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EVALUATION OF MEASUREMENT UNCERTAINTY
ANNEX 2.1

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Annex 2 to Guideline "Evaluation of Measurement Uncertainty"
PA/PH/OMCL (18) 145 (in its current version)

Estimation of measurement uncertainty using Top-down approach

Annex 2.1. Use of data from validation studies for the estimation of measurement uncertainty

The uncertainty of measurement can be estimated using validation data coming either from MAH documentation or from within-laboratory validation studies.

If all relevant uncertainty sources are taken into account in the experimental design of the validation study, the uncertainty of measurement can be estimated from uncertainty contribution associated with precision and bias estimates. The approach described in the Eurachem guide was used in this annex to determine whether the bias could be considered as significantly different from 0 or not. Depending on the significance of the bias and the laboratory policy, a correction to testing results using the mean recovery could be applied or not. In either case, the uncertainty associated with the determination of the bias remains an essential component of overall uncertainty and should be taken into account [1].

Combined standard uncertainty of measurement u_c could be obtained using the next formula:

$$u_c = \sqrt{u(p)^2 + u(b)^2 \ + u(x)^2}$$

Where:
$u(p) =$ uncertainty contribution associated with the precision estimate;
$u(b) =$ uncertainty contribution associated with the bias estimate (recovery);
$u(x) =$ uncertainty contribution associated with any other relevant contributor.

If all relevant uncertainty sources are taken into account in the experimental design of the validation study [3], the combined standard uncertainty of measurement can be expressed as:

$$u_c = \sqrt{u(p)^2 + \ u(b)^2}$$

With

$$u(p) = \sqrt{\left(\frac{{S_g}^2}{k}\right) + \left(\frac{{S_r}^2}{k*n}\right)} \text{ [2]}$$

Where:
S_g and S_r: correspond respectively to between-run and within-run (repeatability) standard deviations.
k and n: correspond respectively to the number of runs and the number of independent replicates per run carried out in the routine of the assay, which affect the precision of the assay reported as mean result.

It must be noted that the contribution due to the precision can be decreased using the most fit for purpose k and n (see table 2), or identifying and reducing the major sources of variability.

And if recovery experiments are used to assess the accuracy of the method:

$$u(b) = \sqrt{\frac{\sum_{i=1}^{q} b_i^2}{q} + u(\text{add})^2} \text{ [4]}$$

Where:
b_i: the difference between the obtained recovery (i^{th}) and either 100% (complete recovery) or the mean recovery (if a correction is applied to the testing results using the mean recovery) ;
q: the number of recovery experiments;
$u(\mathrm{add})$: the uncertainty in the concentration of the added analyte.

In MAH documentation, most often only deviations and RSD obtained from recovery experiments are given with the number of tests performed to assess the bias. The uncertainty in the concentration of the analyte added is rarely mentioned. The uncertainty component related to the bias could usually be estimated based on deviations obtained from recovery experiments, assuming that the uncertainty contribution due to the concentration of the analyte added is negligible. The recovery experiments should be performed with at least 6 different samples of the relevant matrix.

Example: Estimation of the measurement uncertainty in determination of content of active ingredient on solution, using data from method validation

1. Description of the analytical procedure

1.1 Testing procedure

Option A: Assay is performed using **3 replicates within the same run.**
Option B: Assay is performed using **1 replicate on 3 different runs.**

1.2 Available validation data

Data from the method validation (obtained from the validation report given in MAH documentation) analysed by ANOVA
The validation exercise consists in 3 runs performed by 2 operators on 3 different days, with 6 independent replicates. The bias of the method is estimated using spiked preparations. An analysis of variance (ANOVA) is carried out on the experimental data in order to estimate the within-run (S_r) and between-run (S_g) standard deviations. The intermediate precision (S_{IP}^2) is the sum of S_g^2 and S_r^2.

Precision validation data:

- mean value: 50.0 mg/unit
- within-run (repeatability): RSD_r = 1.5%
- between-run: RSD_g = 3.0%

Accuracy validation data:

- **1st Case (bias is negligible):** Estimated Recovery: Mean 99.0%, RSD=1.1%, n=6 (99.8%; 98.7%; 98.1%; 98.9%; 98.0%; 100.7%)
- **2nd Case (bias is not negligible):** Estimated Recovery: Mean 97.0%, RSD=0.8%, n=6 (97.8%; 96.7%; 97.1%; 95.9%; 97.9%; 96.7%)

2. Estimation of the measurement uncertainty

2.1 Specification of measurand

The measurand is the concentration of active ingredient in finished product (mg/unit).

