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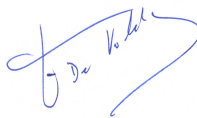

Laboratories Administration

Procedure

ESTIMATING MEASUREMENT UNCERTAINTY IN QUANTITATIVE CHEMICAL ANALYSIS

Comes into force :

- see date of approval for new methods for which an approval is applied for;
- at last on 01/01/2011 for all methods for which approval has been obtained.

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Addressees

All approved laboratories :

- Laboratories of the FASFC
- NRLs
- External laboratories

This procedure is available on the website of the FASFC (<http://www.favv.be> > Business Sectors > Laboratories > Approved Laboratories > Office circular).

For staff of the FASFC the documents are also available on the central server. Only versions of group A are considered valid.

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ESTIMATING MEASUREMENT UNCERTAINTY IN QUANTITATIVE CHEMICAL ANALYSIS

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ESTIMATING MEASUREMENT UNCERTAINTY IN QUANTITATIVE CHEMICAL ANALYSIS

1 Aim

The aim of this document is to present guidelines for estimating the measurement uncertainty in chemical analysis in order to allow all laboratories to estimate the uncertainty in an identical and consistent manner.

2 Scope

This procedure describes how the measurement uncertainty of test results of chemical analyses should be determined.

3 Legal and normative documents

The guidelines have been laid down in accordance with the requirements of standard NBN EN ISO/IEC 17025 clause 5.4.6.

4 Definitions and abbreviations

b and %b	bias / relative bias
C _{spiked}	the value at which a test sample was spiked
C _{cons}	the consensus value in a ring test (interlaboratory comparison exercise)
C _{ref}	the reference value
CRM	certified reference material
%d	relative duplicate difference
k	coverage factor to make the conversion from the relative measurement uncertainty %u to the expanded measurement uncertainty %U
%MS _{bias}	relative mean square of the bias
R	reproducibility (= 2,8 s _R)
%R _{mean}	the relative mean range (for duplicates : the mean value of the relative duplicate differences %d between two paired values)
RSD	the relative standard deviation, calculated as $RSD = 100 * s/X$ where s = standard deviation and X = measured value. RSD is expressed as a %.
RSD _{bias}	the relative standard deviation of the bias
RSD _{CC}	the relative standard deviation as deduced from the control chart
s	the standard deviation
s _{CRM} and RSD _{CRM}	the (relative) standard deviation deduced from the control chart of a CRM
s _r and RSD _r	the (relative) standard deviation under repeatability conditions
s _R and RSD _R	the (relative) standard deviation under reproducibility conditions
u _{bias} and %u _{bias}	the (relative) measurement uncertainty on the bias
u _c	the combined measurement uncertainty
u _{Rw} and %u _{Rw}	the (relative) measurement uncertainty on the within-lab reproducibility
u _{Cref} and %u _{Cref}	the (relative) measurement uncertainty related to the reference value of a CRM
%u	the relative measurement uncertainty, $\%u = 100 * u/X$ with X = the

	measured value. %u is expressed as a %.
%U _{spiked}	the relative measurement uncertainty on the spiked value in a recovery experiment
%U _{Cref, spiked}	the relative measurement uncertainty on the standard solution used in a recovery experiment
%U _{Cons}	the relative measurement uncertainty of the consensus value in a ring test
%U _{ref}	the relative measurement uncertainty of all consensus values in several ring tests
%U _p	the relative bias of the pipette volume as used in a recovery experiment
%U _v	the relative measurement uncertainty of pipetting in a recovery experiment
U and %U	the (relative) expanded measurement uncertainty

5 General considerations

In principle, two approaches may be used when calculating the measurement uncertainty of a test result : the 'Bottom-up' approach in which all possible sources of variation of the result are listed separately and the contribution of each source to the measurement uncertainty is estimated, and the 'Top-down' approach which is based upon a statistical evaluation of the test results from samples that have undergone the entire analytical process.

Since it is difficult in a 'Bottom-up' approach of a chemical analysis to establish all possible sources of variation, the 'Top-down' approach was chosen in this document.

The expanded measurement uncertainty **U** is described as being twice the combined measurement uncertainty **u_c**. This is based on the hypothesis that the combined measurement uncertainty has enough degrees of freedom (= has been deduced from a sufficient number of measured values) to allow the use of the rounded off value of 2,0 of the *t*-distribution for determining the 95 % confidence interval.

