HPTLC – Method Validation

Applicability of validated methods for control purposes

Ingo Schellenberg

Anhalt University of Applied Sciences,
Bernburg
Germany

INTERNATIONAL REQUIREMENTS AND GUIDE-LINES

Compliance with the general criteria
for testing laboratories
ISO/IEC Standard 17025 (1999)

EURACHEM / CITAC Guide
Second Edition

What is a Validated Method of Analysis?

it`s a Method for which we know:

- the applicability
- the reliability
- the performance characteristics

Therefore we need assurance that the laboratory is proficient in the use of the method.

METHODS OF ANALYSIS CRITERIA

They are currently under discussion:

- **Accuracy**
- **Applicability**

  (matrix, concentration range and preference given to 'general' methods)

Detection/determination limits if appropriate for the determination being considered

- **Linearity**
- **Precision**

  - repeatability intra-laboratory (within laboratory),

  - reproducibility inter-laboratory (within laboratory and between laboratories), but generated from collaborative trial data rather than measurement uncertainty considerations

Detection/determination limits if appropriate for the determination being considered

- **Recovery**
- **Selectivity (interference effects etc.)**
- **Sensitivity**
- **Other criteria that may be selected as required**

The precision values

- shall be obtained from a collaborativetrial
- which has been conducted in accordance with an internationally recognised protocol on collaborative trials

(e.g. ISO 5725:1994 or the IUPAC International Harmonised Protocol).

The ***repeatability and reproducibility values***

- shall be expressed in an internationally recognised form (e.g. the 95% confidence intervals as defined by ISO 5725:1994 or IUPAC).
- The results from the collaborative trial shall be published or freely available.

In situations where methods of analysis can only be validated within a single laboratory

- then they should be validated in accordance with IUPAC Harmonised Guidelines.

Methods of analysis adopted under this Regulation

- should be edited in the standard layout for methods of analysis
- recommended by the International Organisation for Standardisation

Will enable performance based approach to be introduced rather than a prescribed method approach – use any method provided:

- Laboratory is proficient
- Method meets the performance criteria identified above

In order to do this

- we do need method performance information,
- laboratory proficiency testing,

Also helps in enabling the measurement uncertainty of a result to be estimated.

Analytical chemists

- coming now more and more under increased pressure
- to be able to demonstrate the quality of their results
- by giving a measure of the confidence
- placed on a particular result
- to demonstrate its fitness for purpose.

This includes the degree

- to which the result would be expected to agree with other results
- irrespective of the method used.

“Measurement Uncertainty” (MU)

- is a useful parameter
- which gives this information,
  and one that is increasingly being discussed.

<u>All quantitative analytical results</u> may be reported to the customer in the form

“a ± b”

where “a”
is the best estimate of the true value of the concentration of the measure (the analytical result)

and “b”
is the range within which the true value is estimated, with a given probability (normally 95%), to fall.

The value of “b”

- is known as the “measurement uncertainty”
- and may be estimated by the analyst in a number of different ways.

Even though this terminology is considered suspect by some, it is now internationally accepted.

The estimation of the **value of "a"** is dependent on:

- the accuracy of the method of analysis used
- how well the analyst uses that method,
  i.e. whether the analytical system is "in control".

The value of the
measurement uncertainty "b" is dependent on:

- the inherent precision of the method of analysis used
- the number of analytical replicates that are carried out.
- The more replicates the less the value of the measurement uncertainty.

What is Measurement Uncertainty?

"A parameter,

- associated with the result of a measurement,
- that characterises the dispersion of the values
- that could reasonably be attributed to the measurand"

(ISO Guide)

The number after the ±

ISO Guide approach

- Specify the measurand
  – including complete equation
- Quantify significant uncertainties in all parameters
  – A: from statistics of repeated experiment
  – B: by any other means (theory, certificates,judgement...)
- Express as standard deviation
- Combine according to stated principles

Find a way for the laboratories to calculate measurement uncertainty for

results from a procedure in routine use

which is:

- **relatively easy and clear (to promote harmonisation)**
- **using existing data if possible**
- **giving a value ≥ correct estimation**

Different ways of estimating uncertainty

Estimates based on:

1. Data from standard method
2. Data from proficiency testing
3. Data from QC and validation
4. Detailed data from all known uncertainty contributions

Within-Laboratory Reproducibility

- Sample inhomogenity
- Sub - sampling
- Sample preparation
- Measurement
- Variation in time, operator, season etc.
  i.e. the whole analytical proces

$$u(R_w)$$

Lab or/and Method Bias

- *Bias or biases itself*
- $u(C_{ref})$ Uncertainty of the nominal/certified value

If a single CRM is used also

- s_{bias} Uncertainty in the bias determination

  In most cases small and already part of repeatability

- **Note: Even if the bias is corrected,**

  u(bias) has to be estimated

Premise

 Measured value = repeatable component
+ random component

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Random component

- includes influence of all factors affecting measurement precision
- causes uncertainty in measured value
- leads to uncertainty in calculated property based on the measured value.

