MOOC: Estimation of measurement uncertainty in chemical analysis (analytical chemistry) course

This is an introductory course on measurement uncertainty estimation, specifically related to chemical analysis.

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Course introduction

This course is offered as a MOOC (Massive Open Online Course) during March 22 – May 03, 2022.
The registration is open and available here.

Course introduction

This is an introductory course on estimation of measurement uncertainty, specifically related to chemical analysis (analytical chemistry). The course gives the main concepts and mathematical apparatus of measurement uncertainty estimation and introduces two principal approaches to measurement uncertainty estimation – the ISO GUM modeling approach (the "bottom-up" or modeling approach) and the single-lab validation approach as implemented by Nordtest (the "top-down" or Nordtest approach). The course contains lectures, practical exercises and numerous tests for self-testing.

Course introduction

http://www.uttv.ee/naita?id=17710

https://www.youtube.com/watch?v=r34Y-gzf62Y

In spite of being introductory, the course intends to offer sufficient knowledge and skills for carrying out uncertainty estimation for most of the common chemical analyses in routine laboratory environment. The techniques for which there are examples or exercises include acid-base titration, Kjeldahl nitrogen determination, UV-Vis spectrophotometry, atomic absorption spectroscopy and liquid chromatography mass spectrometry (LC-MS). It is important to stress, however, that for successful measurement uncertainty estimation experience (both in analytical chemistry as such and also in uncertainty estimation) is crucial and this can be acquired only through practice.

The materials of this course can also be useful for people who do not intend to follow the full course but only want to find answers to some specific questions.

This course has been described in the paper: I. Leito, I. Helm, L. Jalukse. Using MOOCs for teaching analytical chemistry: experience at University of Tartu. *Anal. Bioanal. Chem.* **2015**, DOI: 10.1007/s00216-014-8399-y.

On May 13, 2021 the 8th edition of the course finished successfully. Altogether 950 people registered from 97 countries. 501 participants actually started the course (i.e. tried at least one graded test at least once) and out of them 314 successfully completed the course. The overall completion rate was 33%. The completion rate of participants who actually started the studies was 63%. (Some more statistics).

You can have a preview of the MOOC in Moodle environment as a guest. Guest access allows you to view the course contents, but you cannot read the forums and take quizzes.

Required preliminary knowledge

Introductory level knowledge of analytical chemistry is required. More advanced knowledge of analytical chemistry and introductory knowledge of mathematical statistics is an advantage. Fluency with and access to a spreadsheet software package (MS Excel, OpeOffice, etc) is highly recommended.

Why is measurement uncertainty important

http://www.uttv.ee/naita?id=17711

https://www.youtube.com/watch?v=tn2DLYA72Dk

Study outcomes

The student who has successfully passed the course knows:

the main concepts related to measurement results and measurement uncertainty, including their application to chemical analysis;
the main mathematical concepts and tools in uncertainty estimation;
the main measurement uncertainty sources in chemical analysis;
the main approaches for measurement uncertainty estimation.

The student who has successfully passed the course is able to:

decide what data are needed for uncertainty estimation, understand the meaning of the available data and decide whether the available data are sufficient;
select the uncertainty estimation approach suitable for the available data;
quantify the uncertainty contributions of the relevant uncertainty sources using the available data;
carry out estimation of uncertainty using the main approaches of uncertainty estimation.

Organization of the course material

The course (overall volume 1 ECTS) is organized in 12 sections, of which some are in turn split into smaller subsections. The following parts are found in the sections:

1. The sections (and also many subsection) start with a **brief introduction** stating the main topic(s) and study outcomes of the section.
2. The main topic of the respective section is explained in a short **video lecture**.
3. The lecture is followed by a textual part. This text is in most cases meant to complement, not substitute the lecture (although in some cases the contents of the lecture are also repeated in some extent). It rather gives additional explanations and addresses some additional topics that were not covered by the lecture.
4. Most sections end with a self-test, which enables to test the acquired knowledge and skills. The tests contain questions, as well as calculation problems. The self-tests are on one hand meant for the students to monitor his/her progress. On the other hand, however, they also promote thinking and provide (by the feedback of the questions) additional knowledge about measurement uncertainty estimation in different practical situations. So, the self-tests are an intrinsic component of the course and it is strongly recommended to take all of them.

The printout of the current version of the course materials (including lecture slides) can be downloaded from here.

If you consistently get a message "Server not found" when attempting to watch videos then with high probability the reason is the firewall of your local network. The local network administrators should enable outcoming connections from your network via port 1935. More specifically it is necessary to access the server rtmp://flash.ut.ee:1935.

An additional possibility is to watch the videos in YouTube via channel "ESTIMATION OF MEASUREMENT UNCERTAINTY IN CHEMICAL ANALYSIS".
Direct link: https://www.youtube.com/channel/UCeNhxB_WuTDNcbNHFxsBjUw

Self-testing

Throughout the course there are numerous self-tests for enabling the student to test his/her knowledge and skills in specific topics. Each test is graded as a percentage (100% corresponding to correctly answering all questions and correctly solving all problems).

Feedback is given as:

✓ Correct answer, correctly recognised and marked by the student.

✓ Correct answer, not recognised and not marked by the student.

✗ Incorrect answer, however, considered correct by the student.

Explanatory feedback is displayed when wrong answer is selected. All self-tests can be taken as many times as needed and the success of taking self-tests will not influence the final grade. We recommend that you take all the self-tests and work with them until you achieve score 100% and only then move to next topic.

Course organization

During the six-week course period the work will be organized using the Moodle online environment. For every week a set of tasks will be given - which sections of the material should be studied and which self-tests/exercises should be passed at https://sisu.ut.ee/measurement/uncertainty. The self-tests can be taken unlimited number of times (preferably, until the answers/solutions are fully correct) and their outcomes will not influence the final grade. At the end of each week there will be a graded interim test (in the Moodle environment), which will be counted for assigning the final grade. The participants can choose themselves how they spend their time during the course, because all graded tests are available from the beginning of the course until the end of the course. This enables them to organize their time the way that is best suitable for them. Please note, however, that the difficulty level of the tests increases as the course progresses: tests 3-4 are more difficult than 1-2 and tests 5-6 are still more difficult. Because of the higher difficulty level it may be a good idea you might have to plan more time for the last weeks of the course. How much time it takes for passing the course, is very individual, but we estimate that an average participant needs 3-4 h per week.

Terminology and definitions

Wherever possible, the used terminology adheres to the 3rd edition of the *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*, [1] referred to as "VIM" throughout the course. However, in the interest of better understanding and in order to stress the most important aspects of concepts, in many cases concepts are introduced by definitions that are somewhat simplified compared to the VIM. More deeply interested students are encouraged to consult the VIM.

If you would like to learn more ...

This course is part of the Excellence in Analytical Chemistry (https://www.analyticalchemistry.eu/) *Erasmus Mundus* master's programme, which offers education in all aspects of Analytical chemistry, including metrology in chemistry (measurement uncertainty, method validation, reference materials, etc), as well as economic and legal aspects of chemical analysis.

This course is run within the framework of the Estonian Center of Analytical Chemistry with the aim of offering easily accessible knowledge in analytical chemistry to labs and industries in Estonia and elsewhere.

Main literature sources [2]

(1) JCGM 200:2012, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*, 3rd edition. BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML, **2012**. Available on-line from http://www.bipm.org/en/publications/guides/vim.html
(2) JCGM 100:2008 *Evaluation of measurement data — Guide to the expression of uncertainty in measurement*. JCGM, **2008**. Available on-line from http://www.bipm.org/en/publications/guides/gum.html

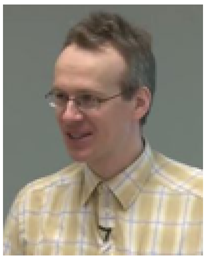
(3) *Quantifying Uncertainty in Analytical Measurement*, 2nd ed.; Ellison, S. L. R.; Williams, A., Eds.; EURACHEM/CITAC, **2012**. Available on-line from http://eurachem.org/index.php/publications/guides

(4) *Measurement Uncertainty Revisited*. Eurolab Technical Report No 1/2007. Eurolab, **2007**. Available on-line from http://www.eurolab.org/documents/1-2007.pdf

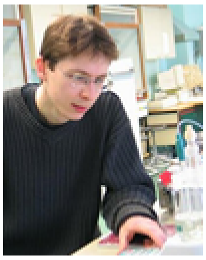
(5) *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*. B. Magnusson, T. Näykki, H. Hovind, M. Krysell, E. Sahlin. Nordtest technical report 537, ed. 4.0. Nordtest, **2017**. Available on-line from http://www.nordtest.info/wp/2017/11/29/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-4/

(6) *Analytical Measurement: Measurement Uncertainty and Statistics*. Eds: N. Majcen, V. Gegevicius. EC-JRC IRMM, **2012**. Available on-line from http://publications.jrc.ec.europa.eu/repository/bitstream/111111111/29537/1/lana2207enn-web.pdf

Course team



Ivo Leito, professor of analytical chemistry at University of Tartu.
Ivo teaches analytical chemistry and metrology in chemistry at all study levels and organizes short training courses for practitioners on different topics of analytical chemistry and metrology in chemistry. His research work embraces a wide area of topics ranging from studies of superacids and superbases to LC-MS analysis. He is the initiator of the master's programme Applied Measurement Science at University of Tartu.



Lauri Jalukse, research fellow in analytical chemistry at University of Tartu.
Lauri teaches analytical chemistry and metrology in chemistry at all study levels. He is continuously introducing innovative and active learning approaches into teaching. His research work is focused on metrological studies of electrochemical and optical sensors, measurements of dissolved oxygen concentration and moisture content, as well as organization of interlaboratory comparisons.

Irja Helm, research fellow in analytical chemistry at University of Tartu.
Irja teaches practical classes of analytical chemistry. She takes care that metrological concepts and approaches are introduced to students at as early stage of analytical chemistry studies as possible.

Technical design: Educational Technology Centre, University of Tartu.

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[1] JCGM 200:2012, *International vocabulary of metrology — Basic and general concepts and associated terms (VIM)*, 3rd edition. BIPM, IEC, IFCC, ILAC, ISO, IUPAC, IUPAP and OIML, **2012**. Available on-line from http://www.bipm.org/en/publications/guides/vim.html

[2] This list of literature references is selecive, not exhauxtive. The references were selected based on the following criteria: (1) Widely used and cited; (2) useful under practical lab conditions (i.e. not too deeply scientific); (3) a fairly recent version is available and (4) the document is preferably available free of charge on the Internet. These references are referred to in the course via superscript numbers in round brackets, e.g.: VIM$^{(1)}$.

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1. The concept of measurement uncertainty (MU)

Brief summary: This section introduces the concepts of **measurand**, **true value**, **measured value**, **error**, **measurement uncertainty** and **probability**.

The concept of measurement uncertainty

http://www.uttv.ee/naita?id=17583

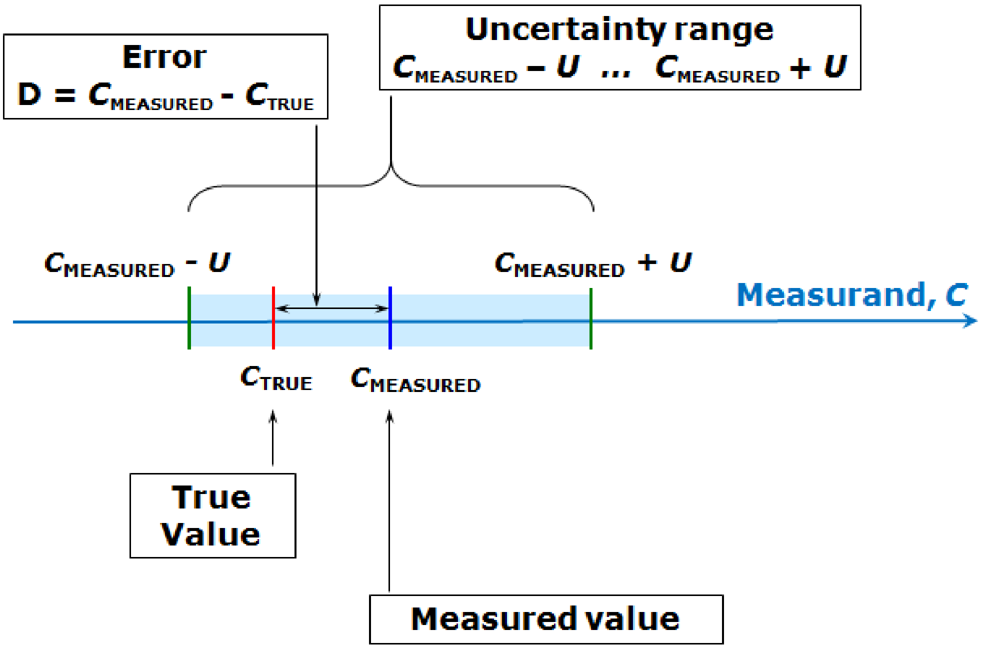
https://www.youtube.com/watch?v=BogGbA0hC3k

Measurement is a process of experimentally obtaining the value of a quantity. The quantity that we intend to measure is called **measurand**. In chemistry the measurand is usually the content (concentration) of some chemical entity (molecule, element, ion, etc) in some object. The chemical entity that is intended be determined is called **analyte**. Measurands in chemistry can be, for example, lead concentration in a water sample, content of pesticide thiabendazole in an orange or fat content in a bottle of milk. In the preceding example lead (element), ascorbic acid (molecule) and fat (group of different molecules) are the analytes. Water, orange and milk are **analysis objects** (or **samples** taken from analysis objects).

In principle, the aim of a measurement is to obtain the **true value** of the measurand. Every effort is made to optimize the **measurement procedure** (in chemistry chemical analysis procedure or analytical procedure [1]) in such a way that the measured value is as close as possible to the true value. However, our measurement result will be just an estimate of the true value and the actual true value will (almost) always remain unknown to us. Therefore, we cannot know exactly how near our measured value is to the true value – our estimate always has some uncertainty associated with it.

The difference between the measured value and the true value is called **error.** Error can have either positive or negative sign. Error can be regarded as being composed of two parts – **random error** and **systematic error** – which will be dealt with in more detail in coming lectures. Like the true value, also the error is not known to us. Therefore it cannot be used in practice for characterizing the quality of our measurement result – its agreement with the true value.

The quality of the measurement result, its **accuracy**, is characterized by **measurement uncertainty** (or simply **uncertainty**), which defines an interval around the measured value C_{MEASURED}, where the true value C_{TRUE} lies with some probability. The measurement uncertainty U itself is the half-width of that interval and is always non-negative. [2] The following scheme (similar to the one in the lecture) illustrates this:



Scheme 1.1. Interrelations between the concepts true value, measured value, error and uncertainty.

Measurement uncertainty is always associated with some probability – as will be seen in the next lectures, it is usually not possible to define the uncertainty interval in such a way that the true value lies within it with 100% probability.

Measurement uncertainty, as expressed here, is in some context also called the **absolute measurement uncertainty**. This means that the measurement uncertainty is expressed in the same units as the measurand. As will be seen in subsequent lectures, it is sometimes more useful to express measurement uncertainty as **relative measurement uncertainty**, which is the ratio of the absolute uncertainty U_{abs} and the measured value y:

$$U_{\text{rel}} = \frac{U_{\text{abs}}}{y} \qquad (1.1)$$

Relative uncertainty is a unitless quantity, which sometimes is also expressed as per cent.

Measurement uncertainty is different from error in that it does not express a difference between two values and it does not have a sign. Therefore it cannot be used for correcting the measurement result and cannot be regarded as an estimate of the error because the error has a sign. Instead measurement uncertainty can be regarded as our estimate, what is the highest probable absolute difference between the measured value and the true value. With high probability the difference between the measured value and the true value is in fact lower than the measurement uncertainty. However, there is a low probability that this difference can be higher than the measurement uncertainty.

Both the true value and error (random and systematic) are abstract concepts. Their exact values cannot be determined. However, these concepts are nevertheless useful, because their **estimates** can be determined and are highly useful. In fact, as said above, our measured value is an estimate of the true value.

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[1] Analytical chemists mostly use the term „analytical method". In this course we use the term „procedure" instead of „method", as this usage is supported by the VIM.

[2] Here and in the lecture the capital U is used to denote a generic uncertainty estimate. The symbol U is picked on purpose, because expanded uncertainty (generally denoted by capital *U*) fits very well with the usage of uncertainty in this section. However, it is not explicitly called expanded uncertainty here, as this term will be introduced in later lectures.

2. The origin of measurement uncertainty

Brief summary: Explanation, on the example of pipetting, where measurement uncertainty comes from. The concept of **uncertainty sources** – effects that cause the deviation of the measured value from the true value – is introduced. The main uncertainty sources of pipetting are introduced and explained: repeatability, calibration, temperature effect. Explanation of random and systematic effects is given. The concept of **repeatability** is introduced.

The first video demonstrates how pipetting with a classical volumetric pipette is done and explains where the uncertainty of the pipetted volume comes from.

Why measurement results have uncertainty? The concept of uncertainty source explained on the example of pipetting

http://www.uttv.ee/naita?id=17577

https://www.youtube.com/watch?v=ufWJB9orWdU

The second video demonstrates pipetting with a modern automatic pipette and explains the uncertainty sources in pipetting with an automatic pipette [1] .

Measurement uncertainty sources of pipetting with an automatic pipette

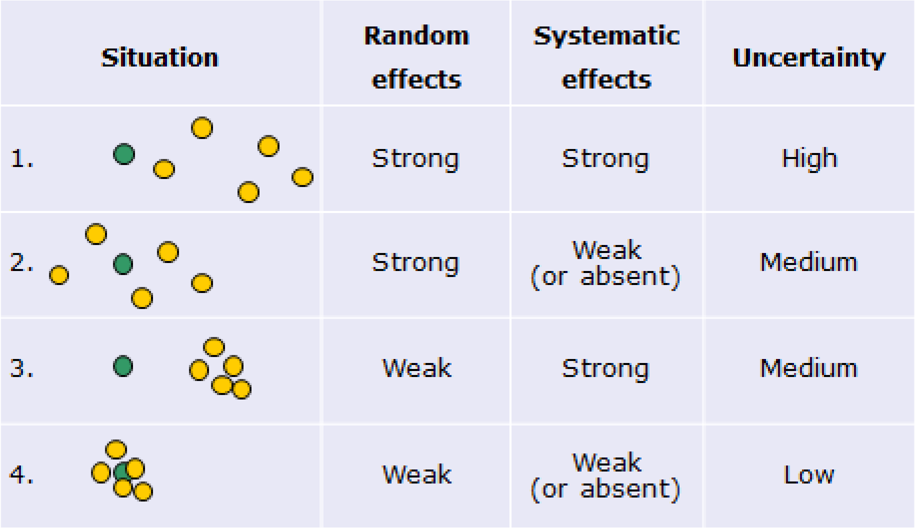
http://www.uttv.ee/naita?id=18164

https://www.youtube.com/watch?v=hicLweJcJWY

Measurement results have uncertainty because there are **uncertainty sources** (effects that cause uncertainty). These are effects that cause deviations of the measured value from the true value. [2] If the used measurement procedure is well known then the most important uncertainty sources are usually also known. Efforts should be made to minimize and, if possible, eliminate uncertainty sources by optimizing the measurement procedure (analysis procedure). The uncertainty sources that cannot be eliminated (and it is never possible to eliminate all uncertainty sources) have to be taken into account in uncertainty estimation.

The magnitudes of the deviations caused by uncertainty sources are usually unknown and in many cases cannot be known. Thus, they can only be estimated. If we can estimate the magnitudes of all important uncertainty sources then we can combine them and obtain the estimate of measurement uncertainty, which in this case will be called **combined measurement uncertainty**. How this combining is mathematically done, will be demonstrated in the coming lectures.

If we make a number of repeated measurements of the same measurand then ideally all these repeated measurements should give exactly the same value and this value should be equal to the true value of the measurand. In reality the results of the repeated measurements almost always differ to some extent and their mean value also usually differs from the true value. The uncertainty sources cause this. In a somewhat simplified way the uncertainty sources (or effects) can be divided into **random effects** and **systematic effects**. [3] The following scheme illustrates this (green circles denote true values, yellow circles denote measured values):



Situation	Random effects	Systematic effects	Uncertainty
1.	Strong	Strong	High
2.	Strong	Weak (or absent)	Medium
3.	Weak	Strong	Medium
4.	Weak	Weak (or absent)	Low

Scheme 2.1. The influence of random and systematic effects on measurement uncertainty.

Random effects cause the difference between the repeated measurement results (and thus, obviously, also from the true value). However, if a large number of repeated measurements are made then the mean value will have little influence from the random effects (situation 2 on the scheme). So, the influence of random effects can be decreased by increasing the number of repetitions. Systematic effects cause deviation of all measurements in the series into the same direction by the same magnitude. [4] Increasing the number of repetitions does not enable decreasing their influence (situation 3 on the scheme).

In principle it is desirable to determine the magnitude and direction of the systematic effects and correct the measurement results for the systematic effects. However, it can often be so difficult and work-intensive, that it becomes impractical. Therefore in many cases, rather than accurately determining the systematic effects and correcting for them their possible magnitudes is estimated and are taken into account as uncertainty sources. In lectures 5.4 and 6 random and systematic effects are treated more comprehensively.

There are in general four main sources of uncertainty in volumetric measurements, i.e. measurements by pipettes, burettes, measuring cylinders and volumetric flasks:

Uncertainty due to the **non-ideal repeatability** of measurement (often called **repeatability uncertainty)**. In the case of pipetting this means that however carefully we try to fill and empty the pipette, we will nevertheless every time get a slightly different volume. This is sometimes referred to as the "human effect" or the "human factor", but in fact, if a machine would do the pipetting then there would also be difference between the volumes (although probably smaller). Repeatability is a typical random effect and contributes to uncertainty with glass pipettes as well as with automatic pipettes. Its influence on the measurement result can be decreased by making repeated measurements but it can never be entirely eliminated.

Uncertainty due to calibration of the volumetric equipment (often called **calibration uncertainty**). In the case of volumetric glassware this is the uncertainty in the positions of the marks on the volumetric glassware. In the case of automatic pipettes this uncertainty is caused by the systematically too high or too low displacement of the piston inside the pipette. In the case of a given pipette it is a typical systematic uncertainty source. This uncertainty source can be significantly reduced by recalibrating the pipette in the laboratory by the person who actually works with it. Accurate weighing of water at controlled temperature is the basis of calibration of volumetric instruments and also the way how usually the repeatability uncertainties of different volumetric instruments are found.

Uncertainty due to the temperature effect (often called as **temperature uncertainty**). All volumetric ware is usually calibrated at 20 °C and volumes usually refer to volumes at 20 °C. The density of the liquid changes (almost always decreases) with temperature. If pipetting is done at a higher temperature than 20 °C then there amount of liquid (in terms of mass or number of molecules) pipetted is smaller than if it were done at 20 °C. Consequently, the volume of that amount of liquid at 20 °C is also smaller than if the pipetting were done at 20 °C. In the case of volumetric glassware temperature affects the dimensions of the volumetric ware (its volume increases with temperature). The effect of liquid density change is ca 10 times stronger. Therefore the volume change of volumetric glassware is almost always neglected. In the case of automatic pipettes the effect of temperature is more complex. If the air inside the pipette warms then the delivered liquid volume can change to some extent. If the temperature of the laboratory and, importantly, temperature of the pipetted liquid, is constant during repeated measurements then the temperature effect is a systematic effect.

Application-specific uncertainty sources. These are not caused by the volumetric equipment but by the liquid that is handled or by the system that is investigated. **Some examples:**

1. If a foaming solution is pipetted, measured by a volumetric flask or a measuring cylinder then it is not clear where exactly the solution “ends”, i.e., there is no well-defined meniscus. This will cause an additional uncertainty. Depending on situation this effect can be random or systematic or include both random and systematic part.
2. If titration is carried out using visual indicator then the end-point of titration, i.e. the moment when the indicator changes color is assumed to match the equivalence point (the stoichiometry point). However, depending on the titration reaction and on the actual analyte that is titrated, the end-point may come earlier or later than the equivalence point. In the case of the given titration this will be a systematic effect. This effect can be minimized by some other means of end-point detection, e.g. potentiometric titration. [5]

There are some other uncertainty sources that usually turn out to be less important, because they can be minimized or eliminated by correct working practices (however, they can be important if these correctly practices are not applied). The remaining effects will usually influence the repeatability of pipetting or its calibration uncertainty and can be taken into account within those uncertainty sources.

If the pipette is not kept vertically (both glass and automatic pipettes), waiting is not long enough after the end of drainage of solution (glass pipette) then the pipetted volume will be lower than the one obtained with correct pipetting. No waiting is needed in the case of automatic pipettes because no liquid film remains (and must not remain) on pipette to inner walls.

When using a glass pipette then there is always some possibility that small residues of the previous solution are still in the pipette. It is therefore a good idea to rinse the pipette before pipetting (e.g. two times) with the solution that will be pipetted (and discarding the rinse solution it into waste, not into the vessel from where the solution is taken). In the case of automatic pipettes it is a good idea to use a new tip every time e new solution is pipetted. In that case such contamination is usually negligible. Also, when pipetting the same solution numerous times with the same pipetteit is a good idea to monitor it for the absence of droplets on the inner walls and replace the tip when the droplets appear.

If the walls of a glass pipette are not clean then droplets may remain on the walls after the pipetted solution has been drained. This leads to a different volume from the case when no

> droplets are left on pipette walls after draining the solution. The obvious thing to do is to clean the pipette.
>
> If the pipetted liquid is very different from water (e.g. some highly viscous liquid, such as vegetable oil) then the pipetted volume may be systematically different from the nominal volume of the pipette. This effect exists both with glass pipettes and with automatic pipettes. In such a case the pipette should either be recalibrated using the liquid under question or weighing should be used instead of volumetry [6] .

