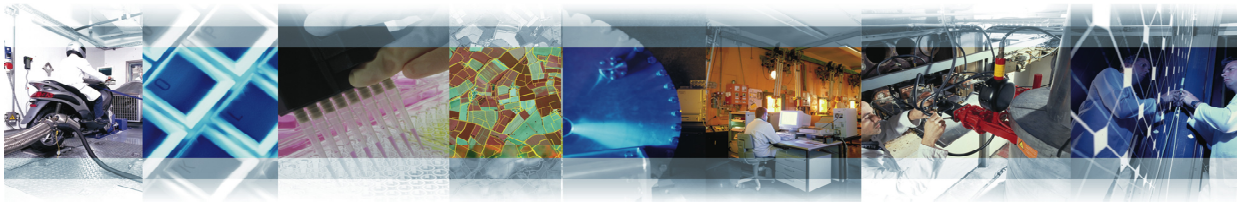

# Practical Examples on Traceability, Measurement Uncertainty and Validation in Chemistry

Volume 1

**Edited by Nineta Majcen and Philip Taylor**

TrainMiC
Training in Metrology in Chemistry

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Practical examples on

# Traceability, Measurement Uncertainty and Validation

in Chemistry

*Volume 1*


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# Practical examples on traceability, measurement uncertainty and validation in chemistry

# Abbreviations

| | |
|---|---|
| CRM | Certified reference materials |
| RM | Reference materials |
| QC | Quality control |
| PT | Proficiency testing |
| ILC | Inter-laboratory comparisons |

# Practical examples on traceability, measurement uncertainty and validation in chemistry

## Foreword


*If you will tell it to me,*
*I will forget*
*If you will show it to me,*
*I will forget*
*If you involve me,*
*I will remember.*

*Xun Zi*
*Chinese philosopher*
*310-237 BC*

This is why having practical examples inside a training course is so important. Participants not only apply what they have learnt in the theoretical part, but the examples increase the degree of interactivity in the course. TrainMiC participants' and trainers' feedback shows that practical exercises are needed and valued most.

Many of those involved in teaching within the TrainMiC programme, use such examples. Nevertheless, preparing and composing the examples take an enormous amount of time. Therefore, the idea grew to also catalogue and share these practical examples, particularly because it is more efficient and allows to more easily address audiences from various sectors.

For this reason, a harmonised format was developed. In this publication, you will find a description of this format, as well as of some of these examples.

*Nineta Majcen and Philip Taylor*
*June 2007, Geel*

# Chapter 1
# Introduction

*Nineta Majcen, Philip Taylor*

From experience gained during several TrainMiC courses in various European countries, a standardised approach of the TrainMiC example session has been developed to

- facilitate exchange of training material that has been prepared and collected by the various trainers
- facilitate exchange of feedback from the participants as well as from the trainers
- improve teaching impact of the course.

Therefore, a structure for a TrainMiC example has been developed and some guidelines on how to conduct a typical TrainMiC example session have been drafted. As this is crucial for proper understanding and conducting of the TrainMiC example sessions, a detailed description is given below.

**How does a standardised TrainMiC example look like?**

Each TrainMiC example includes a part on

a) the input information (description of the analytical procedure, customer's requirement and measurement data)
b) the questions regarding traceability, validation and measurement uncertainty (this part is thus sub-divided in three *exercises*, which are known as 'Traceability exercise', 'Validation exercise' and 'Measurement uncertainty exercise')
c) the solutions for the exercises.

To easily distinguish between different parts of an example, colours have been assigned to each part, as shown in Figure 1.

The input information files, which include a description of the analytical procedure, the customer's requirement and measurement data, all needed for the three exercises, are referred to as ***'yellow pages'***. During the TrainMiC example session they are given to each participant, as well as a booklet of exercises on traceability, validation and measurement uncertainty. The latter

are referred to as ***'white pages'*** and the questions that are to be answered by the trainees are fully aligned with the theoretical presentations. On the other side they are complementary to them in a sense that by presenting theory as well as doing the examples, each of the topics is appropriately addressed and sufficiently covered.

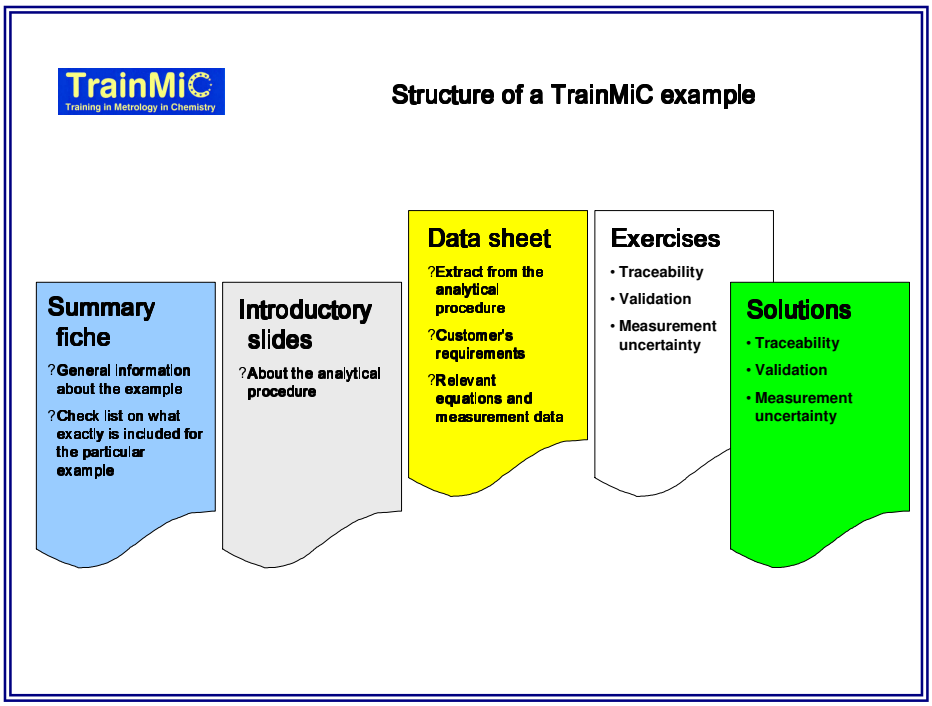



**Figure 1 Harmonised TrainMiC example**

The so called ***'green pages'*** provide answers to the questions asked in all three exercises i.e. traceability, validation and measurement uncertainty. Ideally, for the measurement uncertainty exercise three different approaches to the measurement uncertainty evaluation are presented: a simple arithmetic approach, a spreadsheet solution and result obtained by using professional software e.g. GUMWorkbench. At the moment, the green pages are only for the internal use amongst the trainers (they do not carry the 'TrainMiC' logo) and are not to be handed over to the participants.

On top, as a quality management tool, a summary form (***'blue page'***) is wrapping up each example. It contains all the essential information about each example e.g. analytical procedure, type of the sample, analytes, measurement method, customer's requirements and some other, which help in managing and selecting the examples.

**What is a recommended approach of conducting a TrainMiC example session?**

The TrainMiC ambassador, who is organising a TrainMiC event, decides on the exact format of the TrainMiC example session, taking into account the knowledge and needs of the trainees as well as specific areas that are addressed during the training course e.g. environmental analysis, analysis of food or clinical analysis.

In practice, this means that a TrainMiC example session at a certain TrainMiC event can be conducted in one of the following forms:

- One example, all three exercises
- One example, one or two exercises only
- More than one example, all exercises for each
- More than one example, one or two exercises only
- One example, Measurement uncertainty exercise: comparing different tools for its evaluation

When deciding on which format to choose it is essential not to forget about the time constraints of a certain training event, as it is crucial that the trainees have enough time to do the exercises as well as to dedicate enough time to a properly led discussion after completing the exercises. Based on our experience, we suggest to dedicate about 60 minutes for each of the exercises (group work) and about 30 minutes for a follow-up discussion. The groups should not be bigger than five participants and each group should in the beginning of each exercise *nominate a rapporteur* who afterwards reports on the results and on the questions and the discussion the group had during the exercise. Nominating a rapporteur improves the reporting significantly, so it is higly recommended to give each group a card 'rapporteur' at the start. It is of a vital importance that the trainees are properly briefed before starting with the example session. The slides, which can be used for this purpose are in Appendix 2 and a dynamic process of conducting a TrainMiC example session is schematically shown in Figure 2.

**About the structure of this handbook**

In this handbook, five different analytical procedures are worked out as TrainMiC examples. Following the above described standardised approach, each of them contains

a) A summary form ('blue page')
b) A short introduction to the analytical procedure (as a Power Point presentation) that is given by the trainer
c) All input needed to do the three exercises ('yellow pages') and
d) The solved exercises ('green pages').

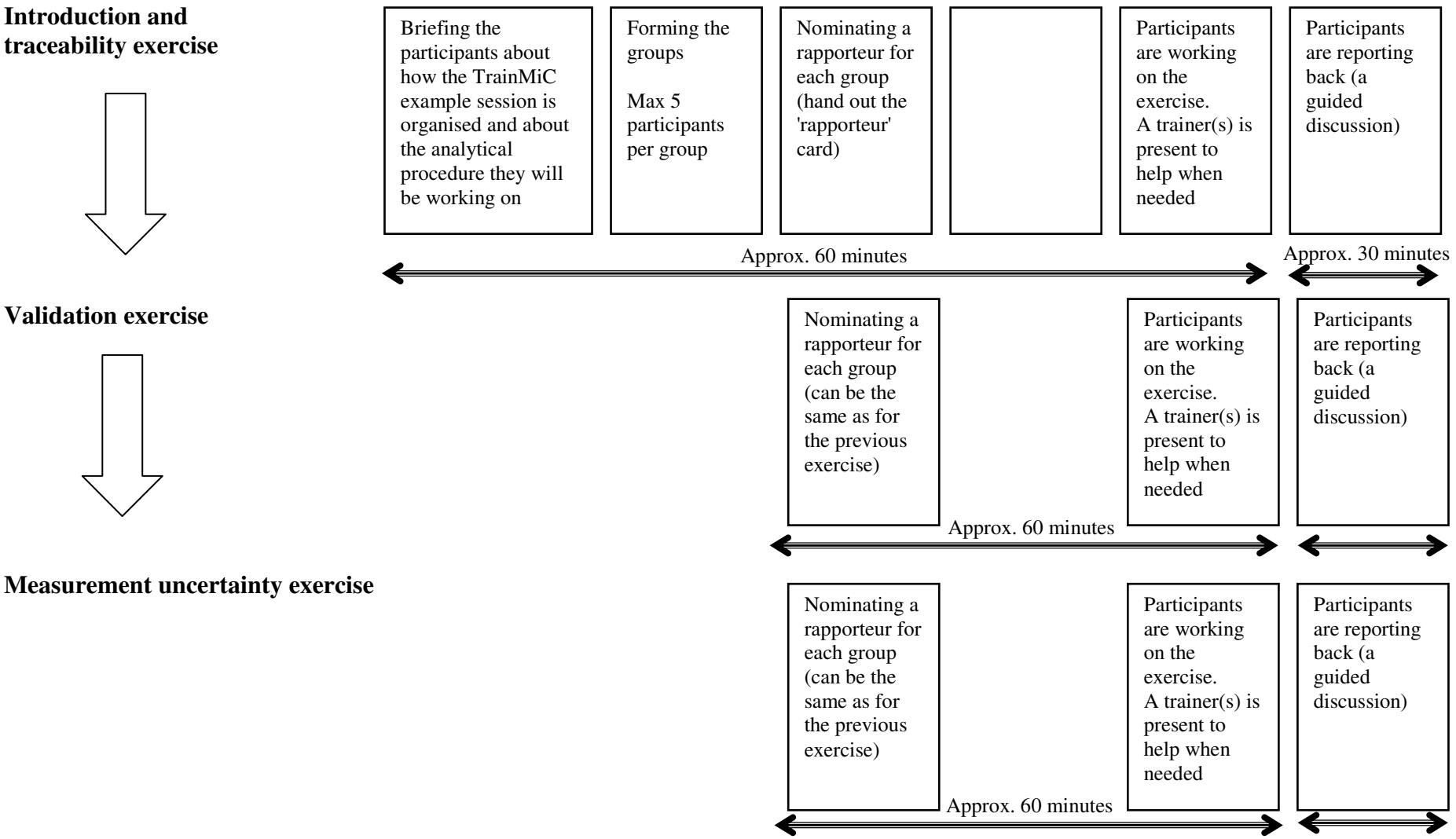



**Figure 2 A process of conducting a completeTrainMiC example**

## Chapter 2

## Analysis of Gold Alloys by Flame Atomic Absorption Spectrometry

*Veselin Kmetov, Emilia Vassileva*

- **TrainMiC example summary form ('blue page')**
- **A short introduction to the analytical procedure ('slides')**
- **All input needed to do the three exercises ('yellow pages')**
- **The solved exercises ('green pages')**

# TrainMiC example summary form

## I. General information about the example

| | |
|---|---|
| **Measurand** | Mass fraction of Au in gold alloys (‰) |
| **Example number** | Ex-06 |
| **Authors of the example** | Veselin Kmetov, Emilia Vassileva |
| **Analytical procedure** | Determination of gold in jewellery gold alloys by flame atomic absorption spectrometry |
| **Customer's requirement** | U = 9‰ (k=3) |

## II. Attached files

| File number, type and name | | Content of the file | | File is attached Yes | File is attached No | Remark |
|---|---|---|---|---|---|---|
| 1 - I | Ex-06-1-I-Au-alloys-FAAS-2006-Ver1.ppt | About the analytical procedure: short introduction | | ✓ | | Given by the lecturer |
| 2 - Yellow | Ex-06-2-Y-Au-alloys-FAAS-2006-Ver1.doc | PART I | Description of the analytical procedure | ✓ | | Each participant receives own copy and may keep it |
| | | PART II | The customer's requirements concerning the quality of the measurement result | ✓ | | |
| | | PART III | Validation of the measurement procedure – relevant equations and measurement data | ✓ | | |
| | | PART IV | Measurement uncertainty of the result – relevant equations and measurement data | ✓ | | |
| 3 - Green | EX-06-3-G-Au-alloys-FAAS-2006-Ver1.doc | PART I | Establishing traceability in analytical chemistry | ✓ | | |
| | | PART II | Single laboratory validation of measurement procedures | ✓ | | |
| | | PART III | Building an uncertainty budget | ✓ | | |
| | | | Addendum 1: By spreadsheet approach | ✓ | | |
| | | | Addendum 2: By dedicated software | | ✓ | |

## III. History of the example

| Version | Uploaded on the webhotel | Short description of the change |
|---|---|---|
| 0 | April 2007 | - |
| 1 | | |
| 2 | | |

# A short introduction to the analytical procedure

## Analysis of Gold Alloys by FAAS

Scope of the presentation

- The analytical procedure and the customer's requirements
- About 'the chemistry' and the measurement method
- Model equation

## The analytical procedure and the customer's requirements

- The traditional internationally recognised method is based on cupellation (fire assay) ***ISO Standard 11426.***
- The fineness of precious metal alloys are specified in **ISO 9202:1991**, according to the purity of gold as ‰: 375, 585, 750 and 916 ( 9, 14, 18 and 22 karats respectively). **One carat is equal to 41,667‰.**
- The Au analysis has to keep the expanded uncertainty (k=3) **less than 9‰**.

## Analytical procedure

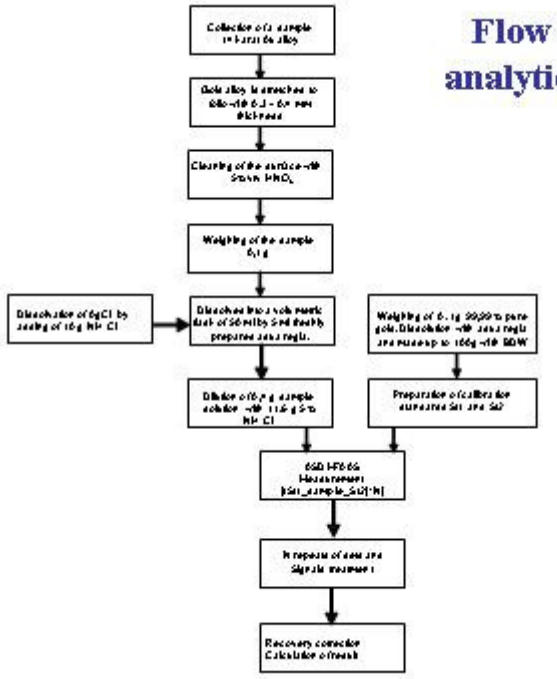
• Gold alloy samples are stretched to folio with 0.3 - 0.4 mm thickness. The surface is washed by 5% v/v $HNO_3$. A dry piece of 0.1 g accurately weighted to $\pm$ 0,0001 g is directly dissolved into a volumetric flask of 50 mL by 5mL freshly prepared aqua regia.

• The flask is heated on ceramic hot plate for 20 min. During this process Ag precipitates as AgCl. AgCl is dissolved by adding of 10 g $NH_4Cl$ to the cooled solution and volume is made up to the mark (50 mL) with BDW at (20°C).

• The solution is diluted additionally by transferring 0,400 mL with micro-pipette to a conical vial adding 5% $NH_4Cl$ in order to keep the solution homogeneous with final weight of 12,000 g gravimetrically controlled.

• Procedural blank is subject to exactly the same sample preparation procedure as the analysed sample.

## Flow chart of the analytical procedure



## More equations (1)

### Calibration standards

An initial Au standard solution C_Au_999,9 mg/L is prepared by dissolution of 0,1 g pure gold 99,99 % (certified from Non-Ferrous Metallurgical Plant Plovdiv) with 5 mL aqua regia filled up to 100 g with 5 % $NH_4Cl$.

$$C_{Au\_999,9} = \frac{m\_pureAu * Au\_purity}{G\_100} * 10^4$$

Two calibration standard solutions with concentration 37 and 43 µg/g respectively are prepared in 5 % $NH_4Cl$.Both calibration standards are obtained in polypropylene vials after further dilution of 0.370 g and 0.430 g from initial standard solution made up with procedural blank solution to 10.000 g (gravimetrically controlled).

$$C_{-St1} = C_{Au\_999,9} * \frac{G_{p\_0,37}}{G_{-10}} \qquad C_{-St2} = C_{Au\_999,9} * \frac{G_{p\_0,43}}{G_{-10}}$$

## More equations (2)

### Calculation of signal standard uncertainty

$$R = \frac{W\_\%}{W\_ref\_\%}$$

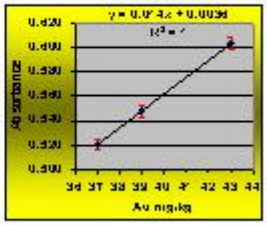
### Recovery correction

$$u_{\_x} = \frac{u_{\_x\_meas}}{\sqrt{n}}$$

### Bracketing calibration

- Two calibration standard solutions used are with Au concentration 37 and 43 mg/kg respectively in 5 % $NH_4Cl$
- The selected calibration range corresponds to the range 13.5 – 14.5 kt recalculated for analyzed alloy.
- It is proved that the S/N ratio is minimum and the calibration interval belongs to the linear range

$$C_x = \frac{C_{-St1}(A_{-St2} - A_x) + C_{-St2}(A_x - A_{-St1})}{A_{-St2} - A_{-St1}}$$



## Model equation

Gold content (‰) in jewellery gold alloys

$$W\_‰ = \frac{1}{1000} * \frac{V\_50}{m\_0{,}1} * \frac{G_{vials\ \_12}}{G_P\ \_0{,}4}.C_x * \frac{1}{R}$$

# All input needed to do the three exercises 'yellow pages'

## Analytical procedure

*Determination of gold in jewellery gold alloys by Flame Atomic Absorption Spectrometry*

**PART I**

**Description of the analytical procedure**

**PART II**

**The customer's requirements concerning the quality of the measurement result**

**PART III**

**Validation of the measurement procedure – relevant equations and measurement data**

**PART IV**

**Measurement uncertainty of the result – relevant equations and measurement data**

# PART I. Description of the analytical procedure

## Task description

The fineness of jewellery gold alloys depends from the Au contend in the used material that must be strictly marked. This mark is given by the Ministry of Finances after testing in accredited for the scope laboratories. The set of marks of precious metal alloys are specified in **ISO 9202:1991**, and are nominated according to the purity for gold m/m as ‰: 375, 585, 750 and 916, which correspond to 9, 14, 18 and 22 carats respectively. One carat is equal to 41,667‰. In a common practice 14 carat (585‰) gold alloys are used for jewellery, therefore such samples are more often a subject of analyses.

Important conclusions and decisions linked to the customer interest protection sphere derive from measurement results that have to be based on reliable data of good quality (e.g. sufficiently small uncertainty). The legislations require the actual fineness of jewellery alloys shall not be less by more than three one-thousandth parts than the fineness indicated by the mark stamped. Therefore the testing method should be able to provide results with high accuracy and low uncertainty (less ***than 3 ‰; k=1***) of analytical measurements. The calculated expanded uncertainty for Au mass fraction should be less than 9 ‰ (k=3).

The traditional and internationally recognised method for gold alloy analysis is based on the cupellation (fire assay) (ISO Standard 11426). Recently other alternative methods based on atomic spectrometry have been suggested.

1. *ISO/TC 174. rev.N71. Gouda 1992 Determination of gold in gold jewelry allows –ICP solution spectrometric method using yttrium as internal standard*
2. *CNR-PRO Art Project (1998) Tecniche spettrometriche alternative alla copellazione per il saggio delle leghe dioro*

## Scope

This example describes a laboratory developed method for determination of gold after *aqua regia* solubilisation and measurements by flame atomic absorption spectrometry (FAAS). The range of application is jewellery alloys containing gold 14±0,5 carats.

The procedure is optimised to fit for purpose by means of a system for air-segmented discrete introduction (ASDI) that allows signals to be accumulated without drift. External procedure for pseudo steady state signal smoothing and ensemble summation is used for bettering the repeatability of the instrumental performance.

The experimental protocol is shown in the figure below.

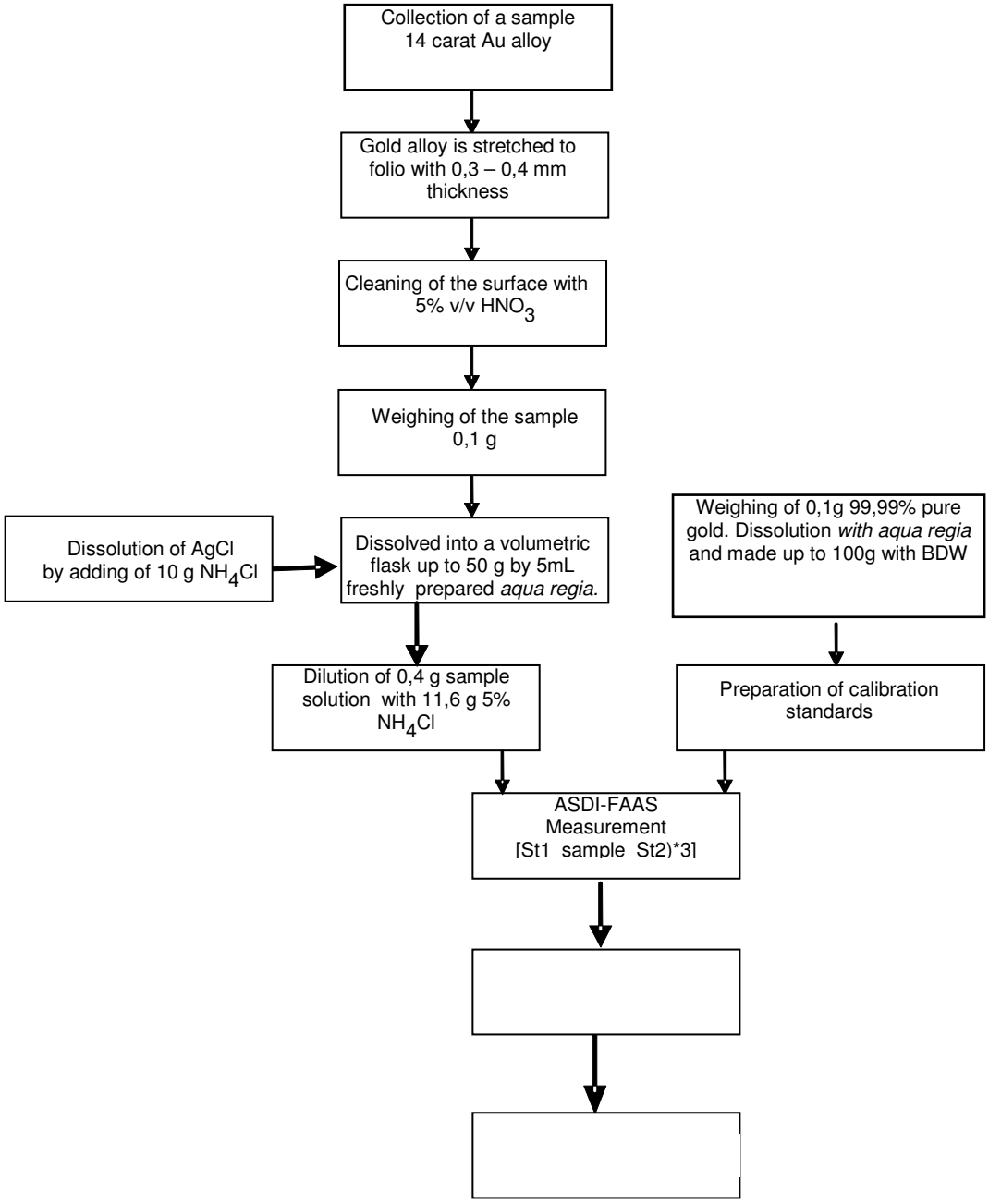



**Figure 3 Flow chart of the analytical procedure for determination of gold in gold alloys**

## Reagents

- E. Merck class p.a. - 5% v/v $HNO_3$; freshly prepared *aqua regia*; $NH_4Cl$ – salt
- Pure Au 99,99 % certified from Non-Ferrous Metallurgical Plant Plovdiv

## Apparatus

- Flame Atomic Absorption Spectrometer equipped with hollow cathode lamp for gold
- Discrete sample introduction system (ASDI)
- Balance d=0.0001 g (certified – BDS EN 45501)
- Hot plate
- Pipette variable 200-1000 µL gravimetrically checked (certified d=0,4% in 200-500 µL range)
- Volumetric flask 50 mL (laboratory glassware class A; certified d=0,02mL for 20 °C)
- Volumetric flask 100 mL (laboratory glassware class A; certified d=0,08 mL for 20 °C)
- Polypropylene vials 12 g (ASDI autosampler kit)

## Description of the analytical procedure

*Sample preparation procedure*

Gold alloy samples are stretched to folio with 0.3 - 0.4 mm thickness. The surface is washed by 5% v/v $HNO_3$. A dry piece of 0,1 g accurately weighed to $\pm$ 0,0001 g is directly dissolved into a volumetric flask of 50 mL by 5mL freshly prepared *aqua regia*.

The flask is heated on ceramic hot plate for 20 min. During this process Ag precipitates as AgCl. AgCl is dissolved by adding of 10 g $NH_4Cl$ to the cooled solution and volume is made up to the mark (50 mL) with ultrapure water at (20$^{\circ}$C).

The solution is diluted additionally by transferring 0,400 g with micro-pipette to a conical vial adding 5% $NH_4Cl$ in order to keep the solution homogeneous with final weight of 12,000 g gravimetrically controlled.

Procedural blank is subject to exactly the same sample preparation procedure as the analysed sample.

*Calibration*

Stock standard solution was made in laboratory by dissolution of 0,1 g Au with purity 99,99 % with 5 mL *aqua regia* and filled up to 100 g with 5 % $NH_4Cl$. Two calibration standards are obtained in polypropylene vials after further dilution of 0,370 g and 0,430 g from stock standard solution with procedural blank solution to 10,000 g (gravimetrically controlled).

The selected calibration range, recalculated for analysed alloy, corresponds to the range 13,5 – 14,5 carats.

The calibration standards are subject to the same sample preparation procedure as the analysed sample. The exact matching of sample and solution used for calibration allows to avoid the influence of matrix effect on obtained signals.

*Atomic absorption measurement*

Gold is determined by air segmented discrete introduction flame atomic absorption spectrometry (ASDI-FAAS) using brackets calibration. In order to improve the repeatability of absorption measurements, the following experimental conditions are respected:

- Working with the best SIGNAL/NOISE ratio according the scedastic curves (signals near 0,6 absorbance units) and in very narrow concentration interval (37 ÷ 43 µg/g) with linear response according the Beer's low.
- Removing the drift by aspiration washing solution between injections and application of standard-sample-standard sequence ($St_1$ _ sample _ $St_2$ )
- Auto zero performance before every injection
- Applying signal smoothing and ensemble summation.

Instrumental parameters are described in Table 1.

Signals are accumulated in the sampling set ($St_1$ _ sample _ $St_2$) by precise time control (0,1 s) and are smoothed by means of external data treatment software. Signal profiles are summated as ensembles from N replicates of the sampling set for the St1, sample and St2 respectively and for each of them an ensemble pseudo plateau profile is obtained.

The stable plateau part (3 s) of summated ensembles is used for calibration and quantitative calculations. Standard uncertainty of the signals repeatability was calculated as standard deviation of absorbance measured in the plateau part (3 s).

**Table 1. Instrumental parameters for ASDI-FAAS determination of Au**

| FAAS parameters | Values | ASDI parameters |
|---|---|---|
| Au spectral line [nm]<br>Au spectral slit [nm] | 242,8<br>0,7 | $Q_l$- aspiration rate 6,4 mL/min checked by BDW<br>Injection time 5 s; Injection volume ≈ 0,530 µL |
| Au hollow cathode lamp current [mA] | 10 | Washing time 10 s; Total replicate time 15 s |
| Air/$C_2H_2$ units<br>Observation high [mm] | 50/18<br>6 | Smoothing Savitzky-Golay 24 points<br>Ensemble summation N signal profiles |
| Working range µg/g<br>Deuterium BG corrector | 37 - 43 OFF | Pseudo plateau 3 s<br>Sampling mode ($St_1$ _ sample _ $St_2$ )* N |
| Readings – points [s] | 50 | Total time for one set 66 s |

## Calculations

*Concentration of initial standard solution made up from pure gold*

$$C_{\_Au\,999,9} = \frac{m_{\_pureAu} * Au_{\_purity}}{G_{-100}} * 10^4$$

| | |
|---|---|
| $C_{\_Au\,999,9}$ | concentration of initial standard solution made up from pure gold [µg/g] |
| $m_{\_pureAu}$ | mass weighed of pure gold [g] |
| $G_{\_100}$ | mass of the solution in the volumetric flask made up to 100 g with 5% $NH_4Cl$ [g] |
| $Au_{\_purity}$ | the purity of gold stated in the certificate [%] |
| $10^4$ | conversion factor from % to µg/g; ρ≈ 1 equalised for standard and samples in 5% $NH_4Cl$ |

*Concentration of calibration standard solutions*

$$C_{-St1} = C_{Au\_999,9} * \frac{G_{\_0,37}}{G_{-100}} \qquad C_{-St2} = C_{Au\_999,9} * \frac{G_{\_0,43}}{G_{-100}}$$

| | |
|---|---|
| $C_{-St1}$ | Concentration of Au working standard solutions [µg/g]: |
| $C_{-St2}$ | $C_{\_St1}$ for low (37 µg/g) and $C_{\_St2}$ for high (43 µg/g) |
| $C_{Au\_999,9}$ | Concentration of Au standard solution Au 999,9 [µg/g] prepared from pure gold |
| $G_{\_0,37}$ | Masses of the initial Au standard solution transferred for the preparation of |
| $G_{\_0,43}$ | calibration solutions $C_{\_St1}$ (37 µg/g) or $C_{\_St2}$ (43 µg/g) [g]: 0,370 g or 0,430 g respectively |
| $G_{\_100}$ | Mass of gravimetrically controlled calibration standard solutions after adding 5% $NH_4Cl$ in polypropylene vials [g] |

*Bracketing calibration*

$$Cx = \frac{C_{-st1}(A\_St2 - A\_X) + C_{-St2}(A_{-x} - A_{-St1})}{A_{-St2} - A_{-St1}}$$

| | |
|---|---|
| $Cx$ | Concentration of Au in the analysed solution [µg/g] |
| $C_{-St1}$ | Concentration of the lower calibration standard solution used for bracketing calibration [µg/g] |
| $C_{-St2}$ | Concentration of the higher calibration standard solution used for bracketing calibration [µg/g] |
| $A_{-St1}$ | Absorbance measured for the lower calibration standard solution $C_{\_St1}$ |
| $A\_St2$ | Absorbance measured for the higher calibration standard solution $C_{\_St2}$ |
| $A\_X$ | Absorbance measured for the analysed sample solution |

*Calculation of Au mass fraction (W_‰) in analysed sample*

$$W\_‰ = \frac{1}{1000}\frac{V\_50}{m\_0{,}1} * \frac{1}{R} * \frac{G_{vials\ -12}}{G_{P\_0,4}}.C_x$$

| | |
|---|---|
| W_‰ | final concentration of Au in tested jewellery gold alloy w/w [‰] |
| $V\_50$ | volume of the solution in the volumetric flask [mL] |
| $m\_0{,}1$ | mass of analysed alloy sample [g] |
| $G_{vials\ -12}$ | weight of final sample solution prepared in vials [g] |
| $G_{P\ -0,4}$ | mass of Au sample solution taken from $V\_50$ [g] |
| R | correction for recovery |

*Combined model equation for calculation of Au content (‰)*

$$W\_‰ = \frac{1}{1000}\left(\frac{V_{-50}}{m_{-0,1}} * \frac{G_{vials\_12}}{G_{P\ -0,4}}\right) * \frac{C_{Au\_999,9}}{G_{\_10}} * \frac{\left(G_{P\_0,37}(A\_St2 - A\_X) + G_{P\_0,43}(A_{-X} - A_{-St1})\right)}{A_{-St2} - A_{-St1}} * \frac{1}{R}$$

*Calculation of signal standard uncertainty estimated as standard deviation*

$$u_{\_A} = \frac{u_{\_A\ -one\_set}}{\sqrt{N}}$$

| | |
|---|---|
| $u_{\_A}$ | calculated standard uncertainty from the plateau part (3s) of the absorbance signal after ensemble averaging of N sets of sampling ($St_1$ _ sample _ $St_2$ ) |
| $u_{\_A\ -one\_set}$ | calculated standard uncertainty from the plateau part (3s) of the absorbance signal obtained from one set of sampling ($St_1$ _ sample _ $St_2$ ) |
| $N$ | number of sets performed and summated as ensemble |

# PART II. The customer's requirements concerning quality of the measurement result

Expanded measurement uncertainty: 9‰ (k=3)

# PART III. Validation of the measurement procedure – relevant equations and measurement data

The procedure has been developed in the laboratory, thus a full validation must be performed.

