Different approaches to estimation of measurement uncertainty in analytical chemistry

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Overview

- The main question of uncertainty evaluation
- The different approaches
  - (Modelling approach)
  - Approach based on validation and QC data
- The role of method performance data
  - Precision
  - Trueness, bias
- Online course „*Measurement Uncertainty Estimation in Analytical Chemistry*“

The main question of uncertainty evaluation in an analytical lab:

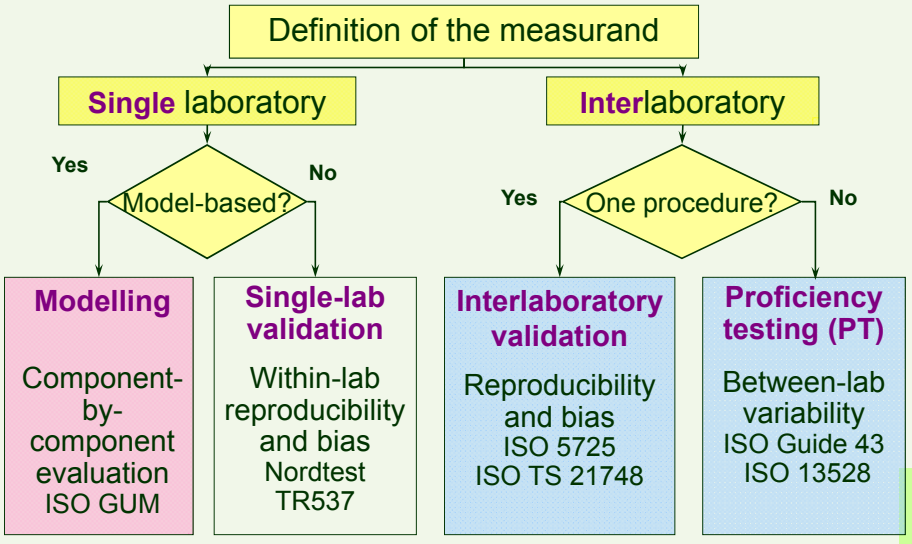
The uncertainty sources are more or less known

There are different data available (control charts, PT results, parallel measurements …)

How to use these data to take these uncertainty sources into account?

Different approaches offer different solutions to this question

Uncertainty estimation approaches



Eurolab Technical Report No 1/2007 Available from: http://www.eurolab.org/

Uncertainty estimates by different approaches

- Modelling (classical ISO GUM)
  - Uncertainty of an **individual result** of a measurement can be obtained
- Single-lab validation
  - Typical uncertainty of results obtained using a **procedure in the laboratory**
- Interlaboratory validation
  - Uncertainty of results obtained using the same **procedure in different laboratories**

These uncertainties refer to different situations!

The Modelling Approach

Component by component evaluation

Essence

- Based on measurement model, identification and quantification of all important uncertainty components
- Has been applied in chemistry, but often with problems

Challenges with Chemical Measurements

- Often not readily modeled
- Uncertainty contributions not readily quantified
  - Analyte losses during sample preparation
  - Interferences from other components of the sample
  - Sample inhomogeneity
  - Often insufficient information available

Danger to underestimate uncertainty!

Example: Moisture Content

- The model:

$$Q_{\text{moisture}} = \frac{m_{\text{sample}} - m_{\text{sample\_after\_heating}}}{m_{\text{sample}}} \times 100\%$$

- Substituting typical balance data yields:
- Q_{moisture} = (12.500 ± 0.013) % (k = 2)

Example: Moisture Content

- If in addition to the balance we try to take into account:
  - Sample inhomogeneity
  - Possibly incomplete drying
- Then, substituting more realistic data, we get:
- Q_{moisture} = (12.50 ± 0.88) % (k = 2)

Example: Moisture Content

The difference between 0.013% and 0.88% is almost 70 times !

The intrinsic balance uncertainty sources are almost insignificant in this case!

Underestimation of uncertainty is not an "intrinsic property" of the Modelling Approach

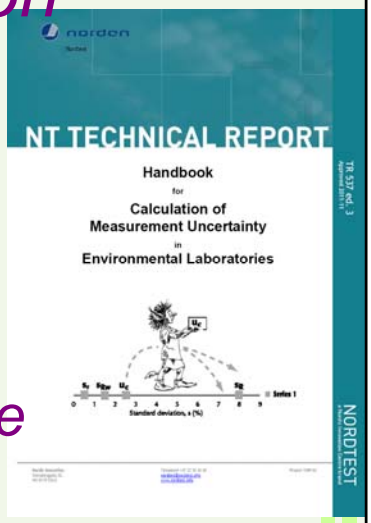
It all depends on the implementation

But can be very work-intensive

Thus, alternative approaches have been developed

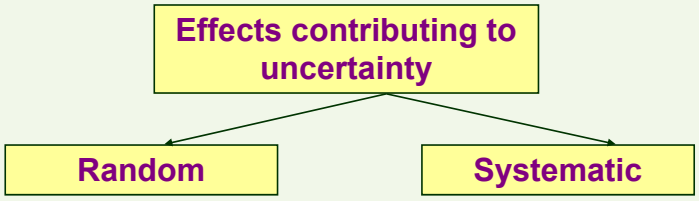
Approach Based on Validation and Quality Control Data

on the example of the Nordtest approach



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

Single-laboratory validation approach



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

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

at „long term“ level!

