Procedures for estimation of uncertainty of results of analytical measurements

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- According to ISO/IEC 17025, testing laboratories shall have and shall apply procedures for estimating uncertainty of measurement.

- The presented procedures should be regarded as practical examples without being prescriptive

- They give reference to correspondent passages of the standards

- Procedures agreed by trading partners will help avoid future conflicts between importing and exporting countries

Types of Analytical Methods

Standard Methods

***Single-laboratory Validated* Methods**

Defining Methods | Rational Methods

Established Methods | Ad-hoc Methods

Defining Standard Methods I

- no intent to obtain an absolute measure of the true amount of analyte
- Corrections for method bias or matrix effects are ignored by convention

Single Laboratory measurement uncertainty

Prerequisite: Verificaton of repeatability standard deviation of collaborative trial

$u_{rel} = cv_R$ relative reproducibility standard deviation of interlaboratory validation

Defining Standard Methods II

- In most cases, the collaborative trials providing homogenised powdered dry material do not cover preparation steps (e.g. grinding, drying)

Single Laboratory measurement uncertainty

uncertainty contributions of sample preparation cv_P must be additionally taken into consideration provided that the contribution is significant i.e. >1/3 cv_R

Multiple preparation of a perfectly homogenised laboratory sample and measurement of the different analytical samples under repeatability conditions.

$$u_{rel} = \sqrt{cv_R^2 + cv_P^2}$$

Defining Standard Methods III

- collaborative trials provide homogenised material

Single Laboratory measurement uncertainty

uncertainty contributions of sample inhomogeneity must be additionally taken into consideration provided that the contribution is significant i.e. >1/3 cv_σ

between-subsamples standard deviation s_s might be estimated by the procedure given in ISO 13528, Annex B1 and using the formula given in ISO 13528, Annex B3 and combined with the uncertainty contributions of preparation if applicable

$$u_{rel} = \sqrt{cv_R^2 + cv_P^2 + cv_S^2}$$

Example: Contribution of Inhomogeneity , ISO 13528

t analytical subsamples of one laboratory sample, selected randomly, each measured in duplicate (two test portions)

Define the sample averages as:

$$x_{t,\cdot} = (x_{t,1} + x_{t,2}) / 2$$

and the between-test-portion ranges as:

$$w_t = |x_{t,1} - x_{t,2}|$$

Calculate the general average:

$$\bar{x}_{\cdot,\cdot} = \sum \bar{x}_{t,\cdot} / g$$

the standard deviation of sample averages:

$$s_x = \sqrt{\sum \left(x_{t,\cdot} - \bar{x}_{\cdot,\cdot}\right)^2 / (g - 1)}$$

and the within-samples standard deviation:

$$s_w = \sqrt{\sum w_t^2 / (2g)}$$

where the summations are over samples ($t = 1, 2, \ldots, g$).

Finally, calculate the between-samples standard deviation as:

$$s_s = \sqrt{s_x^2 - \left(s_w^2 / 2\right)}$$

i.e. the within-samples stddev is deconvolved from the stddev of sample averages

Example: Contribution of Inhomogeneity , ISO 13528

Nine subsamples of one test sample, each measured in duplicate:

X1	X2	mean (x1,x2)	range(x1,x2)
3,70	3,60	3,65	0,100
3,30	3,20	3,25	0,100
3,80	3,70	3,75	0,100
3,30	3,40	3,35	0,100
3,20	3,20	3,20	0,000
3,50	3,60	3,55	0,100
3,10	3,20	3,15	0,100
3,30	3,20	3,25	0,100
3,20	3,10	3,15	0,100
average(mean(x1,x2))		3,37	
stddev samples		0,23	
stddev within samples		0,21	
stddev betweensamples		0,17	
variance average		0,05	

$$cv_S = 0.05$$

Rational Standard Methods I

- Matrix effects are to be considered

Single Laboratory measurement uncertainty

uncertainty contributions of matrix influences must be additionally taken into consideration provided that the contribution is significant i.e. >1/3 cv_σ

Between-matrices standard deviation s_M might be estimated by the procedure given in ISO 13528, Annex B1 and using the formula given in ISO 13528, Annex B3 and combined with the other uncertainty contributions if applicable

$$u_{rel} = \sqrt{cv_R^2 + cv_P^2 + cv_S^2 + cv_M^2}$$

Example: Contribution of Matrix , ISO 13528

Seven different matrices (e.g. spiked with 5 mg/kg) measured in duplicate:

X1	X2	mean (x1,x2)	range(x1,x2)
6,20	6,40	6,30	0,200
4,80	4,90	4,85	0,100
6,00	5,90	5,95	0,100
4,50	4,60	4,55	0,100
5,30	5,20	5,25	0,100
6,50	6,30	6,40	0,200
3,30	3,40	3,35	0,100
average(mean(x1,x2))	5,24		
stddev samples	0,89		
stddev within samples	0,21		
stddev betweensamples	0,88		
variance average	0,17		

Matrix effects: $cv_M = 0.17$

Rational Standard Methods II

- Method bias is to be considered

Single Laboratory measurement uncertainty

Where bias is significant compared to the combined uncertainty, the analytical result might be corrected for the bias