However, for the purposes of this exercise, recovery (R) and repeatability will be calculated only.

1. Equations

*See Part I*

2. Measurement data

*Recovery:*

Cupellation method: 585,1‰

ADI-FAAS: 583,5‰

*Repeatability:*

586,48‰

582,32‰

581,68‰

583,82‰

585,88‰

580,56‰

# PART IV. Measurement uncertainty of the result – relevant equations and measurement data

Calculate combined and expanded uncertainty (k=3) from the following measurement data:

| **Input quantity** | **Value** | **Unit** | **Standard uncertainty** | **Remark** |
|---|---|---|---|---|
| $V_{\_50}$ | 50 | mL | 0,0379 | *Volume of analysed solution* |
| $V_{\_100}$ | 100 | mL | 0,0697 | *Volume of stock standard solution* |
| $m_{\_0,1}$ | 0,1001 | g | 0,0002 | *Mass of analysed alloy sample* |
| $G_{vials\ -12}$ | 12,0030 | g | 0,0008 | *Mass of sample solution prepared in vials* |
| $G_{P\_0,4}$ | 0,4015 | g | 0,0009 | *Mass of Au sample solution taken from* $V_{\_50}$ *flask* |
| $m_{\_pureAu}$ | 0,1004 | g | 0,0002 | *Mass weighed of pure gold* |
| $Au_{\_purity}$ | 99,99 | % | 0,0058 | *The purity of gold stated in the certificate* |
| $G_{p\_0,37}$<br>$G_{p\_0,43}$ | 0,3701<br>0,4302 | g | 0,0006 | *Masses of the stock Au standard solution transferred for the preparation of calibration solutions* $C_{\_St1}$ *and* $C_{\_St2}$ |
| $G_{\_10}$ | 10,0321 | g | 0,0008 | *Mass of calibration standard solutions* |
| $A_{-St1}$<br>$A_{\_St2}$ | 0,5203<br>0,6041 | AU | 0,0010<br>0,0011 | *Absorbance measured for calibration standard solutions* |
| $A_X$ | 0,5488 | AU | 0,0011 | *Absorbance measured for the analysed sample solution* |
| R | 1,002 | - | 0,0025 | *Recovery* |

# The solved exercises 'green pages'

## TRAINMIC EXERCISES

### Analytical procedure

***Determination of gold in jewellery gold alloys by flame atomic absorption spectrometry***

*Exercise 1:*

**Establishing traceability in analytical chemistry**

*Exercise 2:*

**Single laboratory validation of measurement procedures**

- **Part I: General issues**
- **Part II: Parameters to be validated**
- **Part III: Some calculations and conclusions**

*Exercise 3:*

**Building an uncertainty budget**

- **Addendum I: By spreadsheet approach**
- **Addendum II: By dedicated software**

# ESTABLISHING TRACEABILITY IN ANALYTICAL CHEMISTRY
*EXERCISE*

## 1. Specifying the analyte and measurand

| | |
|---|---|
| *Analyte* | Gold |
| *Measurand* | Gold mass fraction in jewellery alloys after *aqua regia* dissolution |
| *Units* | ‰ (g/1000 g) |

## 2. Choosing a suitable measurement procedure with associated model equation

| | | | |
|---|---|---|---|
| *Measurement procedure* | | | |
| *Type of calibration* | *standard curve* ☒ | *standard addition* ☐ | *internal standard* ☐ |

## Model equation

*1. Standard solutions*

*1.1. Stock standard solution - prepared from pure gold*

$$C_{Au\_999,9} = \frac{m_{\_pureAu} * Au_{\_purity}}{G_{\_100}} * 10^4$$

*1.2. Calibration standard solutions*

$$C_{\_St1} = C_{Au\_999,9} * \frac{G_{p\_0,37}}{G_{\_100}} \qquad C_{\_St2} = C_{Au\_999,9} * \frac{G_{p\_0,43}}{G_{\_100}}$$

*2. Bracketing calibration*

$$Cx = \frac{C_{\_St1}(A_{\_St2} - A_{\_X}) + C_{\_St2}(A_{\_x} - A_{\_St1})}{A_{\_St2} - A_{\_St1}}$$

*3. Calculation of Au content (W_‰) in analysed sample*

$$W\_‰ = \frac{1}{1000} \frac{V_{\_50}}{m_{\_0,1}} * \frac{G_{vials\ \_12}}{G_{P\_0,4}} . C_x * \frac{1}{R}$$

*4. Calculation of signal standard uncertainty*

$$u_{\_A} = \frac{u_{\_A\ -one\_set}}{\sqrt{N}}$$

*5. Calculation of recovery*

$$R = \frac{W_{observed}}{W_{ref}}$$

*6. Combined model equation for calculation of Au mass fraction (‰)*

$$W\_‰ = \frac{1}{1000}\left(\frac{V\_50}{m\_0{,}1} * \frac{G_{vials\_12}}{G_{P\_0{,}4}}\right) * \frac{m_{-pureAu} * Au_{purity}}{G_{\_100} * V_{-100}} * 10^4 * \frac{\left(G_{P\_0{,}37}(A_{\_St2} - A_{\_X}) + G_{P\_0{,}43}(A_{\_X} - A_{\_St1})\right)}{A_{\_St2} - A_{\_St1}} * \frac{1}{R}$$

| | |
|---|---|
| $V_{-50}$ | volume of analysed solution [mL] |
| $V_{-100}$ | volume of stock standard solution [mL] |
| $m_{\_0{,}1}$ | mass of analysed alloy sample [g] |
| $G_{vials\ -12}$ | mass of sample solution diluted in vials [g] |
| $G_{P\ -0{,}4}$ | mass of Au sample solution taken from V_50 flask [g] |
| $m_{\_pureAu}$ | mass weighed of pure gold [g] |
| $Au_{\_purity}$ | the purity of gold stated in the certificate [%] |
| $G_{p\_0{,}37}$ *or* $G_{p\_0{,}43}$ | masses of the stock Au standard solution transferred for the preparation of calibration solutions $St_1$ and $St_2$ [g] |
| $G_{\_100}$ | mass of calibration standard solutions [g] |
| $A_{\_St1}$ and $A_{\_St2}$ | absorbance measured for calibration standard solutions 1 and 2 |
| $A_X$ | absorbance measured for the analysed sample solution |
| R | recovery |

**3. List the input quantities according to their influence on the uncertainty of the result of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.**

| | |
|---|---|
| *1* | Recovery - 37,6% to the expanded uncertainty |
| *2* | Absorption of analysed gold sample - contributing 26,1% to the expanded uncertainty |
| *3* | Mass of analysed gold sample - contributing 14,9% to the expanded uncertainty |
| *4* | Mass of stock solution taken for the preparation of first standard solution - contributing 12,1% to the expanded uncertainty |
| *5* | Volume of the analysed solution – contributing 3,4% to the expanded uncertainty |

**4. List the reference standards needed and give also the information regarding traceability of the reference value**

**For the analyte**

| | | |
|---|---|---|
| *1* | *Name/ChemicalFormula/Producer:* | Pure Gold - certified by Non-Ferrous Metallurgical Plant Plovdiv - Bulgaria |
| *2* | *Name/ChemicalFormula/Producer:* | |

**For the other input quantities**

| | | |
|---|---|---|
| *1* | *Quantity/Equipment/Calibration:* *e.g. mass/balance/calibrated by NMI, U=xx (k=2),* | Balance – calibrated by NMI |
| *2* | *Quantity/Equipment/Calibration:* | Volumetric flask - class A quality |
| *3* | *Quantity/Equipment/Calibration:* | Absorbance - relative measurement. Not direct part of the traceability chain. |

**5. Estimating uncertainty associated with the measurement**

| | | |
|---|---|---|
| *Are all important parameters included in the measurement equation?* | *Yes* ☐ | *No* ☒ |
| *Other important parameters are:* | Within-lab reproducibility | |

**6. How would you prove traceability of your result?**

| | |
|---|---|
| *1* | Comparing the results with independent method (cupellation) |

**7. Any other comments, questions…**

# SINGLE LABORATORY VALIDATION OF MEASUREMENT PROCEDURES

*EXERCISE*

## PART I: GENERAL ISSUES

### 1. Specify the measurement procedure, analyte, measurand and units

| | |
|---|---|
| *The measurement procedure* | Analysis of gold alloys by AAS |
| *Analyte* | Gold |
| *The measurand* | Gold in jewellery alloys containing gold 14± 0,5 carats after *aqua regia* dissolution |
| *Unit* | ‰ |

### 2. Specify the Scope

| | |
|---|---|
| *Matrix* | Gold in 5% $NH_4Cl$ |
| *Measuring range* | 37-43 µg/g |

### 3. Requirement on the measurement procedure

| | | | |
|---|---|---|---|
| *Intended use of the results:* | Quality of products from precious metals alloys | | |
| *Mark the customer's requirements and give their values* | ☐ | *LOD* | |
| | ☐ | *LOQ* | |
| | ☐ | *Repeatability* | |
| | ☐ | *Within-lab reproducibility* | |
| | ☒ | *Measurement uncertainty* | 9‰ |
| | ☐ | *Trueness* | |
| | ☐ | *Other-state* | |

### 4. Origin of the measurement procedure

| | | VALIDATION |
|---|---|---|
| *New in-house method* | ☒ | *Full* |
| *Modified validated method* | ☐ | *Partial* |
| *Official standard method* | ☐ | *Confirmation/Verification* |

## PART II: PARAMETERS TO BE VALIDATED

### 5. Selectivity/Interference/Recovery

*Where yes, please give further information e.g. which CRM, reference method*

| | |
|---|---|
| ☐ | *CRM/RM: analysis of available CRM or RM* |
| | *Further information:* |
| ☒ | *Spike of pure substance* |
| | Pure gold 99,99% certified from non-ferrous metallurgical plant Plovdiv, Bulgaria |
| ☒ | *Compare with a reference method* |
| | Comparison with cupellation method |
| ☐ | *Selectivity, interferences* |
| | |
| ☐ | *Test with different matrices* |
| | |
| ☒ | *Other – please specify* |
| | Test for recovery with RM jewellery gold alloy marked 585 |

### 6. Measuring range

| | |
|---|---|
| ☐ | *Linearity* |
| ☐ | *Upper limit* |
| ☐ | *LOD* |
| ☐ | *LOQ* |

### 7. Spread – precision

| | |
|---|---|
| ☒ | *Repeatability* |
| ☐ | *Reproducibility (within lab)* |
| ☐ | *Reproducibility (between lab)* |

## 8. Robustness

| | |
|---|---|
| | *Variation of parameters* |
| | |

## 9. Quality control

| | |
|---|---|
| ☐ | *Control charts* |
| ☐ | *Participation in PT schemes* |

## 10. Other parameters to be tested

| | |
|---|---|
| ☐ | *Working range and testing of homogeneity of variances* |
| ☒ | *Recovery* |
| ☐ | *Residual standard deviation* |
| ☒ | *Standard deviation of the method* |
| ☐ | *Coefficient of variation of the method* |

## PART III: SOME CALCULATIONS AND CONCLUSIONS

### 11. Calculation of parameters requested by the customer

| Parameters requested to be validated | Calculations |
|---|---|
| ☐ *LOD* | |
| ☐ *LOQ* | |
| ☒ *Repeatability* | 2,4‰ |
| ☐ *Within-lab reproducibilty* | |
| ☐ *Trueness* | |
| ☒ *Measurement uncertainty* | 8,3‰ (k=3) |
| ☒ *Other - please state* *Recovery* | 1,0002 ± 0,0025 |

**12. Does the analytical procedure fulfil the requirement(s) for the intended use?**

| **Parameter** | **Value requested by the customer** (the same as stated in question 3) | **Value obtained during validation** | **The requirement is fulfilled Yes/No** |
|---|---|---|---|
| ☐ LOD | | | |
| ☐ LOQ | | | |
| ☐ Repeatability | | | |
| ☐ Within-lab reproducibility | | | |
| ☐ Trueness | | | |
| ☒ Measurement uncertainty | 9‰ (k=3) | 8,3‰ (k=3) | yes |
| ☐ Other | | | |

**The analytical procedure is fit for the intended use:**

☒ *Yes* ☐ *No*

*For measurement uncertainty and traceability refer to the corresponding report-sheets*

# BUILDING AN UNCERTAINTY BUDGET
*EXERCISE*

## 1. Specify the measurand and units

| | |
|---|---|
| *Measurand* | Gold mass fraction in jewellery alloys after *aqua regia* dissolution |
| *Unit* | ‰ (g/1000 g) |

## 2. Describe the measurement procedure and provide the associated model equation

*Measurement procedure:*

Gold alloy samples are stretched to folio with 0,3 - 0,4 mm thickness. The surface is washed by 5% v/v $HNO_3$. A dry piece of 0,1 g accurately weighed to + 0,0001 g is directly dissolved into a volumetric flask of 50 mL by 5mL freshly prepared *aqua regia*.

The flask is heated on ceramic hot plate for 20 min. During this process Ag precipitates as AgCl. AgCl is dissolved by adding of 10 g $NH_4Cl$ to the cooled solution and volume is made up to the mark (50 mL) with BDW at (20°C).

The solution is diluted additionally by transferring 0,400 mL with micro-pipette to a conical vial adding 5% $NH_4Cl$ in order to keep the solution homogeneous with final weight of 12,000 g gravimetrically controlled.

Procedural blank and gold reference material are subject to exactly the same sample preparation and measurement procedures as the analysed sample.

*Model equation:*

*1. Concentration of initial standard solution made up from pure gold*

$$C_{Au\,999,9} = \frac{m_{pureAu} * Au_{purity}}{G_{-100}} * 10^4$$

$$C_{-St\,2} = C_{Au\_999,9} * \frac{G_{\_0,43}}{G_{-100}}$$

*2. Concentration of calibration standard solutions*

$$C_{-St1} = C_{Au\_999,9} * \frac{G_{\_0,37}}{G_{-100}}$$

*3. Bracketing calibration*

$$Cx = \frac{C_{\_St1}(A_{\_St2} - A_{\_X}) + C_{\_St2}(A_{\_x} - A_{\_St1})}{A_{\_St2} - A_{\_St1}}$$

*4. Calculation of Au mass fraction (W_‰) in analysed sample*

$$W\_‰ = \frac{1}{1000}\frac{V_{\_50}}{m_{\_0,1}} * \frac{1}{R} * \frac{G_{vials\_12}}{G_{P\_0,4}}.C_x$$

*5. Calculation of signal standard uncertainty*

$$u_{\_A} = \frac{u_{\_A\_one\_set}}{\sqrt{N}}$$

*6. Calculation of recovery*

$$R = \frac{W_{observed}}{W_{ref}}$$

*7. Combined model equation for calculation of Au mass fraction (‰)*

$$W\_‰ = \frac{1}{1000}\left(\frac{V_{\_50}}{m_{\_0,1}} * \frac{G_{vials\_12}}{G_{P\_0,4}}\right) * \frac{C_{Au\_999,9}}{G_{\_100}} * \frac{\left(G_{P\_0,37}(A_{\_St2} - A_{\_X}) + G_{P\_0,43}(A_{\_X} - A_{\_St1})\right)}{A_{\_St2} - A_{\_St1}} * \frac{1}{R}$$

## 3. Identify (all possible) sources of uncertainty

| | |
|---|---|
| ☐ | *Uncertainty of concentration of reference solutions* |
| ☐ | *Uncertainty of measurements of absorption of standard and sample solutions* |
| ☐ | *Mass of analysed gold sample* |
| ☐ | *Volume of the analysed solution* |
| ☐ | *Recovery* |
| ☐ | *Other:* |
| ☐ | *Other:* |

### 4. Evaluate values of each input quantity

| *Input quantity* | *Value* | *Unit* | *Remark* |
|---|---|---|---|
| $V_{-50}$ | 50 | mL | Volume of analysed solution |
| $V_{-100}$ | 100 | mL | Volume of stock standard solution |
| $m_{\_0,1}$ | 0,1001 | g | Mass of analysed alloy sample |
| $G_{vials\ -12}$ | 12,0030 | g | Mass of sample solution prepared in vials |
| $G_{P\_0,4}$ | 0,4015 | g | Mass of Au sample solution taken from $V_{\_50}$ flask |
| $m_{-pureAu}$ | 0,1004 | g | Mass weighed of pure gold |
| $Au_{\_purity}$ | 99,99 | % | The purity of gold stated in the certificate |
| $G_{P\_0,37}$ ; $G_{P\_0,43}$ | 0,3701; 0,4302 | g | Masses of the stock Au standard solution transferred for the preparation of calibration solutions $C_{\_St1}$ and $C_{\_St2}$ |
| $G_{\_100}$ | 10,0321 | AU | Mass of calibration standard solutions |
| $A_{-St1}$ ; $A_{\_St2}$ | 0,5203; 0,6041 | AU | Absorbance measured for calibration standard solutions |
| $A_X$ | 0,5488 | AU | Absorbance measured for the analysed sample solution |
| R | 1,002 | - | Recovery |

### 5. Evaluate the standard uncertainty of each input quantity

| *Input quantity* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|
| $V_{-50}$ | 0,0379 | mL | Volume of analysed solution |
| $V_{-100}$ | 0,0697 | mL | Volume of stock standard solution |
| $m_{\_0,1}$ | 0,0002 | g | Mass of analysed alloy sample |
| $G_{vials\ -12}$ | 0,0008 | g | Mass of sample solution prepared in vials |
| $G_{P\_0,4}$ | 0,0009 | g | Mass of Au sample solution taken from $V_{\_50}$ flask |
| $m_{-pureAu}$ | 0,0002 | g | Mass weighed of pure gold |
| $Au_{\_purity}$ | 0,0058 | % | The purity of gold stated in the certificate |
| $G_{P\_0,37}$ ; $G_{P\_0,43}$ | 0,0006; 0,0006 | g | Masses of the stock Au standard solution transferred for the preparation of calibration solutions $C_{\_St1}$ and $C_{\_St2}$ |
| $G_{\_10}$ | 0,0008 | g | Mass of calibration standard solutions |
| $A_{-St1}$ ; $A_{\_St2}$ | 0,0010; 0,0011 | AU | Absorbance measured for calibration standard solutions |
| $A_X$ | 0,0011 | AU | Absorbance measured for the analysed sample solution |
| R | 0,0025 | | Recovery |

### 6. Calculate the value of the measurand, using the model equation

583,4849‰

### 7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units

*Using*: ☐ Mathematical solution; ☒ Spreadsheet approach; ☐ Commercial software

| *Input quantity* | *Value* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|---|
| W_‰ | 583,4849 | 2,7664 | ‰ | Au mass fraction in jewellery alloys |
| | | | | |
| | | | | |
| | | | | |

### 8. Calculate expanded uncertainty ($U_c$) & specify the coverage factor k and the units

8,2993 ‰ (k=3)

### 9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$

| | |
|---|---|
| *1* | Recovery – contributing 37,6% to the expanded uncertainty |
| *2* | Absorption of analysed gold sample - contributing 26,1% to the expanded uncertainty |
| *3* | Mass of analysed gold sample - contributing 14,9% to the expanded uncertainty |

### 10. Prepare your uncertainty budget report

(583,4849 ± 8,2993) ‰ (k=3)*

(*) the reported uncertainty is an expanded uncertainty calculated using a coverage factor of k = 3, which gives a level of confidence of approximately 99,7%

# Addendum I. Measurement uncertainty calculation: spreadsheet approach (Excel)

**Determination of gold in jewellery gold alloys by Flame Atomic Absorption Spectrometry (2)**
**(Combined model equation and input quantity description)**

$$W\_‰ = \frac{1}{1000}\left(\frac{V\_50}{m\_0{,}1} * \frac{G_{vials}\_12}{G_P\_0{,}4}\right) * \left(\frac{C_{Au\_999{,}9}}{G\_10}\right) * \frac{\left(G_{P\_0{,}37}(A\_St2 - A\_X) + G_{P\_0{,}43}(A\_X - A\_St1)\right)}{A\_St2 - A\_St1} * \frac{1}{R}$$

| № | Input_Quant | Units | Marck | Values | u_c | Ru_c |
|---|---|---|---|---|---|---|
| 1 | Volume of analysed Au solution | g | V_50 | 50,00 | 0,0379 | 0,00% |
| 2 | gold pice mass | g | m_0,1 | 0,1001 | 0,0002 | 0,16% |
| 3 | Weight of vials | g | Gvials_12 | 12,0030 | 0,0008 | 0,01% |
| 4 | Mass of Au solution | g | Gp_0,4 | 0,4015 | 0,0009 | 0,22% |
| 5 | Concentrationt of stock standard solution | µg/L | C_Au_999,9 | 1003,8996 | 0,1770 | 0,02% |
| 6 | Weight of flask | g | G_10 | 10,0321 | 0,0008 | 0,01% |
| 7 | Mass of stock solution used for preparation of St1 | g | Gp_0,37 | 0,3701 | 0,0006 | 0,16% |
| 8 | Mass of stock solution used for preparation of St2 | g | Gp_0,43 | 0,4302 | 0,0006 | 0,13% |
| 9 | Absorbanse of high St2 | AU | A_St2 | 0,6041 | 0,0010 | 0,16% |
| 10 | Absorbanse of the sample | AU | A_x | 0,5488 | 0,0011 | 0,21% |
| 11 | Abrorbance of the low St1 | AU | A_St1 | 0,5203 | 0,0011 | 0,21% |
| 12 | | numb | 1000 | 1000,0 | 0 | 0,00% |
| 13 | Recovery | | R | 1,0002 | 0,0025 | 0,25% |
| | | | | **583,4849** | **2,7664** | |
| | | | | | k=1 | |

| V_50 | m_0,1 | Gvials_12 | Gp_0,4 | C_Au_999,9 | G_10 | Gp_0,37 | Gp_0,43 | A_St2 | A_x | A_St1 | 1000 | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 50,0379 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 | 50,0000 |
| 0,1001 | 0,1003 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 | 0,1001 |
| 12,0030 | 12,0030 | 12,0038 | 12,0030 | 12,0030 | 12,0030 | 12,0030 | 12,0030 | 12,0030 | 12,0030 | 12,0030 | 12,0030 | 12,0030 |
| 0,4015 | 0,4015 | 0,4015 | 0,4024 | 0,4015 | 0,4015 | 0,4015 | 0,4015 | 0,4015 | 0,4015 | 0,4015 | 0,4015 | 0,4015 |
| 1003,8996 | 1003,8996 | 1003,8996 | 1003,8996 | 1004,0766 | 1004,0000 | 1003,8996 | 1003,8996 | 1003,8996 | 1003,8996 | 1003,8996 | 1003,8996 | 1003,8996 |
| 10,0321 | 10,0321 | 10,0321 | 10,0321 | 10,0321 | 10,0329 | 10,0321 | 10,0321 | 10,0321 | 10,0321 | 10,0321 | 10,0321 | 10,0321 |
| 0,3701 | 0,3701 | 0,3701 | 0,3701 | 0,3701 | 0,3701 | 0,3707 | 0,3701 | 0,3701 | 0,3701 | 0,3701 | 0,3701 | 0,3701 |
| 0,4302 | 0,4302 | 0,4302 | 0,4302 | 0,4302 | 0,4302 | 0,4302 | 0,4308 | 0,4302 | 0,4302 | 0,4302 | 0,4302 | 0,4302 |
| 0,6041 | 0,6041 | 0,6041 | 0,6041 | 0,6041 | 0,6041 | 0,6041 | 0,6041 | 0,6051 | 0,6041 | 0,6041 | 0,6041 | 0,6041 |
| 0,5488 | 0,5488 | 0,5488 | 0,5488 | 0,5488 | 0,5488 | 0,5488 | 0,5488 | 0,5488 | 0,5499 | 0,5488 | 0,5488 | 0,5488 |
| 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5203 | 0,5214 | 0,5203 | 0,5203 |
| 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 | 1000,0 |
| 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0002 | 1,0027 |
| **583,9275** | **582,5346** | **583,5246** | **582,1826** | **583,5878** | **583,4957** | **584,0541** | **583,778** | **583,1355** | **584,7160** | **582,7010** | **583,4849** | **582,0072** |
| diff | -0,44263088 | 0,950324628 | -0,039691195 | 1,302328948 | -0,102882537 | -0,010864538 | -0,5692262 | -0,2933625 | 0,34941893 | -1,23108285 | 0,784 | 0,000 | 1,478 |
| sqrt | 0,1959 | 0,903116899 | 0,001575391 | 1,69606069 | 0,010584816 | 0,000118038 | 0,32401844 | 0,08606155 | 0,12209359 | 1,51556499 | 0,614417952 | 0,000 | 2,184 |

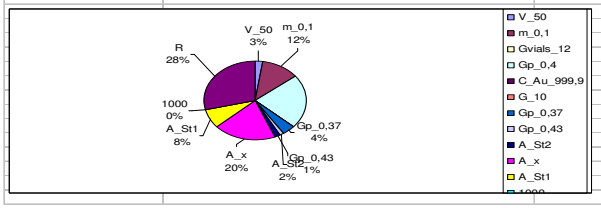
| Requarement | 1 katat | V_50 | m_0,1 | Gvials_12 | Gp_0,4 | C_Au_999,9 | G_10 | Gp_0,37 | Gp_0,43 | A_St2 | A_x | A_St1 | 1000 | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13,5-14,5 karat | | | | | | | | | | | | | | |
| 562.5 – 604.2 ‰ | 41,6‰ | **2,6%** | **11,8%** | **0,0%** | **22,2%** | **0,1%** | **0,0%** | **4,2%** | **1,1%** | **1,6%** | **19,8%** | **8,0%** | **0,0%** | **28,5%** |

**Result**

| k= | 3 | | Target U |
|---|---|---|---|
| U= | 8,3 | ‰ | 9 ‰ |

| SUM |
|---|
| 7,6532 |

**Main contrubutors to combained uncertainty,%**



## Chapter 3

## Determination of Calcium in Serum by Spectrophotometry

*Steluta Duta, Philip Taylor*

- **TrainMiC example summary form ('blue page')**
- **A short introduction to the analytical procedure ('slides')**
- **All input needed to do the three exercises ('yellow pages')**
- **The solved exercises ('green pages')**

# TrainMiC example summary form

## I. General information about the example

| | |
|---|---|
| **Measurand** | Concentration of calcium in human serum (mg/dL) |
| **Example number** | Ex-10 |
| **Authors of the example** | Steluta Duta, Philip Taylor |
| **Analytical procedure** | Standard WHO procedure |
| **Customer's requirement** | Standard WHO procedure |

## II. Attached files

| File number, type and name | | Content of the file | | File is attached Yes | File is attached No | Remark |
|---|---|---|---|---|---|---|
| 1 - I | Ex-10-1-I-Ca-serum-Photometry-2006-Ver1.ppt | About the analytical procedure: short introduction | | ✓ | | Given by the lecturer |
| 2 - Yellow | Ex-10-2-Y-Ca-serum-Photometry-2006-Ver1.doc | PART I | Description of the analytical procedure | ✓ | | Each participant receives own copy and may keep it |
| | | PART II | The customer's requirements concerning the quality of the measurement result | ✓ | | |
| | | PART III | Validation of the measurement procedure – relevant equations and measurement data | ✓ | | |
| | | PART IV | Measurement uncertainty of the result – relevant equations and measurement data | ✓ | | |
| 3 - Green | Ex-10-3-G-Ca-serum-Photometry-2006-Ver1.doc | PART I | Establishing traceability in analytical chemistry | ✓ | | |
| | | PART II | Single laboratory validation of measurement procedures | ✓ | | |
| | | PART III | Bulding an uncertainty budget | ✓ | | |
| | | | Addendum 1: By spreadsheet approach | ✓ | | |
| | | | Addendum 2: By dedicated software | | ✓ | |

## III. History of the example

| Version | Uploaded on the webhotel | Short description of the change |
|---|---|---|
| 0 | April 2007 | |
| 1 | | |

# A short introduction to the analytical procedure

## Determination of calcium in serum by (spectro)photometry

**Scope of the presentation**

- The analytical procedure and the customer's requirements
- About 'the chemistry' and the measurement aspects
- Model equation and more about traceability, validation and uncertainty exercises

## The analytical procedure and the customer's requirements

Analytical procedure:

- Guideline on Standard Operating Procedure for Clinical Chemistry: Calcium-O-Cresolphtalein complexone method (Standard Methods)
- http://w3.whosea.org

Customer's requirements:

- Analytical reproducibility (CV, %): 8% (WHO); 2% (actual state-of-art)
- Intended use: clinical interpretation

## Experimental protocol

prepare buffer reagents (pH = 10.7) and colour reagents

prepare Ca stock solution ➔ calibrants

Sample = serum (no sample treatment)
MIX: water+std+colour reag (+sample)
incubate at $25^0$ C for 15 min

Calibration, check linearity
blank to zero
interpolation ➔ One point calibration

check QC & between days precision

## Model equation

$$c_{Ca} = \left[(m * M_{Ca} * P) * 100 / (V_{500} * M_{CaCO_3})\right] * (V_i / V_{100}) * \left[(A_x - A_{blank}) / (A_{-10} - A_{blank})\right] * \left(V_f / V_{\text{int}}\right)$$

*10 (mg/dL)*: concentration of standard solution

*A_x:* absorbance of serum sample

*A_10*: absorbance of standard solution

$$c\_Ca = \left(A_x / A_{-10}\right) * 10\ (mg / dL)$$

$$c_{Ca} = c_x * \left(V_f / V_{\text{int}}\right)$$

$$c_x = c_{-10}\left(A_x - A_{blank}\right) / \left(A_{-10} - A_{blank}\right)$$

$$c_{stock} = (m * M_{Ca} * P) * 100 / (V_{500} * M_{CaCO_3})$$

$$c_i = c_{stock} \left(\frac{V_i}{V_{100}}\right)$$

# All input needed to do the three exercises 'yellow pages'

## Analytical procedure

***Determination of concentration of calcium in serum by molecular absorption spectrometry.***

***The quality of the results should comply with the requirements in the WHO procedure***

**PART I**

**Description of the analytical procedure**

**PART II**

**The customer's requirements concerning the quality of the measurement result**

**PART III**

**Validation of the measurement procedure – relevant equations and measurement data**

**PART IV**

**Measurement uncertainty of the result – relevant equations and measurement data**

# PART I. Description of the analytical procedure

## Laboratory task

Clinical laboratory has to determine calcium concentration in human serum sample ariving in their laboratory. It is the case when only the analitical part is considered, the laboratory has not responsibility how sample is taken, prepared, transported.

The laboratory should evaluate the analytical procedure reliability (within laboratory reproducibility). The reported results should comply with the clinical interpretation: the expected normal range of calcium concentration in serum is 8,5 – 10,4 mg/dL.

## Principle of the measurement method

Text extract from World Health Organization (WHO) - Standard Operating Procedures for Clinical Chemistry: Determination of calcium concentration by calcium-O-cresolphthalein complexone method (http://w3.whosea.org).

