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EVALUATION OF MEASUREMENT UNCERTAINTY
ANNEX 2.2

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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 2.2 Use of data from control charts for the estimation of measurement uncertainty

Control charts are used as a quality control tool to demonstrate that testing results are distributed according to some random variability around one expected value, e.g. reference or mean value. The use of control charts enables estimation of the uncertainty component for precision and bias within the laboratory.

Repeated runs carried out on stable and homogeneous references (e.g. internal control, standard having similar composition in respect to test samples) can provide a reliable estimation of the uncertainty of measurements, providing that random and systematic (bias) errors can be estimated and then combined.

All available data should be included in the control chart, including data points lying beyond the alert (mean ± 2 standard deviation) and action limits (mean ± 3 standard deviation). Sub-groups of data with clear different random errors and/or means (biases) should be considered separately or modelled using appropriate statistical methods so that the uncertainty of measurements can be correctly calculated.

Example 1: Estimation of measurement uncertainty for determination of the content of a test sample by HPLC

1. Description of the analytical procedure

The laboratory has determined the content of a test sample by HPLC (triplicate determination) using a reference solution as external standard prepared following the same procedure. The results are given in Table 1.

Table 1. Test Results

Rep.	Content (mg/L)	Mean (mg/L)
1	15.55	15.33
2	15.13	
3	15.32	

The same analytical method was applied for the periodic qualification of an HPLC equipment, using certified reference material, gathering data over 15 time points which were included in a control chart. The data used to build the control chart are presented in Table 2 and plotted in Figure 1. As shown, all data points lay within the control limits i.e. Lower Control Limit (LCL) and Upper Control Limit (UCL). Indeed, with a relative standard deviation of 1 % over 15 measurements, the HPLC

equipment showed a limited variability over the period of evaluation. However, the mean result was found to be 15.44 mg/mL i.e. 2.9 % above the target of 15.0 mg/L, as shown in the Figure 1.

Table 2. Control Chart Data of the Reference Solution

Run Order	Content (mg/L)	Run Order	Content (mg/L)	Run Order	Content (mg/L)
1	15.35	6	15.18	11	15.66
2	15.23	7	15.68	12	15.45
3	15.42	8	15.64	13	15.40
4	15.35	9	15.31	14	15.57
5	15.59	10	15.45	15	15.32

Mean 15.44 mg/L; Standard deviation = 0.157 mg/L

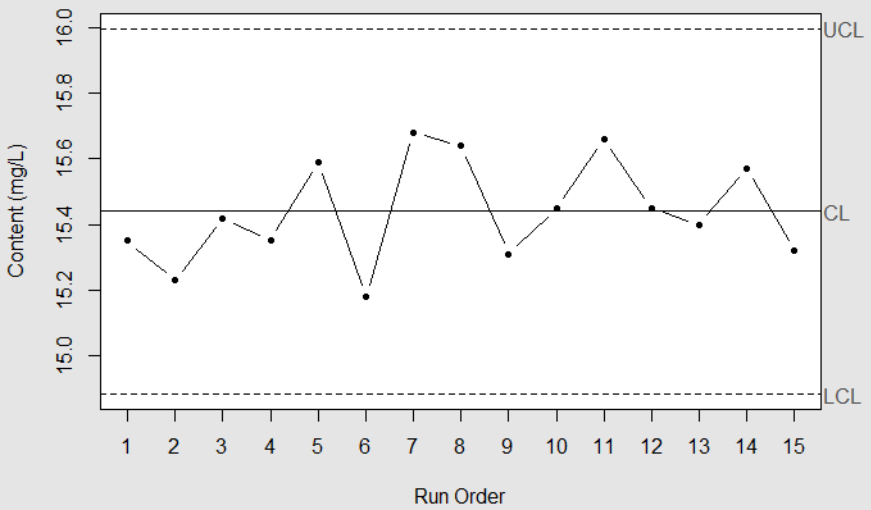

Figure 1. Individual Control Chart for the Reference Preparation

2. Estimation of measurement uncertainty

2.1 Specification of measurand

The measurand is the content of a test sample expressed as mg/L.

