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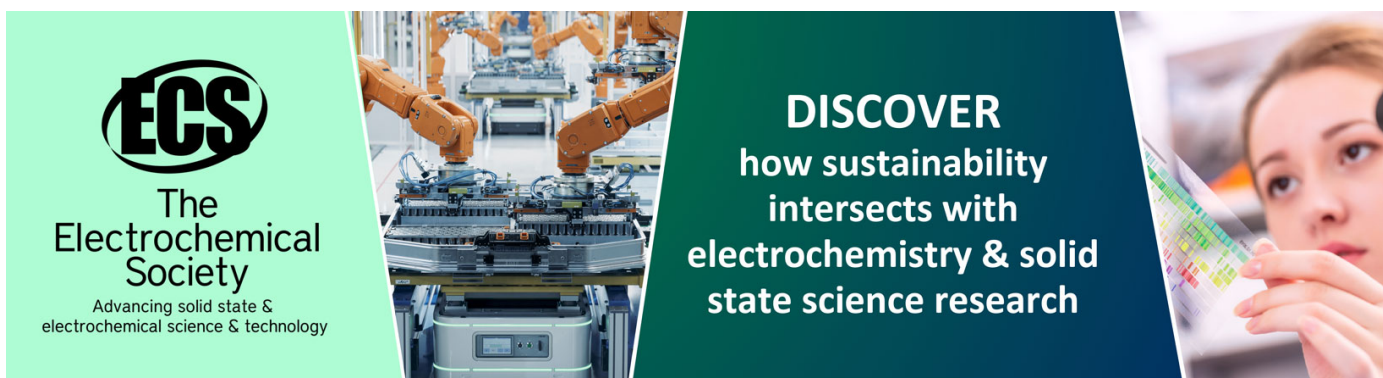
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Method validation for chemical composition determination by electron microprobe with wavelength dispersive spectrometer.

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Abstract. The main goal of method validation is to demonstrate that the method is suitable for its intended purpose. One of the advantages of analytical method validation is translated into a level of confidence about the measurement results reported to satisfy a specific objective. Elemental composition determination by wavelength dispersive spectrometer (WDS) microanalysis has been used over extremely wide areas, mainly in the field of materials science, impurity determinations in geological, biological and food samples. However, little information is reported about the validation of the applied methods. Herein, results of the in-house method validation for elemental composition determination by WDS are shown. SRM 482, a binary alloy Cu-Au of different compositions, was used during the validation protocol following the recommendations for method validation proposed by Eurachem. This paper can be taken as a reference for the evaluation of the validation parameters more frequently requested to get the accreditation under the requirements of the ISO/IEC 17025 standard: selectivity, limit of detection, linear interval, sensitivity, precision, trueness and uncertainty. A model for uncertainty estimation was proposed including systematic and random errors. In addition, parameters evaluated during the validation process were also considered as part of the uncertainty model.

1. Introduction

No matter what it costs, any testing and calibration laboratory need to be recognized for the quality of its results. The ISO/IEC 17025 [1] standard establishes the general requirements for the competence of testing and calibration laboratories. Laboratories accredited with ISO/IEC 17025 must comply its requirements grouped in two main fields: (1) Management Requirements; part 4 of the standard, and



(2) Technical Requirements; described in section 5 of the standard. If the laboratory is certified with ISO 9001 [2] standard, it is complying the section 4 of the ISO/IEC 17025. However, section 5 demands the knowledge and experience in specialized topics, such as, fundamentals of metrology, method validation and estimation of uncertainty. Therefore, method validation is a fundamental topic at any laboratory due to the benefits obtained to demonstrate its technical competence and the validity of its results.

On the other hand, wavelength-dispersive X-ray spectrometers (WDS) have been around for more than 60 years. In this time, they have maintained a central role in microanalysis because of their effectiveness in measuring X-rays reproducibly, and because of their high spectral resolution. By the above the WDS has been in the market for many years and it is still the preferred X-ray analytical system for many, mainly due to its qualifications of resolution spectral. For comparison, an energy dispersive spectrometer (EDS) has an energy resolution of around 130 eV (when measured as the full width at half maximum, FWHM, of Mn K α) as-compared to a traditional wavelength-dispersive spectrometer (WDS), which has an energy resolution of around 16 eV, almost an order of magnitude improvement [3]. The WDS microanalysis advantages can be reinforced having information about validation of the applied method to obtain reliable results. Method validation information is crucial to have a good uncertainty estimation of the result of the measurement [4].

