ANNEX B-4: TECHNICAL NOTE ON THE DETERMINATION OF MEASUREMENT UNCERTAINTY

Appendix 1

1 Protocol for evaluation of measurement uncertainty from in-house quality control measurements

Estimating measurement uncertainty can be done by identifying all possible sources of uncertainty associated with a method, quantifying uncertainty components (estimating the magnitude of the uncertainty associated with each potential source), and calculating total uncertainty by combining the individual uncertainty components following appropriate mathematical rules ("bottom-up" approach, see, e.g., EURACHEM/CITAC Guide (EURACHEM/CITAC, 2000)).

Another approach uses data from routinely undertaken internal quality control measurements, e.g., results of the replicate analyses of certified reference materials (CRM), without identifying all potential sources of uncertainty associated with the method and quantifying uncertainty components ("top-down" approach).

This document provides guidance on how uncertainty estimates for a method can be obtained from replicate quality control measurements of a representative certified reference material. It is assumed that these measurements comprise the total analytical procedure and have been carried out with appropriate frequency and during a sufficiently long time period. In that way, it can be ensured that most relevant uncertainty components associated with the method will be covered (starting from the laboratory sample or analysis sample, excluding contributions associated with sampling and sample handling prior to analysis).

Following these assumptions total uncertainty of the method is composed of:

a contribution from the precision of the method, and
a contribution from trueness of the method (recovery of the analyte from the CRM).

Both contributions can be easily quantified using data from routinely performed quality control measurements.

The mathematical equations 1 to 6, outlined below, can be applied for calculating measurement uncertainty on the condition that the relative uncertainty of measurement of the method expressed in percent is approximately constant within the working range.

This condition might apply in most cases as long as the lower limit of the working range is well above the limit of quantification (see Part 3, below).

This implies further that:

the precision expressed as relative standard deviation (RSD) is approximately constant within the working range considered. This denotes that the absolute standard deviation increases proportionally with increasing concentration of the analyte in the sample;

the relative uncertainty of the recovery of the analyte from the CRM $u(\overline{R}_m)_{rel}$ is independent of the concentration of the analyte. This denotes it is approximately constant, e.g., ± 5% of the determined concentration.

Note: If this condition does not apply, modified mathematical equations adjusted to the specific circumstances need to be used. For details see Barwick and Ellison (2000).

Then, the relative combined uncertainty, $u_c(y)$, of the method is obtained using the following equation:

$$u_c(y) = \sqrt{RSD^2 + u(\overline{R}_m)_{rel}^2} \qquad \text{Eq. 1}$$

The relative standard deviation is given by:

$$RSD = \frac{s_{obs}}{\overline{C}_{obs}} \qquad \text{Eq. 2}$$

where $\overline{C}_{obs}$ is the mean of replicate analyses of the CRM and s_{obs} is the standard deviation of the results from the replicate analyses of the CRM.

The relative uncertainty of the recovery, $u(\overline{R}_m)_{rel}$, is calculated using:

$$u(\overline{R}_m)_{rel} = \frac{u(\overline{R}_m)}{\overline{R}_m} = \sqrt{\left(\frac{s_{obs}^2}{n * \overline{C}_{obs}^2}\right) + \left(\frac{u(C_{CRM})}{C_{CRM}}\right)^2} \qquad \text{Eq. 3}$$

where C_{CRM} is the certified concentration of the analyte in the CRM, n is the number of replicates ($n \geq 10$) and $u(C_{CRM})$ is the standard uncertainty of the certified concentration for the CRM with a mean recovery, $\overline{R}_m$, given by:

$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{CRM}} \qquad \text{Eq. 4}$$

It is assumed that $\overline{R}_m$ does not differ significantly from 1 and, hence, no correction for recovery is made. To determine whether the recovery is significantly different from 1, a significance test is used. The test statistic t is calculated using the following equation:

$$t = \left|1 - \overline{R}_m\right| / u(\overline{R}_m) \qquad \text{Eq. 5}$$

If the degrees of freedom associated with $u(\overline{R}_m)$ are known, t is compared with the two-tailed critical value, t_{crit}, for the appropriate number of degrees of freedom at 95% confidence. If t is less than the critical value then $\overline{R}_m$ is not significantly different from 1.