2.2 Quantification of the uncertainty of measurement using control chart data

2.2.1 Calculation of the combined standard uncertainty and expanded uncertainty

Assuming that all critical sources of variation (e.g. due to preparation of samples and HPLC method) are taken into account, the data included in the control chart can be used to estimate the uncertainty of measurements. Specifically, the relative combined standard uncertainty (u_c/x) is calculated by the formula:

$$\frac{u_c}{x} = \sqrt{\mathrm{RSD}^2 + \mathrm{RBE}^2 + \mathrm{RB}^2 + u(ref)}$$

Where:
RSD is the relative standard deviation of the measured values.

$\mathrm{RSD} = \frac{100 \cdot S}{Mean} = \frac{100 \cdot 0.157}{15.44}$ = 1.0%

RBE is the relative standard error of the estimated mean.

$\mathrm{RBE} = \frac{1.0\ \%}{\sqrt{15}} = 0.26\%$

RB is the relative bias, i.e. the relative difference between the estimated mean and the accepted value of the reference solution.

$$RB = 100 \cdot \left(\frac{Mean - target}{Target}\right) = 100 \cdot \left(\frac{15.44 - 15}{15}\right) = 2.9\%$$

The relative bias (RB = 2.9 %) is tested for significance. Since RB is 11.2 times higher than the standard error (RBE = 0.26 %), it is not negligible. This is also confirmed by the t-test: the observed ratio (t_{obs} = 11.2) is higher than the two-tailed critical value of the t-Student distribution (t_{crit} = 2.1), calculated for n-1 = 14 degrees of freedom at a 95% level of confidence.

$u(ref)$ is the uncertainty of the reference value calculated from the purity (99.5% ± 0.5%) indicated on the certificate of analysis. Considering a uniform (rectangular) distribution:

$$u(ref) = \frac{0.5}{\sqrt{3}} = 0.288\%$$

$$\frac{u(ref)}{99.5} = 0.0029$$

Therefore, the relative combined standard uncertainty (u_c/x) of measurements is equal to:

$$\frac{u_c}{x} = \sqrt{0.01^2 + 0.029^2 + 0.0026^2 + 0.0029^2} = 3.1\%.$$

And the relative expanded uncertainty (U_{rel}) is:

$$U_{rel} = 2 \cdot \frac{u_c}{x} = 2 \cdot 3.1\% = 6.2\%$$ (k = 2, 95% level of confidence).

The expanded uncertainty U is:

$U = 2 \cdot \frac{u_c}{x} \cdot x = 2 \cdot 0.031 \cdot 15.33 mg/L = 0.95046\ mg/L$

The expanded uncertainty can be used during routine analysis to provide the uncertainty of individual measurements of test samples.

When independent measurements are to be taken into consideration (in this example n = 3), the relative combined uncertainty is given by:

$$\frac{u_c}{x} = \sqrt{\frac{0.01^2}{3} + 0.029^2 + 0.0026^2 + 0.0029^2} = 0.03 = 3.0\%$$

The combined uncertainty u_c is

$$u_c = 0.03 \cdot 15.33 = 0.4599$$

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

$$U = 0.4599 \cdot 2 = 0.9198\ mg/L$$

2.3 Reporting of results

Result is reported as: 15.33 mg/L ± 0.92 mg/L, for k =2 and level of confidence 95%.

Comment:

In this example, replicated measurements which affect the precision (random error) only, have a limited contribution, since most of the uncertainty is given by the bias (i.e. RB, systematic error) of the method.

Example 2: Estimation of measurement uncertainty for content of polymer in albumin solution by HPLC-SEC

1. Description of the analytical procedure

The content of polymer in albumin solution is measured by an HPLC-SEC method and calculated by normalisation using an internal control. The set of data used to build up the control chart are presented in Table 3. Two operators carried out several runs over a 3-year period, using different pre-columns, making two measurements per run.

