IDENTIFYING OF UNCERTAINTY SOURCES: A USEFUL TOOL FOR VALIDATION PROCESS OF HEAVY METALS IN WATERS

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The aim of this research was to validate an analytical method using flame atomic absorption spectrometry for determination of some heavy metals in water. The method was validated for linearity, accuracy, precision, repeatability, limit of detection, and limit of quantification. The linearity was validated over the concentration ranges of 0.1 – 5 mg/L with coefficient of correlation higher than 0.99. Limit of detection were found to be between 0.005 - 0.19 mg/L, while limit of quantitation (LOQ) were found to be between 0.01 - 0.66 mg/L. This method was precise for determination of metals in sample which were indicated.

Keywords: linearity, accuracy, precision, repeatability, limit of detection

1. Introduction

The priority of the study has been focused on pollutants like heavy metals. The heaviest metals such as lead, copper, nickel, cadmium, zinc, mercury, arsenic and chromium represent a threat to the environment and public health [1]. It is known that many heavy metals are toxic and tend to accumulate in living organisms because they are not biodegradable [2]. The atomic absorption spectrometry (AAS) can be used to analyze the levels of heavy metals in waters [3, 4, 5]. A property of chemical measurements is comparison of the results under repeatability conditions at different times. In this paper, a protocol regarding the uncertainty assessment as it was calculated and tested in environmental Laboratory from University *POLITEHNICA* of Bucharest**,** Center for Research and Eco-Metallurgical Expertise is presented. All quantitative components of

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uncertainty have been identified according to the requirements from Eurachem guide [6] and SR ISO 8466-1/1999 international standard regarding statistical evaluation of a linear calibration function [7]. Specificity of this protocol consists in type of heavy metals analyzed in order to define internal quality control as tool for laboratory competence according to ISO 17025 requirements [8].

2. Materials and Methods

All used materials were analytical grade and purchased from Merck Company. The stock solution of each metal had a concentration of 1000 mg/L. To obtain standard solutions these solutions were prepared with double distilled deionized water. Standard solutions which used for calibration and samples were examined at atomic absorption spectroscopy flame GBC 932. The equipment has the following parameters [9, 10]:

Table 1

Conditions of atomic absorption spectrometry

Parameter	Cd	Cu	Fe	Pb	Zn
nm	228.8	324.7	248.3	217	213.9
Type of flame	Air-acetylene				

Fig. 1 shows the measurement procedure of metals from water by atomic absorption in flame.

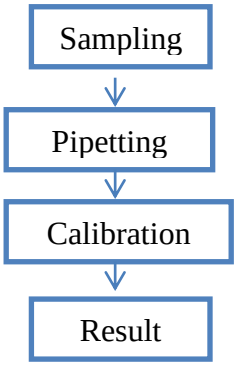


Fig. 1: Measurement procedure of metals;

The concentration of the calibration standard solution depends on the concentration of the metal corresponding to the absorbance of sample to be analyzed, the concentration of the metal corresponding to the absorbance of the blank and the volume of the liquid.
Calculation: the concentration is given by SR ISO 8288 [11]:

$$\text{Concentration} = [(\rho_1 - \rho_b) \text{ x } 100]/V \quad [\text{mg/L}] \qquad (1)$$

Where:

ρ_1 - the concentration of the metal corresponding to the absorbance of sample to be analyzed, mg/L;
ρ_b - the concentration of the metal corresponding to the absorbance of the blank, mg/L;
V – Volume of the liquid;

Fig. 2 shows the sources of uncertainty for the determination of metals in water by atomic absorption technique evaluated according to the internal procedure of the laboratory.

