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EVALUATION OF MEASUREMENT UNCERTAINTY ANNEX 3

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Annex 3 to Guideline “Evaluation of Measurement Uncertainty”
PA/PH/OMCL (18) 145 (in its current version)

Estimation of measurement uncertainty expressed as confidence interval

This Annex gives examples of estimation of the uncertainty of measurement (UoM), expressed as confidence interval using the standard deviation of the results obtained from routine testing (Example 1) and using Combistats software (Example 2). This approach is applicable to compliance testing using well-recognised methods, including methods described in the Market Authorisation Holder (MAH) documentation, whether validation data are accessible or not, and properly validated in-house developed methods, for which the uncertainty of measurement is already established and verified. In addition, the laboratory should demonstrate that all contributions to the uncertainty of measurement are identified and under control: testing is performed by qualified personnel using suitable reference standards and calibrated/qualified equipment, system suitability criteria are fulfilled and the repeatability is evaluated against pre-defined acceptance criteria.

However, unless required by the test method being used or if it has been demonstrated that the main and most relevant component of uncertainty is repeatability, relying exclusively on internal precision data for the estimation of measurement uncertainty is not recommended.

Example 1: Estimation of the measurement uncertainty for determination of content of Phenylephrine Hydrochloride in solution for injection

1. Description of the analytical procedure

The assay of the finished product Phenylephrine Hydrochloride solution for injection (10 mg/mL), with approved product specification limits of 9.5-11.0 mg/mL, is performed by UV-spectrophotometry at 272 nm, using a compendial reference standard. The method is performed according the MAH documentation, but the validation data are not accessible.

Preparation of standard solution

Two standard solutions were prepared using compendial reference standard, with a concentration of 0.05 mg/mL.

Preparation of test solution

Three independent test solutions were prepared, by diluting the sample (finished product) with the solvent to obtain a concentration of 0.05 mg Phenylephrine Hydrochloride / mL.

The absorbance of the standard solutions and test solutions was measured and the active substance content, expressed as mg Phenylephrine Hydrochloride / mL solution for injection, was determined.

2. Estimation of the measurement uncertainty

2.1 Specification of Measurand

The measurand is the concentration of Phenylephrine Hydrochloride in the solution for injection, expressed as mg/mL, calculated by the following formula:

$$X = \frac{A(test) \times m(st) \times V(test) \times P(st)}{A(st) \times V(st) \times V(sample) \times 100\%}$$

where:

X - mg active substance in 1 mL of the solution for injection;

$A(test)$, $A(st)$ - absorbance of the test solution and the standard solution, respectively;

$m(st)$ - mass of the reference standard;

$V(st)$ - volume of the standard solution;

$V(sample)$ - volume of the sample (finished product) taken for the preparation of the test solution;

$V(test)$ - volume of the test solution;

$P(st)$ - purity of the reference standard (%).

2.2 Control of contributions to the uncertainty of measurement

Testing is performed by qualified personnel using suitable reference standards and calibrated/qualified equipment and system suitability criteria are satisfied.

Internal verification

The absorption of the prepared standard solutions was measured and the recovery (similarity) of the standard solutions was determined using the following formula:

$$R = \frac{C(st\ 1)}{C(st\ 2)} \times \frac{A(st\ 2)}{A(st\ 1)} \times 100\%,$$

where

$C(st\ 1)$, $C(st\ 2)$ - concentration (mg/mL) of the first and second reference standard solutions, respectively;

$A(st\ 1)$, $A(st\ 2)$ - absorption of the first and second reference standard solutions, respectively.

The recovery of the reference standard obtained was $R = \frac{0.0504}{0.0507} \times \frac{0.45288}{0.44726} \times 100\% = 100.65\%$,

which fulfilled the established internal quality control criteria of $100 \pm 2\%$.

Following confirmation of the quality control criteria, the testing of the finished pharmaceutical product proceeded.

