Evaluating uncertainty: Practical approaches for testing laboratories

Vicki Barwick
LGC
29 May 2017

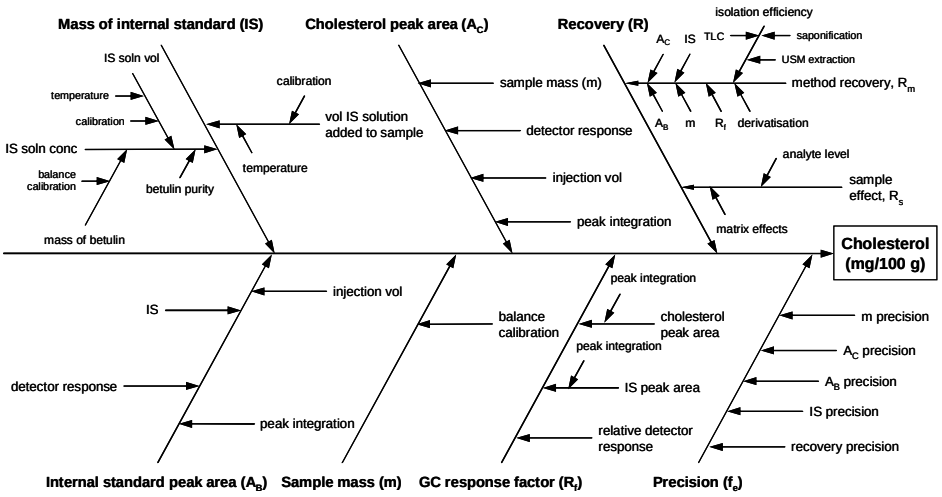
Overview

- 'Bottom-up' vs 'top-down' approach to uncertainty estimation
- Using validation and quality control data in uncertainty estimation
  - requirements for the top-down approach
- Sources of data
- Practical example
- Limitations

ISO approach – 'bottom-up'

- Write an equation that completely describes the measurement system
  - includes all parameters that could influence the measurement result
- Evaluate the uncertainties associated with all parameters in the equation
  - Type A: statistical evaluation, Type B: any other data (certificates, instrument specifications, etc)
- Express all uncertainties as standard deviations
- Combine using mathematical rules for the combination of variances
- Apply a suitable coverage factor

Can the "bottom-up" approach work for analytical chemistry?



Problems

- Difficult to write an equation that includes all influence factors
  - what about sample clean-up conditions, recovery of analyte from matrix, instrument conditions, interferences….
- Challenging to evaluate individual uncertainty components
- Process is too time consuming and unworkable in routine testing laboratories
  - a ‘reasonable estimation’ is required

‘Top-down’ approach

- Use method performance data
  - validation data on precision and bias
    - in-house/interlaboratory studies
  - ongoing internal quality control (IQC) data
  - proficiency testing data
- Capture the effect of a number of sources of uncertainty
- Look at the variation in method *outputs* (i.e. results) rather than method *inputs*
- Cover method scope
  - matrix, analyte concentration

‘Top-down’ requirements

- The best available estimate of precision
  - from validation studies or ongoing QC
- The best available estimate of bias and its uncertainty
  - includes method bias and laboratory bias
- Other significant effects evaluated
  - by experiment, or from existing data

Evaluating precision

- Aim to cover as many sources of variation as possible
  - extended time period, different analysts, different calibration standards, environmental conditions
- A parameter varied representatively during a precision study requires no further evaluation
- Types of data
  - method validation study (intermediate precision)
  - quality control data – repeated analysis of QC materials
  - data from interlaboratory studies (method validation or PT)

Case study – determination of cholesterol in animal and vegetable fats and oils

- Extraction/clean-up followed by quantification by GC-FID
  - calibration via internal standard
- Data from precision study: analysis of different sample types
  - each sample was analysed in triplicate on five different days, by two different analysts
  - fresh internal standard was prepared for the analysis of each sample
- In each case the repeatability standard deviation (s_r) and the intermediate precision (s_I) was calculated
  - analysis of variance (ANOVA)

Precision – estimating uncertainty contribution

Summary of results from precision studies (mg/100 g)

		Repeatability		Intermediate precision	
Sample type	Mean ($\bar{x}$)	s_r	$s_r/\bar{x}$	s_I	$s_I/\bar{x}$
Anhydrous milk fat CRM	269.3	1.69	0.00628	2.93	0.0109
Spiked olive oil	106.2	0.840	0.00791	1.44	0.0135
Spiked corn oil	70.30	0.420	0.00597	0.73	0.0104
Pork & beef fat CRM	128.1	0.935	0.00730	1.62	0.0126
Pooled values		1.07	0.00691	1.86	0.0119

Which precision value?

