**Overview**

- We will look at measurement uncertainty and related concepts (precision, bias, ...):
  - What are the sources of uncertainty?
  - How to quantify them from the available data?
  - What could be done better?

- We will try to mimic the usual situations in laboratories

- Slides contain questions rather than answers
  - We will find the answers in the course of the work

- Preference is given to difficult issues
  - We will not look much at pipettes, volumetric flasks …

**Measurement uncertainty sources**

- What is the uncertainty source?
- Why do we need to know them?
- How can we use this knowledge in uncertainty estimation?
Which of the following are uncertainty sources in chemical analysis? Please explain. Part I

- Spectrophotometric cell pathlength is not exactly 1 cm
- Analyte partially decomposes during sample preparation
- The sample contains a substance that interferes with the derivatisation reaction and leads to less than 100% derivatisation efficiency
- Part of the sample solution was spilled during quantitative transfer
- Subsample is taken for analysis from a sample that is inhomogenous
- There is scatter of data points around the calibration line

Which of the following are uncertainty sources in chemical analysis? Please explain. Part II

- On a chromatogram a small peak is found, which partially interferes with the analyte peak
- Injection volume in GC varies between chromatograms
- In HPLC wavelength 290 nm is accidentally used instead of 280 nm
- A C18 column is used in HPLC, but not the same brand as defined in the procedure
- Unstable analyte partially decomposes in autosampler
- Partial ionization of the analyte at the mobile phase pH

What are the uncertainty sources in photometric NO$_2^-$ determination?

- Sample: wastewater
- Sample preparation: 25 ml of sample (not filtered) is measured with a graduated cylinder then 0.5 ml of sulphanylamide is added and the solution is allowed to stand for 3 minutes. After that 0.5 ml of diamine (NEDA) is added additionally. The absorbance of the sample solution is then measured at 540 nm.
- Calibration graph: A series of calibration standard solutions containing 0.2, 0.7, 1.0, 1.2, 1.4, 1.6 ml of nitrite standard solution were prepared in 50 ml volumetric flasks. The flasks were then made up to the mark with distilled water. Then 25 ml of each solution is measured with graduated cylinder, 0.5 ml of sulphanylamide is added to each graduated cylinder and after the solutions have been left to stand for 3 minutes, 0.5 ml of diamine is added to each graduated cylinder. The absorbances of these solutions are then measured at 540 nm and calibration graph is built.
- Nitrite concentration in sample is determined from the graph
How to take into account the uncertainty sources?

- Uncertainty sources from the previous slide
- Peak overlap in chromatography
- Irreproducible injection volume in GC
- Partial decomposition of the analyte during sample preparation

Uncertainty estimation approaches

What approaches exist?

How to choose, which one to use?

What data are needed?

Precision

What is the meaning of precision?

How is it usually expressed?

- Different precisions:
  - Repeatability ($s_r$)
  - Reproducibility
    - Within-lab reproducibility ($s_{	ext{Rwithin}}$)
    - Between-lab reproducibility ($s_{	ext{Rbetween}}$)
    - ...
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 100 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 repeatability of the procedure as standard deviation of the results.

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

Examples

File: 1.2_Precision.xls

What types of precision are found in these examples?

What conditions need to be met so that the obtained results would indeed give the respective type of precision?

Questions

What variations are taken into account by these standard deviations?

How do we know what type of precision we need?

Can precision be different at different concentrations?

How are the different types of precision related to measurement uncertainty?
Which of these situations describe bias?

1. All the results of a specific day are systematically influenced by the calibration graph of that day
2. Delicate analyte partially decomposes during sample preparation leading to lowered results
3. The titrant concentration determined on a particular day is slightly lower or higher than the true concentration
4. Because of the specifics of the used sample preparation procedure the sample is digested incompletely, leading to lowered values

Does bias depend on the time frame?

1. Yes, bias determined within a single day is different from one determined on different days (and averaged)
2. No
Systematic and random effects

- Random and systematic effects can be grouped differently:
  - Within-day bias
  - Long-term bias
  - Repeatability $s_r$
  - Intermediate precision $s_{RW}$

All effects causing error/uncertainty

The longer is the time frame the more effects change their „status“:
systematic $\rightarrow$ random

Why is lab/method bias more useful than within-day bias?

- Within-day bias should be redetermined every day
  - Long-term bias can be determined less frequently
- It is useful to work with the lowest possible bias
  - $s_{RW}$ can be determined more reliably than bias
  - It is good if most of the uncertainty sources are included into the random component $s_{RW}$

From now on in this session we only address the long-term bias (lab/method bias)

Example: LC-MS determination of a delicate bioactive compound in blood plasma

| Effect                                      | Systematic within day | Systematic in long term |
|---------------------------------------------|-----------------------|-------------------------|
| Calibration graph of a specific day         |                       |                         |
| Injection volume of autosampler is 5% higher than nominal |                       |                         |
| Delicate analyte partially decomposes at room temperature before samples are loaded into cooled autosampler |                       |                         |
| Repeatability of peak integration           |                       |                         |
| Ionization suppression in the ESI source by a co-eluting compound |                       |                         |
| Baseline noise                              |                       |                         |
Trueness / Bias

What do we need in order to assess trueness of a procedure?

How do we express trueness numerically?

How do we choose how to express trueness?

Which are important issues in determining bias?

| Issue                        | Bias | $s_{MW}$ |
|------------------------------|------|----------|
| Sufficient number of replicates |      |          |
| Sufficiently long timeframe  |      |          |
| Homogeneous sample           |      |          |
| Matrix match                 |      |          |
| Concentration range match    |      |          |
| Reliable reference value     |      |          |
| Determination of one can be hindered by the other | | |

Which are the best approaches for determining bias?

| Approach                                             | How good? |
|------------------------------------------------------|-----------|
| Analysing spiked blank matrix                        |           |
| Replicate measurements of a routine sample           |           |
| Using a PT sample and consensus value as reference value |       |
| Analysing a CRM                                       |           |
| Analysing a routine sample with a reference procedure |           |
How to calculate/express bias?

| Way of expressing | Formula | When to use? |
|-------------------|---------|--------------|
| Absolute bias     | $bias = C_{lab.mean} - C_{ref}$ |            |
| Relative bias     | $bias = \frac{C_{lab.mean} - C_{ref}}{C_{ref}}$ |            |
| Recovery          | $R = \frac{C_{uncorrected}}{C_{ref}}$ |            |
| Recovery          | $R = \frac{C_1 - C_0}{\Delta C}$ |            |

Recovery, R

When is recovery an important parameter?

Can recovery be above 1 (above 100%)?
  * If yes, then what could this mean?

How can recovery be determined?

Recovery from spiking

$R = \frac{C_1 - C_0}{\Delta C}$

What are the meanings of the terms in the equation?

How will the equation change if it is possible to obtain a blank sample?
How to conduct a spiking experiment?

- Two analysts determined meropenem (an antibiotic) in blood plasma. Both needed to determine the recovery 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 for protein precipitation. He separated the precipitated proteins by centrifugation and transferred the supernatant into an HPLC vial. 100 μl of meropenem standard solution with suitable concentration was added to the supernatant and the resulting solution was injected into the HPLC system for analysis.

- **Analyst 2** took 500 μl of the blank plasma, added 100 μl of meropenem standard solution and mixed well. She then added 400 μl of methanol for protein precipitation. She separated the precipitated proteins by centrifugation and injected the resulting supernatant into the HPLC system for analysis.

Which analyst did it more correctly? Why?