



UNCERTAINTY

Measurement uncertainty and legal limits in analytical measurements

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1 Introduction

Analytical measurements that are used as a basis for legal decisions must be reliable and therefore demand an appropriate statement of the degree of their uncertainty when evaluating the compliance of suspect matrices with maximum acceptable legal limits. Since most of these limits correspond to a low level of concentration, this necessarily leads to the evaluation of the minimum detectable signal, from which the minimum detectable inadmissible concentration can be obtained. To this end, Type 1 and 2 statistical errors must be considered. Also, estimating measurement uncertainty associated with very low levels of concentration very much depends on the correct identification and quantification of the main sources of uncertainty (due to the calibration, instrument, reference materials, etc.).

The *Guide to the Expression of Uncertainty in Measurement* [1] and *Quantifying Uncertainty in Analytical Measurement* [2] are used as normative references for the evaluation and expression of uncertainty in chemical measurements at all levels of accuracy from basic research and development to routine analysis. But the extent to which measurement uncertainty is taken into account when evaluating the compliance to maximum acceptable legal limits is still a problem under discussion.

The experience of the Romanian National Institute of Metrology (INM) in identifying and estimating measurement uncertainty in legal metrology activities concerning the protection of the environment is presented and reviewed; practical examples of measurement uncertainty estimation in spectro(photo)metric determinations (in particular the case of spectrometers used to determine metal pollutants in water) and the consideration of measurement uncertainty for interpretation of regulatory compliance are both discussed.

Within the framework outlined above, this paper aims to discuss the practical problems involved in met-

rological verification of the analytical spectrophotometers according to OIML Recommendations, mainly regarding detection limits and severity.

2 Brief review of metrological assurance in legal spectrometric measurements

Uniformity of all measurements is the main goal of the legal metrology activities in accordance with the Romanian Law of Metrology and the regulations issued in the field of metrology. Consequently, measurements in trade, in production and testing of pharmaceuticals or in the fields of health, safety and environmental protection are performed in a coherent measurement system within which the consistency of measurements is easily maintained and demonstrated.

Many different types of spectro(photo)meters (starting from the discontinuous wide band absorption photometers to completely automated atomic absorption (AA) spectrometers or inductively coupled plasma (ICP) atomic emission spectrometers) are extensively used to support most of the decisions made on the basis of quantitative chemical results in environmental protection and in public health.

Legal metrology principles clearly apply to such measurements and traditional metrological assurance of measurements implies:

- pattern approval of the instrumentation;
- calibration of the equipment;
- development of a proper system of reference standards (reference materials (RM's) and certified reference materials (CRM's) included); and
- achievement of traceability.

Note that an outline of the metrological assurance of legal analytical measurements is presented in [3].

Accordingly, all spectro(photo)meters used in legal activities are subject to pattern approval of each model

with any variants of that model and also subject to calibration or mandatory verification. Since the suitability of such instruments with regard to legal activity is specified their metrological performances are evaluated using, as a rule, legal metrological norm (NML) methods and appropriate standards and RM's. Several NML's and metrology procedures have been issued on the basis of the OIML Recommendations in the field of environmental protection. These issues provide the requirements for absorption photometers for water analysis (Testing Procedure no. 48/1997), atomic absorption spectrometers for measurement of metal pollutants in water (NML 9-02-94, based on OIML R 100), inductively coupled atomic emission spectrometers for measurement of metal pollutants in water (NML draft based on OIML R 116), as well as the methods and RM's or CRM's to be used for this purpose.

For metrological assurance of such measurements performed in the field of the environment only CRM's are recognized and accepted for use. The national system of CRM's developed by the INM for ensuring the required uniformity and accuracy of analytical measurements is presented in [4].