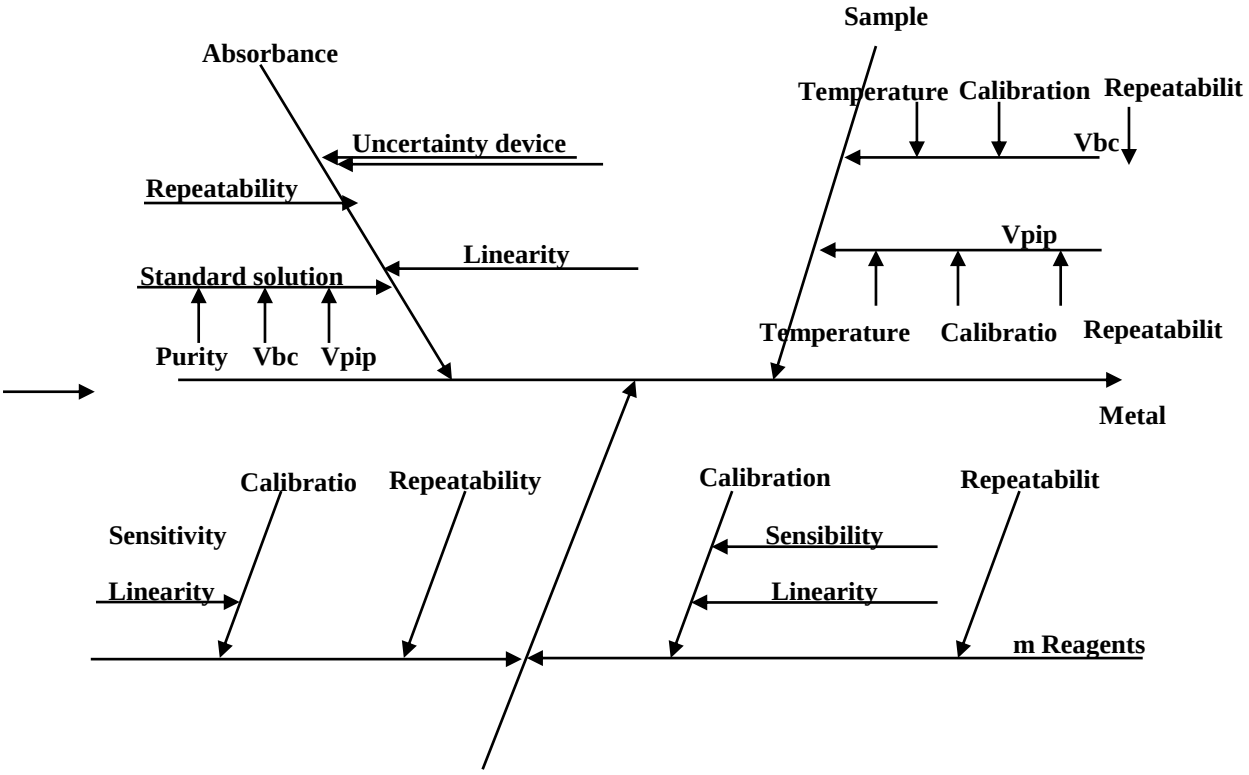


Fig. 2: The sources of uncertainty for the determination of metals in water

- *Quantifying the uncertainty components*

One of the most important sources of uncertainty is the volume

There are three sources of uncertainty for volume measured such as:

- Tolerance
- Standard deviation
- Temperature

The sample is introduced into a 100 mL volumetric flask. Standard solutions are prepared from the basic solution, 1 mL of this solution containing 1 mg metal. Pipettes of 1, 5, 10, 25 mL are used. Four solutions were prepared by dilution of the calibration solution by nitric acid.

1. Pipette of 1 mL, class A

- Calibration:

The manufacturer quotes a volume for the flask of 1±0.006 mL measured at a temperature of 20˚C. The value of the uncertainty is given without a confidence level, so the standard uncertainty area is assigned a triangular distribution:

$$\frac{0.006\text{mL}}{\sqrt{3}} = 0.0034 \text{ mL} \quad (2)$$

- Repeatability:

The uncertainty due to variations in filling of the pipette is given by the calibration certificate which is the value of standard deviation of 0.0015 mL.

- Temperature:

The coefficient of volume expansion for water is 2.1 x 10^{-4} $^{\circ}C^{-1}$, which leads to a volume variation of 1mL ± 4 ˚C x 2.1 x 10^{-4} ˚C = ± 0.00084 mL

The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation:

$$\frac{0.00084\text{mL}}{\sqrt{3}} = 0.00048 \text{ mL} \quad (3)$$

The three contributions are combined to give the standard uncertainty u($V_{1\text{ mL}}$)

$$u(V_{1\text{ mL}}) = \sqrt{(0.00346)^2 + (0.0015)^2 + (0.00048)^2} = 0.0038 \text{ mL} \quad (4)$$

2. Pipette of 5 mL, class A

- Calibration:

The manufacturer quotes a volume for the flask of 5±0.0300 mL measured at a temperature of 20˚C. The value of the uncertainty is given without a confidence level, so the standard uncertainty area is assigned a triangular distribution:

$$\frac{0.0300 \text{ mL}}{\sqrt{3}} = 0.0173 \text{ mL} \quad (5)$$

- Repeatability:

The uncertainty due to variations in filling of the pipette is given by the calibration certificate which is the value of standard deviation of 0.006 mL.