Example

Uncertainty Estimation from In-House Validation Studies.

Determination of Organophosphorus Pesticides in Bread

Aim

Determination of the amount of an organophosphorus pesticide residue in food
by using
extraction and an HPTLC procedure

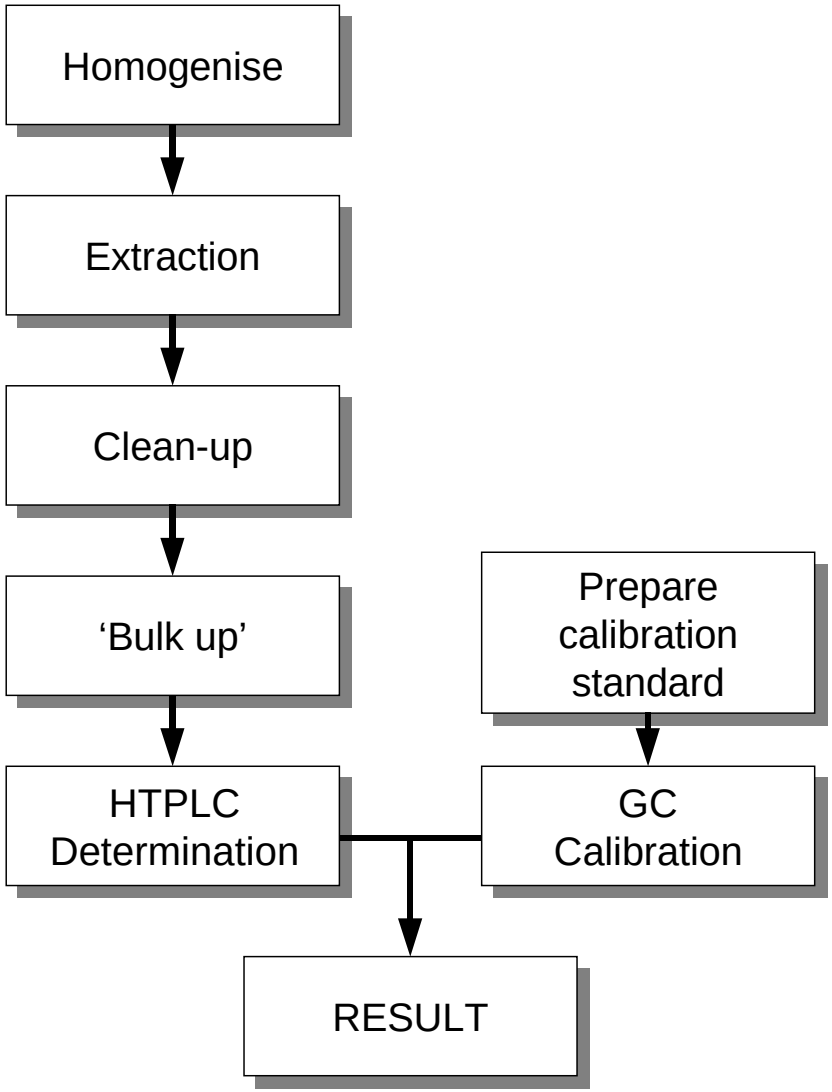
The examples illustrate

- the way in which in-house validation data can be used to quantify the measurement uncertainty.

- The specification of the measurand for more extensive analytical methods is best done
  - by a comprehensive description of the different stages of the analytical method
  - and by providing the equation of the measurand.