Because there is a lack of information about method validation with WDS microanalysis, authors hope to contribute with novel information. A method for the determination of chemical elemental composition of a binary Au-Cu alloy was studied. Evaluation of the following parameters is reported: selectivity, limit of detection, linear interval, sensitivity, precision, trueness and uncertainty.

2. Materials and Measuring Method

2.1. Materials

SRM 482 NIST [5] is a set of 6 wires with different chemical elemental composition of the binary Au-Cu alloy. There are two pure metallic wires, one of Au at 100 % and another one of 100 % Cu and four binary alloys Au-Cu with chemical nominal compositions as follows expressed as mass fraction (%): Au20-Cu80, Au40-Cu60, Au60-Cu40 and Au80-Cu20. The SRM 481 standard was arranged at the same capsule as a condition of practice good laboratory. The alloy Au80-Cu20 was considered as test sample, while the another alloys were used as standard and control sample.

2.2. Equipment

All experiments were done using an electron probe micro analyzer (EPMA) model Super-Probe JXA 8200 JEOL equipped with 3 WDS and one EDS. WDS are from JEOL, each one containing 2 analyzer crystals. JXA-8200, has one EDS Si (Li), window Be Thermo Noran, 130.8 eV at 5.9 keV. Specimen tilt 0°, take off angle 40°, EDS elevation angle 40°, EDS azimuth angle 140°, insertion distance 87 mm, height 11 mm.

The microprobe is controlled with the software JXA-8200 JEOL V01.02. Record and analysis of the data was done with the same software. The EPMA has a UNIX operating system; therefore, it is not possible to obtain spectral files in emsa format.

3. Results and Discussion

3.1. Measurand definition

The basis for quantitative X-ray microanalysis is that, to a first approximation:

$$\frac{C_{sample}}{C_{std}} \propto \frac{I_{sample}}{I_{std}} \cong k \text{ value} \quad (1)$$

Where C is the mass (weight) concentration of an element. I is the measured characteristic X-ray intensity for the element (corrected for background, peak overlap and dead time). The subscript “sample” denotes the concentration and intensity of the element in the sample, and “std” refers to the standard of known concentration, typically it is a pure element. The ratio of the characteristic intensities measured on the standard is known as the “ k ratio” or “ k value”. The proportionality sign in equation (1) indicates that the relationship between concentration and characteristic X-ray intensity is not exact. There exist “matrix effects”, arising from the nature of the electron and X-ray interactions with matter, which modify the measured intensities, and which depend on the unknown composition of the sample. A variety of approaches are used (ZAF, $\phi(\rho)z$, empirical) to calculate correction factors for these matrix effects.

3.2. Selectivity

The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in samples to which specific interferences have been deliberately introduced (those thought likely to be present in samples) [6].

The selectivity of the method was evaluated through the ability of the spectrometer to change the analyzer crystal for the analysis of the same element due to the use of different acceleration voltages: 5 and 25 kV, table 1. The interferences were avoided through the selection of X-ray lines to different level for each element. Table 1 shows the experimental conditions used for the analysis of each one of the elements including type of analyzer crystal, acceleration voltage and working distance. Measurement results of the Au80-Cu20 alloy, taken as a sample, are also included in table. The Au80-Cu20 standard was measured at 5 and 25 kV electron beam. Under these conditions, 7 microanalyses with duration of 40 s each were recorded. The selectivity of the WDS microanalysis method for Au-Cu alloy was through of the estimation of the variation coefficient or relative standard deviation (RSD) 0.49 to 0.7 % to Au and 0.67 to 0.76% to Cu. Thus, we demonstrated the ability of the method to confirm analyte identity and its ability to measure the isolated analyte and with interferences for the effect of the binary alloy, taken as a sample, are also included in table 1

Table 1. Experimental conditions and results for the evaluation of selectivity.

Acceleration voltage	Au80-Cu20 @ 5 kV, 50 μ A		Au80-Cu20 @ 25 kV, 17 nA	
Analyzer crystal	PET	TAP	LiF	LiF
Element-X-ray line	Au-M α	Cu-L α	Au-L α	Cu-K α
Counts	452592.5	279216.8	215934.7	176421.2
% RSD	0.49	0.67	0.7	0.76

3.3. Limits of detection and quantification

Limit of detection (LOD) was based on the analysis of blank samples following the whole measurement procedure. Calculation of the LOD was done through the measurement of pure metallic wires. Pure Cu wire was used for determining the detection limit of Au, considering this wire as a sample blank for Au. Whereas for determining the detection limit of Cu, pure Au wire was measured. Fourteen independent measurements of the sample blank were made under repeatability conditions.