Option A (3 replicates within the same run): Individual assay results are: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit. The mean value is 50.2 mg/unit.
Option B (1 replicate on 3 different runs): Individual assay results are the same: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit. The mean value is 50.2 mg/unit.

2.2 Quantification of measurement uncertainty (1st Case: bias is negligible)

2.2.1 Calculation of the standard uncertainties

2.2.1.1 *Uncertainty contribution associated with the precision estimate ($u(p)$)*

Evaluation of the within-run and between-run contributions to the intermediate precision

Table 1 provides the estimated RSD (obtained from the validation report given in MAH documentation) and their contribution to the intermediate precision. The between-run variability contributes to 80% of the intermediate precision of the method.

Table 1. Contribution of the within-run and between-run variability on the intermediate precision

Variability	RSD (%)	Contribution
Within-run (RSD_r)	1.5	20%
Between-run (RSD_g)	3.0	80%
Intermediate precision (RSD_{IP})	3.4	-
Mean	50.0	-

E.g. $RSD_{IP} = \sqrt{RSD_r^2 + RSD_g^2} = \sqrt{1.5^2 + 3.0^2} = 3.4\%$

Within-run contribution = $100 \times (RSD_r / RSD_{IP})^2 = 100 \times (1.5/3.4)^2 = 20\%$

Choice of the testing design

Before performing tests in routine, it is important to determine the most fit for purpose testing design (choice of k and n) to obtain an acceptable precision. From validation data available, the variability of the assay result for different combinations of n and k has first to be determined calculating the standard uncertainty of the reported mean, $u(p)$, using the following formula:

$$u(p) = Mean\ x \sqrt{\left(\frac{RSD_g^2}{k}\right) + \left(\frac{RSD_r^2}{k * n}\right)}$$

Values obtained are given in table 2.

Table 2. Standard uncertainty (mg/unit) of reported mean $u(p)$

N. of rep (n)	N. of runs (k)			
	1	2	3	4
1	1.68	1.19	**0.97**	0.84
2	1.59	1.13	0.92	0.80
3	**1.56**	1.10	0.90	0.78
4	1.55	1.09	0.89	0.77

E.g.: k = 3, n = 1, $Relative\, u(p) = \sqrt{\left(\frac{3.0^2}{3}\right) + \left(\frac{1.5^2}{3\times1}\right)} = 1.94\%$

Absolute $u(p)$ = Relative $u(p)$ × Mean = 0.0194 × 50.0 mg/unit = 0.97 mg/unit.

In order to decrease the standard uncertainty $u(p)$ of the reported mean of assay results, it is recommended to perform replicates on different runs due to the high contribution of the between-run variability (80%) on the intermediate precision of the method. Indeed, for the same number of replicates (3), $u(p)$ decreases from 1.56 mg/unit if the 3 replicates are performed within the same run (option A) to 0.97 mg/unit if replicates are performed in 3 different runs (option B).

2.2.1.2 *Uncertainty contribution associated with the bias (u(b))*

Estimated Recovery: Mean 99.0%, RSD = 1.1% (i.e. SD =1.0424%), n=6 (99.8%; 98.7%; 98.1%; 98.9%; 98.0%; 100.7%)

The approach used in the Eurachem guideline (Example A4) could be used to determine whether the mean recovery is significantly different from 1 or not [1].

The standard uncertainty of the mean recovery ($u(rec)$) is calculated as:

$$u(rec) = \frac{0.010424}{\sqrt{6}} = 0.004256$$

A Student's t test is used to determine whether the mean recovery is significantly different from 1. The t value is calculated using the following equation:

$$t = \frac{|1 - Rec|}{u(rec)} = \frac{|1 - 0.990|}{0.004256} = 2.350$$

This value is compared with the 2-tailed critical value t_{crit}, for n–1 degrees of freedom at 95% level of confidence (where n = 6 is the number of results used to estimate the recovery). If it is lower than the critical value t_{crit} then the value of the recovery (0.990) cannot be considered as significantly different from 1 and no correction has to be applied to subsequent testing results regarding the bias of the method. Otherwise a correction using the mean recovery could be applied to the subsequent testing results or not.

$$t = 2.350 < t_{crit} = 2.571$$

In this case (1st case), the bias can be considered as not significantly different from 0, therefore no correction has to be applied on subsequent testing results. Nevertheless, the uncertainty associated with the determination of the bias remains a component of the overall uncertainty and should be taken into account.