The calculations are to be made with a sufficient number of significant figures ; there is no rounding off before the final step of the calculation of the expanded measurement uncertainty **U**.

6 Top-down approach based on trueness and reproducibility

6.1 General considerations

Several approaches may be used when calculating the expanded uncertainty **U** depending on the available data. The three main approaches are :

- using data from the original validation of the standardised method applied,
- using data obtained from ring tests,
- using data obtained within the laboratory while applying the method in routine.

The advantage of the first approach is that the expanded measurement uncertainty **U** may be deduced from the results of the ring test used for the initial validation of the standardised

method. In that case, $U = 2s_R$ (s_R being the standard deviation of reproducibility). This means that the contribution of the spread between laboratories is already included in s_R . The disadvantages of this approach are that it may be used only if the method is conducted **exactly** as described in the standard, that it must be possible to prove that the in-house bias is negligible and that for routine tests, the data on the control charts should always be within the limits for the repeatability r (duplicate estimations) and the reproducibility R (in the long term). An extra disadvantage is that the in-house measurement uncertainty may be better than the uncertainty deduced from the validation so that the in-house measurement uncertainty is, in fact, overestimated. In addition, it is obvious that this may be applied only when data of such a validation are available.

The second approach – expanded measurement uncertainty U from ring tests in which the laboratory participated – also has the advantage that the distribution between laboratories is already included in the measurement uncertainty. U is then calculated in a similar way as $U = 2s_R$, with s_R being the standard deviation of the reproducibility from the ring tests. This approach may be used only if the individual laboratory has performed well at the ring tests. Again, the disadvantage is that the in-house measurement uncertainty may be better than the one deduced from the ring tests, so that the individual measurement uncertainty may be overestimated. This may, again, be applied only when ring tests are available for the method, matrix and parameter concerned.

The third approach – expanded measurement uncertainty U based upon data obtained within the individual laboratory – offers some advantages when compared to the other approaches : the result refers to the method such as it is actually applied in the laboratory (i.e. including the changes with respect to the standard) and it is possible to use results that are obtained from routine tests (control charts, duplicate differences, and so on). When it is preferable to include the results of ring tests, this can be done. And finally, this approach makes it possible to have a better understanding of the relative importance of the different sources of measurement uncertainty.

The third approach will be further developed in this procedure.

6.2 Background

Two factors are important when estimating the expanded measurement uncertainty U from data obtained in the individual laboratory : the trueness or bias and the precision or within-lab reproducibility. The bias may be considered as the systematic error and the precision may be considered as the random error of a measurement.

The bias may, in its turn, be divided into b , the value of the bias, on the one hand, and u_{bias} , the uncertainty linked to that value. In addition to these two components linked to the bias, one must also take into account the u_{Rw} , the uncertainty arising from the analytical process itself characterised by the within-lab reproducibility (Rw referring to the within-lab Reproducibility). It is on the basis of these three components – one systematic and two random – that the expanded uncertainty U is calculated.

There are several ways in which the bias components **b** and **u_{bias}** may be calculated.

In the method of quadratic combination the expanded measurement uncertainty **U** is considered as the sum of two random components **u_{bias}** and **u_{Rw}**. This means that, when there is a significant bias, a correction is made for this bias. When there is no significant bias or when the bias is too small or when no correction is made for the bias, the value of it is included in **u_{bias}**, the uncertainty of the bias. The equation for the expanded measurement uncertainty **U** is then (with a coverage factor k=2) :

$$U = 2\sqrt{(u_{bias}^2 + u_{Rw}^2)}.$$

Note : the **u_{bias}** (the uncertainty on the bias) must also be taken into account when the bias does not differ significantly from 0. The bias is estimated and **u_{bias}** characterises the uncertainty of this estimation. Adopting a value of 0 for the bias **b** then results in shifting the reference value and that has no effect on the dispersion around this value.

The estimation of the measurement uncertainty comprises the following steps :

- find the within-lab reproducibility,
- find the bias and the dispersion around the bias,
- calculate the overall measurement uncertainty by adding up all factors.

The calculations may be done with the absolute values of the standard deviations **s** as well as with the relative values (**RSD**, the relative standard deviation). The relation between these two is

$$RSD = 100 * s/X$$

with **X** being the value of the parameter.