If the degrees of freedom associated with $u(\overline{R}_m)$ are unknown, for example, if there is a contribution from the uncertainty in the certified value of a reference material, t is compared

with *k*, the coverage factor that will be used in the calculation of the expanded uncertainty (see Eq. 6) (Barwick and Ellison, 2000).

If $\left|1-\overline{R}_m\right| / u(\overline{R}_m) < k$, the recovery is not significantly different from 1.

If $\left|1-\overline{R}_m\right| / u(\overline{R}_m) > k$, the recovery is significantly different from 1 and results are corrected for recovery. Guidance on how to proceed is given in Barwick and Ellison (2000).

To calculate the combined uncertainty, $u_c(y)$, both relative standard uncertainties RSD and $u(\overline{R}_m)_{rel}$ are combined following Equation 1.

The expanded uncertainty, *U(y)*, is obtained by multiplying the combined standard uncertainty, $u_c(y)$, by an appropriate coverage factor, *k*, (Eq. 6). For most cases, a coverage factor of 2 is recommended, which gives an interval containing approximately 95% of the distribution of values:

$$U(y) = k * u_c(y) = 2 * u_c(y) \qquad \text{Eq. 6}$$

The result *y* of an analytical measurement should be stated together with the corresponding expanded uncertainty, *U(y)*, in the following form:

(result): $x \pm U$ [units]
The stated uncertainty is an expanded uncertainty, calculated using a coverage factor of 2. This corresponds approximately to the 95% confidence interval (EURACHEM/CITAC, 2000).

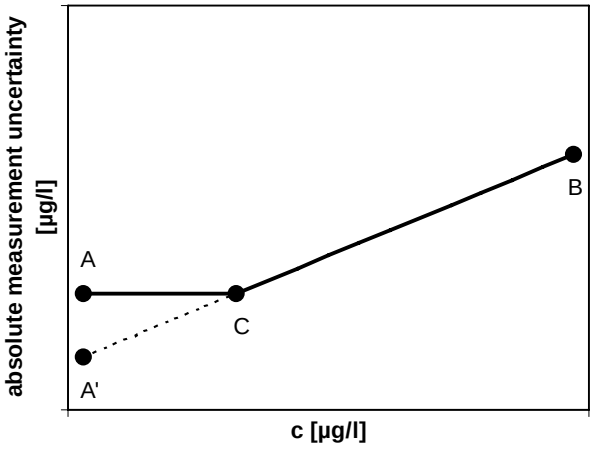
2 Estimation of measurement uncertainty using reproducibility data from interlaboratory studies

In principle, it is possible to use the relative reproducibility standard deviation, CV_R, obtained in intercomparison studies as a basis for estimating the uncertainty of a method in a particular laboratory, if there is no significant difference between the relative repeatability standard deviation seen in the interlaboratory study and that observed in the laboratory. If so, this indicates that the precision achieved in the particular laboratory is similar to that obtained by the participants of the interlaboratory study (EURACHEM/CITAC, 2000).

For estimating the laboratory`s expanded uncertainty, the relative reproducibility standard deviation, CV_R, obtained in the interlaboratory study is assumed to be an estimate of the combined standard uncertainty of the laboratory and multiplied with the coverage factor $k = 2$.

The uncertainty for a method, *U(y)*, obtained in that way can only be considered as a rough estimate for obtaining an idea about the order of uncertainty, but not replace estimating uncertainty from own measurements of, e.g., certified reference materials.

