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Estimating uncertainties of results using information of internal quality control, intercomparison exercises and reference materials

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1. INTRODUCTION

The quality standard ISO/IEC/EN 17025 [1] states that analytical laboratories must ensure the traceability of their results and calculate their uncertainty. The evaluation of uncertainty is an essential part of any quantitative analysis. Without uncertainty, results obtained by different laboratories or by different methods cannot be compared and decisions derived from these analytical results cannot be taken. In the antidoping control field, the estimation of the uncertainty of the measurement of threshold substances is a requisite described in the International Standard for Laboratories [2].

Uncertainty can be obtained either by calculating individually all the sources of uncertainty or by grouping different sources of uncertainty whenever possible. The first way is known as the "bottom-up" approach and was proposed by ISO [3, 4]. However, identifying and calculating all the individual sources of uncertainty is not straightforward and may be very time-consuming, so other more global approaches, based on grouping different sources of uncertainty, have been proposed. The first global approach (known as "top-down") was proposed by the Analytical Methods Committee, AMC [5] and it is based on using data from inter-laboratory collaborative studies. However, since uncertainty is evaluated using information of other laboratories, this evaluation may have little to do with the uncertainty of a given laboratory. To overcome this disadvantage, other global approaches have been developed to estimate uncertainty using "within-laboratory" information, i.e. information obtained during the validation process and from internal quality control (IQC) data [6,7]. These global approaches have been finally accepted by Eurachem [8] as a valid alternative to the "bottom-up" approach.

In this paper, we describe an approach to calculate measurement uncertainty that can be easily applicable to all routine analytical methods. This approach requires little extra-work to calculate uncertainty because it uses information already available in the analytical laboratory, i.e. generated in the processes of method validation and IQC.

2. ESTIMATION OF UNCERTAINTY

All the sources of uncertainty of the analytical procedure have been grouped in four terms:

- uncertainty associated to precision
- uncertainty associated to trueness
- uncertainty associated to pre-processing steps
- uncertainty associated to other sources

In this document, the estimation of all these components of uncertainty is described. Each term is calculated at one concentration level. Assuming that the working concentration range is narrow, the uncertainty values may be extrapolated to other concentrations. If the working range is wide, i.e. covers more than one order of magnitude, ideally all the terms should be estimated at least at two concentration levels to bracket the whole concentration range.

2.1. Uncertainty associated to precision (u_{PREC})

The uncertainty associated to the precision of the analytical procedure (u_{PREC}) is defined as the uncertainty due to experimental variation when the analytical procedure is applied to a future sample to obtain a result. This uncertainty is associated to the variability of the analytical results due to random errors in the different parts of the analytical procedure.

This uncertainty depends on the intermediate precision of the procedure at the level of concentration of the routine sample analysed and it is estimated according to the following expression:

$$u_{PREC}^2 = \frac{s_{b\_run}^2}{p_e} + \frac{s_{w\_run}^2}{n_e p_e} \quad (1)$$

where:

$s^2_{b\_run}$, is the between run variance

p_e, is the number of runs, where the future sample will be analyzed

$s^2_{w\_run}$, is the within run variance (repeatability variance)

n_e, is the number of replicates of the future sample

The result of a future sample is usually obtained by performing different replicates (for example, n_e=3) in the same run (p_e=1).

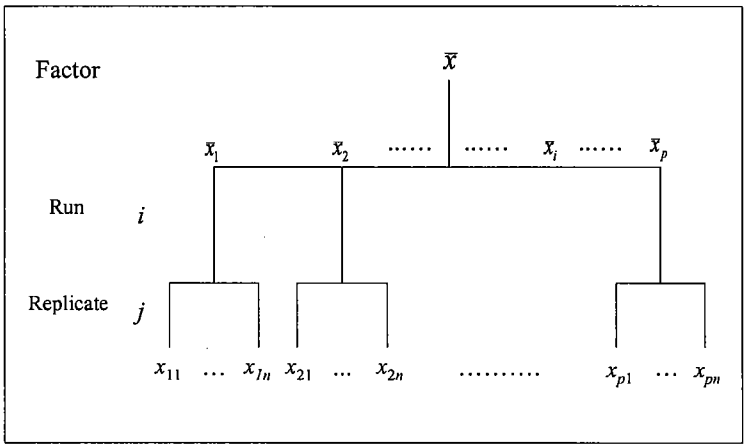

Figure 1. Two-factor fully-nested design proposed to calculate the within-run and the between-run variances. The factors studied are the run and the replicates. p is the number of runs on which the control sample is analysed and n is the number of replicates carried out in each run.

The estimation of u_{PREC} can be performed following the two-factor fully-nested design proposed in Fig. 1. In this design, a quality control sample (QC sample: blank urine sample spiked with the compound of interest) is analysed in p runs and using n replicates per run. Following this design, the within-run variance ($s^2_{w\_run}$), the between-run variance ($s^2_{b\_run}$) and the intermediate variance (s^2_I) of the analytical procedure can be calculated. The typical ANOVA table to calculate these variances is shown in Tables 1 and 2.