- Repeatability or intermediate precision?
  - intermediate precision – covers more sources of uncertainty than repeatability
- Can precision estimates for different samples be combined?
  - yes – if they are similar
- In current example the relative standard deviations are similar
  - precision is approximately proportional to concentration over the range studied
- Use intermediate precision pooled rsd
  - precision estimate is 0.0119 (relative)

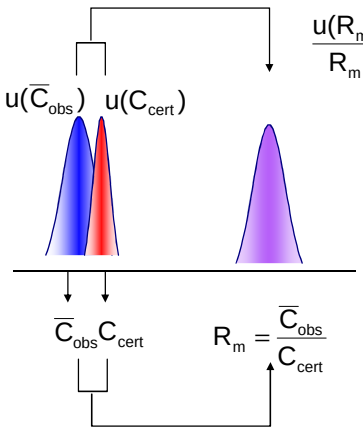
Uncertainty budget

Parameter	Value, x_i	Standard uncertainty, $u(x_i)$	Relative uncertainty, $u(x_i)/ x_i$
Precision, f_e	1.0	-	0.0119
Combined standard uncertainty (relative)			
Expanded uncertainty (relative), 95% confidence			

Evaluating bias

- A reasonable estimate of the bias can be obtained from
  - validation data (using CRMs or spiked samples)
  - PT data (depending on the nature of the scheme/samples)
- Is the bias significant?
  - statistically significant?
  - significant compared to the method precision?
- Bias and its uncertainty should be considered as part of the uncertainty evaluation process
- Need to consider effect of sample matrix on bias/recovery

Including bias in uncertainty estimates (1)
Approaches in chemical analysis

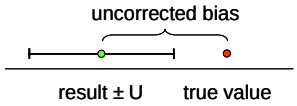


$$\frac{u(R_m)}{R_m} = \sqrt{\left(\frac{u(\overline{C}_{obs})}{\overline{C}_{obs}}\right)^2 + \left(\frac{u(C_{cert})}{C_{cert}}\right)^2}$$

- Estimate of recovery/bias has associated uncertainty
  - uncertainty in reference value $u(C_{cert})$
  - uncertainty in results from analysis of reference $u(\overline{C}_{obs})$
- **Insignificant bias** – recovery not significantly different from 100%
  - assume $R_m = 1$ with an uncertainty, $u(R_m)$
  - uncertainty on bias estimate included, even if bias itself is not significant

Including bias in uncertainty estimates (2)

- **Significant bias**
  - develop method to remove/reduce bias
  - correct results for known significant bias (ISO Guide)
    - include u(R_m) in uncertainty estimate for corrected results
  - correction uncommon in chemical analysis
  - but, uncertainty is a range which includes the true value…..



  - …so significant bias should not be ignored
  - options: report bias and its uncertainty separately OR increase reported uncertainty to take account of the bias

Including bias in uncertainty estimates (3)

- If a separate report of bias or recovery is not appropriate
  - increase reported uncertainty by including a bias uncertainty term
  - bias term combined with precision using “root sum of squares” rule
- Different approaches proposed for estimating bias term
  - root mean square (RMS) of bias estimates
  - mean bias
  - bias divided by coverage factor, k
- Further information in the literature
- However – all have limitations

Case study – determination of cholesterol in animal and vegetable fats and oils

- Recovery
  - results from the replicate analysis of a CRM certified for cholesterol content
  - recovery data for cholesterol from 7 different sample matrices with differing cholesterol levels

Method recovery (R_m) – data

- Results are available from the analysis of a reference material **(anhydrous milk fat reference material CRM 164)**

Mean (mg/100 g) ($\overline{C}_{obs}$)	269.33
Standard deviation (mg/100 g)	1.692
Number of replicates	11
Certified cholesterol content (mg/100 g) (C_{cert})	274.7 ± 9* *Uncertainty in certified value given at the 95% confidence level

Method recovery (R_m) – and estimating uncertainty $u(R_m)$

- Method recovery (R_m)

$$R_m = \frac{\overline{C}_{obs}}{C_{cert}} = \frac{269.33}{274.7} = 0.98$$

- $u(R_m)$ has contributions from:
  - the uncertainty in the certified value of the reference material ($u(C_{cert})$)
  - the uncertainty in the mean of the laboratory results ($u(\overline{C}_{obs})$)

$$u(C_{cert}) = \frac{9.0}{1.96} = 4.59 \qquad u(\overline{C}_{obs}) = 1.692/\sqrt{11} = 0.51$$

Method recovery (R_m) – estimating uncertainty $u(R_m)$

$$u(R_m) = R_m \times \sqrt{\left(\frac{u(C_{cert})}{C_{cert}}\right)^2 + \left(\frac{u(\overline{C}_{obs})}{\overline{C}_{obs}}\right)^2}$$

$$u(R_m) = 0.98 \times \sqrt{\left(\frac{4.59}{274.7}\right)^2 + \left(\frac{0.51}{269.33}\right)^2} = 0.016$$

- The method recovery is therefore estimated as 0.98 with a standard uncertainty of 0.016.