Under the Romanian Law of Metrology, any spectrometer used in a legal activity should be calibrated and/or verified by an authorized metrological laboratory. This laboratory, in turn, should calibrate its standards to INM, which owns the national standards of different physical and chemical quantities, and inter-compares them in a worldwide frame. The above chain characterizes the traceability of the result. To reach this target, in analytical measurements two aspects are to be considered:

- the instrument should be calibrated in a traceable manner; and
- RM's should assure traceability to the SI.

At this point, it should be noted that the above-mentioned legal metrology activities are more concerned with errors (for comparison with maximum permissible errors) as well as with other metrological characteristics (of the spectrometer, CRM's, measurement standards, etc.). However, approval or rejection of a spectrometer on a "wrong" basis or based on a hazardous decision has serious economic implications. This is why uncertainties introduced in verification or calibration of the spectrometer need to be taken into account when evaluating the compliance with legal limits stated in the NML or other procedures.

In addition, the increased use of certification/accreditation with reference to ISO standards on requirements, measurement and test methods, measurement equipment, quality control (ISO 9000 and 14000 series) have major legal implications: use of these leads to a harmonized common approach to metrology based on measurement uncertainty. In such laboratories

the evaluation of compliance is particularly challenging when a completely unknown sample, different from those routinely analyzed, has to be evaluated and compared to maximum acceptable legal limits. Since most of these limits correspond to a low level of concentration, this necessarily leads to the importance of the evaluation of minimum detectable instrumental signal, from which the minimum detectable admissible concentration can be obtained, and of the appropriate instrumental sensitivity for the specific type of measurement.

3 Instrumental detection limits and sensitivity in NML's and the consequences for analytical measurements

Spectrometers used to measure metal pollutants in water are tested by measuring detection limits, optimum working range, short term precision and accuracy at minimum five representative analyte wavelengths that cover the complete spectral range of the instrument (AA spectrometer or ICP spectrometer). Additionally, AA spectrometers are tested for characteristic concentration and during pattern approval tests they are tested at the nominal limits of exploitation too.

Both AA and ICP spectrometric methods used in this field are relative methods of measurement. Therefore, to determine a certain concentration (which can be detected with reasonable uncertainty) or a characteristic concentration, two concentrations are compared via their instrumental random signals. In the case of detection limit tests, the first instrumental signals correspond to the analyte-free (blank) solution, and the second ones to a very low concentration of the specified analyte. Poor precision is obtained at the detection limit due to the significant percentage of the noise. Thus, a higher level of concentration (for instance of one hundred times the detection limit level) is more appropriate for this comparison.

To determine detection limits in accordance with the NML's specifications, spectrometers are tested in a standard configuration and under standard operating conditions as defined in the operator's manual. Calibration is required. Depending on the manufacturer's procedure, the functional relationship between signal and concentration is usually obtained by a linear least square regression using up to five reference solutions containing the specified analyte. Four series of ten measurements are performed on the blank solution. The standard deviation of the mean values is determined and then multiplied by three. Note that the definition of the detection limit given in OIML R 100 was adopted in NML 9-02-94. Starting from the definition and the

general verification procedure, the detection limit is a statistical measure of the smallest concentration of a particular analyte that can be distinguished from the baseline noise signal.

Using the slope (b) information, characteristic concentrations are determined from the equation:

$$C_{\text{char}} = \frac{0.0044}{b} \quad (1)$$

and provide useful information on the instrument sensitivity. Although the definition of the characteristic concentration is different from that of the sensitivity given in the VIM [7], for routine activities the second formulation is still widely used for this specification.

Some results on initial verification of the above instrument specifications with various different spectrometers used to measure metal pollutants in water are discussed below.

Usually, after installation any pattern approved AA spectrometer model is subject to an initial metrological verification when detection limits, characteristic concentrations, accuracy and short-term repeatability are tested using no more than five analytes. Copper is compulsory in this verification and single element CRM type 13.01, containing (1.000 ± 0.002) mg/L Cu, produced by the INM, is used to prepare the required diluted solution in a range of concentration of $(0.5 \dots 10)$ mg/L. The spectrometer is calibrated as far as possible according to the calibration procedure agreed in the manufacturer's instructions.