3 Transition to constant absolute uncertainty of measurement at low concentrations



A: lower limit of the working range
B: upper limit of the working range
C: threshold to be defined, below that the assumption of constant absolute uncertainty of measurement is accepted
--- shape of the function below C on the condition that relative uncertainty of measurement is constant over the whole working range

Figure B-4.1. Graphical representation of the absolute uncertainty of measurement as a function of analyte concentration.

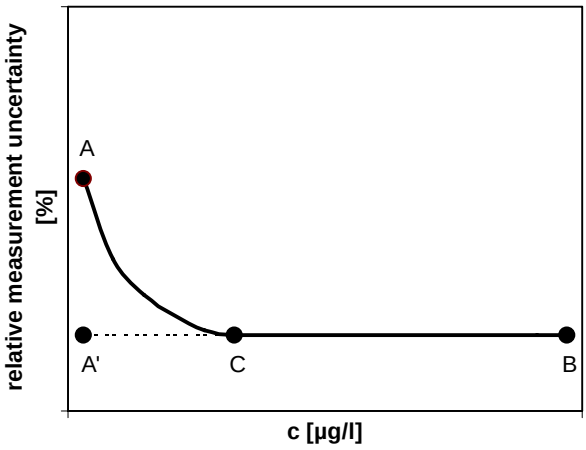


Figure B-4.2. Graphical representation of the relative uncertainty of measurement as a function of analyte concentration.

4 Introduction to additional uncertainty component in case of significant deviation from 1 of the recovery of the analyte from a CRM

If the recovery $\overline{R}_m$ of the analyte from the reference material differs significantly from 1 (*t*-test, $t \geq t_{crit}$), an additional uncertainty component is introduced.[1] Instead of Eq. 1, Eq. 7 and 8 apply.

$$u_c(y) = \sqrt{RSD^2 + u(\overline{R}_m)^2_{rel}} \qquad \text{Eq. 1}$$

[1] If the recovery $\overline{R}_m$ of the analyte from the reference material differs significantly from 1, the analytical procedure is to be checked for the reason of the bias and, where applicable, the method has to be modified. But, in some cases, if the uncertainty of the certified concentration of the analyte in the CRM is extremely small, significant differences in the *t*-test can be observed even when the recovery, $\overline{R}_m$, is close to 1.

$$u_c(y) = \sqrt{RSD^2 + u(\overline{R}_m)^2_{rel} + \Delta^2} \qquad \text{Eq. 7}$$

$$\Delta = \frac{\overline{C}_{obs} - C_{CRM}}{C_{CRM}} \qquad \text{Eq. 8}$$

where Δ is the relative deviation of the measured concentration in the CRM from the reference value.

Example 1: Estimation of measurement uncertainty using the results of replicate analyses of a CRM

During routine analyses of phosphate in seawater samples, a certified reference material was regularly analysed (30 times) as AQC sample over a period of three months. The certified phosphate concentration in the reference material was 2.43 ± 0.41 µmol l^{-1} and assumed to be representative for the working range of the method.

According to manufacturer's specifications, the confidence interval of the phosphate concentration in the CRM was calculated using the reproducibility standard deviation obtained in the certification interlaboratory study multiplied by three. Hence, the standard uncertainty of the phosphate concentration, $u_c(PO_4)$, in the CRM is given by 0.41 µmol l^{-1} / 3 = 0.14 µmol l^{-1}.

Note: Be aware that, depending on the producer of the CRM, different modes of calculation for the confidence interval of the certified concentration are in use. This must be taken into account when calculating the standard uncertainty of the certified concentration, $u_c(y)$, in the CRM.

