27/06/2012

Quality assurance, analytical measurement uncertainty and scrutinising analytical data

Stefan Voorspoels
Agenda

» Quality assurance
  » ISO 17025 in a research environment: nightmare or added value
  » Method validation: a quick intro
  » QA in practice

» Measurement uncertainty
  » What
  » Why
  » How

» Scrutinising analytical results
No absolute truth – opinions differ

The truth is out there ... Anybody know the URL?
But at least the aim is the same ...
ISO 17025

General requirements for the competence of testing and calibration (laboratories)
= ISO 9001 (management)
  + details on technical requirements:
    - equipment
    - sampling
    - technical competency
    - ...

GLP (OECD)

Specific to safety studies
  - planning
  - execution
  - monitoring
  - data recording
  - data storage
  - reporting

Pharma, chemicals, pesticides, food and feed additives, ...

Assuring quality of data
ISO 17025 - accreditation
ISO 17025

→ Useful guidelines to assist/help your quest for quality

→ Be careful for overkill, time consuming

→ Accreditation ≠ guarantee for quality
Method validation

"The process of providing documented evidence that something does what it is intended to do."

» Method validation is completed to ensure that an analytical methodology is accurate, reproducible and rugged over the specific range that an analyte will be analyzed.
   » Method validation provides assurance of reliability and comparability
   » BELAC, UKAS, FDA, etc.

» In short: know what it can do and what to expect

**PERFORMANCE CRITERIA**

- Precision: < 7 %
- Accuracy: < 10 %
- Uncertainty: < 20 %
Method validation

Analytical performance characteristics:

» Precision

» Accuracy (trueness)

» Limit of Detection (LOD) / Limit of Quantification (LOQ)

» Specificity / Selectivity

» Linearity and Range

» Robustness

» Method uncertainty
Terminology

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search:dbbmm26

Huh - Houston we have a problem
Terminology

Depends on source: VIM, ISO, ...

Precision:
The closeness of agreement between independent test results obtained under stipulated conditions (random errors)

Accuracy:
Closeness of agreement between a measured quantity value and a true quantity value of a measurand.

Trueness:
Closeness of agreement between the average of an infinite number of replicate measured quantity values and a reference quantity value.
## Precision and accuracy

<table>
<thead>
<tr>
<th></th>
<th>Accurate</th>
<th>Inaccurate (systematic error)</th>
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</thead>
<tbody>
<tr>
<td>Precise</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imprecise (reproducibility error)</td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
</tr>
</tbody>
</table>

![Diagram](image)
Method validation - Precision

The measure of the degree of agreement among test results when the method is applied repeatedly to multiple samplings of a homogeneous sample

Expressed as % RSD for a statistically significant number of samples

Precision should (ideally) be performed at three levels (increasing ‘complexity’)
- Repeatability
- Intermediate precision
- (Reproducibility) – cfr. Session on proficiency testing (J. de Boer)

In practise: 1-3-5 → 1 sample - 3 replicates a day - 5 days
Method validation – accuracy (trueness)

- The closeness of test results obtained by the method to the true value.

- Established across the range (ideally)

- How to assess:
  - Analysis of (certified) reference material
  - Compare results to a second, well-characterized method
  - Analysis of spiked samples with known quantities of components (recovery)
Method validation – selectivity - specificity

» Selectivity refers to the extent to which a method can determine particular analytes in mixtures or matrices without interferences from other components.

» Environmental analysis: no specific methods

» Pharma: highly selective (~specific) methods are preferred

» Increasing selectivity: Column chrom → LC → GC → GC-MS → GC-MS/MS

» IUPAC: “Specificity is the ultimate selectivity” and “Sometimes the term specificity is used. This usage of specificity suggests that no component other than the analyte contributes to the result. Hardly any method is that specific (sic!) and, in general, the term should be avoided”
Method validation – LOD/LOQ

Detection Limit (LOD)

» Lowest concentration of analyte in a sample that can be detected (not necessarily quantitated)
» $3 \times S/N; 3 \times SD/S; 3 \times SD_{\text{blank}}$ (blank subtraction); etc.
» Not useful in practice

Quantification Limit (LOQ)

» Lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy
» $10 \times S/N; 10 \times SD/S; 10 \times SD_{\text{blank}}$ (blank subtraction); etc.
Method validation – Linearity and range

» **Linearity:** The ability of the method to elicit test results that are directly proportional to the mass fraction within a given range

» Expressed as the variance of the slope of the regression Line

» **Range:** Interval between upper and lower levels of analyte demonstrated by the method

» In practice mostly LOQ – xx ng/g

Fit-for-purpose
Method validation – range

Injected amount – quality of result – LOD/LOQ

![Graph showing RSD (%) against pg on column with a 2 % cut-off at 5 pg on column]
Method validation – robustness

» Measure of the capacity to remain unaffected by small (deliberate) variations in method parameters

» Indication of reliability during normal use

» If measurements are susceptible to variations in analytical procedures, these conditions should be controlled and a precautionary statement included

» Sampling
   » Weighing
      » Extraction (10 mL – 10,0 mL – 10,00 mL)
         » Clean-up (e.g. silica 2 cm, 5 g, 6 g, etc.)
            » ...
            » ...
            » Use common sense ...

vito
vision on technology
Method validation – robustness

Robustness ≠ Ruggedness

» Ruggedness refers to parameters *external* to the method
  - Operator
  - Day of week

» Robustness refers to parameters *internal* to the method
  - % organic in mobile phase
  - Temperature
## Analytical performance parameters

<table>
<thead>
<tr>
<th>Analytical Performance Parameter</th>
<th>Assays</th>
<th>Quant.</th>
<th>Limit test</th>
<th>Specific tests</th>
<th>I.D.</th>
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<td>*</td>
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<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

+ measurement uncertainty

» * May be required, depending on the nature of the specific test.
QA in practice – analytical

» Selection of reference materials (“standards”)
  » Internal standard(s)
  » External standard(s)
  » Syringe standard(s)
  » Calibration standards
  » ...
» (Certified) reference materials
» QC-samples
» QC-charts
» Proficiency tests
QA in practice – analytical

» Sample prep:
  » Record everything that happens
  » Balance: calibration and handling!
  » Samples (randomise)
  » Method blanks (1/5, 1/10, ...)
  » Control samples (replicates, (C)RM, ...)

» Instrumental analysis:
  » Instrument condition (tune file, peak shape, baseline, ...?)
  » Group calibration – group “samples”
  » RANDOMISE within each group !!!
  » Include QC-steps:
    » Solvent blanks
    » Replicate standard injections (drift control) – whole set/only one
QA in practice – Reference material (RM)

Material, sufficiently **homogeneous and stable** with respect to one or more specified properties, which has been established to be **fit for its intended use in a measurement process**.

Notes: 1) RM is a generic term.

2) Properties can be quantitative or qualitative, e.g. identity of substances or species.

3) Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

4) An RM can only be used for a single purpose in a given measurement.

*ISO Guide 35 (2006)*
QA in practice – Certified Reference Material (CRM)

Reference material characterized by a metrologically valid procedure for one or more specified properties, accompanied by a certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability.

Notes: 1) The concept of values includes qualitative attributes such as identity or sequence. Uncertainties for such attributes may be expressed as probabilities.

2) Metrologically valid procedures for the production and certification of reference materials are given in, among others, ISO Guides 34 and 35.

3) ISO Guide 31 gives guidance on the contents of certificates.
QA in practice – CRMs

- Vitamins
- Proximates
- Veterinary drugs
- BFRs
- Enzymes
- Hormones
- Proteins
- DNA
- Size
- Impact toughness
- Microorganisms
- PAHs
- Hydrocarbons
- Prions
- Mycotoxins
- TSE QCMs
- Food CRMs
- Clinical CRMs
- GMO CRMs
- Engin. Mat. CRMs
- Biological CRMs
- Water CRMs
- Soil/Sediment CRMs
- Microbiol. CRMs
- Metal species
- Isotopes
- Cations
- Impact toughness
<table>
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<th>Reference standard</th>
<th>Measurement standard</th>
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<td>Calibration material</td>
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<td>Analytical standard</td>
<td>Laboratory standard</td>
</tr>
<tr>
<td>Reference material</td>
<td>Laboratory reference material</td>
</tr>
<tr>
<td>Proficiency testing material</td>
<td>Reference substance</td>
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</table>
QA in practice - Analytical processes of RMs

Sampling, Processing

Conservation

Sample preparation

Analyte identification & Quantitative measurement

Evaluation

Assessment

Matrix RMs → Quality control

RM for qualitative analysis
• identity

• as close as possible to real sample

Calibrants → for calibration
• often pure analyte
It’s all relative ...

