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HOW THE ESTIMATION OF UNCERTAINTY CAN BE HELPFUL FOR THE DETERMINATION OF Cu AND Zn IN SERUM SAMPLES

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ABSTRACT

Essential metals play a vital role in physiological processes like the metabolism of proteins, lipids, and enzymatic reaction. The precise knowledge of the levels of some metals such as Cu and Zn in human serum is crucial for the diagnosis, prognosis and monitoring of a disease. The uncertainty budget helps making a result analytically reliable. This paper describes the parameters needed for the evaluation of the measurement uncertainty for Cu and Zn determined by HR-ICPMS. The expanded uncertainty was obtained with 95% confidence level (k = 2).

Keywords: Metals, uncertainty, human serum, HR-ICPMS

1. INTRODUCTION

Careful evaluation of the uncertainty parameters helps to ensure the quality of the results of an analytical laboratory promoting its competence in the marketplace. The uncertainty is defined as a parameter associated to the result of a measurement that characterizes the dispersion of values that could be attributed to the measurand [2].

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The identification of the main uncertainties sources in the analytical procedure provides the understanding of the factors that may influence the measured result correcting possible "doubts" of the obtained value [3].

During the measuring process, each parameter can be expressed in a cause-effect diagram, that details the measuring protocol to track most of the sources of uncertainty and also to avoiding duplication of errors. [3] From there on, each source of uncertainty is quantified and classified as type A, when measurements are obtained under conditions of repeatability and type B, when each input variable has a distribution within a range of dispersion that can be uniform, rectangular, triangular or normal [4].

When data related safety and health are considered it is customary define an expanded uncertainty interval for a result [4].

2 MATERIAL and METHODS

2.1 Sampling

50 Blood samples were collected the São Paulo Hospital using 6 mL BD additiveless Vacutainer tubes. After resting for 30 min to allow coagulation, the tubes were centrifuged at 4500rpm during 15 minutes. The serum was separated and stored at -20 °C until needed for analysis. The serum samples were diluted 1:20 in Milli-Q water containing 1% of HNO_3. Indium (In) was added as internal standard at the final concentration 10µg/L.

2.2 Equipment

The analysis was made using a sector field ICP-MS with a magnetic sector followed by an electrostatic analyzer (ESA) in reverse Nier_Johnson geometry, running in the mean-resolution mode. The measurements were done using a cyclonic spray chamber and a

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Meinhard type nebulizer. The operational conditions of the HR ICP-MS were 1.24 kW r.f. power, 0.56 L/min auxiliary gas and 16 L/min coolant gas. The signal was collected in a dual detector.

3. RESULTS AND DISCUSSION

Table 1 shows two results obtained in the determination of Zn and Cu in a sample of human serum in order to identify the components of uncertainty for the analysis.

1. Results obtained in the determination of Zn and Cu in human serum

Samples	Conc.(ug/g) ± U (ug/g)
Zn (C70)	0.80± 0.17
Cu (C70)	0.87± 0.24

Expanded uncertainty with 95% confidence

3.1 Identification of sources of uncertainty

The figure 1 shows the cause-effect diagram of the sources of uncertainty associated with the process of measuring copper and zinc in human serum samples, used to identify possible sources of uncertainty in the measurement. The central vector represents the measurand and Its ramifications are the contributions of the factors that may affect the outcome of the analysis. [5]

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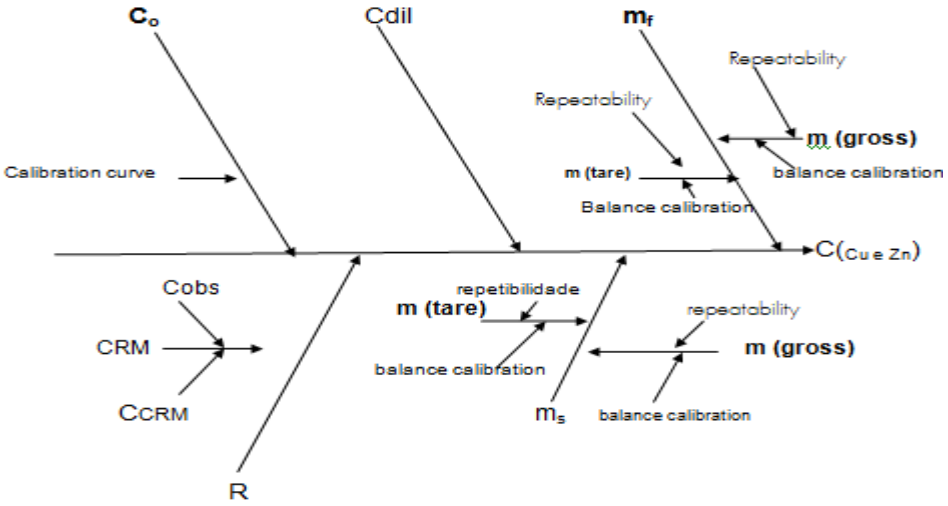

Figure 1. Cause-effect diagram of the sources of uncertainty associated with the measurement of Cu and Zn.