Certified concentration of the phosphate in the reference material C_{CRM} *= 2.43 µmol* l^{-1}
Standard uncertainty of the certified phosphate concentration $u(C_{CRM})$ *= 0.14 µmol* l^{-1}

From the results of the replicate analyses of the CRM, the following values can be determined directly:

Mean of replicate analyses of the CRM, $\overline{C}_{obs}$ *= 2.34 µmol* l^{-1}
Standard deviation of the results from the replicate analyses of the CRM, s_{obs} *= 0.12 µmol* l^{-1}

Then, the relative standard deviation of the mean of the phosphate concentration, RSD_{PO4}, is given by:

$$RSD = \frac{s_{obs}}{\overline{C}_{obs}} = \frac{0.12 \ \ \mu mol l^{-1}}{2.34 \ \ \mu mol l^{-1}} = 0.051$$

and the recovery, $\overline{R}_m$, is given by:

$$\overline{R}_m = \frac{\overline{C}_{obs}}{C_{CRM}} = \frac{2.34 \ \ \mu mol l^{-1}}{2.43 \ \ \mu mol l^{-1}} = 0.963$$

To calculate the relative standard uncertainty of the recovery, $u(\overline{R}_m)_{rel}$, Equation 3 is used:

$$u(\overline{R}_m)_{rel} = \frac{u(\overline{R}_m)}{\overline{R}_m} = \sqrt{\left(\frac{s^2_{obs}}{n * \overline{C}^2_{obs}}\right) + \left(\frac{u(C_{CRM})}{C_{CRM}}\right)^2} = \sqrt{\left(\frac{0.12^2}{30 * 2.34^2}\right) + \left(\frac{0.14}{2.43}\right)^2} = 0.058$$

To test whether the observed recovery is significantly different from 1, a statistical significance test (*t*-test) is performed following Equation 5:

$$t = \frac{\left|1 - \overline{R}_m\right|}{u(\overline{R}_m)} = \frac{1 - 0.963}{0.056} = 0.661$$

If $t < k$ (coverage factor), it can be assumed that the recovery is not significantly different from 1. Since 0.661 is less than 2, the significance test indicates no significant difference between the observed recovery (0.963) and 1.

The relative combined standard uncertainty $u_c(PO_4)$ is than estimated as:

$$u_c(PO_4) = \sqrt{RSD^2 + u(\overline{R}_m)^2_{rel}} = \sqrt{0.051^2 + 0.058^2} = 0.077$$

Using the recommended coverage factor $k = 2$, the expanded uncertainty, $U(PO_4)$, is given by:

$$U(PO_4) = k * u_c(y) = 2 * 0.077 = 0.154$$

Result: the relative expanded uncertainty, $U(PO_4)$, for the determination of phosphate in seawater samples within the considered working range is 0.154 and 15.4%, respectively.

This denotes for a theoretical result of 10.0 µmol l^{-1} phosphate:

„Phosphate concentration: 10.0 ± 1.5 µmol l^{-1},
the stated uncertainty is an expanded uncertainty, calculated using a coverage factor of 2 (this corresponds approximately to the 95% confidence interval).”

Example 2: Estimation of measurement uncertainty using reproducibility data from interlaboratory studies

The results of the three QUASIMEME exercises on the determination of phosphate in seawater carried out in 2001 were as follows:

Relative reproducibility standard deviation: 4.67 / 4.47 / 6.30 %

Phosphate concentration - Assigned value: *9.71 / 13.08 / 1.88 µmol l^{-1}*

Using this information, the averaged relative reproducibility standard deviation expressed as coefficient of variation, $CV(PO_4)$, for the intercomparison study on phosphate determination in seawater is, which can be equated with combined standard uncertainty, $u_c(PO_4)$, is given by:

$$CV(PO_4) = \sqrt{\Sigma x_i^2 / n} = \sqrt{\left(4.67^2 + 4.47^2 + 6.30^2\right)/3} = 5.2\%$$

Using the recommended coverage factor $k = 2$, the expanded uncertainty, $U(PO_4)$, is 10.4%.

This result is in satisfactory agreement with the estimated expanded uncertainty, $U(PO_4)$, of 15.4% obtained by using the results of replicate analyses of a certified reference material.