In section 4 the uncertainty sources of pipetting (the same pipetting experiment that was performed in the video) will be quantified and combined into the measurement uncertainty estimate of pipetted volume. Sections 4.1 to 4.5 present the uncertainty calculation using a factory-calibrated pipette. Section 4.6 presents an example of measurement uncertainty calculation of pipetted volume using a self-calibrated pipette. In section 5 an overview of the majority of uncertainty sources that are encountered in chemical analysis will be given.

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[1] This second video explains how to pipet with an automatic pipette if accurate volume is desired. In many routine, high-volume applications (e.g. in biochemistry), however, speed is more impoirtant than accuracy and in such cases some steps shown here, most importantly, rinsing, can be omitted. Also, in some cases there is a very limited volume available of the solution that is pipetted – in such case also rinsing is not possible. On the other hand, if still higher accuracy is desired then the so-called reverse pipetting technique can be used. Reverse pipetting is more accurate than the commonly used forward pipetting, which is the technique demonstrated and explained in this video.

[2] These sources also cause the existence of error and could therefore also be called error sources.

[3] It is in principle not wrong to call them random and systematic sources of uncertainty, but this is not usually done. This is largely because, as we will see in a coming lecture, the concept of measurement uncertainty stresses that random and systematic effects should be treated the same way.

[4] It is more correct to say „by a predictable magnitude‟. This means that the magnitude is not necessarily always the same – it can vary, e.g. as the magnitude of the measurand value varies – but it can be predicted, i.e. it is not random.

[5] In fact, even if the end-point is determined potentiometrically, it can still have some systematic deviation from the equivalence point. However, this effect is usually so small that it can be neglected.

[6] In the case of automatic pipettes also reverese pipetting instead of the more common forward pipetting can be used to decrease the uncertainty when pipetting viscous liquids.

3. The basic concepts and tools

Brief summary: This section presents the most basic concepts and tools for practical estimation of measurement uncertainty. First, the concepts of random quantities and distribution functions are introduced. Then the Normal distribution – the most important distribution function in measurement science – is explained and its two main parameters – the mean value and standard deviation – are introduced (3.1). Based on standard deviation the concept of standard uncertainty is introduced (3.1, 3.2). Thereafter the A type and B type uncertainty estimation is introduced (3.3). The mean value of random quantities is also a random quantity and its reliability can be described by the standard deviation of the mean (3.4). Besides the normal distribution three more distribution functions are introduced: rectangular and triangular distribution (3.5) as well as the Student distribution (3.6).

3.1. The Normal distribution
3.2. Mean, standard deviation and standard uncertainty
3.3. A and B type uncertainty estimates
3.4. Standard deviation of the mean
3.5. Rectangular and triangular distribution
3.6. The Student distribution

3.1. The Normal distribution

Brief summary: This lecture starts by generalizing that all measured values are **random quantities** from the point of view of mathematical statistics. The most important distribution in measurement science – the **Normal distribution** – is then explained: its importance, the parameters of the Normal distribution (**mean** and **standard deviation**). The initial definitions of **standard uncertainty** (*u*), **expanded uncertainty** (*U*) and **coverage factor** (*k*) are given. A link between these concepts and the Normal distribution is created.

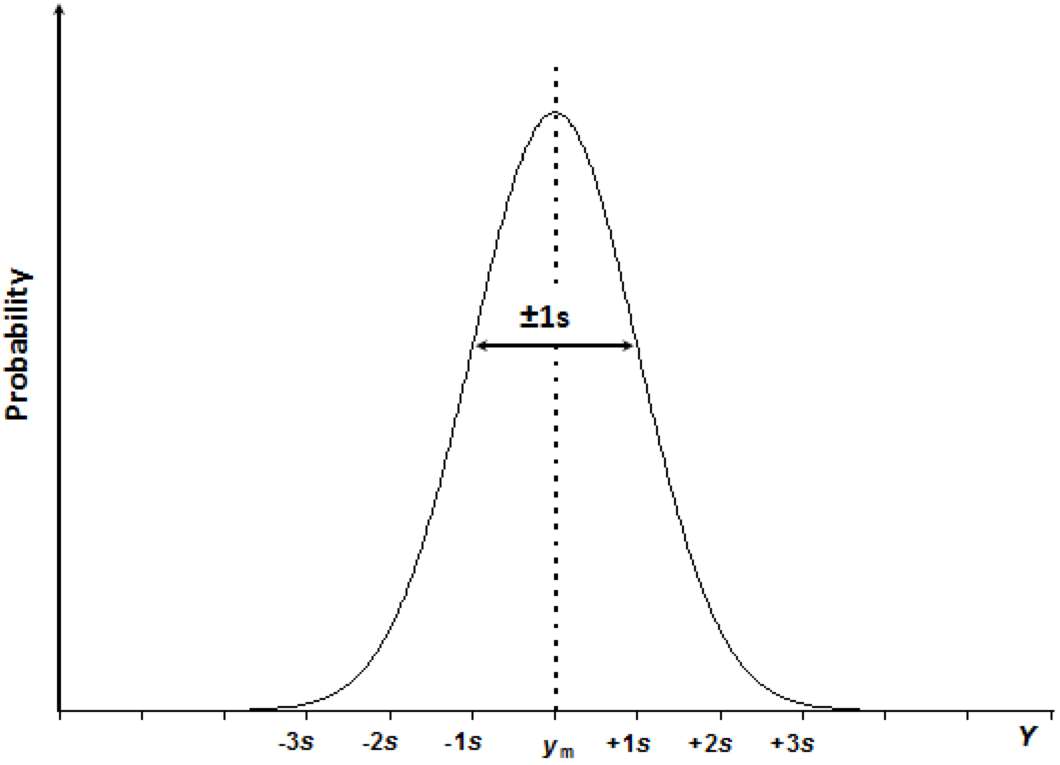
The Normal distribution

http://www.uttv.ee/naita?id=17589

https://www.youtube.com/watch?v=N-F6IeWyNZk

All measured quantities ~~(measurands)~~ are from the point of view of mathematical statistics random quantities. Random quantities can have different values. This was demonstrated in the lecture on the example of pipetting. If pipetting with the same pipette with nominal volume 10 ml is repeated multiple times then all the pipetted volumes are around 10 ml, but are still slightly different. If a sufficiently large number of repeated measurements are carried out and if the pipetted volumes [1] are plotted according to how frequently they are encountered then it becomes evident that although random, the values are still governed by some underlying relationship between the volume and frequency: the maximum probability of a volume is somewhere in the range of 10.005 and 10.007 ml and the probability gradually decreases towards smaller and larger volumes. This relationship is called **distribution function**.

There are numerous distribution functions known to mathematicians and many of them are encountered in the nature, i.e. they describe certain processes in the nature. In measurement science the most important distribution function is the **normal distribution** (also known as the Gaussian distribution). Its importance stems from the so-called **Central limit theorem**. In a simplified way it can be worded for measurements as follows: if a measurement result is simultaneously influenced by many uncertainty sources then if the number of the uncertainty sources approaches infinity then the distribution function of the measurement result approaches the normal distribution, irrespective of what are the distribution functions of the factors/parameters describing the uncertainty sources. In reality the distribution function of the result becomes indistinguishable from the normal distribution already if there are 3-5 (depending on situation) significantly contributing [2] uncertainty sources. This explains, why in so many cases measured quantities have normal distribution and why most of the mathematical basis of measurement science and measurement uncertainty estimation is based on the normal distribution.



Scheme 3.1. The normal distribution curve of quantity *Y* with mean value y_m and standard deviation *s*.

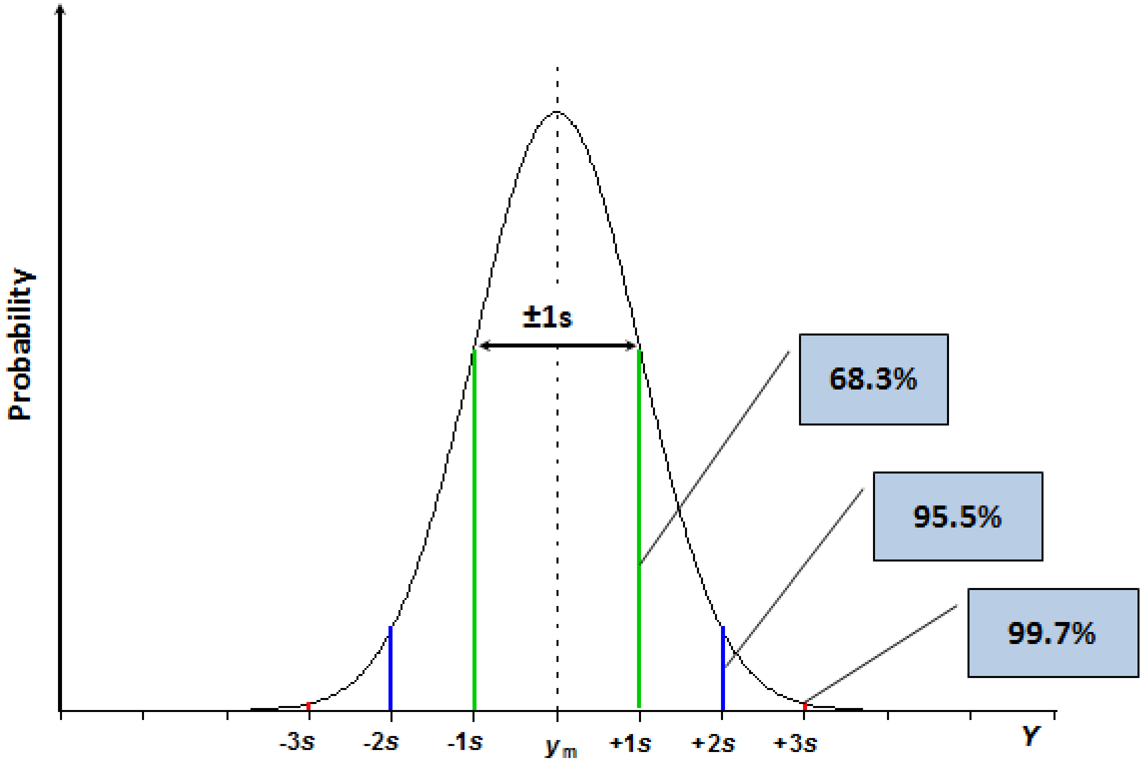
The normal distribution curve has the bell-shaped appearance (Scheme 3.1), and is expressed by equation 3.1:

$$f(y) = \frac{1}{s\sqrt{2\pi}}\, e^{-\frac{(y-y_m)^2}{2s^2}} \tag{3.1}$$

In this equation $f(y)$ is the probability that the measurand *Y* has value *y*. y_m is the **mean** value of the **population** and *s* is the **standard deviation** of the population. y_m characterizes the position of the normal distribution on the *Y* axis, *s* characterizes the width (spread) of the distribution function, which is determined by the scatter of the data points. The mean and standard deviation are the two parameters that fully determine the shape of the normal distribution curve of a particular random quantity. The constants 2 and p are normalization factors, which are present in order to make the overall area under the curve equal to 1.

The word "population" here means that we would need to do an infinite number of measurements in order to obtain the true y_m and *s* values. In reality we always operate with a limited number of measurements, so that the mean value and standard deviation that we have from our experiments are in fact **estimates** of the true mean and true standard deviation. The larger is the number of repeated measurements the more reliable are the estimates. The number of parallel measurements is therefore very important and we will return to it in different other parts of this course.

The normal distribution and the standard deviation are the basis for definition of **standard uncertainty**. Standard uncertainty, denoted by *u*, is the uncertainty expressed at standard deviation level, i.e., uncertainty with roughly 68.3% coverage probability (i.e. the probability of the true value falling within the uncertainty range is roughly 68.3%). The probability of 68.3% is often too low for practical applications. Therefore uncertainty of measurement results is in most cases not reported as standard uncertainty but as **expanded uncertainty**. Expanded uncertainty, denoted by *U*, is obtained by multiplying standard uncertainty with a **coverage factor**,[3] denoted by *k*, which is a positive number, larger than 1. If the coverage factor is e.g. 2 (which is the most commonly used value for coverage factor) then in the case of normally distributed measurement result the coverage probability is roughly 95.5%. These probabilities can be regarded as fractions of areas of the respective segments from the total area under the curve as illustrated by the following scheme:



Scheme 3.2. The same normal distribution curve as in Scheme 3.1 with 2*s* and 3*s* segments indicated.

Since the exponent function can never return a value of zero, the value of *f* (*y*) (eq 3.1) is higher than zero with any value of *y*. This is the reason why uncertainty with 100% coverage is (almost) never possible.

It is important to stress that these percentages hold only if the measurement result is normally distributed. As said above, very often it is. There are, however, important cases when measurement result is not normally distributed. In most of those cases the distribution function has "heavier tails", meaning, that the expanded uncertainty at e.g. $k = 2$ level will not correspond to coverage probability of 95.5%, but less (e.g. 92%). The issue of distribution of the measurement result will be addressed later in this course.

***

[1] It is fair to ask, how do we know the individual pipetted volumes if the pipette always „tells" us just that the volume is 10 ml? In fact, if we have only the pipette and no other (more accurate) measurement possibility of volume then we cannot know how much the volumes differ form each other or from the nominal volume. However, if a more accurate method is available then this is possible. In the case of pipettig a very suitable and often used more accurate method is weighing. It is possible to find the volume of the pipetted water, which is more accurate than that obtained by pipetting, by weighing the pipetted solution (most often water) and divided the obtained mass by the density of water at the temperature of water. Water is used in such experiments because the densities of water at different temperatures are very accurately known (see e.g. http://en.wikipedia.org/wiki/Properties_of_water#Density_of_water_and_ice).

[2] Significantly cointributing uncertainty sources are the important uncertainty sources. We have already qualitatively seen in section 2 that different uncertainty sources have different „importance". In the coming lectures we will also see how the „importance" of an uncertainty source (its uncertainty contribution) can be quantitatively expressed.

[3] This definition of expanded uncertainty is simplified. A more rigorous definition goes via the *combined* standard uncertainty and is introduced in section 4.4.

3.2. Mean, standard deviation and standard uncertainty

Brief summary: the lecture explains calculation of **mean** (V_m) and **standard deviation** (s). Illustrates again the 68% probability of s. Explains how the **standard uncertainty** of repeatability u (V, REP) can be estimated as standard deviation of parallel measurement results. Stresses the importance of standard uncertainty as the key parameter in carrying out uncertainty calculations: uncertainties corresponding to different sources (not only to repeatability) and to different distribution functions are converted to standard uncertainties when uncertainty calculations are performed.

Mean, standard deviation and standard uncertainty

http://www.uttv.ee/naita?id=17554

https://www.youtube.com/watch?v=ND3iryaVQ68

One of the most common approaches for improving the reliability of measurements is making replicate measurements of the same quantity. In such a case very often the measurement result is presented as the **mean value** of the replicate measurements. In the case of pipetting n times with the same pipette volumes V_1, V_2, …, V_n are obtained and the mean value V_m is calculated as follows:

$$V_{\mathrm{m}} = \frac{V_1 + V_2 + \ldots + V_n}{n} = \frac{\sum_{i=1}^{n} V_i}{n} \qquad (3.2)$$

As explained in section 3.1, the mean value calculated this way is an estimate of the true mean value (which could be obtained if it were possible to make an infinite number of measurements).

The scatter of values obtained from repeated measurements is characterized by **standard deviation** of pipetted volumes, which for the same case of pipetting is calculated as follows:

$$s(V) = \sqrt{\frac{\sum_{i=1}^{n} (V_i - V_{\mathrm{m}})^2}{n-1}} \qquad (3.3)$$

The $n - 1$ in the denominator is often called **number of degrees of freedom**. We will see later that this is an important characteristic of a set or repeated measurements. The higher it is the more reliable mean and standard deviation can be from the set.

Two important interpretations of the standard deviation:

1. If V_m and s (V) have been found from a sufficiently large number of measurements (usually 10-15 is enough) then the probability of every next measurement (performed under the same conditions) falling within the range $V_m \pm s$ (V) is roughly 68.3%.
2. If we make a number of repeated measurements under the same conditions then the standard deviation of the obtained values characterized the uncertainty due to non-ideal repeatability (often called as repeatability standard uncertainty) of the measurement: u (V, REP) = s(V). Non-ideal repeatability is one of the uncertainty sources in all measurements. [1]

Standard deviation is the basis of defining **standard uncertainty** – uncertainty at standard deviation level, denoted by small u. Three important aspects of standard uncertainty are worth stressing here:

1. Standard deviation can be calculated also for quantities that are not normally distributed. This enables to obtain for them standard uncertainty estimates.
2. Furthermore, also uncertainty sources that are systematic by their nature and cannot be evaluated by repeating measurements can still be expressed numerically as standard uncertainty estimates.
3. Converting different types of uncertainty estimates to standard uncertainty is very important, because as we will see in section 4, most of the calculations in uncertainty evaluation, especially combining the uncertainties corresponding to different uncertainty sources, are carried out using standard uncertainties.

Standard uncertainty of a quantity (in our case volume V) expressed in the units of that quantity is sometimes also called absolute standard uncertainty. Standard uncertainty of a quantity divided by the value of that quantity is called **relative standard uncertainty**, u_{rel} (similarly to eq 1.1). In the case of volume V:

$$u_{\mathrm{rel}}(V) = \frac{u(V)}{V} \quad (3.4)$$

***

[1] We will see later that standard deviation of measurements repeated under conditions that changer in predefined way (i.e. it is not repeatability) is also extremely useful in uncertainty calculation, as it enables taking a number of uncertainty sources into account simultaneously.

3.3. A and B type uncertainty estimates

Carrying out the same measurement operation many times and calculating the standard deviation of the obtained values is one of the most common practices in measurement uncertainty estimation. Either the full measurement or only some parts of it can be repeated. In both cases useful information can be obtained. The obtained standard deviation (or the standard deviation of the mean, explained in section 3.4) is then the standard uncertainty estimate. Uncertainty estimates obtained as standard deviations of repeated measurement results are called **A type** uncertainty estimates. If uncertainty is estimated using some means other than statistical treatment of repeated measurement results then the obtained estimates are called **B type** uncertainty estimates. The other means can be e.g. certificates of reference materials, specifications or manuals of instruments, estimates based on long-term experience, etc.

Uncertainty estimates of A- and B-type

http://www.uttv.ee/naita?id=18165

https://www.youtube.com/watch?v=jdbx5UMQD9k

3.4. Standard deviation of the mean

Brief summary: Like the individual values, the mean value calculated from them is also a random quantity and for it also a standard deviation can be calculated. It is possible to calculate it from the standard deviation of the individual value. It is explained when to use the standard deviation of the individual value and when to use the standard deviation of the mean: whenever the individual result is used in further calculation the standard deviation of the individual result has to be used; whenever the mean value is used in further calculations, the standard deviation of the mean has to be used.

Standard deviation of the mean

http://www.uttv.ee/naita?id= 17580

https://www.youtube.com/watch?v=GLsHHIW1yjo

The standard deviation $s(V)$ calculated using the formula 3.3 is the standard deviation of an *individual* pipetting result (value). When the mean value is calculated from a set of individual values which are randomly distributed then the mean value will also be a random quantity. As for any random quantity, it is also possible to calculate standard deviation for the mean $s(V_\mathrm{m})$. One possible way to do that would be carrying out numerous measurement series, find the mean for every series and then calculate the standard deviation of all the obtained mean values. This is, however, too work-intensive. However, there is a very much simpler approach for calculating $s(V_\mathrm{m})$, simply divide the $s(V)$ by square root of the number of repeated measurements made:

$$s(V_\mathrm{m}) = \frac{s(V)}{\sqrt{n}} \qquad (3.5)$$

So, for a set of repeated pipetting values we have in fact two standard deviations: standard deviation of the single value $s(V)$ and standard deviation of the mean $s(V_\mathrm{m})$. It is important to ask: when we use one and when another of them?

The general rule of thumb is the following: when the measured value reported or used in subsequent calculations is a single value then we use standard deviation of the single value; when it is the mean value then we use the standard deviation of the mean.

Let us illustrate this by two examples:

1. **Pipetting**. When we deliver a certain volume by a pipette then pipetting is a one-time operation: we cannot repeat the pipetting with the same liquid amount. So we use the standard deviation of single pipetting as pipetting repeatability uncertainty.
2. **Weighing**. When we weigh a certain amount of a material then we can weigh it repeatedly. So, if we need to minimize the influence of weighing repeatability in our measurement then we can weigh the material repeatedly and use in our calculations the mean mass. In this case the repeatability standard deviation of this mean mass is the standard deviation of the mean. If, on the other hand, it is not very important to have the lowest possible repeatability uncertainty of mass then we weigh only once and use the mass value from the single weighing and as its repeatability uncertainty we will use the standard deviation of a single value. [1]

In the case of single pipetting or single weighing the repeatability uncertainty of course cannot be estimated from this single operation. In these cases repeatability is determined separately and then used for the concrete measurements.

***

[1] As we will see later, modern balances are highly accurate instruments and uncertainty due to weighing is seldom among the important uncertainty sources. So, unless some disturbing effects interfere with weighing, it is usually not necessary to weigh materials with many repetitions.

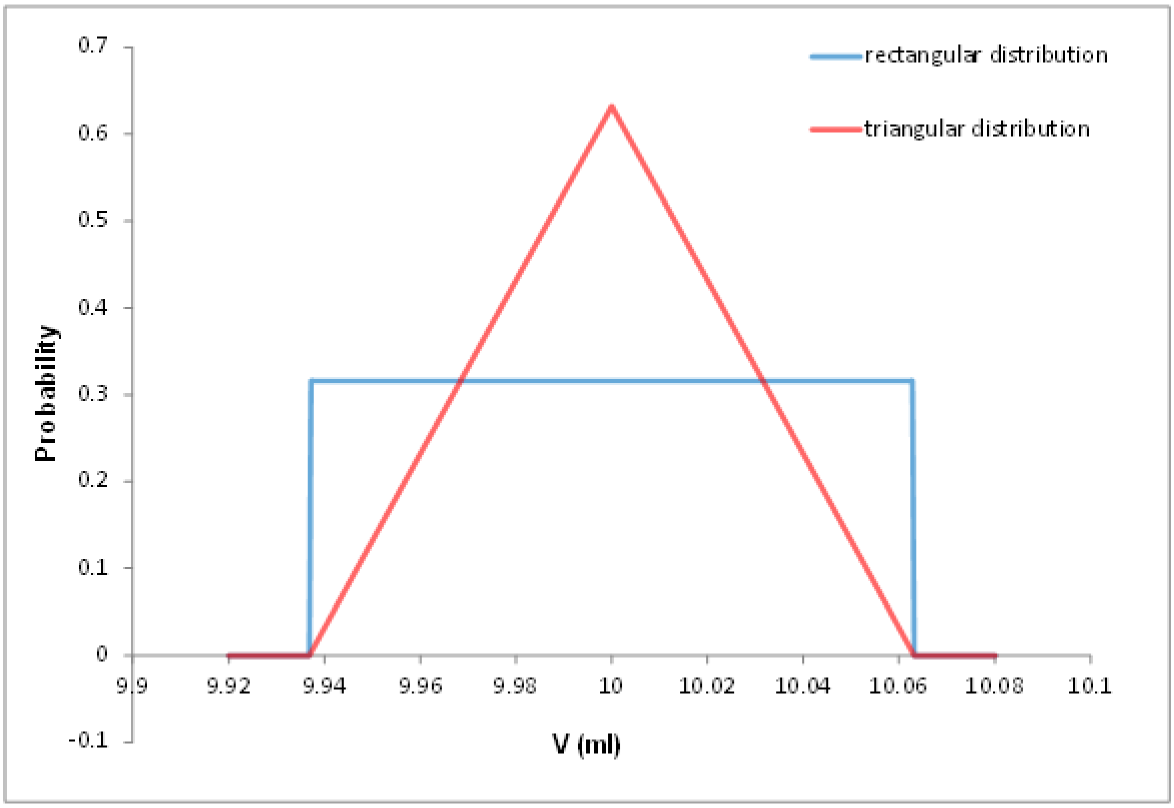
3.5. Rectangular and triangular distribution

Brief summary: Rectangular distribution and **triangular distribution** are explained, as well as how the uncertainties corresponding to rectangular or triangular distribution can be converted to standard uncertainties. Often the information on distribution function is missing and then usually some distribution function is assumed or postulated. Rectangular and triangular distributions are among the most common postulated distribution functions. Recommendations are given, which of these distributions to assume.

Other distribution functions: rectangular and triangular distribution

http://www.uttv.ee/naita?id=17584

https://www.youtube.com/watch?v=g_PefybO2Ao



Scheme 3.3. Rectangular and triangular distributions. Both of them correspond to the situation (10.000 ± 0.063) ml.

In measurement uncertainty estimation situations often occur where it is necessary to make choice between two alternatives of which one may possibly lead to somewhat overestimated uncertainty and the other one to somewhat underestimated uncertainty. In such situation it is usually reasonable to rather somewhat overestimate than underestimate the uncertainty.