In a similar way, pattern approved models of ICP spectrometers are subject to initial metrological verification when detection limits, accuracy and short and long term repeatability are tested immediately after cali-

bration. In this case, multi-element CRM's type 15.02 (containing zinc, manganese, copper, sodium, lithium and potassium), also produced by the INM, in the range of concentration recommended in OIML R 116 [8], are used.

The instrumental accuracy is determined as being the difference between the mean of read values (usually rounded to three decimals) of concentration on a standard solution of the specified analyte and the certified value of that solution. For AA spectrometers a limit of 7 % from the certified value is admitted. In the case of ICP spectrometers the uncertainty limits of the CRM's are considered.

The repeatability error is determined as being the relative standard deviation of 5 measurements performed on the standard solution of the specified analyte. A maximum 3.5 % relative error of repeatability is permitted for the AA spectrometers and 2 % for the ICP spectrometers.

Table 1 shows some results obtained on verification of common spectrometers (SOLAR 939/959, AAS 3/30/5, Baird ICP types) used to measure copper in water. Note that the spectrometers included a system for data acquisition and processing. The instrumental accuracy and repeatability of the flame AA spectrometers (instruments 1-7) were evaluated against a (2.00 ± 0.05) mg/L copper. A multi-element solution containing (10.00 ± 0.20) mg/L was used against the ICP spectrometer (instrument 8).

Since the NML requires a detection limit of 0.003 mg/L for copper, instrument 4 (SOLAR 939 type) should be rejected. Also, if a characteristic concentration of copper of 0.03 mg/L is admitted several instruments (1 – AAS 5 Anal. Jena type, 4-6 – SOLAR 939/959 types and 7 – AAS 30 Zeiss Jena type) should also be rejected on initial verification. On the other hand, since the instrumental error of instrument 2 (SOLAR 959

Table 1 Some results on the verification of instrument specifications of spectrometers used to measure metal pollutants in water

	Detection limit (c_{Ld}), mg/L	Characteristic concentration (c_{char}), mg/L	Instrumental relative error (e), %	Repeatability error (R), %
Instrument 1	0.0026	0.0318	- 1.00	1.17
Instrument 2	0.0028	0.0297	- 7.50	0.77
Instrument 3	0.0015	0.0290	+ 7.00	0.33
Instrument 4	0.0036	0.0420	+ 0.50	0.25
Instrument 5	0.0030	0.0429	- 1.00	0.70
Instrument 6	0.0032	0.0432	- 1.00	0.10
Instrument 7	0.0030	0.0432	- 0.50	0.50
Instrument 8	0.0024	-	- 1.20	0.87

type) exceeded the limit of 7 % it should be rejected. It is obvious that such decisions have to take into account the instrumental calibration, uncertainties of the standard solutions used, uncertainty of the determination of a particular specification, as well as additional means to optimize instrumental working conditions (such as bandpass, lamp current, burner height, burner alignment, fuel flow, flame type or impact bead adjustment). Note that both detection limit and characteristic concentration values provide useful information about the intrinsic performance of the spectrometers (background precision, signal amplitude, sensitivity, signal to noise ratio, etc.), particularly if measurements include the acquisition of additional data. But in a real analytical measurement process, the lowest amount of an analyte in a natural matrix, which can be detected, may be adversely affected. Also, knowing the expected characteristic concentration allows the operator to predict the absorbance range, which will be observed for a specific concentration range of the analyte of interest.

To compare the calibration of AA spectrometers, Table 2 presents information on:

- the intercept, a , of the regression line;
- the slope, b , of the regression line;
- the uncertainties associated with them, s_a , s_b ;
- the number of standard solutions used for the calibration, N ;
- the number of replicate measurements, n ; and
- the standard deviation of the points about the regression line, s .