Based on the three measurements, the repeatability of the obtained results was calculated and expressed as relative standard deviation (RSD). The precision complies with the internal acceptance criterion ($\leq 2.0\%$).

2.3 Quantification of the uncertainty of measurement

The obtained results were used for calculation of the mean, standard deviation, relative standard deviation and two-sided confidence interval, as shown in Table 1. The results presented in the table are displayed with three decimal places, but all of the calculations were performed without rounding of intermediate results.

Table 1. Summary of testing results and calculations.

NOTE: The data reported are not rounded, however they are displayed with 3 decimal places for convenience.

Determination	Formula	Result
1	see 2.1	10.172 mg/mL
2	see 2.1	10.160 mg/mL
3	see 2.1	10.203 mg/mL
Mean	$\bar{X} = \frac{X1 + X2 + X3}{3}$	10.178 mg/mL
S	$S = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{(n-1)}}$	0.022 mg/mL
RSD %	$RSD = \frac{S \times 100}{\bar{X}}$	0.2 %
Two-sided Confidence interval (CI)*	$(\bar{X} \pm \Delta\bar{X}) = \bar{X} \pm \frac{t_{((1-\frac{\alpha}{2}), n-1)} \times S}{\sqrt{n}}$ <ul style="list-style-type: none"> ▪ 95% level of confidence, $\alpha = 0.05$ ▪ $n = 3$ (2 degrees of freedom) ▪ $S = 0.022$ ▪ $t_{(95\%, 2)} = 4.30265$ 	Half-width of CI
		± 0.055 mg/mL
		Lower 95%CI
		10.123 mg/mL
		Upper 95%CI
10.233 mg/mL		

2.4 Reporting of result

Summary of the results and comparison with the specification limits is given in Table 2. The final result should be reported with the same number of decimal places as the specification limits. However, according to the Eurachem guideline [2] it is seldom necessary to give more than two significant digits for the uncertainty. Therefore, the result should also be rounded to be consistent with the uncertainty given.

Table 2 Summary of the results and comparison with the specification limits

Assay (mg/mL)	Specification limits	Reported results (95% confidence interval)
	9.5 - 11.0	10.2 ± 0.1 mg/mL

The statement of conformity with a specification shall be based on a decision rule (as described in the core document). In this particular case, the decision rule is inherent to the specification limits (compliance testing using well-recognised method described in the MAH documentation). Therefore, no further tolerances should be applied to the specification limits prescribed to determine whether the sample being examined complies with the requirements. The mean reported value together with the confidence interval lies within the specification limits.

Example 2: Estimation of the measurement uncertainty for *in-vitro* assay of three hepatitis B vaccines against a standard, using the CombiStats software

The approach for estimation of measurement uncertainty using the CombiStats software is explained using the example "5.1.4 Five-dose multiple assay with completely randomised design, an *in-vitro* assay of three hepatitis B vaccines against a standard", described in the general chapter of Eur. Ph. 5.3: "Statistical analysis of results of biological assays and tests".

1. Description of the analytical procedure

Three independent two-fold dilution series (replicates) of 5 dilutions were prepared from each of the vaccines. After some additional steps in the assay procedure, absorbances were measured (shown in Table 3).

Table 3: Optical densities obtained for the standard and the three preparations

Dilution	Standard S			Preparation T		
1:16 000	0.043	0.045	0.051	0.097	0.097	0.094
1:8000	0.093	0.099	0.082	0.167	0.157	0.178
1:4000	0.159	0.154	0.166	0.327	0.355	0.345
1:2000	0.283	0.295	0.362	0.501	0.665	0.576
1:1000	0.514	0.531	0.545	1.140	1.386	1.051
Dilution	Preparation U			Preparation V		
1:16 000	0.086	0.071	0.073	0.082	0.082	0.086
1:8000	0.127	0.146	0.133	0.145	0.144	0.173
1:4000	0.277	0.268	0.269	0.318	0.306	0.316
1:2000	0.586	0.489	0.546	0.552	0.551	0.624
1:1000	0.957	0.866	1.045	1.037	1.039	1.068

2. Estimation of the measurement uncertainty

2.1 Specification of Measurand

The measurand is the concentration of μg protein/mL. The standard assigned potency is 20 μg protein/mL.