Protagoras: Truth is relative. It is only a matter of opinion.

Socrates: You mean that truth is mere subjective opinion?

Protagoras: Exactly. What is true for you is true for you, and what is true for me, is true for me. Truth is subjective.

Socrates: Do you really mean that? That my opinion is true by virtue of its being my opinion?

Protagoras: Indeed I do.

Socrates: My opinion is: Truth is absolute, not opinion, and that you, Mr. Protagoras, are absolutely in error. Since this is my opinion, then you must grant that it is true according to your philosophy.

Protagoras: You are quite correct, Socrates.
... it’s all about the reference

Protagoras: Any value is relative. It is only a matter of reference.

Socrates: You mean that a so-called true value is mere subjectivity.

Protagoras: Exactly. What is true for you is true for you, and what is true for me, is true for me. Truth is subjective.

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COMMON REFERENCE
Traceability: Wikipedia - ‘Traceability refers to the completeness of the information about every step in a process chain.’

Regulation (EC) No 178/2002 - ‘The ability to trace and follow food, feed, and ingredients through all stages of production, processing and distribution.’

BIPM - ‘Traceability means that the result of a measurement, no matter where it is made, can be related to a national or international measurement standard …’
Metrological traceability

Definition ‘International Vocabulary of Metrology’ – VIM 2007

ISO/IEC Guide 99

‘Property of a measurement result whereby the result can be related to a stated metrological reference through a documented unbroken chain of comparisons, all having stated measurement uncertainties.’

Why?

Only measurement results where the metrological traceability is established can be compared, independent from when and where they were obtained.

- IUPAC
The gravimetrical reference

SI-units:
- Kg (mass)
- m (length)
- s (time)
- A (electric current)
- K (Temperature)
- mol (amount of substance)
- cd (luminous intensity)
Traceability – key points

• **Property of a measurement result** – methods are not traceable
• Closely linked to measurement **uncertainty**
  
  ! Uncertainty + traceability = 2 main factors assuring measurement reliability

• Traceability to a common reference allows **comparability** of results and ensures defined and constant measurement conditions
• **Traceability chain** = the sequence of standards and comparisons that relate the result to the stated reference
Traceability – final reference

» Important: traceability chain shall be linked to a **final reference**:

» - a measurement unit

» - a measurement procedure

» - a reference material ("artefact")

ISO/IEC Guide 99 (VIM)
Traceability – final reference

» Important: traceability chain shall be linked to a final reference:

» - a measurement unit

» - a measurement procedure

» - a reference material (“artefact”)

ISO/IEC Guide 99 (VIM)
Traceability to a unit

- Preferably to the SI unit
  - e.g. “mass: 5 kg” means: the measurement result of the property “mass” has been obtained by comparing the mass of the item with the mass of the kg in Paris

- Exists for most physical measurements - independent of the method

- Not (yet) available for many chemical measurements:
  - “kg ≠ “kg”; may depend on the method employed
  - SI-unit is not always relevant for the application (mass vs. catalytic concentration of an enzyme)
Traceability – final reference

Important: traceability chain shall be linked to a final reference:

- a measurement unit
- a measurement procedure
- a reference material (“artefact”)

ISO/IEC Guide 99 (VIM)
Traceability to a procedure

A problem?
No! These **methods define the analytes**
– “dietary fibre” has no real meaning
– a certified value without statement of the method is meaningless
– the only useful reference is the procedure
Traceability – final reference

Important: traceability chain shall be linked to a final reference:

- a measurement unit
- a measurement procedure
- a reference material ("artefact")

ISO/IEC Guide 99 (VIM)
Traceability to a reference material ("artefact")