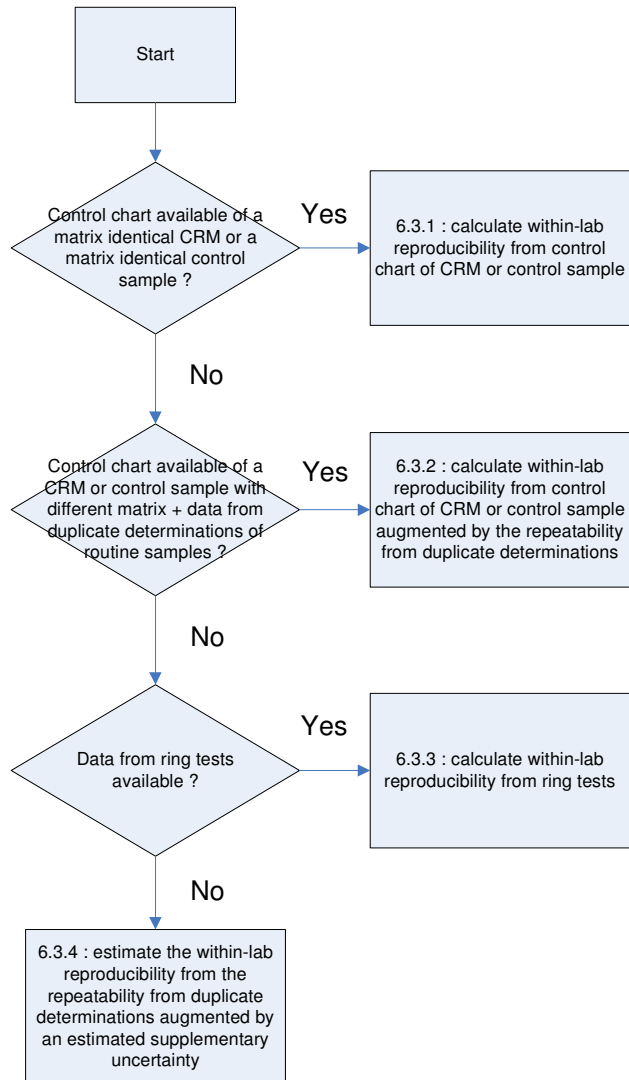
Example : if the standard deviation **s** of a measurement is 2,4 mg/kg at level **X** = 200 mg/kg, then $RSD = 100 * 2,4/200 = 1,2\%$.

The advantage of using relative values is that results obtained at different concentration levels (e.g. different CRM) are easy to combine with each other. That is the reason why the relative values will be used further on.

6.3 Within-lab reproducibility

The relative **within-lab reproducibility** $\%u_{RW}$ is a measure of the dispersion of the test results within one laboratory over a longer period of time. This longer period of time is important since the effect of some factors (different analysts, different apparatus if available, different batches of reagents/standards, different times, different environmental conditions,...) on the measurement uncertainty become only apparent after a longer period of time.

Depending on the data that are available there are different ways to estimate this parameter. These different ways are presented below, **in order of decreasing importance**, which implies that one should use the first procedure for which the data are available.



6.3.1 Within-lab reproducibility from the control chart of a CRM or from a matrix identical control sample

The CRM or the control sample must undergo the entire analytical process and **be representative for the matrix of the routine samples**. When these requirements are met, the relative variance of the within-lab reproducibility is equal to RSD_{CC}^2 , the relative variance as it is deduced from the control chart of the CRM or the control sample :

$$\%u_{Rw}^2 = RSD_{CC}^2$$

Example : from the control chart of a CRM a mean value $\bar{X} = 30,5$ mg/kg and a standard deviation $s = 1,52$ mg/kg were calculated.

The relative standard deviation $RSD_{CC} = 100 \cdot 1,52 / 30,5 = 4,98\%$ and the relative variance $RSD_{CC}^2 = (4,98)^2 = 24,84 = \%u_{Rw}^2$.

6.3.2 Within-lab reproducibility from the control chart of a CRM or from a control sample completed by data of duplicate analyses on routine samples

This method is appropriate when the CRM or the control sample used **are not representative for the matrices of the routine samples**. Since in such cases the within-lab reproducibility deduced from the control chart may underestimate the actual dispersion, the within-lab reproducibility is increased by the repeatability estimated from duplicate determinations of routine samples. The way to proceed is as follows :

- quantify the RSD_{CC}^2 , the relative variance of the control sample from the control chart,
- calculate the relative range $\%d$ (relative duplicate differences) of each routine sample as follows :

$$\%d = 100 \cdot |x_1 - x_2| / \bar{X}$$

where x_1 and x_2 are the individual measured values of the duplicate determination and \bar{X} is the average of these values.