$$u(b) = \sqrt{\frac{\sum_{i=1}^{q} b_i^2}{q}}$$

$$u(b) = \sqrt{\frac{(1-0.998)^2+(1-0.987)^2+(1-0.981)^2+(1-0.989)^2+(1-0.980)^2+(1-1.007)^2}{6}} = 0.01356.$$

2.2.2 Calculation of combined standard uncertainty and expanded uncertainty

Combined standard Uncertainty (u_c)

$$u_c = \sqrt{u(p)^2 + u(b)^2}$$

<u>Option A</u>: If we assume performing assays using **3 replicates** within the **same run** and individual assay results are: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit, the mean value is 50.2 mg/unit.

k=1, n=3, $Relative\, u(p) = \sqrt{\left(\frac{3.0^2}{1}\right) + \left(\frac{1.5^2}{1\times3}\right)} = 3.122\%$, $u(p) = 0.03122$

$$u_c = \sqrt{(0.0312)^2 + (0.01356)^2} = 0.034021\ (\text{eq. } 3.4\%)$$

The corresponding *expanded uncertainty (U)*, k = 2 (95% level of confidence) is:

U = 2 × (0.034021 × 50.2 mg/unit) = 3.4 mg/unit.

<u>Option B</u>: If now we perform assays using **1 replicate on 3 different runs** and individual assay results are the same: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit, the mean value is 50.2 mg/unit.

K = 3, n = 1, $Relative\, u(p) = \sqrt{\left(\frac{3.0^2}{3}\right) + \left(\frac{1.5^2}{3\times1}\right)} = 1.94\%$, $u(p) = 0.0194$

$$u_c = \sqrt{(0.0194)^2 + (0.01356)^2} = 0.023672\ (\text{eq. } 2.4\%)$$

The corresponding *expanded uncertainty (U)*, k = 2 (95 % level of confidence) is:

U = 2 × (0.023672 ×50.2 mg/unit) = 2.4 mg/unit.

2.2.3 Reporting of result

The assay result may be expressed as:

Reported result = Mean value ± U

Option A (3 replicates within the same run):
The result is reported as 50.2 ± 3.4 mg/unit, for k = 2 and level of confidence 95%.

Option B (1 replicate on 3 different runs):
The result is reported as 50.2 ± 2.4 mg/unit, for k = 2 and level of confidence of 95%.

2.3 Quantification of measurement uncertainty (2nd Case: bias is not negligible)

2.3.1 Calculation of the standard uncertainties

2.3.1.1 *Uncertainty contribution associated with the precision estimate ($u(p)$)*

The procedure is the same as described in 2.2.1.1.

2.3.1.2 *Uncertainty contribution associated with the bias (u(b))*

Estimated Recovery: Mean 97.0%, RSD = 0.8% (i.e. SD = 0.8%), n=6 (97.8%; 96.7%; 97.1%; 95.9%; 97.9%; 96.7%)

The standard uncertainty of the mean recovery ($u(rec)$) is calculated as:

$$u(rec) = \frac{0.008}{\sqrt{6}} = 0.00308$$

The t value is calculated using the following equation:

$$t = \frac{|1 - Rec|}{u(rec)} = \frac{|1 - 0.970|}{0.00308} = 9.74$$

The 2-tailed critical value t_{crit} is:

$t = 9.74 > t_{crit} = 2.571$

In this case (2nd case), the bias can not be considered as not significantly different from 0. Therefore, the laboratory can decide whether to apply a correction to the subsequent testing results using the mean recovery (Choice 1) or not (Choice 2: in which case, the bias component will be taken into account in the calculation of the uncertainty).

Choice 1: **the laboratory decides to apply a correction to testing results using the mean recovery**

The uncertainty contribution associated with the bias estimate *u(b)* is calculated using the difference between the obtained recovery and the mean recovery:

$$u(b) = \sqrt{\frac{(0.970-0.978)^2+(0.970-0.967)^2+(0.970-0.971)^2+(0.970-0.959)^2+(0.970-0.979)^2+(0.970-0.967)^2}{6}} = 0.00689$$

<u>Choice 2</u>: the laboratory decides to not apply a correction to testing results

The uncertainty contribution associated with the bias estimate *u(b)* is calculated using the difference between the obtained recovery values and the absolute recovery value (100%):

$$u(b) = \sqrt{\frac{(1-0.978)^2 + (1-0.967)^2 + (1-0.971)^2 + (1-0.959)^2 + (1-0.979)^2 + (1-0.967)^2}{6}} = 0.03062$$

