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COMBINED STANDARD UNCERTAINTIES AND COVARIANCES OF OUTPUT ESTIMATES

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Abstract: Metrology, in general, and issues such as traceability and measurement uncertainty, in particular, are increasingly demanded when checking the safety of a wide range of food and agricultural products, in chemical manufacturing, environment protection, clinical chemistry as well for regulatory purposes.

In many chemical quantitative analysis, the evaluation of measurement uncertainty requires the practician to look closely at all possible sources of uncertainty. Among them, data provided in calibration and correlation between the input quantities call for insight based on experience and general knowledge. Some aspects related to the estimation of measurement uncertainty and of calibration uncertainty in this field are presented.

Despite the greater attention given in the recent years to covariances and correlations in metrology, they still seem to be undervalued in chemical measurements based on relative or comparative methods of measurement. Consequently, the paper describes the applicability of the variance- and covariance-propagation law in the particular case of spectrometric analysis. An example showing the magnitude of the correlation between output estimates is discussed.

Keywords: IMEKO, World Congress, Estimation of Uncertainty and Errors in measurements

1 INTRODUCTION

Lately, Romanian chemist community pays an increasingly attention to uncertainty mainly for two reasons: (a) it is an indispensable part of any reported result, and (b) the dispersion between measurement results of different analytical laboratories is usually much greater than that between the repeated results within one laboratory. Therefore, the need to consider the full list of uncertainty sources and their contributions is stressed. Also, some aspects of covariances and correlations in analytical metrology require a special attention in chemical measurements based on relative and comparative methods of measurement.

For the purpose of this paper, only estimates outcoming from molecular absorption method after the complexation of the sample (as mass concentration) and from atomic emission spectrometry of solid samples (as mass fraction) will be considered.

2 COMBINED STANDARD UNCERTAINTY IN CHEMICAL MEASUREMENTS

Recently, the ISO Guide to the Expression of Uncertainty in Measurement [1] has been adopted as a national standard (SR 13434:July 1999) and stands as the main reference to estimate and express uncertainty in chemical measurements at all levels of accuracy, from basic research to routine analysis performed in any industrial or commercial application, as well as in the areas of health and safety. Also, the growing interest for national and international comparability of all chemical (referred from this point forward as analytical) measurement results, determined an increased attention toward the adequate application of the standard in calibration, laboratory accreditation, metrology services, standardization and routine measurements.

Every analytical result of a specific quantitative analysis (a particular type of chemical measurement), denoted with c, is a combination of i intermediate measurement results (weighings - m, volumes - V, instrument readings - S, temperature - T etc.) with other parameters (molecular weights - M etc.) through a functional relationship f:

$$c = f(m, V, S, T, M, \ldots) \tag{1}$$

A simple relationship is that relating the absorbance measured, A and the molar absorbance coefficient, ε to determine mass concentration in molecular spectrophotometry. A more complex one is

that describing the determination of the same measurand by a relative method using braketing technique [2]. In this case several input estimates are correlated to a certain extend. Regardless the measurement method (analytical process) used, the output estimate may be described as the following sum:

$$\mu_c + \delta_w + U \tag{2}$$

where: μ_c is the the accepted value for the concentration measured, usually the average of several independent determinations, δ_w - laboratory bias and U - expanded uncertainty.

Note that the identification of laboratory bias is an important prerequisite when estimating measurement uncertainty and several standards describes how to determine it [2].

Expanded uncertainty is obtained by multiplying a coverage factor, k, with the combined standard uncertainty of the measurement result, $u_c(c)$, calculated from the standard uncertainties and covariances associated with the input quantities (x_i , x_j), upon which the concentration depends, according to the equation:

$$U = k \cdot u_c(c) = k \cdot \left(\sum_1^N \left[\frac{\partial c}{\partial x_i} \right]^2 \cdot u^2(x_i) + 2 \sum_1^{N-1} \sum_2^N \frac{\partial c}{\partial x_i} \cdot \frac{\partial c}{\partial x_j} \cdot u(x_i, x_j) \right) \tag{3}$$

An example of estimation of combined uncertainty associated with four mass concentration results of iron, obtained against the method described in the SR ISO 6322:96, is detailed in the table 1.