The formulae described in [5] to obtain the regression line and the associated error estimates, presented in Table 3, were used.

One may note that the values of the slope of the regression line were homogenous enough and the standard deviation of the points about the regression line were dispersed within a range of (0.0002 ... 0.0100). It is

therefore very important to highlight the role of spectrometer calibration to achieve an appropriate uncertainty of such determination.

4 Uncertainty of the determination of the detection limit and characteristic concentration

The continuous increase in demand for the number of measurements supporting environmental policy and the continuous decrease in tolerance levels result in a necessary improvement of measurements and specifications. Since a specification such as instrumental detection limit, or characteristic concentration is used as a characterization of an intended function of the spectrometer, it is therefore an integrated part of metrology in the same manner as the definition of the measurand. Also, to compare a measured characteristic with a given specification (specification limit) measurement results are used. For instance, the mean result of several series of repeatable measurements on a blank solution and the slope of the calibration line are necessary to determine a specific result on the detection limit.

According to [1], any measurement result should be associated with a parameter that characterizes the dispersion of the values that could reasonably be attributed to the measurand, i.e. measurement uncertainty. Consequently measurement results performed in tests for pattern approval or for verification as well as calibrations carried out for legal metrology purposes should have an indication of measurement uncertainty, which have to be evaluated starting from:

- incomplete knowledge of the true value of CRM's (measurement standard);
- the use of CRM's or RM's in testing;

Table 2 Instrumental calibration of AA spectrometers tested

	a	s_a	b	s_b	N	n	s
Instrument 1	- 0.001	0.0002	0.1385	0.0001	1	10	0.0002
Instrument 2	0.027	0.0007	0.1375	0.0016	4	3	0.0100
Instrument 3	0.036	0.0030	0.1515	0.0005	3	3	0.0040
Instrument 4	- 0.002	0.0003	0.1034	0.0002	1	2	0.0003
Instrument 5	0.013	0.0030	0.1025	0.0005	3	2	0.0040
Instrument 6	0.003	0.0005	0.1018	0.0001	6	3	0.0005
Instrument 7	0.002	0.0005	0.1018	0.0005	4	3	0.0005

Table 3 Summary of the formulae used to obtain the regression line and the associated error estimates

<p>Intercept:</p> $a_{(c)} = \bar{y} - b_{(c)} \cdot \bar{c}$ <p>where: $\bar{y} = \frac{1}{N \cdot n} \cdot \sum_{i=1}^N \sum_{j=1}^n y_{ij}$ and: $\bar{c} = \frac{1}{N} \cdot \sum_{i=1}^N c_i$</p>
<p>Standard deviation of the intercept:</p> $s_a = \sqrt{\frac{s_b^2}{N} \cdot \sum_{i=1}^N c_i^2}$
<p>Slope:</p> $b_{(c)} = \frac{\sum_{i=1}^N (c_i - \bar{c}) \cdot (y_i - \bar{y})}{\sum_{i=1}^N (c_i - \bar{c})^2}$
<p>Standard deviation of the slope:</p> $s_b = \sqrt{\frac{s^2}{\sum_{i=1}^N (c_i - \bar{c})^2}}$
<p>Std. deviation of the points about the regression line:</p> $s = \sqrt{\frac{\sum_{i=1}^N (y_i - \hat{y})^2}{N - 2}}$
<p>Precision of the estimation of a predicted value:</p> $s_c = \frac{s}{b} \cdot \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(y_{meas} - \bar{y})^2}{b^2 \cdot \sum_{i=1}^N (c_i - \bar{c})^2}}$
<p>Equation of the regression line:</p> $\hat{y} = a_{(c)} + b_{(c)} \cdot c$

- the assumption of linearity;
- the instrument being tested (random variations in the instrumental measurement affect both the response reference and the measured response).

In the particular case of detection limit verification, the author considers that variances due to the variability of the calibration of the instrument and due to the certified value of the RM's used should be combined in a square root way to estimate the overall uncertainty.