3.6. The Student distribution

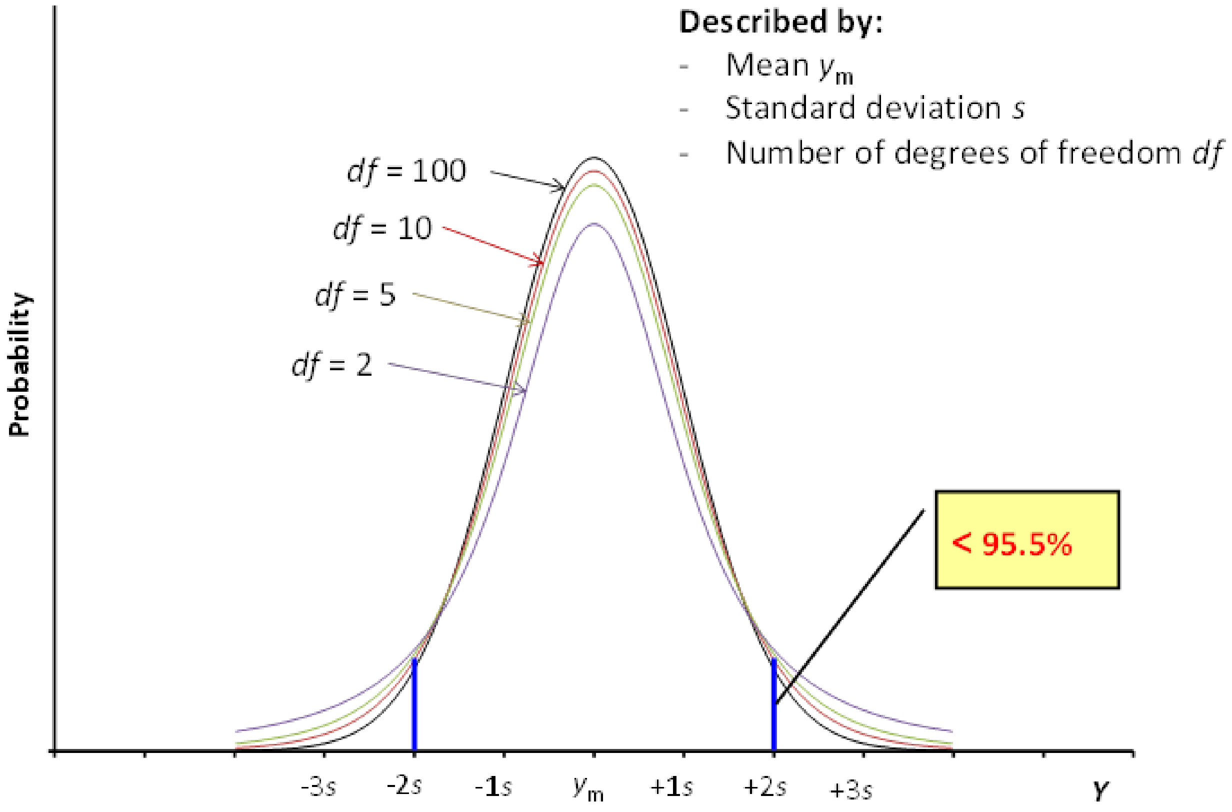
Brief summary: Like the individual values, the mean value calculated from them is also a random quantity. If the individual values are distributed according to the Normal distribution then the mean value calculated from them is distributed according to the **Student distribution** (also called as ***t*-distribution**). The properties of the *t*-distribution compared to the Normal distribution are explained. Importantly, the shape of the t-distribution curve depends on the number of **degrees of freedom**. If the number of individual values approaches infinity then the shape of the t-distribution curve approaches the Normal distribution curve.

Other distribution functions: the Student distribution

http://www.uttv.ee/naita?id=17708

https://www.youtube.com/watch?v=CWU8KM2z59I

If a measurement is repeated and the mean is calculated from the results of the single individual measurements then, just as the individual results, their mean will also be a random quantity. If the individual results are normally distributed then their mean is distributed according to the **Student distribution** (also known as the **t-distribution**). Student distribution is presented in Scheme 3.5.



Scheme 3.5. the Student distribution.

Similarly to the normal distribution the Student distribution also has mean value y_m and standard deviation s. y_m is the mean value itself, [1] and standard deviation is the standard deviation of the mean, calculated as explained in section 3.4. But differently from the normal distribution there is in addition a third characteristic – the number of **degrees of freedom** *df*. This number is equal to the number of repeated measurements minus one. So, the four Student distribution graphs in Scheme 3.5 correspond to 101, 11, 6 and 3 repeated measurements, respectively.

If *df* approaches infinity then the t-distribution approaches the normal distribution. In reality 30-50 degrees of freedom is sufficient for handling the t-distribution as the normal distribution. So, the curve with *df* = 100 in Scheme 3.5 can be regarded as the normal distribution curve.

The lower is the number of degrees of freedom the "heavier" are the tails of the Student distribution curve and the more different is the distribution from the normal distribution. This means that more probability resides in the tails of the distribution curve and less in the middle part. Importantly, the probabilities pictured in Scheme 3.2 for the ±1*s*, ±2*s* and ±3*s* ranges around the mean do not hold any more, but are all lower.

So, if a measurement result is distributed according to the t-distribution and if expanded uncertainty with predefined coverage probability is desired then instead of the usual coverage factors 2 and 3 the respective **Student coefficients** [2] should be used. Measurement result can be distributed according to the Student distribution if there is a heavily dominating [3] A type uncertainty source that has been evaluated as a mean value from a limited number of repeated measurements. More common, however, is the situation that there is an influential but not heavily dominating A-type uncertainty source. In such a case the distribution of the measurement result is a convolution [4] of the normal distribution and the t-distribution. What to do in this case is explained in section 9.8.

***

[1] It may look strange at first sight that while the mean value y_m is the only mean value we have, we immediately take it as the mean value of the distribution of mean values. However, if we had more mean values, we would anyway pool them into a single mean value (with a much higher *df*!) and use that value.

[2] Student coefficients (i.e. *t*-distribution values) for a given set of coverage probability and number of degrees of freedom can be easily obtained from special tables in statistical handbooks (use two-sided values!), from calculation or data treatment software, such as MS Excel or Openoffice Calc or from the Internet, e.g. from the address https://en.wikipedia.org/wiki/Student_distribution

[3] The contributions of different uncertainty sources can be expressed numerically. This is explained in section 9.9 and the respective calculations are shown in 9.7. In this context the phrase „heavily dominating" means that the contribution (uncertainty index) of the respective input quantity is above 75%.

[4] Convolution of two distribution functions in mathematical statistics means a combined of distribution function, which has a shape inbetween the two distribution functions that are convoluted.

4. The first uncertainty quantification

Brief summary: In this section the basic concepts and tools of the previous sections are put into practice on the example of a simple analytical chemistry operation – pipetting. The uncertainty sources identified in section 2 are now quantified (4.1), the obtained individual uncertainty estimates are (when needed) converted to standard uncertainties and are then combined into the combined standard uncertainty (4.2). The uncertainty components making up the combined standard uncertainty are compared and some conclusions are made (4.3). The combined standard uncertainty is converted into expanded uncertainty (4.4) and the result is presented (4.5). This approach is then practiced one calculation example (calibration of pipette, 4.6).

4.1. Quantifying uncertainty components
4.2. Calculating the combined standard uncertainty
4.3. Looking at the obtained uncertainty
4.4. Expanded uncertainty
4.5. Presenting measurement results
4.6. Practical example

4.1. Quantifying uncertainty components

Brief summary: The same pipetting as in lecture 2 is now examined from the point of view of quantifying the uncertainty sources. All the important sources of uncertainty are quantitatively expressed as **uncertainty components** – uncertainty estimates quantitatively describing the respective uncertainty source. The uncertainty components are quantified. An example is given on converting an uncertainty estimate with (assumedly) rectangular distribution into a standard uncertainty estimate.

Introduction to quantifying measurement uncertainty

http://www.uttv.ee/naita?id=17555

https://www.youtube.com/watch?v=CaYJGxtQBzo

The main uncertainty sources are the same as explained in lecture 2 and here the uncertainty components corresponding to them are quantified.

Uncertainty due to the **non-ideal repeatability**, which in the case of pipetting means that however carefully to fill and empty the pipette, we will nevertheless every time get a slightly different pipetted volume. Repeatability is a typical random effect. The standard uncertainty due to repeatability *u*(*V*, REP) can be calculated as standard deviation [1] of accurate volumes delivered by the pipette. [2] . According to the data for the used pipette is

$$u(V, \mathrm{REP}) = 0.006\ \mathrm{ml} \qquad (4.1)$$

Uncertainty due to calibration of the volumetric equipment (often called **calibration uncertainty** or uncertainty of the nominal volume). This is the uncertainty in the positions of the marks on the volumetric ware. In the case of a given pipette it is a typical systematic effect. Calibration uncertainty of the pipette used in this example is specified by the producer as ± 0.03 ml. [3] There is no information on the distribution or coverage of this uncertainty estimate. This is very common if uncertainty estimates are obtained from instrument documentation. In such case it is the safest to assume that the uncertainty estimate corresponds to rectangular distribution. In order to carry out uncertainty calculation we have to convert this uncertainty to standard uncertainty. For doing this, as explained in section 3.5, we have to divide it by square root of 3:

$$u(V, \mathrm{CAL}) = \frac{0.03\ \mathrm{ml}}{\sqrt{3}} = 0.017\ \mathrm{ml} \qquad (4.2)$$

Uncertainty due to the temperature effect (often called as **temperature uncertainty**). All volumetric ware is usually calibrated at 20 °C and volumes usually refer to volumes at 20 °C [4] . Temperature change affects first of all the density of the liquid (the effect of expansion/contraction of glass is significantly smaller). If pipetting is done at a higher temperature than 20 °C then there amount of liquid (in terms of mass or number of molecules) pipetted is smaller than corresponds to the volume at 20 °C. Consequently, the volume of that amount of liquid at 20 °C is also smaller than if the pipetting were done at 20 °C. If the temperature of the laboratory and, importantly, temperature of the pipetted liquid, is constant during repeated measurements then the temperature effect is a systematic effect. The following video explains calculating the standard uncertainty of liquid volume due to the temperature *u*(*V*, TEMP):

Quantifying the uncertainty due to temperature effect in volumetric measurement

http://www.uttv.ee/naita?id= 17825

https://www.youtube.com/watch?v=CDjX8K_Vsds

u(*V*, TEMP) is dependent on the volume *V* of liquid delivered, [5] the maximum possible temperature difference from 20°C (Δ*t*) and the thermal expansion coefficient of water *γ*. It is calculated as follows:

$$u(V,\text{TEMP}) = \frac{V \cdot \Delta t \cdot \gamma}{\sqrt{3}} = \frac{10.00\,\text{ml} \cdot 4\,^{\circ}\text{C} \cdot 0.00021\,^{\circ}\text{C}^{-1}}{\sqrt{3}} 0.0049\ \text{ml} \qquad (4.3)$$

Dividing by square root of 3 is for transforming the uncertainty estimate into standard uncertainty (assuming rectangular distribution of Δ*t*). It is important to note that the *V* in eq 4.3 always refers to the actual measured volume, not the full capacity of the volumetric device. For example, if 21.2 ml of solution was measured with a 50 ml burette then the volume to use is 21.2 ml, not 50 ml.

In the case of more complex uncertainty estimations some of the sources can be presented also with expanded uncertainty. For example, the analyte content in reference material: (0.2314 ± 0.0010) mg/kg, *k*=2. In this case this uncertainty also needs to be converted to standard uncertainty for further calculations. For that the expanded uncertainty is divided by the presented coverage factor. So in the given example the combined standard uncertainty is 0.0005 mg/kg.

***

[1] Since pipetting for delivering a certain liquid volume is done only once and cannot be averaged (i.e. it is not possible to pipet several times and then “average” the volumes) the suitable estimate of repeatability uncertainty is the standard deviation of a single measurement, not standard deviation of the mean.

[2] The accurate volumes can be measured by weighing the water delivered by the pipette and converting it into volume by using accurate density data.

[3] This uncertainty can be significantly reduced if the pipette is recalibrated in laboratory by weighing the delivered water.

[4] If volumetric glassware is calibrated in the same laboratory then a different temperature can be used.

[5] In the case of a volumetric pipette the nominal volume is the same as the delivered volume but in the case of a burette it is usually not. So, if from a 25 ml burette 12.63 ml of solution is delivered then the volume that has to be used for temperature effect calculation is 12.63 ml, not 25 ml.

4.2. Calculating the combined standard uncertainty

The uncertainty components that were quantified in the previous lecture are now combined into the **combined standard uncertainty** (u_c) – standard uncertainty that takes into account contributions from all important uncertainty sources by combining the respective uncertainty components. The concept of **indirect measurement** – whereby the value of the **output quantity** (measurement result) is found by some function (model) from several **input quantities** – is introduced and explained. The majority of chemical measurements are indirect measurements. The general case of combining the uncertainty components into combined standard uncertainty as well as several specific cases are presented and explained.

The first video lecture explains in a simple way how the uncertainty components are combined in the particular example of pipetting. The second video lecture presents the general overview of combining the uncertainty components.

Combining the uncertainty components into the combined standard uncertainty in the case of pipetting

http://www.uttv.ee/naita?id=17556

https://www.youtube.com/watch?v=S5v58VQ4zSg

In all cases where combined standard uncertainty is calculated from uncertainty components all the uncertainty components have to be converted to standard uncertainties.

In the example of pipetting the combined standard uncertainty is calculated from the uncertainty components found in the previous section as follows:

$$u_{\mathrm{C}}(V) = \sqrt{u(V,\mathrm{REP})^2 + u(V,\mathrm{CAL})^2 + u(V,\mathrm{TEMP})^2} = \sqrt{0.006^2 + 0.017^2 + 0.005^2} = 0.019\ \mathrm{ml} \qquad (4.4)$$

This is the typical way of calculating combined standard uncertainty if all the uncertainty components refer to the same quantity and are expressed in the same units. It is often used in the case of **direct measurements** – measurements whereby the measurement instrument (pipette in this case) gives immediately the value of the result, without further calculations needed.

Combining the uncertainty components into the combined standard uncertainty: simple cases and the general case

http://www.uttv.ee/naita?id=17826

https://www.youtube.com/watch?v=FJ4hn9LgGmw

An indirect measurement is one where the **output quantity** (result) is found ba calculation (using a **model equation**) from several input quantities. A typical example is titration. In case of titration with 1:1 mole ratio the analyte concentration in the sample solution C_S (the output quantity) is expressed by the input quantities – volume of sample solution taken for titration (V_S), titrant concentration (C_T) and titrant volume consumed for titration (V_T) – as follows:

$$C_{\mathrm{S}} = \frac{V_{\mathrm{T}} \cdot C_{\mathrm{T}}}{V_{\mathrm{S}}} \qquad (4.5)$$

In the general case if the output quantity Y is found from input quantities X_1, X_2, … X_n according to some function F as follows

$$Y = F(X_1, X_2, \dots, X_n) \qquad (4.6)$$

then the combined standard uncertainty of the output quantity $u_c(y)$ can be expressed via the standard uncertainties of the input quantities $u(x_i)$ as follows:

$$u_c(y) = \sqrt{\left[\frac{\partial Y}{\partial X_1} u(x_1)\right]^2 + \left[\frac{\partial Y}{\partial X_2} u(x_2)\right]^2 + \dots + \left[\frac{\partial Y}{\partial X_n} u(x_n)\right]^2} \qquad (4.7)$$

The terms $\left[\frac{\partial Y}{\partial X_i} u(x_i)\right]$ are the uncertainty components. The terms $\frac{\partial Y}{\partial X_i}$ are partial derivatives. At first sight the eq 4.7 may seem very complex but it is in fact not too difficult to use – the uncertainty components can be calculated numerically using the Kragten's spreadsheet method (as is demonstrated in section 9.7).

In specific cases simpler equations hold. If the output quantity is expressed via the input quantities as follows

$$Y = X_1 - X_2 + \dots + X_n \qquad (4.8)$$

then $$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + \dots + u(x_n)^2} \qquad (4.9)$$

Importantly, irrespective of whether the input quantities are added or subtracted, the squared standard uncertainties under the square root are always added.

This way of combining uncertainty components is in principle the same as used above for the case of pipetting.

If the measurement model is

$$Y = \frac{X_1 \cdot X_2}{X_3 \cdot X_4} \qquad (4.10)$$

then $$\frac{u_c(y)}{y} = \sqrt{\left(\frac{u(x_1)}{x_1}\right)^2 + \left(\frac{u(x_2)}{x_2}\right)^2 + \left(\frac{u(x_3)}{x_3}\right)^2 + \left(\frac{u(x_4)}{x_4}\right)^2} \qquad (4.11)$$

As can be seen, here it is the relative standard uncertainties that are combined and the squared summing gives us the relative combined standard uncertainty of the output quantity. The absolute combined standard uncertainty of the output quantity is found as follows:

$$u_c(y) = y \cdot \sqrt{\left(\frac{u(x_1)}{x_1}\right)^2 + \left(\frac{u(x_2)}{x_2}\right)^2 + \left(\frac{u(x_3)}{x_3}\right)^2 + \left(\frac{u(x_4)}{x_4}\right)^2} \qquad (4.12)$$

The file used in second video can be downloaded from here.

combining_u_components.pdf 22 KB

Combined Standard Uncertainty

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Institute of Chemistry

2013

Combined Standard Uncertainty

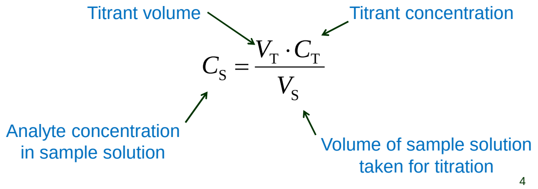
- When estimating the standard uncertainty of an **output quantity** then the standard uncertainties of all **input quantities** are taken into account
- The standard uncertainty of the output quantity obtained in this way is called **combined standard uncertainty**
- Is denoted by $u_c(y)$

Indirect Measurement

- If the result is obtained as an **output quantity** by calculations from one or several **input quantities** then we speak about **indirect measurement**
- Example: Titration
  - Titrant concenration
  - Volume of sample solution taken for titration
  - Volume of titrant consumed for titartion

Measurement model

- Expression that enables calculating the output quantity value (result value) from the input quantity values
- Titration with 1:1 stoichiometry:



Titrant volume

Titrant concentration

$$C_S = \frac{V_T \cdot C_T}{V_S}$$

Analyte concentration
in sample solution

Volume of sample solution
taken for titration

Finding u_c

- If the model is:

$$Y = X_1 - X_2 + ... + X_n$$

- then:

$$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + ... + u(x_n)^2}$$

- All uncertainties have to be converted to **standard uncertainties** before calculations!

Finding u_c

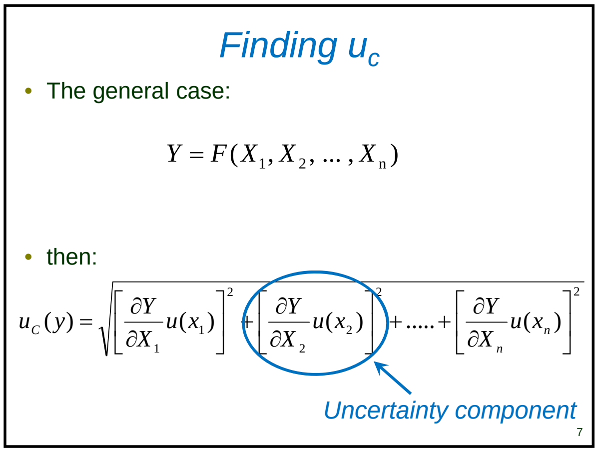
- If the model is:

$$Y = \frac{X_1 \cdot X_2}{X_3 \cdot X_4}$$

- Then:

$$u_c(y) = y \cdot \sqrt{\left(\frac{u(x_1)}{x_1}\right)^2 + \left(\frac{u(x_2)}{x_2}\right)^2 + \left(\frac{u(x_3)}{x_3}\right)^2 + \left(\frac{u(x_4)}{x_4}\right)^2}$$

$$\frac{u_c(y)}{y} = \sqrt{\left(\frac{u(x_1)}{x_1}\right)^2 + \left(\frac{u(x_2)}{x_2}\right)^2 + \left(\frac{u(x_3)}{x_3}\right)^2 + \left(\frac{u(x_4)}{x_4}\right)^2}$$



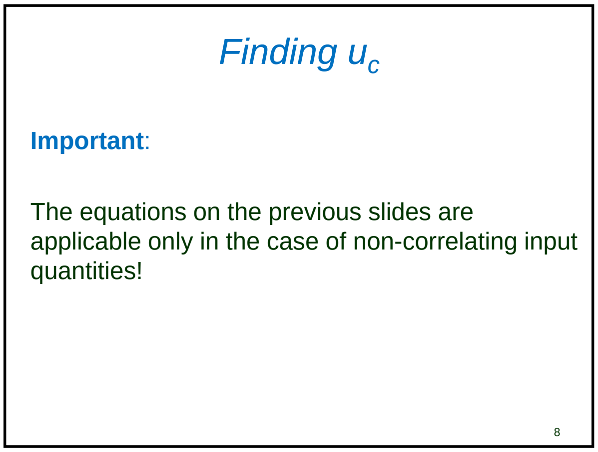
Finding u_c

- The general case:

$$Y = F(X_1, X_2, \ldots, X_n)$$

- then:

$$u_C(y) = \sqrt{\left[\frac{\partial Y}{\partial X_1}u(x_1)\right]^2 + \underbrace{\left[\frac{\partial Y}{\partial X_2}u(x_2)\right]^2}_{\text{Uncertainty component}} + \ldots.. + \left[\frac{\partial Y}{\partial X_n}u(x_n)\right]^2}$$



Finding u_c

Important:

The equations on the previous slides are applicable only in the case of non-correlating input quantities!

4.3. Looking at the obtained uncertainty

Brief summary: The uncertainty components of the previous lecture are compared. The property of squared summing – suppressing the less influential uncertainty components – is explained. The meaning of the obtained combined standard uncertainty estimate is explained in terms of probability (the probability of the true value of pipetted volume being within the calculated uncertainty range).

Comparing the uncertainty components

http://www.uttv.ee/naita?id=17578

https://www.youtube.com/watch?v=Xnq2-7nq_bg

We can see the uncertainty component with the largest magnitude is the calibration uncertainty $u(V, \text{CAL}) = 0.017$ ml. The combined standard uncertainty is in fact quite similar to it: $uc(V) = 0.019$ ml. If the summing were made not by the squaring and square root approach but by simple arithmetic sum then the value would be 0.028. This is a good illustration of the property of the squared summing: the smaller uncertainty components are suppressed by larger uncertainty components.

The idea of the squared summing of the components is that the different effects causing uncertainty influence the result in different directions (thus partially canceling) and their magnitudes are not necessarily equal to the values of the uncertainty estimates but can also be smaller (see section 1).

Looking at the uncertainty contributions is very useful if one wants to reduce the uncertainty. In order to reduce the uncertainty of a particular measurement it is always necessary to focus on decreasing the uncertainties caused by the largest components. So, in this case it is not very useful to buy a more expensive air conditioner for the room because the resulting uncertainty improvement will be small. It will also not be possible to improve the uncertainty markedly by reducing the repeatability component. Clearly, whatever is done with these two components the combined standard uncertainty $u_c(V)$ (eq 4.4) cannot decrease from 0.019 ml to lower than 0.017 ml, which is not a significant decrease. Thus, if more accurate pipetting is needed, then the way to go is to calibrate the pipette in the laboratory. This way it is realistic to achieve threefold lower calibration uncertainty, which leads to two times lower combined uncertainty of the pipetted volume. See section 4.6 for an example.

4.4. Expanded uncertainty

Brief summary: The probability of roughly 68% that is provided by the standard uncertainty is often too low for the users of measurement uncertainty. Therefore measurement uncertainty is presented to customers mostly as **expanded uncertainty**, U. Expanded uncertainty is calculated from the standard uncertainty by multiplying it with a **coverage factor**, k.

In the case of the pipetting example the $k = 2$ expanded uncertainty is found as follows:

$$U(V) = u\mathrm{c}(V) \cdot k = 0.019\ \mathrm{ml} \cdot 2 = 0.038\ \mathrm{ml} \quad (4.13)$$

Expanded uncertainty at $k = 2$ level is the most common way of expressing uncertainty of measurement and analysis results.

Calculating the expanded uncertainty

http://www.uttv.ee/naita?id=17579

https://www.youtube.com/watch?v=tBwkuL0ap14

4.5. Presenting measurement results

Brief summary: The pipetting result – the value and expanded uncertainty – is presented. It is stressed that it is important to clearly say, what was measured. The correct presentation of measurement result includes value, uncertainty and information about the probability of the uncertainty. It is explained that in simplified terms we can assume that k = 2 corresponds to roughly 95% of coverage probability. It is explained how to decide how many decimals to give when presenting a measurement result and the uncertainty.

The correct presentation of the measurement result in this case would look as follows:

$$\text{The volume of the pipetted liquid is } V = (10.000 \pm 0.038)\ \text{ml},\ k = 2,\ \text{norm.} \quad (4.14)$$

The parentheses (brackets) mean that the unit “ml” is valid both for the value and the uncertainty. “norm.” means that the output quantity is expected to be approximately normally distributed. This, together with coverage factor value 2, means that the presented uncertainty is expected to corresponds to approximately 95% coverage probability (see section 3.1 for details).

When can we assume that the output quantity is normally distributed? That is, when can we write “norm.” besides the coverage factor? Rigorous answer to this question is not straightforward, but a simple rule of thumb is that when there are at least three main uncertainty sources of comparable influence (i.e. the smallest and largest of the uncertainty components differ by ca 3 times or less) then we can assume that the distribution function of the output is sufficiently similar to the normal distribution. [1]

Section 9.8 presents a more sophisticated approach of calculating expanded uncertainty that corresponds to a concrete coverage probability.

Presenting of measurement result

http://www.uttv.ee/naita?id=17576

https://www.youtube.com/watch?v=x9l1jIVqT7k

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[1] In this particular case there is a dominating quantity with assumedly rectangular distribution, which leads to a distribution function with very „weak tails“ (meaning: this in fact not exactly a normal distribution). So, 95% coverage probability is achieved already by expanded uncertainty of 0.0034 ml (as evidenced by Monte Carlo simulations). Thus, the presented uncertainty of 0.0038 ml is a conservative estimate (which is not bad as explained in section 3.5).

4.6. Practical example

This is an example of calculating the volume and its uncertainty of liquid delivered from a self-calibrated volumetric pipette

The uncertainty of the pipetted volume u(*V*) has three main uncertainty components: uncertainty due to repeatability, u(*V*,rep); uncertainty due to pipette calibration, u(*V*,cal) and uncertainty due to the temperature difference from 20 °C, u(*V*,temp).