Table 1 Results on estimation of measurement uncertainty of iron mass concentration in water

Measurement method:	Addition of 1,10 phenantroline solution to a test portion and photometric measurement of the orange red complex at a wavelength of 510 nm against a double-beam grating spectrophoto-meter, bandwidth 2 nm UV2 UNICAM with 10 mm rectangular cuvettes							
Measurement equation:	$c = \left(\bar{c}_{cal} + \frac{\left(A_{meas} - \bar{A}_{cal} \right)}{b} \right) \cdot f_m \cdot f_{RM}$ where: $\bar{c}_{cal}$ is the mean concentration of the calibration curve; A_{meas} - absorbance measured on the sample; $\bar{A}_{cal}$ - absorbance corresponding to the mean concentration of the calibration curve; b - slope of the calibration curve, f_m - correction factor for method sensitivity; f_{RM} - correction factor for the concentration of the reference solution used for calibration;							
Experimental steps:	Sampling	$Fe^{3+} \rightarrow Fe^{2+}$ oxidation	Interference elimination	Fe^{2+} reduction	Complex develop.	Reference soln. prep.	Calibrat. curve	Sample meas.
Input quantities and their standard uncertainties	b, 1/mg.l^{-1}:	0.1796	0.1796	0.1796	0.1796	$s_b = s_0 / \sqrt{\sum (c_i - \bar{c}_{cal})^2}$ =0.00		
	A_{meas}, 1:	0.009	0.018	0.183	0.452	10		
	$\bar{A}_{cal}$, 1:	0.315	0.315	0.315	0.315	$s_{A_{meas}} = s_0 / \sqrt{n}$ =0.002/√3		
	$\bar{c}_{cal}$, mg.l^{-1}	1.60	1.60	1.60	1.60			
	f_m , 1:	0.994	0.994	0.994	0.994	$s_{\bar{A}_{cal}} = s_0 / \sqrt{N}$=0.002/√5		
	f_{RM}, 1:	1.000	1.000	1.000	1.000			
Output results		0.0498	0.0996	1.0128	2.5016			
Combined standard uncertainties	$u_c(c) = c \cdot \sqrt{\frac{s_0^2}{c^2 \cdot b^2} \cdot \left(\frac{1}{N} + \frac{1}{n} + \frac{\left(A_{meas} - \bar{A}_{cal} \right)^2}{b^2 \cdot \sum \left(c - \bar{c}_{cal} \right)^2} \right) + \left(\frac{u_{RM}}{c_{RM}} \right)^2 + \left(\frac{u_m}{f_m} \right)^2}$							
		0.0119	0.0069	0.0315	0.0304			

Note that s_0 is the standard deviation of the calibration curve; n - number of replicates; N - number of RMs used in calibration. Also, note that two correction factors have been introduced in the measurement equation to consider the standard uncertainties evaluated by scientific judgement based on general knowledge of the behavior of the analytic method and from data provided in the linear calibration of the instrument. These standard uncertainties are rather difficult to estimate in the above considered example.

3 ESTIMATION AND OPTIMIZATION OF THE CALIBRATION UNCERTAINTY

Calibration, as defined in [3], is increasingly demanded and implemented in the world of chemistry to assure the required metrological traceability. Since, both physical measurements and reference materials (RMs) of a properly described chemical compozition are used in any analytical measurements, usually, it is necessary to calibrate: (a) mass and/or volume measurements, (b) the

system for measuring correction parameters applied to the foregoing measurements (e.g. temperature, pressure, relative humidity, etc.) and (c) the instrument used to compare the sample being analyzed with a set of calibration samples. It should be stressed here that calibration itself is only one aspect of the entire analytical process - a complex and multistep process.

Usually, any user of a spectrophotometric device calibrates it according to a specific internal procedure. Thus, the relationship (within a specified range and within a period of time during which relevant measurement operations are maintained under control with acceptable repeatability of the measurand), between the values on the quantity indicated by the instrument and the corresponding values assigned to calibration samples of known chemical composition or RMs at a stated uncertainty is determined. Against this relationship, by interpolation, the unknown value of a sample is evaluated.