When measuring the characteristic concentration described by equation (1), variances due to the variability of the calibration slope and the certified value of the RM's may be considered. From the above observation it results that the magnitude of the calibration uncertainty is very important when evaluating the overall uncertainty of such tests.

Calibration of the spectrometers used to measure metal pollutants in water usually means the set of operations that establish, under specific conditions, the relationship, within a specified range, between values indicated by the instrument and the corresponding values assigned to calibration samples of known chemical composition or RM's at a stated uncertainty. As a common practice for such instruments, the regression line and its limits of confidence, illustrated in Fig. 1, synthesize the relationship between the two variables. Linear regression is based on the method of least squares to obtain the best-fitting straight line through the data and both the intercept (*a*) and the slope (*b*) are estimated with certain uncertainties. It also assumes certain conditions: the values of concentration are error free, and all errors are contained in the y-values (instrumental signals) only. However, even if CRM's are used in calibration they are associated with some uncertainties (usually smaller than those of the spectrometer).

During calibration, 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 - α - β) probability. Note that α is the probability of a false positive error and β is the probability of a false negative error. The experimental variance of signals, the degree of uncertainty on the real location of the calibration line, and the precision of estimation will determine the magnitude of the confidence range. Uncertainties associated with y are not constantly homogeneous to the line and depend on the position of the center of this line, as is illustrated in Fig. 1. On the one hand confidence limits of the regression line allow a probable range to be predicted within which 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 - α - β). In the particular situation where the upper confidence

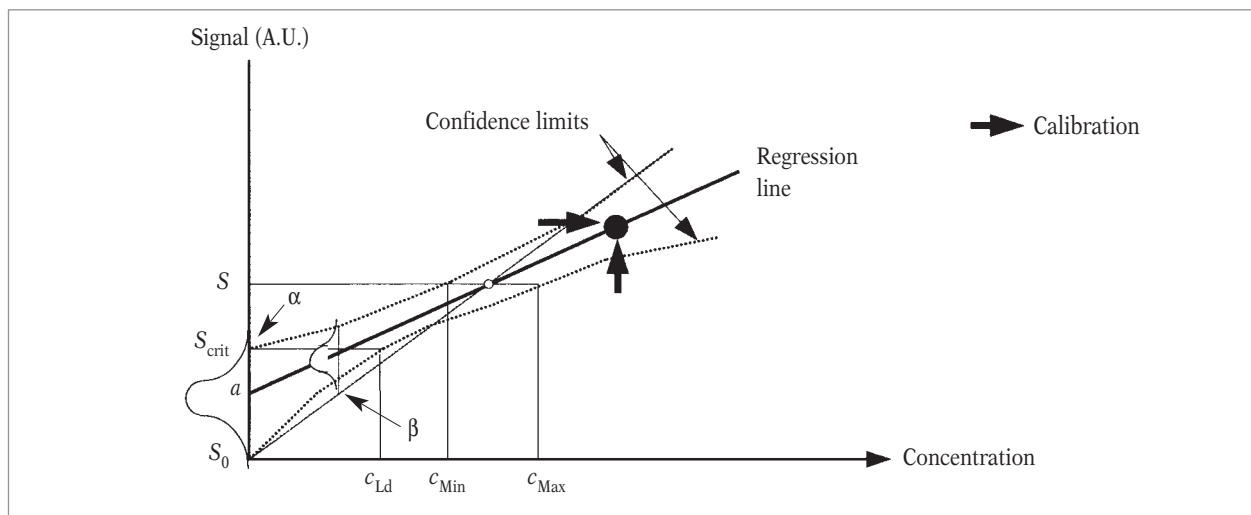


Fig. 1 Linear regression based on the method of least squares

limit intersects the y-axis (noted with S_{crit}), the concentration is equal to zero, corresponding to a blank solution. The standard deviation of the blank is given by s_a , and the limit of the confidence interval for the intercept, assuming that the instrumental signals follow a Student distribution, is $t_{(p; n-2)} \cdot s_a$. Since the detection limit is taken at a certain distance from a , it is the concentration c_{Ld} (at the intersection of the horizontal line from S_{crit} with a lower confidence limit). Near c_{Ld} , and quite far away from the center of the regression line, the lower confidence limit may be approximated by the corresponding asymptote (dotted line in Fig. 1) described by the general equation (2):