- important for **arbitrary units**

  » use the reference material directly as comparison
  » can be method-dependent or -independent
  » e.g. WHO standard: “by definition, this sample has 5000 international units”
Traceability is split into:

- **Identity** (measurand)
  - Structurally defined
  - Operationally defined

- **Quantity value** (number and unit)
  - SI reference
  - Artefact reference

<table>
<thead>
<tr>
<th>Quantity value</th>
<th>Structurally defined</th>
<th>Operationally defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>SI</td>
<td>e.g. BDE 47 mass fraction</td>
<td>e.g. dietary fibre</td>
</tr>
<tr>
<td>Artefact</td>
<td>Rare !</td>
<td>e.g. coagulation time (WHO material)</td>
</tr>
</tbody>
</table>
On certificates, reporting of traceability is split into “identity” and “quantity”

<table>
<thead>
<tr>
<th><strong>BODY/MATRIX</strong></th>
<th>Certified quantity (e.g. Mass Fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certified value (^2) [unit]</td>
</tr>
<tr>
<td></td>
<td>Uncertainty (^3) [unit]</td>
</tr>
<tr>
<td>Certified properties (^1)</td>
<td></td>
</tr>
</tbody>
</table>

1) Only relevant for procedurally defined identity and quantity (e.g. “as defined by the procedure according to ISO 12345” or “as obtained by Soxhlet extraction and subsequent analysis by GC/MS”).
2) “Traceable to SI” or “traceable to XXX”
3) Description of the type of uncertainty value given
Traceability to institutes?

One-product unambiguous synthesis
Uncertainty is the maximum combined relative uncertainty of weights and volumes
**All weights and volumes are traceable to NIST**
HRGC/HRMS mass spectrum attached, see Figure 1
HRGC/HRMS SIR data attached (10,000 RP) see Figure 2
Structure confirmed by HRGC retention time comparison to native isomer
Traceability to institutes?

<table>
<thead>
<tr>
<th>Component</th>
<th>CAS #</th>
<th>Purity %</th>
<th>Prepared Concentration</th>
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</thead>
<tbody>
<tr>
<td>Desiccant 1</td>
<td>12345</td>
<td>98.5%</td>
<td>12.3%</td>
</tr>
<tr>
<td>Desiccant 2</td>
<td>67890</td>
<td>97.6%</td>
<td>11.2%</td>
</tr>
</tbody>
</table>

1. All weights are traceable through National Institute of Standards & Technology.

2. Certified Analytical Concentration = Purity x Prepared Concentration.
Traceability to institutes?

‘The results are traceable to NIST’

Incorrect! - what is meant is for example:

“The mass obtained is traceable to the kg in Paris, because it has been compared with a weight from NIST, which in turn has been compared to the kg in Paris.”

What matters is the end-point of the traceability chain!
Metrological traceability in practice ... more complex?

Example of extraction protocol for serum analysis:

“Weigh 100 mg of sample in a 2 mL tube, add 5 mL of hexane and 25 µL of enzyme solution X, mix by shaking and incubate at 60 °C for 30 min; centrifuge for 10 min at 13000 rpm, then add the supernatant to 500 µL of chloroform, ...”

- Analytical system: complete set of operations
  - Weighing, milling, heating (water bath), centrifuging, timing, pipetting,...

- Each operation has to be calibrated
  - Mass for balances, temperature for water bath,...
  - Not all operations are critical for a particular measurement result, i.e.
    required accuracy of the reference depends on the impact of the operation on the final analytical result

→ analyst’s (competent) judgement
CRMs for **short-cutting the traceability chain**

- CRM = RM + certificate
  + certified value with uncertainty
  + stated *traceability* (to a final reference)

- CRMs to ensure traceability of
  1. measurement conditions, classical ‘calibration’
  2. results of final quantification (through calibration)
  3. results of overall method (through validation)
  4. result in daily work
Traceability in sample preparation

- Problem: every sample preparation step breaks the traceability chain
  - one is not entirely sure that what went into the sample preparation step is the same that came out
- Method assumes correctness, absence of losses, etc.
  - Any evidence?
- Therefore: ways to “restore” the traceability chain are needed: method validation
- Matrix CRMs can be used for method validation
Restoring the traceability chain