Determination of calcium in human serum is performed by molecular absorption (spectro)photometry method. Calcium forms a purple-coloured complex with orto-cresolphthalein complexone in an alkaline medium. The inclusion of HCl helps to release calcium bound to proteins, and 8-hydroxy-quinoline eliminates the interference by magnesium. Additional reagents as 2-amino,2-methyl,1-propanol (AMP) provides the proper alkaline medium for the colour reaction. The intensity of the colour is measured at 540 nm.

## Analytical procedure

*Serum sample preparation and storage*

No serum preparation is performed by the laboratory in charge with the analytical work. Serum sample arives in the laboratory after separation from the blood cells during the preanalytical step performed by another department. Haemolysed and heparinised samples are unsuitable for this method.

Calcium in serum is stable for 12 hours at room temperature (25-35)℃, one week at (2-8)℃ and for a longer period up to 3 months at -20℃.

*Reagents*

AMP Buffer pH 10,7

In 37,8 mL of AMP reagent add 150 mL of distilled water and mix. Adjust the pH to 10,7 with HCl 6N and make up to 250 mL with distilled water. Store in the refrigerator in a brown coloured glass bottle. Stable for 3 weeks.

Colour reagents

Add 15 mL concentrated HCl to a 250 mL volumetric flask containing about 25 mL of distilled water. Transfer 25 mg O-cresolphthalein complexone power into it, mix to dissolve. Then add 250 mg of 8-hydroxy-quinoline, dissolve and than make up to 250 mL with distilled water. Store in a brown coloured glass bottle at room temperature (25-35)℃. Stable for about one month.

Calcium standard solutions

***Stock calcium standard solution***

Calcium carbonate is dried at $100^0$C for 2 hours. Allow to cool in a desicator. Dissolve 625 mg of dried calcium carbonate in 50 mL of distilled water taken in a 500 mL volumetric flask and add 3,5 mL HCl conc. Mix to dissolve and make up to 500 mL with distilled water. Store in brown bottle at room temperature (25-35)℃. Stable for 6 months. The calcium concentration in this solution is 50 mg/dL.

***Calibration calcium standard solutions***

The calibration calcium standard solutions are prepared by dilution of stock calcium standard solution: into four 100 mL volumetric flasks transfer 10 mL, 15 mL, 20 mL and 25 mL of stock calcium standard solution and dilute each to 100 mL with benzoic acid. The working standards contain S5/5 mg/dL, S7,5/7,5 mg/dL, S10/10 mg/dL, and S12,5/12,5 mg/dL calcium, respectively. Store in brown bottles at room temperature (25-35)℃. Stable for 2 months.

*Instrumentation*

A photometer or spectrophotometer is used in the visible range, in text it is called (spectro)photometer. The instrumental performances: spectral range: 190-850 nm; absorbance accuracy: 0,003 at 0,1 A. The instrument has the absorbance scale, as a concequence for concentration measurements the calibration graph should be established by laboratory itself.

*Experimental protocol*

The experimental steps of the measurement procedure are described in the table bellow: a defined volumes of calibration solutions and serum sample (0,1 mL) are mixed with 2,0 mL of colour reagent. Mixed than with 2 mL of buffer solution.

| | **Blank** | **S5** | **S7,5** | **S10** | **S12,5** | **Serum** | **QC** |
|---|---|---|---|---|---|---|---|
| Distilled water (mL) | 0,1 | - | - | - | - | - | - |
| Standard (mL) | - | 0,1 | 0,1 | 0,1 | 0,1 | - | - |
| Serum/QC (mL) | - | - | - | - | - | 0,1 | 0,1 |
| Colour reagent (mL) | 2,0 | 2,0 | 2,0 | 2,0 | 2,0 | 2,0 | 2,0 |
| | | Mix | well | | | | |
| Buffer solution (mL) | 2,0 | 2,0 | 2,0 | 2,0 | 2,0 | 2,0 | 2,0 |
| | | Mix | well | | | | |

After 15 minutes incubation at room temperature (25-35)℃, the absorbance is measured at 540 nm against distilated water as procedural blank. By plotting the absorbance of the standards against their respective concentrations, the calibration graph is plotted. Once linearity is proved, it is just enough if a single standard as S10 (10 mg/dL) is used to determine the calcium concentration in the sample.

The measurable range with this graph is from 1,0 to 12,0 mg/dL. It is advisable to plot a calibration graph whenever the reagents are freshly prepared.

## Calculation of result

The following equation is indicated in WHO procedure:

$c_{Ca} = (A_x / A_{-10}) * 10$ mg/dL

where: $c_{Ca\_}$ concentation of calcium in serum sample

$A_x$-absorbance of serum sample

$A_{-10}$-absorbance of the calibration solution (10 mg/dL)

## Analytical reliability

Include one internal quality control sample (QC) in every batch of samples analysed each day irrespective of the number of samples in a batch. Since calcium is analysed single batch in a day in an intermediate laboratory, it will not be possible to analyse several QC samples and calculate within-day precision. However even if only a single QC sample is analysed in a day, this value can be pooled with the preceding 10 or 20 values obtained in the previous days and between-day precision can be calculated and express as % CV. Ensure that this is well within the acceptable limit, ( i.e. 8%, actual performance even 2%)

At least once a week analyse another QC serum from either a low or high QC pool.

# PART II. The customer's requirements concerning quality of the measurement result according to WHO*

**Clinical interpretation:**

Calcium concentration in serum: 8,5…10,4 mg/dL - normal range

Calcium concentration in serum: 12,5…16,1 mg/dL - pathological range

* World Health Organization

# PART III. Validation of the measurement procedure – relevant equations and measurement data

*Within-laboratory reproducibility (between day precision)*

**Model equation**

Coefficient of variation (CV)

$$CV = \frac{\sqrt{\frac{\sum_{1}^{5} (c_{i,obs} - c_{QC})^2}{n(n-1)}}}{c_{QC}} * 100$$

$CV$ coefficient as variation [%]

$c_{obs}$ observed calcium concentration in QC serum in $i^{th}$ day (i=1…5) [mg/dL]

$c_{QC}$ target calcium concentration in QC serum [mg/dL]

n number of reproducible measurements

**Measurement data**

| Input quantity | Value ± standard deviation (3 replicates) | Mean value ± standard deviation | Unit |
|---|---|---|---|
| $c_{i,obs}$ (i=1…5) day 3 replicates/day | 1st day: 9,28 ± 0,021 | 9,16 ± 0,05 | mg/dL |
| | 2nd day: 8,99 ± 0,057 | | |
| | 3rd day: 9,21 ± 0,105 | | |
| | 4th day: 9,23 ± 0,086 | | |
| | 5th day: 9,11 ± 0,120 | | |
| $c_{QC}$ | 8,24 …10,52 | 9,38 ± 0,38 | mg/dL |
| n | 5 | | no units |

*CV = 1,27%*

# PART IV. Measurement uncertainty of the result: relevant equations and measurement data[2]

## IV.1. Preparation of standard solutions

### *IV.1.1 Preparation of calcium stock standard solution*, $c_{stock}$

$$c_{stock} = (m * M_{Ca} * P) * 100 / (V_{500} * M_{CaCO_3})$$

| | |
|---|---|
| $c_{stock}$ | concentration of calcium stock solution [mg/dL] |
| m | mass of $CaCO_3$ [mg] |
| $M_{Ca}$ | molar mass of calcium [g/mol] |
| P | purity of $CaCO_3$ [mass fraction] |
| $V_{500}$ | final volume of calcium stock solution [mL] |
| $M_{CaCO3}$ | molar mass of $CaCO_3$[g/mol] |

Measurement data

| Input quantity | Value | Standard uncertainty | Unit |
|---|---|---|---|
| m | 625,0 | 0,2 | mg |
| $M_{Ca}$ | 40,078 | 0,002 | g/mol |
| P | 0,9999 | 0,0058 | mass fraction |
| $V_{500}$ | 500,00 | 0,15 | mL |
| $M_{CaCO3}$ | 100,0869 | 0,0024 | g/mol |

### *IV.1.2 Preparation of calibration standard solutions, $c_i$:*

$$c_i = c_{stock} \left( \frac{V_i}{V_{100}} \right)$$

| | |
|---|---|
| $c_{stock}$ | Concentration of calcium stock solution [mg/dL] |
| $V_i$ | Intake stock solution for dilution ($V_i = V_{-20}$ corresp. to $c_{-10}$) [mL] |
| $V_{100}$ | Volume of calibration solution [mL] |

where $V_i=V_{-10}$; $V_{-15}$; $V_{-20}$; $V_{-25}$ corresponding to $c_i=c_{-5}$; $c_{-7,5}$; $c_{-10}$; $c_{-12,5}$

[2] Here you may also want to include the relevant certificates

Measurement data:

| Input quantity | Value | Standard uncertainty | Unit |
|---|---|---|---|
| $c_{stock}$ | 50,05 | 0,02 | mg/dL |
| $V_i$ | 20,00 | 0,043 | mL |
| $V_{100}$ | 100,00 | 0,058 | mL |

### 4.2 Calibration – one point calibration

Model equation ($c_{-10}$ – the point of calibration standard solution):

$$c_x = c_{-10}\left(A_x - A_{blank}\right)/\left(A_{-10} - A_{blank}\right)$$

| | |
|---|---|
| $c_x$ | Concentration of serum sample from calibration data [mg/dL] |
| $c_{-10}$ | Concentration of calcium calibration solution (10 mg/dL) [mg/dL] |
| $A_x$ | Absorbance corresponding to serum sample |
| $A_{-10}$ | Absorbance corresponding to calibration solution (10 mg/dL) |
| $A_{blank}$ | Absorbance corresponding to blank solution |

Measurement data:

| Input quantity | Value | Standard uncertainty | Unit |
|---|---|---|---|
| $c_{\_10}$ | 10,01 | 0,023 | mg/dL |
| $A_x$ | 0,323 | 0,004 | no units |
| $A_{-10}$ | 0,338 | 0,002 | no units |
| $A_{blank}$ | 0,052 | 0,004 | no units |

### IV.3 Calculation of calcium concentration in serum sample

$$c_{Ca} = c_x * \left(V_f / V_{\text{int}}\right)$$

| | |
|---|---|
| $c_{Ca}$ | calcium concentration in serum sample [mg/dL] |
| $c_x$ | concentration of serum sample from calibration data [mg/dL] |
| $V_f$ | volume of serum sample under investigation [mL] |
| $V_{int}$ | intake volume from serum sample [mL] |

Measurement data:

| Input quantity | Value | Standard uncertainty | Unit |
|---|---|---|---|
| $c_x$ | 9,486 | 0,303 | mg/dL |
| $V_f$ | 0,100 | 0,002 | mL |
| $V_{int}$ | 0,100 | 0,002 | mL |

# The solved exercises 'green pages'

## TRAINMIC EXERCISES

### Analytical procedure

***Determination of calcium concentration in human serum by molecular absorbtion (spectro)photometry***

***The quality of results should comply with WHO[3] procedure requirements***

*Exercise 1:*

**Establishing traceability in analytical chemistry**

*Exercise 2:*

**Single laboratory validation of measurement procedures**

**Part I: General issues**

**Part II: Parameters to be validated**

**Part III: Some calculations and conclusions**

*Exercise 3:*

**Building an uncertainty budget**

**Addendum I: By spreadsheet approach**

**Addendum II: By dedicated software**

[3] World Health Organization

# ESTABLISHING TRACEABILITY IN ANALYTICAL CHEMISTRY

## 1. Specifying the analyte and measurand

| | |
|---|---|
| *Analyte* | Calcium |
| *Measurand* | Total concentration of calcium in human serum |
| *Units* | mg/dL |

## 2. Choosing a suitable measurement procedure with associated model equation

| | | | |
|---|---|---|---|
| *Measurement procedure* | To determine the calcium concentration in human serum, a serum sub-sample is mixed with reagent colour and buffer solution, according to WHO standard operation procedure. The absorbance of calcium calibration solutions and serum sample are measured by visible spectrophotometry at 540 nm. From the calibration data the concentration of calcium in human serum is calculated. | | |
| *Type of calibration* | *standard curve* ☒ | *standard addition* ☐ | *internal standard* ☐ |

**Model equation: calcium concentration in serum**

$$c_{Ca} = \left[\left(m * M_{Ca} * P\right) * 100 / \left(V_{500} * M_{CaCO_3}\right)\right] * \left(V_i / V_{100}\right) * \left[\left(A_x - A_{blank}\right) / \left(A_{-10} - A_{blank}\right)\right] * \left(V_f / V_{\text{int}}\right)$$

| | |
|---|---|
| $c_{Ca}$ | total calcium concentration in serum sample [mg/dL] |
| M | mass of $CaCO_3$ [mg] |
| $M_{Ca}$ | molar mass of calcium [g/mol] |
| P | purity of $CaCO_3$ [mass fraction] |
| $V_{500}$ | final volume of calcium stock standard solution [mL] |
| $M_{CaCO3}$ | molar mass of $CaCO_3$ [g/mol] |
| $V_i$ | intake stock solution for dilution ($V_i = V_{-20}$ corresp. to $c_{-10}$) [mL] |
| $V_{100}$ | final volume of calibration solution [mL] |
| $A_x$ | absorbance corresponding to serum sample |
| $A_{-10}$ | absorbance corresponding to calibration solution (10 mg/dL) |
| $A_{blank}$ | absorbance corresponding to blank |
| $V_f$ | volume of serum sample under investigation [mL] |
| $V_{int}$ | intake volume from serum sample [mL] |

**3. List the input quantities according to their influence on the uncertainty of the result of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.**

| | |
|---|---|
| *1* | Matrix effect - recovery |
| *2* | Instrumental signal (absorbance) |
| *3* | Concentration of standard solutions - purity of $CaCO_3$ |
| *4* | Volume of the glassware (pipettes, volumetric flasks) |
| *5* | Mass |

**4. List the reference standards needed and state the information regarding traceability of the reference value**

**For the analyte**

| | | |
|---|---|---|
| *1* | *Name/ChemicalFormula/Producer:* | $CaCO_3$ purity, Merck, min. 99,99 % |
| *2* | *Name/ChemicalFormula/Producer:* | $CaCO_3$ molar masses/IUPAC |

**For the other input quantities**

| | | |
|---|---|---|
| *1* | *Quantity/Equipment/Calibration:* *e.g. mass/balance/calibrated by NMI, U=xx (k=2),* | Absorbance/(Spectro)photometer/Calibrated against traceable optical standard (i.e PTB) |
| *2* | *Quantity/Equipment/Calibration:* | Volume/Laboratory glassware (pipettes, volumetric flasks/ calibrated by manufacturer (i.e. Hirschmann Laborgerate ) |
| *3* | *Quantity/Equipment/Calibration:* | Mass/Analytical balance/calibrated by manufacturer against traceable mass standards |

**5. Estimating uncertainty associated with the measurement**

| | | |
|---|---|---|
| *Are all important parameters included in the model equation?* | *Yes* ☐ | *No* ☒ |
| *Other important parameters are:* | Matrix effect | |

**6. How would you prove traceability of your result?**

| | |
|---|---|
| *1* | Via traceable calibration data |
| *2* | Via traceable volumetric measurements |
| *3* | Via traceable mass measurements |

**7. Any other comments, questions…**

# SINGLE LABORATORY VALIDATION OF MEASUREMENT PROCEDURES

## PART I: GENERAL ISSUES

### 1. Specify the measurement procedure, analyte, measurand and units

| | |
|---|---|
| *The measurement procedure* | To determine the calcium concentration in human serum, a serum sub-sample is mixed with reagent colour and buffer solution, according to WHO standard operation procedure. The absorbance of calcium calibration solutions and serum sample are measured by visible spectrophotometry at 540 nm. From the calibration data the concentration of calcium in human serum is calculated. |
| *Analyte* | Calcium |
| *The measurand* | Total calcium concentration in human serum |
| *Unit* | mg/dL |

### 2. Specify the scope

| | |
|---|---|
| *Matrix* | Human serum |
| *Measuring range* | 1,0…12,0 mg/dL |

### 3. Requirement on the measurement procedure

| | | | |
|---|---|---|---|
| *Intended use of the results* | Calcium concentration in serum result is intended to be used for clinical interpretation | | |
| *Mark the customer's requirements and give their values* | *Parameters to be validated* | | *Value requested by the customer* |
| | ☐ | *LOD* | |
| | ☐ | *LOQ* | |
| | ☐ | *Repeatability* | |
| | ☒ | *Within-lab reproducibility* | 8% as CV, by WHO procedure<br>2% as CV, the actual state-of-art |
| | ☐ | *Trueness* | |
| | ☐ | *Measurement uncertainty* | |
| | ☐ | *Other-state* | |

### 4. Origin of the measurement procedure

| | | |
|---|---|---|
| | | VALIDATION |
| *New in-house method* | ☐ | *Full* |
| *Modified validated method* | ☐ | *Partial* |
| *Official standard method* | ☒ | *Confirmation/Verification* |

## PART II: PARAMETERS TO BE VALIDATED

### 5. Selectivity/Interference/Recovery

Where yes, please give further information e.g. which CRM, reference method

| | |
|---|---|
| ☒ | *CRM/RM: analysis of available CRM or RM* |
| | *Further information: ROCHE-Control serum type Precipath U* |
| ☐ | *Spike of pure substance* |
| | |
| ☐ | *Compare with a reference method* |
| | |
| ☐ | *Selectivity, interferences* |
| | |
| ☐ | *Test with different matrices* |
| | |
| ☐ | *Other – please specify* |
| | |

### 6. Measuring range

| | |
|---|---|
| ☒ | *Linearity* |
| ☐ | *Upper limit* |
| ☐ | *LOD* |
| ☐ | *LOQ* |

### 7. Spread – precision

| | |
|---|---|
| ☐ | *Repeatability* |
| ☒ | *Reproducibility (within lab)* |
| ☐ | *Reproducibility (between lab)* |

## 8. Robustness

| | |
|---|---|
| ☐ | *Variation of parameters* |

## 9. Quality control

| | |
|---|---|
| ☐ | *Control charts* |
| ☐ | *Participation in PT schemes* |

## 10. Other parameters to be tested

| | |
|---|---|
| ☐ | *Working range and testing of homogeneity of variances* |
| ☐ | *R squared* |
| ☐ | *Residual standard deviation* |
| ☐ | *Standard deviation of the analytical procedure* |
| ☒ | *Coefficient of variation of the analytical procedure* |
| ☒ | *Measurement uncertainty* |

## PART III: SOME CALCULATIONS AND CONCLUSIONS

### 11. Calculation of parameters requested by the customer

| Parameters requested to be validated | Calculations |
|---|---|
| ☐ *LOD* | |
| ☐ *LOQ* | |
| ☐ *Repeatability* | |
| ☒ *Within-lab reproducibilty* | $CV = \frac{\sqrt{\frac{\sum_{1}^{5} (c_{i,obs} - c_{QC})^2}{n(n-1)}}}{c_{QC}} * 100 = 1{,}27\%$ |
| ☐ *Trueness* | |
| ☐ *Measurement uncertainty* | |
| ☐ *Other - please state* | |

**12. Does the analytical procedure fulfil the requirement(s) for the intended use?**

| **Parameter** | **Value requested by the customer** (the same as stated in question 3) | **Value obtained during validation** | **The requirement is fulfilled Yes/No** |
|---|---|---|---|
| ☐ LOD | | | |
| ☐ LOQ | | | |
| ☐ Repeatability | | | |
| ☒ Within-lab reproducibility | 8% as CV, by WHO procedure | 1,27 % | YES |
| ☐ Trueness | | | |
| ☐ Measurement uncertainty | | | |
| ☐ Other | | | |

**The analytical procedure is fit for the intended use:**

☒ *Yes* ☐ *No*

*For measurement uncertainty and traceability refer to the corresponding sheets*

# BUILDING AN UNCERTAINTY BUDGET

## 1. Specify the measurand and units

| | |
|---|---|
| *Measurand* | Total calcium concentration in human serum |
| *Unit* | mg/dL |

## 2. Describe the measurement procedure and provide the associated model equation

Measurement procedure

To determine the calcium concentration in human serum, a serum sub-sample is mixed with reagent colour and buffer solution, according to WHO standard operation procedure. The absorbance of calcium calibration solutions and serum sample are measured by visible spectrophotometry at 540 nm. From the calibration data the concentration of calcium in human serum is calculated.

Model equation: calcium concentration in serum

$$c_{Ca} = \left[(m * M_{Ca} * P) * 100 / (V_{500} * M_{CaCO_3})\right] * (V_i / V_{100}) * \left[(A_x - A_{blank}) / (A_{-10} - A_{blank})\right] * \left(V_f / V_{\text{int}}\right)$$

| | |
|---|---|
| $c_{Ca}$ | total calcium concentration in serum sample [mg/dL] |
| m | mass of $CaCO_3$ [mg] |
| $M_{Ca}$ | molar mass of calcium [g/mol] |
| P | purity of $CaCO_3$ [mass fraction] |
| $V_{500}$ | final volume of calcium stock standard solution [mL] |
| $M_{CaCO3}$ | molar mass of $CaCO_3$ [g/mol] |
| $V_i$ | intake stock solution for dilution ($V_i = V_{-20}$ corresp. to $c_{-10}$) [mL] |
| $V_{100}$ | final volume of calibration solution [mL] |
| $A_x$ | absorbance corresponding to serum sample |
| $A_{-10}$ | absorbance corresponding to calibration solution (10 mg/dL) |
| $A_{blank}$ | absorbance corresponding to blank |
| $V_f$ | volume of serum sample under investigation [mL] |
| $V_{int}$ | intake volume from serum sample [mL] |

### 3. Identify (all possible) sources of uncertainty

| | |
|---|---|
| ☒ | *Uncertainty of concentration of reference solutions* |
| ☐ | *Uncertainty of measurements of peak area* |
| ☒ | *Method bias* |
| ☒ | *Matrix effect* |
| ☒ | *Other: Uncertainty of absorbance measurements* |
| ☒ | *Other: Uncertainty of volume measurements* |

### 4. Evaluate values of each input quantity

| *Input quantity* | *Value* | *Unit* | *Remark* |
|---|---|---|---|
| m | 625,0 | mg | |
| $M_{Ca}$ | 40,078 | g/mol | |
| P | 0,9999 | mass fraction | |
| $V_{500}$ | 500,00 | mL | |
| $M_{CaCO3}$ | 100,0869 | g/mol | |
| $V_i$ | 20,00 | mL | |
| $V_{100}$ | 100,00 | mL | |
| $A_x$ | 0,323 | no units | |
| $A_{-10}$ | 0,338 | no units | |
| $A_{blank}$ | 0,052 | no units | |
| $V_f$ | 0,100 | mL | |
| $V_{int}$ | 0,100 | mL | |

### 5. Evaluate the standard uncertainty of each input quantity

| *Input quantity* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|
| m | 0,2 | mg | |
| $M_{Ca}$ | 0,002 | g/mol | |
| P | 0,0058 | mass fraction | |
| $V_{500}$ | 0,15 | mL | |
| $M_{CaCO3}$ | 0,0024 | g/mol | |
| $V_i$ | 0,043 | mL | |
| $V_{100}$ | 0,058 | mL | |
| $A_x$ | 0,004 | no units | |
| $A_{-10}$ | 0,002 | no units | |
| $A_{blank}$ | 0,004 | no units | |
| $V_f$ | 0,002 | mL | |
| $V_{int}$ | 0,002 | mL | |

## 6. Calculate the value of the measurand, using the model equation

$$c_{Ca} = \left[(m * M_{Ca} * P) * 100 / (V_{500} * M_{CaCO_3})\right] * (V_i / V_{100}) * \left[(A_x - A_{blank}) / (A_{-10} - A_{blank})\right] * \left(V_f / V_{int}\right)$$

$c_{Ca}$ = 9,486 mg/dL

## 7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units

*Using*: ☐ Mathematical solution; ☒ Spreadsheet approach; ☐ Commercial software

| *Input quantity* | *Value* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|---|
| m | 625,0 | 0,2 | mg | |
| $M_{Ca}$ | 40,078 | 0,002 | g/mol | |
| P | 0,9999 | 0,0058 | mass fraction | |
| $V_{500}$ | 500,00 | 0,15 | mL | |
| $M_{CaCO3}$ | 100,0869 | 0,0024 | g/mol | |
| $V_i$ | 20,00 | 0,043 | mL | |
| $V_{100}$ | 100,00 | 0,058 | mL | |
| $A_x$ | 0,323 | 0,004 | no units | |
| $A_{-10}$ | 0,338 | 0,002 | no units | |
| $A_{blank}$ | 0,052 | 0,004 | no units | |
| $V_f$ | 0,100 | 0,002 | mL | |
| $V_{int}$ | 0,100 | 0,002 | mL | |

u ($c_{Ca}$) = 0,303 mg/dL

## 8. Calculate expanded uncertainty ($U_c$) & specify the coverage factor k and the units

**U($c_{ca}$) = k * u ($c_{Ca}$) = 0,606 mg/dL, k=2**

## 9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$

| | |
|---|---|
| *1* | Volume serum measurements |
| *2* | Concentration of serum sample from calibration data |

## 10. Prepare your uncertainty budget report

# Addendum I: Measurement uncertainty calculation: spreadsheet approach (Excel)

## Preparation of the standard solution

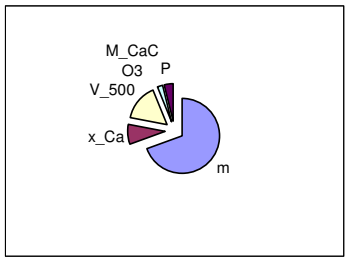
**Preparation of calcium stock standard solution**

| | value | std-unc | Rsu | m | M_Ca | V-500 | M_CaCO3 | P | |
|---|---|---|---|---|---|---|---|---|---|
| m | 625.0 | 0.2 | 0.032% | **625.2** | 625 | 625 | 625 | 625 | |
| M_Ca | 40.078 | 0.002 | 0.005% | 40.078 | **40.08** | 40.078 | 40.078 | 40.078 | |
| V_500 | 500.00 | 0.15 | 0.030% | 500 | 500 | **500.15** | 500 | 500 | |
| M_CaCO3 | 100.0869 | 0.0024 | 0.002% | 100.0869 | 100.0869 | 100.0869 | **100.0893** | 100.087 | |
| P | 0.9999 | 0.0058 | 0.005801 | 0.9999 | 0.9999 | 0.9999 | 0.9999 | 1.0057 | |
| **c_stock** | **0.5005** | **0.0002** | **0.05%** | 0.5007 | 0.5006 | 0.5004 | 0.5005 | 0.5005 | functic |
| mg/ml | | (k = 1) | | -0.0002 | -0.0001 | 0.0001 | 0.0000 | 0.0000 | diff |
| | | | | 4.42E-08 | 5.63E-09 | 1.00E-08 | 1.45E-09 | 2.46E-09 | diff^2 |
| **c_stock** | **50.05** | **0.02** | | | | | 6.13E-08 | 1.95E-08 | sum(d |
| **mg/dl** | | | | m | x_Ca | V_500 | M_CaCO3 | P | |
| | | | | 72.1% | 9.2% | 16.3% | 2.4% | 4.0% | index |
| | | | | | | | 100% | | sum(ir |

check

c_stock = m*A_Ca*P/(V_500*M_CaCO3)
multiplication / division

rsu(y) = sqrt(sum(rsu(xi)^2) 0.04%

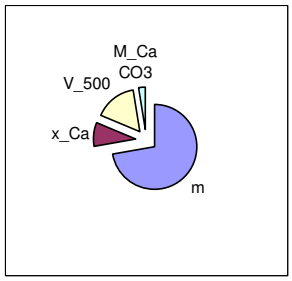



| | value | std-unc | Rsu | M_Ca | M_C | M_O | | |
|---|---|---|---|---|---|---|---|---|
| M_Ca | 40.0780 | 0.00023 | 0.001% | **40.07823** | 40.0780 | 40.0780 | | |
| M_C | 12.0107 | 0.00046 | 0.004% | 12.0107 | **12.01116** | 12.0107 | | |
| M_O | 15.9994 | 0.00017 | 0.001% | 15.9994 | 15.9994 | **15.99957** | | |
| **M_CaCO3** | **100.0869** | **0.0007** | **0.0%** | 100.087 | 100.087 | 100.087 | | function |
| | | (k = 1) | | 0.000 | 0.000 | -0.001 | | diff |
| | | | | 5.29E-08 | 2.12E-07 | 2.60E-07 | | diff^2 |
| | | | | | | | 5.25E-07 | sum(diff^2) |
| | | | | M_Ca | M_C | M_O | | |
| | | | | 10.1% | 40.3% | 49.6% | 100.0% | index |
| | | | | | | | | sum(index) |

check

M_CaCO3=M_Ca+M_C+3*C20M_O
multiplication / division

rsu(y) = sqrt(sum(rsu(xi)^2) 0.0%



**Preparation of calibration calcium standard solutions**

| | | value | std-unc | cert |
|---|---|---|---|---|
| 10 | V_10 | 10 | 0.029 | 0.05 |
| 15 | V_15 | 15 | 0.035 | 0.06 |
| 20 | V_20 | 20 | 0.043 | 0.075 |
| 25 | V_25 | 25 | 0.058 | 0.1 |
| | V_100 | 100 | 0.058 | 0.1 |
| | | | | [B, rect] |

**c_i = c_stock * V_i / V_100**

| | | value | std-unc | Rsu |
|---|---|---|---|---|
| | c_stock | 50.050 | 0.020 | 0.04% |
| **20** | V_20 | 20 | 0.043 | 0.22% |
| | V_100 | 100.000 | 0.058 | 0.06% |

| c-stock | V1 | V_100 |
|---|---|---|
| 50.070 | 50.050 | 50.050 |
| 20.000 | 20.043 | 20.000 |
| 100.000 | 100.000 | 100.058 |

| | | |
|---|---|---|
| c_10 | 10.01 | 0.0228 |
| | | (k=1) |

| | | | |
|---|---|---|---|
| 10.014 | 10.03167 | 10.00422 | function |
| 0.004 | 0.021672 | -0.00578 | diff |
| 1.6E-05 | 0.00046969 | 3.3361E-05 | diff^2 |
| | | 0.000519 | sum(diff^2) |
| C_stock | V1 | V_100 | |

| **C, mg/dl** | value | std-unc |
|---|---|---|
| c_5 | 5.005 | 0.015 |
| c_7.5 | 7.510 | 0.018 |
| c_10 | 10.010 | 0.023 |
| c_12.5 | 12.512 | 0.030 |

## Calibration

**4. 2. One point calibration**

| | value | std-unc | Rsu | c_10 | A_10 | A_x | A_blank | |
|---|---|---|---|---|---|---|---|---|
| **c_10** | 10.010 | 0.023 | 0.2% | 10.033 | 10.010 | 10.010 | 10.010 | |
| **A_10** | 0.338 | 0.002 | 0.005124 | 0.338 | 0.340 | 0.338 | 0.338 | |
| **A_x** | 0.323 | 0.004 | 0.011721 | 0.323 | 0.323 | 0.327 | 0.323 | |
| **A_blank** | 0.052 | 0.004 | 0.085347 | 0.052 | 0.052 | 0.052 | 0.056 | |
| | | | | | | | | |
| **c_x** | **9.486** | **0.146** | 1.5% | 9.507406 | 9.428577 | 9.617965 | 9.477409 | function |
| | mg/dL | | | 0.021795 | -0.05703 | 0.132354 | -0.0082 | diff |
| | | | | 0.000475 | 0.00325 | 0.017517 | 6.73E-05 | diff^2 |
| | | | | | | | 0.02131 | sum(dif^2) |
| | | | | c_10 | A_10 | A_x | A_blank | |
| | | | | 2.229% | 15.263% | 82.193% | 0.316% | index |
| | | | | | | | 100% | sum(index) |

## Calculation of calcium concentration in serum sample

**c_Ca = c_x*V_f/V_int**

| | value | std-unc | RSU | c_x | V_f | V_int |
|---|---|---|---|---|---|---|
| c_x | 9.486 | 0.146 | 1.5% | 9.632 | 9.486 | 9.486 |
| V_f | 0.1 | 0.002 | 2.0% | 0.1 | 0.102 | 0.1 |
| V_int | 0.1 | 0.002 | 2.0% | 0.1 | 0.1 | 0.102 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **c_Ca** | **9.486** | **0.303** | **3.2%** | **9.632** | **9.676** | **9.300** | function |
| | mg/dL | (k=1) | | -0.146 | -0.190 | 0.186 | diff |
| | | | | 0.0213 | 0.0360 | 0.0346 | diff^2 |
| | | | | | | 0.0919 | sum(diff^2) |
| | | | | 23.2% | 39.2% | 37.6% | index |
| | | | | | | 100.0% | sum(index) |

## Chapter 4

## Determination of Radium in Water by α-Spectrometry

*Ljudmila Benedik, Urška Repinc, Monika Inkret*

- **TrainMiC example summary form ('blue page')**
- **A short introduction to the analytical procedure ('slides')**
- **All input needed to do the three exercises ('yellow pages')**
- **The solved exercises ('green pages')**

# TrainMiC example summary form

## I. General information about the example

| | |
|---|---|
| **Measurand** | Activity concentration of Ra-226 in water (Bq/L) (by α-spectrometry) |
| **Example number** | Ex-08 |
| **Authors of the example** | Ljudmila Benedik, Urška Repinc, Monika Inkret |
| **Analytical procedure** | Determination of radium isotopes by $BaSO_4$ coprecipitation for the preparation of alpha-spectrometric sources<br>J.C. Lozano, F. Fernandez and J.M.G. Gomez, Journal of Radioanalytical and Nuclear Chemistry **223** (1997) 1-2, 133-137 |
| **Customer's requirement** | Directive 98/83/EC on the quality of water intended for human consumption |

## II. Attached files

| File number, type and name | | Content of the file | | File is attached Yes | No | Remark |
|---|---|---|---|---|---|---|
| **1-I** | EX-08-1-I-Ra226-water-AS-2006-Ver1.ppt | About the analytical procedure: short introduction | | ✓ | | Given by the lecturer |
| **2 - Yellow** | EX-08-2-Y-Ra226-water-AS-2006-Ver1.doc | PART I | Description of the analytical procedure | ✓ | | Each participant receives own copy and may keep it |
| | | PART II | The customer's requirements concerning the quality of the measurement result | ✓ | | |
| | | PART III | Validation of the measurement procedure – relevant equations and measurement data | ✓ | | |
| | | PART IV | Measurement uncertainty of the result – relevant equations and measurement data | ✓ | | |
| **3 - Green** | EX-08-3-G-Ra226-water-AS-2006-Ver1.doc | PART I | Establishing traceability in analytical chemistry | ✓ | | |
| | | PART II | Single laboratory validation of measurement procedures | ✓ | | |
| | | PART III | Building an uncertainty budget | ✓ | | |
| | | | Addendum 1: By spreadsheet approach | ✓ | | |
| | | | Addendum 2: By dedicated software | ✓ | | |

## III. History of the example

| Version | Uploaded on the webhotel | Short description of the change |
|---|---|---|
| 0 | April 2007 | |
| 1 | | |

# A short introduction to the analytical procedure

## Determination of Ra-226 in water by α-spectrometry

Scope of the presentation

- The analytical procedure and the customer's requirements
- About 'the chemistry' and the measurement method
- Model equation

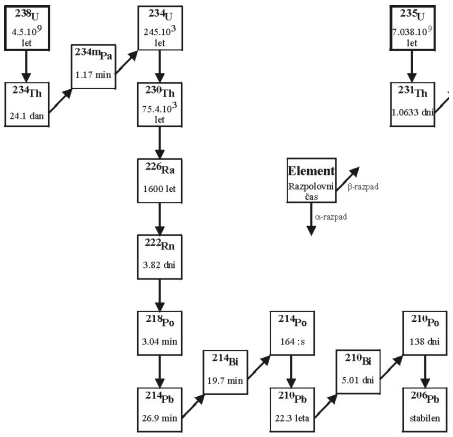
Introduction to TrainMiC
example session

## The analytical procedure and the customer's requirements

- **Determination of radium isotopes by $BaSO_4$ coprecipitation for the preparation of alpha-spectrometric sources**
  J.C. Lozano, F. Fernandez and J.M.G. Gomez
  Journal of Radioanalytical and Nuclear Chemistry **223**
  (1997) 1-2, 133-137
- ***The quality of the results should comply with the requirement in the revised Directive 98/83/EC on the quality of water intended for human consumption*** *(draft annex 2005/04/20)*

Introduction to TrainMiC
example session

## Ra-226




- Similar chemical properties as Ca
- 20 known isotopes with mass 206–234
- 4 are found in nature
- Emitted alpha particles have high potential for causing biological damage

Introduction to TrainMiC
example session

## Measurement methods

The most widely used methods are:

- Emanation methods
- High resolution gamma spectrometry
- Liquid scintillation counting
- High resolution alpha spectrometry
  - preparation of standards and samples
  - calibration of a-spectrometer
  - counting of sources
  - calculation of results
  - quality control.