$$Y = a + b \cdot c + 3 \cdot t_{p;n-2} \cdot s_b \cdot (c - \bar{c}) \quad (2)$$

Thus, we may describe the detection limit in terms of intercept, slope, standard deviation of the intercept and slope respectively, as well as the mean concentration of the regression line, accordingly to equation (3):

$$c_{Ld} = \frac{3 \cdot (s_a + \bar{c} \cdot s_b)}{b + 3 \cdot s_a} \quad (3)$$

From this equation one may note that the uncertainty of determination of the detection limit may be estimated by combining in a square root way the relative standard deviation of the intercept, the standard deviation of the slope and the standard deviation of the certified concentration of the calibration sample corresponding to the center of the regression line. Also, note that in this approach the null hypothesis and Type 1 (α) and Type 2 (β) statistical errors have been considered.

Using same approach the uncertainty of determination of the characteristic concentration is estimated by

combining in a square root way the relative standard deviation of the slope and the standard deviation of the certified concentration of the calibration sample corresponding to the center of the regression line.

Starting from the results presented in Table 2, the estimated overall uncertainty ($k = 1$) and its components are illustrated in Fig. 2. Note that the first three values presented under each instrument represent the components described above and the fourth value is the estimated relative combined uncertainty. The lowest measurement uncertainty was estimated in the case of the verification of instrument 3 (13.02 %) and the highest for instrument 2 (5.08 %). These results concord with the fact that a precision of 11 % ($k = 1$) is practically expected for these concentration levels and instrumental conditions of measurements.

5 Evaluation of compliance to legislative limits

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 the conformity of an unknown sample to certain legal limits means a statistical comparison of the instrumental signals obtained on the unknown sample to the instrumental signals obtained on a known sample having c_{Max} concentration using the t (Student) test. For a selected confidence level ($1 - \alpha$), and supposing homogeneous variances, it is possible to