When a good knowledge of the uncertainty of calibration is required for a further estimation of measurement result uncertainty, a calibration, performed by a metrological authority according to a legal metrology procedure, is needed. During this activity two operations are considered. First, it is acomplished the verification of wavelength and absorbance scale calibration by means of proper wavelength and absorbance RMs [4], traceable to national standards. Second, the above mentioned relationship and its limits of confidence are established, under well specified condition. The case of a linear regression based on the method of least squares to obtain the best-fitting straight line through data, the intercept (a) and slope (b), estimated with certain uncertainties, are shown in the figure 1.

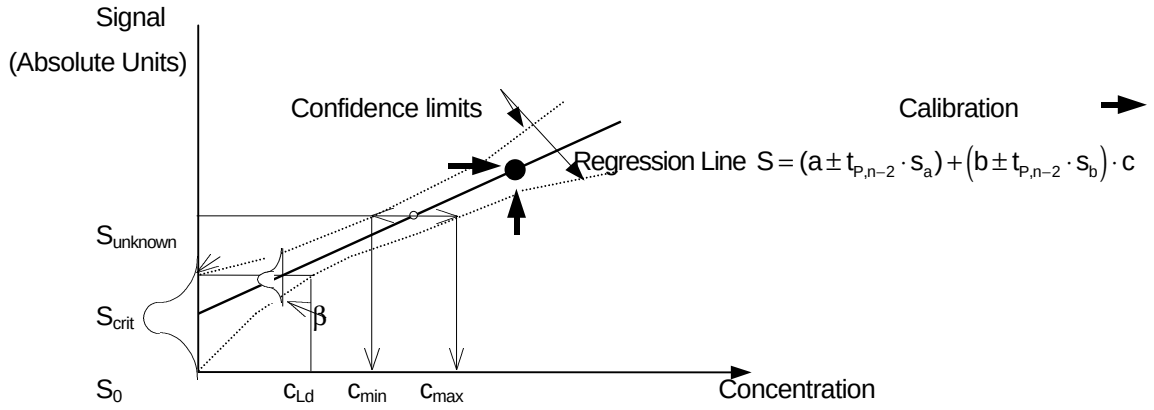

Figure 1. Linear regression based on the method of least squares

Each input concentration will produce a repartition related to instrument signals around the estimated mean from the regression line, within a confidence range of $(1 - \alpha - \beta)$ probability. Note that α is the probability of false positive error and β is the probability of false negative error. The experimental variance of signals, the degree of uncertainty of the real location of the calibration line, and the precision of estimation will determine the magnitude of the confidence range. Uncertainties associated to signal inputs are not constant homogeneous to the curve and depend on the position to the center of the curve, as illustrated in the figure 1. On one-hand, confidence limits of the regression line allow to predict a probable range within the signal value is expected to lie for a certain concentration. On the other hand, for any signal value (*S*) measured against an unknown concentration, it is possible to predict the corresponding concentration range of (c_{min}- c_{max}) with a probability of $(1 - \alpha - \beta)$.

Concentration determination using a spectrometric device relies itself on a comparison of the measured quantity (absorbance for instance) in the unknown sample with the same quantity in a 'standard', i.e. a RM, according to a specific measurement equation, usually, after calibrating the instrument. Consequently, if the value indicated by the instrument is in concentration units, calibration will provide the necessary correction factors. Some results on the estimation of the calibration function of a specialized photometer (DR 890 Hach type) and the use of the analysis of variance to compare the adjustment default and the pure error are presented in the table 2. Note that the procedure described in the table 2 to estimate calibration uncertainty components followed the recommendation given in [5], and the CRMs used were issued by the INM.