As suggested by the Eurachem method validation working group [7], the LOD was calculated using equation (2) through the standard deviation of the measurement of 100 % Cu standards and 100 % Au.

$$LOD = 3 s'_0 \quad (2)$$

where:

$$s'_0 = \frac{s_0}{\sqrt{n}} \quad (3)$$

s_0 - is the estimated standard deviation of n single results at or near zero concentration.

s'_0 - is the standard deviation used for calculating LOD.

n – is the number of replicate observations.

Table 2 shows information considered in the calculation of the method detection limit. Signal to Noise (S/N) ratio is also included.

Table 2. LOD and LOQ information for Au and Cu.

25 kV, 17 nA									
Au-L α					Cu-K α				
Signal (S)	Noise (N)	Ratio S/N	LOD	LOQ	Signal (S)	Noise (N)	Ratio S/N	LOD	LOQ
291301.7	2963.3	98.3	0.87	0.23	740750.1	727	1018.9	1.15	0.33

Limit of quantification (LOQ) is a parameter associated to the LOD. LOQ is the lowest level of analyte that can be determined with an acceptable level of confidence. In practice, according to the Eurachem working group, LOQ is calculated by most conventions to be the analyte concentration corresponding to the obtained standard deviation (s_0) at low levels multiplied by a factor, k_Q . The IUPAC default value for k_Q is 10 [8] and if the standard deviation is approximately constant at low concentrations this multiplier corresponds to a relative standard deviation (RSD) of 10 %.

3.4. Working Range

In order to establish the instrument working range, Au-100, Au60-Cu40, Au40-Cu60, Au20-Cu80 and Cu100 standards were measured 10 times on the surface of each wire. Measurements were obtained with an electron beam of 5 and 25 kV respectively. The average value of the measurements was used to build the response curve. Figure 1 show the calibration function obtained through four calibration points. A high correlation factor (r) above than 0.99 was obtained indicating the fitness for purpose of the calibration curves. Parameters of the least square linear calibration curve are summarized in figure 1 and table 3.

The working range was assessed with a calibration function. The calibration curve for Au has a slope lower than Cu for both conditions (5 and 25 kV). Thus, Cu measurements point out more sensitivity that the Au measurements.

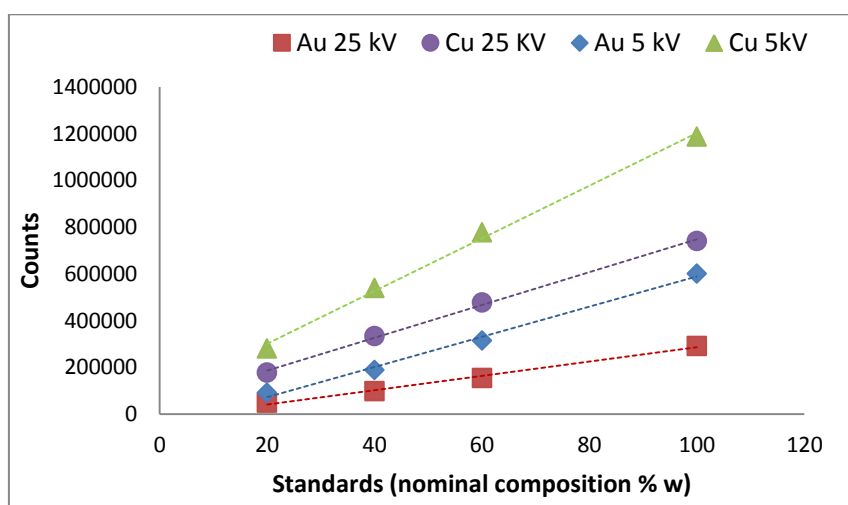


Figure 1. Performance of the calibration function.

Table 3. Parameters working range

	5 kV, 17 nA		25 kV, 50 μ A	
	Au	Cu	Au	Cu
Intercept	-56898	75463	-21167	45994
Slope	6458.1	11276	3067.1	7016.8
Correlation coefficient, r	0.9937	0.9967	0.994	0.9983

3.5. Sensitivity

Analytical sensitivity is the change in instrumental response which corresponds to a change in the measured quantity (for example an analyte concentration), i.e. the gradient of the response curve [9,10]. The prefix ‘analytical’ is recommended to avoid confusion with ‘diagnostic sensitivity’ used in laboratory medicine [11].