Introduction to TrainMiC
example session

## Preparation of standards and samples

**Analytical procedure**

**Ra-226 standard disc**

For determination of efficiency of alpha spectrometer

**Ba-133 standard disc**

For determination recovery of radiochemical separation

**Sample**

Addition of Ba-133 tracer

$Pb(Ra)(Ba)SO_4$ precipitation

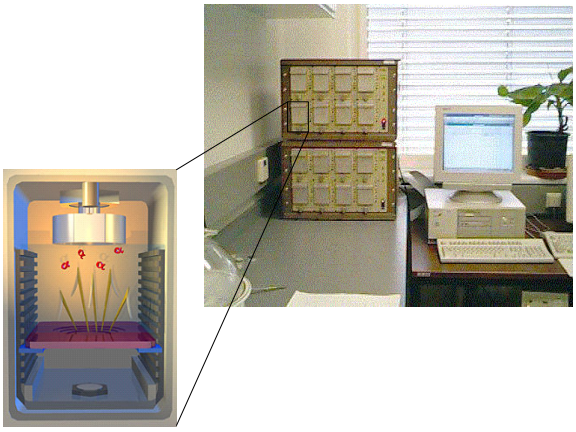
Washing the precipitate
Dissolution
Re-precipitation
Microfiltration

Gamma counting for determination of recovery

Alpha counting for determination of Ra-226

Introduction to TrainMiC example session

## α-spectrometry



$^{4}_{2}He^{2+}$ **α-particle**

- A very large mass
- A high charge
- α-particles are easily stopped by a few microns of air.
- A very thin source allows adequate transmission of the α-particles to the detector surface

Introduction to TrainMiC example session

## Model equation

Measurand: Activity concentration of Ra-226 in water ($A_{Ra-226}$)

Unit: Bq/L

$$A_{Ra-226} = \frac{P_{Ra-226}}{t_{Ra-226} \cdot \varepsilon_{\alpha det} \cdot V_{sample}} \cdot \frac{1}{R_{chem}}$$

$$R_{chem} = \frac{P_{Ba-133sample}}{t_{Ba-133sample} \cdot m_{Ba-133sample}} \cdot \frac{t_{Ba-133Std} \cdot m_{Ba-133Std}}{P_{Ba-133Std}}$$

$$\varepsilon_{\alpha det} = \frac{P_{Ra-226Std}}{t_{Ra-226Std} \cdot m_{Ra-226SS} \cdot A_{Ra-226SS} \cdot R_{Ra-226Std}}$$

Introduction to TrainMiC
example session

## Model equation and equation for measurement uncertainty calculation

$$A_{Ra-226} = \frac{P_{Ra-226}}{t_{Ra-226} \cdot \varepsilon_{\alpha det} \cdot V_{sample}} \cdot \frac{1}{R_{chem}}$$

$$\frac{u(A_{Ra-226})}{A_{Ra-226}} = \sqrt{\left(\frac{u(P_{Ra-226})}{P_{Ra-226}}\right)^2 + \left(\frac{u(\varepsilon_{\alpha det})}{\varepsilon_{\alpha det}}\right)^2 + \left(\frac{u(V)}{V}\right)^2 + \left(\frac{u(R_{chem})}{R_{chem}}\right)^2}$$

$$U = k \times u(A_{Ra-226})$$

Introduction to TrainMiC
example session

# All input needed to do the three exercises 'yellow pages'

## Analytical procedure

*Determination of activity concentration of Ra-226 in drinking water.*

*The quality of the results should comply with the requirements in the revised Directive 98/83/EC on the quality of water intended for human consumption*

**PART I**

**Description of the analytical procedure**

**PART II**

**The customer's requirements concerning the quality of the measurement result**

**PART III**

**Validation of the measurement procedure – relevant equations and measurement data**

**PART IV**

**Measurement uncertainty of the result – relevant equations and measurement data**

# PART I. Description of the analytical procedure

For the determination of Ra-226 in water the following published procedure is used:

**Determination of radium isotopes by $BaSO_4$ coprecipitation for the preparation of alpha-spectrometric sources**

J.C. Lozano, F. Fernandez and J.M.G. Gomez

Journal of Radioanalytical and Nuclear Chemistry **223** (1997) 1-2, 133-137.

## 1. Scope

1.1 General

The procedure is specified for the determination of Ra-226 in water with activity concentration (drinking water, rain water, ground water and surface water) in range of 0,01 Bq/L to 10 Bq/L. In certain cases, the range of application may be changed by variations in the working conditions (e.g. sample volume, pre-concentration techniques, sensitivity ranges of detectors, etc.).

1.2 Interferences

In the case of Ra-226 measurement by α-spectrometry extensive chemical separation prior to counting to remove peak interferences from other alpha emitters is required.

## 2. Principle

A coprecipitation procedure for the preparation of alpha spectrometric source for radium, using $BaSO_4$ as carrier for determination of Ra-226 in water, is used. The use of Ba-133 as a suitable tracer for determination of recovery of the radiochemical procedure by gamma spectrometry is applied. Experimental protocol is schematically shown on the figure below.

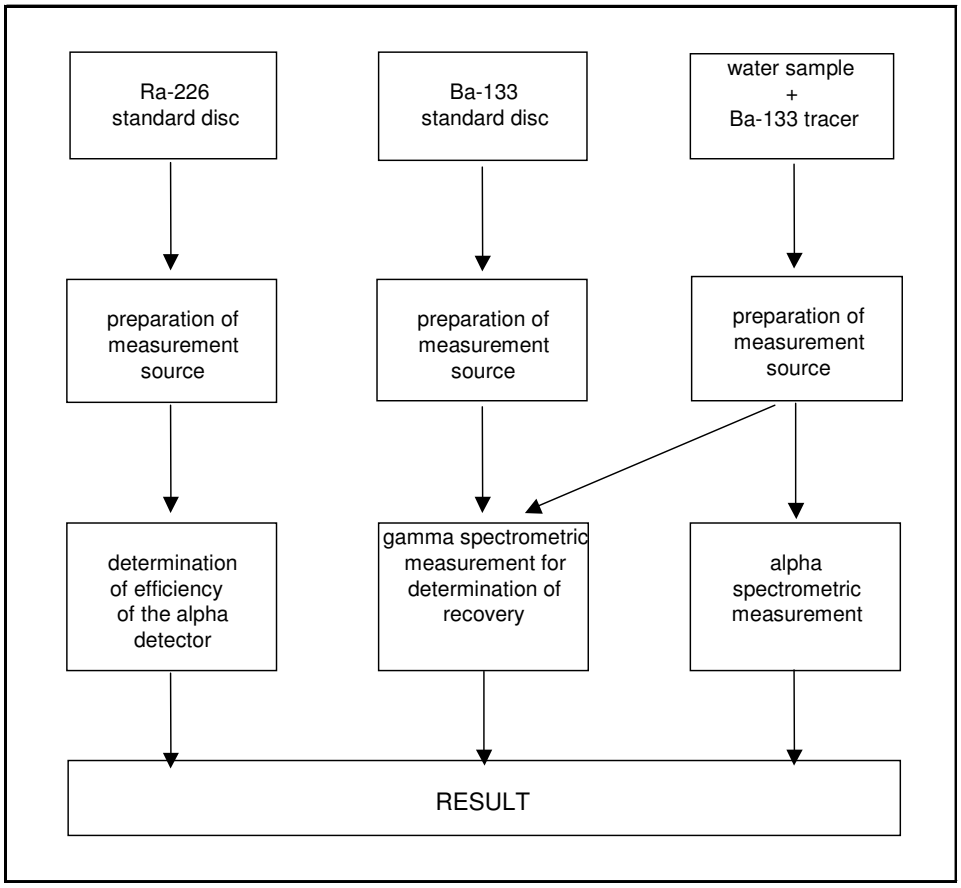



**Figure 4 Experimental protocol for determination Ra-226 in water**

## 3. Apparatus

- Alpha spectrometer with low background silicon surface detector
- HP Ge gamma detector
- Analytical balance: **d = 0,001**
- Centrifuge and 50 mL centrifuge tubs
- Fume hood
- Hot plate
- Magnetic stirrer plate, bars and retriever
- Filter apparatus
- Glass beakers, volumetric flask and graduated cylinders, assorted sizes
- Pipettes, assorted sizes
- 0,1 µm, 25 mm diameter polypropylene filters
- Stainless steel disks
- Petrislides, watch glasses

## 4. Reagents

- Mixed α standard source for detector calibration: 398 dpm ± 3% (k=2)
- Ra-226 standard solution: NIST SRM 4967
- Activity: 2729 Bq/g ± 1,18% (overall, k=3)
- Ba-133 tracer
- Activity: 124,9 kBq/g ± 0,4% (k=2)
- Working solution: 100 Bq/g
- $H_2SO_4$ concentrated
- $Pb^{2+}$ solution (50 mg/mL)
- Ba carrier solution (30 mg/mL)
- 0,1M EDTA / 0,5M NaOH
- $Na_2SO_4$ solution (saturated)
- Indicator pH 3-5
- Acetic acid 1:1
- $BaSO_4$ seeding suspension

## 5. Sample preparation procedure

*The radiochemical separation procedure of Ra-226 with lead coprecipitation*

1. Measure 1000 mL of the water into a beaker
   Graduated cylinder **1000 mL ± 5 mL** (BLAUBRAND® tolerance)
2. Add 0,3 g of Ba-133 tracer (working solution)
3. Add 0,3 mL of $Ba^{2+}$ carrier solution
4. Add 10 mL of conc. $H_2SO_4$
5. Precipitate $Pb(Ra)(Ba)SO_4$ by adding 0,5 mL $Pb^{2+}$ solution through a dripper while stirring. Keep stirring for 1-2 minutes
6. Remove stirrer bar, cover beaker with watch glass and allow to settle overnight
7. Decant supernatant liquor to as low volume as possible. Discard decanted supernatant
8. Wash precipitate into 50 mL centrifuge tube and centrifuge at 3500 rpm for five minutes
9. Pour out supernatant
10. Repeat steps 7 and 8 once

11. Wash sides and the walls of the centrifuge tube with Mili-Q and centrifuge at 3500 rpm for five minutes
12. Pour out supernatant (take care not to disturb the precipitate)
13. Add 2 mL 0,1M EDTA / 0,5M NaOH
14. Vortex to dissolve the precipitate
15. Add 4 mL of saturated $Na_2SO_4$
16. Add 1:1 acetic acid to adjust pH (4-5): $Pb^{2+}$ ions remain in solution
17. Add 0,3 mL of Ba seeding solution
18. Allow to sit at least 30 minutes
19. Filter the colloidal suspension of $(Ra)(Ba)SO_4$ through a pre-wetted 0,1 µm pore size, 25 mm polypropylene filter
20. After the sample has filtered rinse the centrifuge walls and the filter holder with ultrapure water
21. Remove filter and allow to air dry
22. Mount the filter on a stainless steel disc, using double-sided tape or glue stick or cover the source with 6% VYNS foil

## 6. Preparation of standard discs

### 6.1 Preparation of a Ba-133 standard disc

A Ba-133 standard disc is prepared to determine the recovery of prepared source. It is made by adding a known amount of Ba-133 as a sulphate in the same manner as samples are prepared.

1. Measure into 50 mL centrifuge tube approximatelly the same amount of Ba-133 standard as used per sample
2. Add 0,3 mL of Ba carier solution
3. Add appropriate volume of conc. $H_2SO_4$
4. Add 0,5 mL of $Pb^{2+}$ solution
5. Centrifuge at 3500 rpm for five minutes
6. Pour out supernatant
7. Wash sides and the walls of the centrifuge tube with ultrapure water and centrifuge at 3500 rpm for five minutes

8. Pour out supernatant liquid (take care not to disturb the precipitate)
9. Add 2 mL 0,1M EDTA / 0,5M NaOH
10. Disolve the precipitate
11. Add 4 mL of saturated $Na_2SO_4$
12. Add 1:1 acetic acid to adjust pH (4-5)
13. Add 0,3 mL of Ba seeding solution
14. Micro-filtration of suspension
15. Washing filter with water, followed by drying
16. Mount the filter on a stainless steel disc, using double-sided tape or glue stick or cover the source with 6% VYNS foil

The filtrate and washing from the Ba-133 standard source preparation are collected in a bottle, counted on a gamma detector and compared to an equivalent, known activity of Ba-133 in the same geometry. The losses incured in mounting the source are calculated from these measurements, allowing the efficiency of mounting, and therefore the fractional recovery of the Ba-133 standard disc, to be calculated.

6.2 Preparation of a Ra-226 standard disc

A Ra-226 standard disc is prepared to determine the efficiency and perform an energy calibration of each alpha spectrometer. The disc is made by mounting a known amount of Ra-226 calibration solution and Ba-133 solution, as a sulphate in the same manner as samples are prepared.

Disc should be prepared with an activity of 25 – 50 Bq.

1. Measure into 50 mL centrifuge tube 0,01 g of Ra-226 standard solution
2. Add approximatelly the same amount of Ba-133 standard as used per sample
3. Add 0,3 mL of Ba carier solution
4. Add appropriate volume of concentrated $H_2SO_4$
5. Add 0,5 mL of $Pb^{2+}$ solution
6. Centrifuge at 3500 rpm for five minutes
7. Pour out supernatant liquid

8. Wash sides and the walls of the centrifuge tube with ultrapure water and centrifuge at 3500 rpm for five minutes
9. Pour out supernatant (take care not to disturb the precipitate)
10. Add 2 mL 0,1M EDTA / 0,5M NaOH
11. Disolve the precipitate
12. Add 4 mL of saturated $Na_2SO_4$
13. Add 1:1 acetic acid to adjust pH (4-5)
14. Add 0,3 mL of Ba seeding solution
15. Micro-filtration of suspension
16. Washing filter with water, followed by drying
17. Mount the filter on a stainless steel disc, using double-sided tape or glue stick or cover the source with 6% VYNS foil

## 7. Preparation of blank filters

7.1 Preparation of blank filter

A blank filter is prepared to check if 0,1 µm pore size, 25 mm polypropylene filter contains any traces of Ra-226.

- Mount the filter on a stainless steel disc, using double-sided tape or glue stick or cover the source with 6% VYNS foil

7.2 Making a reagent blank filter

A reagent blank filter is prepared to determine if there are any traces of Ra-226 in used chemical reagents.

- A reagent blank filter is made in the same manner as samples (steps 1-22, from point 5). Ultrapure water is used instead of sample water.

## 8. Gamma and alpha counting

**Gamma**

1. Measure the background of gamma detector
2. Measure Ba-133 (Ba-133 standard disc) on a gamma spectrometer
3. Measure Ba-133 (sample) on a gamma detector

**Alpha**

4. Measure the background of alpha detector
5. Measure the blank filter
6. Measure the reagent blank filter
7. Measure the Ra-226 standard disc for efficiency determination
8. Measure the filter with Ra-226 (sample) on alpha spectrometer

## 9. Calculation

### 9.1 Sample recovery calculation

Determination of recovery by gamma spectrometry is calculated as follows:

$$R_{chem} = \frac{P_{Ba-133sample}}{t_{Ba-133sample} \cdot m_{Ba-133sample}} \cdot \frac{t_{Ba-133Std} \cdot m_{Ba-133Std}}{P_{Ba-133Std}}$$

| | |
|---|---|
| $R_{chem}$ | radiochemical yield (recovery) |
| $P_{Ba\text{-}133\ sample}$ | peak area of Ba-133 in the sample |
| $t_{Ba\text{-}133\ sample}$ | time of the sample measurement [s] |
| $m_{Ba\text{-}133\ sample}$ | mass of added Ba-133 in the sample [g] |
| $P_{Ba\text{-}133Std}$ | peak area of Ba-133 in barium standard disc |
| $t_{Ba\text{-}133Std}$ | time of measurement of Ba-133 in barium standard disc [s] |
| $m_{Ba\text{-}133Std}$ | mass of added Ba-133 in barium standard disc [g] |

### 9.2 Alpha spectrometer efficiency determination

$$\varepsilon_{\alpha\,det} = \frac{P_{Ra\text{-}226Std}}{t_{Ra-226Std} \cdot m_{Ra-226SS} \cdot A_{Ra-226SS} \cdot R_{Ra-226Std}}$$

| | |
|---|---|
| $\varepsilon_{\alpha\,det}$ | efficiency of alpha detector |
| $R_{Ra\text{-}226Std}$ | radium standard disc recovery |
| $P_{Ra\text{-}226Std}$ | peak area of Ra-226 in standard disc |
| $t_{Ra\text{-}226Std}$ | time of measurement of standard disc [s] |
| $m_{Ra\text{-}226SS}$ | mass of added Ra-226 in standard solution [g] |
| $A_{Ra\text{-}226SS}$ | activity concentration of Ra-226 in standard solution [Bq/g] |

9.3 Activity concentration of Ra-226 in the sample (Bq/L)

$$A_{Ra-226} = \frac{P_{Ra-226}}{t_{Ra-226} \cdot \varepsilon_{\alpha\,det} \cdot V_{sample} \cdot R_{chem}}$$

| | |
|---|---|
| $A_{Ra-226}$ | Activity concentration of Ra-226 in the sample in [Bq/L] |
| $P_{Ra-226}$ | peak area of Ra-226 |
| $t_{Ra-226}$ | time of measurement [s] |
| $V_{sample}$ | volume of the sample [L] |
| $\varepsilon_{\alpha\,det}$ | corrected efficiency of alpha detector |
| $R_{chem}$ | radiochemical yield (recovery) |

# PART II. The customer's requirements concerning quality of the measurement result

*Extract from the Directive 98/83/EC, Draft annex 2005/04/20 on the quality of water intended for human consumption*

Reference concentration for radioactivity in drinking water*

| Origin | Nuclide | Reference concentration |
|---|---|---|
| Natural | Ra-226 | 0,5 Bq/L |

** This table includes the most common natural and artificial radionuclide Reference concentrations for other radionuclides can be calculated using the dose coefficients for adults laid down in Annex III, Table A of Directive 96/29/Euratom, or more recent information recognised by the competent authorities in the Member State, and by assuming an intake of 730 litres per year.*

Performance characteristics and methods of analysis

For the following radioactivity parameters, the specified performance characteristics are that the method of analysis used must, as a minimum, be capable of measuring concentrations equal to the parametric value with a limit of detection specified.

| Parameter | Limit of detection | Notes |
|---|---|---|
| *Ra-226* | *0,04 Bq/L* | *Note 1*<br>*Note 2* |

*Note 1: the limit of detection should be calculated according to ISO 11929-7, Determination of the detection limit and decision thresholds for ionizing radiation measurements - Part 7: Fundamentals and general applications, with probabilities of errors of 1st and 2nd kind of 0,05 each*
*Note 2: measurement uncertainties should be calculated and reported as complete standard uncertainties, or as expanded standard uncertainties with an expansion factor of 1,96, according to the ISO Guide for the Expression of Uncertainty in Measurement (ISO, Geneva 1993, corrected reprint Geneva, 1995)*

# PART III. Validation of the measurement procedure – relevant equations and measurement data

In the present case study, methodology for validation of measurement procedure for determination of Ra-226 in water by α-spectrometry is presented. For the calculation part, the emphasis is on the parameters that are required by the customer. In this particular case, these parameters are:

- LOD
- within-laboratory reproducibility.

For the purpose of this exercise, LOD (LLD) will be calculated only.

Equation

$$LLD = \frac{2{,}71 + 4{,}65\sqrt{Bkg}}{t_{Bkg} \times \varepsilon_{\alpha\,\mathrm{det}} \times R_{chem} \times V_{sample}}$$

Measurement data

| Input quantity | | Unit | Value |
|---|---|---|---|
| $\mathbf{R_{chem}}$ | radiochemical yield (recovery) | - | 0,803 |
| $\boldsymbol{\varepsilon_{\alpha\,det}}$ | efficiency of alpha detector | - | 0,2453 |
| **Bkg** | peak area of background of alpha detector at the Ra-226 alpha energy | - | |
| $\mathbf{t_{Bkg}}$ | time of measurement of background | s | |
| $\mathbf{V_{sample}}$ | volume of the sample | L | |

# PART IV. Measurement uncertainty of the result – relevant equations and measurement data

In the present case study, methodology for evaluation of measurement uncertainty of result of Ra-226 determination in drinking water is presented. Ra-226 was determined using α-spectrometry. The necessary relevant information was obtained from the method validation data, the quality control data and equipment calibration certificates. The method of measurement is described together with the measurement equation, selected traceable reference standards and the associated measurement uncertainty. The major sources of uncertainty of the result of measurement were identified and the combined uncertainty was calculated. Identification of the main uncertainty sources represent basis for target operation for reducing the measurement uncertainty of this determination.

## Equations

$$\frac{u(A_{Ra\text{-}226})}{A_{Ra\text{-}226}} = \sqrt{\left(\frac{u(P_{Ra-226})}{P_{Ra-226}}\right)^2 + \left(\frac{u(\varepsilon_{\alpha det})}{\varepsilon_{\alpha det}}\right)^2 + \left(\frac{u(V_{sample})}{V_{sample}}\right)^2 + \left(\frac{u(R_{chem})}{R_{chem}}\right)^2}$$

$$\frac{u(R_{chem})}{R_{chem}} = \sqrt{\left(\frac{u(P_{Ba-133Std})}{P_{Ba-133Std}}\right)^2 + \left(\frac{u(m_{Ba-133Std})}{m_{Ba-133Std}}\right)^2 + \left(\frac{u(P_{Ba-133sample})}{P_{Ba-133sample}}\right)^2 + \left(\frac{u(m_{Ba-133sample})}{m_{Ba-133sample}}\right)^2}$$

$$\frac{u(\varepsilon_{\alpha det})}{\varepsilon_{\alpha det}} = \sqrt{\left(\frac{u(P_{Ra-226Std})}{P_{Ra-226Std}}\right)^2 + \left(\frac{u(m_{Ra\text{-}226SS})}{m_{Ra\text{-}226SS}}\right)^2 + \left(\frac{u(A_{Ra\text{-}226SS})}{A_{Ra\text{-}226SS}}\right)^2 + \left(\frac{u([R_{Ra-226Std})}{R_{Ra-226Std}}\right)^2}$$

$$u(A_{Ra-226}) = \quad k \cdot (A_{Ra-226})$$

Measurement data

| Input quantity | | Unit | Value | Standard uncertainty (u) | Type of uncertainty | Type of distribution | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | normal | rectangular | triangular |
| $V_{sample}$ | volume of the sample | L | 1,0 | 0,002 | B | | | X |
| $m_{Ba\text{-}133\ sample}$ | mass of added Ba-133 in the sample | g | 0,301 | 0,001 | B | | X | |
| $m_{Ba\text{-}133Std}$ | mass of added Ba-133 in barium standard disc | g | 0,112 | 0,001 | B | | X | |
| $m_{Ra\text{-}226\ SS}$ | mass of added Ra-226 in standard solution | g | 0,010 | 0,001 | B | | X | |
| $A_{Ra\text{-}226\ SS}$ | activity concentration of Ra-226 in standard solution | Bq/g | 2729 | - | B | X | | |
| $t_{Ra\text{-}226}$ | time of measurement | s | 300 000 | - | - | | - | |
| $t_{Ba\text{-}133\ sample}$ | time of the sample measurement (s) | s | 3 000 | - | - | | - | |
| $t_{Ba\text{-}133Std}$ | time of measurement of Ba-133 in barium standard disc | s | 3 000 | - | - | | - | |
| $P_{Ra\text{-}226}$ | peak area of Ra-226 | - | 7516 | 87 | A | X | | |
| $P_{Ba\text{-}133\ sample}$ | peak area of Ba-133 in the sample | - | 10914 | 104 | A | X | | |
| $P_{Ba\text{-}133\ Std}$ | peak area of Ba-133 in barium standard disc | - | 5090 | 71 | A | X | | |
| $P_{Ra\text{-}226\ Std}$ | peak area of Ra-226 in standard disc | - | 12785 | 113 | A | X | | |
| $R_{chem}$ | radiochemical yield (recovery) | - | - | - | A | X | | |
| $\varepsilon_{\alpha\ det}$ | efficiency of alpha detector | - | - | - | A | X | | |
| $R_{Ra\text{-}226Std}$ | radium standard disc recovery | - | - | - | A | X | | |

# The solved exercises 'green pages'

## TRAINMIC EXERCISES

### Analytical procedure

***Determination of activity concentration of Ra-226 in drinking water.***

***The quality of the results should comply with the requirement in the revised Directive 98/83/EC on the quality of water intended for human consumption***

*Exercise 1:*

**Establishing traceability in analytical chemistry**

*Exercise 2:*

**Single laboratory validation of measurement procedures**

**Part I: General issues**

**Part II: Parameters to be validated**

**Part III: Some calculations and conclusions**

*Exercise 3:*

**Building an uncertainty budget**

**Addendum I: By spreadsheet approach**

**Addendum II: By dedicated software**

# ESTABLISHING TRACEABILITY IN ANALYTICAL CHEMISTRY

## 1. Specifying the analyte and measurand

| | |
|---|---|
| *Analyte* | Ra-226 |
| *Measurand* | Activity concentration of Ra-226 in water (drinking, surface, waste…) |
| *Units* | Bq/L |

## 2. Choosing a suitable measurement procedure with associated model equation

| | | | |
|---|---|---|---|
| *Measurement procedure* | Determination of radium isotopes by $BaSO_4$ coprecipitation for the preparation of alpha-spectrometrical sources<br>Lozano et al.: Journal of Radioanalytical and Nuclear Chemistry | | |
| *Type of calibration* | *mixed standard source* ☒ | *standard addition* ☐ | *internal standard* ☐ |

Model equation

The activity concentration of Ra-226 in sample (Bq/L), is calculated by

$$A_{Ra-226} = \frac{P_{Ra-226}}{t_{Ra-226} \cdot \varepsilon_{\alpha\,det} \cdot V_{sample} \cdot R_{chem}}$$

| | |
|---|---|
| $A_{Ra\text{-}226}$ | activity concentration of Ra-226 in the sample [Bq/L] |
| $P_{Ra\text{-}226}$ | peak area of Ra-226 |
| $t_{Ra\text{-}226}$ | time of measurement [s] |
| V | volume of the sample [L] |
| $\varepsilon_{\alpha\,det}$ | corrected efficiency of alpha detector |
| $R_{chem}$ | radiochemical yield (recovery) |

Recovery obtained by gamma spectrometry is calculated as follows:

$$R_{chem} = \frac{P_{Ba-133sample}}{t_{Ba-133sample} \cdot m_{Ba-133sample}} \cdot \frac{t_{Ba-133Std} \cdot m_{Ba-133Std}}{P_{Ba-133Std}}$$

| | |
|---|---|
| $R_{chem}$ | radiochemical yield (recovery) |
| $P_{Ba\text{-}133\ sample}$ | peak area of Ba-133 in the sample |
| $t_{Ba\text{-}133\ sample}$ | time of the sample measurement [s] |
| $m_{Ba\text{-}133\ sample}$ | mass of added Ba-133 in the sample [g] |
| $P_{Ba\text{-}133Stda}$ | peak area of Ba-133 in barium standard disc |
| $t_{Ba\text{-}133Std}$ | time of measurement of Ba-133 standard disc [s] |
| $m_{Ba\text{-}133Std}$ | mass of added Ba-133 in barium standard disc [g] |

Alpha spectrometer efficiency is calculated as follows:

$$\varepsilon_{\alpha\,det} = \frac{P_{Ra\text{-}226Std}}{t_{Ra-226Std} \cdot m_{Ra-226SS} \cdot A_{Ra-226SS} \cdot R_{Ra-226Std}}$$

| | |
|---|---|
| $\varepsilon_{\alpha\,det}$ | efficiency of alpha detector |
| $R_{Ra\text{-}226Std}$ | radium standard disc recovery |
| $P_{Ra\text{-}226Std}$ | peak area of Ra-226 of standard disc |
| $t_{Ra\text{-}226Std}$ | time of measurement of Ra-226 standard disc [s] |
| $m_{Ra\text{-}226SS}$ | mass of added Ra-226 standard solution [g] |
| $A_{Ra\text{-}226SS}$ | Ra-226 activity concentration in standard solution [Bq/g] |

### 3. List the input quantities according to their influence on the uncertainty of the result of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.

| | |
|---|---|
| *1* | Uncertainty of concentration of reference solutions |
| *2* | Uncertainty of volumes |
| *3* | Uncertainty of weighing |
| *4* | Uncertainty of measurement, using alpha and gamma detectors |

### 4. List the reference standards needed and state the information regarding traceability of the reference value

**For the analyte**

| | | |
|---|---|---|
| *1* | *Name/Chemical Formula/Producer:* | Standard Radionuclide Source, Analytics, SRS 67978-121 |
| *2* | *Name/Chemical Formula/Producer:* | Ba-133 standard solution, Czech Metrological Institute, Cert. No: 931-OL-137/99 |
| *2* | *Name/Chemical Formula/Producer:* | Ra-226 standard solution, NIST SRM 4967 |