Measurement procedure

The stages needed to determine the amount of organophosphorus pesticide residue



The 6 steps to be followed:

1. Specify measurand
2. Quantify within-lab reproducibility
3. Quantify bias
4. Convert components to standard uncertainties $u(R_W)$ and u(bias)
5. Calculate combined standard uncertainty
6. Calculate expanded uncertainty

1. Measurand:

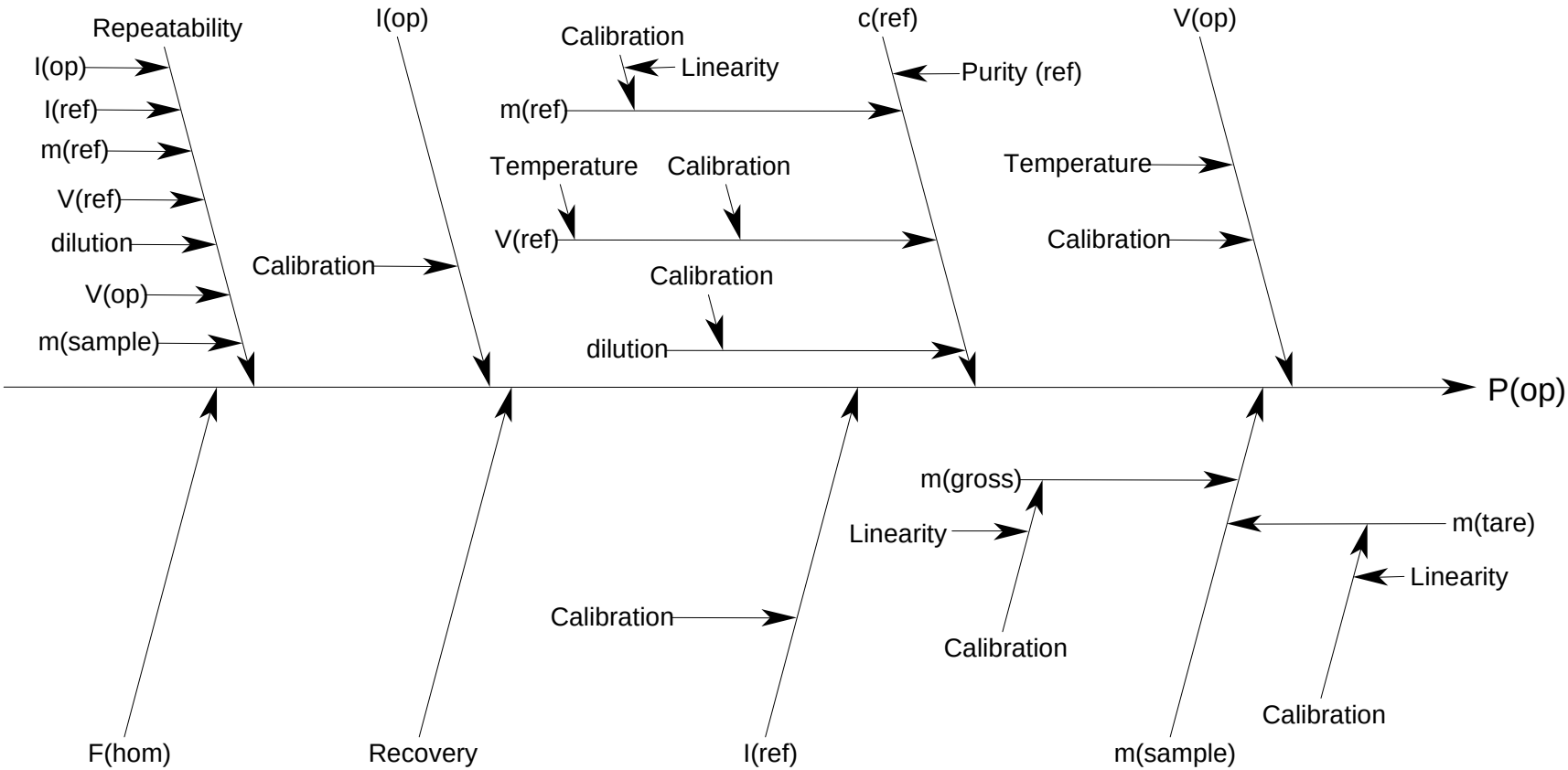
$$P_{op} = \frac{I_{op} \cdot c_{ref} \cdot V_{op}}{I_{ref} \cdot Rec \cdot m_{sample}} \cdot F_{hom}$$

where

P_{op} : Level of pesticide in the sample [mg/kg-1]
I_{op} : Peak intensity of the sample extract
C_{ref} : Mass concentration of the reference standard [μg/ ml-1]
V_{op} : Final volume of the extract [ml]
I_{ref} : Peak intensity of the reference standard
R_{ec} : Recovery
M_{sample} : Mass of the investigated sub-sample [g]
F_{hom} : Correction factor for sample inhomogeneity