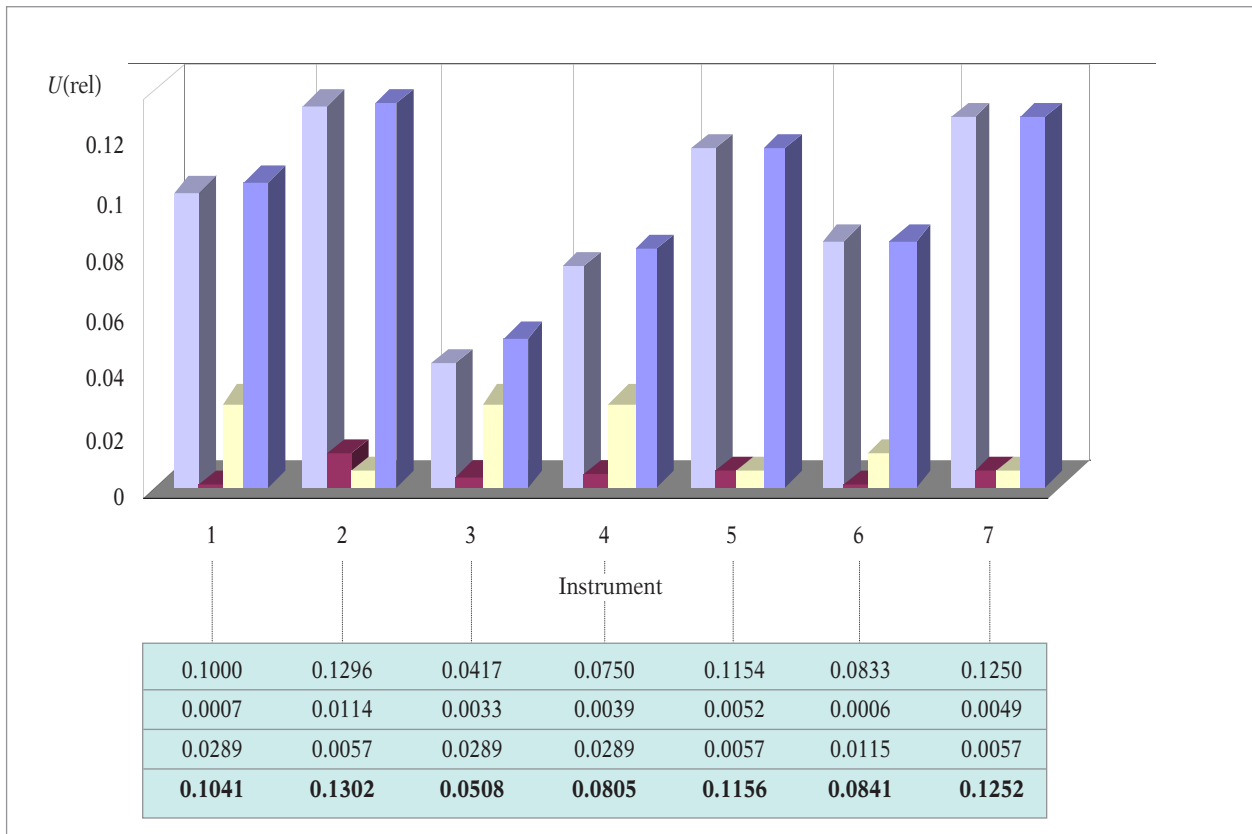


Fig. 2 Attempt to evaluate the measurement uncertainty components of the detection limit determination for AA spectrometers

state that the signal of the unknown sample S_x is significantly larger than S_{Max} if it is larger than (4):

$$S_{Max} + 2t_{(1-\alpha, \nu)} S_{Max} \quad (4)$$

where:

s_{Max} is the relevant standard deviation;

ν is the degree of freedom; and

t is the Student coverage factor.

Consequently, the Student test may be used to perform this evaluation:

$$t_{exp} = \frac{(S_x - S_{Max}) \cdot \sqrt{n_x}}{s_x} \quad (5)$$

where n_x 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.

The following example is given to illustrate the above approach.

Assume that a maximum concentration of 0.05 mg/L copper is accepted in a drinking water sample. An unknown sample containing approximately this concentration is measured against a flame AA spectrometer (instrument 7 type) using the calibration parameters described in Table 2. Three repeated measurements on the unknown sample gave an average response of 0.052 mg/L (for a mean absorbance of 0.0075 measured), a relative standard deviation of 1 %. Using the 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 whether the concentration in the suspect sample is significantly higher.

For an average signal on the solution containing the limit level of concentration of 0.0072 and a standard deviation of 0.0002, 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 ($k = 2$) was used.

Such a result still requires an additional set of measurements using another calibration within a lower concentration range. In this frame, a higher character-

istic concentration specification for the instrument would be inappropriate to solve this measurement problem.

Conclusions

This paper has examined a number of problems concerning the uncertainty of determination of two metrological characteristics of spectrometers used to measure metal pollutants in water (detection limit and characteristic concentration), and how to consider this when evaluating the compliance against decision limits.

Clearly defined instrument specifications, knowledge of main sources of uncertainty affecting the measurement and adequate application of the Guide [1] requirements are the main targets.

Calibrating spectrometers for routine measurements against suitable CRM's considerably reduces the risk of the wrong decision being made when rejecting/accepting legal measurement results. ■

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