**For the other input quantities**

| | | |
|---|---|---|
| *1* | *Quantity/Equipment/Calibration:* *e.g. mass/balance/calibrated by NMI, U=xx (k=2),* | Graduated and mixing cylinders, volumetric flask /with established traceability BLAUBRAND® tolerance |
| *2* | *Quantity/Equipment/Calibration:* | Mass/calibrated balance/with established traceability Sartorius |

## 5. Estimating uncertainty associated with the measurement

| | | |
|---|---|---|
| *Are all important parameters included in the model equation?* | *Yes* ☐ | *No* ☒ |
| *Other important parameters are:* | Uncertainty of measured background of detector, uncertainty of measured blank reagents (minor contributions) | |

## 6. How would you prove traceability of your result?

| | |
|---|---|
| *1* | Analysis of matrix CRM |
| *2* | Participation in a proficiency testing scheme |
| *3* | - |

## 7. Any other comments, questions…

# SINGLE LABORATORY VALIDATION OF MEASUREMENT PROCEDURES

## PART I: GENERAL ISSUES

### 1. Specify the measurement procedure, analyte, measurand and units

| | |
|---|---|
| *The measurement procedure* | Determination of radium isotopes by $BaSO_4$ coprecipitation for the preparation of alpha-spectrometric sources<br>J.C. Lozano, F. Fernandez and J.M.G. Gomez<br>Journal of Radioanalytical and Nuclear Chemistry **223** (1997) 1-2, 133-137. |
| *Analyte* | Ra-226 |
| *The measurand* | Activity concentration of Ra-226 in drinking water |
| *Unit* | Bq/L |

### 2. Specify the scope

| | |
|---|---|
| *Matrix* | Drinking water |
| *Measuring range* | 0,01 Bq/L – 10 Bq/L |

### 3. Requirement on the measurement procedure

| *Intended use of the results* | *Compliance to the requirements in the revised water directive 98/83/EC on the quality of water intended for human consumption* | | |
|---|---|---|---|
| *Mark the customer's requirements and give their values* | *Parameters to be validated* | | *Value requested by the customer* |
| | ☒ | *LOD* | 0,04 Bq/L |
| | ☐ | *LOQ* | |
| | ☐ | *Repeatability* | |
| | ☐ | *Within-lab reproducibility* | |
| | ☐ | *Trueness* | |
| | ☐ | *Measurement uncertainty* | |
| | ☐ | *Other-state* | |

### 4. Origin of the measurement procedure

| | | VALIDATION |
|---|---|---|
| *New in-house method* | ☒ | *Full* |
| *Modified validated method* | ☐ | *Partial* |
| *Official standard method* | ☐ | *Confirmation/Verification* |

## PART II: PARAMETERS TO BE VALIDATED

### 5. Selectivity/Interference/Recovery

Where yes, please give further information e.g. which CRM, reference method

| | |
|---|---|
| ☐ | *CRM/RM: analysis of available CRM or RM* |
| | *Further information:* |
| ☒ | *Spike of pure substance* |
| | spiking of samples with pure substances and calculation of recovery |
| ☐ | *Compare with a reference method* |
| | |
| ☐ | *Selectivity, interferences* |
| | |
| ☐ | *Test with different matrices* |
| | |
| ☐ | *Other – please specify* |
| | |

### 6. Measuring range

| | |
|---|---|
| ☒ | *Linearity* |
| ☒ | *Upper limit* |
| ☒ | *LOD* |
| ☒ | *LOQ* |

### 7. Spread – precision

| | |
|---|---|
| ☒ | *Repeatability* |
| ☒ | *Reproducibility (within lab)* |
| ☐ | *Reproducibility (between lab)* |

## 8. Robustness

| | |
|---|---|
| ☐ | *Variation of parameters* |

## 9. Quality control

| | |
|---|---|
| ☒ | *Control charts* |
| ☒ | *Participation in PT schemes* |

## 10. Other parameters to be tested

| | |
|---|---|
| ☒ | *Working range and testing of homogeneity of variances* |
| ☒ | *R square* |
| ☒ | *Residual standard deviation* |
| ☒ | *Standard deviation of the analytical procedure* |
| ☒ | *Coefficient of variation of the analytical procedure* |
| ☒ | *Measurement uncertainty* |

## PART III: SOME CALCULATIONS AND CONCLUSIONS

### 11. Calculation of parameters requested by the customer

| Parameters requested to be validated | Calculations |
|---|---|
| ☒ *LOD* | $LLD = \frac{2,71 + 4,65\sqrt{14,26092744}}{420730 \times 0,2453 \times 0,803 \times 1} = 0,000245 Bq / L$ |
| ☐ *LOQ* | |
| ☐ *Repeatability* | |
| ☐ *Within-lab reproducibilty* | |
| ☐ *Trueness* | |
| ☐ *Measurement uncertainty* | |
| ☐ *Other - please state* | |

**12. Does the analytical procedure fulfil the requirement(s) for the intended use?**

| **Parameter** | **Value requested by the customer** (the same as stated in question 3) | **Value obtained during validation** | **The requirement is fulfilled Yes/No** |
|---|---|---|---|
| ☒ LOD | 0,04 Bq/L | 0,00025 Bq/L | YES |
| ☐ LOQ | - | | |
| ☐ Repeatability | - | | |
| ☐ Within-lab reproducibility | | | |
| ☐ Trueness | - | | |
| ☐ Measurement | | | |
| ☐ Other | - | | |

**The analytical procedure is fit for the intended use:**

☒ *Yes* ☐ *No*

*For measurement uncertainty and traceability refer to the corresponding sheets*

# BUILDING AN UNCERTAINTY BUDGET

## 1. Specify the measurand and units

| | |
|---|---|
| *Measurand* | Activity concentration of Ra-226 in water (drinking, surface, waste…) |
| *Unit* | Bq/L |

## 2. Describe the measurement procedure and provide the associated model equation

Measurement procedure

Determination of radium isotopes by $BaSO_4$ coprecipitation for the preparation of alpha-spectrometric sources

J.C. Lozano, F. Fernandez and J.M.G. Gomez

Journal of Radioanalytical and Nuclear Chemistry **223** (1997) 1-2, 133-137.

Model equation:

The activity concentration of Ra-226 in the sample (Bq/L), is calculated by

$$A_{Ra-226} = \frac{P_{Ra-226}}{t_{Ra-226} \cdot \varepsilon_{\alpha\,det} \cdot V_{sample} \cdot R_{chem}}$$

| | |
|---|---|
| $A_{Ra-226}$ | Activity concentration of Ra-226 in the sample [Bq/L] |
| $P_{Ra-226}$ | peak area of Ra-226 |
| $t_{Ra-226}$ | time of measurement [s] |
| V | volume of the sample [L] |
| $\varepsilon_{\alpha\,det}$ | corrected efficiency of alpha detector |
| $R_{chem}$ | radiochemical yield (recovery) |

Recovery measured by gamma spectrometry is calculated as follows:

$$R_{chem} = \frac{P_{Ba-133sample}}{t_{Ba-133sample} \cdot m_{Ba-133sample}} \cdot \frac{t_{Ba-133Std} \cdot m_{Ba-133Std}}{P_{Ba-133Std}}$$

| | |
|---|---|
| $R_{chem}$ | radiochemical yield (recovery) |
| $P_{Ba\text{-}133\ sample}$ | Peak area of Ba-133 in the sample |
| $t_{Ba\text{-}133\ sample}$ | time of the sample measurement [s] |
| $m_{Ba\text{-}133\ sample}$ | mass of added Ba-133 in the sample [g] |
| $P_{Ba\text{-}133Std}$ | peak area of Ba-133 in barium standard disc |
| $t_{Ba\text{-}133Std}$ | time of measurement of Ba-133 in barium standard disc [s] |
| $m_{Ba\text{-}133Std}$ | mass of added Ba-133 in barium standard disc [g] |

Alpha spectrometer efficiency determination is calculated as follows:

$$\varepsilon_{\alpha\,det} = \frac{P_{Ra\text{-}226Std}}{t_{Ra-226Std} \cdot m_{Ra-226SS} \cdot A_{Ra-226SS} \cdot R_{Ra-226Std}}$$

| | |
|---|---|
| $\varepsilon_{\alpha\ det}$ | efficiency of alpha detector |
| $R_{Ra\text{-}226Std}$ | radium standard disc recovery |
| $P_{Ra\text{-}226Std}$ | peak area of Ra-226 in standard disc |
| $t_{Ra\text{-}226Std}$ | time of measurement of Ra-226 standard disc [s] |
| $m_{Ra\text{-}226SS}$ | mass of added Ra-226 standard solution [g] |
| $A_{Ra\text{-}226SS}$ | Ra-226 activity concentration in standard solution [Bq/g] |

### 3. Identify (all possible) sources of uncertainty

| | |
|---|---|
| ☒ | *Uncertainty of concentration of reference solutions* |
| ☒ | *Uncertainty of measurements of peak area (alpha and gamma detectors)* |
| ☐ | *Method bias* |
| ☐ | *Matrix effect* |
| ☒ | *Other: Uncertainty of volume measurements* |
| ☒ | *Other: Uncertainty of weighing* |
| ☒ | *Other: Uncertainty of measured background of alpha and gamma detectors* |
| ☒ | *Other: Uncertainty of measured blank reagents, filters, discs* |

### 4. Evaluate values of each input quantity

| *Input quantity* | *Value* | *Unit* | *Remark* |
|---|---|---|---|
| $P_{Ra\text{-}226}$ | 7516 | - | |
| $t_{Ra\text{-}226}$ | 300 000 | s | |
| $\varepsilon_{\alpha det}$ | 0,2453 | - | |
| $V_{sample}$ | 1,0 | L | |
| $R_{chem}$ | 0,803 | - | |

### 5. Evaluate the standard uncertainty of each input quantity

| *Input quantity* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|
| $P_{Ra\text{-}226}$ | 87 | - | |
| $t_{Ra\text{-}226}$ | 0 | s | Constant |
| $\varepsilon_{\alpha det}$ | 0,01392 | - | |
| $V_{sample}$ | 0,0020 | L | |
| $R_{chem}$ | 0,0142 | - | |

### 6. Calculate the value of the measurand, using the model equation

$$A_{Ra-226} = \frac{P_{Ra-226}}{t_{Ra-226} \cdot \varepsilon_{\alpha\, det} \cdot V_{sample}} \cdot \frac{1}{R_{chem}}$$

$$A_{Ra-226} = \frac{7516}{300000 \cdot 0.2453 \cdot 1} \cdot \frac{1}{0.803} = 0.127\ Bq/L$$

### 7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units

*Using*: ☒ Mathematical solution; ☒ Spreadsheet Approach; ☒ Commercial Software

| *Input quantity* | *Value* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|---|
| $P_{Ra\text{-}226}$ | 7516 | 87 | - | |
| $t_{Ra\text{-}226}$ | 300 000 | 0 | s | |
| $\varepsilon_{\alpha det}$ | 0,2453 | 0,01392 | - | |
| $V_{sample}$ | 1,0 | 0,0020 | L | |
| $R_{chem}$ | 0,803 | 0,0142 | - | |

$$\frac{u(A_{Ra\text{-}226})}{A_{Ra\text{-}226}} = \sqrt{\left(\frac{u(P_{Ra-226})}{P_{Ra-226}}\right)^2 + \left(\frac{u(\varepsilon_{\alpha det})}{\varepsilon_{\alpha det}}\right)^2 + \left(\frac{u(V_{sample})}{V_{sample}}\right)^2 + \left(\frac{u(R_{chem})}{R_{chem}}\right)^2}$$

$$\frac{u(A_{Ra\text{-}226})}{A_{Ra\text{-}226}} = \sqrt{\left(\frac{87}{7516}\right)^2 + \left(\frac{0{,}01392}{0{,}2453}\right)^2 + \left(\frac{0{,}0020}{1}\right)^2 + \left(\frac{0{,}0142}{0{,}803}\right)^2} = 0{,}00806$$

## 8. Calculate expanded uncertainty ($U_c$) & specify the coverage factor k and the units

$$u(A_{Ra-226}) = \quad k \cdot (A_{Ra-226})$$

$$U = 2 \cdot 0{,}00806 = 0{,}016 \text{ Bq/L}$$

## 9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $u_c$

| | |
|---|---|
| *1* | Mass of Ra-226 standard solution |
| *2* | Peak area of Ba-133 in the standard disc |
| *3* | Peak area of Ra-226 of the sample |

## 10. Prepare your uncertainty budget report

*See the attached Excel calculations and calculations done using the software GumWorkbench*

## Addendum I: Measurement uncertainty calculation, spreadsheet approach (Excel)

**Efficiency of alpha detector**

| Stdev | Value | | $P_{Ra\text{-}226Std}$ | $t_{Ra\text{-}226Std}$ | $m_{Ra\text{-}226SS}$ | $A_{Ra\text{-}226SS}$ | $R_{Ra226Std}$ |
|---|---|---|---|---|---|---|---|
| 113 | 12785 | $P_{Ra\text{-}226Std}$ | 12898 | 12785 | 12785 | 12785 | 12785 |
| | 2000 | $t_{Ra\text{-}226Std}$ | 2000 | 2000 | 2000 | 2000 | 2000 |
| 0,00058 | 0,01022 | $m_{Ra\text{-}226SS}$ | 0,01022 | 0,01022 | 0,01080 | 0,01022 | 0,01022 |
| 11 | 2729 | $A_{Ra\text{-}226SS}$ | 2729 | 2729 | 2729 | 2740 | 2729 |
| 0,015 | 0,9344 | $R_{Ra226Std}$ | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9494 |
| 0,0139 | 0,2453 | $\varepsilon_{\alpha det}$ | 0,2475 | 0,2453 | 0,2322 | 0,2443 | 0,2414 |
| | | diff | -0,002168 | 0,000000 | 0,013116 | 0,000985 | 0,003875 |
| | | sumsq(diff) | 0,000193 | | | | |
| | | index | 2,4 | 0,0 | 89,3 | 0,5 | 7,8 |

## Radiochemical yield (recovery)

| Stdev | Value | | $P_{Ba\text{-}133sample}$ | $t_{Ba\text{-}133sample}$ | $m_{Ba\text{-}133sample}$ | $t_{Ba\text{-}133Std}$ | $m_{Ba\text{-}133Std}$ | $P_{Ba\text{-}133Std}$ |
|---|---|---|---|---|---|---|---|---|
| 104 | 10914 | $P_{Ba\text{-}133sample}$ | 11018 | 10914 | 10914 | 10914 | 10914 | 10914 |
| | 3000 | $t_{Ba\text{-}133sample}$ | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| 0,000577 | 0,30120 | $m_{Ba\text{-}133sample}$ | 0,301200 | 0,301200 | 0,301777 | 0,301200 | 0,301200 | 0,301200 |
| | 3000 | $t_{Ba\text{-}133Std}$ | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| 0,000577 | 0,11280 | $m_{Ba\text{-}133Std}$ | 0,112800 | 0,112800 | 0,112800 | 0,112800 | 0,113377 | 0,112800 |
| 71 | 5090 | $P_{Ba\text{-}133Std}$ | 5090 | 5090 | 5090 | 5090 | 5090 | 5161 |
| 0,0141 | 0,8030 | $R_{chem}$ | 0,8107 | 0,8030 | 0,8015 | 0,8030 | 0,8071 | 0,7920 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| diff | -0,0076519 | 0,0000000 | 0,0015363 | 0,0000000 | -0,0041101 | 0,0110470 |
| sumsq(diff) | 0,000200 | | | | | |
| | | | | | | |
| index | 29,3 | 0,0 | 1,2 | 0,0 | 8,5 | 61,1 |

## Activity of Ra-226 in water

| Stdev | Value | | $P_{Ra-226}$ | $t_{Ra-226}$ | $\varepsilon_{\alpha det}$ | $V_{sample}$ | $R_{chem}$ |
|---|---|---|---|---|---|---|---|
| 86 | 7516 | $P_{Ra-226}$ | 7602 | 7516 | 7516 | 7516 | 7516 |
| | 300000 | $t_{Ra-226}$ | 300000 | 300000 | 300000 | 300000 | 300000 |
| 0,0139 | 0,2453 | $\varepsilon_{\alpha det}$ | 0,2453 | 0,2453 | 0,2592 | 0,2453 | 0,2453 |
| 0,002 | 1,000 | $V_{sample}$ | 1,000 | 1,000 | 1,000 | 1,002 | 1,000 |
| 0,01414 | 0,80301 | $R_{chem}$ | 0,80301 | 0,80301 | 0,80301 | 0,80301 | 0,81715 |
| 0,00731 | 0,12719 | $A_{Ra-226}$ | 0,12865 | 0,12719 | 0,12038 | 0,12693 | 0,12499 |

| | | | | | |
|---|---|---|---|---|---|
| diff | -0,00146 | 0,00000 | 0,00681 | 0,00026 | 0,00220 |
| sumsq(diff) | 5,34434E-05 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| index | 4,0 | 0,0 | 86,9 | 0,1 | 9,1 |

### Activity of Ra-226 in water

| Stdev | Value | | $P_{Ra\text{-}226}$ | $t_{Ra\text{-}226}$ | $P_{Ra\text{-}226Std}$ | $t_{Ra\text{-}226Std}$ | $m_{Ra\text{-}226SS}$ | $A_{Ra\text{-}226SS}$ | $R_{Ra226Std}$ | $V_{sample}$ | $P_{Ba\text{-}133sample}$ | $t_{Ba\text{-}133sample}$ | $m_{Ba\text{-}133sample}$ | $t_{Ba\text{-}133Std}$ | $m_{Ba\text{-}133Std}$ | $P_{Ba\text{-}133Std}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 86 | 7516 | $P_{Ra\text{-}226}$ | 7602 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 | 7516 |
| | 300000 | $t_{Ra\text{-}226}$ | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 | 300000 |
| 113 | 12785 | $P_{Ra\text{-}226Std}$ | 12785 | 12785 | 12898 | 12785 | 12785 | 12785 | 12785 | 12785 | 12785 | 12785 | 12785 | 12785 | 12785 | 12785 |
| | 2000 | $t_{Ra\text{-}226Std}$ | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 | 2000 |
| 0,00058 | 0,01022 | $m_{Ra\text{-}226SS}$ | 0,01022 | 0,01022 | 0,01022 | 0,01022 | 0,01080 | 0,01022 | 0,01022 | 0,01022 | 0,01022 | 0,01022 | 0,01022 | 0,01022 | 0,01022 | 0,01022 |
| 11 | 2729 | $A_{Ra\text{-}226SS}$ | 2729 | 2729 | 2729 | 2729 | 2729 | 2740 | 2729 | 2729 | 2729 | 2729 | 2729 | 2729 | 2729 | 2729 |
| 0,0150 | 0,9344 | $R_{Ra226Std}$ | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9494 | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9344 | 0,9344 |
| 0,002 | 1,000 | $V_{sample}$ | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,002 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 | 1,000 |
| 104 | 10914 | $P_{Ba\text{-}133sample}$ | 10914 | 10914 | 10914 | 10914 | 10914 | 10914 | 10914 | 10914 | 11018 | 10914 | 10914 | 10914 | 10914 | 10914 |
| | 3000 | $t_{Ba\text{-}133sample}$ | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| 0,00058 | 0,30120 | $m_{Ba\text{-}133sample}$ | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30120 | 0,30178 | 0,30120 | 0,30120 | 0,30120 |
| | 3000 | $t_{Ba\text{-}133Std}$ | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 | 3000 |
| 0,00058 | 0,11280 | $m_{Ba\text{-}133Std}$ | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11280 | 0,11338 | 0,11280 |
| 71 | 5090 | $P_{Ba\text{-}133Std}$ | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5090 | 5161 |
| 0,00803 | 0,12719 | $A_{Ra\text{-}226}$ | 0,12864794 | 0,12719257 | 0,12607823 | 0,12719257 | 0,134377958 | 0,1277053 | 0,1292344 | 0,126933468 | 0,125991986 | 0,12719257 | 0,127436377 | 0,1271926 | 0,126545 | 0,128967 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| diff | -0,00145537 | 0 | 0,00111434 | 0 | -0,00718539 | -0,0005127 | -0,0020418 | 0,000259102 | 0,001200583 | 0 | -0,000243807 | 0 | 0,000648 | -0,001774 | |
| sumsq(diff) | 6,45569E-05 | | | | | | | | | | | | | | |
| index | 3,3 | 0,0 | 1,9 | 0,0 | 80,0 | 0,4 | 6,5 | 0,1 | 2,2 | 0,0 | 0,1 | 0,0 | 0,6 | 4,9 | |

## Final result:

| | |
|---|---|
| **$A_{Ra\text{-}226}$ [Bq/L]** | **0,127** |
| **Expanded uncertainty (k=2) [Bq/L]** | **0,016** |

# Addendum II: Measurement uncertainty calculation – GumWorkbench

## Model equation:

$A_{Ra266}=(P_{Ra266}/(t_{Ra266}*\varepsilon_{\alpha det}*V_{sample}))*(1/R_{chem});$

$R_{chem}=(P_{Ba133sample}/(t_{Ba133sample}*m_{Ba133sample}))*((t_{Ba133Std}*m_{Ba133Std})/P_{Ba133Std});$

$\varepsilon_{\alpha det}=P_{Ra226Std}/(t_{Ra226Std}*m_{Ra226SS}*A_{Ra226SS}*R_{Ra226Std});$

## List of quantities:

| Quantity | Unit | Definition |
|---|---|---|
| $A_{Ra266}$ | Bq/L | Activity of Ra-266 in sample |
| $P_{Ra266}$ | | Area of Ra-266 |
| $t_{Ra266}$ | s | Time of measurement |
| $\varepsilon_{\alpha det}$ | | Efficiency for alfa detector |
| $V_{sample}$ | L | Volume of the sample |
| $R_{chem}$ | | Radiochemical yield (recovery) |
| $P_{Ba133sample}$ | | Area of Ba-133 in sample |
| $t_{Ba133sample}$ | s | Time of measurement of the sample |
| $m_{Ba133sample}$ | g | Mass of Ba-133 in the sample |
| $t_{Ba133Std}$ | s | Time of measurement of Ba-133 standard disc |
| $m_{Ba133Std}$ | g | Mass of Ba-133 standard disc |
| $P_{Ba133Std}$ | | Area of Ba-133 in standard disc |
| $P_{Ra226Std}$ | | Area of Ra-266 in standard disc |
| $t_{Ra226Std}$ | s | Time of measurement of the standard disc |
| $m_{Ra226SS}$ | g | Mass of Ra-226 standard solution |
| $A_{Ra226SS}$ | | Activity of Ra-226 in standard solution |
| $R_{Ra226Std}$ | | Radium standard disc recovery |

**$P_{Ra266}$:**
Type A summarised
Mean: 7516
Standard Uncertainty: 86

**$t_{Ra266}$:**
Constant
Value: 300000 s

**$V_{sample}$:**
Type B triangular distribution
Value: 1 L
Halfwidth of limits: 0,005 L

**$P_{Ba133sample}$:**
Type A summarised
Mean: 10914
Standard Uncertainty: 104

**$t_{Ba133sample}$:**
Constant
Value: 3000 s

**$m_{Ba133sample}$:**
Type B rectangular distribution
Value: 0,30120 g
Halfwidth of limits: 0,001 g

**$t_{Ba133Std}$:**
Constant
Value: 3000 s

**$m_{Ba133Std}$:**
Type B rectangular distribution
Value: 0,11280 g
Halfwidth of limits: 0,001 g

**$P_{Ba133Std}$:**
Type A summarised
Mean: 5090
Standard Uncertainty: 71

**$P_{Ra226Std}$:**
Type A summarised
Mean: 12785
Standard Uncertainty: 113

**$t_{Ra226Std}$:**
Constant
Value: 2000 s

**$m_{Ra226SS}$:**
Type B rectangular distribution
Value: 0,01022 g
Halfwidth of limits: 0,001 g

**$A_{Ra226SS}$:**
Type A summarised
Mean: 2729
Standard Uncertainty: 10,734

**$R_{Ra226Std}$:**
Type A summarised
Mean: 0,9344
Standard Uncertainty: 0,015

## Uncertainty budgets:

### $A_{Ra266}$: Activity of Ra-266 in sample

| Quantity | Value | Standard Uncertainty | Distribution | Sensitivity Coefficient | Uncertainty Contribution | Index |
|---|---|---|---|---|---|---|
| $P_{Ra266}$ | 7516,0 | 86,0 | normal | $17 \cdot 10^{-6}$ | $1,5 \cdot 10^{-3}$ Bq/L | 3,3 % |
| $t_{Ra266}$ | $300,0 \cdot 10^{3}$ s | | | | | |
| $V_{sample}$ | 1,00000 L | $2,04 \cdot 10^{-3}$ L | triangular | -0,13 | $-260 \cdot 10^{-6}$ Bq/L | 0,1 % |
| $P_{Ba133sample}$ | 10914 | 104 | normal | $-12 \cdot 10^{-6}$ | $-1,2 \cdot 10^{-3}$ Bq/L | 2,3 % |
| $t_{Ba133sample}$ | 3000,0 s | | | | | |
| $m_{Ba133sample}$ | 0,301200 g | $577 \cdot 10^{-6}$ g | rectangular | 0,42 | $240 \cdot 10^{-6}$ Bq/L | 0,0 % |
| $t_{Ba133Std}$ | 3000,0 s | | | | | |
| $m_{Ba133Std}$ | 0,112800 g | $577 \cdot 10^{-6}$ g | rectangular | -1,1 | $-650 \cdot 10^{-6}$ Bq/L | 0,7 % |
| $P_{Ba133Std}$ | 5090,0 | 71,0 | normal | $25 \cdot 10^{-6}$ | $1,8 \cdot 10^{-3}$ Bq/L | 4,9 % |
| $P_{Ra226Std}$ | 12785 | 113 | normal | $-9,9 \cdot 10^{-6}$ | $-1,1 \cdot 10^{-3}$ Bq/L | 2,0 % |
| $t_{Ra226Std}$ | 2000,0 s | | | | | |
| $m_{Ra226SS}$ | 0,010220 g | $577 \cdot 10^{-6}$ g | rectangular | 12 | $7,2 \cdot 10^{-3}$ Bq/L | 79,9 % |
| $A_{Ra226SS}$ | 2729,0 | 10,7 | normal | $47 \cdot 10^{-6}$ | $500 \cdot 10^{-6}$ Bq/L | 0,4 % |
| $R_{Ra226Std}$ | 0,9344 | 0,0150 | normal | 0,14 | $2,0 \cdot 10^{-3}$ Bq/L | 6,5 % |
| $A_{Ra266}$ | 0,12719 Bq/L | $8,04 \cdot 10^{-3}$ Bq/L | | | | |

### $\varepsilon_{\alpha det}$: Efficiency of alfa detector

| Quantity | Value | Standard Uncertainty | Distribution | Sensitivity Coefficient | Uncertainty Contribution | Index |
|---|---|---|---|---|---|---|
| $P_{Ra226Std}$ | 12785 | 113 | normal | $19 \cdot 10^{-6}$ | $2,2 \cdot 10^{-3}$ | 2,2 % |
| $t_{Ra226Std}$ | 2000,0 s | | | | | |
| $m_{Ra226SS}$ | 0,010220 g | $577 \cdot 10^{-6}$ g | rectangular | not valid! | -0,014 | 90,1 % |
| $A_{Ra226SS}$ | 2729,0 | 10,7 | normal | $-90 \cdot 10^{-6}$ | $-960 \cdot 10^{-6}$ | 0,4 % |
| $R_{Ra226Std}$ | 0,9344 | 0,0150 | normal | -0,26 | $-3,9 \cdot 10^{-3}$ | 7,2 % |
| $\varepsilon_{\alpha det}$ | 0,2453 | 0,0146 | | | | |

### $R_{chem}$: Radiochemical yield (recovery)

| Quantity | Value | Standard Uncertainty | Distribution | Sensitivity Coefficient | Uncertainty Contribution | Index |
|---|---|---|---|---|---|---|
| $P_{Ba133sample}$ | 10914 | 104 | normal | $74 \cdot 10^{-6}$ | $7{,}7 \cdot 10^{-3}$ | 28,8 % |
| $t_{Ba133sample}$ | 3000,0 s | | | | | |
| $m_{Ba133sample}$ | 0,301200 g | $577 \cdot 10^{-6}$ g | rectangular | -2,7 | $-1{,}5 \cdot 10^{-3}$ | 1,2 % |
| $t_{Ba133Std}$ | 3000,0 s | | | | | |
| $m_{Ba133Std}$ | 0,112800 g | $577 \cdot 10^{-6}$ g | rectangular | 7,1 | $4{,}1 \cdot 10^{-3}$ | 8,3 % |
| $P_{Ba133Std}$ | 5090,0 | 71,0 | normal | $-160 \cdot 10^{-6}$ | -0,011 | 61,7 % |
| $R_{chem}$ | 0,8030 | 0,0143 | | | | |

## Result:

| Quantity | Value | Expanded Uncertainty | Coverage factor | Coverage |
|---|---|---|---|---|
| $A_{Ra266}$ | 0,127 Bq/L | 0,016 Bq/L | 2,00 | 95% |

## Chapter 5

## Determination of Polar Pesticides by Liquid Chromatography Mass Spectrometry

*Allan Künnapas, Koit Herodes, Ivo Leito*

- **TrainMiC example summary form ('blue page')**
- **A short introduction to the analytical procedure ('slides')**
- **All input needed to do the three exercises ('yellow pages')**
- **The solved exercises ('green pages')**

# TrainMiC example summary form

## I. General information about the example

| | |
|---|---|
| **Measurand** | Concentration of imazalil and thiabendazole in tangerines by liquid chromatography-mass spectrometry |
| **Example number** | Ex-04 |
| **Authors of the example** | Allan Künnapas, Koit Herodes, Ivo Leito |
| **Analytical procedure** | Determination of concentration of imazalil and thiabendazole in tangerines by liquid chromatography-mass spectrometry. The sample preparation procedure is modified AOAC 985.22 procedure. The measurement procedure is an in-house developed procedure. |
| **Customer's requirement** | The quality of the results should comply with the requirements given in the EU Directives 93/58/EEC and 00/42/EEC on pesticide residues analysis |

## II. Attached files

| File number, type and name | | Content of the file | | File is attached Yes | File is attached No | Remark |
|---|---|---|---|---|---|---|
| **1 - I** | Ex-04-1-I-Pesticides-Food-LCMS-2006-Ver1.ppt | About the analytical procedure: short introduction | | | ✓ | Given by the leacturer |
| **2 - Yellow** | Ex-04-2-Y-Pesticides-Food-LCMS-2006-Ver1.doc | PART I | Description of the analytical procedure | ✓ | | Each participant receives own copy and may keep it |
| | | PART II | The customer's requirements concerning the quality of the measurement result | ✓ | | |
| | | PART III | Validation of the measurement procedure – relevant equations and measurement data | ✓ | | |
| | | PART IV | Measurement uncertainty of the result – relevant equations and measurement data | ✓ | | |
| **3 - Green** | Ex-04-3-G-Pesticides-Food-LCMS-2006-Ver1.doc | PART I | Establishing traceability in analytical chemistry | ✓ | | |
| | | PART II | Single laboratory validation of measurement procedures | ✓ | | |
| | | PART III | Bulding an uncertainty budget | ✓ | | |
| | | | Addendum 1: By spreadsheet approach | | ✓ | |
| | | | Addendum 2: By dedicated software | | ✓ | |

## III. History of the example

| Version | Uploaded on the webhotel | Short description of the change |
|---|---|---|
| 0 | April 2007 | - |
| 1 | | |

# A short introduction to the analytical procedure

## Determination of Polar Pesticides by Liquid Chromatography Mass Spectrometry

Scope of the presentation

- The Customer's Requirements
- The Analytical Procedure
  - Sample preparation
  - LC-MS analysis
- Model Equation

Introduction to TrainMiC
example session

## The Customer's Requirements

Post-registration control and monitoring of pesticides based on MRLs set by the EU Directives 93/58/EEC and 00/42/EEC for **imazalil** and **thiabendazole** respectively:

- LOD = 0.02 mg/kg (imazalil), LOD = 0.05 mg/kg (thiabendazole)
- Recovery between 70 – 110 %
- Identity confirmed by MS/MS experiments

Introduction to TrainMiC
example session

## LC-MS as an Analytical Tool

Unites two powerful methods:

**LC** (liquid chromato-graph) **separates** the analytes from each other

**MS** (mass spectrometer) **detects, identifies** and **quantifies** the analytes

## The Analytical Procedure

- Sample preparation procedure is a modification of the AOAC official method 985.22
  - Modifications were made to cut down sample size and solvent consumption
  - Changes were made in the solvent of final extract to suite LC-MS system
- Analysis is carried out on an LC-MS system using a self-developed chromatographic method

**Partial Validation Required!**

Introduction to TrainMiC
example session

## Sample Preparation

- 50 g of homogenized sample is extracted with 100 ml of acetone using high speed blender
- Mixture is filtered and the volume of extract is measured
- 50 mL of extract is extracted with 100 mL dichloromethane petroleum ether mixture (1:1), organic layer is filtered through a layer of sodium sulphate (for drying purpose)
- Water phase is saturated with NaCl and extracted twice with 50 mL of dichloromethane
- Organic extracts are dried
- Solvent is evaporated to almost dryness and the sample is dissolved in 10 - 20 mL of methanol
- Sample is filtered through a syringe filter and analysed using LC-MS system

**Complex Sample Preparation!**

Introduction to TrainMiC
example session

## The LC-MS Analysis Procedure

- In the LC-MS system the samples are separated chromatographically
  - Eluent: acetontrile (B) and buffer solution (1mM ammonium acetate, 0.1 % formic acid) (A) as eluent
  - The gradient program : B 20 –> 100 % 15 min, B 100 % 17 min at 0.8 mL/min
  - Analysed substances were then ionized using the ESI procedure and analysed with the ion-trap MS using fragmentation of quasimolecular ions ($[M+H]^+$)
- Using the 20 mg/kg standard solution and other dilutions the calibration solutions are prepared in methanol in the concentration range of 5 – 0.003 mg/kg
- Calibration graphs are compiled using peak areas of certain characteristic fragment ions on different concentrations

Introduction to TrainMiC
example session

## Model Equation

$$C = \frac{C_c \cdot V_{10} \cdot \rho \cdot V_e}{V_{50} \cdot m}$$

| | |
|---|---|
| $C$ | concentration of extractable pesticide in sample (mg of pesticide per kg of sample) [mg/kg] |
| $C_c$ | concentration of extractable pesticide in analysed extract [mg/kg] (found from the calibration graph) |
| $V_{10}$ | the volume of final extract in methanol [mL] |
| $\rho$ | density of methanol [g/mL] |
| $V_e$ | the full volume of acetone extract [mL] |
| $V_{50}$ | the volume of acetone extract to be purified [mL] |
| $M$ | mass of homogenised sample to be extracted [g] |

Introduction to TrainMiC
example session

# All input needed to do the three exercises 'yellow pages'

## Analytical procedure

***Determination of concentration of imazalil and thiabendazole in tangerines by liquid chromatography mass spectrometry.***

***The quality of the results should comply with the requirements in the EU directives 93/58/EEC and 00/42/EEC/ on pesticide residues analysis***

**PART I**

**Description of the analytical procedure**

**PART II**

**The customer's requirement concerning quality of the measurement result**

**PART III**

**Validation of the measurement procedure – relevant equations and measurement data**

**PART IV**

**Measurement uncertainty of the result – relevant equations and measurement data**

# PART I. Description of the analytical procedure

The objective of this analysis is post-registration control and monitoring of imazalil and thiabendazole (polar pesticides) based on Maximum Residue Limits (MRLs) set by the EU Directives 93/58/EEC and 00/42/EEC.