The estimate of the probable maximum difference of the pipette volume from the nominal volume, expressed as ±x is often used as the estimate of calibration uncertainty of the pipette (as was done in section 4.1). It is usually given by the manufacturer without any additional information about its coverage probability or distribution function. In such a case it is the safest to assume that rectangular distribution holds and to convert the uncertainty estimate to standard uncertainty by dividing it with square root of three.

Usually in the case of high-accuracy work the pipette is calibrated in the laboratory in order to obtain lower calibration uncertainty. As was seen in sections 4.2 and 4.3, if the uncertainty due to factory calibration is used, then this calibration uncertainty component is the most influential one. So, reducing it would also reduce the overall uncertainty. It is very important, that calibration and pipetting are performed under the same conditions and preferably by the same person. From the calibration data we can obtain two important pieces of information: (1) the correction term for the pipette volume $V_{\text{correction}}$ with uncertainty *u* (*V*,cal) and (2) repeatability of pipetting *u* (*V*,rep). The example presented here explains this.

For calibration of a pipette water is repeatedly pipetted (at controlled temperature in order to know its density), the masses of the pipetted amounts of water are measured and the pipetted volumes of water are calculated (using density of water at the calibration temperature). Here are the calibration data of a 10 ml pipette:

The standard deviation is calculated according to the equation:

$$s = \sqrt{\frac{\sum_i^n (V_i - V_m)^2}{n-1}} = 0.0057\ \text{ml} \qquad (4.15)$$

Uncertainty due to repeatability of pipetting *u* (*V*, REP) is equal to this standard deviation 0.0057 ml. Pipetting is often used in titration analysis. If the solution that is titrated is pipetted then this repeatability contribution is already accounted for in the repeatability of the titration results and is not separately taken into account in the uncertainty of pipette volume.

Uncertainty of the calibration (in fact the uncertainty of the correction) has to be always taken into account. In many cases, the uncertainty due to repeatability of obtaining the correction is the only important uncertainty source and other sources can be left out of the consideration. This uncertainty is expressed (when calibration is done [1]) as the standard deviation of the mean:

$$u(V,\text{CAL}) = s(V_m) = \frac{s(V)}{\sqrt{n}} = \sqrt{\frac{\sum_i^n (V_i - V_m)^2}{n(n-1)}} = 0.0018\ \text{ml} \qquad (4.16)$$

In this example the correction is -0.0080 ml and its standard uncertainty is 0.0018 ml.

temperature of water	23.8	°C
density of pure water	0.9973	g/cm³

	m(water) (g)	**V(water) (ml)**
1	9.96037	9.98734
2	9.96454	9.99152
3	9.9632	9.99017
4	9.97152	9.99852
5	9.9683	9.99529
6	9.97413	10.00113
7	9.96806	9.99505
8	9.96165	9.98862
9	9.96397	9.99095
10	9.9544	9.98135
average	9.965014	**9.9920**
Correction		**-0.0080**
Experimental standard deviation, $s(V)$		**0.0057**
Relative standard deviation, $\frac{s(V)}{V}$		**0.058%**
Standard deviation of the mean, $s(V_m)$		**0.0018**

When there is a possibility that pipetting is performed at a different temperature from the calibration (and this possibility exists almost always), then an additional uncertainty source due to temperature change is introduced and it has to be taken into account.

In this case we assume that pipette's using temperature does not differ from the calibration temperature by more than 4 °C (Δt = ±4°C, assuming rectangular distribution). Water's density depends on temperature, therefore we have to consider also the thermal expansion coefficient of water, which is $\gamma_W = 2.1 \cdot 10^{-4}$ 1/°C. So,

$$u(V_{temp}) = \frac{V_p \cdot \Theta \cdot \gamma_w}{\sqrt{3}} = \frac{9.992\text{ml} \cdot 4°\text{C} \cdot 2.1 \cdot 10^{-4} \frac{1}{°C}}{\sqrt{3}} = 0.0048\text{ml} \qquad (4.17)$$

Now, when we will perform a single pipetting, the volume is 9.992 ml and its combined standard uncertainty is

$$u(V) = \sqrt{0.0057^2 + 0.0018^2 + 0.0048^2} = 0.0077\text{ ml} \qquad (4.18)$$

The k = 2 expanded uncertainty [2] of the pipetted volume can be found as follows:

$$U(V) = u_c(V) \cdot k = 0.0077 \cdot 2 = 0.0154\text{ ml}$$

(4.19)

As explained in section 4.5 if the first significant digit of the uncertainty is 1... 4 then uncertainty should be presented with two significant digits. Thus we can write the result:

The volume of the pipetted liquid is:

$$V = (9.992 \pm 0.015)\text{ ml},\ k = 2,\text{ norm.} \qquad (4.20)$$

It is interesting to compare now this expanded uncertainty with the expanded uncertainty obtained in section 4.5 (eq 4.14). We see that when the pipette is calibrated in our laboratory then the uncertainty of the volume is more than two times lower. We also see that the uncertainty component due to pipette calibration, which back then was the largest uncertainty component, is now the smallest.

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[1] When the calibration is not performed in the laboratory, the uncertainty of the calibration can be taken into account according to the manual information, that is usually given on the pipette as the tolerance range. For example, if the manufacturer has provided the tolerance range ±0.03 mL (in case of 10 mL pipette), then the standard uncertainty of the pipette correction is calculated as $\frac{0.03\text{mL}}{\sqrt{3}} = 0.017\text{mL}$. Correction value on this case is 0.00 mL.

[2] Later in this course (section 9.8) we will see, how to rigorously find, whether we can say that the k = 2 expanded uncertainty in a particular case corresponds to 95% (this depends on the so-

called effective number of degrees of freedom). And if not then what *k* should be used to achieve approximately 95% coverage probability.

In the case of this example the effective number of degrees of freedom is 26 and the respective coverage factor (actually the Student coefficient) with the probability of 95% is 2.06, which is only very slightly different from 2 (the expanded uncertainty would increase from 0.015 ml to 0.016 ml).

5. Principles of measurement uncertainty estimation

Brief summary: The main principles of measurement uncertainty estimation – the so-called GUM principles – are presented on the example of determination of pesticides in oranges. These principles have been laid down in the ISO GUM [1] and they are now universally accepted as being the common foundation of all the different uncertainty estimation approaches. These principles are the following:

1. The basis of any measurement (thus obviously also measurement uncertainty evaluation) is the definition of the measurand;
2. The used measurement procedure has to correspond to the measurand definition;
3. All relevant sources of uncertainty have to be carefully considered and those that are important have to be taken into account;
4. The random and systematic effects are treated the same way when estimating measurement uncertainty – both are evaluated as standard uncertainties, which thereafter are combined into the combined standard uncertainty.

5.1. Measurand definition
5.2. Measurement procedure
5.3. Sources of measurement uncertainty
5.4. Treatment of random and systematic effects

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[1] ISO GUM originally refers to the *Guide To The Expression of Uncertainty in Measurement*, ISO, Geneva, Switzerland, 1993 (Reprinted 1995). In 2008 this document was revised and reissued as ISO JCGM 100:2008 *Evaluation of measurement data - Guide to the expression of uncertainty in measurement*. The latter document is available on-line from http://www.bipm.org/en/publications/guides/gum.html

5.1. Measurand definition

Brief summary: The first principle of measurement uncertainty is: the measurand must be correctly and unambiguously defined. The importance of measurand definition is explained on the example of pesticide determination in oranges.

Defining the measurand

http://www.uttv.ee/naita?id=17585

https://www.youtube.com/watch?v=Bf_0EssHQ60

Defining the measurand in the case of pesticide determination in oranges is not trivial. On one hand it is important to define whether the result is applied to a single orange or few oranges that were taken as the **sample** or whether it is applied to the **whole lot** of oranges (the whole analysis object, also called sampling target). On the other hand, oranges are not homogenous. Pesticides are applied on orange surface, not inside. At the same time pesticide can diffuse from the orange peel to the inside. So, a number of different possibilities exist: whole orange, whole peel, outside part of the peel, only orange flesh.

Combining together we get 8 possibilities, in what exactly we can determine pesticides. Measuring pesticide content according to any of these will lead to different and mutually non-comparable results.

In addition (not explained in the video) instead of defining the measurand via the total analyte content in the sample (or part of the sample) it is often more practical to look at some part of the analyte only.

A good example is phosphorus determination in soil. Although it is possible to determine the **total** phosphorus content in soil it is in fact more interesting to determine only the part that is available to plants – the **bioavailable** phosphorus – because it is this part of the total phosphorus content that contributes to the fertility of the soil and is therefore of interest in agriculture.

Total phosphorus content and bioavailable phosphorus content are different measurands and their values for the same soil differ strongly. This has important implications for the measurement procedure. This is explained in section 5.2.

5.2. Measurement procedure

Brief summary: The main steps of a measurement/analysis procedure are presented on the example of pesticide measurement are presented: Sample preparation (in this case: homogenization, extraction(s), extract purification), instrument calibration, actual analysis. It is stressed that the measurement procedure must correspond to measurand definition.

Measurement procedure

http://www.uttv.ee/naita?id=17586

https://www.youtube.com/watch?v=BKIB_iB4wp4

This scheme of a chemical analysis procedure is very general. In specific cases there can be deviations from this scheme (more steps or less steps). In particular, sampling is not introduced here as a step of chemical analysis procedure. This holds if samples are brought to the laboratory for analysis and the laboratory itself does not do sampling (see section 5.3 for more details).

It is worth stressing the importance of **sample preparation as** a step in analytical procedure. The majority of analytical procedures need that the sample is converted into a solution which contains as large as possible share of the analyte from the sample (ideally all of it) and as little as possible of the other components of the sample **matrix**. In analytical chemistry sample matrix is the term for describing jointly all sample components except the analyte(s). The matrix components often act as **interfering** compounds, which can artificially increase or decrease the result. Therefore it is important to minimize their content in the solution obtained from the sample. If the interfering compounds cannot be fully eliminated and the interference cannot be corrected (which is quite usual in chemical analysis) then their effect has to be taken into account in measurement uncertainty estimation.

Sample preparation is often the most work-intensive part of chemical analysis and in most cases it is also the part, which has the largest uncertainty contribution. Sample preparation usually involves either of the two approaches:

1. Essentially destroying the sample matrix so that a solution containing the analyte(s) and few matrix components is obtained. This is often done by digestion with acids or fusing with alkalies or salts. This approach is suitable for determining elements.
2. Separating the analyte(s) from the sample matrix so that a solution containing the analyte(s) is obtained where the amount of matrix components is as small as possible. This is usually done by a set of extractions. This approach is suitable for organic analytes.

Obviously the choice of sample preparation procedure depends on whether the measurand corresponds to the total analyte content in the sample or some part of it, e.g. the bioavailable analyte content (see the text in section 5.1). In the case of e.g. total phosphorus content determination in soil the analyst can make the choice of the sample preparation procedure. All procedures that lead to determination of the total phosphorus content (often involving complete destruction of the matrix) are suitable. In the case of determining of e.g. bioavailable phosphorus content in soil the sample preparation procedure must mimic the way the plants get phosphorus from soil. So, sample preparation involves leaching at predefined conditions. Such sample preparation procedures are often standardized and whenever the results are meant to be mutually comparable they must be obtained with the same procedure. Thus, in this latter case the sample preparation procedure becomes part of the measurand definition.

5.3. Sources of measurement uncertainty

Brief summary: The overview of possible uncertainty sources, on the example pesticide analysis, is presented. Although the uncertainty sources are presented on the example of pesticide analysis, the same uncertainty sources hold for the majority of other analytical methods. Most of the uncertainty sources are linked to specific steps in the analysis procedure. It is stressed that sample preparation is usually the biggest contributor to measurement uncertainty. When performing chemical analysis then every care should be taken to minimize (preferably eliminate) the influence of the uncertainty sources, as far as possible. And what cannot be eliminated, has to be taken into account. It is not necessary to quantify every uncertainty source individually. Instead, it is often more practical to quantify several uncertainty sources jointly.

Measurement uncertainty sources

http://www.uttv.ee/naita?id=17587

https://www.youtube.com/watch?v=4y2cjJ8Jpsg

5.4. Treatment of random and systematic effects

Brief summary: Although within a measurement series random and systematic effects influence measurement results differently, they are mathematically taken into account the same way – as uncertainty components presented as standard uncertainties.

Treatment of random and systematic effects

http://www.uttv.ee/naita?id=17712

https://www.youtube.com/watch?v=hdh5xVVZTbg

In the case of pipetting (demonstrated and explained in sections 2 and 4.1) there are three main sources of uncertainty: repeatability, calibration uncertainty of the pipette and the temperature effect. These effects influence pipetting in different ways.

1. Repeatability is a typical random effect. Every pipetting operation is influenced by random effects that altogether cause the differences between the volumes that are pipetted under identical conditions;
2. The uncertainty due to calibration of the pipette is a typical systematic effect: If instead of 10.00 ml the mark on the pipette is, say, at 10.01 ml then the pipetted volume will be systematically too high. This means that although individual pipetting results can be lower than 10.01 ml (and in fact even below 10.00 ml), the average volume will be higher than 10.00 ml: approximately 10.01 ml.
3. The temperature effect can be, depending on the situation, either systematic or random effect or (very commonly) mixture of the two. Which way it is depends on the stability of the temperature during repetitions (which is influenced by the overall duration of the experiment).

Although the three uncertainty sources influence pipetting results in different ways they are all taken into account the same way – via uncertainty contributions expressed as standard uncertainties.

In principle, it is possible to investigate the systematic effects, determine their magnitudes and take them into account by correcting the results. When this is practical, this should be done. If this is not done then the results will be biased, i.e. will be systematically shifted from the true value.

An example where systematic effect can be determined and correction introduced with reasonable effort is calibration of pipette, explained in the example in section 4. Two cases were examined: without correcting and with correcting:

1. In subsection 4.1 the calibration uncertainty of ± 0.03 ml as specified by the producer is used. This corresponds to the situation that there is possibly a systematic effect – the *possible* [1] difference of the true pipette volume from its nominal volume, but it is not closely investigated or corrected and the uncertainty ± 0.03 ml is assigned to it, which with very high probability covers this effect. As a result the standard uncertainty of calibration was u (V, CAL) = 0.017 ml (rectangular distribution is assumed).
2. In subsection 4.6 it was explained how to determine the actual volume of pipette by calibration. It was found that the pipette volume was 10.006 ml. The calibration that was carried out in the laboratory still has uncertainty, but this uncertainty now is due to the repeatability during calibration (i.e. random effects) and is by almost 10 times smaller: u (V, CAL) = 0.0018 ml.

Thus, when correcting for systematic effects can be done with reasonable effort then it can lead to significant decrease of measurement uncertainty. However, in many cases accurate determination of a systematic effect (accurate determination of bias) can involve a very large effort and because of this can be impractical. It can also happen that the uncertainty of correction is not much smaller than the uncertainty due to possible bias. In fact, with reasonable (i.e. not very large) effort the outcome of bias determination often is that there may be a systematic effect and may not be. In such cases correction cannot be done and the uncertainty due to the effect has to cover the *possible* systematic effect (*possible* bias).

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[1] The word *possible* means here that in fact there may be no systematic effect – the actual pipette volume can be 10.00 ml. We simply do not know.

6. Random and systematic effects revisited

Brief summary: This section explains that whether an effect will influence the measurement result as a random or as a systematic effect depends on the conditions. Effects that are systematic in short term can become random in long term. This is the reason why repeatability is by its value smaller than within-lab reproducibility and the latter is in turn smaller than the combined standard uncertainty. This section also explains that the A and B type uncertainty estimates do not correspond one to one to the random and systematic effects.

How a within-day systematic effect can become a long-term random effect?

http://www.uttv.ee/naita?id=17713

https://www.youtube.com/watch?v=qObLSS7mfDo

Random and systematic effects in the short term and in the long term

An effect that within a short time period (e.g. within a day) is systematic can over a longer time period be random. Examples:

1. If a number of pipetting operations are done within a day using the same pipette then the difference of the actual volume of the pipette from its nominal volume (i.e. calibration uncertainty) will be a systematic effect. If pipetting is done on different days and the same pipette is used then it is also a systematic effect. However, if pipetting is done on different days and different pipettes are used then this effect will change into a random effect.
2. An instrument is calibrated daily with calibration solutions made from the same stock solution, which is remade every month. In this case the difference of the actual stock solution concentration and its nominal concentration is a systematic effect within a day and also within few weeks. But over a longer time period, say, half a year, [1]It cannot be strictly defined, how long is „long-term". An approximate guidance could be: one year is good, „several months" (at least 4-5) is minimum. Of course it also depends on the procedure. this effect becomes random, since a number of different sock solutions will have been in use during that time period.

Conclusions:

1. An effect, which is systematic in short term can be random in long term;
2. The longer is the time frame the more effects can change from systematic into random.

As explained in a past lecture if the measurement of the same or identical sample is repeated under identical conditions (usually within the same day) using the same procedure then the standard deviation of the obtained results is called **repeatability standard deviation** and denoted as $\boldsymbol{s_r}$. If the measurement of the same or identical sample is repeated using the same procedure but under changed conditions whereby the changes are those that take place in the laboratory under normal work practices then the standard deviation of the results is called **within-lab reproducibility** or **intermediate precision** and it is denoted as $\boldsymbol{s_{RW}}$. [2]

The conclusions expressed above are the reason why s_r is smaller than s_{RW}. Simply, some effects that within day are systematic and are not accounted for by s_r become random over a longer time and s_{RW} takes them into account. The combined standard uncertainty u_c is in turn larger that the intermediate precision, because it has to take into account all significant effects that influence the result, including those that remain systematic also in the long term. This important relation between these three quantities is visualized in Scheme 6.1.

Repeatability < Within-lab reproducibility < Combined uncertainty

$s_r < s_{RW} < u_c$

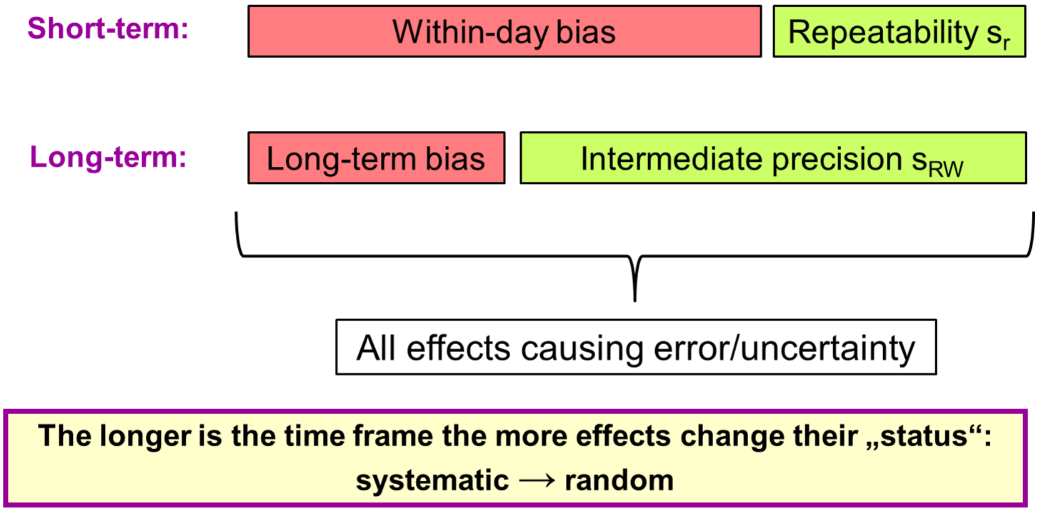
Scheme 6.1. Relations between repeatability, within-lab reproducibility and combined uncertainty.

The random and systematic effects cannot be considered to be in one-to-one relation with type A and B uncertainty estimation. These are categorically different things. The effects refer to the intrinsic causal relationships, while type A and B uncertainty estimation refers rather to approaches used for quantifying uncertainty. Table 6 illustrates this further.

Table 6.1. Interrelations between random and systematic effects and A and B types of uncertainty estimates.

Effect	**Type A estimation**	**Type B estimation**
Random	The usual way of estimating uncertainties caused by random effects	Type B estimation of the uncertainty caused by random effects is possible if no repeated measurements are carried out and the data/information on the magnitude of the effect is instead available from different sources.
Systematic	This is only possible if the effect will change into a random effect in the long term	The usual way of estimating uncertainties caused by the systematic effects

So, depending on the timeline, all the effects causing uncertainty can be grouped as pictured in Scheme 6.2. In the short-term view, most effects act as systematic and the random effects can be quantified via repeatability. In the long term more (usually most) effects are random and can be quantified via within-lab reproducibility (intermediate precision).



Scheme 6.2. Two ways of grouping effects that cause uncertainty (short-term and long-term).

As is explained in section 8, the two main uncertainty estimation approaches addressed in this course use the above ways as follows: The modelling (ISO GUM) approach tends to follow to the short-term view (estimating uncertainty of one concrete result on one concrete day), while the Single-lab approach (Nordtest) always follows the long-term view (estimating an average uncertainty of the procedure). See sections 8-11 for more information.

Determining repeatability and within-lab reproducibility in practice

The typical requirements for determining s_r and s_{RW} are presented in Table 6.2.

Table 6.2. Typical requirements for determining *s*r and *s*RW of an analytical procedure.

Repeatability s_r	**Within-lab reproducibility s_{RW}**
There is a **sufficient amount** of a **stable** and **homogeneous** sample (control sample)	
The control sample has to be **similar to the routinely analysed samples** by analyte content and by **difficulty level**	
Sample has to be stable within a day	Sample has to be **stable for months**
Measurements with subsamples of the control sample are carried out on the same day under the same conditions	On days when the analysis procedure is used, in addition to calibrants and customer samples also a subsample of the control sample is analysed
The subsample of the control sample has to **through all the steps of the procedure**, including the sample preparation steps	
s_r or s_{RW} is found as standard deviation of the results obtained with subsamples of the control sample	

When estimating the uncertainty contributions due to random effects, then it is important that a number of repeated measurements are carried out. On the other hand, if, e.g. repeatability of some analytical procedure is estimated then each repetition has to cover all steps in the procedure, including sample preparation. For this reason making extensive repetitions is very work-intensive. In this situation the concept of **pooled standard deviation** becomes very useful. Its essence is pooling standard deviations obtained from a limited number of measurements. The following video explains this:

Pooled standard deviation

http://www.uttv.ee/naita?id=18228

https://www.youtube.com/watch?v=xsltS41PZW0

Depending on how the experiments are planned, the pooled standard deviation can be used for calculating of either repeatability s_r or within-lab reproducibility s_{RW}. The experimental plan and calculations when finding repeatability s_r are explained in the following video:

Pooled standard deviation in practice: estimating repeatability

http://www.uttv.ee/naita?id=18232

https://www.youtube.com/watch?v=DM_zf85PYic

The experimental plan and calculations when finding within-lab reproducibility s_{RW} are explained in the following video:

Pooled standard deviation in practice: estimating within-lab long-term reproducibility

http://www.uttv.ee/naita?id=18234

https://www.youtube.com/watch?v=nPJY8HfPxNs

***

[1] It cannot be strictly defined, how long is „long-term". An approximate guidance could be: one year is good, „several months" (at least 4-5) is minimum. Of course it also depends on the procedure.

[2] The terms „within-lab reproducibility" and „intermediate precision" are synonyms. The VIM(1) prefers intermediate precision. The Nordtest handbook(5) uses within-lab reproducibility (or

reproducibility within laboratory). In order to stress the importance of the „long-term“, in this course we often refer to s_{RW} as the within-lab long-term reproducibility.

***

The slides of the presentation and the calculation files – with initial data only, as well, as with calculations performed – are available from here:

pooled_standard_deviation.pdf	18 KB
pooled_standard_deviation_repeatability_initial.xlsx	16 KB
pooled_standard_deviation_repeatability_solved.xlsx	16 KB
pooled_standard_deviation_reproducibility_initial.xlsx	15 KB
pooled_standard_deviation_reproducibility_solved.xlsx	16 KB

Pooled Standard Deviation

Ivo Leito
University of Tartu
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2013

Pooled Standard Deviation

- If it is impossible to make many repeated measurements with the same sample
- Then precision can be estimated during longer time in the form of **pooled standard deviation**

- Pooled standard deviation can be used to calculate:
  - Repeatability
  - Within-lab reproducibility

Pooled Standard Deviation

- General formula for the case when experiment is done with different samples, each measured repeatedly:

$$s_{\text{pooled}} = \sqrt{\frac{(n_1-1)s_1^{\ 2}+(n_2-1)s_2^{\ 2}+\ldots+(n_k-1)s_k^{\ 2}}{n_1+n_2+\ldots+n_k-k}}$$

- Symbols:
  - *k* number of samples
  - s_1, s_2, etc are within sample standard deviations
  - n_1, n_2, etc are numbers of measurements made for different samples

---

Pooled Standard Deviation

- If the number of measurements made with each sample was the same:

$$s_{\text{pooled}} = \sqrt{\frac{s_1^{\ 2}+s_2^{\ 2}+\ldots+s_k^{\ 2}}{k}}$$

- Symbols:
  - *k* number of samples
  - s_1, s_2, etc are within sample standard deviations
  - n_1, n_2, etc are numbers of repeated measurements with every sample

---

Pooled standard deviation in practice

- How to set up experiment for **repeatability** s_r evaluation using s_{pooled}?

- How to set up experiment for **within-lab long-term reproducibility** s_{RW} evaluation using s_{pooled}?