Sensitivity was estimated as the slope of the response curve in the table 4, shown the results for each element in term of the counts and K value.

Table 4. Sensitivity values for counts and k value

Slope	Au (counts)	K value Au	Cu (counts)	K value Cu
5 kV	6422.31	0.0104	11469.5	0.0095
25 kV	3025.76	0.0106	7041.46	0.0097

3.6. Precision

Approaches to simultaneous determination of repeatability and intermediate precision are described in ISO 5725-3 [12]. In addition, a design based on the harmonized guidelines for single-laboratory validation of methods of analysis [13] offers the possibility to determine repeatability and intermediate precision from a single study. Subsamples of the selected test material are analyzed in replicate under repeatability conditions across a number of different runs, with maximum variation in conditions between the runs (different days, different analysts, different equipment, etc.). Via one-way ANOVA [14,15], repeatability can be calculated as the within-group precision, while the intermediate precision is obtained as the square root of the sum of squares of the within group and between-group precision. This type of design can provide an efficient way of obtaining sufficient degrees of freedom for estimates of repeatability and between-group precision. The precision was quantified through of assessment of the repeatability and reproducibility.

3.6.1. Repeatability Test

The repeatability was based on 8 measurements obtained under repeatability conditions. Repeatability conditions were alloy Au80-Cu20 as test sample, electron beam of 25 KV, beam current 17 nA, distance working 11 mm, the same spot size and assumptions of the high homogeneity of the sample, thus, the analysis was done in different points of the surface of the alloy. Table 5 shows the measurement results under repeatability conditions. The variation coefficient was the parameter use for assessment the measurement repeatability.

Table 5. Repeatability results as variation coefficient

25 KV	5 KV	25kV	5 kv
Cu	Cu	Au	Au
20.65	19.53	79.35	80.47
20.55	19.13	79.46	80.87
20.71	18.87	79.29	81.13

	20.55	18.72	79.47	81.28
	20.52	18.61	79.48	81.39
	20.57	18.50	79.43	81.50
	20.60	18.90	79.40	81.10
Average	20.6	18.9	79.4	81.1
Std Desv.	0.06	0.32	0.06	0.32
Var. Coef.	0.29	1.70	0.078	0.39

3.6.2.Reproducibility Test

The reproducibility assessment was done with an electron beam of 5kV and 25 kV as a reproducibility condition, thus, a change in the beam current was used, analyzer crystal and X-ray line. The test sample was Au80-Cu20 and variance analysis (F test) was applied for reproducibility evaluation. Measurements were performed applying the two different electron beam voltage and the k value data were used in the F test. F test results are shown in the table 6 for each element.

Table 6. Data and F test results as reproducibility

Cu L and K		Au M and L	
5kV	25 kv	5 kv	25 kv
k values	k values	k values	k values
19.53	20.65	80.47	79.35
19.13	20.55	80.87	79.46
18.87	20.71	81.13	79.29
18.72	20.55	81.28	79.47
18.61	20.52	81.39	79.48
18.50	20.57	81.50	79.43
19.53	20.65	80.47	79.35

F test parameters	Cu L and K		Au M and L	
Electron beam	Cu 25 kV	Cu 5 kV	Au 25 kV	Au 5 kV
Average	20.5906667	18.893	79.41266667	81.107
Variance	0.00501467	0.1457628	0.005374667	0.1457628
Data	6	6	6	6
Degrees of freedom	5	5	5	5
F	0.034		0.037	
P(F<=f) one-way	0.001		0.0012	
Value F critical (one-way)	0.198		0.198	

3.7. Trueness

The bias criterion states that if Δv (absolute difference) $> \Delta_c$ (uncertainty associated absolute difference), then the difference is greater than can be explained as an instrument correction with a calibration standard. However, if the situation is opposite, spectrometer is accurate and any correction is not required. Equation (4), indicates how to estimate, and Δ_c equation (5) for Δv calculated.

The evaluation of the trueness was realized as a bias (Δ_v). The bias calculated following the equation (5).

$$\Delta_c = (t_{n-1} 0,95) \left(\frac{s}{\sqrt{n}} \right) + U \quad (4)$$

$$\Delta v = |\bar{x} - \mu| \quad (5)$$

Where: μ is the certified value for each element of the sample alloy; $\bar{x}$ is mean of the measurements, s is standard deviation, n is the number of measurement and u is uncertainty standard.

Equations (4) and (5) were considered to obtain the results included in table 7. According to the results, significant bias was not obtained.