2.2 Control of contributions to the uncertainty of measurement

Testing is performed by qualified personnel using suitable reagents and calibrated/qualified equipment. System suitability criteria are satisfied. Bias component is considered negligible and the repeatability is known to be the main contributor to the uncertainty.

2.3 Quantification of the uncertainty of measurement

The potency estimates of the three vaccines are calculated with CombiStats 5.0 using the following options:

- Parallel lines model;
- Completely randomised design;
- Optical densities are transformed with the neperian logarithm function;
- Residual variance estimated using observed residuals.

Validity criteria of the analysis of variance are satisfied:

- Probability for regression is significant ($p = 0.000 < 0.05$)
- Probability for the non-parallelism is non-significant ($p = 0.434 > 0.05$)
- Probability for the global non-linearity is non-significant ($p = 0.531 > 0.05$)
- Probability for the Standard non-linearity is non-significant ($p = 0.475 > 0.05$)
- Probability for the Vaccine T non-linearity is non-significant ($p = 0.254 > 0.05$)
- Probability for the Vaccine U non-linearity is non-significant ($p = 0.456 > 0.05$)
- Probability for the Vaccine V non-linearity is non-significant ($p = 0.645 > 0.05$)

All the calculations performed are not detailed in this document since they are presented in Ph. Eur. Chapter 5.3.

The uncertainty of measurement (in μg protein/mL) is expressed as 95% confidence interval (Lower and Upper limits, see table 4).

The output of calculations for this example performed using CombiStats is presented in Figure 1.