Method recovery: is there a significant bias?

- Is the recovery significantly different from 1?
  - Ratio $|1-R_m|/u(R_m)$ is compared with **k.** In most cases, **k** is taken to be 2 to give a confidence level of approximately 95%

$$\frac{|1-0.98|}{0.016} = 1.19$$

- 1.19 < 2: there is no evidence that the recovery is significantly different from 1 and no reason to correct experimental results for incomplete recovery

Matrix effects $u(R_s)$

Summary of recovery data for cholesterol in different sample matrices

Sample matrix	Mean recovery
Anhydrous milk fat	0.98
Turkey-chicken fat blend	0.98
Beef-pork fat blend	0.96
Animal fat (others)	0.97
Trout Flesh	0.95
Spiked olive oil	1.03
Corn oil	1.06
mean	0.99
sample standard deviation	0.040
relative standard deviation	0.0404

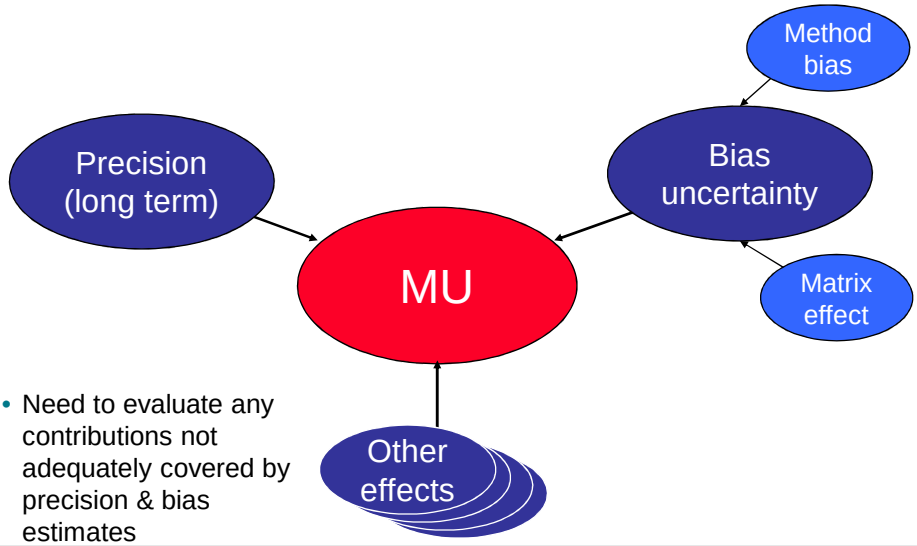
Assume $R_s = 1$

$u(R_s) = 0.040/1$

Uncertainty budget

Parameter	Value, x_i	Standard uncertainty, $u(x_i)$	Relative uncertainty, $u(x_i)/ x_i$
Method recovery, R_m	1.0	0.016	0.016
Matrix effect, R_s	1.0	0.040	0.040
Precision, f_e	1.0	-	0.012
Combined standard uncertainty (relative)			0.045
Expanded uncertainty (relative), 95% confidence (k=2)			0.089

Any other significant contributions?



- Need to evaluate any contributions not adequately covered by precision & bias estimates

Limitations of top-down approach

- No information on main sources of uncertainty
- Uncertainty will apply to any future result obtained within scope of method
  - uncertainty estimate needs to address effects of sample matrix/analyte level
- Single estimate may not be possible if MU varies with level/matrix
- Including effect of uncorrected bias
  - different approaches exist

Summary

- The ‘bottom-up’ approach is impractical for many test methods
- The ‘top-down’ approach utilises method performance data
  - requires a reliable estimate of method precision and information on bias
  - available from method validation studies, QC and PT
- ‘Fit for purpose’ for testing laboratories
- …but no information on main sources of uncertainty

Further reading

- *Measurement uncertainty revisited: Alternative approaches to uncertainty evaluation*, Eurolab Technical Report 1/2007, 2007 (available at www.eurolab.org)
- NORDTEST Report TR 537, *Handbook for calculation of measurement uncertainty in environmental laboratories* (available from www.nordtest.info)
- ISO 21748 *Guidance for the use of repeatability, reproducibility and trueness estimates in measurement uncertainty evaluation*
- ISO 11352 *Water quality -- Estimation of measurement uncertainty based on validation and quality control data*
- B. Magnusson, S. L. R. Ellison, *Treatment of uncorrected measurement bias in uncertainty estimation for chemical measurements*, Anal. Bioanal. Chem., 390, 201-213, 2008.
- G. E. O'Donnell, D. Bryn Hibbert, *Treatment of bias in estimating measurement uncertainty*, Analyst, 130, 721-729, 2005.