Sample preparation procedure is modified AOAC official method 985.22. Analysis was carried out on an LC-MS system using a self-developed chromatographic method.

## 1. Scope

A modified AOAC 985.22 sample preparation procedure was used, to suite LC-MS-MS analysis. The analysis was carried out using liquid chromatographic separation and atmospheric pressure electrospray ionisation with tandem mass spectrometric detection (AP-ESI-LC-MS-MS).

Sample preparation procedure is suitable for berries, fruits and vegetables containing less then 2% of fat and more than 70% of water (water can be added if its content is insufficient). All in all 14 residues of polar pesticides are analysed in this analytical procedure. In this example, only two of them will be discussed in detail: imazalil and thiabendazole.

## 2. Principle

An aliquot of homogenised sample is extracted with acetone and filtered. A portion of the extract is subjected to liquid-liquid clean-up step consisting of one extraction with petroleum ether (40 – 60°C)-dichloromethane mixture and two extractions with dichloromethane from saturated NaCl solution. Organic extracts are dried using anhydrous sodium sulphate. Then the solvent is exchanged to methanol through evaporation and dissolving. The obtained extract is analysed using LC-MS.

In the LC-MS system the samples are separated chromatographically using acetontrile (B) and buffer solution (1mM ammonium acetate, 0,1 % formic acid) (A) as eluent. The gradient program was as follows: B 20 –> 100 % 15 min, B 100 % 17 min at 0,8 mL/min. Analysed substances were then ionised through the ESI procedure and analysed with the ion-trap MS

using fragmentation of quasimolecular ions ($[M+H]^+$). Calibration graphs are compiled using peak areas of certain characteristic fragment ions on different concentrations.

The result is calculated in mg of pesticide residue per kg of sample, or ppm.

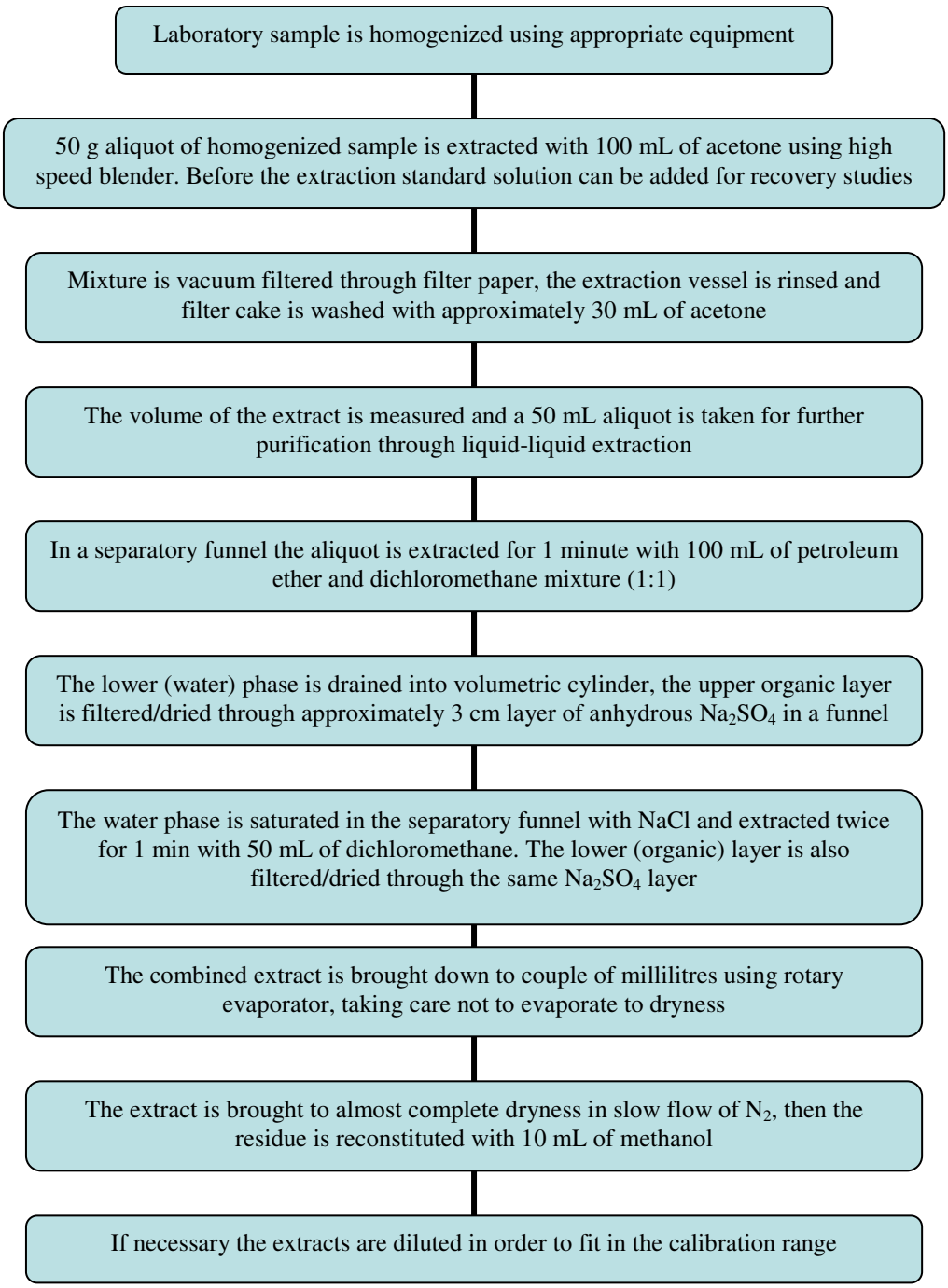



**Figure 5 Flow chart of the analytical procedure**

## 3. Interferences

ESI procedure is dependent on ionization efficiencies of the species. The ionisation efficiencies can be affected by co-eluting polar matrix components. Thus sample preparation and in most part chromatographic separation should be able to cope with these circumstances. For this reason retention times should be reasonably large compared to the dead volume of the column. In addition suitable buffer solution should be used.

The best ways to correct these effects are using matrix matched calibration, standard addition or labelled internal standards. However these means will make the analysis procedure significantly more complex and are not used in the current procedure.

## 4. Reagents

*1000 mg/kg individual pesticide standard solutions*

Prepare pesticide standard solutions by dissolving 10 mg of substance in 10 g of acetone (1000 mg/kg) in 15 mL vials.

*20 mg/kg combined pesticide standard solution*

Weigh 0,2 g of each individual pesticide standard solution into 15 mL vial and fill it up with methanol (9,6 g in the case of two components)

*Calibration solutions*

Using the 20 mg/kg standard solution and other dilutions the calibration solutions can be prepared in methanol. Suitable number of solutions should be prepared in the range of 5 – 0,003 mg/kg.

*Solvents/eluent:*

Gradient grade methanol, ultra pure water, ammonium acetate and formic acid (suitable for LC-MS buffer), petrol ether, dichloromethane and acetone (for residue analysis or GC/HPLC grade if suitability checked)

*Other:*

NaCl, $MgSO_4$ pure for pesticide analysis (e.g. heated before use)

## 5. Sampling and pre-treatment

Sampling shall be carried out in accordance with European Commission Directive 2002/63/EC. While getting a laboratory/analytical sample one has to obtain homogenous and representative sample, also a great care has to be taken in order to avoid cross-contamination before or during or after sample preparation. Standard solutions should be kept separate from samples.

## 6. Calculation

The residue content *C* in the sample is found according to the following equation.

$C_c$ is found from the calibration graph.

$$C = \frac{C_c * V_{10} * \rho * V_e}{V_{50} * m}$$

| | |
|---|---|
| C | concentration of extractable pesticide in sample (mg of pesticide per kg of sample) [mg/kg] |
| $C_c$ | concentration of extractable pesticide in analysed extract [mg/kg] |
| $V_{10}$ | the volume of final extract in methanol [mL] |
| ρ | density of methanol (extract) [g/mL] |
| $V_e$ | the full volume of acetone extract [mL] |
| $V_{50}$ | the volume of acetone extract to be purified [mL] |
| m | mass of homogenised sample to be extracted [g] |

## 7. Results

Calculations are performed using calibration graph and the model equation given above. Obtained results are compared against MRLs set by EU Council – 5 mg/kg for both pesticides. The samples at or over MRL must be reanalysed and/or otherwise confirmed.

## PART II. The customer's requirement concerning quality of the measurement result

The laboratory should provide at least the following LODs for pesticide residues:

- Imazalil 0,02 mg/kg (for citrus) (Directive 93/58/EEC)
- Thiabendazole 0,05 mg/kg (for citrus) (Directive 00/42/EC)

*Extract from the EU Quality Control Procedures for Pesticide Residues Analysis, SANCO/10232/2006*

**********

58. The method must be tested to assess for sensitivity, mean recovery (as a measure of trueness or bias) and precision. This effectively means that spiked recovery experiments to check the accuracy of the method should be undertaken. A minimum of 5 replicates is required

Mean recovery range should be within 70 – 110 %. In that case no recovery correction is performed.

Exceptionally, where recovery is low but consistent (i.e. demonstrating good precision) and the basis for this is well established (e.g. due to pesticide distribution in partition), a mean recovery below 70% may be acceptable. However, a more accurate method should be used, if practicable.

In the case of low recovery one has to take it into account when making decisions at or above MRL.

78. EI-MS or MS/MS, performed with acquisition of spectra, may provide good evidence of identity and quantity in many cases. In other cases, as with mass spectra produced by other processes (e.g. CI, API) that can be too simple for absolute confirmation of identity, further evidence may be required. If the isotope ratio of the ion(s), or the chromatographic profile of isomers of the analyte, is highly characteristic it may provide sufficient evidence. Otherwise, the evidence may be sought using:

(i) a different chromatographic separation system;

(ii) a different ionisation technique;

(iii) MS/MS;

(iv) medium/high resolution MS; or

(v) inducing "in-source" fragmentation in LC-MS.

**Table 3. Recommended maximum permitted tolerances for relative ion intensities using a range of spectrometric techniques**

| Relative intensity (% of base peak) | EI-GC-MS (relative) | CI-GC-MS, GC-MS$^n$, LC-MS, LC-MS$^n$ (relative) |
|---|---|---|
| > 50% | ± 10% | ± 20% |
| > 20% to 50% | ± 15% | ± 25% |
| > 10% to 20% | ± 20% | ± 30% |
| ≤ 10% | ± 50% | ± 50% |

# PART III. Validation of the measurement procedure – relevant equations and measurement data

Equations

$$R = \frac{C_{\exp}}{C_{theor}} * 100\%$$

$$STDEV = \sqrt{\frac{n\sum x^2 - \left(\sum x\right)^2}{n(n-1)}}$$

$$AVERAGE = \frac{\sum x}{n}$$

$$RSD = \frac{STDEV}{AVERAGE} * 100\%$$

| | |
|---|---|
| $R$ | recovery of the method [%] |
| $C_{\exp}$ | experimentally measured concentration of the pesticide residue in the sample, in the recovery studies the pesticide is spiked into the sample homogenate [mg/kg] |
| $C_{\text{theor}}$ | theoretically calculated concentration of the pesticide residues in the spiked sample [mg/kg] |
| $n$ | the number of data points in the set |
| $x$ | Individual data points (in our case x denotes $R$) in the set |
| STDEV | standard deviation [%] |
| AVERAGE | average value of the data set [%] |
| RSD | relative standard deviation [%] |

Measurement data

| Imazalil | | | Thiabendazole | | | Imazalil | Thiabendazole | |
|---|---|---|---|---|---|---|---|---|
| $C_{exp}$ (mg/kg) | $C_{theor}$ (mg/kg) | $R$ (%) | $C_{exp}$ (mg/kg) | $C_{theor}$ (mg/kg) | $R$ (%) | Peak area | Peak area | |
| 0,06427 | 0,05597 | | 0,03120 | 0,04244 | | 3996669 | 300802 | |
| 0,07516 | 0,05871 | | 0,03281 | 0,04452 | | 3459066 | 281164 | |
| 0,04812 | 0,05821 | | 0,03181 | 0,04413 | | 3838651 | 230775 | |
| 0,10238 | 0,07342 | | 0,04095 | 0,05567 | | 3727188 | 274366 | |
| 0,04201 | 0,06088 | | 0,03400 | 0,04616 | | 3414893 | 296724 | |
| 0,05741 | 0,06241 | | 0,03331 | 0,04732 | | 3553740 | 258916 | |
| **AVERAGE recovery** | | | **AVERAGE recovery** | | | **AVERAGE concentration** | | |
| **STDEV of recovery** | | | **STDEV of recovery** | | | **STDEV of concentration** | | |
| **RSD of recovery ($u_{rel\_rec}$)** | | | **RSD of recovery ($u_{rel\_rec}$)** | | | **RSD of concentration ($u_{rel\_meth}$)** | | |

@* The recovery determinations were carried out two per day on three consecutive days.

# PART IV. Measurement uncertainty of the result – relevant equations and measurement data

Equations

$$u_c = \sqrt{u_{sys}^2 + u_{rnd}^2}$$

$$u_{rnd} = \frac{\sqrt{u_{rel\_rec}^2 + u_{rel\_meth}^2}}{100\%} * c$$

$$d = c - c_{ref}$$

$$u_{ref} = \frac{s}{\sqrt{n_l}}$$

$$u_{dev} = \sqrt{\frac{d^2}{n}}$$

$$u_{sys} = \sqrt{u_{ref}^2 + u_{dev}^2}$$

| | |
|---|---|
| $u_c$ | standard uncertainty of concentration of pesticide [mg/kg] |
| $u_{sys}$ | systematic component of uncertainty [mg/kg] |
| $u_{rnd}$ | random component of uncertainty [mg/kg] |
| $u_{rel\_rec}$ | relative uncertainty of recovery [mg/kg] |
| $u_{rel\_meth}$ | relative uncertainty of analysis method [mg/kg] |
| $C$ | pesticide concentration in standard sample as obtained with the measurement procedure [mg/kg] |
| $d$ | difference in concentration between our laboratory and reference value (laboratory bias) [mg/kg] |
| $c_{ref}$ | reference concentration of pesticide in the reference sample [mg/kg] |
| $S$ | the standard deviation of the results of the participants of the interlaboratory comparison [mg/kg] |
| $n_l$ | the number of laboratories who took part in interlaboratory comparison (ILC) |
| $N$ | number of completed ILCs |

Measurement data

| | Imazalil | Thiabendazole | Comments |
|---|---|---|---|
| $u_{rel\_rec}$ | 27% | 2% | The relative standard deviation of recovery calculated from parallel measurement results (two measurements per day on three consecutive days) |
| $u_{rel\_meth}$ | 10% | 6% | The relative standard deviation of results obtained for the same solution from repeated injections of the same solution |
| $c$ | 1,3350 mg/kg | 3,5230 mg/kg | |
| $c_{ref}$ | 1,2975 mg/kg | 3,2863 mg/kg | consensus value of interlaboratory comparison measurement |
| $s$ | 0,0530 mg/kg | 0,5571 mg/kg | |
| $n_1$ | 2 | 3 | |
| $n$ | 1 | 1 | |

# The solved exercises 'green pages'

## TRAINMIC EXERCISES

### Analytical procedure

***Determination of concentration of imazalil and thiabendazole in tangerines by liquid chromatography-mass spectrometry.***

***The quality of the results should comply with the requirements in pesticide residues analysis directives and guidelines***

*Exercise 1:*

**Establishing traceability in analytical chemistry**

*Exercise 2:*

**Single laboratory validation of measurement procedures**

- **Part I: General issues**
- **Part II: Parameters to be validated**
- **Part III: Some calculations and conclusions**

*Exercise 3:*

**Building an uncertainty budget**

- **Addendum I. By spreadsheet approach**
- **Addendum II. By dedicated software**

# ESTABLISHING TRACEABILITY IN ANALYTICAL CHEMISTRY

## 1. Specifying the analyte and measurand

| | |
|---|---|
| *Analyte* | Residues of imazalil and thiabendazole |
| *Measurand* | Acetone-extractable imazalil and thiabendazole residues in tangerines |
| *Units* | mg/kg (ppm) |

## 2. Choosing a suitable measurement procedure with associated model equation

| | | | |
|---|---|---|---|
| *Measurement procedure* | 50 g of homogenized sample is extracted with 100 mL of acetone using high speed blender. Mixture is filtered and the volume of extract is measured. 50 mL of the extract is extracted with 100 mL dichloromethane petroleum ether mixture (1:1), the organic layer is filtered through a layer of sodium sulphate (for drying purpose).Water phase is saturated with NaCl and extracted twice with 50 mL of dichloromethane. Organic extracts are dried as before. Solvent is evaporated to almost dryness and the sample is dissolved in 10 - 20 mL of methanol. Sample is filtered through a syringe filter and analysed using LC-MS system.<br>Sample preparation procedure is based on the AOAC official method 985.22 'Organochlorine and Organophosphorus Pesticide Residues Gas Chromatographic Method'. The modifications were made in order to cut down sample size and thus solvent consumption. Also changes were made in the solvent of final extract to suite LC-MS system. LC-MS analysis method was developed within laboratory. | | |
| *Type of calibration* | *standard curve* ☒ | *Standard addition* ☐ | *internal standard* ☐ |

Model equation

$$C = \frac{C_c * V_{10} * \rho * V_e}{V_{50} * m}$$

$C$ concentration of extractable pesticide in sample (mg of pesticide per kg of sample) [mg/kg]

$C_c$ concentration of extractable pesticide in analysed extract [mg/kg] (found from the calibration curve)

$V_{10}$ the volume of final extract in methanol [mL]

$\rho$ density of methanol [g/mL]

$V_e$ the full volume of acetone extract [mL]

$V_{50}$ the volume of acetone extract to be purified [mL]

$m$ mass of homogenised sample to be extracted [g]

**3. List the input quantities according to their influence on the uncertainty of the result of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.**

| | |
|---|---|
| *1* | Concentration of extractable pesticide in analysed extract ($C_c$, mg/kg) |
| *2* | The full volume of acetone extract ($V_e$, mL) |
| *3* | The volume of final extract in methanol ($V_{10}$, mL) |
| *4* | The volume of acetone extract to be purified ($V_{50}$, mL) |
| *5* | The density of methanol ($\rho$, g/mL) |
| *6* | The mass of homogenized sample ($m$, g) |

**4. List the reference standards needed and state the information regarding traceability of the reference value**

**For the analyte**

| | | |
|---|---|---|
| *1* | *Name/ChemicalFormula/ Producer:* | Imazalil (solid substance)/$C_{14}H_{14}Cl_2N_2O$/Dr. Ehrenstorfer<br>Value including uncertainty (with units):<br>Imazalil: purity 97,5% (tolerance ±0,5%) (data obtained from corresponding Certificate of Analysis) |
| *2* | *Name/ChemicalFormula/ Producer:* | Thiabendazole (solid substance)/$C_{10}H_7N_3S$/Dr. Ehrenstorfer<br>Value including uncertainty (with units):<br>Thiabendazole: purity 99,0% (tolerance ±0,5%) (data obtained from corresponding Certificate of Analysis) |

**For the other input quantities**

| | | |
|---|---|---|
| *1* | *Quantity/Equipment/Calibration:*<br>*e.g. mass/balance/calibrated by NMI, U=xx (k=2),* | None |

**5. Estimating uncertainty associated with the measurement**

| | | |
|---|---|---|
| *Are all important parameters included in the model equation?* | *Yes* ☒ | *No* ☐ |
| *Other important parameters are:* | | |

**6. How would you prove traceability of your result?**

| | |
|---|---|
| *1* | Participate in EU proficiency testing programme |
| *2* | Analyse a CRM (in future, when such CRM becomes available) |

**7. Any other comments, questions…**

# SINGLE LABORATORY VALIDATION OF MEASUREMENT PROCEDURES

## PART I: GENERAL ISSUES

### 1. Specify the measurement procedure, analyte, measurand and units

| | |
|---|---|
| *The measurement procedure* | Sample preparation procedure is modified AOAC official method 985.22. Analysis was carried out on an LC-MS system using a self-developed chromatographic method. |
| *Analyte* | Residues of imazalil and thiabendazole (polar basic pesticides) |
| *The measurand* | Acetone-extractable pesticides in tangerines.<br>Results are not recovery corrected, thus extractable pesticides are determined, not total amounts. |
| *Unit* | mg/kg (ppm) |

### 2. Specify the scope

| | |
|---|---|
| *Matrix* | Tangerines |
| *Measuring range* | imazalil 0,004 – 0,9 mg/kg<br>thiabendazole 0,003 – 0,7 mg/kg. |

### 3. Requirement on the measurement procedure

| | | | |
|---|---|---|---|
| *Intended use of the results* | Post-registration control and monitoring of pesticides based on MRLs set by the EU Directives 93/58/EEC and 00/42/EEC for imazalil and thiabendazole respectively. | | |
| *Mark the customer's requirements and give their values* | *Parameters to be validated* | | *Value requested by the customer* |
| | ☒ | *LOD* | LOD < 0,02 mg/kg (imazalil), LOD < 0,05 mg/kg (thiabendazole) |
| | ☐ | *LOQ* | |
| | ☐ | *Repeatability* | |
| | ☐ | *Within-lab reproducibility* | |
| | ☒ | *Trueness* | Recovery between 70 – 110% |
| | ☐ | *Measurement uncertainty* | |
| | ☒ | *Other - state* | Identity/confirmation: retention time (compared with standard) + $MS^{\wedge 2}$ fragmentation: imazalil (297 → 201), thiabendazole (202 → 175) + additional qualifier ion comparison if necessary. Guidance document refers to sufficient confirmation when $MS^{\wedge 2}$ is used and ion ratios in standard and sample agree within the limits specified in Table 3 (Yellow sheet, Part II). |

### 4. Origin of the measurement procedure

| | | VALIDATION |
|---|---|---|
| *New in-house method* | ☐ | *Full* |
| *Modified validated method* | ☒ | *Partial* |
| *Official standard method* | ☐ | *Confirmation/Verification* |

## PART II: PARAMETERS TO BE VALIDATED

### 5. Selectivity/Interference/Recovery

Where yes, please give further information e.g. which CRM, reference method

| | |
|---|---|
| ☐ | *CRM/RM: analysis of available CRM or RM* |
| | *Further information:* |
| ☒ | *Spike of pure substance* |
| | At approximate concentration level of 0,05 mg/kg |
| ☐ | *Compare with a reference method* |
| | |
| ☒ | *Selectivity, interferences* |
| | Chromatographic separation and mass-spectrometric identification (including $MS^{\wedge 2}$ confirmation of identity) |
| ☒ | *Test with different matrices* |
| | The method has been proved via ILC to perform with tangerine, orange and tomato |
| ☒ | *Other – please specify* |
| | Confirmation of identity: chromatographic retention time and $MS^{\wedge 2}$ confirmation of identity |

### 6. Measuring range

| | |
|---|---|
| ☒ | *Linearity*<br>Imazalil: 0,004 – 0,9 mg/kg; Thiabendasole: 0,003 – 0,7 mg/kg |
| ☒ | *Upper limit*<br>Imazalil: 0,9 mg/kg; Thiabendasole: 0,7 mg/kg |
| ☒ | *LOD*<br>Imazalil: 0,004 mg/kg; Thiabendazole: 0,003 mg/kg |
| ☐ | *LOQ* |

### 7. Spread – precision

| | |
|---|---|
| ☒ | *Repeatability*<br>Instrumental: standard deviation of the measurement method: 10 % for imazalil and 6 % for thiabendazole (repeated injection of the same standard solution). |
| ☒ | *Reproducibility (within Lab)*<br>Full procedure: standard deviation of recovery experiments carried out on three consecutive days – 27% for imazalil, 2% for thiabendazole (full sample preparation included ) |
| ☒ | *Reproducibility (between Lab)*<br>in ILC the difference between results were 5,6 and 4,4% for imazalil and thiabendazole respectively |

## 8. Robustness

| | |
|---|---|
| ☒ | *Variation of parameters*<br>Variation of some of the parameters: during method development 2 different columns were used (C18 250 x 4,6 5µ, C18 150 X 150 2,1), mobile phase composition and velocity were changed in increments and obtained data analysedd, final extract volumes of 10 and 20 mL were utilized. |

## 9. Quality control

| | |
|---|---|
| ☐ | *Control charts* |
| ☒ | *Participation in PT schemes* |

## 10. Other parameters to be tested

| | |
|---|---|
| ☐ | *Working range and testing of homogeneity of variances* |
| ☐ | *R square* |
| ☐ | *Residual standard deviation* |
| ☐ | *Standard deviation of the analytical procedure* |
| ☐ | *Coefficient of variation of the analytical procedure* |
| ☐ | *Measurement uncertainty* |
| ☒ | *Other-state:* Confirmation of identity: in accordance with requirements in section 3. |

## PART III: SOME CALCULATIONS AND CONCLUSIONS

### 11. Calculation of parameters requested by the customer

| Parameters requested to be validated | Calculations |
|---|---|
| ☒ *LOD* | Since the method permits in principle to obtain significantly lower LOD values than requested by the customer, LOD was estimated in a conservative way by taking the lowest points on the respective calibration graphs as LOD estimates. Values obtained:<br>Imazalil: 0,004 mg/kg<br>Thiabendazole: 0,003 mg/kg |
| ☐ *LOQ* | |
| ☐ *Repeatability* | |
| ☐ *Within-lab reproducibility* | |
| ☒ *Trueness* | Average recovery; for data and equations see first document<br>Imazalil 104%<br>Thiabendazole 73%<br>Recovery is found according to the following equation:<br>$R = \frac{C_{\exp}}{C_{theor}} * 100\%$<br>$R$ – recovery of the method [%]<br>$C_{\exp}$ – experimentally measured concentration of the pesticide residue in the sample, in recovery studies the pesticide sis spiked into the sample homogenate [mg/kg]<br>$C_{theor}$ – theoretically calculated concentration of the pesticide residues in the spiked sample [mg/kg] |
| ☐ *Measurement uncertainty* | |
| ☐ *Other - please state* | |

**12. Does the analytical procedure fulfil the requirement(s) for the intended use?**

| Parameter | Value requested by the customer (the same as stated in question 3) | Value obtained during validation | The requirement is fulfilled Yes/No |
|---|---|---|---|
| ☒ LOD | Imazalil < 0,02 mg/kg<br>Thiabendazole < 0,05 mg/kg | 0,004 mg/kg<br>0,003 mg/kg | Yes<br>Yes |
| ☐ LOQ | | | |
| ☐ Repeatability | | | |
| ☐ Within-lab reproducibility | | | |
| ☒ Trueness | 70 – 110% | Imazalil 104%<br>Thiabendazole 73% | Yes<br>Yes |
| ☐ Measurement | | | |
| ☒ Other | Confirmation based on similarity to standard (MS^2 spectrum) | MS^2 spectrum in sample is similar to standard | Yes |

**The analytical procedure is fit for the intended use:**

☒ *Yes* ☐ *No*

*For measurement uncertainty and traceability refer to the corresponding sheets*

# BUILDING AN UNCERTAINTY BUDGET

## 1. Specify the measurand and units

| | |
|---|---|
| *Measurand* | Extractable pesticide content in fruit/vegetable |
| *Unit* | mg/kg |

## 2. Describe the measurement procedure and provide the associated model equation

Measurement procedure

50 g of homogenised sample is extracted with 100 mL of acetone using high speed blender. Mixture is filtered and the volume of extract is measured. 50 mL of extract is extracted with 100 mL dichloromethane – petroleum ether (40 - 60°C) mixture (1:1), organic layer is filtered through a layer of sodium sulphate (for drying purpose).Water phase is saturated with NaCl and extracted twice with 50 mL of dichloromethane. Organic extracts are dried as before. Solvent is evaporated to almost dryness and the sample is dissolved in 10 - 20 mL of methanol (volume might be even larger if the concentration does not fit in the calibration graph range). Sample is filtered through a syringe filter and analysed using LC-MS system.

Sample preparation procedure is modified AOAC official method 985.22. Analysis was carried out on an LC-MS system using self developed method.

Model equation

$$C = \frac{C_c * V_{10} * \rho * V_e}{V_{50} * m}$$

$C$ concentration of pesticide in sample [mg/kg]
$C_c$ concentration of pesticide in analysed extract [mg/kg]
$V_{10}$ the volume of final extract in methanol [mL]
$\rho$ density of methanol [g/mL]
$V_e$ the full volume of acetone extract [mL]
$V_{50}$ the volume of acetone extract to be purified [mL]
$m$ mass of homogenised sample to be extracted [g]

Uncertainty estimation is carried out using the Nordtest method[4]. In principle the Nordtest approach can be regarded as a special case of the ISO method where very general uncertainty sources are considered.

The main equation:

$$u_c = \sqrt{u_{sys}^2 + u_{rnd}^2}$$

$u_c$ standard uncertainty of concentration of pesticide [mg/kg]
$u_{sys}$ systematic component of uncertainty [mg/kg]
$u_{rnd}$ random component of uncertainty [mg/kg]

The two main uncertainty sources are:
1. Uncertainty $u_{sys}$, which is due to the systematic effects – laboratory bias and method bias. In the original document it is denoted also as $u(bias)$. This component is found from analysis of CRM-s or from participating in ILC-s.
2. Uncertainty $u_{rnd}$, which is due to the random effects – within-laboratory between-day reproducibility. In the original document it is denoted also as $u(R_w)$. This component is found from routine between-day reproducibility monitoring of the method (using e.g. a control chart).

The component $u_{sys}$ in turn is found according to the following formula:

$$u_c = \sqrt{u_{dev}^2 + u_{ref}^2}$$

where $u_{dev}$ denotes uncertainty manifested by the deviation of the laboratory's result from the reference value (denoted by $RMS_{bias}$ in the original document) and $u_{ref}$ denotes the uncertainty of the reference value (denoted as $u(c_{ref})$ in the original document).