Matrix CRM

sample

weigh the sample

X

eXtraction/digestion

clean-up

X

dilution to

a certain volume

quantification

balance calibration

impossible to link sample to final extract

calibrated glassware

pure standards
QA in practise – CRMs in daily work – QC charts

• Demonstration that conditions of validation study apply for this particular measurement: quality control charts

• In practice: CRMs often used
  » guaranteed homogeneity
  » Better/documented stability compared to normal samples
  » ultimate test of accuracy and traceability of results in each measurement series

  » (C)RM in control charts prove that the method has been applied correctly and that the conditions from the validation study apply
QA in practise – QC charts

![Individuals chart with 2σ and 3σ control limits](chart.png)
Measurement uncertainty – Use of measurements

Result → Conclusion → Action

Out of specification → re-work batch
Within legal limits → permit granted
CO₂ rising → Kyoto protocol
Elevated testosterone → lose Tour de France
Two results disagree → find correct result

43.263 ↔ 43.264
Measurement uncertainty

Analytical result: \( \bar{x} \pm u \)

• Fit-for-decision: by adequate uncertainty
Measurement uncertainty – What

Degree of doubt about the correctness of a measurement result

» Standard uncertainty ("small u")
  » format of a standard deviation

» Expanded uncertainty ("capital U"): interval which includes the true value with a high probability
  » if interval incorrectly too small: wrong conclusions
  » if interval incorrectly too large: useless result

Therefore: correct estimation matters!
Uncertainty vs error

» Don’t confuse *uncertainty* with *error*!

» **Uncertainty** is the quantification of the doubt about the measurement result

» **Error** is the difference between the *measured value* and the *true value* of the object being measured
Connection with CRMs?

Use of CRMs

Validation → proving method trueness

Use of CRMs requires uncertainty information

Calibration → contributes to uncertainty

CRMs contribute to uncertainty information
Measurement uncertainty – Definitions

Uncertainty – parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand

Standard uncertainty – uncertainty of the result of a measurement, expressed as a standard deviation

Combined standard uncertainty (u) – several uncertainty contributions: u is equal to the positive square root of the sum of variances, weighted accordingly

Coverage factor (k) – numerical factor; used as multiplier for u to obtain U; typically in the range of 2-3 (same purpose as t-factor for confidence interval!)

Expanded uncertainty (U) – interval about the result of a measurement that may be expected to encompass a large fraction of the distribution of values that could reasonably be attributed to the measurand
Measurement uncertainty – Prerequisites

Know your measurement!

Measurement has been corrected for all recognised significant systematic effects!
Measurement uncertainty – Why

Knowledge of the uncertainty of a measurement result is essential for the correct interpretation of this result

- In-depth knowledge of procedure and transparency
- Demonstration of competence: procedure is under control
- Identification of (the main) variation sources in a procedure: hints for improvements
- Confidence: “with x% probability my result is \( y \pm U \)”
- Necessary for ISO 17025 accreditation
- Comparability of data
- End user has obtained result with adequate confidence interval
- Audits
- Required by external contractors
Meaurement uncertainty – 2 approaches

- Method validation
  - Know normal reliability of the method
    - Method shows normal performance?
      - Normal reliability applies
        - Result and its uncertainty
          - Quantify influences
            - Know all influences on the result
              - Know method in great detail

Top-down

Bottom-up
Measurement uncertainty – standard uncertainty

Calculate **standard uncertainty** for each parameter that can have an influence on the final result.

Two uncertainty types:

- **Type A**: the best estimate of input quantity has been obtained by repeat measurements

- **Type B**: obtained by other means (certificate of analysis, previous measurement data, manufacturer’s specifications, etc.)
Measurement uncertainty – Type A evaluation

Estimate for input quantity $q$:

average of $n$ repeated observations (=measurements)

Standard uncertainty: standard deviation of the mean

$$u_q = \frac{\text{stddev}(q_1, q_2, q_3, \ldots)}{\sqrt{n}}$$

$$u_q = \frac{0.22}{\sqrt{10}} = 0.069$$

<table>
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<tbody>
<tr>
<td>1</td>
<td>10.31</td>
</tr>
<tr>
<td>2</td>
<td>10.35</td>
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<td>3</td>
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<td>9</td>
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<td>10</td>
<td>10.29</td>
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<tr>
<td>mean</td>
<td>10.41</td>
</tr>
<tr>
<td>SD</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Measurement uncertainty – Type B evaluation