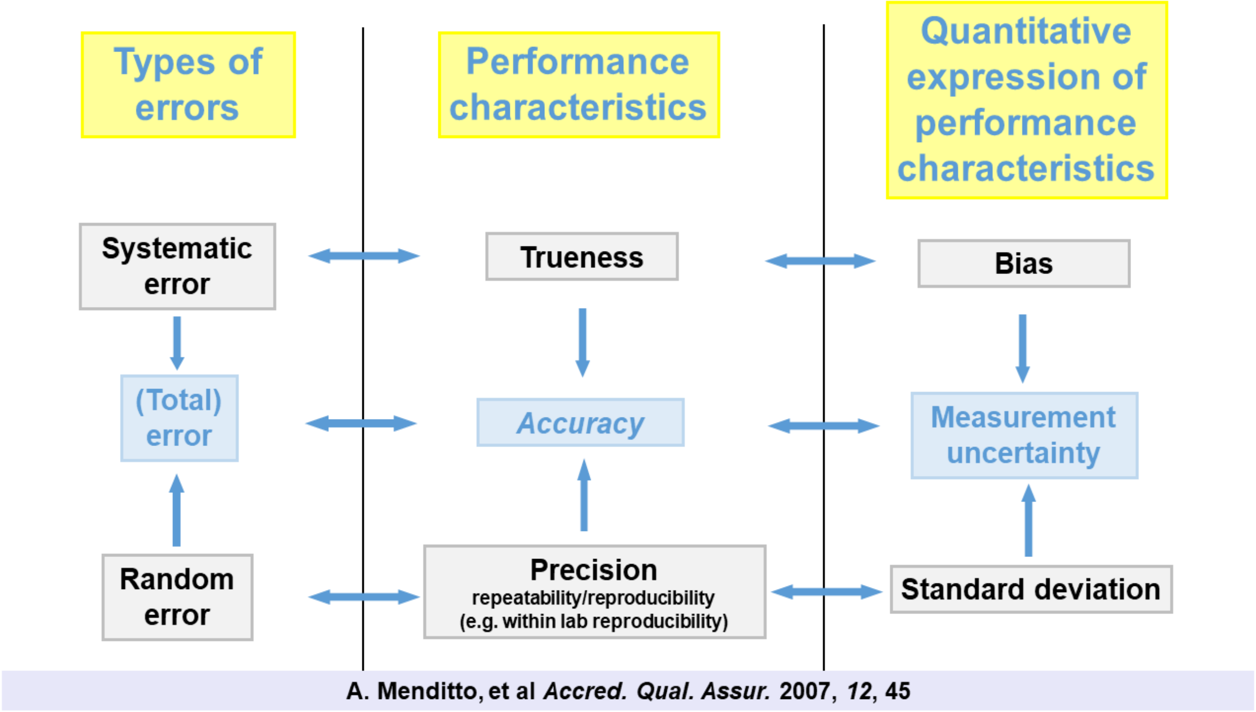
7. Precision, trueness, accuracy

Brief summary: Interrelations between the different error types (**random**, **systematic**, **total**), their corresponding performance characteristics (**precision**, **trueness**, **accuracy**) and the parameters for quantitatively expressing these performance parameters (**standard deviation**, **bias**, **measurement uncertainty**) are explained in this section.

Interrelation between the concepts of precision, trueness, accuracy and measurement uncertainty

http://www.uttv.ee/naita?id=17824

https://www.youtube.com/watch?v=NdDK03f0wew



Scheme 7.1. Interrelations between the different error types, the performance characteristics used to estimate them and the ways of expressing the estimates quantitatively. This type of scheme was originally published in article A. Menditto, et al Accred. Qual. Assur. 2007, 12, 45**.**

The difference between the measured value and the true value is called error or *total* error (see section 1). This error can be divided into two parts – **random error** (having different magnitude and sign in the case of repeated measurements) and **systematic error** (having the same or systematically changing magnitude and sign in the case of repeated measurements). As seen in section 1 errors cannot be known exactly. Therefore instead of errors themselves we operate with *estimates* of errors – the performance characteristics.

Thus, **trueness** is the estimate of the systematic error. For determining trueness we do not need to know the true value but we need to know a **reference value**. Reference value (differently from the true value) has uncertainty, but usually a small one. Different types of **precision** are estimates of the random error. For obtaining the "true" precision we would need to make an infinite number of repeated measurements. There are different types of precision, depending on the conditions under which precision is determined, e.g. repeatability (section 1) and intermediate precision (section 6). Accuracy embraces both trueness and precision and be considered as describing the total error.

These performance characteristics can be quantitatively expressed. **Bias** – difference between the measured value obtained from multiple repeated measurements with the same sample and the reference value – is the quantitative expression of trueness. **Standard deviation** – again obtained from multiple measurements with the same sample – is the quantitative expression of precision. These two can be combined into a **measurement uncertainty** estimate, which can be regarded as the quantitative expression of accuracy.

8. Overview of measurement uncertainty estimation approaches

Brief summary: In this section an overview is given about the main types of approaches that can be used for estimation of measurement uncertainty.

Overview of the approaches for estimating measurement uncertainty

http://www.uttv.ee/naita?id=17704

https://www.youtube.com/watch?v=Ed8S8KN1GlU

The two main approaches that are addressed in this course – the modelling (ISO GUM) and the within-lab validation (Nordtest) approach differ by the extent of detail, by the way the random effects are taken into account and by the status of the obtained uncertainty. Table 8.1 outlines the differences. See also Table 11.1 in section 11.

Table 8.1 Some key differences between the modelling and within-lab validation approach of measurement uncertainty estimation.

Aspect	Modelling (ISO GUM)	Within-lab validation (Nordtest)
Taking random effects into account	Typically random effects are addressed at within-day (short-term) level (see Section 6), using repeatabilities. All effects that within a day are systematic, are taken into account separately. So, usually quite many uncertainty sources are quantified separately.	Random effects are addressed at long-term level, with the aim of incorporating as many effects as possible into the random component of uncertainty, usually quantified as within-lab reproducibility s_{RW} (termed as $u(R_W)$ in section 10). This is an important advantage of this approach as explained in section 10.2.
Extent of information needed (or obtained) on different uncertainty sources	As a result of quantifying a number of uncertainty sources separately, the extent of information on different uncertainty sources is usually quite high.	As a result of quantifying a large number of uncertainty sources as within-lab reproducibility, the level of information needed/obtained on different uncertainty sources is low.
Status of the obtained uncertainty	The obtained uncertainty applies to a specific analysis result obtained on a specific day.	The obtained uncertainty is an average uncertainty estimate of the analysis procedure across several a time period of several months.

Although ISO GUM (2) is the de facto standard document of measurement uncertainty estimation, especially in physics, the within-lab validation (Nordtest) approach is also gaining popularity. For example, the Nordest approach forms the foundation of the ISO 11352 standard *Water quality — Estimation of measurement uncertainty based on validation and quality control data*.[1]

***

[1] ISO 11352, *Water quality — Estimation of measurement uncertainty based on validation and quality control data,* **2012.**

9. The ISO GUM Modeling approach

This section presents step by step the modeling approach to measurement uncertainty estimation. This approach is described in detail in the ISO GUM(2) and has been interpreted for chemistry in the Eurachem measurement uncertainty guide(3). It is often called also the "bottom-up" approach. This means that the uncertainties of the input quantities are found and thereafter combined into the combined standard uncertainty. The uncertainty estimation carried out in section 4 in principle also used this approach. The presentation in this section is based on a practical example – determination of ammonium nitrogen in water.

9.1. Step 1– Measurand definition
9.2. Step 2 – Model equation
9.3. Step 3 – Uncertainty sources
9.4. Step 4 – Values of the input quantities
9.5. Step 5 – Standard uncertainties of the input quantities
9.6. Step 6 – Value of the output quantity
9.7. Step 7 – Combined standard uncertainty
9.8. Step 8 – Expanded uncertainty
9.9. Step 9 – Looking at the obtained uncertainty

The slides used in this section can be downloaded from here:

uncertainty_of_photometric_nh4_determination_iso_gum_modeling.pdf 82 KB

Estimation of measurement uncertainty in chemical analysis (analytical chemistry)

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2013

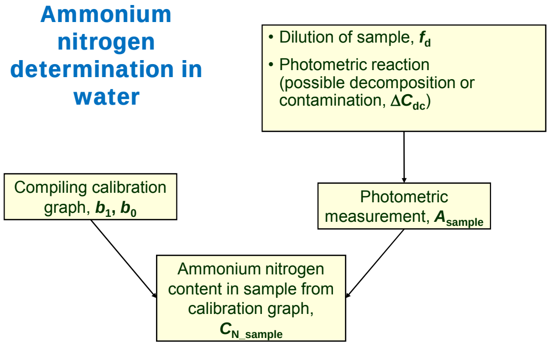
Measurement uncertainty by the modeling approach:

Determination of NH_4^+ in water

- A dye (photometric complex) is formed quantitatively from NH_4^+ and its absorbance is measured at 640-660 nm by a photometer
- The concentration of ammonium nitrogen is found from calibration graph

---

Procedure



Ammonium nitrogen determination in water

- Dilution of sample, f_d
- Photometric reaction (possible decomposition or contamination, ΔC_{dc})

Photometric measurement, A_{sample}

Compiling calibration graph, b_1, b_0

Ammonium nitrogen content in sample from calibration graph, C_{N_sample}

Step 1 – defining the measurand

Measurand = The quantity intended to be measured

Our measurand:
Concentration of NH_4^+ expressed as ammonium concentration C_{N_sample} mg/l in the water sample

---

Step 2 – Model

Model is the equation which enables calculating the measurand (**output quantity Y**) value from the values of directly measured quantities (**input quantities X_1 .. X_n**) :

$$Y = f(X_1, X_2, \ldots\ldots, X_n)$$

Step 2 – Model

- Model:

$$C_{\mathrm{N\_sample}} = \frac{(A_{\mathrm{sample}} - b_0)}{b_1} \times f_{\mathrm{d}} + \Delta C_{\mathrm{dc}}$$

- A_{sample} – absorbance of the dye solution obtained from the sample
- b_1 and b_0 – slope and intercept of the calibration graph
- f_d – dilution factor
- ΔC_{dc} – component taking into account uncertainty originating from possible decomposition or contamination

Step 3 – Uncertainty sources

- **All possible uncertainty sources need to be considered**
  - **The important ones need to be accounted for**
  - **This can be done individually or by grouping**
- For this the source has to be linked with some input quantity in the model
- If an important uncertainty source exists that cannot be linked with any input quantity then the model has to be modified

Step 3 – Uncertainty sources

- **Sampling**
  - Sample non-representativeness

The result is expressed for sample, sampling is not included

- **Sample preparation**
  - Inhomogeneity
  - Separation of analyte incomplete
  - Analyte adsorbs

The sample is homogenous, the analyte is not separated and does not adsorb

  - Analyte or photometric complex decomposes
  - Analyte volatilizes
  - Incomplete reaction
  - Contamination

Analyte or the photometric complex can decompose or get contaminated: ΔC_{dc}

Step 3 – Uncertainty sources

- Preparation and dilution of solutions

f_d accounts for this

- Weighing

Is included in b_1 and b_0 uncertainty

- Calibration of instrument
  - Standard substance purity
  - Solution preparation

b_1 and b_0 uncertainty

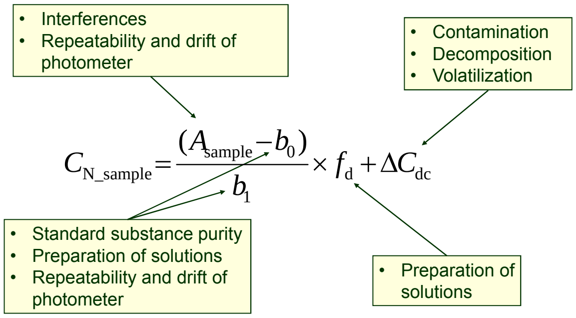
- Measurement of sample
  - Interferences
  - Repeatability of reading
  - Drift of reading

A_{sample} accounts for these

  - Memory effects

Absent in our example

Step 3 – Uncertainty sources



- Interferences
- Repeatability and drift of photometer

- Contamination
- Decomposition
- Volatilization

$$C_{N\_sample}=\frac{(A_{sample}-b_0)}{b_1}\times f_d+\Delta C_{dc}$$

- Standard substance purity
- Preparation of solutions
- Repeatability and drift of photometer

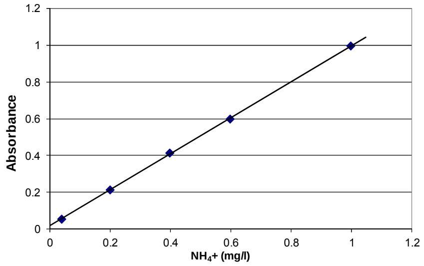
- Preparation of solutions

Step 4 – Finding values of input quantities

$A = b_0 + b_1\times C$

Calibration graph

$A = 0.017 + 0.981\times C$



Step 4 – Finding values of input quantities

Quantity	Value	Unit
A_{sample}	0.1860	AU*
b_0	0.0171	AU*
b_1	0.9808	AU×l/mg
f_d	1.2500	–
ΔC_{dc}	0.0000	mg/l

* Absorbance in fact does not have a unit, AU is used for clarity

Step 5 – Standard uncertainties of the input quantities: A_{sample}

- Absorbance of the sample solution A_{sample}:

$u(A_{\text{sample}}$, rep) =	0.0010	AU
$u(A_{\text{sample}}$, drift) =	0.0012	AU
$u(A_{\text{sample}}$, chem) =	0.0030	AU

$$u(A_{\text{sample}}) = \sqrt{\begin{aligned}&u(A_{\text{sample}},\text{rep})^2 + u(A_{\text{sample}},\text{drift})^2\\ &+ u(A_{\text{sample}},\text{chem})^2\end{aligned}} = 0.0034\,\text{AU}$$

Step 5 – Standard uncertainties of the input quantities: b_0 and b_1

- Standard deviations of b_0 and b_1 as found from regression statistics are used as standard uncertainty estimates

$u(b_0)$ = 0.0025 AU

$u(b_1)$ = 0.0046 AU×l/mg

- This is an approximation!

Step 5 – Standard uncertainties of the input quantities: f_d

- The standard uncertainty of dilution factor is estimated here as 0.5% of the dilution factor value
- This is a safe estimate if volumetric operations are performed correctly

$u(f_d)$ = 1.25 / 200 = 0.0063

Step 5 – Standard uncertainties of the input quantities: ΔC_{dc}

- The *possible* contribution of decomposition or contamination at this concentration level is estimated from the experience of the laboratory as follows:

$u(\Delta C_{\text{dc}})$ = 0.004 mg/l

Step 5 – Standard uncertainties of the input quantities: summary

- **The uncertainties of the input quantities have to be used as standard uncertainties (*u*)**

Quantity	Value	*u*	Unit
A_{sample}	0.1860	0.0034	AU
b_0	0.0171	0.0025	AU
b_1	0.9808	0.0046	AU×l/mg
f_d	1.2500	0.0063	–
ΔC_{dc}	0.0000	0.0040	mg/l

Step 6 – Calculating the measurand value

$$\boxed{C_{\text{N\_sample}} = \frac{(A_{\text{sample}} - b_0)}{b_1} \times f_{\text{d}} + \Delta C_{\text{dc}}}$$

$$C_{\text{N\_sample}} = \frac{(0.1860 - 0.0171)}{0.9808} \times 1.25 + 0$$

$C_{\text{N\_sample}}$ = 0.215 mg/l

Step 7 – Finding combined standard uncertainty (1)

- In the case on non-correlating input quantities:

$$u_c(y)=\sqrt{\left[\frac{\partial Y}{\partial X_1}u(x_1)\right]^2+\left[\frac{\partial Y}{\partial X_2}u(x_2)\right]^2+.....+\left[\frac{\partial Y}{\partial X_n}u(x_n)\right]^2}$$

$u_c(y)$ = combined standard uncertainty of the output quantity
$u(x_i)$ = standard uncertainties of the input quantities

Step 7 – Finding combined standard uncertainty (2)

$$u_c(C_{\mathrm{N\_sample}})=\sqrt{\begin{array}{l}\left(\frac{\partial C_{\mathrm{N\_sample}}}{\partial A_{\mathrm{sample}}}u(A_{\mathrm{sample}})\right)^2+\left(\frac{\partial C_{\mathrm{N\_sample}}}{\partial b_0}u(b_0)\right)^2+\left(\frac{\partial C_{\mathrm{N\_sample}}}{\partial b_1}u(b_1)\right)^2\\+\left(\frac{\partial C_{\mathrm{N\_sample}}}{\partial f_{\mathrm{d}}}u(f_{\mathrm{d}})\right)^2+\left(\frac{\partial C_{\mathrm{N\_sample}}}{\partial \Delta C_{\mathrm{dc}}}u(\Delta C_{\mathrm{dc}})\right)^2\end{array}}$$

Step 7 – Finding combined standard uncertainty (3)

$$u_c(C_{\mathrm{N\_sample}})=\sqrt{\begin{array}{l}(0.00429)^2+(-0.00324)^2+(-0.0010)^2\\+(0.00108)^2+(0.00400)^2\end{array}}$$

$u_c(C_{\mathrm{N\_sample}})$ = 0.00686 mg/l

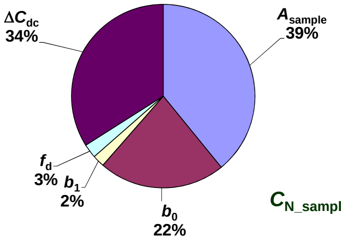
Step 8 – Finding expanded uncertainty

- Expanded uncertainty ***U*** is found by multiplying $\boldsymbol{u_c}$ with coverage factor ***k***
  - Very often *k* = 2, which in the case of normal distribution corresponds to ca 95% probability

U = 0.00686 x 2 = 0.014 mg/l

Result: **$C_{\mathrm{N\_sample}}$ = (0.215 ± 0.014) mg/l**
***k* = 2**

Step 9 – Contributions of uncertainty sources



<u>The most important uncertainty sources</u>:

- Interference
- Contamination
- Instability of the dye

$C_{\mathrm{N\_sample}}$ = (0.215 ± 0.014) mg/l
***k* = 2, norm.**

$$C_{\mathrm{N\_sample}}=\frac{(A_{\mathrm{sample}}-b_0)}{b_1}\times f_{\mathrm{d}}+\Delta C_{\mathrm{dc}}$$

Step 9 – Contributions of uncertainty sources

- Uncertainty contributions (indexes) are found as follows:

$$Index(x_1)=\frac{\left[\frac{\partial Y}{\partial X_1}u(x_1)\right]^2}{\left[\frac{\partial Y}{\partial X_1}u(x_1)\right]^2+\left[\frac{\partial Y}{\partial X_2}u(x_2)\right]^2+.....+\left[\frac{\partial Y}{\partial X_n}u(x_n)\right]^2}$$

9.1. Step 1– Measurand definition

Definition of the measurand

http://www.uttv.ee/naita?id=17636

https://www.youtube.com/watch?v=P_i268pDgvM

The measurand definition is the most basic step of any measurement. In this step it is defined what is actually measured and this definition is also the basis for the measurement procedure and model equation.

The measurand in this case is concentration of NH_4^+ expressed as ammonium ion concentration *C*N_sample [mg/l] in the water sample.

9.2. Step 2 – Model equation

Model equation

http://www.uttv.ee/naita?id=17637

https://www.youtube.com/watch?v=N45YNwToyac

The model equation (equation 9.1) enables calculating the **output quantity** value (result value) from the **input quantity** values. Input quantities are the directly measured quantities (or are calculated from directly measured quantities). In addition, the model equation has to enable accounting for all important measurement uncertainty sources. In the case of this analysis the model equation is the following:

$$C_{\mathrm{N\_sample}} = \frac{(A_{\mathrm{sample}} - b_0)}{b_1} \times f_{\mathrm{d}} + \Delta C_{\mathrm{dc}} \qquad (9.1)$$

The output quantity is $C_{\mathrm{N\_sample}}$ – ammonium ion concentration in the water sample.

The input quantities are:

A_{sample} – absorbance of the dye solution obtained from the sample;
b_1 and b_0 – slope and intercept of the calibration graph;
f_{d} – dilution factor;
ΔC_{dc} – component taking into account uncertainty originating from possible decomposition or contamination.

The parameters A_{sample}, b_1, b_0 and f_{d} in the equation account for the directly measured input quantities. In contrast, ΔC_{dc} is introduced only for taking into account uncertainty due to possible decomposition of the photometric complex and due to possible contamination. Its value is zero as will be seen in section 9.4, so, it does not contribute to the value of $C_{\mathrm{N\_sample}}$. However, its uncertainty is different from zero and therefore will contribute to the uncertainty of $C_{\mathrm{N\_sample}}$.

9.3. Step 3 – Uncertainty sources

All possible uncertainty sources have to be considered and those that are likely to be influential have to be taken into account. The schemes in this section show the uncertainty sources that influence the result of this analysis. Please note, however, that not necessarily all of them need to be individually taken into account in uncertainty estimation. Some may be small so that they can be neglected. Some may be grouped into one uncertainty component.

Uncertainty sources: in general

http://www.uttv.ee/naita?id=17638

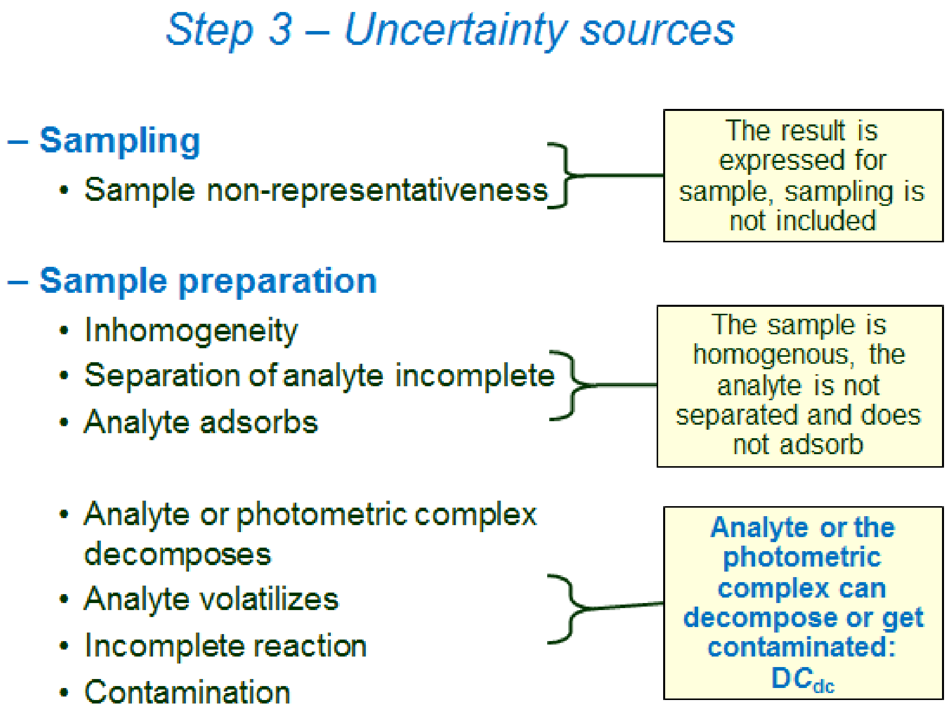
https://www.youtube.com/watch?v=EWYNWtRgvmI

Uncertainty sources: one by one

http://www.uttv.ee/naita?id=17639

https://www.youtube.com/watch?v=kEfhKCj44N0

The following schemes list the main uncertainty sources in chemical analysis and comment on the presence or absence of the respective uncertainty sources in our case. [1]



Step 3 – Uncertainty sources

– **Sampling**
  - Sample non-representativeness

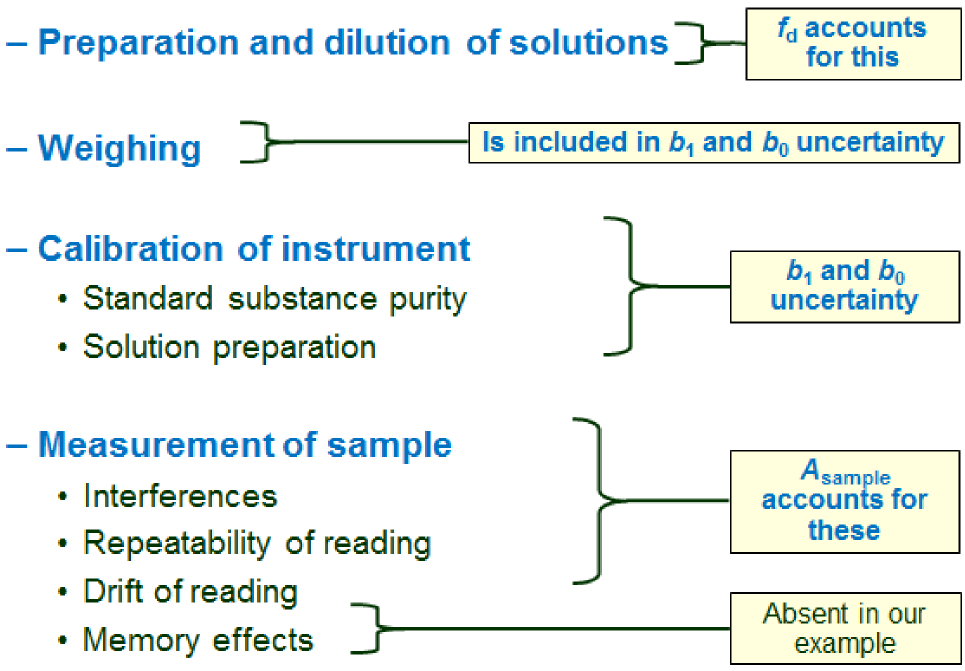
The result is expressed for sample, sampling is not included

– **Sample preparation**
  - Inhomogeneity
  - Separation of analyte incomplete
  - Analyte adsorbs

The sample is homogenous, the analyte is not separated and does not adsorb

  - Analyte or photometric complex decomposes
  - Analyte volatilizes
  - Incomplete reaction
  - Contamination