Table 2. Calibration of a water analysis specialized photometer

$c_{Fe\ (CRM)}$, mg/L	$c_{Fe(measured)}$, mg/L 1	2	3	4	5	6	7	8	9	10	Mean, mg/L	$c_{Fe(adjusted)}$
(0.50±0.03)	0.50	0.55	0.56	0.58	0.59	0.59	0.58	0.58	0.56	0.55	0.564	0.566
(1.00±0.05)	1.06	1.04	1.02	1.03	1.03	1.03	1.05	1.05	1.06	1.05	1.042	1.052
(1.50±0.08)	1.50	1.52	1.58	1.59	1.57	1.57	1.58	1.59	1.59	1.57	1.566	1.539
(2.00±0.10)	2.00	1.97	1.98	1.99	2.00	2.00	2.03	2.04	2.05	2.05	2.011	2.025
(3.00±0.15)	2.96	2.97	2.95	2.90	2.98	3.03	2.96	3.04	3.10	3.08	2.997	2.997

For the calibration function: $\hat{c}_{Fe(meas)} = 0.0360 \pm 0.9998 \cdot c_{Fe(CRM)}$

Source of variation	Degree of freedom, DF	Sum of squares, SS	SS/DF	F
Calibration function	1	*SST - SSE* = 34.988 37 $\sum_1^5\sum_1^{10}(c_{nk}-\bar{c})^2 - \sum_1^5\sum_1^{10}(c_{nk}-\hat{c})^2$		
Rezidual	50 - 2	*SSE* = 0.070 83 $\sum_1^5\sum_1^{10}(c_{nk}-\hat{c})^2$	$\hat{\sigma}^2 = \frac{SSE}{48}$ 0.001 475	
Adjustment default	5 - 2	*SSE - SSP* = 0.010 29 $\sum_1^5\sum_1^{10}(c_{nk}-\hat{c})^2 - \sum_1^5\sum_1^{10}(c_{nk}-c_n)^2$	$\hat{\sigma}_1^{\,2} = \frac{SSE-SSP}{3}$ 0.003 431	$\frac{\hat{\sigma}_1^2}{\hat{\sigma}_p^2} = 2.55 < F_{tab;0.95(3,45)}$
Pure error	50 - 5	*SSP* = 0.060 54 $\sum_1^5\sum_1^{10}(c_{nk}-c_n)^2$	$\hat{\sigma}_p^{\,2} = \frac{SSP}{45}$ 0.001 345	
Overall	50 - 1	*SST* = 35.059 20 $\sum_1^5\sum_1^{10}(c_{nk}-\bar{c})^2$		

For a typical example of copper determination in water by flame atomic absorption spectrophotometry (SOLAR 939 type, of a 0.04 mg/l concentration characteristic), the steps considered when evaluating calibration uncertainty components are illustrated in the table 3.

Table 3**.** Typical components of calibration uncertainty of a flame atomic absorption spectrometer

Uncertainty Components:	Evaluation of the Uncertainty Component	Mathematical relationship	Uncertainty (rel)
due to the photometric measurement	Starting from Calibration Uncertainty and run-to-run variation	$s_1 = \sqrt{u_{NF}^2 + u_{rep}^2 + u_{cor}^2}$	0.010
due to the CRMs	from Calibration Certificate	$s_2 = U_{CRM}/\sqrt{3}$	0.004
due to the linear adjustment	Starting from the random errorr associated with the slope and intercept of the regression line	$s_{\hat{x}_c} = \frac{s_{y_{(x)}}}{b}\sqrt{\frac{1}{n}+\frac{1}{N}+\frac{(\hat{y}-\bar{y})^2}{b\sum_1^n(x_i-\bar{x})^2}}$	0.027
Combined uncertainty	As square sum of above uncertainty components	$u_{cal} = \sqrt{s_1^2 + s_2^2 + s_{\hat{x}_c}}$	0.029
Overall uncertainty	k = 2	$U_{cal} = 2 \cdot u_{cal}$	0.058

In this table, the uncertainty components, their magnitude and the way to evaluate them are also summarized. Note that u_{NF} is the uncertainty of the neutral filters used, u_{rep}- uncertainty due to the repeated measurements of absorbance on neutral filters, u_{cor}- uncertainty of the correction factor for absorbance values, U_{CRM} - uncertainty of the CRM (stated in the certificate of the CRM), $s_{\hat{x}_c}$ -

uncertainty of the predicted value of concentration from the calibration curve, $s_{y_{(x)}}$ - standard deviation of the points about the regression line, b, n, and N - were defined in the table 1, x_i - concentration values used to determine the regression line and $\hat{y}$ - the given value of *y* from which the unknown value is to be determined. Note that the calibration uncertainty did not exceed 6 % for a value of 2 mg/L Cu. Also, this way of evaluating calibration uncertainty, starting from potential sources of error was a real aid to identify those components that have a large contribution in the overall uncertainty and, thus, to minimize them as much as possible.