- calculate the mean relative range $\%R_{mean}$, the mean of the relative duplicate differences $\%d$,
- calculate from $\%R_{mean}$ the relative standard deviation of the repeatability RSD_r :

$$RSD_r = \%R_{mean} / 1,128$$

- the relative variance of the measurement uncertainty associated with the within-lab reproducibility then equals the sum of both variances :

$$\%u_{Rw}^2 = RSD_{CC}^2 + RSD_r^2$$

Example : a control chart of a CRM is available for the same analytical parameter as for the routine samples but with a different matrix. The statistical parameters calculated from this control chart are : mean value $\bar{X} = 40,5$ mg/kg and standard deviation $s = 0,84$ mg/kg. From these values it follows that $RSD_{cc} = 100 \cdot 0,84 / 40,5 = 2,07\%$ and $RSD_{cc}^2 = (2,07)^2 = 4,30$.

From duplicate determinations of routine samples the relative duplicate differences %d are calculated (cfr. table, x_1 and x_2 are duplicate values, \bar{X} is the mean value of the duplicates, $|x_1 - x_2|$ is the absolute difference between the duplicates and %d is the relative difference $100 \cdot |x_1 - x_2| / \bar{X}$).

Remark : in practice use more duplicates than in the table below !

x_1	x_2	\bar{X}	$ x_1 - x_2 $	%d
45,2	40,1	42,65	5,1	11,96
62,8	60,4	61,60	2,4	3,90
83,5	87,6	85,55	4,1	4,79
59,0	53,3	56,15	5,7	10,15
39,1	43,5	41,30	4,4	10,65
25,5	28,4	26,95	2,9	10,76
Relative mean range % R_{gem} :				8,70

The relative mean range % R_{gem} (the mean of column %d) = 8,70%.

RSD_r then becomes % $R_{gem} / 1,128 = 8,70 / 1,128 = 7,72\%$ and $RSD_r^2 = (7,72)^2 = 59,51$.

% u_{Rw}^2 is the sum of the two variances : % $u_{Rw}^2 = 4,30 + 59,51 = 63,82$.

6.3.3 Within-lab reproducibility from ring tests

This method may be used *when there is no internal information on the basis of which the within-lab reproducibility may be estimated*. As an estimation of the within-lab reproducibility the relative value of the reproducibility of the ring test(s), RSD_R , may then be used:

$$\%u_{Rw}^2 = RSD_R^2$$

If the results of several ring tests have to be combined, it is preferable to take the weighted average of all RSD_R^2 :

- determine the RSD_R^2 for each ring test and multiply it by (m-1), the number of degrees of freedom for that ring test (m-1 is the number of participating laboratories -1),
- calculate the combined RSD_R^2 as the sum of all terms and divide it by the total number of degrees of freedom (= total number of participating laboratories - number of ring tests).

Example : the laboratory participated in 6 ring tests with the following results (Conc. = reference value, s_R = standard deviation of the reproducibility, m = number of participants, $RSD_R = 100 \cdot s_R / \text{Conc.}$) :

Conc. (mg/kg)	s_R (mg/kg)	m	RSD_R (%)	$RSD_R^2 \cdot (m-1)$
42,3	5,6	20	13,24	3330
51,1	7,8	18	15,26	3961
65,9	9,0	15	13,66	2611
55,3	8,1	21	14,65	4291
72,8	6,3	18	8,65	1273
31,2	8,2	21	26,28	13815
Total number of participants :		113	Sum :	29281

The number of degrees of freedom is the total number of participants minus the number of ring tests = $113 - 6 = 107$.

The combined $RSD_R^2 = 29281/107 = 273,65 = \%u_{RW}^2$.

6.3.4 Within-lab reproducibility from routine samples only

When ***no control sample is available*** and there are ***no data from ring tests*** either, the within-lab reproducibility may, in the last resort, be estimated on the basis of the duplicate differences of routine samples. This method should be avoided whenever possible !

Starting from the duplicate differences the relative range $\%d$ is calculated first followed by the mean relative range $\%R_{\text{mean}}$. Then, the relative standard deviation of the repeatability RSD_r is deduced from the results (see above).