- Temperature:

The coefficient of volume expansion for water is 2.1 x $10^{-4}\ ^{\circ}C^{-1}$, which leads to a volume variation of 5 mL $\pm$ 4 ˚C x 2.1 x 10^{-4} ˚C = $\pm$ 0.0042 mL
The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation:

$$\frac{0.0042\ mL}{\sqrt{3}} = 0.00242\ mL \quad (6)$$

The three contributions are combined to give the standard uncertainty $u(V_{5\ mL})$

$$u(V_{5\ mL}) = \sqrt{(0.0173)^2 + (0.0060)^2 + (0.00242)^2} = 0.01847\ mL \quad (7)$$

3. Pipette of 10 mL, class A

- Calibration:

The manufacturer quotes a volume for the flask of 10±0.0500 mL measured at a temperature of 20 ˚C. The value of the uncertainty is given without a confidence level, so the standard uncertainty area is assigned a triangular distribution:

$$\frac{0.0500\ mL}{\sqrt{3}} = 0.029\ mL \quad (8)$$

- Repeatability:

The uncertainty due to variations in filling of the pipette is given by the calibration certificate which is the value of standard deviation of 0.0099 mL:

- Temperature:

The coefficient of volume expansion for water is 2.1 x $10^{-4}\ ^{\circ}C^{-1}$, which leads to a volume variation of 10 mL $\pm$ 4 ˚C x 2.1 x 10^{-4} ˚C = $\pm$ 0.0084 mL.

The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation:

$$\frac{0.0084\ mL}{\sqrt{3}} = 0.0048\ mL \quad (9)$$

The three contributions are combined to give the standard uncertainty $u(V_{10mL})$

$$u(V_{10\ mL}) = \sqrt{(0.029)^2 + (0.0099)^2 + (0.0048)^2} = 0.03101\ mL \quad (10)$$

4. Pipette of 25 mL, class A

- Calibration:

The manufacturer quotes a volume for the flask of 25±0.1mL measured at a temperature of 20˚C. The value of the uncertainty is given without a confidence level, so the standard uncertainty area is assigned a triangular distribution:

$$\frac{0.1\ \text{mL}}{\sqrt{3}} = 0.0578\ \text{mL} \quad (11)$$

- Repeatability:

The uncertainty due to variations in filling of the pipette is given by the calibration certificate which is the value of standard deviation of 0.0152 mL

- Temperature:

The coefficient of volume expansion for water is 2.1 x $10^{-4}\ ^{\circ}C^{-1}$, which leads to a volume variation of 25 mL ± 4 ˚C x 2.1 x 10^{-4} ˚C = ± 0.021 mL
The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation:

$$\frac{0.021\ \text{mL}}{\sqrt{3}} = 0.0121\ \text{mL} \quad (12)$$

The three contributions are combined to give the standard uncertainty $u(V_{25mL})$

$$u(V_{25\ mL}) = \sqrt{(0.0578)^2 + (0.0152)^2 + (0.0121)^2} = 0.06\ \text{mL} \quad (13)$$

5. Pipette of 100 mL, class A

- Calibration:

The manufacturer quotes a volume for the flask of 100 ± 0.07 mL measured at a temperature of 20 ˚C. The value of the uncertainty is given without a confidence level, so the standard uncertainty area is assigned a triangular distribution:

$$\frac{0.07\ \text{mL}}{\sqrt{3}} = 0.040\ \text{mL} \quad (14)$$

- Repeatability:

The uncertainty due to variations in filling of the pipette is given by the calibration certificate which is the value of standard deviation of 0.02 mL.

- Temperature:

The coefficient of volume expansion for water is 2.1 x $10^{-4}{}^{\circ}C^{-1}$, which leads to a volume variation of 100 mL x ± 4 ˚C x 2.1 x 10^{-4} ˚C = ± 0.084 mL. The standard uncertainty is calculated using the assumption of a rectangular distribution for the temperature variation:

$$\frac{0.084\ mL}{\sqrt{3}} = 0.048\ mL \quad (15)$$

The three contributions are combined to give the standard uncertainty $u(V_{100mL})$

$$u(V_{100mL}) = \sqrt{0.040^2 + 0.02^2 + 0.048^2} = 0.066\ mL \quad (16)$$

Table 2

Calculation of the combined standard uncertainty for the determination of metals by atomic absorption