In photometric measurements, the ratio between the uncertainty of a photometric result and the uncertainty of the upper standard used in calibration, is very important, being a measure of the strength of traceability chain. Usually, this ratio should be minimum of 3. For physical standards used to calibrate the photometric systems this ratio is most commonly achieved. This rule generally applies also for weight and volume measurements performed in conjunction with the photometer. But in some chemical measurements of molar or mass concentration using photometric systems, often this ratio did not exceed 1 or 1.2 [6].

4 PROPAGATION OF VARIANCES AND COVARIANCES OF ANALYTICAL RESULTS

Any output quantities c_k and c_l obtained in analytical measurements using the same instrument and depending on the same set of input quantities, are correlated to some extend. In a way analogous to the usual derivation of the variance-propagation law [1], any two output estimates c_k and c_l may be represented as the first-order term of a Taylor expansion about the expectations of the input quantities (x_1, x_2, x_3, ...).

$$c_k = f_k(\mu_1, \mu_2, \mu_3, \ldots) + \sum_1^n \left.\frac{\partial f}{\partial x_i}\right|_{x_i = \mu_{x_i}} (x_i - \mu_i) + \ldots \quad \text{and} \tag{4}$$

$$c_l = f_l(\mu_1, \mu_2, \mu_3, \ldots) + \sum_1^n \left.\frac{\partial f}{\partial x_j}\right|_{x_j = \mu_{x_i}} (x_j - \mu_j) + \ldots \tag{5}$$

Considering the definition of covariance given in [1] and assuming that the expectation of the difference $E(x_i - \mu_l)$ is equal to 0, then:

$$\operatorname{cov}(c_k, c_l) = \sum_1^n \frac{\partial c_k}{\partial x_i} \cdot \frac{\partial c_l}{\partial x_j} \cdot \sigma^2(x_i) + 2\sum_1^{n-1}\sum_2^n \frac{\partial c_k}{\partial x_i} \cdot \frac{\partial c_l}{\partial x_j} \cdot \operatorname{cov}(x_i, x_j) \tag{6}$$

Equation (6) provides the elements k and l of the covariance matrix of output estimates in terms of the elements of the covariance matrix of input estimates.

Following the example described in tables 1 and 2, we consider that two output estimates c_k and c_l were obtained by comparing n times the unknown sample against a reference solution of ($\bar{c}_{cal} \pm u_{\bar{c}_{cal}}$), by means of a calibrated photometric device. Taking into account the equation of measurement described in the table 1, then the second term of the sum can be expresed as a difference Δc_k depending on photometric sensitivity. The two output estimates will therefore become:

$$c_k = \bar{c}_{cal} - \frac{1}{n}\overline{\Delta c}_k \quad \text{and} \tag{7}$$

$$c_l = \bar{c}_{cal} - \frac{1}{n}\overline{\Delta c}_l \tag{8}$$

The input estimates are now $\bar{c}_{cal}$, Δc_k, Δc_l. Considering that they are uncorrelated, from (7) and (8), the combined standard uncertainties of the estimates are:

$$u_c^2(c_k) = u_{\bar{c}_{cal}}^2 + u^2\left(\overline{\Delta c}_k\right) \quad \text{and} \tag{9}$$

$$u_c^2(c_l) = u_{\bar{c}_{cal}}^2 + u^2\left(\overline{\Delta c}_l\right) \tag{10}$$

Both $u^2(\Delta c_k)$ and $u^2(\Delta c_l)$ depend on the instrument sensivity (slope) and on the instrument capability to determine an absorbance difference with a known standard deviation $s_{\Delta A}$. Then, their covariance is:

$$\operatorname{cov}(c_k, c_l) = u_{\bar{c}_{cal}}^2 \tag{11}$$

Therefore, the concentration outputs estimates are correlated by their comon uncertainty arising from the estimate of the reference solution, thus a correlation coefficient may be defined as:

$$r(c_k, c_l) = \mathrm{cov}(c_k, c_l) / u_{c_k} \cdot u_{c_l} \quad (12)$$

In this case the correlation coefficient depends on the ratio of variance of the photometric comparator to that of the reference standard.