Table 1. ANOVA table for a two-factor fully-nested design.

Source	Mean Squares	Degrees of freedom
run	$MS_{b\_run} = \frac{n \cdot \sum_{i=1}^{p} (\bar{x}_i - \bar{\bar{x}})^2}{p-1}$	p-1
replicate	$MS_{w\_run} = \frac{\sum_{i=1}^{p} \sum_{j=1}^{n} (x_{ij} - \bar{x}_i)^2}{p \cdot (n-1)}$	$p \cdot (n$-1)

Table 2. Calculation of variances for a two-factor fully-nested design.

Variance	Expression	Degrees of freedom
Within-run variance $s^2_{w\_run}$	MS_{w_run}	$p \cdot (n$-1)
Between-run variance $s^2_{b\_run}$	$\frac{MS_{b\_run} - MS_{w\_run}}{n}$	
Intermediate variance $s_I^2 = u^2_{PREC}$	$s^2_{w\_run} + s^2_{b\_run}$	

Results of QC samples obtained during method validation or results of QC samples analyzed during routine work, as IQC, can be used to estimate u_{PREC} . The sources of uncertainty included in the term u_{PREC} will depend on the type of QC sample and the treatment of the QC sample. If the QC samples are analysed by varying all the factors that can affect the analytical procedure, the term u_{PREC} will include all the sources of variability of the analytical method. However, if it is not the case, the uncertainty of the factors not representatively varied should be included in other terms (u_{OTHER}).

It is assumed that the standard uncertainty u_{PREC} is proportional to analyte concentration, within the concentration range. To be able to apply the uncertainty to different concentrations, the relative standard uncertainty ($u_{PREC(\%)}$) should be calculated by dividing the standard uncertainty by the concentration used to calculate it and multiplying by 100.

2.2. Uncertainty associated to trueness (u_{TRAC})

The trueness or absence of bias of an analytical procedure may be assessed through the verification of traceability. The traceability of results generated by an analytical procedure should always be assessed before application of the procedure to routine samples. The traceability is the property of the result that connects it with a known reference.

The traceability is evaluated by verifying the absence of bias between the analytical procedure and a known reference. The traceability level will depend on the metrological quality of the reference used. If bias is not statistically significant, the procedure is traceable to the reference, and no systematic error exists. However, a source of uncertainty associated to the assessment of the traceability still remains (u_{TRAC}), because due to random errors no one can be 100% sure of the result of any statistical test.

Different references can be used to assess traceability:

- Certified Reference Materials (CRM)
- Intercomparison exercises
- Quality control samples (spiked samples)

The highest level of traceability is obtained using CRM and data from intercomparison exercises. In antidoping control, only CRM for a few analytes are available. Thus, participation in intercomparison exercises is strongly recommended to have data of high metrological quality. When even intercomparison exercises are not available, QC samples prepared in the laboratory can be used as references, although the metrological quality of the data is lower

In the following pages, the estimation of u_{TRAC} considering the use of CRM and data from intercomparison exercises is described.

2.2.1. Use of certified reference materials

The traceability is assessed by comparing the reference value of the CRM with the mean value of concentration obtained by the laboratory after repeated analysis of the CRM, using a Student *t*-test. If the difference is not statistically significant, the results obtained by the laboratory are traceable to the reference used and, hence, no significant bias is detected.

The u_{TRAC} is estimated by combining the variance of the reference value and the variance of the laboratory mean:

(2) $$u_{\mathrm{TRAC}}^2 = u_{\mathrm{CRM}}^2 + u_x^2$$

The u_{CRM} is calculated from the data of the certificate of analysis of the CRM: $u_{CRM} = U_{\mathrm{CRM}}/k$, where U is the expanded uncertainty of the reference value and k is the coverage factor (normally k=2). To have a best estimate of the intermediate precision, u^2_x may be calculated using Eq (1), where p_e and n_e are substituted by p and n, meaning the number of runs and replicates per run in which the CRM has been analyzed.

The standard uncertainty u_{TRAC} may be expressed as relative standard uncertainty by dividing by the concentration and multiplying by 100, $u_{TRAC(\%)}$.

2.2.2. Use of the results of intercomparison exercises

When using data from intercomparison exercises, the assessment of traceability is performed by comparing the mean value obtained by the laboratory with the consensus mean of the intercomparison exercise by means of hypothesis tests (Student *t*-test).