It's possible to see that the contributors to the uncertainties of this process are: The concentration of the aliquot analyzed (Co), the dilution of the stock standard solution (C_{DIL}), final mass (m_s), recovery of reference material (R), and sample mass (m_a). [5]

3.2 Calculation of uncertainties

3.2.1 C_o- Analytical curve

The concentrations of Cu and Zn were measured using standard curves diluted gravimetrically. The calculation of the uncertainty associated with analytical curve for this procedure was calculated as follows:

$$u(c_o) = \frac{S}{B_1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{(c_o - \bar{c})^2}{Q_{xx}}} \qquad (1)$$

S = standard deviation; B1 = slope of the curve, p = number of measurements to determine Co, n = number of measurements for calibration, Co = concentration of the analyte in the

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solution of analysis, average c = average concentration of the solutions of the curve; Qxx = sum of concentrations.

For this experiment the value of the uncertainty associated with the analytical curve was:

$u(c_o)$ Zn = 2.807

$u(c_o)$ Cu = 5.230

3.2.2 C_{dill} - Concentration of dilute standard

Was prepared two dilutions of the stock standard solution which covers Concentration = 2002 were respectively C_{dill_10} $\mu g.g^{-1}$ and C_{dill_100} = 400,367, the uncertainty in the determination of diluted solution corresponding to μ (C_{dill_10}) solution for 10 0.0048781 $\mu g.g^{-1}$. And for the solution to 100 $\mu g.Kg^{-1}$ uncertainty was μ (C_{dill_100}) 0.9766, for the elements Cu and Zn, was calculated from the equation below.

$$\mu_{C_{dil\_10}} = C_{dil\_10} \times \sqrt{\left(\frac{\mu_{estoque}}{C_{estoque}}\right)^2 + \left(\frac{\mu_{m_p}}{m_p}\right)^2 + \left(\frac{\mu_{m_f}}{m_f}\right)^2} \quad (2)$$

3.2.3 m_a -Mass initial and final mass

The uncertainty associated with the initial mass of the samples of Cu and Zn and final mass of the dilution was associated with the calibration certificate balance its repeatability demonstrated by measuring the standard deviation of the balance obtained through successive measurements of daily check, the balance results from the sum of these two variables was used as the uncertainty of the masses given by the equation:

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$$u(m) = \sqrt{(u_1)^2 + (u_2)^2} \tag{3}$$

$\mu(m) = 0.0011$

3.2.4 R- Recovery of reference material

The uncertainty associated with method recovery was obtained by preparation of reference material by combining the uncertainty of the material provided by the certificate obtained, by the media and the uncertainty of observations made.

$$\mu_{sol} = C_{sol} \times \sqrt{\left(\frac{Inc_{padrão}}{C_{padrão}}\right)^2 + (Inc_{balança})^2} \tag{4}$$

$\mu_{sol\ (Cu)} = 0.280$

$\mu_{sol\ (Zn)} = 0.385$

$R_m = \frac{C_{obs}}{C_{MRC}}$ C_{obs} = is the average of the results obtained by replicate;

C_{crm} = Is the concentration obtained by replicate analysis of.

$R_{m\ (Cu)} = 1.06$

$R_{m\ (Zn)} = 1.15$

$$\mu_{Rm} = R_m \times \sqrt{\left(\frac{\mu_{sol}}{C_{sol}}\right)^2 + \frac{S_{obs}^2}{n \times C_{obs}^2}} \tag{5}$$

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$\mu_{rm\ (Cu)=}$ 0.06

$\mu_{rm\ (Zn)=}$ 0.09

3.2.3 Expanded uncertainty

$$u_{C_{Zn,Cu}} = C_{Zn,Cu} \times \sqrt{\left(\frac{u(C_o)}{C_o}\right)^2 + \left(\frac{u(Sol_{100\,ppb})}{Sol_{100\,ppb}}\right)^2 + \left(\frac{u(m_f)}{m_f}\right)^2 + \left(\frac{u(R)}{R}\right)^2 + \left(\frac{u(m_a)}{m_a}\right)^2} \qquad (6)$$

$uC_{Zn,} =$ 0.17µg.g^{-1}

$uC_{Cu,} =$ 0.24µg.g^{-1}

3.2.4 Uncertainty of the components considered

2. Estimation of uncertainty components considered in the determination of Cu in samples of human serum

Relative uncertainty		
	measures	%
u(Co)/Co	0.1182	11.82
u(Cdil100)/Cdil100	0.0024	0.24
u(mf)/mf	0.0010	0.10
u(Rec)/Rec	0.0613	6.13
u(ma)/ma	0.0020	0,20