2.3.2 Calculation of combined standard uncertainty and expanded uncertainty

Combined Uncertainty (u_c)

$$u_c = \sqrt{u(p)^2 + u(b)^2}$$

<u>Choice 1 (correction is applied)</u>:

<u>Option A</u>: If we assume performing assays using **3 replicates** within the **same run** and individual assay results are: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit, the mean value is 50.2 mg/unit.

$$\overline{X_{Cor}} = \frac{50.2}{0.970} = 51.8\, mg/unit$$

with

$$u_c = \sqrt{(0.0312)^2 + (0.00689)^2} = 0.03195\ (\text{eq. } 3.2\%)$$

The corresponding *expanded uncertainty (U)*, k = 2 (95% level of confidence) is:

U = 2 × (0.03195 × 50.2 mg/unit) = 3.2 mg/unit.

<u>Option B</u>: If now we perform assays using **1 replicate on 3 different runs** and individual assay results are the same: 51.2, 50.3 and 49.2 mg/unit, the mean value is 50.2 mg/unit.

$$\overline{X_{Cor}} = \frac{50.2}{0.970} = 51.8\, mg/unit$$

with

$$u_c = \sqrt{(0.0194)^2 + (0.00689)^2} = 0.02059\ (\text{eq. } 2.1\ \%)$$

The corresponding *expanded uncertainty (U)* or half-width of 95% confidence interval is:

U = 2 × (0.02059 × 50.2 mg/unit) = 2.1 mg/unit.

<u>Choice 2 (no correction)</u>:

<u>Option A</u>: If we assume performing assays using **3 replicates** within the **same run** and individual assay results are: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit, the mean value is 50.2 mg/unit.

$$u_c = \sqrt{(0.0312)^2 + (0.03062)^2} = 0.04371\ (\text{eq. } 4.3\%)$$

The corresponding expanded uncertainty (U), k = 2 (95 % level of confidence) is:

U = 2 × (0.04371 ×50.2 mg/unit) = 4.4 mg/unit.

Option B: If now we perform assays using **1 replicate on 3 different runs** and individual assay results are the same: 51.2 mg/unit, 50.3 mg/unit and 49.2 mg/unit, the mean value is 50.2 mg/unit.

$$u_c = \sqrt{(0.0194)^2 + (0.03062)^2} = 0.036247 \text{ (eq. } 3.6\ \%)$$

The corresponding expanded uncertainty (U), k = 2 (95 % level of confidence) is:

U = 2 × (0.036247 × 50.2 mg/unit) = 3.6 mg/unit.

2.3.3 Reporting of result

Choice 1 (correction is applied):

The assay result is expressed as:

Reported result = corrected Mean value ± CI

$$\overline{X_{Cor}} = \frac{50.2}{0.970} = 51.8\ mg/unit$$

Option A (3 replicates within the same run):
The result is reported as 51.8 ± 3.2 mg/unit, for k = 2 and level of confidence of 95%.

Option B (1 replicate on 3 different runs):
The result is reported as 51.8 ± 2.1 mg/unit, for k = 2 and level of confidence of 95%.

Choice 2 (no correction):

The assay result is expressed as:

Reported result = Mean value ± U

Option A (3 replicates within the same run):
The result is reported as 50.2 ± 4.4 mg/unit, for k = 2 and level of confidence of 95%.

Option B (1 replicate on 3 different runs):
The result is then reported as 50.2 ± 3.6 mg/unit, for k = 2 and level of confidence of 95%.

2.4 Summary of results

In Table 3 a summary of results for estimation of measurement uncertainty for the same individual tests results (i.e.: 51.9 mg/unit, 50.1 mg/unit and 48.9 mg/unit), using different approaches is provided.

Table 3 The summary of reporting of results

	1st Case : bias is negligible *Estimated Recovery: Mean 99.0%, RSD=1.1%, n=6*		**2nd Case :** bias is not negligible *Estimated Recovery: Mean 97.0%, RSD=0.8%, n=6*	
	Option A 3 replicates within the same run	**Option B** 1 replicate on 3 different runs	**Option A** 3 replicates within the same run	**Option B** 1 replicate on 3 different runs
Choice 1: A correction to testing results using the mean recovery is applied	/	/	51.8 +/- 3.2 mg/unit	51.8 +/- 2.1 mg/unit
Choice 2: No correction to testing results using the mean recovery is applied	50.2 +/- 3.4 mg/unit	50.2 +/- 2.4 mg/unit	50.2 +/- 4.4 mg/unit	50.2 +/- 3.6 mg/unit

3. References

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