In case of correction, the relative uncertainty of the bias cv_B must be estimated by recovery experiments and combined with the other uncertainty contributions if applicable (EURACHEM Example A4):

$$u_{rel} = \sqrt{cv_R^2 + cv_P^2 + cv_S^2 + cv_M^2 + cv_B^2}$$

Single-laboratory Validated Methods I

- In case that the Method is a modification of a standard method with no significant influence on precision:

$u_{rel} = cv_R$ relative reproducibility standard deviation of standard method

- Otherwise two approaches:

1. The combination of the precision ***of all single steps of analysis*** (e.g. weighing, drying, extracting)

2. Intermediate Precision estimated ***by series of analysis*** as far as possible over an extended time period allowing natural variation of all impact factors.

Single-laboratory Validated Methods II

1. The combination of the precision ***of all single steps of analysis*** (e.g. weighing, drying, extracting)

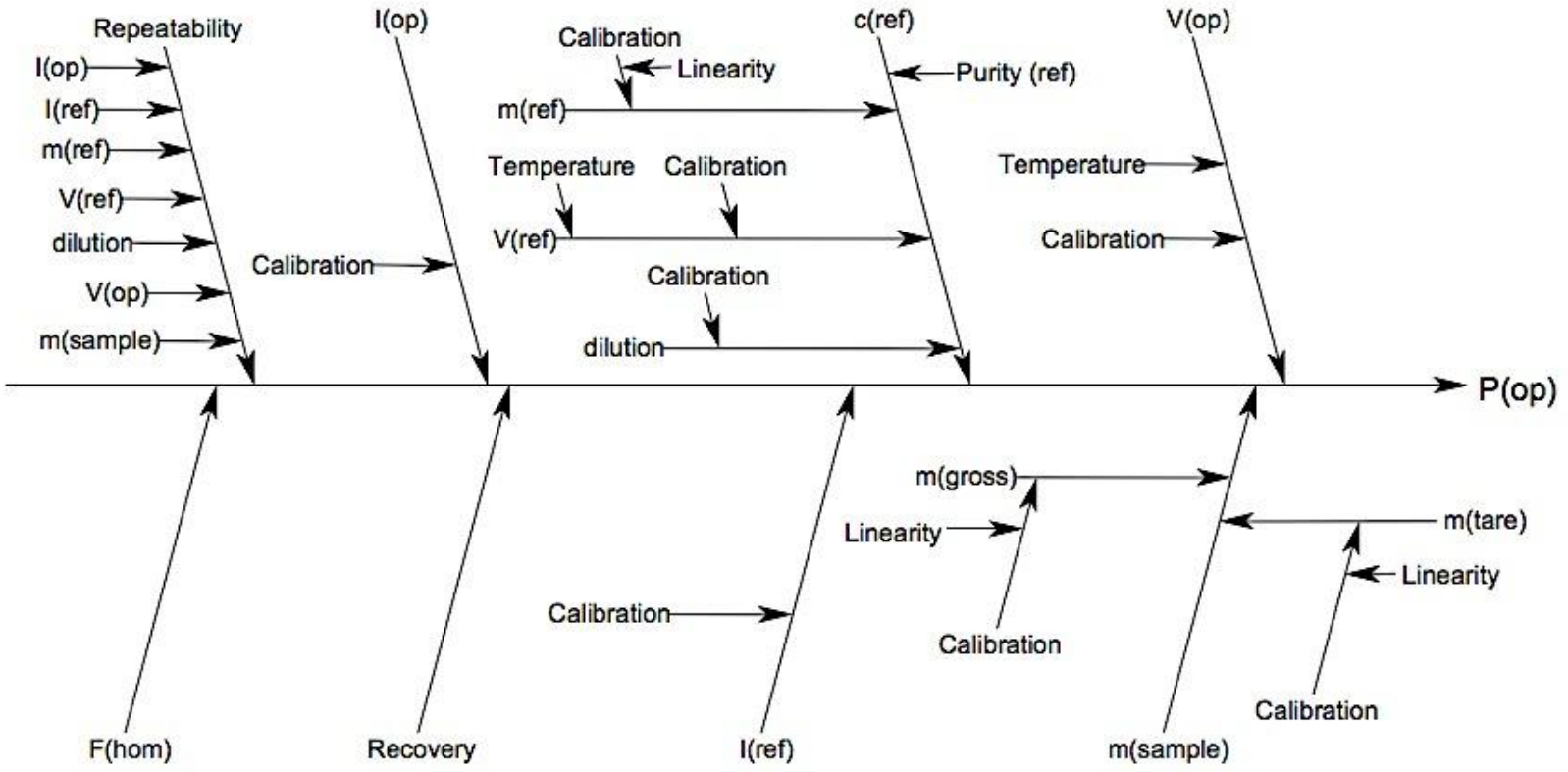
potential sources of uncertainty

- Standard substances (certified uncertainty/purity)
- Physical/chemical variability (extraction, derivatisation, stoichiometry)
- Application of measuring devices for preparation of the test samples (balances, pipettes, thermometers etc.)
- Application of analytical instruments (stability, calibration, contamination etc.)
- Different experience of test personnel