Analyte or the photometric complex can decompose or get contaminated: DC_{dc}



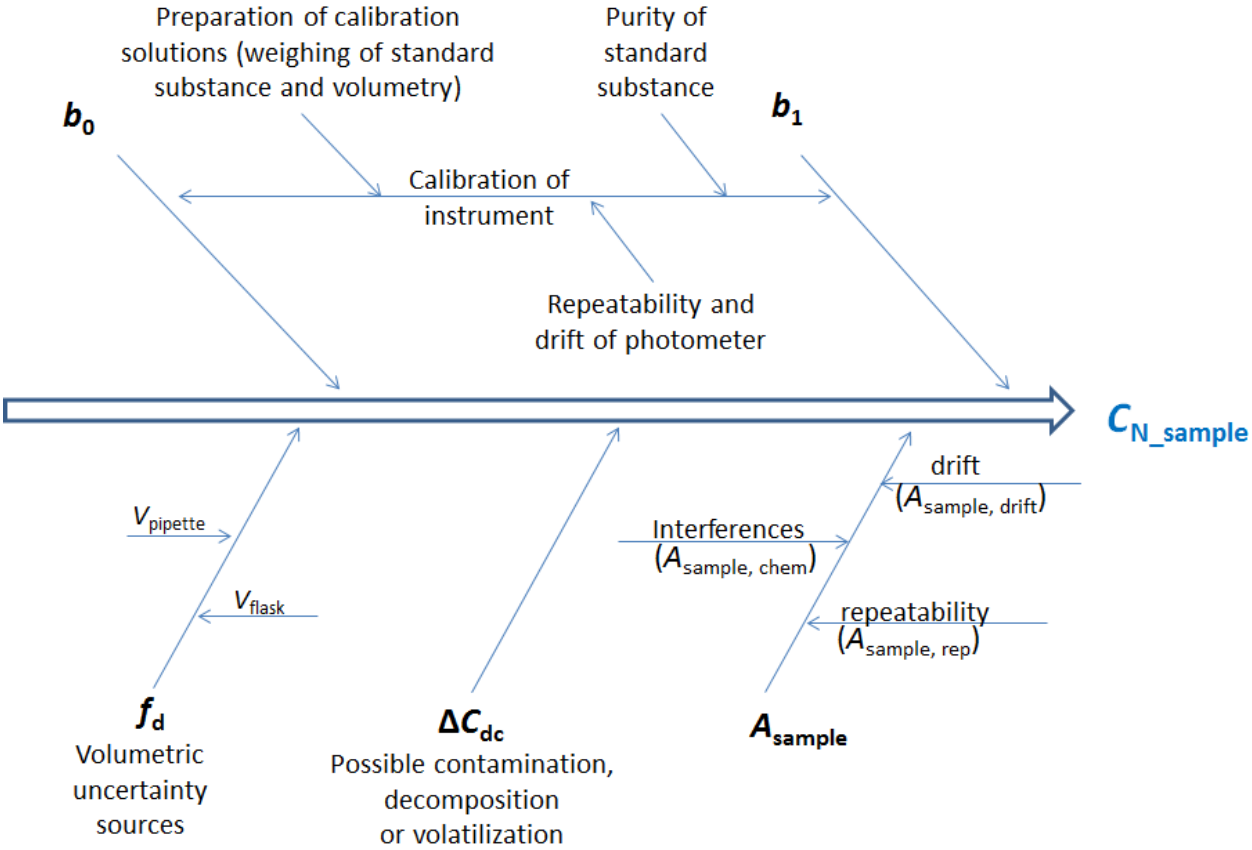
- Preparation and dilution of solutions — f_d accounts for this
- Weighing — Is included in b_1 and b_0 uncertainty
- Calibration of instrument
  - Standard substance purity
  - Solution preparation

  — b_1 and b_0 uncertainty
- Measurement of sample
  - Interferences
  - Repeatability of reading
  - Drift of reading
  - Memory effects

  Interferences, Repeatability of reading, Drift of reading — A_{sample} accounts for these

  Drift of reading, Memory effects — Absent in our example

The influence of measurement uncertainty sources, grouped according to input quantities, on the result can be schematically presented in the form of the so-called "fish-bone" diagram:



Scheme 9.1. Cause effect diagram: Photometric ammonia determination

About modifying the model

As is explained in the video, this model equation

$$C_{\mathrm{N\_sample}} = \frac{(A_{\mathrm{sample}} - b_0)}{b_1} \times f_{\mathrm{d}} + \Delta C_{\mathrm{dc}} \tag{9.2}$$

, identical to equation 9.1 (in section 9.2) can be regarded as modified model: the term ΔC_{dc} is brought in to account for some uncertainty sources (possible decomposition, possible contamination). This term is an *additive term*, i.e. its influence on $C_{\mathrm{N\_sample}}$ is constant, irrespective of the value of $C_{\mathrm{N\_sample}}$. The value of ΔC_{dc} is 0 mg/l and its uncertainty expresses the *absolute* (i.e. expressed in mg/l) uncertainty corresponding to the possible effects of decomposition and contamination. Bringing in such additive terms is one typical possibility of modifying the model for taking into account additional uncertainty sources. Additive term is suitable if the additional uncertainty is not very sensitive to analyte concentration (or if analyte concentration does not vary much).

Another typical possibility is to introduce a *multiplicative term* (or *multiplicative factor*). In the case of such modification the equation would look as follows:

$$C_{\mathrm{N\_sample}} = \frac{(A_{\mathrm{sample}} - b_0)}{b_1} \times f_{\mathrm{d}} \times f_{\mathrm{dc}} \tag{9.3}$$

In this case the value of f_{dc} would be 1 and its uncertainty would express the *relative* (i.e. unitless) uncertainty corresponding to the possible effects of decomposition and contamination. Multiplicative term is suitable if the additional uncertainty is roughly proportional to analyte concentration and if analyte concentration varies significantly. In the case of the data of this example the suitable value of $u(f_{\mathrm{dc}})$ would be $u(f_{\mathrm{dc}}) = u(\Delta C_{\mathrm{dc}}) / C_{\mathrm{N\_sample}} = 0.019$.

***

[1] Comment on the memory effect as uncertainty source: Memory effect is problematic first of all in the case of trace analysis and secondly, if the analyte is specifically prone to adsorption on glass or plastic surfaces. In this case, although the concentration of ammonium in the sample is quite low, it is still not yet a true trace analysis. Ammonium ion is by no means a strongly adsorbing species. Therefore we can leave the memory effect as uncertainty source out of consideration.

9.4. Step 4 – Values of the input quantities

Finding the values of the input quantities

http://www.uttv.ee/naita?id=17640

https://www.youtube.com/watch?v=TP8AguK1jSo

The values of Asample, b1, b0 and fd are found from the measured data (b1 and b0 are found from regression analysis). The value of ΔCdc is zero.

9.5. Step 5 – Standard uncertainties of the input quantities

Standard uncertainties of the input quantities

http://www.uttv.ee/naita?id=17641

https://www.youtube.com/watch?v=ZIEhfCoNyxU

Finding standard uncertainties of the input quantities

The standard uncertainty of A_{sample} is found from the following three uncertainty components:

(1) The uncertainty due to the repeatability of photometric measurement:

$$u\,(A_{\text{sample}},\,\text{rep}) = 0.0010\ \text{AU} \qquad (9.2)$$

This uncertainty includes the repeatability of the instrument, repeatability of positioning the cell in the instrument and possible disturbances, such as a random dust particle on the optical windows of the cell.

(2) The uncertainty due to the possible drift of the spectrophotometer parameters:

$$u\,(A_{\text{sample}},\,\text{drift}) = 0.0012\ \text{AU} \qquad (9.3)$$

(3) The uncertainty due to the possible interfering effects:

$$u\,(A_{\text{sample}},\,\text{chem}) = 0.0030\ \text{AU} \qquad (9.4)$$

These can be due to some other compound (interferent) absorbing (or scattering) light at the same wavelength that is used for measurement (leading to increase of the absorbance value) or due to some disturbance in forming the photometric complex (leading to decrease of the absorbance value).

As a result:

$$u(A_{\text{sample}}) = \sqrt{u(A_{\text{sample}},\text{rep})^2 + u(A_{\text{sample}},\text{drift})^2 + u(A_{\text{sample}},\text{chem})^2} = 0.0034\ \text{AU} \qquad (9.5)$$

We see that uncertainty due to possible interference dominates the uncertainty budget of sample absorbance. Although in this example accounting for possible interferences is easy, in reality quantitative evaluation of uncertainty due to possible interference is difficult. Some practical advice on this is given below in the section "Evaluating uncertainty due to possible interference".

The standard uncertainties of the slope b_1 and intercept b_0 are in this example found as standard deviations of the respective regression coefficients (see the XLS files in section 9.7). This is an approximate way of taking into account the uncertainty due to calibration graph and linear regression analysis, because it (1) neglects the systematic effects affecting all the points on the regression line (e.g. weighing and purity of the standard substance) and (2) neglects the negative correlation between b_1 and b_0 (which always exists). The first of these effects leads to some underestimation of uncertainty and the second one leads to some overestimation of uncertainty. So, this approach should only be used if it is not expected that calibration graph and linear regression analysis will be among the main contributors to uncertainty. This usually holds if the standard substance has high purity, can be weighed accurately and if the scatter of data points around calibration graph is not high. [1]

The $u(f_d)$ is found on an assumption that the relative combined standard uncertainty of all involved volumetric operations is not higher than 0.5%. If volumetric operations are carried out carefully then this is a safe assumption under usual laboratory conditions. Considering that the value of f_d is 1.25 (unitless) we get the following:

$$u\ (f_d) = 1.25 \cdot 0.5\% \ / \ 100\% = 0.0065 \text{ (unitless)} \qquad (9.6)$$

The uncertainty of ΔC_{dc} accounts for *possible* decomposition of the photometric complex and *possible* contamination of the sample. The word "*possible*" is stressed here: it is well possible that actually there is neither decomposition of the photometric complex nor contamination of the sample. However, in order to rigorously establish this, extensive research would be needed. Therefore in this example we use an estimate based on experience from our laboratory:

$$u\ (\Delta C_{dc}) = 0.004 \text{ mg/l} \qquad (9.7)$$

The standard uncertainties of the input quantities are summarized in table:

Table 9.1. Standard uncertainties of the input quantities in the measurement model (eq 9.1).

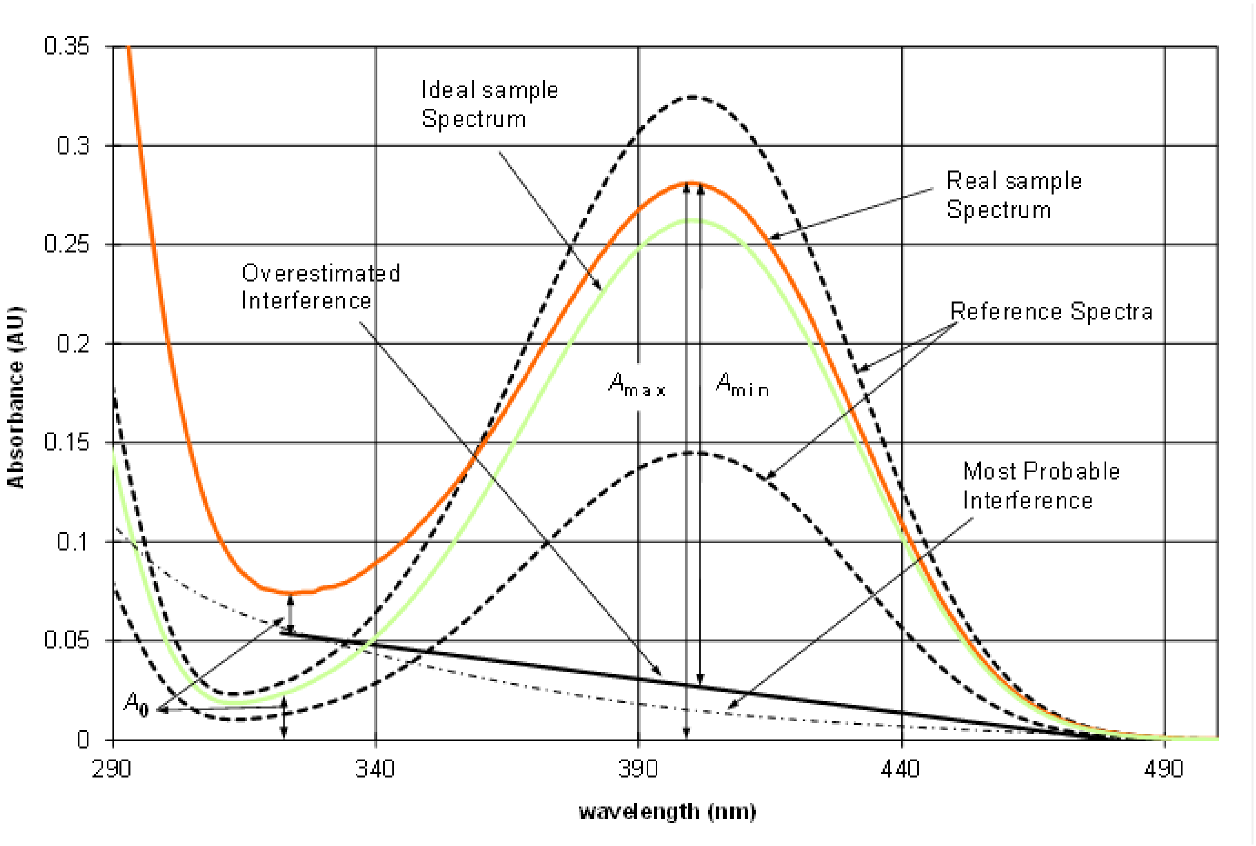
Quantity	Value	u	Unit
A_{sample}	0.1860	0.0034	AU
b_0	0.0171	0.0025	AU
b_1	0.9808	0.0046	AU×l/mg
f_d	1.2500	0.0063	-
ΔC_{dc}	0.0000	0.0040	mg/l

Evaluating uncertainty due to possible interference

There is no universally applicable way of evaluating uncertainty due to possible interference. The most rigorous approach is separately determining the content of the interfering compounds in the sample and correcting the result (then of course the uncertainty of correction has to be evaluated but that is easier). However, in most cases running separate analysis for determining the (possible) interferents is far too labor-intensive for being practical. The matter is further complicated by the limited information that is usually available about the interferents: it is usually not known what the compounds that cause interference are. For this reason, if the interference is not too strong then the usual approach is to try to take it into account by increasing the measurement uncertainty [2] .

The following are two examples how the uncertainty due to (possible) interfering compounds can be taken into account in measurement uncertainty estimation.

1. Obtaining and using interference information from spectra. The following scheme presents a situation where interference is clearly present and can be seen from the appearance of the spectra:



Scheme 9.2. UV-Vis absorbance spectra of calibration solutions (dotted lines), sample solution with interference (red line) and ideal (theoretical) sample solution where no interference is present.

The dotted spectra are the analyte spectra in the calibration solutions (reference spectra) where the correct shape of the spectrum can be seen. The green line corresponds to the "theoretical" – i.e. devoid of any interference – spectrum of the sample solution. Red line corresponds to the actual (experimental) spectrum of the sample solution. The most probable spectrum of the interferent [3] is presented by the dash-dot line. Its shape suggests that it is a sum of a large number of different organic compounds – quite common situation.

The absorbance at 400 nm (wavelength of the absorbance maximum) is the analytical signal. It is obvious from the scheme that when simply measured from the "red" spectrum (A_{max}) it leads to overestimated absorbance. Using the "green" spectrum or the spectrum of the interferent is impossible, because neither of the two is available.

In this situation one can try to estimate the probable maximum and minimum absorbance values (A) of the analyte, corresponding to the probable minimum and maximum, respectively, interference. A good estimate of the maximum A is A_{max} (it corresponds to the not very likely situation that there is no interference at 400 nm and the whole A is due to analyte). For estimating the minimum A we assume linear relation between the interfering effect and wavelength as presented by the solid line tagged "Overestimated interference". It is overestimated in the sense that the absorbance at 400 nm is higher than the probable interference. This line is defined as follows. It is fixed to zero at 480 nm (it is clear that there is no interference at that wavelength). At wavelength 322 nm the line is fixed in such a way that the A difference from the spectrum with interference is the same as the difference of the (estimated) spectrum without interference from zero at 322 nm (both denoted as A_0 on scheme 9.2).

From the values of A_{min} and A_{max} the corrected absorbance (to be used for calculation of the result), as well as its uncertainty component due to interference can be found as follows:

$$A_{\mathrm{corrected}} = \frac{A_{\mathrm{min}} + A_{\mathrm{max}}}{2} \qquad (9.8)$$

$$u(A_{\mathrm{corrected}}, \mathrm{interference}) = \frac{A_{\mathrm{max}} - A_{\mathrm{min}}}{2 \cdot \sqrt{3}} \qquad (9.9)$$

This approach is approximate but its advantage is that it can be used in the case of unknown interferents.

2. Using interference data from previous studies. If validation included interference studies and the interference was quantitatively described then these data can be used for correcting the result and obtaining the uncertainty estimate due to possible interference. As an example, let us look at selectivity data in the standard method ISO 7150:1984 of spectrophotometric ammonium nitrogen determination. Table 9.2. presents the data about the influence of selected interferents.

Table 9.2. Influence of selected interferents on ammonium nitrogen determination according to ISO 7150:1984 (data from ISO 7150:1984).

Interferent, B	C_B (mg/l)	Influence of B on C_N (mg/l) at the following C_N (mg/l) values:		
		0	0.2	0.5
Cl^-	1000	+0.002	+0.013	+0.033
CN^-	5	+0.002	+0.019	+0.016
PO_4^{3-}	100	0	-0.001	-0.015
Ethanolamine	1	+0.16	+0.11	—

Let us take interference from chloride as an example. Suppose we know that our sample may contain chloride and its content is certainly below 800 mg/l and we have found C_N in the sample around 0.2. In that case the maximum interference would be +0.013 x 800 / 1000 = 0.0104 mg/l. If we do not know the amount of chloride in the sample and if determining it separately would be impractical then we can assume that the chloride content is (400 ± 400) mg/l. This uncertainty range embraces the whole concentration range from 0 to 800 mg/l. The interference corresponding to this chloride content would be +0.0052 mg/l and it can be used for correcting the obtained C_N value (by subtracting it from C_N). Its standard uncertainty, if rectangular distribution is assumed is 0.0052 / sqrt(3) = 0.0030 mg/l. this uncertainty should be additionally included in the uncertainty budget.

3. Using interference data via model equation. Sometimes the measurement model can be used directly for taking interference into account. An example of this situation can be found in Self-test 9 C.

***

[1] The same example solved with full rigor is available from http://www.ut.ee/katsekoda/GUM_examples/. Please look at the example "Ammonium by Photometry" with elaboration level "High (uncertainty estimated at full rigor, suitable for experts)". Comparison of the obtained combined uncertainties: 0.00686 mg/l obtained here (section 9.7) and 0.0065 mg/l obtained with full rigor shows that this approach is acceptable, especially since it leads rather to uncertainty overestimation than underestimation.

[2] If the interference is really strong for a specific matrix then analysis procedure should be modified and revalidated.

[3] Approximately this shape of the spectrum is usually observed, if a large number of different compounds are present, each one at very low level. Furthermore, the way the analyte spectrum in the sample is distorted implies exactly this shape of interferent spectrum.

9.6. Step 6 – Value of the output quantity

Calculating the value of the output quantity

http://www.uttv.ee/naita?id=17642

https://www.youtube.com/watch?v=OKzpnlMsjJM

The output quantity value (the measurand value) is calculated from the input quantity values (section 9.4) using the mathematical model (section 9.2). The measurand value in this example is: CN_sample = 0.215 mg/l.

9.7. Step 7 – Combined standard uncertainty

Calculating the combined standard uncertainty

http://www.uttv.ee/naita?id=17643

https://www.youtube.com/watch?v=v6JZT2PkE-M

Numerical calculation of the uncertainty components: the Kragten method

http://www.uttv.ee/naita?id=17721

https://www.youtube.com/watch?v=qRx8cFVitgk

The initial XLS file (i.e. containing only the data but not the calculations) used in this example and the XLS file containing the also the combined standard uncertainty (and expanded uncertainty) calculation according to the Kragten's approach can be downloaded from here:

uncertainty_of_photometric_nh4_determination_kragten_initial.xls	49 KB
uncertainty_of_photometric_nh4_determination_kragten_solved.xls	53 KB

9.8. Step 8 – Expanded uncertainty

The expanded uncertainty can be found at two different levels of sophistication. The simpler approach uses simply a preset *k* value (most often 2) and the actual coverage probability is not discussed. This approach is presented in the first video lecture.

Finding the expanded uncertainty (simpler approach)

http://www.uttv.ee/naita?id=17644

https://www.youtube.com/watch?v=KomDnLRArDs

The second approach is more sophisticated. It is an approximation approach based on the assumption that the distribution function of the output quantity can be approximated by a Student distribution with the effective number of degrees of freedom found by the so-called Welch-Satterthwaite method. This enables then to use the Student coefficient corresponding to a desired level of confidence (coverage probability) as the coverage factor. This approach is explained in the second video lecture.

Finding the expanded uncertainty (the Welch-Satterthwaite method)

http://www.uttv.ee/naita?id=17916

https://www.youtube.com/watch?v=CylWJjG_8ck

The XLS file containing the combined standard uncertainty and expanded uncertainty calculation and the XLS file containing the expanded uncertainty calculation using coverage factor found using the effective number of degrees of freedom form the Welch-Satterthwaite approach can be downloaded from here:

uncertainty_of_photometric_nh4_determination_kragten_solved.xls	53 KB
uncertainty_of_photometric_nh4_determination_kragten_solved_df.xls	48 KB

9.9. Step 9 – Looking at the obtained uncertainty

Calculation of the uncertainty components (uncertainty indexes) is explained and demonstrated in section 9.7.

Analysis of the uncertainty sources

http://www.uttv.ee/naita?id=17645

https://www.youtube.com/watch?v=zE5yi6NwAvI

Conclusions 1

http://www.uttv.ee/naita?id=17647

https://www.youtube.com/watch?v=7VqqLGLQHgg

Conclusions 2

http://www.uttv.ee/naita?id=18096

https://www.youtube.com/watch?v=ENg2aTyBwKg

10. The single-lab validation approach

Brief summary: This section explains the so-called single-lab validation approach. We will look at the formalization of this approach published by Nordtest. [1] Therefore in this course this approach is often called "the Nordtest approach". The single-lab validation approach, contrary to the ISO GUM modeling approach, does not go deeply into the measurement procedure and does not attempt to quantify all uncertainty sources individually. Instead uncertainty sources are quantified in large "batches" via components that take a number of uncertainty sources into account. Most of the data that are used come from validation of the analytical procedure. This is the reason for the word "validation" in the name of the approach. This type of approach is also sometimes called the "top-down" approach.

Sections 10.1 to 10.3 present the Nordtest approach step by step and explain the way of obtaining necessary data. Section 10.4 gives a "roadmap" of the Nordtest approach. Section 10.5 presents a practical example of applying the Nordtest approach in the case of determination of acrylamide in snacks by liquid chromatography mass spectrometry (LC-MS).

- 10.1. Principles
- 10.2. Uncertainty component accounting for random effects
- 10.3. Uncertainty component accounting for systematic effects
- 10.4. Roadmap
- 10.5. Determination of acrylamide in snacks by LC-MS

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[1] *Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories*. B. Magnusson, T. Näykki, H. Hovind, M. Krysell, E. Sahlin. Nordtest technical report 537, ed. 4.0. Nordtest, **2017**. Available on-line from http://www.nordtest.info/wp/2017/11/29/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-4/

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The slides presented in this section are available from here:

single-lab_validation_nordtest_uncertainty.pdf 33 KB

Uncertainty estmation approach based on validation and Quality Control Data “the Nordtest approach"

Ivo Leito
University of Tartu
Institute of Chemistry

2013

Nordtest Technical Report 537, 3rd ed (2011)
http://www.nordtest.info/

Single-laboratory validation approach

Effects contributing to uncertainty

Random | **Systematic**

The two groups of uncertainty contributions are quantified separately and then combined:

$$u_c = \sqrt{u_1^2 + u_2^2}$$

Uncertainty arising from random effects

Uncertainty accounting for *possible* bias

at „long term“ level!

Single lab validation approach: in practice (1)

The main equation:

$$u_C = \sqrt{u(R_W)^2 + u(bias)^2}$$

Within-laboratory reproducibility

This component accounts for the random effects

Uncertainty of the estimate of the laboratory and the method bias

This component accounts for the systematic effects

This and subsequent equations work with absolute and relative values

Nordtest Technical Report 537, 3rd ed (2011)
http://www.nordtest.info/

Absolute vs relative uncertainties: Rules of Thumb

- **At low concentrations (near detection limit, trace level) use absolute uncertainties**
  - Uncertainty is not much dependent on analyte level
- **At medium and higher concentrations use relative uncertainties**
  - Uncertainty is roughly proportional to analyte level
- **In general: whichever is more constant**

Appendix E.4 from Quantifying Uncertainty in Analytical Measurement, EURACHEM/CITAC Guide, Second Edition (2000)
Available from : http://www.eurachem.org/

Single lab validation approach: in practice

Steps of the process:

1. Specify measurand
2. Quantify R_w component ***u*(*R*w)**
3. Quantify bias component ***u*(*bias*)**
4. Convert components to standard uncertainties ***u*(*x*)**
5. Calculate combined standard uncertainty $\boldsymbol{u_c}$
6. Calculate expanded uncertainty ***U***

$u(R_w)$

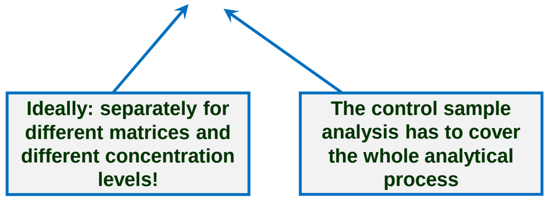
- $u(R_w)$ is the uncertainty component that takes into account long-term variation of results within lab, that means: within-lab reproducibility (s_{Rw})
- Ideally:
  - The same sample
    - Sample similar to test samples – matrix, concentration, homogeneity
  - The same lab
  - The same procedure
  - Different days (preferably over 1 year)
  - Different persons
  - Different reagent batches
  - …

Include sample preparation!