Because the repeatability is an underestimation of the within-lab reproducibility, an additional component, RSD_{Rb} , must be taken into account which is related to the between days variation, a.s.o.. To estimate this component, all possible additional data on the analysis must be used; in their absence (e.g. when a new analysis is being introduced) one may, if need be, use an 'educated guess' based upon experiences with similar methods etc.

The relative variance of the within-lab reproducibility then equals the sum of the two variances:

$$\%u_{RW}^2 = RSD_{Rb}^2 + RSD_r^2$$

Example : from duplicate determinations (see table in 6.3.2) values of $\%R_{\text{gem}} = 8,70$ and there from $RSD_r = 8,70/1,128 = 7,72\%$.

From a control chart of a similar analysis the value of the additional uncertainty component is estimated as $RSD_{Rb} = 2,5\%$.

From this it follows that $\%u_{RW}^2 = (7,72)^2 + (2,5)^2 = 65,76$.

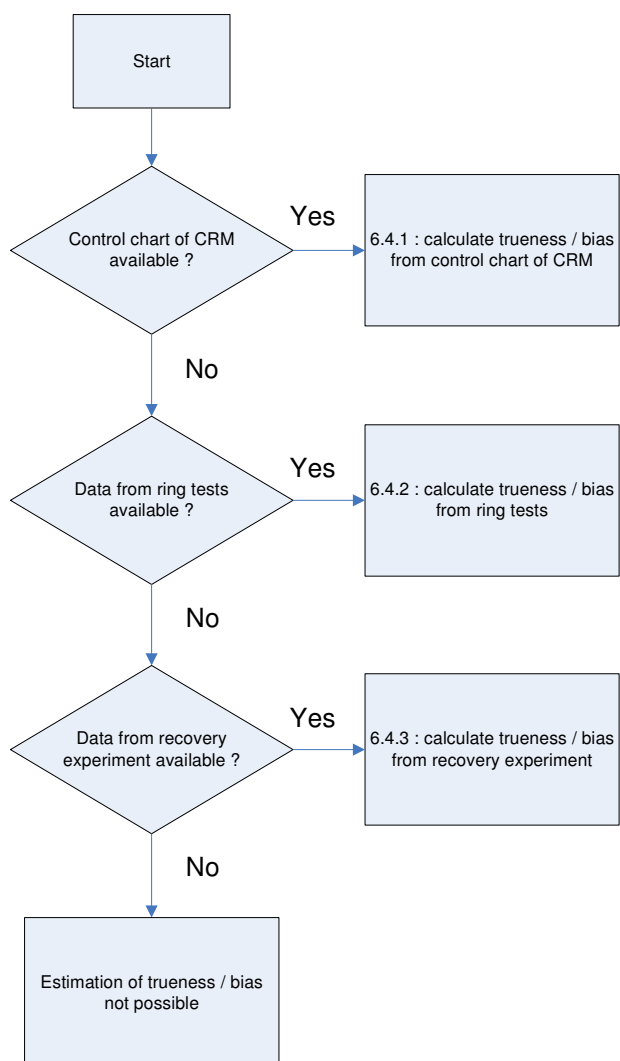
6.4 Trueness / bias

The **trueness** is the closeness of agreement between the average value obtained from a large number of measured values and the 'true' value. This last parameter is however not known and is preferably estimated by means of a certified reference material (CRM); the certified value is then considered as the 'true' value. The results obtained from the analysis of the CRM are compared to the certified values and the bias is calculated on the basis thereof.

When no CRM is available, the bias may be deduced from the data on ring tests. In that case, the 'consensus value' of the material that was analysed is considered to be the 'true' value.

Another method consists of estimating the bias on the basis of recovery experiments of spiked samples. The spiked amount shall then be a surrogate 'true' value.

These possibilities are summarised in the flow sheet alongside.



6.4.1 Estimating trueness / bias from the control chart of a certified reference material (CRM)

A CRM is characterised by 2 parameters : C_{ref} , the certified reference value and U_{Cref} , the expanded measurement uncertainty of this reference value. The reference value is used to determine the value of the bias, U_{Cref} must be taken into account when estimating the uncertainty of the bias.