Use data from any other source available

- *certificates* (CRMs, balance calibration, …)
- *manufacturer’s specifications* (balances, volumetric flasks, …)
- *previous measurements* (validation data, …)
- *uncertainties of reference data*
- *experience, general knowledge*
- …

- If available as standard uncertainty, use as such
- If not, use appropriate conversion factor
Measurement uncertainty – Conversion factors

Specification: 100 ± x

Any value between 100-x and 100+x equally likely
- Rectangular distribution
  - standard uncertainty: $x/\sqrt{3}$
- Typically: weighings, purity values
  - from a certificate

Values close to 100 more likely
- Triangular distribution
  - standard uncertainty: $x/\sqrt{6}$
- Typically: volumetric flasks
Meaurement uncertainty – Combined uncertainty

Simplified cases for uncorrelated input quantities
sensitivity coefficients are 1.0 if:

Models involving only a sum or difference of quantities
\[ y = a + b - c - d \]
\[ u_c = \sqrt{u_a^2 + u_b^2 + u_c^2 + u_d^2} \]

Models involving only a product or quotient of quantities
\[ y = p \times q / r \]
\[ u_c = \sqrt{\left(\frac{u_p}{p}\right)^2 + \left(\frac{u_q}{q}\right)^2 + \left(\frac{u_r}{r}\right)^2} \]
Measurement uncertainty – summary bottom-up

• Modelling of the measurement
• Identification of all uncertainty sources
• Evaluation of uncertainties of the input quantities, type A and B
• Combination of uncertainties
Measurement uncertainty – Top-down approach

In a measurement, some effects concern

• only the individual measurement
  – dilutions, pipetting, injections, weighing...

• all measurements in a particular series
  – measurement standard, temperature in the lab, batch of reagents…

• all measurements with a particular method
  – deviation from the “true” value
Measurement uncertainty – Top-down approach

Repeatability

– immediately visible
– does not include effects from standard preparation, performance of instrument and reagents
– estimate $u_r$ either from measurements (if $n>6$) or take $s_r$ from validation study

$$u_r = \frac{s_r}{\sqrt{n}}$$

$n = \text{replicates for the measurement}$
Intermediate precision

- covers all series-to-series effects
- best estimated from the validation study (ANOVA) or quality control charts
- can eliminate repeatability influence

\[ u_{ip} = \frac{s_d}{\sqrt{d}} \]

- \( s_d \): day-to-day variation
- \( d \): number of measurement days for this particular measurement
Meaurement uncertainty – Top-down approach

Trueness

Question: How certain am I that the method gives on average the correct result?

Two influences
1. uncertainty of trueness estimation \[ \frac{s_t}{\sqrt{n_t}} \]
   \( s_t, n_t \) from the trueness determination

2. uncertainty of assigned value of material to estimate trueness
   \( u_{\text{mat}} \) standard uncertainty of the value
   \[ u_t = \sqrt{\frac{s_t^2}{n_t} + u_{\text{mat}}^2} \]
Measurement uncertainty – Top-down approach

Combined uncertainty:

\[ u_c = \sqrt{u_r^2 + u_{ip}^2 + u_t^2} \]

– easier to calculate if relative uncertainties are used

Important: check that all variations are included!

Combination of approaches possible
Measurement uncertainty – Bottom-up vs top-down

**Bottom-up**
- + guide for method improvement
- + evidence that method is fully understood
- + scientifically elegant
- - difficult to know all factors
- - difficult to define equation comprising all factors

**Top-down**
- + easy to implement
- + easier to cover all effects
- - no information for method improvement
- - crude method

Both are valid approaches in line with the GUM (Guide to the Expression of Uncertainty in Measurement)
Measurement uncertainty – Expanded uncertainty

Combined uncertainty $u_c$ : format of a standard deviation

» range ± $s$ covers 2/3 of possible outcomes

» need expansion factor to cover “a large fraction of” cases

Expansion factor is called “coverage factor” $k$

» $k$ same purpose as t-factor for confidence intervals

Expanded uncertainty $U = k \times u_c$
Measurement uncertainty – Expanded uncertainty

Choose coverage factor $k$ according to level of confidence required

for normally distributed values and sufficient degrees of freedom: $k=2$ (95% conf.), $k=3$ (99% conf.)