Table 3. Albumin (%) Control Chart Data

Run	Date	Precolumn	Operator	Meas.1	Meas.2
1	10-Feb-2015	P1	O1	4.70	4.77
2	23-Mar-2015	P1	O2	4.62	4.80
3	13-Apr-2015	P1	O1	4.83	4.91
4	23-Apr-2015	P1	O1	5.43	5.58
5	19-May-2015	P1	O2	5.30	5.47
6	28-May-2015	P1	O1	5.51	5.53
7	01-Jun-2015	P1	O2	4.93	5.05
8	29-Jun-2015	P1	O2	5.06	5.02
9	08-Jul-2015	P1	O1	4.70	4.83
10	03-Aug-2015	P1	O2	4.17	4.24
11	25-Aug-2015	P1	O1	4.14	4.20
12	27-Aug-2015	P1	O1	4.11	4.21
13	14-Sep-2015	P1	O2	4.61	4.59
14	04-Nov-2015	P2	O2	4.95	4.91
15	24-Nov-2015	P2	O2	4.94	5.03
16	17-Dec-2015	P2	O1	4.89	4.99
17	02-Feb-2016	P2	O2	4.67	4.79
18	24-Feb-2016	P2	O2	4.58	4.41
19	23-May-2016	P2	O2	5.11	5.22
20	08-Jun-2016	P2	O2	5.11	5.26
21	08-Aug-2016	P2	O2	5.20	5.33
22	07-Sep-2016	P2	O2	5.17	5.34
23	19-Oct-2016	P2	O1	5.17	5.00
24	09-Nov-2016	P2	O2	4.98	5.12
25	12-Dec-2016	P2	O2	5.29	5.45
26	11-Jan-2017	P2	O2	5.49	5.46
27	17-Jan-2017	P2	O1	5.31	5.27
28	24-Jan-2017	P3	O2	5.31	5.33
29	02-Feb-2017	P3	O2	5.33	5.28
30	14-Mar-2017	P3	O1	4.88	4.91
31	03-May-2017	P3	O2	4.01	3.89
32	16-May-2017	P3	O2	3.63	3.50
33	21-Jun-2017	P3	O2	5.30	4.89
34	01-Aug-2017	P3	O2	4.92	4.85

Using the same method, an unknown sample was analysed by one of the operators in two days, two runs per day, obtaining the following results: 5.30%, 5.32% (run 1) and 5.11%, 5.11% (run 2) (mean of 5.21%).

2. Estimation of measurement uncertainty

2.1 Specification of measurand

The measurand is the content of polymer contained in albumin solution expressed as percentage, calculated by normalisation using an internal control.

2.2 Quantification of the uncertainty of measurement using control chart data

2.2.1 Calculation of the combined standard uncertainty and expanded uncertainty

To estimate the uncertainty of the measurement of the result for the unknown sample, using control chart data, the first step is to calculate the combined standard uncertainty by combining the within- and between-run standard deviations (S_w and S_i respectively), using a one-way random analysis of variance, which provides the intermediate precision of the method [1].

Table 4 provides the values of S_w and S_i together with their contribution to the intermediate precision. Most of the uncertainty (contribution of 96%) is due to the between-run variability, which is somewhat expected considering the various sources of variation that S_i encompasses (i.e. days, operators, pre-columns).

Table 4. One-Way Random Anova - Standard Deviation Estimates

Source of variation	Standard Deviation	Estimated value (%)	Contribution
Measurements within a run	Within-run (S_W)	0.092	4%
Days, operators, pre-columns	Between-run (S_i)	0.462	96%
Intermediate precision	$\sqrt{S^2_W + S^2_i}$	0.471	-

The results in Table 4 indicate that the combined standard uncertainty (standard deviation) associated with individual measurement is:

$$u_{c(1)} = \sqrt{{S_W}^2 + {S_i}^2}$$

$$u_{c(1)} = \sqrt{0.092^2 + 0.462^2} = 0.471\%$$

The test consists in two measurements in one run, therefore the combined standard uncertainty (standard error) associated with the mean reported value is calculated as follow:

$$u_{c(2)} = \sqrt{\frac{{S_W}^2}{2} + {S_i}^2}$$

$$u_{c(2)} = \sqrt{\frac{0.092^2}{2} + 0.462^2} = 0.467\%$$

The expanded uncertainty is:

$$U = u_{c(2)} \cdot 2 = 0.934\ \%$$ (k = 2, 95 % level of confidence).