The u_{TRAC} is estimated by combining the variance of the consensus value and the variance of the laboratory mean:

(3) $$u_{\mathrm{TRAC}}^2 = \frac{s_{\mathrm{cons}}^2}{n_{\mathrm{cons}}} + u_x^2$$

where, s_{cons} is the standard deviation of the results of the intercomparison exercise and n_{cons} is the number of participating laboratories. To have a best estimate of the intermediate

precision, u^2_x may be calculated using Eq (1), where p_e and n_e are substituted by p and n, meaning the number of runs and replicates per run in which the sample of the intercomparison exercise has been analyzed.

The standard uncertainty u_{TRAC} may be expressed as relative standard uncertainty by dividing by the concentration and multiplying by 100, $u_{TRAC(\%)}$.

2.3. Uncertainty associated to preprocessing steps

This term should include the sources of uncertainty of the pre-processing steps that are applied to the routine samples but have not been applied to the QC sample and, hence, have not been included in the term u_{PREC}.

2.4. Uncertainty associated to other sources

The uncertainty associated to other sources not previously considered should be included in this term. For example, uncertainty associated to sample inhomogeneity and instability, matrix variability and uncertainty due to factors that have not been representatively varied during estimation of u_{PREC}.

2.5. Combined standard uncertainty:

After the estimation of the individual components of uncertainty, the next step is to combine the standard uncertainties to obtain the combined standard uncertainty.

(4) $$u^2_{total} = u^2_{PREC} + u^2_{TRAC} + u^2_{PRE-PRO} + u^2_{OTHER}$$

Equation 4 may be expressed in relative terms:

(5) $$u^2_{total}\ (\%) = u^2_{PREC}(\%) + u^2_{TRAC}(\%) + u^2_{PRE-PRO}(\%) + u^2_{OTHER}(\%)$$

2.6. Expanded uncertainty.

The combined standard uncertainty has to be multiplied by a coverage factor (k) to calculate the expanded uncertainty (U). The expanded uncertainty defines the interval Result±U which is expected to include a large fraction of the values reasonably attributable to the measurand. For most purposes it is recommended that k is set to 2. For a normal distribution and assuming that the number of degrees of freedom associated with the estimation of the individual components of uncertainty is reasonable, k=2 gives a 95% confidence that the true value of the measurand is within the interval Result±U.

For analytes with threshold concentrations, we only would like to know whether or not the concentration in a sample exceeds the threshold. In this case, the coverage factor may be obtained from the one-tailed table of critical values for the Student t-test. Thus, to calculate the decision limits (concentrations to consider the sample as exceeding the threshold), a coverage factor k=1.65 should be used for a level of confidence of 95% .

3. EXAMPLES

Uncertainties of different analytical methods have been estimated using the procedure described in this paper. As an example of standard uncertainties at different concentration ranges, standard uncertainties for caffeine and 19-norandrosterone analysis are listed in Table 3.

For caffeine analysis, u_{PREC} was estimated using results of a QC sample with a nominal concentration of caffeine of 14 µg/ml, and the term u_{TRAC} was estimated using two types of references: intercomparison exercises (WAADS 2002 and Reaccreditation IOC 2002) and a spiked QC sample. For 19-norandrosterone analysis, u_{PREC} was estimated using results of a QC sample with a nominal concentration of 5 ng/ml, and the u_{TRAC} was estimated using two types of references: intercomparison exercises (WAADS 2001) and a spiked QC sample. In both cases, the uncertainty due to other factors (u_{OTHER}) was considered to be negligible.

As can be observed in Table 3, different values of u_{TRAC} were obtained depending on the reference used. When different possibilities to estimate u_{TRAC} are available, it is recommended

to use the values of u_{TRAC} obtained with the reference of highest metrological quality. In these cases, they are those obtained using data from intercomparison exercises.

Our results show that the standard uncertainty values of analytical procedures applied routinely by antidoping control laboratories are much lower than those proposed by other authors using Horwitz equation [9].

Table 3. Standard uncertainty values calculated for the measurement of caffeine and 19-noradrosterone.

Analyte	Standard uncertainty			
	u_{PREC} (%)	u_{TRAC} (%)		u_{TOTAL} (%)
		Source	Value	
Caffeine	3,4	WAADS 2002	3,5	4,9
		Re-accreditation IOC 2002	3,8	5,1
		QC sample	1,7	3,8
19-Norandrosterone	3,5	WAADS 2001	7,4	8,1
		QC sample	2,1	4,0

4. CONCLUSIONS

A global approach to evaluate the uncertainty (U) of measurement has been described.
The approach is compliant with ISO/IEC 17025 and it uses data from internal quality control, intercomparison exercises or certified reference materials.

Due to the lack of CRM in antidoping control, the existence of intercomparison exercises is important to have reference data of high metrological quality.

The procedure has been applied to different analytical methods and reasonable estimates of U have been obtained.

Results show that the overall U is dependent on the data considered.

A common protocol for estimating U should be implemented in antidoping control laboratories in order to have comparable data.

A common reporting of results should also be implemented to avoid a wrong impression of U.

5. REFERENCES

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