The procedure consists of the following steps :

- Calculate from the given expanded measurement uncertainty U_{Cref} the relative measurement uncertainty $\%u_{\text{Cref}}^2$ as

$$\%u_{\text{Cref}} = (100 * U_{\text{Cref}}) / (k * C_{\text{ref}})$$

where **k** is the coverage factor used for the calculation of **U_{Cref}** (this is mentioned on the certificate and is in most cases 2) and **C_{ref}**, the certified reference value. The relative measurement uncertainty **%u_{Cref}** is then a measure of the uncertainty related to the fact that the 'true' value of the CRM is not known but was estimated on the basis of analyses.

- conduct at least 6 analyses on the CRM and calculate from the results the average value **X** and the standard deviation **s_{CRM}**; then find the relative standard deviation **RSD_{CRM}**,
- calculate the relative bias **%b** from the certified value **C_{ref}** as well as the average value **X** as

$$\%b = 100 * (X - C_{ref})/C_{ref}$$

- calculate the relative variance of the bias as

$$RSD_{bias}^2 = RSD_{CRM}^2/n$$

where **n** is the number of values from which the average value **X** was calculated.

- if the relative bias **%b** is not significant or if it is small (what remains of the bias after correction by a constant value), it is included in **%u_{bias}**² :

$$\%u_{bias}^2 = \%b^2 + RSD_{bias}^2 + \%u_{Cref}^2$$

Note : if the CRM used is not representative for the matrix of the routine samples, one should see to it that the dispersion caused by the various matrices is included in **%u_{Rw}** . Otherwise, the measurement uncertainty will be underestimated !

Example : for the determination of the bias a CRM is used for which the certificate states: **C_{ref}** = 425,0 mg/kg and **U_{Cref}** = 9,0 mg/kg using **k** = 2.

From these data **%u_{Cref}** is calculated as $(100*9,0)/(2*425,0) = 1,06\%$.

This CRM was analysed 12 times; the mean value of the results was **X** = 427,5 mg/kg and **s_{CRM}** = 18,2 mg/kg, from which it follows that **RSD_{CRM}** = $100*18,2/427,5 = 4,26\%$

The relative bias **%b** = $100*(427,5 - 425,0)/425,0 = 0,59\%$.

The relative variance of the bias **RSD_{bias}**² = **RSD_{CRM}**²/n = $(4,26)^2/12 = 1,51$.

The relative bias **%b** of 0,59% is considered to be small and so can be included in the formula for **%u_{bias}**² : **%u_{bias}**² = $(0,59)^2 + 1,51 + (1,06)^2 = 2,98$.

6.4.2 Estimating trueness / bias from ring tests

The results of at least 6 ring tests are required to calculate the bias with a sufficient level of confidence.

Each ring test is characterised here by 3 parameters : the consensus value C_{cons} given by the organiser of the test, the value x that the individual laboratory has obtained and the reproducibility standard deviation s_R as deduced from the ring test. The bias $\%b$ is then estimated from the differences between the consensus value and the result of the individual laboratory.

The procedure consists of the following steps :

- find for each ring test the relative bias $\%b_i$ as

$$\%b_i = 100 * (x_i - C_{cons, i}) / C_{cons, i}$$

- calculate the relative mean square of the bias $\%MS_{bias}$ as :

$$\%MS_{bias} = \Sigma \%b_i^2 / n$$

where n is the number of ring tests.

- calculate for each ring test the relative uncertainty on the consensus value C_{cons} from the reproducibility of the ringtest RSD_R and the number of participants of this ring test m :

$$\%u_{cons} = RSD_R / \sqrt{m}$$

- calculate the mean relative uncertainty on all n consensus values as

$$\%u_{ref} = \Sigma \%u_{cons} / n$$

- the relative variance of the bias, $\%u_{bias}^2$, is then calculated as :

$$\%u_{bias}^2 = \%MS_{bias} + \%u_{ref}^2$$

Example : the laboratory participated in 7 ring tests with the following results (C_{cons} is the consensus value as given in the report of the organiser; x is the result of the laboratory):

nr.	C_{cons} (mg/kg)	x	bias	$\%b$	$\%b^2$
1	1,05	1,10	0,05	4,76	22,68
2	2,23	2,18	-0,05	-2,24	5,03
3	1,48	1,54	0,06	4,05	16,44
4	1,66	1,65	-0,01	-0,60	0,36
5	2,46	2,50	0,04	1,63	2,64
6	2,03	1,99	-0,04	-1,97	3,88
7	1,88	1,95	0,07	3,72	13,86
Sum :					64,89

The sum of the squares $\Sigma \%b^2$ amounts to 64,89; the mean sum of squares $\%MS_{\text{bias}} = 64,89/7 = 9,27$.