Considering the numerical example given in the table 2, correlation of output estimates is as following: r(0,5;1,00)=0.913; r(1,00;1,50)=0.764; r(1,50; 2,00)=0.815; r(2,00; 3,00)=0.978. If one neglects the covariance between two estimates r(1,00;1,50) or r(1,50; 2,00), when combining them in a subsequent experiment, then a significant underestimation of uncertainty may be obtained.

5 MEASUREMENT UNCERTAINTY AND COMPLIANCE TO ACCEPTABLE LIMITS

The extent to which measurement uncertainty is taken into account when evaluating the compliance to maximum acceptable limits is still a problem under debate.

Several national standards indicate the upper limit of the concentration that can be accepted for different analytes currently determined in soil, water or atmospheric air, as well as the corresponding analytical procedures. For a maximum acceptable concentration of a certain analyte c_{Max}, that has been set by the regulatory authority, the evaluation of conformity of an unknown sample to certain limits means a statistical comparison between instrument signals obtained on the unknown sample and instrument signals obtained on a known sample having c_{Max} concentration, using t (Student) test. For a selected confidence level $(1 - \alpha)$, and supposing homogeneous variances, it is possible to state that the signal of the unknown sample S_X is significantly larger than S_{Max} if it is larger than:

$S_{Max} + 2t_{(1-á,í)}s_{Max}$ (13)

where: s_{Max} is the relevant standard deviation, ν - degree of freedom and t - Student coverage factor.

Using the Student test, one can estimate:

$$t_{exp} = \frac{(S_{unknown} - S_{Max}) \cdot \sqrt{n_{unknown}}}{S_{Max}} \quad (14)$$

where $n_{unknown}$ is the number of observations made on the unknown sample. If t_{exp} is larger than the critical one, the sample is not compliant at the selected confidence level. Once compliance has been evaluated, the concentration of the analyte in the suspected sample can be evaluated from the calibration curve.

Following example is given to illustrate the above mentioned approach.

Lets assume that it is accepted a maximum concentration of 0.05 mg/L copper in a drinking water sample. An unknown sample containing approximate this concentration is measured against a flame AA spectrometer using the calibration parameters described in the table 3. Three repeated measurements on the unknown sample gave an average response of 0.052 mg/L (for a mean absorbance of 0.0075 measured), with a relative standard deviation of 1 %. Using the above calibration results an uncertainty of 0.009 mg/L was estimated. Since the considered legal regulation does not indicate any information on the uncertainty of the maximum accepted limit, the problem is to evaluate if the concentration in the suspect sample is significantly higher. For an average signal of 0.0072 corresponding to the solution containing the limit level of concentration and a standard deviation of 0.000 2, the t test gives the experimental t-value of 2.598, while the critical one-tailed value for a 0.95 confidence level is 2.920. Since $t_{exp} < t_{crit}$ the sample may be considered compliant, and a result of (0.052 ± 0.018) mg/L may be reported. Note that a coverage factor of two was used. Such result still needs an additional set of measurements using another calibration within a lower concentration range. A higher characteristic concentration specification for the instrument would be inappropriate to solve this measurement problem.

6 CONCLUSIONS

This paper has examined the importance of an appropriate evaluation of measurement uncertainty in chemical laboratory in order to assure the requirements for traceability. To produce results which are accurate and reliable within a stated uncertainty, several aspects, such as RMs, calibration or photometric system, were carefully considered. Further, the covariances of output estimates were deduced and a correlation coefficient, depending on the ratio of variance of photometric comparator to that of reference standard, was determined. Practical examples of measurement uncertainty estimation and spectrometric calibration in routine measurements demonstrated the possibility to reduce, considerably, the risk of wrong decisions on rejecting/accepting legal measurement results.

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