This and subsequent equations work with both absolute and relative values

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

Precision

Precision component $u(R_w)$

$u(R_w) = s_{Rw}$ is usually found from:

Include sample preparation!

- the warning limits of X chart
  - using a stable control sample
- long term pooled standard deviation

Ideally: separately for different matrices and different concentration levels!

The control sample analysis has to cover the whole analytical process

24. 16

Precision

How to determine precision?

- Example:

An analyst analysed a food sample by HPLC. He carefully homogenized the sample in a blender and took a subsample. With the subsample he carried out sample preparation (consisting of extraction, precipitation and centrifugation). As a result he obtained a clear solution. He transferred it into a 50 ml volumetric flask and filled it up to the mark with the mobile phase. He analysed 10 aliquots of this solution during the same day and calculated the within-lab reproducibility as standard deviation of the results.

Did he do it right? | If not, what should he do differently?

Precision

Determining precision when sample is stable for a long time

Precision
Determination of fat content

Date	Sample	Result (g/100g)
10.02.2008	27	22.5
16.02.2008	27	21.8
26.02.2008	27	22.4
7.03.2008	27	23.6
17.03.2008	27	23.9
27.03.2008	27	23.4
6.04.2008	27	23.7
16.04.2008	27	23.9
26.04.2008	27	22.1
6.05.2008	27	25.8
16.05.2008	27	22.1
26.05.2008	27	23.2
5.06.2008	27	22.2

Mean:	23.1 g/100g
St Dev:	1.1 g/100g
DF:	12

Within-lab reproducibility s_{RW}

Pooled Standard Deviation

Precision

- General formula:

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

- Symbols:
  - k number of groups (in this case samples)
  - s_1, s_2, etc are within group standard deviations
  - n_1, n_2, etc are numbers of measurements made with different samples

Determining precision when sample is not stable for a long time

Precision

Pooled Std Dev
Determination of protein content

Date	Sample	Result (g/100g)
10.02.2008	1	10.2
10.02.2008	2	13.4
10.02.2008	3	17.6
13.02.2008	1	10.7
13.02.2008	2	14.2
13.02.2008	4	16.9
18.02.2008	2	12.9
18.02.2008	3	16.7
18.02.2008	6	12.1
25.02.2008	6	13.5
25.02.2008	4	17.2
25.02.2008	5	19.2
4.03.2008	2	13.1
4.03.2008	6	12.9
8.03.2008	3	17.4
8.03.2008	4	18.2
8.03.2008	5	13.2
8.03.2008	6	13.5

Sample	St dev g/100g	Comp.
1	0.354	0.125
2	0.572	0.980
3	0.473	0.447
4	0.681	0.927
5	0.707	0.500
6	0.663	1.320

s_pooled = 0.598 g/100g

DF: 12

Within-lab reproducibility s_{RW}

Different sample matrixes!

But less "long-term"

u(bias)

Trueness, 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)

Trueness, bias

$$bias_i = C_{lab\_i} - C_{ref}$$

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

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

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}

How to conduct a spiking experiment?

- *Two analysts determined meropenem (an antibiotic) in blood plasma. Both needed to determine the bias of the procedure. They obtained blank plasma samples and did the following:*
- ***Analyst 1** took 500 µl of the blank plasma and added 400 µl of methanol. He separated the precipitated proteins by centrifugation and transferred the supernatant into an HPLC vial. He then added 100 µl of meropenem standard solution with suitable concentration to the supernatant and injected the resulting solution into the HPLC system for ar*

Analyte has to be added at as early stage as possible!

- ***Analyst 2** took 500 µl of the blank plasma and added 100 µl of meropenem standard solution. She then added 500 µl of methanol. She separated the precipitated proteins by centrifugation and injected the resulting supernatant into the HPLC system for analysis.*

Which analyst did it correctly? Why?

Trueness, bias

Roadmap:

Possible bias

$u(Cref_i)$ from certificates

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

$$bias_i = Clab_i - Cref_i$$

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

Uncertainty due to random effects

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

Combined standard uncertainty

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

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.5 from Quantifying Uncertainty in Analytical Measurement, EURACHEM/CITAC Guide, Third Edition (2012)
Available from: http://www.eurachem.org/

Nordtest approach in practice:

Determination of acrylamide in snacks by LC-MS

- Concentration level 998 µg/kg
- Laboratory has analysed two certified reference materials (CRMs) with similar matrixes
  - Potato chips and crisp bread
  - The crisp bread CRM is also used as a control sample

Certified reference material (CRM)