Table 7. Criteria bias results

	Δv		Δc	
	Au	Cu	Au	Cu
25 kV	0.25	0.15	0.74	0.76
5 kV	0.29	0.19	0.96	0.94

Of agree with table 7 it not bias was observed, so, the spectrometer fits accuracy.

3.8 Uncertainty

Uncertainty is an interval associated with a measurement result, which expresses the range of values that can reasonably be attributed to the quantity being measured. An uncertainty estimate should take account of all recognized effects operating on the result. The uncertainties associated with each effect are combined according to well-established procedures. Several approaches to obtaining an uncertainty estimate for the results from chemical measurements are described [16, 17, 18, 19, 20]. The uncertainty model approved is the figure 2. The model included source of variation systematic as uncertainty of the standard and the correction for matrix effect with use the software and random sources as repeatability and intermedia precision at figure 3.

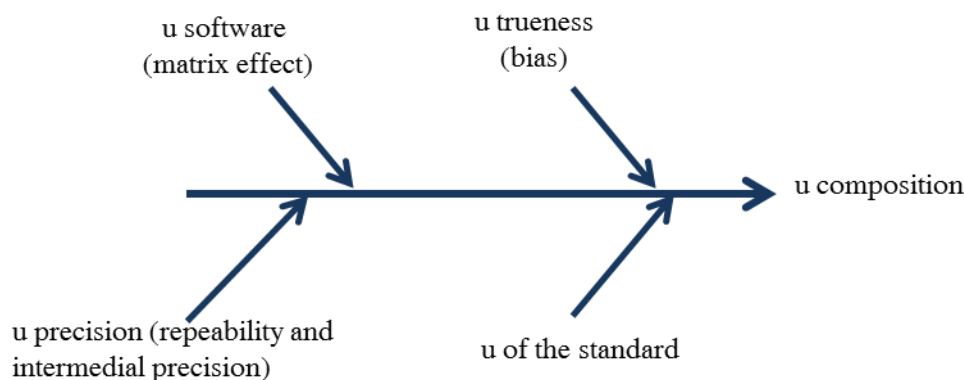


Figure 2. Uncertainty diagram proposed for the determination of element composition of the alloy.

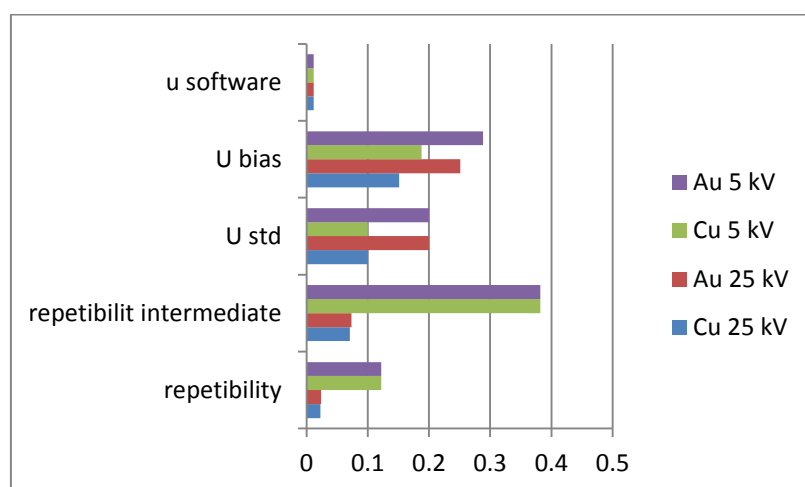


Figure 3. Diagram of the uncertainty budget.

The values for each element composition and their expanded uncertainty are reported in Table 8.

Table 8. Values of the uncertainty expanded for each component

kV	25 KV	5 KV	25kV	5 kv
Element	Cu (kg/kg)	Cu (kg/kg)	Au (kg/kg)	Au (kg/kg)
Value	20.59	18.89	79.41	81.11
Uncertainty expanded	0.39	0.91	0.66	1.07

4. Conclusions

The use of microprobe (EPMA) or electron scanning microscope coupled with WDS has been extended in testing laboratories. Method validation and uncertainty estimation are the main technical requirements to achieve accreditation. This paper shows a way to achieve this goal and it can be considered as a guide for laboratories involved in the accreditation process.

Method validation was performed to determinate the chemical composition by elemental wavelength dispersive spectrometry (WDS). The validation of the method includes uncertainty estimation using a simple model considering and estimation of precision components (repeatability and intermediate precision as source of random variation. The model also includes systematic sources of variation such as the uncertainty due to the software used for matrix effect correction and uncertainty of the standard.

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