In the next step the uncertainty of the consensus value C_{cons} has to be calculated for each ring test. This requires for each ring test the values of s_R and of the number of participants m (both given in the report from the organiser):

nr.	C_{cons} (mg/kg)	s_R	m	RSD_R	RSD_R/\sqrt{m}
1	1,05	0,35	15	33,33	8,61
2	2,23	0,42	18	18,83	4,44
3	1,48	0,38	20	25,68	5,74
4	1,66	0,38	20	22,89	5,12
5	2,46	0,56	23	22,76	4,75
6	2,03	0,72	18	35,47	8,36
7	1,88	0,39	20	20,74	4,64
Mean = $\%u_{\text{ref}}$:					5,95

$$\%u_{\text{bias}}^2 = \%MS_{\text{bias}} + \%u_{\text{ref}}^2 = 9,27 + (5,95)^2 = 44,67.$$

6.4.3 Estimating trueness / bias from recovery experiments

The results of recovery experiments involving spiked samples are treated in a similar way as those involving ring tests except that the uncertainty on the spiked value must be calculated by means of a bottom-up method.

At least 6 results of recovery experiments are required to calculate the bias with a sufficient level of confidence.

Note : when experiments are conducted at various concentration levels that show important differences, it is preferable to calculate the bias for each level separately and to examine afterwards if a mean value can be used.

Each recovery experiment is characterised here by 3 parameters: the spike value C_{spiked} , the uncertainty on that value and value x of the analysis. The bias is then estimated from the differences between the value of the spike and the result of the analysis.

The procedure consists of the following steps :

- calculate for each recovery experiment i the relative bias $\%b_i$ as

$$\%b_i = 100 * (x_i - C_{\text{spiked}, i}) / C_{\text{spiked}, i}$$

- calculate the relative mean square of the bias $\%MS_{\text{bias}}$ as :

$$\%MS_{\text{bias}} = \Sigma \%b_i^2 / n$$

where **n** is the number of recovery experiments.

- In principle, $\%u_{\text{spiked}}^2$, the uncertainty on the spike volume and $\%u_{\text{Cref,spiked}}^2$, the uncertainty of the standard solution used for spiking, must be determined. Both components are usually small when compared to $\%MS_{\text{bias}}$ and may then be neglected , in which case :

$$\%u_{\text{bias}}^2 = \%MS_{\text{bias}}$$

- Note : if both components must be determined, this shall be done as follows :
 - ⇒ calculate for each recovery experiment $\%u_{\text{spiked}}^2$, the relative uncertainty on the spike volume, from the bias of the pipette volume $\%u_p^2$ (the systematic deviation of the pipette volume, to be deduced from the specifications of the manufacturer) and the precision (repeatability) of pipetting $\%u_v^2$:

$$\%u_{\text{spiked}}^2 = \%u_p^2 + \%u_v^2$$

- ⇒ calculate $\%u_{\text{Cref,spiked}}^2$, the uncertainty of the standard solution used for spiking, from the certificate of the supplier,
 - ⇒ then the variance of the bias, $\%u_{\text{bias}}^2$, is calculated as

$$\%u_{\text{bias}}^2 = \%MS_{\text{bias}} + \%u_{\text{spiked}}^2 + \%u_{\text{Cref,spiked}}^2$$

Example : a series of 7 recovery experiments was performed with the following results (C_{spiked} is the value of the spike; x is the laboratory result):

nr.	C_{spiked} (mg/kg)	x	bias	%b	%b ²
1	4,9	5,1	0,2	4,08	16,66
2	5,1	5,0	-0,1	-1,96	3,84
3	5,0	5,1	0,1	2,00	4,00
4	5,0	5,1	0,1	2,00	4,00
5	4,9	4,8	-0,1	-2,04	4,16
6	5,0	5,1	0,1	2,00	4,00
7	5,0	5,0	0,0	0,00	0,00
Sum :					36,67

The sum of the squares $\Sigma \%b^2$ is 36,67; the mean sum of squares $\%MS_{\text{bias}} = 36,67/7 = 5,24$.

As this was a first series of tests, it is not known yet what the values of $\%u_{\text{spiked}}^2$ and $\%u_{\text{Cref,spiked}}^2$ are and whether they are negligible. Therefore both have to be calculated with the bottom-up method.