2. Identification of the uncertainty sources:

The relevant uncertainty sources are shown in the cause and effect diagram



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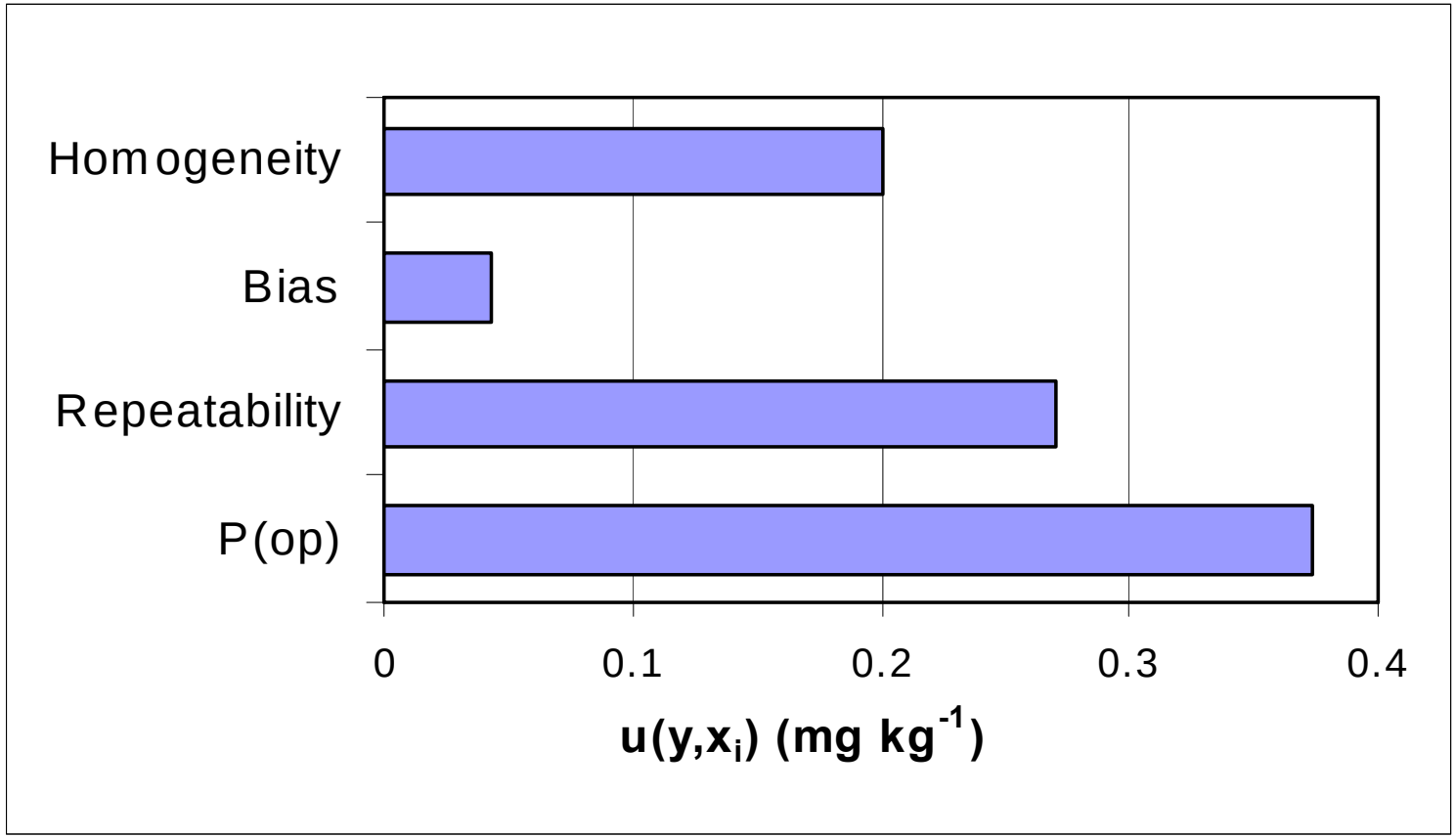
Quantification of the uncertainty components



Based on an in house validation data

Conclusions

- Measurement Uncertainty arises from Effects on the measurement process
- Validation and measurement uncertainty are views of the same problem
- EURACHEM-CITAC guide is the only Measurement Uncertainty guide explicitly using validation data!