Repeatability <	Within-lab reproducibility <	Combined uncertainty
s_r <	s_{Rw} <	u_c

$u(R_w)$

$$u(R_w) = s_{Rw}$$



Ideally: separately for different matrices and different concentration levels!

The control sample analysis has to cover the whole analytical process

---

u(bias)

- The *possible* **bias** of lab's results from the best estimate of true value is taken into account

Include sample preparation!

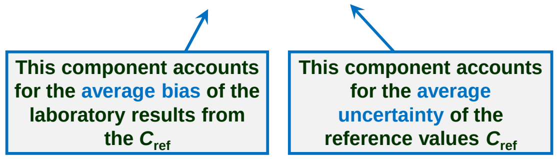
- *u*(*bias*) can be found:
  - From **repeated** analysis of the same samples with a reference procedure
  - From **repeated** analysis of certified reference materials (CRMs)
  - From **repeated** interlaboratory comparison measurements
  - From **repeated** spiking experiments

Ideally: several reference materials, several PTs because the bias will in most cases vary with matrix and concentration range

---

u(bias)

$$u(bias) = \sqrt{RMS_{\text{bias}}{}^2 + u(Cref)^2}$$



This component accounts for the average bias of the laboratory results from the C_{ref}

This component accounts for the average uncertainty of the reference values C_{ref}

---

u(bias)

- The averaging is done using the **root mean square:**

$$bias_i = Clab_i - Cref_i \qquad RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}}$$

$$u(Cref_i) = \frac{s_i}{\sqrt{n_i}} \qquad u(Cref) = \sqrt{\frac{\sum u(Cref_i)^2}{n}}$$

- *n*: the number of bias estimates used
  - If *n* is too small then the bias component will include a large share of random effects and may be overestimated

---

u(bias): only one CRM

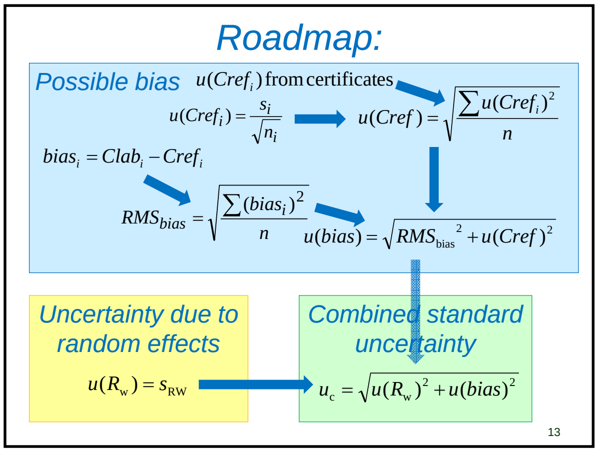
- If only one single CRM is used:

$$u(bias) = \sqrt{RMS_{\text{bias}}{}^2 + s_{\text{bias}}^2 / n + u(Cref)^2}$$

Uncertainty due to possible bias

Evaluation of uncertainty due to bias, ideally:

- **Separately for different sample matrices**
- **Separately for different concentration levels**



Roadmap:

Possible bias

$u(Cref_i)$ from certificates

$$u(Cref_i) = \frac{s_i}{\sqrt{n_i}}$$

$$u(Cref) = \sqrt{\frac{\sum u(Cref_i)^2}{n}}$$

$$bias_i = Clab_i - Cref_i$$

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}}$$

$$u(bias) = \sqrt{RMS_{bias}{}^2 + u(Cref)^2}$$

Uncertainty due to random effects

$$u(R_w) = s_{RW}$$

Combined standard uncertainty

$$u_c = \sqrt{u(R_w)^2 + u(bias)^2}$$

10.1. Principles

In the Nordtest approach the uncertainty is regarded as being due to two components:

1. The **within-lab reproducibility** (intermediate precision) component. This uncertainty component takes into account all uncertainty sources that are **random in the long term** (i.e. several months, preferably one year). So, quite some uncertainty sources that are systematic within a day will become random in the long term. [1]
2. The **bias component**. This component takes into account the systematic effects that cause long-term bias (but not those that just cause bias within a given day). The **long-term bias** can be regarded as sum of procedure bias (bias inherent in the nature of the procedure) and laboratory bias (bias caused by the way how the procedure is implemented in the laboratory).

Introduction to uncertainty estmation based on validation and quality control data (the Nordtest approach)

http://www.uttv.ee/naita?id=17909

https://www.youtube.com/watch?v=9oOX4CUsWjI

The main equation of the Nordtest approach is here:

$$u_c = \sqrt{u(R_w)^2 + u(bias)^2} \qquad (10.1)$$

Here u (R_W) stands for the within-lab reproducibility component of uncertainty and u (*bias*) stands for the uncertainty component taking into account possible bias. The resulting measurement uncertainty u_C is not directly related to any specific result, because it is calculated using data from the past measurements. Therefore it can be said that the uncertainty obtained with the Nordtest approach characterizes the analysis procedure rather than a concrete result. If the uncertainty of a concrete result is needed then it is *assigned* to the result.

Because of this it is necessary to decide whether to express the uncertainty in absolute terms (i.e. in the units of the measured quantity) or in the relative terms (i.e. as a ratio of uncertainty to the value of the measured quantity or a percentage of the value of the measured quantity). The rules of thumb:

At low concentrations (near detection limit, trace level) use absolute uncertainties
Uncertainty is not much dependent on analyte level

At medium and higher concentrations use relative uncertainties
Uncertainty is roughly proportional to analyte level

In general: use whichever is more constant with changing concentration.

The main equation of the Nordtest approach. Absolute and relative quantities

http://www.uttv.ee/naita?id=17911

https://www.youtube.com/watch?v=MH8CixjySjI

Overview of the practical implementation of the Nordtest approach

http://www.uttv.ee/naita?id=17912

https://www.youtube.com/watch?v=hPrncfXr7Ok

The main steps of the process of measurement uncertainty evaluation with the Nordtest approach:

1. Specify measurand
2. Quantify R_W component $\boldsymbol{u(R_\mathrm{W})}$
3. Quantify bias component $\boldsymbol{u(bias)}$
4. Convert components to standard uncertainties $\boldsymbol{u(x)}$
5. Calculate combined standard uncertainty $\boldsymbol{u}_\mathrm{c}$
6. Calculate expanded uncertainty $\boldsymbol{U}$

***

[1] A typical example is titration if new titrant is prepared weekly. Within a given week the titrant concentration is a systematic effect, but in the long term it becomes random, because many batches of titrant will be involved. A similarly typical example is calibration graph if it is prepared daily: every day the possible bias in calibration is a systematic effect, but in the long term it becomes random.

10.2. Uncertainty component accounting for random effects

Estimating the within-lab reproducibility component of uncertainty

http://www.uttv.ee/naita?id=17913

https://www.youtube.com/watch?v=9-803-VViKo

The within-lab reproducibility (intermediate precision, s_{RW}, denoted as $u(R_{\mathrm{W}})$ in the Nordtest guide) component takes into account all uncertainty sources that are random in the long term (i.e. several months, preferably one year). So, quite some uncertainty sources that are systematic within a day will become random in the long term.

This is one of the key points of the within-lab validation approach of uncertainty estimation: **trying to account for as many as possible uncertainty sources via random effects**. This is also an important advantage of the approach, because **as a rule estimating random effects can be done more reliably than estimating systematic effects**. The reason is that for evaluating random effects there is no need for a reference value, but for evaluating systematic effects there is (see section 10.3).

The simplest way to find $u\ (R_{\mathrm{W}})$ is from a number of repeated measurements of a control sample, organized, e.g. as a control chart. Alternatively, the pooled standard deviation approach as explained in section 6 can be used. If this is done then the $u\ (R_{\mathrm{W}})$ can be found based on several different control samples, so that it will be an average value of all of them.

The number of values used for evaluation of $u\ (R_{\mathrm{W}})$ must be sufficiently large. An initial estimate of $u\ (R_{\mathrm{W}})$ can be obtained with 10-15 values but thereafter more data should be collected. Even more importantly, the time period during which the data are collected, must be sufficiently long (at least several months, preferably around a year) so that all the sources of variability in the procedure are taken into account. So, 10 values collected over a five-month time period is a better option than 20 values collected during 1.5 months. If the measurements are done with the same control sample, then it must be available in sufficient amount and must be stable during the time period. Table 6.2 in section 6 gives a compact overview of the requirements of $u(R_{\mathrm{W}})$ (s_{RW}) determination.

Depending on situation $u\ (R_{\mathrm{W}})$ can be used as absolute or relative value.

It is important to stress that $u\ (R_{\mathrm{W}})$ should be estimated separately for different matrixes and different concentration levels.

10.3. Uncertainty component accounting for systematic effects

Estimating the uncertainty component due to possible bias

http://www.uttv.ee/naita?id=17910

https://www.youtube.com/watch?v=hLGZsW_o81o

The component *u* (*bias*) takes into account *possible* bias of the measurement procedure.

Reliably determining the bias of the procedure is not easy for the following reasons:

1. If the random effects are strong then this needs a very large number of measurements. When a limited number of measurements are made then the bias estimate will always contain a contribution from random effects, which will make the bias estimate artificially higher. Even more so – the procedure can actually have no bias at all.
2. Even with formally similar matrixes the bias can differ by magnitude and even by sign (e.g. when determining pesticides in different varieties of apples, determining drug residues in blood plasma from different patients, etc). This means that having determined the bias in one variety of apples this bias is not automatically applicable for another variety and we can speak about unceteinty in applying the bias to another variety.
3. Bias is always determined against a reference value, which also has an uncertainty.

This is why we speak about *possible* bias and the uncertainty component *u* (*bias*) quantifies our limited knowledge about bias.

Bias refers to difference between our measured value and a reference value. Therefore, for finding *u* (*bias*) we need a sample or a material with a reference value. In broad terms there are four different possibilities how the *u* (*bias*) can be determined:

Possibility of determining *u* (*bias*)	**Pros**	**Cons**
Analysing the sample with a reference analysis procedure	Bias can be determined very reliably, because the determined bias corresponds to the bias with exactly the same sample matrix as is in the real sample.	It is usually very difficult to find a suitable reference procedure. Therefore this possibility is not often used at routine labs.
Certified reference material (CRM) [1]	Bias can be determined quite reliably, because the reference values of CRM-s are generally quite reliable.	Availability of certified reference materials is limited and their matrixes are often better homogenised than in the case of real samples, leading to somewhat optimistic bias estimates.
Using samples of interlaboratory comparisons as reference samples	Is often usable at routine labs, because labs usually participate in interlaboratory comparisons with the procedures that	The reference values are mostly derived from participant results and have therefore low reliability (high uncertainty). This leads to overestimated *u* (*bias*).
Using spiking studies	Can be done at the laboratory and can be done with the real samples, thereby exactly matching the matrix.	The main problem in bias determination by spiking is dispersing the analyte in the sample in the same way as the native analyte in the sample. In the case of inhomogenous matrixes this can be very difficult.

In the case of all four possibilities it is critical to include also sample preparation in bias determination.

In the case of all four possibilities it is necessary to make an as large as possible number of replicate measurements in order to separate the bias from random effects as efficiently as possible. Bias generally changes from matrix to matrix and usually is different at different concentration levels. So, it is important to use several CRMs, several interlaboratory comparisons, etc.

The lower is the reliability of the reference value the higher is the *u* (*bias*) estimate.

The *u* (*bias*) component is found according to the following equation:

$$u(bias) = \sqrt{RMS_{\text{bias}}{}^{2} + u(Cref)^{2}} \qquad (10.2)$$

RMS_{bias} is the average (root mean square) bias and is found as follows:

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}} \qquad (10.3)$$

Where n is the number of bias determinations carried out and each $bias_i$ is a result of an individual bias determination and is found as follows:

$$bias_i = Clab_i - Cref_i \qquad (10.4)$$

Where $Clab_i$ is a mean of the results of analyte determination in the reference sample (e.g. in the CRM) obtained by the laboratory and $Cref_i$ is the reference value of the reference sample. It is important that $Clab_i$ corresponds to a number of replicates.

u (*Cref*) is the average standard uncertainty of the reference values of the reference samples and is found as follows:

$$u(Cref) = \sqrt{\frac{\sum u(Cref_i)^2}{n}} \qquad (10.5)$$

Here u ($Cref_i$) is the standard uncertainty of the i-th reference value. In the case of CRM analysis, spiking or analysis with a reference procedure the u ($Cref_i$) can usually be reasonably found. However, in the case of interlaboratory comparisons where the consensus value of the participants is used as the reference value a reliable uncertainty of the reference value cannot be found. The best estimate in that case would be the standard deviation of the average value after elimination of outliers:

$$u(Cref_i) = \frac{s_i}{\sqrt{n_i}} \qquad (10.6)$$

Here s_i is the standard deviation of the participants in the i-th intercomparison after elimination of outlayers and n_i is the number of participants (again after eliminating the outliers) in the i-th intercomparison.

In the special case if a number of bias determinations were carried out using one single CRM the equation 10.2 changes into the following form:

$$u(bias) = \sqrt{RMS_{\text{bias}}{}^{2} + s_{\text{bias}}^{2} / n + u(Cref)^{2}} \qquad (10.7)$$

Where s_{bias} is the standard deviation of the bias estimates obtained and n is the number of bias estimates obtained.

Depending on situation the *u*(*bias*) can be used as absolute or as relative value.

Finally, it is important to stress that the bias uncertainty component should be estimated separately for different matrixes and different concentration levels.

***

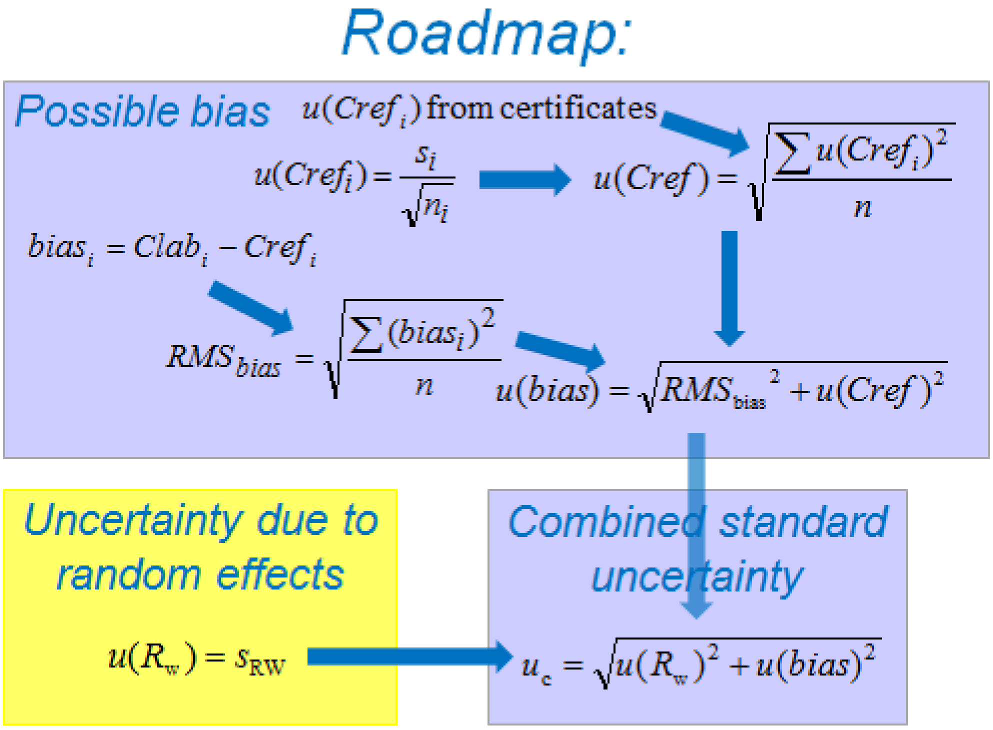
[1] In simplified terms certified reference material is a material, in which the content of the analyte (or analytes) is reliably known (the material has a certificate). If the certified reference material's matrix is similar to real samples (i.e. it is not a pure compound or a solution but is e.g. milk powder, soil or blood plasma) then we call it certified matrix reference material.

10.4. Roadmap

Roadmap of uncertainty estimation using the Nordtest approach

http://www.uttv.ee/naita?id=17914

https://www.youtube.com/watch?v=KNjUAiq5mEQ



Roadmap:

Possible bias

$u(Cref_i)$ from certificates

$$u(Cref_i) = \frac{s_i}{\sqrt{n_i}} \qquad u(Cref) = \sqrt{\frac{\sum u(Cref_i)^2}{n}}$$

$$bias_i = Clab_i - Cref_i$$

$$RMS_{bias} = \sqrt{\frac{\sum (bias_i)^2}{n}} \qquad u(bias) = \sqrt{RMS_{bias}^{\ 2} + u(Cref)^2}$$

Uncertainty due to random effects

$$u(R_w) = s_{RW}$$

Combined standard uncertainty

$$u_c = \sqrt{u(R_w)^2 + u(bias)^2}$$

Scheme 10.1. Roadmap of measurement uncertainty estimation using the single-lab validation approach (the Nordtest approach).

10.5. Determination of acrylamide in snacks by LC-MS

Nordtest approach in practice: Determination of acrylamide in snacks by LC-MS [1]

http://www.uttv.ee/naita?id=18163

https://www.youtube.com/watch?v=P94xWjC6Og0

Some comments on this example:

1. We use relative uncertainties in this example. The reason is that the concentration in these samples is quite high and in LC-MS analysis variability of the results is often roughly proportional to the values of the results.

2. In this example we assume that the matrixes of the used CRMs – potato chips and crisp bread – are sufficiently similar. This means that both reproducibility and bias obtained with these matrixes are similar. In such case the calculated uncertainty is applicable to both of these matrixes. If afterwards we will analyse some matrix that is different from potato chips and crisp bread then we cannot apply to that result the uncertainty estimate that we obtained here. Assessing whether a new matrix is sufficiently similar to the one used for bias evaluation is usually based on experience.

3. The uncertainty estimate $u_{\mathrm{c}}\mathrm{rel}$ that we have obtained is a relative uncertainty, so it is assigned to a result C_{A} by the following formula:

$$u_{\mathrm{c}}(C_{\mathrm{A}}) = C_{\mathrm{A}} \frac{u_{\mathrm{c}}\mathrm{rel}}{100\,\%} \qquad (10.8)$$

In principle it can be assigned to a result of any magnitude, however, it would not be correct to apply this uncertainty to analysis results that are very different from the one presented here. The difference should not be higher than 3-4 times.

When we use the Nordtest approach for uncertainty estimation then in general we can assume that the number of degrees of freedom is sufficiently large so that the k = 2 uncertainty can be assumed to have roughly 95% coverage probability.

***

[1] Please note that in the tables of the slide "Measurements with the CRMs" the measurement units must be μg/kg, not mg/l.

The slides of this example and the calculation files – both the initial and the solved file – are available from here:

nordtest_uncertainty_example_acrylamide_lc-ms.pdf	153 KB
nordtest_uncertainty_example_acrylamide_lc-ms_initial.xls	32 KB
nordtest_uncertainty_example_acrylamide_lc-ms_solved.xls	33 KB

11. Comparison of the approaches

Brief summary: This section summarized the main properties of the uncertainty estimation approaches, their advantages and drawbacks.

Whenever possible, one of the so-called single-lab approaches should be used. The interlaboratory approaches are only suitable for getting very crude uncertainty estimates. We recommend using them only in case when the laboratory actually does not have the measurement in place yet and wants to know, approximately what uncertainty can be obtained.

If the laboratory has competence and time to carry out investigations of the analytical procedures the the modeling approach is often suitable. If the laboratory has limited time, but has validation and quality control data then the single-lab validation approach is the most suitable.

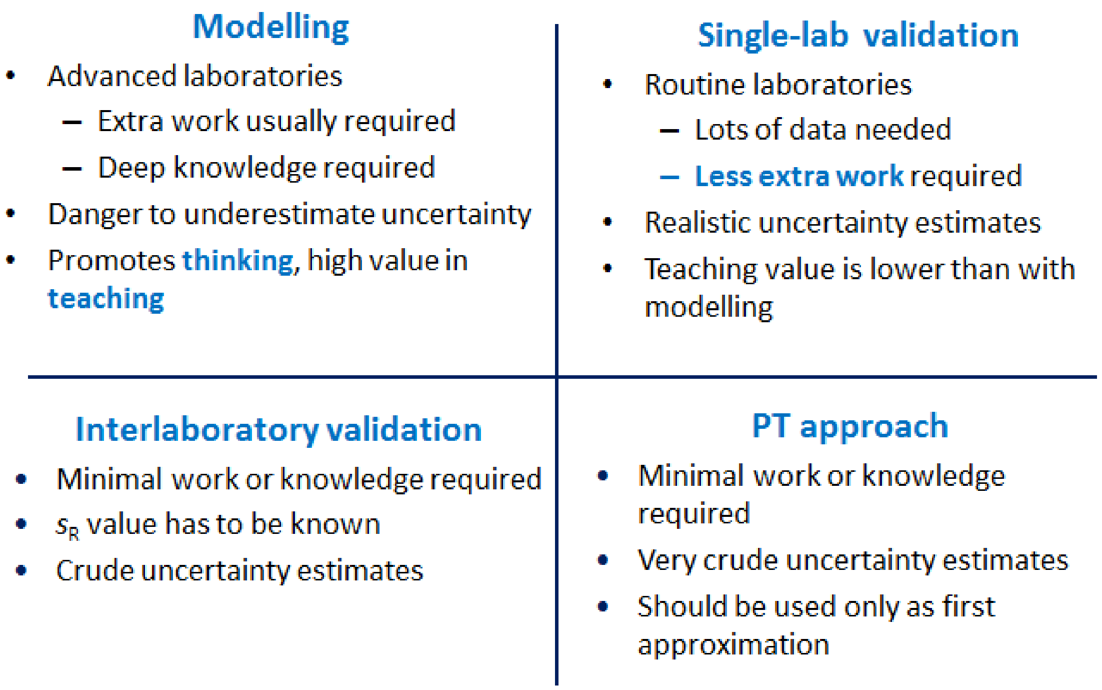
Comparison of measurement uncertainty estimation approaches

http://www.uttv.ee/naita?id=17917

https://www.youtube.com/watch?v=TlpJ1c-9Rx8

Table 11.1 summarizes the pros and cons of the approaches. See also Table 8.1 in section 8 for some key differences.

Table 11.1. Comparison of the measurement uncertainty estimation approaches.



Modelling

- Advanced laboratories
  - Extra work usually required
  - Deep knowledge required
- Danger to underestimate uncertainty
- Promotes **thinking**, high value in **teaching**

Single-lab validation

- Routine laboratories
  - Lots of data needed
  - **Less extra work** required
- Realistic uncertainty estimates
- Teaching value is lower than with modelling

Interlaboratory validation

- Minimal work or knowledge required
- s_R value has to be known
- Crude uncertainty estimates

PT approach

- Minimal work or knowledge required
- Very crude uncertainty estimates
- Should be used only as first approximation

12. Comparing measurement results

Brief summary: This section explains that measurement uncertainty estimates are indispensable if we want to compare two measurement results, both having uncertainty.

Comparing measurement results using measurement uncertainty estimates

http://www.uttv.ee/naita?id=18095

https://www.youtube.com/watch?v=l6nYn6Pe7f0

Additional information. The above presented approach works well when comparing two results, both having uncertainty, with the aim of determining agreement between them. The approach can be to some extent used also in the case when one of the results has uncertainty estimate available but the second one does not. In that case the uncertainty of that second result can be taken as zero. This makes the test stricter. I.e., if the obtained zeta score still shows agreement then the results are in agreement, whatever the actual uncertainty of the second result. If the test shows disagreement or doubtful situation, then it is impossible to say whether there is agreement or not.

However, the presented approach is not suitable for another very important kind of comparison: deciding, whether a measured value (having uncertainty) is above or below a legal limit. In other words: compliance assessment. Probably the best approach to assess compliance of a result having uncertainty with permissible limits is the one presented in this Eurachem guide: Use of uncertainty information in compliance assessment. (2nd ed. 2021).

Very briefly, for deciding if an item can be accepted (is compliant) or rejected (is not compliant) it is necessary to:

1. Have analysis result with measurement uncertainty;
2. Have a specification giving the upper and/or lower permitted limits;
3. Define decision rules that describe how the uncertainty will be taken into account in compliance assessment. Such rules typically define acceptance and rejection zone limits and the respective probabilities of the actual values of measurands being within or outside the limits.

In some fields, for helping compliance assessment, the so-called target measurement uncertainty is defined. This is the maximum permissible uncertainty for the specific type of measurement or analysis.

13. Additional materials and case studies

Brief summary: This section collects some additional materials, examples and case studies on specific chemical analysis techniques.

13.1. Different analytical techniques
13.2. Measurement uncertainty estimation in dissolved oxygen determination
13.3. Coulometric KF titration

Related materials:
"100+ Interesting experiments in chemistry"

13.1. Different analytical techniques

A large number of measurement uncertainty estimation examples (example uncertainty budgets) is available from the following address: http://www.ut.ee/katsekoda/GUM_examples/

13.2. Measurement uncertainty estimation in dissolved oxygen determination

Dissolved oxygen (DO) is one of the most important dissolved gases in water. Sufficient concentration of DO is critical for the survival of most aquatic plants and animals [3] as well as in waste water treatment. DO concentration is a key parameter characterizing natural and wastewaters and for assessing the state of environment in general. Besides dissolved CO_2, DO concentration is an important parameter shaping our climate. It is increasingly evident that the concentration of DO in oceans is decreasing [4 - 7].

