty is also relevant to understand if the determination has adequately low uncertainty.

## 2. Measurement uncertainty evaluation

This work describes the detailed assessment of dissolved oxygen determinations' performance aiming at the 'bottom-up' quantification and optimisation of the measurement uncertainty. The visual end-point detection's uncertainty was estimated by the difference between observed measurement precision and combined models of all precision components except the end-point detection [1-3]. A user-friendly MS-Excel spreadsheet that allows applying the developed uncertainty evaluation procedure was developed.

## 3. Results and conclusion

The determination of dissolved oxygen from analytical portions not lower than 50 mL is fit for environmental monitoring. It allows measurements between 0.3 mg $L^{-1}$ and 14.6 mg $L^{-1}$ with an expanded uncertainty between 0.36 mg $L^{-1}$ and 0.74 mg $L^{-1}$ for a 95% confidence level. This uncertainty allows differentiating dissolved oxygen values between 0.51 mg $L^{-1}$ and 1.0 mg $L^{-1}$ with less than a 5% probability of being wrongly assumed a relevant difference [1]. The described uncertainty evaluation strategy can also be used in other titrimetric determinations [1].

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