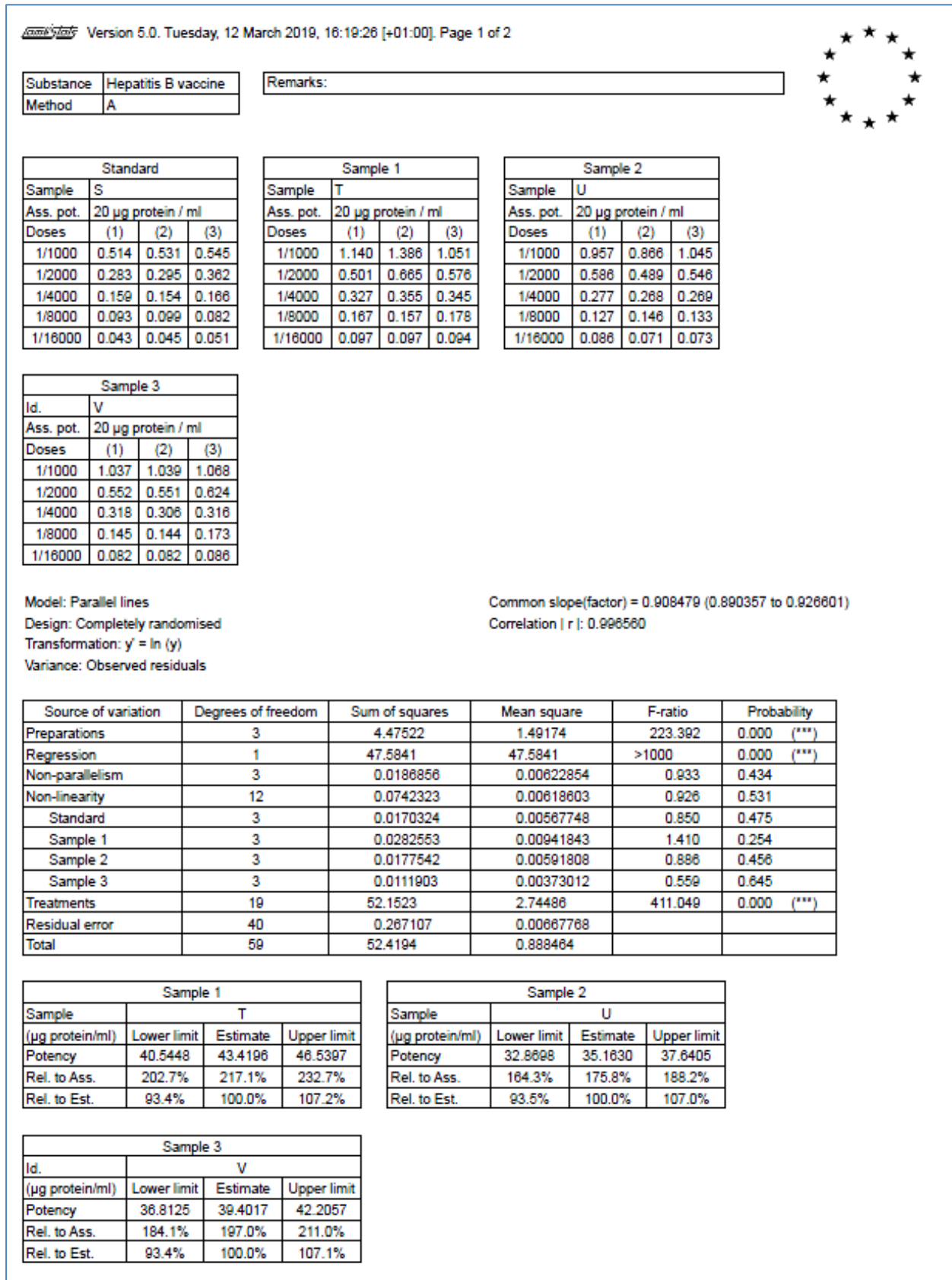
2.4 Reporting of result

Table 4 presents the estimates (in μg protein/mL) obtained for the three vaccines preparations (T, U and V) and the 95% confidence interval (lower and upper limits).

Table 4: Final potency estimates and 95% confidence intervals of the test vaccines (in μg protein/mL)

	Lower limit	Estimate	Upper limit
Vaccine T	40.5	43.4	46.5
Vaccine U	32.9	35.2	37.6
Vaccine V	36.8	39.4	42.2

Figure 1: Output of CombiStats for the example of a titration of Hepatitis B Vaccines



The relative MU can be derived from the “Rel to Est.” line of the output, by the following:

Example for Sample T: *Upper Confidence Limit* – 100% = 107.2% – 100% = 7.2%

Therefore, based on the upper confidence limits calculated for the various samples, the relative MU is about 7%, with a level of confidence of 95% (it should be noticed that, due to the log-transformation, the confidence intervals are asymmetrical around the potency estimates, when converted back in the original unit of measurement, i.e.: µg protein/mL).

If the uncertainty is derived by several assays, it is expressed by the 95% confidence interval for the averaged estimate, which can be obtained by the “Combine Assay” function of the software. In this “scenario”, if the intermediate precision cannot be neglected, the use of the semi-weighted combination is recommended. Further details are provided in [3] and in chapter 5 of the User Manual [4].

3. References

1. OFS.1.1.0013.15 Statistical analysis of chemical test results, State Pharmacopoeia of the Russian Federation, XIV edition
2. S L R Ellison and A Williams (Eds). Eurachem/CITAC guide: Quantifying Uncertainty in Analytical Measurement, Third edition, (2012) ISBN 978-0-948926-30-3. Available from www.eurachem.org.
3. Council of Europe, European pharmacopoeia, current edition 5.3: “Statistical analysis of results of biological assays and tests”
4. The CombiStats 5.0 User Manual, EDQM – Council of Europe, March 2013