Considering the high contribution of S_i, $u_{c(2)}$ can be decreased by:

a) increasing the number of runs, e.g. 2 measurements in 2 runs:

$$u_{c(2,2)} = \sqrt{\frac{{S_W}^2}{2 \times 2} + \frac{{S_i}^2}{2}}$$

$$u_{c(2,2)} = \sqrt{\frac{0.092^2}{2 \times 2} + \frac{0.462^2}{2}} = 0.330\%$$

and the expanded uncertainty is U = 0.660% (k = 2, 95% level of confidence). Therefore, by increasing the number of runs up to 2, u_c is reduced approximately 1.5 times.

b) estimating the contribution (standard deviation) of each variable of S_i. The results of the nested random analysis of variance, presented in Table 5, indicate that the standard deviation between days is the major contributor (88%) to the intermediate precision.

Table 5. Nested Random Anova - Standard Deviation Estimates

Source of variation	Standard Deviation	Estimated value (%)	Contribution
Measurements within Run	S_W	0.092	4%
Day	S_D	0.449	88%
Operator	S_O	0	0%
Pre-column	S_P	0.135	8%
Intermediate precision	S_{Total}	0.478	-

The combined standard uncertainty (standard deviation) associated with individual measurements is:

$$u_{c(1)} = \sqrt{{S_W}^2 + {S_D}^2 + {S_O}^2 + {S_P}^2}$$

$$u_{c(1)} = \sqrt{0.092^2 + 0.449^2 + 0^2 + 0.135^2} = 0.478\%$$

The combined standard uncertainty calculated by decomposition in four components is more accurate than the one calculated by two components presented in Table 4, however the final uncertainty value is similar (0.478% *vs* 0.471%).

The combined standard uncertainty (standard error) associated with the mean value of two measurements in one run is:

$$u_{c(2)} = \sqrt{\frac{{S_W}^2}{2} + {S_D}^2 + {S_O}^2 + {S_P}^2}$$

$$u_{c(2)} = \sqrt{\frac{0.092^2}{2} + 0.449^2 + 0^2 + 0.135^2} = 0.473\%$$

The expanded uncertainty is U = 0.946% (k = 2, 95 % level of confidence).

In order to reduce U considering that S_D is the major contributor (88%), the test procedure should be modified e.g. by performing several runs on different days regardless the operator and the

batch of pre-column. For example, with 2 runs (performed on different days) of 2 measurements, the standard uncertainty (standard error) associated with the reported mean value is:

$$u_{c(2,2)} = \sqrt{\frac{{S_W}^2}{2 \times 2} + \frac{{S_D}^2}{2} + {S_P}^2}$$

$$u_{c(2,2)} = \sqrt{\frac{0.092^2}{2 \times 2} + \frac{0.449^2}{2} + 0.135^2} = 0.348\%$$

The expanded uncertainty is U = 0.696% (k = 2, 95 % level of confidence).

2.3 Reporting of result

The mean reported result is 5.21 ± 0.70%, for k = 2 and level of confidence 95%.

Comment: It must be noted that the uncertainty covers the precision component only. In cases where the internal control is not a reference solution of known concentration, the systematic error (bias) cannot be evaluated. The uncertainty of measurements may be then underestimated, unless the routine method and an orthogonal method can be run in parallel at several occasions to evaluate the bias.

3. Summary

In general, it is necessary to identify, evaluate and control the uncertainty contributors e.g. by decomposition exercise, in order to understand the major sources of uncertainty. The test procedure can be modified to decrease the uncertainty of measurements e.g. by increasing the number of measurements or runs.

4. References

1. ISO/TR 22971: 2005 Accuracy (trueness and precision) of measurement methods and results - Practical guidance for the use of ISO 5725-2:1994 in designing, implementing and statistically analysing inter-laboratory repeatability and reproducibility results.