For each experiment the same pipette was used for distributing 5 ml. The bias on the pipette volume u_p is 0,05 ml and pipetting has a precision (standard deviation) u_v of 0,02 ml.

From these data it follows that $\%u_p = 100 \cdot 0,05/5 = 1,0\%$ and $\%u_v = 100 \cdot 0,02/5 = 0,4\%$. This results in $\%u_{\text{spiked}}^2 = \%u_p^2 + \%u_v^2 = (1,0)^2 + (0,4)^2 = 1,16$.

When preparing the spiking solution a standard solution of 5,00 mg/kg was used; according to the certificate the uncertainty (standard deviation) on this value is 0,02 mg/kg. It follows that $\%u_{\text{Cref,spiked}} = 100 \cdot 0,02/5 = 0,40\%$ and $\%u_{\text{Cref,spiked}}^2 = (0,40)^2 = 0,16$.

$$\%u_{\text{bias}}^2 = \%MS_{\text{bias}} + \%u_{\text{spiked}}^2 + \%u_{\text{Cref,spiked}}^2 = 5,24 + 1,16 + 0,16 = 6,56.$$

In this case $\%u_{\text{spiked}}^2 + \%u_{\text{Cref,spiked}}^2 = 1,16 + 0,16 = 1,32$ is not negligible with reference to $\%MS_{\text{bias}}$.

6.5 Estimating the expanded measurement uncertainty U

For calculating the expanded measurement uncertainty $\%U$ the basic equation is :

$$\%U = 2\sqrt{(\%u_{\text{bias}}^2 + \%u_{\text{RW}}^2)}.$$

where $\%u_{\text{bias}}^2$ has been determined in 6.4 and $\%u_{\text{RW}}^2$ has been determined in 6.3.

Example : in 6.3.2 a value $\%u_{\text{RW}}^2 = 63,82$ was found and in 6.4.1 a value of 2,98 for $\%u_{\text{bias}}^2$. The expanded (relative) measurement uncertainty then becomes $\%U = 2\sqrt{(2,98 + 63,82)} = 16,35\%$ or rounded 16%.

6.6 Measurement uncertainty at different concentrations

Calculate the measurement uncertainty at low, medium and high values.

	Range	How ?
low	From LOQ to 0,5x norm	Report uncertainty at LOQ level as an absolute value (= $\%U \cdot \text{LOQ}/100$)
medium	From 0,5x norm to 1,5x norm	Report as an absolute value (= $\%U \cdot \text{value}/100$)
high	Above 1,5x norm	Report as an absolute value (= $\%U \cdot \text{value}/100$)

When there is no norm, the same approach is followed with the data (3 validation levels) from the validation report of the method.

7 Checking the calculation

The information in the table below makes it possible to check if the measurement uncertainty obtained is realistic :

Data to be checked	Measurement uncertainty
Imposed by performance criteria	$\%U < 1 - \text{max recovery} + 2 \times \text{RSD}_{\text{Rmax}}$
Method comparing or laboratory comparing studies (take into consideration only laboratories with identical method)	Is only exceptionally more than twice as low as the interlaboratory reproducibility for that method.
A random laboratory comparing study	Is only exceptionally considerably lower than the interlaboratory reproducibility obtained.
Repeatability data or duplicate data	Is exceptionally lower than $4.5 \times \text{RSD}_r$
Reproducibility data or control charts	Is exceptionally lower than $3 \times \text{RSD}_{\text{RW}}$
Z scores in interlaboratory comparisons	<ul style="list-style-type: none"> The average of the z scores is 0 and is never higher than 2-3 : The order of magnitude of the measurement uncertainty is 2 x the target RSD of the organiser. The average of the z scores is significantly different from 0 and regularly higher than 1 or 2 : The measurement uncertainty is never lower than 2 x the target RSD of the organiser.
Qualification data for critical devices, operators	Is considerably higher than the prescribed repeatability / reproducibility criteria.

8 Reference to relevant procedures, guidelines, documents, forms or lists

- Template for the calculations according to the procedure above : LAB-P-508-Measurement-uncertainty-v.01-annex-F-001_2008-11-04_en.xls
- Syllabus : 'Determination of the measurement uncertainty for analysis of food and feed stuffs' P. Vermaercke (5 and 7 September 2007)
- Nordtest Report TR 537.