Few values ($n<6$) only: recommendation is to set $k$ equal to the two-tailed value of Student’s test for the number of degrees of freedom associated with that contribution, and for the level of confidence required (normally 95%).
Measurement uncertainty – formula example

\[ U = k \sqrt{ \frac{s_r^2}{n_{rep}} + \frac{s_{ip}^2}{n_{days}} + u_t^2 + u_{cal}^2} \]

- **Repeatability**
  - CRM not necessary

- **Reproducibility**
  - CRM not necessary

- **Trueness (bias)**
  - CRM necessary
Reporting uncertainty

\[ m_s = (100.02147 \pm 0.00079) \ g \ (k=2) \]

Expanded uncertainty
estimated in accordance with the Guide to the
Expression of Uncertainty in Measurement
(GUM)
with a coverage factor \( k=2 \) corresponding to a level of confidence of about 95%
Measurement uncertainty – the practical use

» comparison of the average of measurements with a certified value / another value

\[ \Delta = \bar{x} - \mu \]

\(\Delta\)...bias

\(\mu\)...true value (certificate)

\(\bar{x}\)...measured value (average)
Measurement uncertainty – the practical use

taking into account

» uncertainty of the certified value

» uncertainty of the measurement result

\[ u_\Delta = \sqrt{u_{\text{meas}}^2 + u_{\text{CRM}}^2} \]

if \( \Delta < 2 \times u_\Delta \rightarrow \text{no difference} \)

\[ x - \mu < 2 \times \sqrt{u_{\text{meas}}^2 + u_{\text{CRM}}^2} \]
Measurement uncertainty – the practical use

» How to estimate $u_{CRM}$?
  » Certificate (convert expanded uncertainty to standard uncertainty)

» How to estimate $u_{meas}$?
  » Full uncertainty budget 😊
  » Intermediate precision (method validation, control chart)
  » Reproducibility (collaborative study, certification report)
  » Repeatability 😞
Measurement uncertainty – the practical use

» Divide

» expanded uncertainty given on certificate by coverage factor (usually k=2)

\[ u_{CRM} = \frac{U_{CRM}}{k} \]

Half-width of 95% confidence interval by appropriate t-factor

\[ u_{CRM} = \frac{U_{CRM}}{t_{\alpha,p-1}} \]
Scrutinising analytical data

To scrutinise:

a) to look at critically or searchingly, or in minute detail
b) to examine carefully for accuracy with the intent of verification

Step 1: Determine your need
Step 2: See what you have
Step 3: Use common sense
Scrutinising analytical data – FIT-FOR-PURPOSE

» 2.1236 vs 6.2365
» Measurement uncertainty ~ 15 % ➔ what differentiating power is relevant?
» 1.5 % - 0.15 % - ??? The more the better (statistics) but don’t overshoot

» 2.1236 ➔ x.xxx6 = 0.00282539084573365982294217366735733 % of value
» Skip at least 2 decimals, taking into account measurement uncertainty you cold skip 3

» Rule of thumb : “ A factor contributing to the measurement uncertainty that is less than 1/3 of the biggest contributing factor has a negligible contribution to the total uncertainty”

» 15 % + 7 % + 3 % = 16.8 % vs 15 % + 7 % = 16.6 %
Scrutinising analytical data

» 0.000,000,000,05 grams of clenbuterol per mL (very very very low level)
» Or not: 50 pg/mL – 50000 fg/mL
» Or: 45.5 – 54.5 pg/mL
» If limit value = 10 pg/mL → round result accordingly !!
» Method validation, LOQ, QC during analysis and uncertainty!
References

» International Vocabulary of Metrology – Vocabulaire International de Métrology (VIM)
» ISO guide 17025
» ISO guide 5725
» Hauck et al., Pharmacopeial Forum, 2008
» ISO/TS 21748:2004: Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty estimation
» http://www.nordtest.org
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