- The **crisp bread** CRM has the following acrylamide content:

$$C_{\text{acrylamide}} = (1179 \pm 68)\ \mu g/kg \quad (k = 2, \text{norm.})$$

- The **potato chips** CRM has the following acrylamide content:

$$C_{\text{acrylamide}} = (860 \pm 42)\ \mu g/kg \quad (k = 2, \text{norm.})$$

Measurements with the CRMs

Crisp bread

Days	*C* (mg/l)
5.01.2008	1172
6.03.2008	1186
3.04.2008	1153
8.01.2009	1151
18.03.2009	1181
3.04.2009	1147
11.04.2009	1097
16.04.2009	1102
25.04.2009	1162
3.08.2009	1138
28.08.2009	1122
27.11.2009	1191

Mean:	1150 µg/kg
Std Dev:	31 µg/kg

Potato chips

Days	*C* (mg/l)
3.04.2008	845
3.04.2008	832
3.04.2008	802
27.04.2008	829
27.04.2008	851
27.04.2008	834

Mean:	832 µg/kg
Std Dev:	17 µg/kg

Roadmap:

Possible bias

$u(Cref_i)$ from certificates

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

$$bias_i = Clab_i - Cref_i$$

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

Uncertainty due to random effects

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

Combined standard uncertainty

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

Finding $u(R_W)$

$u(R_w)$ = s_{RW} = 31 µg/kg

$u(R_w)$_rel = s_{RW}_rel = 31/1150·100 = 2.70 %

Finding u(bias)

Ref value µg/kg	U (k=2) µg/kg	u_c µg/kg	Lab result µg/kg	u_c_rel %	$bias_i$ µg/kg	bias_rel %
1179	68	34	1150	2.88	-29	-2.45
860	42	21	832	2.44	-28	-3.24

u(Cref)_rel=	2.67 %
RMS_{bias}_rel=	2.87 %
u(bias)_rel =	3.92 %

Result:

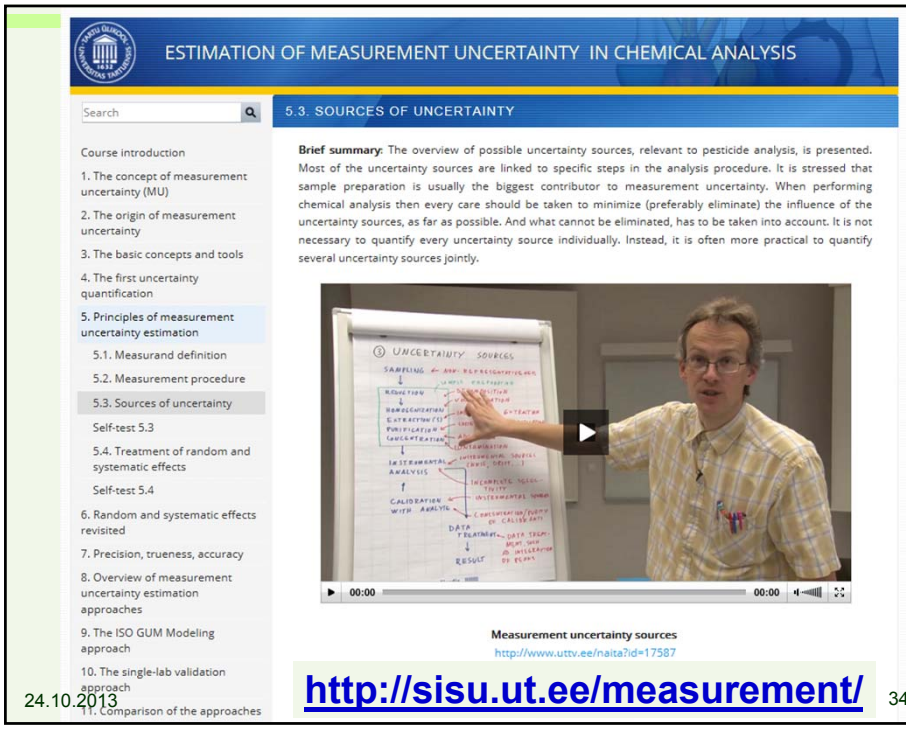
u_c_rel =	4.8 %
u_c =	48 µg/kg
U_rel (k=2) =	9.5 %
U (k=2) =	95 µg/kg

- Acrylamide content in the sample

$$C_{acrylamide} = (998 \pm 95)\ \mu g/kg \quad (k = 2, \text{norm.})$$

Choosing the approach

- If you have
  - Competence and time
  - Data on all important influencing quantities
    - Use the Modeling approach
- If you have
  - Quality control data and results of participation in ILC-s or CRM analysis
    - Use the Single-lab validation approach
- Interlab approaches are not generally recommended



Credits

- Some slides from this presentation have been created in collaboration with **Bertil Magnusson** (SP, Sweden) and used in the

TrainMiC
Training in Metrology in Chemistry

training materials

Thank you for your attention!

- The Online Course of Measurement Uncertainty Estimation in Analytical Chemistry is available from:

sisu.ut.ee/measurement/

- You are always welcome to contact me:

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