Single-laboratory Validated Methods III

The consideration ***of all single steps of analysis*** (e.g. weighing, drying, extracting):

Cause and effect diagram



Single-laboratory Validated Methods IV

The combination of the coefficients of variation ***of all single steps of sample preparation*** (e.g. weighing, homogenising drying, extracting, diluting) provided the sensitivity of the measurand to the particular parameters is one and the parameters are not correlated:

$$cv_P = \sqrt{(cv_{weigh}^2 + cv_{hom}^2 + cv_{dry}^2 + cv_{extr}^2 + cv_{dil}^2)}$$

Single-laboratory Validated Methods V

2. Intermediate Precision estimated by ***series of analysis***

This type of estimation should be performed as far as possible under reproducibility conditions allowing natural variation of all impact factors. Basically this should include all conjectural components (subsampling, matrices, preparation and analysis).

A typical test sample containing an appropriate amount of analyte (e.g. homogenised and dried for stability) might be analysed several times over a period of time, using different analysts and equipment where possible (e.g. the results of measurements on quality control samples).

The measure of precision of the following approaches is the so-called intermediate Measurement uncertainty, which is smaller than the reproducibility standard deviation based on inter-laboratory method validation.

Single-laboratory Validated Methods VI

2.1 ISO 5725-2 and ISO 5725-3 Approach

- A typical test sample (homogenised and dried) is analysed over a period of time on *n* different days by different analysts (with a new extraction/digestion, recalibration).
- Each of the days, a number of *k* replicates of the particular extract/digest are measured with the results $x_{j=1\ldots k}$ under repeatability conditions with $s_{r\,i} = stddev\,(x_{j=1\ldots k})$, the repeatability standard deviation of each day

with $s_{r\,mean} = \sqrt{(1/n \sum s_{r\,i=1\ldots n}^2)}$, the mean repeatability standard deviation of the different days and $s_d = stddev(\,x_{i=1\ldots n})$, the "between-days" standard deviation

the intermediate standard deviation is given by: $s_{int} = \sqrt{(s_{r\,mean}^2 + s_d^2)}$

Example: ISO 5725-2 and ISO 5725-3 Approach

- A typical test sample (homogenised and dried) is analysed over a period of time on *9* different days by different analysts (with a new extraction/digestion, recalibration).
- Each of the days, a number of *3* replicates of the particular extract/digest are measured with the results *x1, x2, x3* under repeatability conditions

X1	X2	X3	mean	stddev
1,70	1,60	1,60	1,63	0,06
1,30	1,20	1,30	1,27	0,06
1,80	1,70	1,60	1,70	0,10
1,30	1,40	1,40	1,37	0,06
1,20	1,20	1,30	1,23	0,06
1,50	1,60	1,50	1,53	0,06
1,10	1,10	1,20	1,13	0,06
1,30	1,20	1,30	1,27	0,06
1,20	1,10	1,20	1,17	0,06
average		1,37		
mean stddev days		0,06		
stddev between days		0,21		
interemediate stddev		0,22		
interemediate variance		0,16		

$$cv_{int} = 0.16$$

Single-laboratory Validated Methods VII

2.2 Duplicate Approach (EURACHEM 7.7.2 and A4.4).

- a number *n* of duplicate tests (homogenised samples each divided into two test samples, each of the test samples subjected to complete extraction/digestion and determination procedure including recalibration)

with $s_{\delta rel} = stddev(\delta_{rel\ i=1...n})$, the standard deviation (stddev) of the relative differences of the particular duplicate results, the relative intermediate standard deviation is given by:

$$cv_{int} = s_{\delta rel} / \sqrt{2}$$

Example: Duplicate Approach (EURACHEM 7.7.2 and A4.4)

- a number *10* of duplicate tests (homogenised samples each divided into two test samples, each of the test samples subjected to complete extraction/digestion and determination procedure including recalibration)

X1	X2	mean (X1,X2	diff(X1,X2)	diff rel(X1,X2)
5,30	5,20	5,25	0,100	0,02
4,70	4,90	4,80	-0,200	-0,04
4,10	4,40	4,25	-0,300	-0,07
5,20	5,00	5,10	0,200	0,04
4,50	4,40	4,45	0,100	0,02
4,90	4,80	4,85	0,100	0,02
5,00	5,10	5,05	-0,100	-0,02
4,60	4,40	4,50	0,200	0,04
4,70	4,80	4,75	-0,100	-0,02
5,20	5,30	5,25	-0,100	-0,02
stddev diff rel		0,04		
intermediate variance		0,03		

$cv_{int} = 0.03$

Single-laboratory ad-hoc Methods

In most cases, ad-hoc methods are based on standard or well-established ***Single laboratory validated*** methods.

They are expanded substantially (e.g. to other analytes or matrices) and will not generally require complete revalidation, but the examination of the altered parts of the analysis only.

The precision data of the standard or well-established method, which is the basis of the ad-hoc method, might be combined with the additional uncertainty contribution of the ad-hoc modification (if significant).

Thank you for your kind attention