The quantification of the uncertainty components is carried out according to the following formulae:

[4] Nordtest Report TR 537. Handbook for Calculation of Measurement Uncertainty in Environmental Laboratories. B. Magnusson, T. Näykki, H. Hovind, M. Krysell. Available on the web at http://www.nordicinnovation.net/nordtestfiler/tec537.pdf

$$u_{\text{rnd}} = \frac{\sqrt{u_{\text{rel\_rec}}^2 + u_{\text{rel\_meth}}^2}}{100\,\%} * c$$

$$d = c - c_{\text{ref}}$$

$$u_{\text{ref}} = \frac{s}{\sqrt{n_{\text{l}}}}$$

$$u_{\text{dev}} = \sqrt{\frac{d^2}{n}}$$

| | |
|---|---|
| $u_{\text{rel\_rec}}$ | relative uncertainty of recovery [%] |
| $u_{\text{rel\_meth}}$ | relative uncertainty of analysis method [%] |
| $C$ | pesticide concentration in a reference sample as determined by the measurement procedure [mg/kg] |
| $c_{\text{ref}}$ | reference concentration of pesticide in the reference sample [mg/kg] |
| $D$ | difference in concentration between our laboratory and reference value [mg/kg] |
| $S$ | the standard deviation for reference value [mg/kg] |
| $n_{\text{l}}$ | the number of laboratories who took part in ILC |
| $n$ | number of completed ILCs ($n$ = 1 in our case) |

### 3. Identify (all possible) sources of uncertainty

| | |
|---|---|
| ☐ | *Uncertainty of concentration of reference solutions* |
| ☐ | *Uncertainty of measurements of peak area* |
| ☐ | *Method bias* |
| ☒ | *Matrix effect:* matrix effects on ionisation of pesticides (repeatability) |
| ☒ | *Other:* repeatability of extraction of the pesticides |
| ☒ | *Other:* stability of standard solutions, integration |
| ☒ | *Other:* calibration graph linearity |

### 4. Evaluate values of each input quantity

| *Input quantity* | *Value* | | *Unit* | *Remark* |
|---|---|---|---|---|
| | Imazalil | Thiabendazole | | |
| $C_c$ | 2,801 | 7,398 | mg/kg | Concentration of residue in extract, calculated based on calibration |
| $V_{10}$ | 10 | 10 | mL | Volume of final methanol extract |
| $\rho$ | 0,791 | 0,791 | g/mL | Density of methanol |
| $V_e$ | 150 | 150 | mL | Volume of extract after filtration |
| $V_{50}$ | 50 | 50 | mL | Volume of extract taken for further cleaning |
| $m$ | 49,8003 | 49,8003 | g | Sample amount taken for extraction |

### 5. Evaluate the standard uncertainty of each input quantity[5]

| | *Standard uncertainty* | | *Unit* | *Remark* |
|---|---|---|---|---|
| | Imazalil | Thiabendazole | | |
| $u_{ref}$ | 0,0375 | 0,3216 | mg/kg | systematic uncertainty component evaluated based on the results of ILC |
| $u_{dev}$ | 0,0375 | 0,2367 | mg/kg | random component of uncertainty, calculated using relative uncertainty (repeatability) of recovery and method |
| $u_{rel\_rec}$ | 27 | 2 | % | relative standard deviation of recoveries calculated using addition experiments |
| $u_{rel\_meth}$ | 10 | 6 | % | relative standard deviation of measuring method (repeated analysis of the same solution) |

### 6. Calculate the value of the measurand, using the model equation

$$C = \frac{C_c * V_{10} * \rho * V_e}{V_{50} * m}$$

$C$ concentration of pesticide in sample [mg/kg]

$C_c$ concentration of pesticide in analysed extract [mg/kg]

$V_{10}$ the volume of final extract in methanol [mL]

$\rho$ density of methanol [g/mL]

$V_e$ the full volume of acetone extract [mL]

$V_{50}$ the volume of acetone extract to be purified [mL]

$m$ mass of homogenised sample to be extracted [g]

[5] The Nordtest method does not require separate uncertainty evaluation for each input quantity

$$C(imazalil) = \frac{2{,}801 * 10 * 0{,}791 * 150}{50 * 49{,}8003} = 1{,}335\, mg / kg$$

$$C(thiabendazole) = \frac{7{,}398 * 10 * 0{,}791 * 150}{50 * 49{,}8003} = 3{,}523\, mg / kg$$

### 7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units

*Using*: ☐ Mathematical solution; ☒ Spreadsheet approach; ☐ Commercial software

| *Uncertainty components* | *Value* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|---|
| $u_{sys}$ (imazalil) | - | 0,0530 | mg/kg | systematic uncertainty component evaluated based on the results of ILC |
| $u_{rnd}$ (imazalil) | - | 0,3844 | mg/kg | random component of uncertainty, calculated using relative uncertainty (repeatability) of recovery and method |
| $u_{sys}$ (thiabendazole) | - | 0,3993 | mg/kg | systematic uncertainty component evaluated based on the results of ILC |
| $u_{rnd}$(thiabendazole) | - | 0,2228 | mg/kg | random component of uncertainty, calculated using relative uncertainty (repeatability) of recovery and method |

Uncertainty is found according to the the Nordtest approach using the following set of equations:

$$u_c = \sqrt{u_{sys}^2 + u_{rnd}^2}$$

$$u_{rnd} = \frac{\sqrt{u_{rel\_rec}^2 + u_{rel\_meth}^2}}{100\%} * c$$

$$d = c - c_{ref}$$

$$u_{ref} = \frac{s}{\sqrt{n_1}}$$

$$u_{dev} = \sqrt{\frac{d^2}{n}}$$

$$u_{sys} = \sqrt{u_{ref}^2 + u_{dev}^2}$$

$u_c$ standard uncertainty of concentration of pesticide [mg/kg]

$u_{sys}$ systematic component of uncertainty [mg/kg]

$u_{rnd}$ random component of uncertainty [mg/kg]

$u_{rel\_rec}$ relative uncertainty of recovery [%]

$u_{rel\_meth}$ relative uncertainty of analysis method [%]

$c$ pesticide concentration in sample [mg/kg]

$d$ difference in concentration between our laboratory and reference value [mg/kg]

$c_{ref}$ reference concentration of pesticide in sample [mg/kg]

$s$ the standard deviation for reference value [mg/kg]

$n_1$ the number of laboratories who took part in ILC

$n$ number of completed ILCs

## 8. Calculate expanded uncertainty ($U_c$) & specify the coverage factor k and the units

***Imazalil***

$$u_c = \sqrt{0{,}05303^2 + 0{,}3844^2} = 0{,}388\, mg/kg$$

$$u_{rnd} = \frac{\sqrt{27\%^2 + 10\%^2}}{100\%} * 1{,}3350 = 0{,}3844\, mg/kg$$

$$d = 1{,}3350 - 1{,}2975 = 0{,}0375\, mg/kg$$

$$u_{ref} = \frac{0{,}0530}{\sqrt{2}} = 0{,}0375\, mg/kg$$

$$u_{dev} = \sqrt{\frac{0{,}0375^2}{1}} = 0{,}0375\, mg/kg$$

$$u_{sys} = \sqrt{0{,}0375^2 + 0{,}0375^2} = 0{,}05303\, mg/kg$$

***Thiabendazole***

$$u_c = \sqrt{0{,}3993^2 + 0{,}2228^2} = 0{,}457\, mg/kg$$

$$u_{rnd} = \frac{\sqrt{2\%^2 + 6\%^2}}{100\%} * 3{,}2860 = 0{,}2228\, mg/kg$$

$$d = 3{,}5230 - 3{,}2863 = 0{,}2367\, mg/kg$$

$$u_{ref} = \frac{0{,}5571}{\sqrt{2}} = 0{,}3216\, mg/kg$$

$$u_{dev} = \sqrt{\frac{0{,}2367^2}{1}} = 0{,}2367\, mg/kg$$

$$u_{sys} = \sqrt{0{,}3216^2 + 0{,}2367^2} = 0{,}3993\, mg/kg$$

$U$(imazalil) = 2 · $u_c$(imazalil) = 2 · 0,388 mg/kg = 0,676 mg/kg (norm, $k$ = 2)

$U$(thiabendazole) = 2 · $u_c$(thiabendazole) = 2 · 0,457 mg/kg = 0,914 mg/kg (norm, $k$ = 2)

## 9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$

| | |
|---|---|
| *1* | $u_{rnd}$ contribution: 98,13% (imazalil), 23,74% (thiabendazole) |
| *2* | $u_{sys}$ contribution: 1,87% (imazalil), 76,27% (thiabendazole) |

## 10. Prepare your uncertainty budget report

The Nordtest approach does not permit to obtain an uncertainty budget similar to that obtained with the ISO GUM approach. This is because it is not a model-based approach to uncertainty estimation: although the model equation is used for calculating the value of the result, it is not used to calculate the uncertainty.

It is however possible to separate the overall uncertainty into random and systematic contributions. From the above table it can be deduced that the uncertainty of determination of imazalil is mostly attributable to random sources and the uncertainty of determination of thiabendazole is mostly attributable to systematic sources. This conclusion should however be treated with care. The reason is that the systematic uncertainty component of thiabendazole is significantly larger than that of imazalil. Both systematic components were determined from the results of a single interlaboratory comparison and the result of thiabendazole deviated more from the reference value of the ILC than the result of imazalil and also the reliability of the thiabendazole reference value was significantly lower (see calculations in section 7). Since all the information is based on a single ILC, far-reaching conclusions are not possible at this stage and the uncertainty estimate should be regarded as the "first estimate". As the work of the laboratory evolves and more data become available, better uncertainty estimates can be found using the same calculation scheme.

## Chapter 6

## Determination of Ammonium in Water by Flow Analysis (CFA) and Spectrometric Detection

*Bertil Magnusson*

- **The summary form ('blue page')**
- **A short introduction to the analytical procedure ('slides')**
- **All input needed to do the three exercises ('yellow pages')**
- **The solved exercises ('green pages')**

# The TrainMiC example summary form

## I. General information about the example

| | |
|---|---|
| **Measurand** | Concentration of ammonium in drinking water in mg/L |
| **Example number** | Ex-07 |
| **Author(s) of the example** | Bertil Magnusson |
| **Analytical procedure** | Determination of concentration of ammonium in drinking water by flow analysis (CFA) and spectrometric detection (ISO 11732: 2005) |
| **Customer requirement** | Directive 98/83/EC on the quality of water intended for human consumption |

## II. Attached files

| File number, type and name | | Content of the file | | File is attached Yes | File is attached No | Remark |
|---|---|---|---|---|---|---|
| 1 - I | Ex-07-1-I-NH4-water-Photometry-2006-Ver1.ppt | About the analytical procedure: short introduction | | ✓ | | Given by the leacturer |
| 2 - Yellow | Ex-07-2-Y-NH4-water-Photometry-2006-Ver1.doc | PART I | Description of the analytical procedure | ✓ | | Each participant receives own copy and may keep it |
| | | PART II | The customer's requirements concerning the quality of the measurement result | ✓ | | |
| | | PART III | Validation of the measurement procedure – relevant equations and measurement data | ✓ | | |
| | | PART IV | Measurement uncertainty of the result – relevant equations and measurement data | ✓ | | |
| 3 - Green | Ex-07-3-G-NH4-water-Photometry-2006-Ver1.doc | PART I | Establishing traceability in analytical chemistry | ✓ | | |
| | | PART II | Single laboratory validation of measurement procedures | ✓ | | |
| | | PART III | Bulding an uncertainty budget | ✓ | | |
| | | | Addendum 1: By spreadsheet approach | - | | |
| | | | Addendum 2: By dedicated software | ✓ | | |

## III. History of the example

| Version | Uploaded on the webhotel | Short description of the change |
|---|---|---|
| 0 | April 2007 | - |
| 1 | | |

# A short introduction to the analytical procedure

## Photometric determination of ammonium in drinking water

Scope of the presentation

- The analytical procedure and the customer's requirements
- About 'the chemistry' and the measurement method
- Model equation

Introduction to TrainMiC
example session

## The analytical procedure and the customer's requirements

- **Water quality -- Determination of ammonium nitrogen -- Method by flow analysis (CFA and FIA) and spectrometric detection**
  ISO 11732 (2005)
- ***The quality of the results should comply with the requirement in the Directive 98/83/EC on the quality of water intended for human consumption***

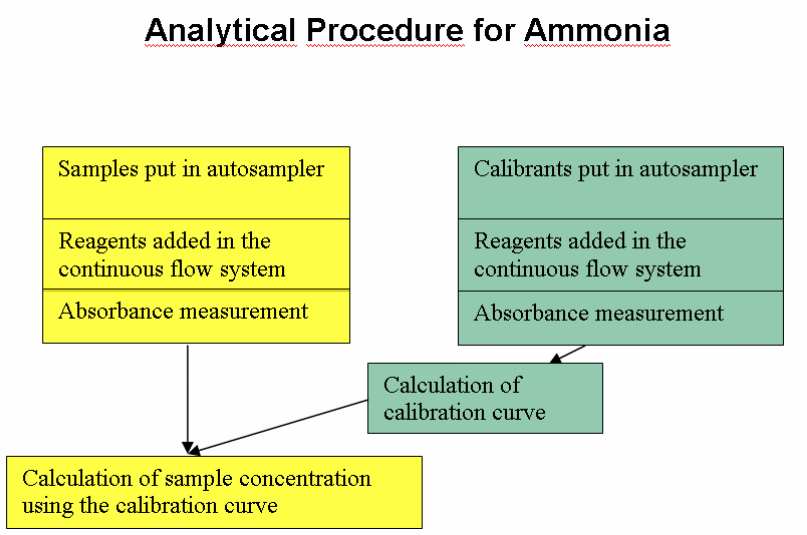
Introduction to TrainMiC
example session

## Measurement procedure

- Ammonium present in the sample reacts in alkaline solution with hypochlorite to form chloramines.
- The chloramines formed reacts under catalysis of nitroprusside with salicylate at a temperature of 37°C to 50°C to form a blue-green indophenol dye.
- The nitroprusside is quantitatively measured in a flow photometer at 640 nm to 660 nm by comparing with a calibration curve.

Introduction to TrainMiC
example session

## Flow chart of the analytical procedure




Introduction to TrainMiC
example session

## Model equation

$$C = [(A\_sample - b\_0) / b\_1] *( f\_dil / R)$$

| Quantity | Unit | Definition |
|---|---|---|
| C | mg/l N | Concentration of $NH_4^+$ in the sample solution |
| $A_{sample}$ | AU | Absorbance of the sample solution |
| $b_0$ | AU | Intercept of calibration line |
| $b_1$ | AU*l/mg N | Slope of calibration line |
| $f_{dil}$ | unitless | Dilution factor |
| R | unitless | Recovery factor of the analysis |

Introduction to TrainMiC
example session

## Model equation and equation for measurement uncertainty calculation

$$C = [(A\_sample - b\_0) / b\_1] *( f\_dil / R)$$

$$U = k \times u(NH_4)$$

Introduction to TrainMiC
example session

# All input needed to do the three exercises 'yellow pages'

## Analytical procedure

***Determination of concentration of ammonium in drinking water by flow analysis (CFA and FIA) and spectrometric detection.***

***The quality of the results should comply with the requirements in the Directive 98/83/EC on the quality intended for human consumption***

**PART I**

**Description of the analytical procedure**

**PART II**

**The customer's requirement concerning quality of the measurement result**

**PART III**

**Validation of the measurement procedure – relevant equations and measurement data**

**PART IV**

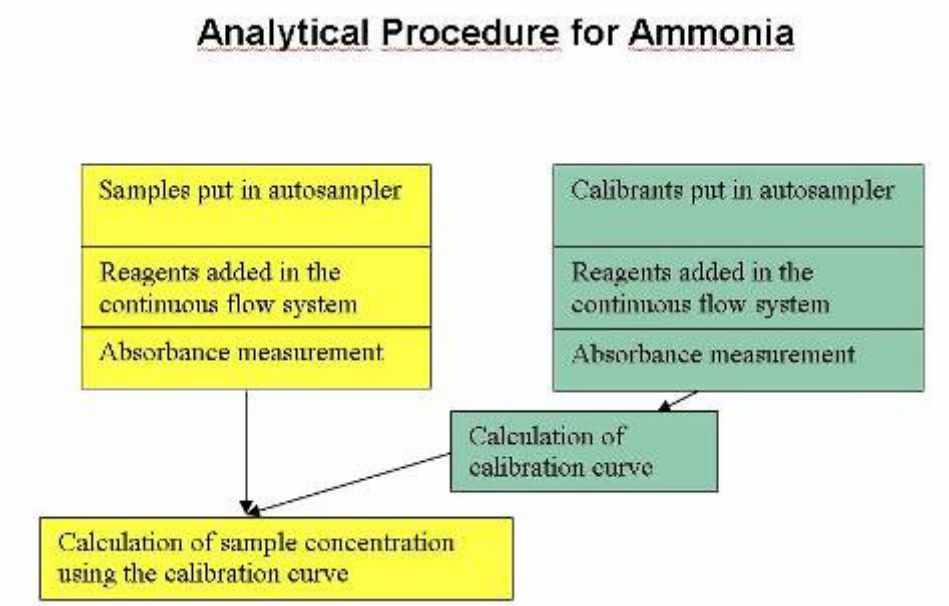
**Measurement uncertainty of the result – relevant equations and measurement data**

# PART I. Description of the analytical procedure

## 1. Scope

This International Standard specifies methods suitable for the determination of ammonium nitrogen in various types of waters (ground, drinking, surface and waste waters) in mass concentration ranging from 0,1 mg/L to 1 mg/L (in the undiluted sample), applying either FIA (Clause 3) or CFA (Clause 4). Here the CFA is presented.

## 2. Principle CFA – Continuous Flow Analysis




In a continuously flowing, gas-segmented carrier stream, ammonium present in the sample reacts in alkaline solution with hypochlorite, which has previously been liberated from dichloroisocyuanurate.

The chloramines formed reacts under catalysis of nitroprusside with salicylate at a temperature of 37°C to 50°C to form a blue-green indophenol dye which is quantitatively measured in a flow photometer at 640 nm to 660 nm.

## 3. Interferences – CFA method

Low-molecular amines react similarly to ammonia and their presence will consequently lead to erroneously high results. Interferences may also occur with strong acidic or ad buffered samples as well as samples with particles or high concentration of metals forming hydroxide precipitates.

## 4. Reagents – here only calibrant is described

*Ammonium stock solution, $C_N$ = 1000 mg/L*

Dissolve in a 1000 mL volumetric flask 3,819 g of ammonium chloride (pa min 99%, dried at 105 °C ± 2°C to constant mass) in approximately 900 mL of water, acidify to pH 2 by drop wise addition of dilute sulphuric acid and make up to a volume with water.

*Standard solutions, 10 mg/L*

Pipette 1 mL of the ammonium stock solution into a 100 mL graduated flask, add approximately 80 mL of water, acidify by drop wise addition of dilute sulphuric acid and make up to a volume with water.

*Calibration solutions*

Prepare calibration solutions by diluting the ammonium standard solutions. At least five calibration standards per working range are recommended. As an example, if six standard are applied proceed for the working ranges 0,1 to 1,0 mg/L N, as follows.

*Working solutions 0,1 to 1,0 mg/L*

Pipette into a series of 100 mL graduated flasks 1, 2, 4, 6 and 10 mL respectively of the ammonium standard solution 10 mg/L and make up to volume with water.

Prepare all calibration solutions freshly before use.

Other chemicals needed – see ISO standard 11732:2005

## 5. Sampling and pre-treatment

Sampling shall be carried out in accordance with ISO 5667-1, ISO 5667-2, ISO 5667-3. Containers of glass, PE, PP and PTFE are suitable for sample collection. Clean all containers with HCl (1 M, 0,1 and 0,01 M) and rinse with water. Analyse the samples immediately after collection. For preservation up to 24 h, add dilute sulphuric acid to pH about 2 and store at 2 to 5°C in the dark.

## 6. Procedure

*Instrument set-up*

Prior to measurement, continuously run the reagent solutions for approximately 10 min through the system, and record the baseline. The system is operational when the baseline does not show any drift. A satisfactory signal-to-noise relation should be obtained.

Perform the calibration with the blank solutions and 4 to 5 equidistant calibration solutions for an appropriate concentration range. It should be stressed that the linearity of the calibration curve is often limited. Correct the absorbance values of the calibration solutions by subtracting the absorbance value of the blank calibration solution. For plotting of a calibration curve or for calculation of the calibration function, use the resulting values together with the analyte concentrations of the calibration solutions.

Analyse the test samples in the same way as the calibration solutions with the continuous flow system.

## 7. Method of calculation

Read the values of the analyte concentrations of the test sample solutions, the reagent blank solution and the blank test solution from the calibration graph or calculate them from the calibration function. Correct the analyte concentrations of the test sample solutions by subtracting the analyte concentrations of the reagent blank solutions or the blank test solution. Correct for dilution steps, if appropriate. Report results ammonia expressed as nitrogen in mg/L.

# PART II. The customer's requirement concerning quality of the measurement result

*Extract from the Directive 98/83/EC (Draft annex 2005/04/20), on the quality of water intended for human consumption*

The parametric value (max limit) for ammonium in drinking water is 0,5 mg/L.

The requirements for the analyses are the following:

| Parameter | Trueness of parametric value (Note 1) | Precision of parametric value (Note 2) | Limit of detection of parametric value (Note 3) |
|---|---|---|---|
| *Ammonium* | *10%* | *10%* | *10%* |

*Note 1* (*): Trueness is the systematic error and is the difference between the mean value of the large number of repeated measurements and the true value

*Note 2* (*): Precision is the random error and is usually expressed as the standard deviation (within and between batch) of the spread of results about the mean. Acceptable precision is twice the relative standard deviation.
(*) These terms are further defined in ISO 5725.

*Note 3:* Limit of detection is either:
— three times the relative within batch standard deviation of a natural sample containing a low concentration of the parameter, or
— five times the relative within batch standard deviation of a blank sample.

# PART III. Validation of the measurement procedure – relevant equations and measurement data

## Limit of Detection

Equation

Calculate detection limit as 5 standard deviations – see Directive 98/83/EC.

Measurement data

A synthetic control sample at a level of 0,020 mg/L has been run for over a period of 7 months. The results in mg/L are given in the table below.

| | | |
|---|---|---|
| 0,021 | 0,032 | 0,023 |
| 0,023 | 0,023 | 0,024 |
| 0,023 | 0,025 | 0,022 |
| 0,024 | 0,026 | 0,024 |
| 0,026 | 0,022 | 0,025 |
| 0,015 | 0,021 | 0,022 |
| 0,015 | 0,019 | 0,021 |
| 0,017 | 0,025 | 0,020 |
| 0,016 | 0,026 | 0,021 |
| 0,014 | | |

## Internal quality control

The standard deviations obtained on these control samples are estimates on the within-laboratory reproducibility.

Measurement data

Results from two control samples are given in the table below

| | Unit | QC1 | QC2 |
|---|---|---|---|
| Mean value | mg/L | 0,114 | 0,605 |
| s | mg/L | 0,005 | 0,021 |
| n | - | 27 | 28 |
| Time period | months | 7 | 7 |
| Nominal value | mg/L | 0,100 | 0,600 |

**External quality control – participating in PT studies**

| Year/Exercise | Nominal value $x_{ref}$ mg/L | Laboratory result $x_i$ mg/L | Bias % | $s_R$ % | Number of labs |
|---|---|---|---|---|---|
| 1999/1 | 81 | 83 | 2,4 | 10 | 31 |
| 1999/2 | 73 | 75 | 2,7 | 7 | 36 |
| 2000/1 | 264 | 269 | 1,9 | 8 | 32 |
| 2000/2 | 210 | 213 | 1,4 | 10 | 35 |
| 2001/1 | 110 | 112 | 1,8 | 7 | 36 |
| 2001/2 | 140 | 144 | 2,9 | 11 | 34 |

# PART IV. Measurement uncertainty of the result – relevant equations and measurement data

The relevant equations

$$C = (A_{sample} - b_0) / b_1 * f_{dil} / R$$

Measurement data

| Input quantity | | Unit | Value | Standard uncertainty (u) | Relative standard uncertainty (%) | Type of uncertainty | Type of distribution | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | normal | rectangular | triangular |
| C | Concentration of $NH^{4+}$ in the sample solution | mg/L | 0,2465 | 0,0031 | 1,3 | | x | | |
| $A_{sample}$ | Absorbance of the sample solution | AU | 0,2560 | 0,0015 | 0,58 | | x | | |
| $b_0$ | Intercept of calibration line | AU | 0,0143 | 0,0021 | 14 | | x | | |
| $b_1$ | Slope of calibration line – unit AU divided by mg N/l | AU*L/ mg | 0,9902 | 0,0070 | 0,71 | | x | | |
| $f_{dil}$ | Dilution factor | unitless | 1 | 0,00 | 0,00 | | | | |
| R | Recovery factor of the analysis | unitless | 0,9900 | 0,0058 | 0,59 | | | x | |

# The solved exercises 'green pages'

## TRAINMIC EXERCISES

### Analytical procedure

***Determination of concentration of ammonium in drinking water by flow analysis (CFA) and spectrometric detection***

***The quality of the results should comply with the requirements in the Directive 98/83/EC on the quality of water intended for human consumption***

*Exercise 1:*

**Establishing traceability in analytical chemistry**

*Exercise 2:*

**Single laboratory validation of measurement procedures**

**Part I: General issues**

**Part II: Parameters to be validated**

**Part III: Some calculations and conclusions**

*Exercise 3:*

**Building an uncertainty budget**

**Addendum I: By spreadsheet solution**

**Addendum II: By dedicated software**

# ESTABLISHING TRACEABILITY IN ANALYTICAL CHEMISTRY

## 1. Specifying the analyte and measurand

| | |
|---|---|
| *Analyte* | Ammonium |
| *Measurand* | Dissolved ammonium in water sample arriving in the laboratory |
| *Units* | mg/L |

## 2. Choosing a suitable measurement procedure with associated model equation

| | | | |
|---|---|---|---|
| *Measurement procedure* | ISO 11732:2005 using the continuous flow analysis and photometric detection | | |
| *Type of calibration* | *standard curve* ☒ | *standard addition* ☐ | *internal standard* ☐ |

Model equation

C = (A_sample - b_0) / b_1 * f_dil / R;

| | |
|---|---|
| C | Concentration of $NH_4^+$ in the sample solution [mg/L] |
| $A_{sample}$ | Absorbance of the sample solution [AU] |
| $b_0$ | Intercept of calibration line [AU] |
| $b_1$ | Slope of calibration line [AU divided by mg/L] |
| $f_{dil}$ | Dilution factor |
| R | Recovery factor of the analysis |

The calibration line evaluated by linear regression is based on five standards in the range 0,04 to 1 mg/L.

The dilute standard solution of 10 mg/L is prepared from a stock solution of 1000 mg/L. This stock solution is prepared from ammonium chloride.

## 3. List the input quantities according to their influence on the uncertainty of the result of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.

| | |
|---|---|
| *1* | Recovery factor – contributing 30 % to the expanded uncertainty |
| *2* | Absorbance of the sample - here the main source is the drift contributing about 20% |
| *3* | Calibration – standard solution – purity of ammonium chloride |
| *4* | Calibration – volumetric flasks and pipettes |

## 4. List the reference standards needed and state the information regarding traceability of the reference value

**For the analyte**

| | | |
|---|---|---|
| *1* | *Name/ChemicalFormula/Producer:* | Ammonium chloride, $NH_4Cl$, Merck pa min 99 % |
| *2* | *Name/ChemicalFormula/Producer:* | |

**For the other input quantities**

| | | |
|---|---|---|
| *1* | *Quantity/Equipment/Calibration:* *e.g. mass/balance/calibrated by NMI, U=xx (k=2),* | Absorbance – relative measurement. Not direct part of the traceability chain |
| *2* | *Quantity/Equipment/Calibration:* | Volumetric flasks – Class A quality |
| *3* | *Quantity/Equipment/Calibration:* | Volumetric pipettes – calibrated by producer and regularly checked by the laboratory |
| *4* | *Quantity/Equipment/Calibration:* | |

## 5. Estimating uncertainty associated with the measurement

| | | |
|---|---|---|
| *Are all important parameters included in the model equation?* | *Yes* ☐ | *No* ☒ |
| *Other important parameters are:* | Within-lab reproducibility, contamination | |

## 6. How would you prove traceability of your result?

| | |
|---|---|
| *1* | Participating in PT rounds |
| *2* | |
| *3* | |

## 7. Any other comments, questions…

# SINGLE LABORATORY VALIDATION OF MEASUREMENT PROCEDURES

## PART I: GENERAL ISSUES

### 1. Specify the measurement procedure, analyte, measurand and units

| | |
|---|---|
| *The measurement procedure* | Measurement procedure is based on EN/ISO11732 |
| *Analyte* | Ammonium |
| *The measurand* | Dissolved ammonium in water sample arriving in the laboratory |
| *Unit* | mg/L |

### 2. Specify the Scope

| | |
|---|---|
| *Matrix* | Drinking water |
| *Measuring range* | up to 1 mg/L for undiluted samples |

### 3. Requirement on the measurement procedure

| | | | |
|---|---|---|---|
| *Intended use of the results* | To analyse drinking water according to the EU requirements in the EU directive | | |
| *Mark the customer's requirements and give their values* | *Parameters to be validated* | | *Value requested by the customer* |
| | ☒ | *LOD* | LOD 0,05 mg/L: - 3*s* on a natural sample, 5*s* on a blank: s is repeatability |
| | ☐ | *LOQ* | |
| | ☐ | *Repeatability* | |
| | ☒ | *Within-lab reproducibility* | at 0,5 mg/L, s = 0,025 mg/L:<br>at 0,2 mg/L *s* the demand estimated to be s = 0,010 mg/L or 5% |
| | ☒ | *Trueness* | at 0,5 mg/L less than 0,05 mg/L or less than 10% relative |
| | ☐ | *Measurement uncertainty* | |
| | ☐ | *Other-state* | |

### 4. Origin of the measurement procedure

| | | |
|---|---|---|
| | | VALIDATION |
| *New in-house method* | ☐ | *Full* |
| *Modified validated method* | ☐ | *Partial* |
| *Official standard method* | ☒ | *Confirmation/Verification* |

## PART II: PARAMETERS TO BE VALIDATED

### 5. Selectivity/Interference/Recovery

Where yes, please give further information e.g. which CRM, reference method

| | |
|---|---|
| ☐ | *CRM/RM: analysis of available CRM or RM* |
| | *Further information:* |
| ☒ | *Spike of pure substance* |
| | |
| ☐ | *Compare with a reference method* |
| | |
| ☐ | *Selectivity, interferences* |
| | |
| ☒ | *Test with different matrices* |
| | |
| ☐ | *Other – please specify* |
| | |

### 6. Measuring range

| | |
|---|---|
| ☐ | *Linearity* |
| ☒ | *Upper limit* |
| ☒ | *LOD* |
| ☐ | *LOQ* |

### 7. Spread – precision

| | |
|---|---|
| ☒ | *Repeatability* |
| ☒ | *Reproducibility (within lab)* |
| ☐ | *Reproducibility (between lab)* |

## 8. Robustness

| | |
|---|---|
| ☐ | *Variation of parameters* |

## 9. Quality control

| | |
|---|---|
| ☒ | *Control charts* |
| ☒ | *Participation in PT schemes* |

## 10. Other parameters to be tested

| | |
|---|---|
| ☐ | *Working range and testing of homogeneity of variances* |
| ☐ | *R square* |
| ☐ | *Residual standard deviation* |
| ☐ | *Standard deviation of the analytical procedure* |
| ☐ | *Coefficient of variation of the analytical procedure* |
| ☒ | *Measurement uncertainty* |

## PART III: SOME CALCULATIONS AND CONCLUSIONS

### 11. Calculation of parameters requested by the customer

| Parameters requested to be validated | Calculations |
|---|---|
| ☒ *LOD* | s= 0,004 mg/L<br><br>LOD= 5s = 0,02 mg/L |
| ☐ *LOQ* | |
| ☐ *Repeatability* | |
| ☒ *Within-lab reproducibilty* | At a level of 0,1 mg/L $s_{Rw}$ is 4,4 % and at a level of 0,6 mg/L $s_{Rw}$ is 3,5%. |
| ☒ *Trueness* | From PT results the trueneness is estimated to be less than 3%. The trueness is probably around 2% - then mean value of the PT results for levels over 0,08 mg/l. |
| ☒ *Measurement uncertainty* | The measurement uncertainty at a level of 0,2 mg/L is estimated to be 2,5%. According to EA guideline this value should be rounded off to 3%. |
| ☐ *Other - please state* | |

**12. Does the analytical procedure fulfil the requirement(s) for the intended use?**

| Parameter | Value requested by the customer (the same as stated in question 3) | Value obtained during validation | The requirement is fulfilled Yes/No |
|---|---|---|---|
| ☒ LOD | 0,05 mg/L | 0,02 mg/L | Yes |
| ☐ LOQ | | | |
| ☐ Repeatability | | | |
| ☒ Within-lab reproducibility | 5% at a level of 0,2 mg/L | 4% | Yes |
| ☒ Trueness | 10% | 2-3% | Yes |
| ☐ Measurement | | | |
| ☐ Other | | | |

**The analytical procedure is fit for the intended use:**

☒ *Yes* ☐ *No*

*For measurement uncertainty and traceability refer to the corresponding sheets*

# BUILDING AN UNCERTAINTY BUDGET

## 1. Specify the measurand and units

| | |
|---|---|
| *Measurand* | Dissolved ammonium in water sample arriving in the laboratory |
| *Unit* | mg/L |

## 2. Describe the measurement procedure and provide the associated model equation

Measurement procedure:

Ammonium present in the sample reacts in alkaline solution with hypochlorite. The chloramines formed reacts under catalysis of nitroprusside with salicylate at a temperature of 37°C to 50°C to form a blue-green indophenol dye which is quantitatively measured in a flow photometer at 640 nm to 660 nm.