Accurate measurements of DO concentration are very important for studying these processes, understanding their role and predicting climate changes.

Electrochemical and optical sensors are the most widespread means of DO concentration measurement. Both are widely used but the effects of different uncertainty sources on the results are remarkably different and estimation of uncertainty is not straightforward. In order to help practitioners with this, **comprehensive comparative validation for these two different types of dissolved oxygen (DO) analyzers, amperometric and optical**, was recently carried out on the basis of two representative commercial DO analyzers and published: I. Helm, G. Karina, L. Jalukse, T. Pagano, I. Leito, *Environ Monit Assess* **2018**, *190*:313 (ref [1]).

A number of performance characteristics were evaluated including drift, intermediate precision, accuracy of temperature compensation, accuracy of reading (under different measurement conditions), linearity, flow dependence of the reading, repeatability (reading stability), and matrix effects of dissolved salts. The matrix effects on readings in real samples were evaluated by analyzing the dependence of the reading on salt concentration. The analyzers were also assessed in DO measurements of a number of natural waters. The uncertainty contributions of the main influencing parameters were estimated under different experimental conditions. It was found that the uncertainties of results for both analyzers are quite similar but the contributions of the uncertainty sources are different. The results imply that the optical analyzer might not be as robust as is commonly assumed, however, it has better reading stability, lower stirring speed dependence, and typically requires less maintenance. On the other hand, the amperometric analyzer has a faster response and wider linear range. The approach described in this work will be useful to practitioners carrying out DO measurements for ensuring reliability of their measurements.

The Winkler titration method is considered the most accurate method for DO concentration measurement. Careful analysis of uncertainty sources relevant to the Winkler method was carried out and the results are presented as a **„Report on improved high-accuracy Winkler method for determination of dissolved oxygen concentration“**.

In that report it is described how the Winkler method was optimized for minimizing all uncertainty sources as far as practical. The most important improvements were: gravimetric measurement of all solutions, pre-titration to minimize the effect of iodine volatilization, accurate amperometric end point detection and careful accounting for dissolved oxygen in the reagents. As a result, the developed method is possibly the most accurate method of determination of dissolved oxygen available. Depending on measurement conditions and on the dissolved oxygen concentration the combined standard uncertainties of the method are in the range of 0.012 – 0.018 mg dm^{-3} corresponding to the k = 2 expanded uncertainty in the range of 0.023 – 0.035 mg dm^{-3} (0.27 – 0.38%, relative). This development enables more accurate calibration of electrochemical and optical dissolved oxygen sensors for routine analysis than has been possible before. Most of this report is based on the article I. Helm, L. Jalukse, I. Leito, Anal. Chim. Acta. **2012**, *741*:21-31 (ref [2]).

The contents of this on-line course can be used as basis for carrying out the measurement uncertainty evaluation described in the above mentioned report. In particular, the Self-test 9.2 A and Self-test 9 B are directly related to DO concentration measurement.

Preparation of the above mentioned report was supported by the European Metrology Research Programme (EMRP), project ENV05 "Metrology for ocean salinity and acidity".

EMRP
European Metrology Research Programme
▶ Programme of EURAMET

The EMRP is jointly funded by the EMRP participating countries
within EURAMET and the European Union

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g_winkler_report_280613.pdf 1.07 MB

13.3. Coulometric KF titration

Active ingredients in pharmaceuticals, carbon-fiber composites, polymers, novel cellulose-based active paper, food powders, biomass – all of these and many other solid materials are highly affected by moisture when processing into various products. Errors and inconsistencies in moisture measurement and control in industrial processes lead to decreased process speed/throughput and increased wastage, shortened durability of biomaterials, increased energy consumption in drying and increased fine particle emissions in biomass combustion.

Coulometric Karl Fischer method is currently the most accurate method of moisture measurement. A **Survey of the factors determining the uncertainty of coulometric Karl Fischer titration method** has been carried out.

This survey gives an overview of the factors that determine the uncertainty of coulometric Karl Fischer (cKF) method for water determination. Distinction is made between uncertainty sources originating from the cKF method itself and uncertainty sources due to sample handling. The "compound" uncertainty sources – repeatability, reproducibility and bias that actually incorporate the contributions from these two classes of uncertainty sources – are also briefly discussed.

Based on the literature data the most influential uncertainty sources of coulometric KF titration method are possible chemical interferences, instrument instability and the accuracy of the end point determination. The uncertainty sources due to sample handling are more problematic with solid samples than with liquid samples. The most important sample preparation related uncertainty sources are change of water content in the sample before the measurement and incomplete transfer of water from the sample to the reaction vessel.

The general conclusion is that although the uncertainty sources of the cKF method are in general rather well known the discussion is almost always either qualitative or limited to the compound uncertainty sources and there is very limited quantitative information available on the contributions of the actual uncertainty sources.

Preparation of this report was supported by the European Metrology Research Programme (EMRP), project SIB64 METefnet "Metrology for Moisture in Materials".

EMRP
European Metrology Research Programme
Programme of EURAMET

The EMRP is jointly funded by the EMRP participating countries within EURAMET and the European Union

coulometric_kf_titration_measurement_uncertainty_sources_survey.pdf 227 KB

14. Tests and Exercises

This section contains a compilation of all the tests and exercises of this course.

The concept of measurement uncertainty (MU) - Self-test 1
The origin of measurement uncertainty - Self-test 2
The Normal distribution - Self-test 3.1
Mean, standard deviation and standard uncertainty - Self-test 3.2
Standard deviation of the mean - Self-test 3.4
Rectangular and triangular distribution - Self-test 3.5
The Student distribution - Self-test 3.6
Quantifying uncertainty components - Self-test 4.1
Calculating the combined standard uncertainty - Self-test 4.2
Looking at the obtained uncertainty - Self-test 4.3
Expanded uncertainty - Self-test 4.4
Presenting measurement results - Self-test 4.5
Sources of uncertainty - Self-test 5.3
Treatment of random and systematic effects - Self-test 5.4
Random and systematic effects revisited - Self-test 6
Precision, trueness, accuracy - Self-test 7
Overview of measurement uncertainty estimation approaches - Self-test 8
Model equation - Self-test 9.2 A and Self-test 9.2 B
Standard uncertainties of the input quantities - Self-test 9.5
The ISO GUM Modeling approach - Self-test 9 A and Self-test 9 B
Uncertainty component accounting for random effects - Self-test 10.2
Uncertainty component accounting for systematic effects - Self-test 10.3
Determination of acrylamide in snacks by LC-MS - Self-test 10.5 A and Self-test 10.5 B
Comparison of the approaches - Self-test 11

Frequently asked questions

0. Please see this paper: Leito, I., Helm, I. Metrology in chemistry: some questions and answers. *J.Chem.Metrol.* **2020**, 14:2, 83-87 **for a number of questions and answers relevant to practical chemical analysis situations.**

---

1. How many decimal places should we leave after comma when presenting results?

The number of decimals after the comma depends on the order of magnitude of the result and can be very different. It is more appropriate to ask, how many significant digits should be in the uncertainty estimate. This is explained in the video in section 4.5. The number of decimals according to that video is OK for the results, unless there are specific instructions given how many decimals after the point should be presented. When presenting result together with its uncertainty then the number of decimals in the result and in uncertainty must be the same.

---

2. If we need to find standard deviation of those wihtin-lab reproducibility measurments, then we need certainly use the pooled one? We can not take the simpliest standard deviation, which is calculated by standard deviationformula?

The within-lab reproducibility standard deviation s_{RW} characterises how well can the measurement procedure reproduce the same results on different days with the same sample. If the sample is not the same (as in this self-test) then if you just calculate the standard deviation of the results then the obtianed standard deviation includes both the reproducibility of the procedure and also the difference between the samples. The difference between the samples is in the case of this self-test much larger than the within-lab reproducibility. So, if you simply calculate the standard deviation over all the results then you will not obtain within-lab reproducibility but rather the variability of analyte concentrations in samples, whith a (small) within-lab reproducibility component added.

---

3. In estimation of uncertainty via the modelling approach: When when we can use the Kragten approach and when we just use the combination of uncertainties?

In principle, you can always use the Kragten approach. However, if the relative uncertainties of the input quantities are large, and especially if such a quantity happens to be in the denominator, then the uncertainty found with the Kragten approach can differ from that found using equation 4.11. This is because the Kragten approach is an approximative approach.

---

4. Exactly what is human factor? I thought that it may be for example person's psychological conditions and personal experience and so on? This will definitely influence measurement, but is this taken into account then?

The "human factor" is not a strict term. It collectively refers to different sources of uncertainty that are due to the person performing the analysis. These uncertainty sources can either cause random variation of the results or systematic shift (bias). In the table below are some examples, what uncertainty sources can be caused by the "human factor". In correct measurement uncertainty estimation the "human factor" will be automatically taken into account if the respective uncertainty sources are taken into account.

Uncertainty source	Type	Taken into account by
Variability of filling a volumetric flask to the mark, variability of filling the pipette to the mark	Random	Repeatability of filling the flask/pipetting
Systematically titrating until indicator is very strongly coloured	Systematic (causes systematically	Uncertainty of the titration end-point

	higher titration results)	determination
Systematically grinding the sample for shorter time than should be done, leading to less dispersed sample and lowered recovery	Systematic	Uncertainty due to sample preparation (uncertainty due to recovery)

5. Can we report as V = (10.006 ± 0.016) mL at 95 % CL at coverage factor of 2?

We use in this course the conventional rounding rules for uncertainty. Therefore uncertainty ±0.0154 ml is rounded to ±0.015 ml. Sometimes it is recommended to round uncertainties only upwards (leading in this case to ±0.016 ml). However, in the graded test quizes please use the conventional rounding rules.

6. How can I attach my photo into Moolde profile?

This is done from your profile in Moodle. Click on your name on the right on the status bar, then click "Profile", then "Edit profile".

7. In case of a simple titration, if replicate titrations are carried out then in the uncertainty of pipetting the uncertainty contribution of repeatability is omitted. Why we ignore the repeatability effect in this case when calculating the result of the titration?

In this case we have results of repeated titrations. Their scatter is caused among other effects also by pipetting repeatability. I.e. one of the reasons why different amounts of titrant were consumed in replicate titrations is the fact that the amount of pipetted acidic liquid slightly differed from titration to titration. For this reason, the repeatability of consumed titrant volume automatically takes into account also pipetting repeatability. If we would take it into account in the uncertainty of pipetted volume, we would account for it two times.

8. Can systematic effects really count as uncertainty sources? The GUM says that the recognized systematic effects should be corrected for and the uncertainties of the resulting corrections should be taken into account.

Indeed, systematic effects (sources of bias) can often be reduced significantly by determining corrections and applying them. The corrections are never perfect and have uncertainties themselves. However, the resulting uncertainties from corrections will be mostly caused by different random effects.

However, the fact that systematic effects influence measurement results, automatically means that they cause uncertainty and are thus uncertainty sources. Furthermore, although the GUM (https://www.bipm.org/en/publications/guides/gum.html) says that known systematic effects should preferably be corrected, in many cases – in particular in chemistry and especially at routine lab level – correcting for the systematic effects is either impossible to do reliably or is not practical, as it would make the measurement much more expensive. It also is often unclear whether a systematic effect exists at all – in this course we often speak about possible systematic effects. As a conclusion, it is often more practical to include the possible systematic effects as additional uncertainty components, rather than try to correct for all of them. Probably the best practical guide on this issue is the Eurachem leaflet Treatment of observed bias (https://www.eurachem.org/index.php/publications/leaflets/bias-trt-01).

9. What is the difference between confidence interval and measurement uncertainty?

Measurement uncertainty defines a range (also called interval), around the measured value where the **true value** of the measurand lies in with some predefined probability. This interval is called **coverage interval** and measurement uncertainty is (usually) its half-width. Coverage interval has to take into account all possible effects that cause uncertainty, i.e. **both to random and systematic effects**.

Confidence interval is somewhat similar to coverage interval. It typically refers to some statistical interval estimate. It expresses the level of confidence that the true value of a certain statistical parameter resides within the interval. A typical example is the confidence interval of a **mean value** found from a limited number of replicates, which is calculated from the standard deviation of the mean and the respective Student coefficient. The main difference is that we speak only of the mean value, not the true value, and **only random effects** are accounted for – i.e. all replicate measurements can be biased but the confidence interval does not account for that in any way.

---

10. What is the basis for the rule (explained in Section 4.5) that when the first significant digit of uncertainty is 1 .. 4 then it is presented with 2 significant digits and when it is 5 .. 9 then it is presented with one significant digit?

The rationale behind this rule is that the uncertainty should change by less than 10%, relative, when rounding it. If uncertainty would be e.g. 0.15 g then rounding it to 0.2 would change it by 33%. At the same time if it is e.g. 0.55 g then by rounding it to 0.6 g would change it by 9% relative.

---

11. The true value lies within the uncertainty range with some probability. Therefore, is it OK if it is sometimes outside that range?

The situation that the true value is outside the uncertainty range is not impossible, but its probability is low. If it is strongly outside (i.e. far from the uncertainty range) or if it is outside for several measurement results obtained with the same method during a short period then the most probable reason is underestimated uncertainty.

Of course, we (almost) never know the true value, so instead of true values we usually operate with their highly reliable estimates, such as e.g. certified values of certified reference materials.

---

12. Why do we used two-tailed t values in calculating expanded uncertainty, not one-tailed values?

One-tailed t values would be justified if we would know for sure that the true value is smaller or larger than our measured value. This is usually not the case and thus it is not justified to use one-tailed values. One-tailed values are also smaller than two-tailed values (for example: ca 1.7 vs ca 2.0, in the case of large number of degrees of freedom and 95% coverage probability), so that the use of one-tailed t values would artificially decrease the uncertainty estimate, possibly leading to underestimated uncertainty.

---

13. When converting from rectangular or triangular distribution to the Normal distribution, where do the rules of dividing by SQRT(3) and SQRT(6) come from?

This is clearly beyond the scope of our course. This derivation can be found in specialised books, e.g.: Rein Laaneots, olev Mathiesen An Introduction to Metrology Tallinn University of Technology press, Tallinn, 2006.

Unfortunately I do not have a freely available source in English. There is one in Estonian: http://tera.chem.ut.ee/~ivo/metro/Room/II_vihik.pdf The derivation is on pages 12-13. You will probably understand the mathematical equations and you can try to translate the text with Google translator.

---

14. Please explain regarding triangular and rectangular distribution function with some real laboratory examples. What is the concept that this is triangular and this is rectangular distribution?

There are in broad terms two types of situation where rectangular or triangular distribution are used:

--1-- When the quantity under question is indeed distributed according to these distributions. In chemistry this occurs first of all in the case of rounding of digital reading. Example: if a thermometer shows 22 °C then, because of rounding, the value could be anywhere between 21.5 and 22.5 °C. If rounding uncertainty is the dominant uncertainty component, then we could say that this temperature is distributed according to rectangular distribution. It can be shown that if two rectangularly distributed quantities (with equal uncertainty) are added or subtracted then the resulting quantity is distributed according to triangular distribution.
These were examples of situations when these distribution functions are “real”.

--2-- It is, however, much more common that these distribution functions are “assumed” or “postulated” (see Section 3.5). This need comes whenever you need to use some uncertainty estimate that is presented in the form “± X” and we have no knowledge of the underlying distribution of that quantity. In such a case we usually recommend to assume rectangular distribution, as it is safer (lower probability of underestimating uncertainty) than assuming triangular distribution. Examples can be: calibration uncertainties of volumetric ware, uncertainties of purchased standard solution concentrations, uncertainty due to possible interferents (see Section 9.5), uncertainties of educated guesses/expert opinions, uncertainties of various systematic effects of measurement instruments, etc. The course materials contain quite some examples on the use of these distributions, as well as self-tests. Please see sections 3.5, 4.1, 9.5 and self-tests 3.5, 9 A, 9 B.

---

15. Does failing at even one graded test quiz means total failure (eventhough the rest of the quizzes are successful) and the participant do not receive the digital certificate of completion?

Exactly, failing one graded test means failing the whole course and not getting the certificate of completion for this edition of the course. But of course, you are welcome to attend again next year.

Failing one test means, that you have not acquired the whole knowledge that you should have acquired form this course and the learning outcomes are not fulfilled. For an analogous example, should you get the driver license, when you know really well how to change the gear, but steering the wheel would be an obstacle for you? Probably not – for successful driving you need to be able to handle all aspects of controlling the car. It is the same with uncertainty.

Therefore, we strongly suggest not to waste attempts. For this, before starting a new attempt, please try with the last dataset to obtain the answer provided by the system and find your mistake.

---

16. Is it always preferable to use your own calibration data of volumetric instruments?

This depends on how high accuracy of volumetric measurement you need.

If high accuracy of volumetric measurements is needed, then it is more correct to calibrate it by herself/himself. Why? Because the uncertainty of the calibration consists to a large extent of the so-called “human factor”. So calibration and working manners should be the same. If more people use the same glassware, then everyone should calibrate it for herself/himself, e.g. person X should not use the pipette with the calibration data, obtained by person Y.

If high accuracy of volumetric measurement is not needed (i.e. if in the used method much more uncertainty comes from other sources than volumetric measurement) then usually the uncertainties assigned to glassware by manufacturers are sufficiently low.

---

17. Is "Incomplete sample matrix decomposition during digestion" a systematic or a random effect?

“Incomplete sample matrix decomposition during digestion” causes a systematic effect. Your result will always be somewhat lower than it should be, because you effectively lose some analyte.

But it is important to add, that the "extent of incompleteness" will almost certainly vary from sample to sample. So, you always get a lower result (and there is a systematic effect), but sometimes it is "more lower" sometimes "less lower". This means that there is additionally a random effect "sitting on top" of the systematic effect. This is actually quite common that with analyte losses by decomposition or incomplete extraction, etc or analyte addition by contamination and other similar systematic effects there are accompanying (and often quite large) random effects.

18.How can we check the validity of our uncertainty estimates? Can we use $s_{RW} < u_c$ or PT z-score < 2 as criteria?

The best check for the validity of your uncertainty estimate is to compare with an independent result obtained for the same sample. Very common is, e.g. analyzing a CRM and then comparing your result with the reference value of the CRM, e.g. using the zeta score as described in Section 12. Also, if you participate in a PT then comparing your result with the PT consensus value is useful. In the case of a PT the consensus values usually do not have uncertainty estimates. Then a simple, although not 100% rigorous, approach is to see, whether the consensus value is within the k = 2 uncertainty range of your result.

Concerning the two ways proposed by you: Just the fact that $s_{RW} < u_c$ does not say that u_c has been correctly estimated. It can still be underestimated (or overestimated). And z-scores of PTs do not say anything about the validity of your uncertainty estimate. But of course z-scores are still useful for getting an idea, how similar your measurement result is compared to other laboratories.

---

19. I understand the different sources of uncertainty well in the example, but it strikes me that the standard deviation value is used to calculate the repeatability uncertainty and then re-taken into account in calculating the calibration uncertainty, it is an over estimate of uncertainty?

Repeatability indeed influences pipetting two times: once when the pipette is calibrated and the second time when actual pipetting is done. So, indeed, it has to be accounted for in both cases.

However, as you could see, repeatability is taken into account differently. In the case of the actual pipetting you take it into account as the standard deviation of an individual measurement. In the case of calibration – as standard deviation of the mean. The more individual measurements have been done for a pipette calibration, the more reliable is the correction value. Therefore, also the uncertainty of calibration is smaller: we use the standard deviation of the mean for calibration uncertainty and standard deviation of the mean is dependent on the number of individual measurements. Moreover, calibration uncertainty given by the manufacturer is usually much higher: in our example in Section 4.6 it is approximately 10 times higher than the one we have obtained.

---

20. I cannot figure out how the standard deviation of b_1 and b_0 are calculated. the solved excel file in section 9.7 has the same formula for the $s(b_1)$ and $s(b_0)$ in all of the cells. "=LINEST(C7:C11,B7:B11,1,1)"

The calculation in the original file is carried out with the LINEST function. It is a peculiar function in that it returns a matrix (i.e. a small table of values), not a single value.

Its usage is quite well described in the Excel help. Let me give here just the main steps:

(1) Mark the matrix area – two columns, three rows.

(2) Immediately start typing the function, a la "=LINEST(C7:C11,B7:B11,1,1)" (without quotation marks). Instead of commas "," you may need to use dot-commas ";" as separators, depending on your language settings. C7:C11 stand for analytical signals, B7:B11 stand for concentrations. The "1" and "1" are for not forcing intercept to zero and giving full set of data about the regression.

(3) While typing, the typed text will go to just one of the marked 6 cells and this is OK. It is also not important, to which of them.

(4) Press CTRL-SHIFT-ENTER. (Not just ENTER!)

(5) The sample file uncertainty_of_photometric_nh4_determination_kragten_initial.xls in Section 9.7 shows, which parameters are in which cells.

Now you have an “automatic” function which is linked to the calibration data: every time you change something in the calibration data, all regression parameters are immediately recalculated.

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21. Does the density of most liquids decrease with temperature? From the context, would the "density" parameter refer to the amount (in mass) of the liquid or to its volume that affects the pipette's delivered volume?

Yes, in case of most liquids, the density decreases when the temperature increases. The main idea behind “uncertainty of volume due to temperature” is that in almost all practical cases in analytical chemistry liquid volume is defined as volume at 20°C. I.e., if a 10.00 ml pipette is calibrated at 20 °C, then when using it, for example, at 25 °C, the pipetted volume of liquid at 25 °C is indeed 10.00 ml (in the range of these temperatures the volume of glassware changes so little that it can be neglected), but the amount of liquid (in terms of mass or number of molecules) is smaller compared to the amount pipetted at 20 °C, although the pipetted volume is the same at both temperatures. So that if the volume of liquid that was 10.00 ml at 20 °C, would be cooled to 20 °C, its volume would be 9.99 ml.
Since temperature differences from 20 °C are usually small, the changes in density are not very large and therefore, the bias is also relatively small in most situations. Therefore, in most cases we do not have to correct the volume, but we take this small effect into account as a measurement uncertainty component.

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22. It is still unclear for me, when to use standard deviation of the mean and when to use standard deviation of an individual value in uncertainty evaluation. For example if I have these values in an experiment: 3.2, 3.6, 3.4, 3.0, 3.9 and I calculate the standard deviation and I get a value of 0.349, can I report any individual value as 3.4 and standard deviation of 0.349?

This question is explained in Section 3.4, but let me try to give some additional explanations here.

The general rule: whenever it is feasible to make replicate measurements of the quantity you are measuring, please do it. And in such cases for the quantity value you should use the mean value and for estimating repeatability you should use the standard deviation of the mean.

Thus, in the example that you are giving, you should certainly report the *mean value*, not a random individual value and as repeatability (assuming you did the measurements on the same day) you should use the *standard deviation of the mean*.

Now, when do you use the standard deviation of an individual value? This is done in such cases, when *for your concrete measurement with your concrete object* you cannot do replicates (or it is not feasible or reasonable). Therefore, you do your measurement just once. And the repeatability of your measurement you estimate from some other experiment that can be repeated.

Two examples:

--- Pipetting. if you need to pipet 10 ml of some solution during your analysis then you cannot do averaging: you cannot pipet 5 times and then somehow “average” the volume. Instead you do pipetting in the course of that analysis *just once* and you estimate repeatability separately (e.g. by pipetting the same amount of water numerous times). In this case, since *you pipetted just once* in your analysis, you will use *standard deviation of an individual result*.

--- Overall repeatability or within-lab reproducibility of an analysis: If you typically analyze your routine samples without replicates then you can estimate the repeatability (or within-lab reproducibility) separately with some control sample which you analyze several times. If that control sample is sufficiently similar to your routine samples then the obtained standard deviation can also be applied to your routine samples (this approach is, for example, used in the Nordtest uncertainty approach). Since you *analyze your routine samples just once*, you should use *standard deviation of a single analysis*, not standard deviation of the mean for quantifying repeatability (or within-lab reproducibility).

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23. How many repetitions we should perform for estimating uncertainty due to the non-ideal repeatability?

It depends on your setup, needs and possibilities. When using some standard procedure, the number of replicates required may be given in the standard. If your aim is to achieve very low uncertainty level using the mean value and (importantly) if uncertainty due to repeatability is an important uncertainty source, then the more replicates you can do, the better result you obtain.

However, in practice, we cannot usually perform many replicate measurements for several practical reasons: we do not have a sufficient amount of sample, we are limited time and/or finances etc. Therefore, as soon as you are able to calculate the standard deviation, e.g. already with just 3 measurements, you can have the first rough estimate the repeatability. But do not stop there! You should collect more data, e.g. on similar samples. And you can pool the data using the pooled standard deviation approach.

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24. What is the uncertainty of a measurement result that is an average of two results of which both have uncertainties?

This is a more difficult issue than one might think. What is presented here is a simplistic and conservative approach.

If the individual results are X_1 and X_2 with combined standard uncertainties $u_c(X_1)$ and $u_c(X_2)$ then, unless the uncertainties are not too different, the value to be presented as the final result can be the simple average of the values X_1 and X_2: $X = (X_1 + X_2) / 2$. If the uncertainties are very different then weighted average should be used whereby $1/u_c(X_1)^2$ and $1/u_c(X_2)^2$are used as weights.

The combined standard uncertainty $u_c(X)$ can be conservatively estimated as follows: Take the highest of the values X_1 and X_2 and add to it its combined standard uncertainty. What you get is the upper uncertainty limit L_U. Then take the lowest of the values and subtract from it its combined standard uncertainty. This way you the lower uncertainty limit L_L. Calculate the distances of the limits from X. The larger of the two distances can be used as combined standard uncertainty estimate.

Example (data with arbitrary units): $X_1 = 154$; $u_c(X_1) = 7$; $X_2 = 160$; $u_c(X_2) = 9$. In this case $X = 157$ and $u_c(X) = 12$.