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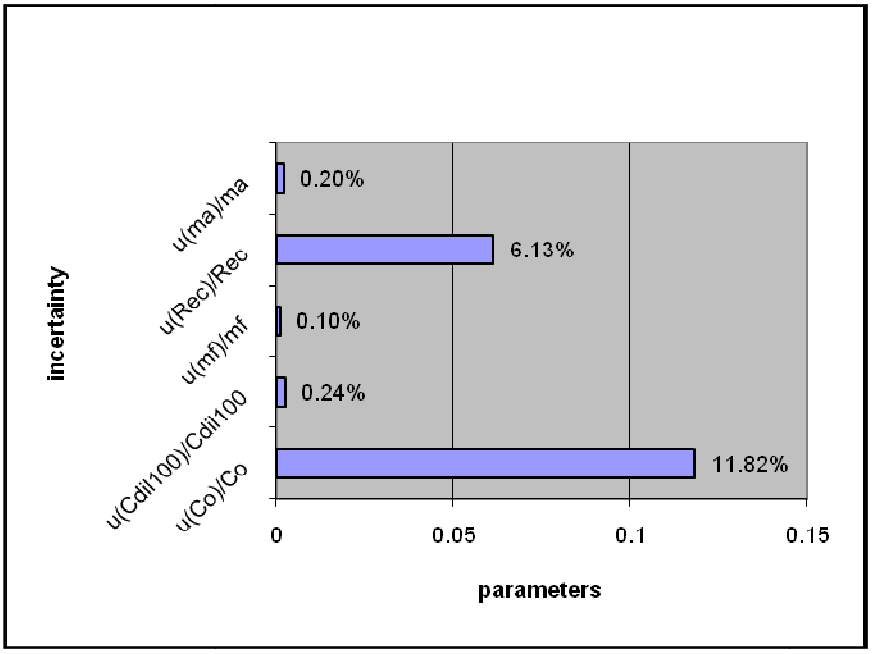

Figure 1. Comparison of parameters of uncertainty that influence the overall uncertainty of determination of Cu in samples of human serum.

Table 3. Estimation of uncertainty components considered in the determination of Zn in human serum samples

Relative uncertainty		
	Measures	%
u(Co)/Co	0.0697	6.97
u(Cdil100)/Cdil100	0.0024	0.24
u(mf)/mf	0.0010	0,10
u(Rec)/Rec	0.0798	7.98
u(ma)/ma	0.0020	0.20

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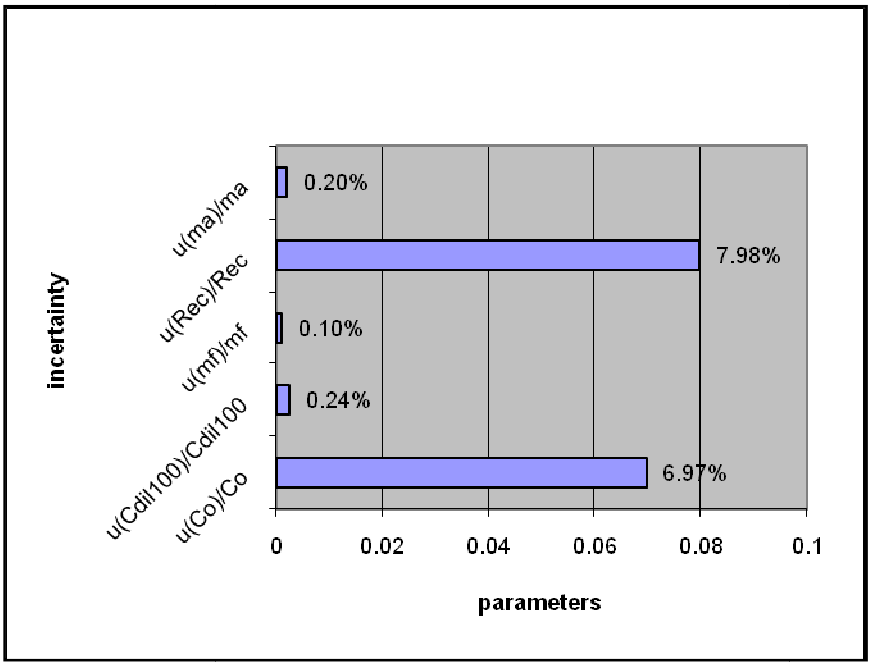

Figure 2. Comparison of parameters of uncertainty that influence the overall uncertainty for the determination of Zn.

By analysis of the uncertainties considered that the dominant component of uncertainty for the determination of Zn was the recovery of reference material, followed uncertainty in the concentration of the aliquot analyzed.

For quantification the uncertainty in the determination of Cu, was found as a dominant component of overall uncertainty, the measured concentration in the aliquot analyzed.

In both cases the increased, of the uncertainty in the concentration of the analyzed rate is justified by the fact that were used only one measure of the analytical curve, when the literature suggests the use of at least, one triplicate to ensure the linearity of the curve. [5]

In the case of reference material should be remembered that the certificate itself brings with its own uncertainty associated and the preparation of the same tends to increase the final uncertainty, would soon be interesting the preliminary study of all reference materials for the matrix analyzed, order of choose the one that best fits the process.

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5. CONCLUSION

The percentage of uncertainty associated with the determination of Cu corresponds to 26.9%, and for the determination of Zn 21.2%, considering these numbers it is clear that the uncertainty estimate provides a broad overview of traceability, for the parameters should receive more attention in the whole sample preparation until the time of analysis, so that the values are within the lower range of uncertainty to ensure the quality of the laboratory.

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