Model equation

*C = (A_sample - b_0) / b_1 * f_dil / R*

| | |
|---|---|
| C | concentration of $NH_4^+$ in the sample solution [mg/L] |
| $A_{sample}$ | absorbance of the sample solution [AU] |
| $b_0$ | intercept of calibration line [AU] |
| $b_1$ | slope of calibration line [AU divided by mg/L] |
| $f_{dil}$ | dilution factor |
| R | recovery factor of the analysis |

## 3. Identify (all possible) sources of uncertainty

| | |
|---|---|
| ☒ | *Uncertainty of concentration of reference solutions* |
| ☐ | *Uncertainty of measurements of peak area* |
| ☒ | *Method bias* |
| ☐ | *Matrix effect* |
| ☒ | *Other: measurement of sample* |
| ☒ | *Other: Preparation, measurement of calibration solutions and constructing the calibration graph* |
| ☐ | *Other:* |

### 4. Evaluate values of each input quantity

| *Input quantity* | *Value* | *Unit* | *Remark* |
|---|---|---|---|
| $A_{sample}$ | 0,256 | AU | |
| $b_0$ | 0,01734 | AU | |
| $b_1$ | 986,3 | AU*L/mg | |
| $f_{dil}$ | 1 | unitless | |
| R | 0,99 | unitless | |

### 5. Evaluate the standard uncertainty of each input quantity

| *Input quantity* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|
| $A_{sample}$ | 1,49 10E-3 | AU | Takes into account repeatability, drift and rounding |
| $b_0$ | 0,00207 | AU | |
| $b_1$ | 0,0070 | AU*l/mg | Takes into account reference solution (0,3% relative uncertainty, preparation and measurement of calibration standards and constructing the calibration graph |
| $f_{dil}$ | 0 | unitless | Dilution of sample – in this case the sample was not diluted |
| R | 0,0058 | unitless | A rough estimate of recovery of 99 ± 1% |

### 6. Calculate the value of the measurand, using the model equation.

*C = (A_sample - b_0) / b_1 * f_dil / R;*

$$C = \frac{0,256 - 0,01734}{986,3} \times 1 \times 0,99 = 0,247 mg / L$$

### 7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units

*Using*: ☒ Mathematical solution; ☒ Spreadsheet approach; ☒ Commercial software

| *Input quantity* | *Value* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|---|
| $A_{sample}$ | 0,256 | 1,49 10E-3 | AU | |
| $b_0$ | 0,01435 | 0,00207 | AU | From calibration graph – note regression without weights and a slight curvature. A too high estimate but here we are interested in higher concentrations. |
| $b_1$ | 0,9902 | 0,0070 | AU*L/mg | |
| $f_{dil}$ | 1 | 0 | Unitless | Sample was not diluted |
| R | 0,99 | 0,01 | Unitless | |

*C = (A_sample - b_0) / b_1 * f_dil / R*

The combined standard uncertainty is 0,0031 mg/L.

## 8. Calculate expanded uncertainty ($U_c$) & specify the coverage factor k and the units

$U = k \times u = 2 \times 0{,}0031 = 0{,}006$ mg/L

The expanded uncertainty using a coverage factor of 2 is $0{,}006_2$ mg/L N or 2,5% relative.

## 9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$

| | |
|---|---|
| *1* | Recovery factor – contributing 20% to the expanded uncertainty |
| *2* | Absorbance of the sample - here the main source is the drift contributing about 20% to the expanded uncertainty |
| *3* | Preparation of standard solution 10 mg/L ± 0,13 mg/L (k=2) - main components dilution using a 1 mL pipette and purity – contribution about 25% |

## 10. Prepare your uncertainty budget report

# Addendum: Measurement uncertainty calculation - GumWorkbench

The values of uncertainty components of volumetric ware and photometric equipment are taken according to experience, experiments carried out in the lab and data from equipment manufacturers. The calculations are here based on a manual method for a clearer view of the calculations but an automated method will give similar or lower uncertainty.

## Model equation:

*{ The main equation }*
$C = (A_{sample} - b_0) / b_1 * f_{dil} / R;$

*{ Nitrogen- Ammonium ion stock solution - 1 000 mg N/L. Prepared from ammonium chloride.}*
$C_{st\_0} = m_{NH4Cl} / V_{1000} * P_{NH4Cl} * f_{NH4Clconv} * 1000;$

*{ Ammonium standard solution - 10 mg N/L. Prepared from ammonium stock solution. The standard solution is further used for preparation of the calibration standard solutions. }*
$C_{st} = C_{st\_0} * V_1 / V_{100};$

*{ Concentrations of calibration standard solutions 0,1 to 1 mg N/L.*
*1 to 10 mL of the standard solution is transferred to 100 mL volumetric flasks.*
*The reagents are added and the solution is made up to the mark. The solution is left to stand for 60 min and then the absorbance at 655 nm is measured. }*
$C_1 = C_{st} * (V_{1\_st} / V_{1\_100});$
$C_2 = C_{st} * (V_{2\_st} / V_{2\_100});$
$C_3 = C_{st} * (V_{3\_st} / V_{3\_100});$
$C_4 = C_{st} * (V_{4\_st} / V_{4\_100});$
$C_5 = C_{st} * (V_{5\_st} / V_{5\_100});$
$f_{dil} = 1;$
*{in this case the sample was not diluted}*
*{ Photometric measurements*
*It is assumed that the uncertainty of all photometric measurements consists of three components (on the example $A_{sample}$):*
*- Repeatability uncertainty (included in $A_{sample\_rep}$);*
*- Uncertainty due to drift ($A_{sample\_drift}$)*
*- Uncertainty due to rounding of the reading ($A_{sample\_round}$) (The photometer use din this example has three decimal places)*
*The absorbance of blank is not subtracted but all the measurements are made against blank}*
*{ Absorbance of sample solution }*
$A_{sample} = A_{sample\_rep} + A_{sample\_drift} + A_{sample\_round};$
*{ The regression equations for finding the slope ($b_1$) and intercept ($b_0$) of the calibration line }*
$\Sigma AC = C_1*A_1 + C_2*A_2 + C_3*A_3 + C_4*A_4 + C_5*A_5;$
$AvgC=(C_1+C_2+C_3+C_4+C_5)/n;$
$AvgA=(A_1+A_2+A_3+A_4+A_5)/n;$
$\Sigma CC=C_1*C_1+C_2*C_2+C_3*C_3+C_4*C_4+C_5*C_5;$
$b_1=(\Sigma AC-n*AvgC*AvgA)/(\Sigma CC-n*AvgC*AvgC);$
$b_0=AvgA-b_1*AvgC$

## List of quantities:

| Quantity | Unit | Definition |
|---|---|---|
| C | mg N/L | Concentration of $NH_4^+$ in the sample solution |
| $A_{sample}$ | AU | Absorbance of the sample solution |
| $b_0$ | AU | Intercept of calibration line |
| $b_1$ | AU*L/mg | Slope of calibration line |
| $f_{dil}$ | unitless | Dilution factor |
| R | unitless | Recovery factor of the analysis |
| $C_{st\_0}$ | mg N/mL | Concentration of $NH_4^+$ in calibration stock solution |
| $m_{NH4Cl}$ | g | Weight of $NH_4Cl$ |
| $V_{1000}$ | mL | Volume of 1 L volumetric flask |
| $P_{NH4Cl}$ | unitless | Purity of $NH_4Cl$ |
| $f_{NH4Clconv}$ | unitless | Conversion factor for converting the amount of ammonium chloride ($NH_4Cl$) to the amount of nitrogen |
| $C_{st}$ | mg N/L | Concentration of $NH_4^+$ in the ammonium standard solution |
| $V_1$ | mL | Volume of 1 mL pipette |
| $V_{100}$ | mL | Volume of 100 mL volumetric flask |
| $C_1$ | mg N/L | Concentration of the 1st ammonium calibration standard solution |
| $V_{1\_st}$ | mL | Volume of ammonium standard solution taken for preparing the 1st ammonium calibration standard solution |
| $V_{1\_100}$ | mL | Volume of the 1st ammonium calibration standard solution |
| $C_2$ | mg N/L | Concentration of the 2nd ammonium calibration standard solution |
| $V_{2\_st}$ | mL | Volume of ammonium standard solution taken for preparing the 2nd ammonium calibration standard solution |
| $V_{2\_100}$ | mL | Volume of the 2nd ammonium calibration standard solution |
| $C_3$ | mg N/L | Concentration of the 3rd ammonium calibration standard solution |
| $V_{3\_st}$ | mL | Volume of ammonium standard solution taken for preparing the 3rd ammonium calibration standard solution |
| $V_{3\_100}$ | mL | Volume of the 3rd ammonium calibration standard solution |

| Quantity | Unit | Definition |
|---|---|---|
| $C_4$ | mg N/L | Concentration of the 4th ammonium calibration standard solution |
| $V_{4\_st}$ | mL | Volume of ammonium standard solution taken for preparing the 4th ammonium calibration standard solution |
| $V_{4\_100}$ | mL | Volume of the 4th ammonium calibration standard solution |
| $C_5$ | mg N/L | Concentration of the 5th ammonium calibration standard solution |
| $V_{5\_st}$ | mL | Volume of ammonium standard solution taken for preparing the 5th ammonium calibration standard solution |
| $V_{5\_100}$ | mL | Volume of the 5th ammonium calibration standard solution |
| $A_{sample\_rep}$ | | |
| $A_{sample\_drift}$ | | |
| $A_{sample\_round}$ | | |
| ΣAC | - | Interim quantity for regression statistics calculation |
| $A_1$ | AU | Absorbance of the 1. ammonium calibration standard solution |
| $A_2$ | AU | Absorbance of the 2. ammonium calibration standard solution |
| $A_3$ | AU | Absorbance of the 3. ammonium calibration standard solution |
| $A_4$ | AU | Absorbance of the 4. ammonium calibration standard solution |
| $A_5$ | AU | Absorbance of the 5. ammonium calibration standard solution |
| AvgC | mg N/L | Interim quantity for regression statistics calculation |
| n | unitless | Number of points on the calibration line |
| AvgA | AU | Interim quantity for regression statistics calculation |
| ΣCC | - | Interim quantity for regression statistics calculation |

**R:**
Type B rectangular distribution
Value: 0,99 unitless
Halfwidth of limits: 0,01 unitless

**$m_{NH4Cl}$:**
Type B rectangular distribution
Value: 3,819 g
Halfwidth of limits: 0,002 g

**$V_{1000}$:**
Type B rectangular distribution
Value: 1 mL
Halfwidth of limits: 0,001 mL

**$P_{NH4Cl}$:**
Type B rectangular distribution
Value: 0,995 unitless
Halfwidth of limits: 0,005 unitless

**$f_{NH4Clconv}$:**
Constant
Value: =14,0067/(14,0067+4*1,0079+35,4527)

**$V_1$:**
Type B rectangular distribution
Value: 1 mL
Halfwidth of limits: 0,01 mL

**$V_{100}$:**
Type B rectangular distribution
Value: 100 mL
Halfwidth of limits: 0,1 mL

**$V_{1\_st}$:**
Type B rectangular distribution
Value: 1 mL
Halfwidth of limits: 0,01 mL

**$V_{1\_100}$:**
Type B rectangular distribution
Value: 100 mL
Halfwidth of limits: 0,1 mL

**$V_{2\_st}$:**
Type B rectangular distribution
Value: 2 mL
Halfwidth of limits: 0,05 mL

**$V_{2\_100}$:**
Type B rectangular distribution
Value: 100 mL
Halfwidth of limits: 0,1 mL

**$V_{3\_st}$:**
Type B rectangular distribution
Value: 4 mL
Halfwidth of limits: 0,1 mL

**$V_{3\_100}$:**
Type B rectangular distribution
Value: 100 mL
Halfwidth of limits: 0,1 mL

**$V_{4\_st}$:**
Type B rectangular distribution
Value: 6 mL
Halfwidth of limits: 0,015 mL

**$V_{4\_100}$:**
Type B rectangular distribution
Value: 100 mL
Halfwidth of limits: 0,1 mL

**$V_{5\_st}$:**
Type B rectangular distribution
Value: 10 mL
Halfwidth of limits: 0,025 mL

**$V_{5\_100}$:**
Type B rectangular distribution
Value: 100 mL
Halfwidth of limits: 0,1 mL

**$A_{sample\_rep}$:**
Type A summarized
Mean: 0,256
Standard Uncertainty: $654 \cdot 10^{-6}$
Degrees of Freedom: 50

**$A_{sample\_drift}$:**
Type A summarized
Mean: 0
Standard Uncertainty: $1{,}3 \cdot 10^{-3}$
Degrees of Freedom: 50

**$A_{sample\_round}$:**
Type B rectangular distribution
Value: 0
Halfwidth of limits: 0,0005

**$A_1$:**
Type B rectangular distribution
Value: 0,108 AU
Halfwidth of limits: 0,001 AU

**$A_2$:**
Type B rectangular distribution
Value: 0,214 AU
Halfwidth of limits: 0,001 AU

**$A_3$:**
Type B rectangular distribution
Value: 0,412 AU
Halfwidth of limits: 0,0015 AU

**$A_4$:**
Type B rectangular distribution
Value: 0,606 AU
Halfwidth of limits: 0,002 AU

**$A_5$:**
Type B rectangular distribution
Value: 0,9979 AU
Halfwidth of limits: 0,002 AU

**n:**
Constant
Value: 5 unitless

## Uncertainty budgets:

C: Concentration of $NH_4^+$ in the sample solution

| Quantity | Value | Standard uncertainty | Distribution | Sensitivity coefficient | Uncertainty contribution | Index |
|---|---|---|---|---|---|---|
| $A_{sample}$ | 0,25600 AU | $1{,}48 \cdot 10^{-3}$ AU | | | | |
| $b_0$ | 0,01435 AU | $2{,}07 \cdot 10^{-3}$ AU | | | | |
| $b_1$ | 0,99023 AU*l/mg | $7{,}01 \cdot 10^{-3}$ AU*l/mg | | | | |
| $f_{dil}$ | 1,0 unitless | 0,0 unitless | | | | |
| R | 0,99000 unitless | $5{,}77 \cdot 10^{-3}$ unitless | rectangular | -0,25 | $-1{,}4 \cdot 10^{-3}$ mg/l | 21,3% |
| $C_{st\_0}$ | 995,01 mg/mL | 2,96 mg/mL | | | | |
| $m_{NH4Cl}$ | 3,81900 g | $1{,}15 \cdot 10^{-3}$ g | rectangular | 0,065 | $75 \cdot 10^{-6}$ mg/l | 0,0% |
| $V_{1000}$ | 1,000000 mL | $577 \cdot 10^{-6}$ mL | rectangular | -0,25 | $-140 \cdot 10^{-6}$ mg/l | 0,2% |
| $P_{NH4Cl}$ | 0,99500 unitless | $2{,}89 \cdot 10^{-3}$ unitless | rectangular | 0,25 | $720 \cdot 10^{-6}$ mg/l | 5,3% |
| $f_{NH4Clconv}$ | 0,261851526425 01 unitless | | | | | |

| Quantity | Value | Standard uncertainty | Distribution | Sensitivity coefficient | Uncertainty contribution | Index |
|---|---|---|---|---|---|---|
| $C_{st}$ | 9,9501 mg/L | 0,0649 mg/L | | | | |
| $V_1$ | 1,00000 mL | $5{,}77 \cdot 10^{-3}$ mL | rectangular | 0,25 | $1{,}4 \cdot 10^{-3}$ mg/L | 20,9% |
| $V_{100}$ | 100,0000 mL | 0,0577 mL | rectangular | $-2{,}5 \cdot 10^{-3}$ | $-140 \cdot 10^{-6}$ mg/L | 0,2% |
| $C_1$ | 0,099501 mg/L | $868 \cdot 10^{-6}$ mg/L | | | | |
| $V_{1\_st}$ | 1,00000 mL | $5{,}77 \cdot 10^{-3}$ mL | rectangular | 0,035 | $200 \cdot 10^{-6}$ mg/L | 0,4% |
| $V_{1\_100}$ | 100,0000 mL | 0,0577 mL | rectangular | $-350 \cdot 10^{-6}$ | $-20 \cdot 10^{-6}$ mg/L | 0,0% |
| $C_2$ | 0,19900 mg/L | $3{,}15 \cdot 10^{-3}$ mg/L | | | | |
| $V_{2\_st}$ | 2,0000 mL | 0,0289 mL | rectangular | 0,031 | $900 \cdot 10^{-6}$ mg/L | 8,4% |
| $V_{2\_100}$ | 100,0000 mL | 0,0577 mL | rectangular | $-620 \cdot 10^{-6}$ | $-36 \cdot 10^{-6}$ mg/L | 0,0% |
| $C_3$ | 0,39800 mg/l | $6{,}31 \cdot 10^{-3}$ mg/l | | | | |
| $V_{3\_st}$ | 4,0000 mL | 0,0577 mL | rectangular | 0,023 | $1{,}3 \cdot 10^{-3}$ mg/L | 17,9% |
| $V_{3\_100}$ | 100,0000 mL | 0,0577 mL | rectangular | $-910 \cdot 10^{-6}$ | $-53 \cdot 10^{-6}$ mg/L | 0,0% |
| $C_4$ | 0,59701 mg/l | $4{,}00 \cdot 10^{-3}$ mg/L | | | | |
| $V_{4\_st}$ | 6,00000 mL | $8{,}66 \cdot 10^{-3}$ mL | rectangular | 0,014 | $120 \cdot 10^{-6}$ mg/L | 0,2% |
| $V_{4\_100}$ | 100,0000 mL | 0,0577 mL | rectangular | $-850 \cdot 10^{-6}$ | $-49 \cdot 10^{-6}$ mg/L | 0,0% |
| $C_5$ | 0,99501 mg/L | $6{,}67 \cdot 10^{-3}$ mg/l | | | | |
| $V_{5\_st}$ | 10,0000 mL | 0,0144 mL | rectangular | $-2{,}7 \cdot 10^{-3}$ | $-39 \cdot 10^{-6}$ mg/L | 0,0% |
| $V_{5\_100}$ | 100,0000 mL | 0,0577 mL | rectangular | $270 \cdot 10^{-6}$ | $16 \cdot 10^{-6}$ mg/L | 0,0% |
| $A_{sample\_rep}$ | 0,256000 | $654 \cdot 10^{-6}$ | normal | 1,0 | $670 \cdot 10^{-6}$ mg/L | 4,6% |
| $A_{sample\_drift}$ | 0,0 | $1{,}30 \cdot 10^{-3}$ | normal | 1,0 | $1{,}3 \cdot 10^{-3}$ mg/L | 18,2% |
| $A_{sample\_round}$ | 0,0 | $289 \cdot 10^{-6}$ | rectangular | 1,0 | $290 \cdot 10^{-6}$ mg/L | 0,9% |
| ΣAC | 1,5720 - | 0,0108 - | | | | |
| $A_1$ | 0,108000 AU | $577 \cdot 10^{-6}$ AU | rectangular | -0,36 | $-210 \cdot 10^{-6}$ mg/L | 0,4% |
| $A_2$ | 0,214000 AU | $577 \cdot 10^{-6}$ AU | rectangular | -0,32 | $-180 \cdot 10^{-6}$ mg/L | 0,3% |
| $A_3$ | 0,412000 AU | $866 \cdot 10^{-6}$ AU | rectangular | -0,23 | $-200 \cdot 10^{-6}$ mg/L | 0,4% |

| Quantity | Value | Standard uncertainty | Distribution | Sensitivity coefficient | Uncertainty contribution | Index |
|---|---|---|---|---|---|---|
| $A_4$ | 0,60600 AU | $1{,}15 \cdot 10^{-3}$ AU | rectangular | -0,14 | $-170 \cdot 10^{-6}$ mg/L | 0,3% |
| $A_5$ | 0,99790 AU | $1{,}15 \cdot 10^{-3}$ AU | rectangular | 0,027 | $31 \cdot 10^{-6}$ mg/L | 0,0% |
| AvgC | 0,45771 mg/L | $3{,}27 \cdot 10^{-3}$ mg/L | | | | |
| n | 5,0 unitless | | | | | |
| AvgA | 0,467580 AU | $404 \cdot 10^{-6}$ AU | | | | |
| ΣCC | 1,5544 - | 0,0211 - | | | | |
| C | 0,24650 mg/L | $3{,}11 \cdot 10^{-3}$ mg/L | | | | |

**Results:**

| Quantity | Value | Expanded uncertainty | Coverage factor | Coverage |
|---|---|---|---|---|
| C | 0,2465 mg/L | 2,5% (relative) | 2,00 | manual |

# Appendix 1

## TRAINMIC Exercises ('white pages')

# TRAINMIC EXERCISES

## Analytical procedure:

*Exercise 1:*

**Establishing traceability in analytical chemistry**

*Exercise 2:*

**Single laboratory validation of measurement procedures**

**Part I: General issues**

**Part II: Parameters to be validated**

**Part III: Some calculations and conclusions**

*Exercise 3:*

**Building an uncertainty budget**

**Addendum I: By spreadsheet approach**

**Addendum II: By dedicated software**

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# ESTABLISHING TRACEABILITY IN ANALYTICAL CHEMISTRY

## 1. Specifying the analyte and measurand

| | |
|---|---|
| *Analyte* | |
| *Measurand* | |
| *Units* | |

## 2. Choosing a suitable measurement procedure with associated model equation

| | | | |
|---|---|---|---|
| *Measurement procedure* | | | |
| *Type of calibration* | *standard curve* ☐ | *standard addition* ☐ | *internal standard* ☐ |

**Model equation**

**3. List the input quantities according to their influence on the uncertainty of the result of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.**

| | |
|---|---|
| *1* | |
| *2* | |
| *3* | |
| *4* | |
| *5* | |

**4. List the reference standards needed and state the information regarding traceability of the reference value**

**For the analyte**

| | | |
|---|---|---|
| *1* | *Name/ChemicalFormula/Producer:* | |
| *2* | *Name/ChemicalFormula/Producer:* | |

**For the other input quantities**

| | | |
|---|---|---|
| *1* | *Quantity/Equipment/Calibration:* *e.g. mass/balance/calibrated by NMI, U=xx (k=2),* | |
| *2* | *Quantity/Equipment/Calibration:* | |
| *3* | *Quantity/Equipment/Calibration:* | |
| *4* | *Quantity/Equipment/Calibration:* | |

**5. Estimating uncertainty associated with the measurement**

| | | |
|---|---|---|
| *Are all important parameters included in the model equation?* | *Yes* ☐ | *No* ☐ |
| *Other important parameters are:* | | |

**6. How would you prove traceability of your result?**

| | |
|---|---|
| *1* | |
| *2* | |
| *3* | |

**7. Any other comments, questions…**

# SINGLE LABORATORY VALIDATION OF MEASUREMENT PROCEDURES

## PART I: GENERAL ISSUES

### 1. Specify the measurement procedure, analyte, measurand and units

| | |
|---|---|
| *The measurement procedure* | |
| *Analyte* | |
| *The measurand* | |
| *Unit* | |

### 2. Specify the Scope

| | |
|---|---|
| *Matrix* | |
| *Measuring range* | |

### 3. Requirement on the measurement procedure

| | | | |
|---|---|---|---|
| *Intended use of the results* | | | |
| *Mark the customer's requirements and give their values* | *Parameters to be validated* | | *Value requested by the customer* |
| | ☐ | *LOD* | |
| | ☐ | *LOQ* | |
| | ☐ | *Repeatability* | |
| | ☐ | *Within-lab reproducibility* | |
| | ☐ | *Trueness* | |
| | ☐ | *Measurement uncertainty* | |
| | ☐ | *Other - state* | |

### 4. Origin of the measurement procedure

| | | VALIDATION |
|---|---|---|
| *New in-house method* | ☐ | *Full* |
| *Modified validated method* | ☐ | *Partial* |
| *Official standard method* | ☐ | *Confirmation/Verification* |

## PART II: PARAMETERS TO BE VALIDATED

### 5. Selectivity/Interference/Recovery

Where yes, please give further information e.g. which CRM, reference method

| | |
|---|---|
| ☐ | *CRM/RM: analysis of available CRM or RM* |
| | *Further information:* |
| ☐ | *Spike of pure substance* |
| | |
| ☐ | *Compare with a reference method* |
| | |
| ☐ | *Selectivity, interferences* |
| | |
| ☐ | *Test with different matrices* |
| | |
| ☐ | *Other – please specify* |
| | |

### 6. Measuring range

| | |
|---|---|
| ☐ | *Linearity* |
| ☐ | *Upper limit* |
| ☐ | *LOD* |
| ☐ | *LOQ* |

### 7. Spread – precision

| | |
|---|---|
| ☐ | *Repeatability* |
| ☐ | *Reproducibility (within lab)* |
| ☐ | *Reproducibility (between lab)* |

## 8. Robustness

| | |
|---|---|
| ☐ | *Variation of parameters* |

## 9. Quality control

| | |
|---|---|
| ☐ | *Control charts* |
| ☐ | *Participation in proficiency testing schemes* |

## 10. Other parameters to be tested

| | |
|---|---|
| ☐ | *Working range and testing of homogeneity of variances* |
| ☐ | *R squared* |
| ☐ | *Residual standard deviation* |
| ☐ | *Standard deviation of the analytical procedure* |
| ☐ | *Coefficient of variation of the analytical procedure* |
| ☐ | *Measurement uncertainty* |

## PART III: SOME CALCULATIONS AND CONCLUSIONS

### 11. Calculation of parameters requested by the customer

| Parameters requested to be validated | Calculations |
|---|---|
| ☐ *LOD* | |
| ☐ *LOQ* | |
| ☐ *Repeatability* | |
| ☐ *Within-lab reproducibilty* | |
| ☐ *Trueness* | |
| ☐ *Measurement uncertainty* | |
| ☐ *Other - please state* | |

**12. Does the analytical procedure fulfil the requirement(s) for the intended use?**

| **Parameter** | **Value requested by the customer** (the same as stated in question 3) | **Value obtained during validation** | **The requirement is fulfilled Yes/No** |
|---|---|---|---|
| ☐ LOD | | | |
| ☐ LOQ | | | |
| ☐ Repeatability | | | |
| ☐ Within-lab reproducibility | | | |
| ☐ Trueness | | | |
| ☐ Measurement | | | |
| ☐ Other | | | |

**The analytical procedure is fit for the intended use:**

☐ *Yes* ☐ *No*

*For measurement uncertainty and traceability refer to the corresponding sheets*

# BUILDING AN UNCERTAINTY BUDGET

## 1. Specify the measurand and units

| | |
|---|---|
| *Measurand* | |
| *Unit* | |

## 2. Describe the measurement procedure and provide the associated model equation

*Measurement procedure:*

*Model equation:*

## 3. Identify (all possible) sources of uncertainty

| | |
|---|---|
| ☐ | *Uncertainty of concentration of reference solutions* |
| ☐ | *Uncertainty of measurements of peak area* |
| ☐ | *Method bias* |
| ☐ | *Matrix effect* |
| ☐ | *Other:* |
| ☐ | *Other:* |
| ☐ | *Other:* |

### 4. Evaluate values of each input quantity

| *Input quantity* | *Value* | *Unit* | *Remark* |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

### 5. Evaluate the standard uncertainty of each input quantity

| *Input quantity* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

### 6. Calculate the value of the measurand, using the model equation

### 7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units

*Using*: ☐ Mathematical solution; ☐ Spreadsheet approach; ☐ Commercial software

| *Input quantity* | *Value* | *Standard uncertainty* | *Unit* | *Remark* |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**8. Calculate expanded uncertainty ($U_c$) & specify the coverage factor k and the units**

**9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$**

| | |
|---|---|
| *1* | |
| *2* | |
| *3* | |

**10. Prepare your uncertainty budget report**

## Addendum I: Measurement uncertainty calculation: spreadsheet approach (Excel)

## Addendum II: Measurement uncertainty calculation – GumWorkbench

# Appendix 2
# Briefing of the trainees on the example session

TrainMiC
Training in Metrology in Chemistry

# TrainMiC example session

## Introduction

Introduction to TrainMiC example session

TrainMiC
Training in Metrology in Chemistry

## How is TrainMiC example session organised?

1. About a TrainMiC example: how is it designed?
2. How are we going to organise ourselves for the example session?
3. Introduction to the analytical procedure we will be working on

Introduction to TrainMiC example session

## TrainMiC example includes:

- All the **input information** needed to do the three exercises i.e.
  - About the analytical procedure
  - About the customer's requirement(s) on the quality of the measurement results
  - Measurement data.

*This is known as 'yellow pages'.*

Introduction to TrainMiC example session

## TrainMiC example also includes:

- A list of questions for which you should find the answers in the 'yellow' pages (sometimes some calculations are be needed too)
- Questions are grouped as follows:
  - **Exercise 1.** Establishing traceability in analytical chemistry
  - **Exercise 2.** Single laboratory validation of measurement procedures
  - **Exercise 3.** Building an uncertainty budget

*Exercises are known as 'white pages'.*

Introduction to TrainMiC example session

## Exercise 1: Establishing traceability in analytical chemistry

1. Specifying the analyte and the measurand
2. Choosing a suitable measurement procedure with associated model equation
3. List the input quantities according to their influence on the uncertainty of the measurement (first the most important ones). At this point, your judgement should be based on your previous experience only.
4. List the reference standards needed and state the information regarding traceability of the reference value
5. Estimating uncertainty associated with the measurement
6. How would you prove traceability of your result
7. Any other comments, questions, ....

Introduction to TrainMiC example session

## How to organise ourselves for the TrainMiC example session?

- Forming the groups, each consisting of maximum 5 participants
- Each group nominates a reporter
- Each of you will get
  - 'the yellow page' (=input information) and
  - 'the white page' (Exercises on traceability, validation and measurement uncertainty)

Introduction to TrainMiC example session

## How to organise ourselves for the TrainMiC example session time wise?

- 30(+15) minutes are available for the group work per exercise
- Afterwards, all the reporters will be asked to report the answers and to explain to the others about the questions and comments his/her group was discussing during the exercise (30 minutes are available for a discussion)

IMPORTANT:

the reporting & discussion panel is not about correct/wrong answers, but about sharing your questions, experiences, ideas, suggestions and comments you would be having during the exercise session with all the attendees, including the trainers.

Introduction to TrainMiC example session

## Exercise 3: Building an uncertainty budget

1. Specify the measurand and units
2. Describe the measurement procedure and provide the associated model equation
3. Identify (all possible) sources of uncertainty
4. Evaluate values of each input quantity
5. Evaluate the standard uncertainty of each input quantity
6. Calculate the value of the measurand, using the model equation
7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units
8. Calculate the expanded uncertainty ($U_c$) & specify the coverage factor k and the units
9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$

Introduction to TrainMiC example session

## Exercise 3: Building an uncertainty budget

1. Specify the measurand and units
2. Describe the measurement procedure and provide the associated model equation
3. Identify (all possible) sources of uncertainty
4. Evaluate values of each input quantity
5. Evaluate the standard uncertainty of each input quantity
6. Calculate the value of the measurand, using the model equation
7. Calculate the combined standard uncertainty ($u_c$) of the result & specify units
8. Calculate the expanded uncertainty ($U_c$) & specify the coverage factor k and the units
9. Analyse the uncertainty contribution & specify the main three input quantities contributing the most to $U_c$

Introduction to TrainMiC
example session

## Introduction to the analytical procedure we will be working on today

- Measurements of xxxx in xxxx by xxxx

see the corresponding presentation for the selected analytical procedure

Introduction to TrainMiC
example session

*Notes*

*Notes*

*Notes*

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**Abstract**

Case studies on traceability, measurement uncertainty and validation for measurements of gold in gold alloys, calcium in serum, radium in water, polar pesticides in food and ammonium in water are presented in this report. Additonally, the idea and structure of the TrainMiC examples, which complement the TrainMiC theoretical presentations, are described in detail to give a complete overview of the TrainMiC teaching material.

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