	Description	Value x	standard uncertainty u(x)	The relative standard uncertainty, u(x)/x
Ap.	The combined uncertainty of the device, mg/l	2.110	0.045	0.02133
V_{1mL}	Volume pipetted from standard solution	1	0.0038	0.0038
V	Volume of the flask, ml	100	0.066	0.00066 x 5
V_{5mL}	Volume pipetted from standard solution	5	0.01846	0.0037
V_{10mL}	Volume pipetted from standard solution	10	0.03101	0.0031
V_{25mL}	Volume pipetted from standard solution	25	0.06	0.0024

The uncertainties associated with each component are calculated as follows:

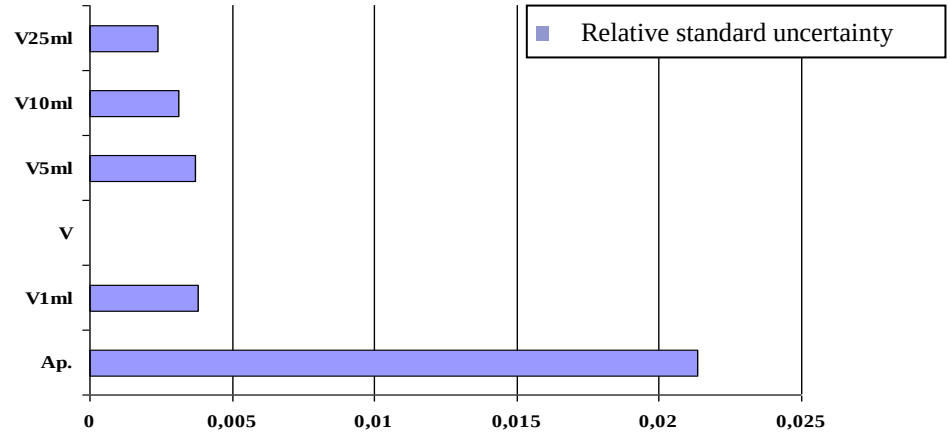
$$\frac{u_c(C_{metal})}{C_{metal}} = \sqrt{\left(\frac{u(ap)}{mg/l}\right)^2 + \left(\frac{u(V_{1ml})}{V_{pipetat}}\right)^2 + \left(\frac{u(V_{100ml})}{V_{balon}}\right)^2 + \left(\frac{u(V_{5ml})}{V_{pipetat}}\right)^2 + \left(\frac{u(V_{10ml})}{V_{pipetat}}\right)^2 + \left(\frac{u(V_{25ml})}{V_{pipetat}}\right)^2} =$$

$$= \sqrt{(0.02133)^2 + (0.0038)^2 + (0.0033)^2 + (0.0037)^2 + (0.0031)^2 + (0.0024)^2}$$

$$\frac{u_C(C_{metal})}{C_{metal}} = 0.03103\ mg/L \quad (17)$$

Fig.3: The uncertainty in the preparation of the standard for determination of metals in water

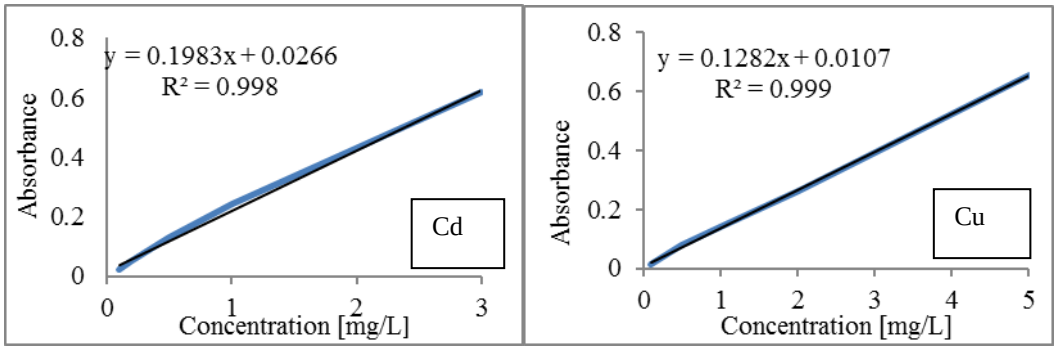
Metal concentration is expressed as:
U(c) = 2.110 mg/L x 0.03103 = 0.065 mg/L
The expanded uncertainty:
Ue = ± 2 U(c) = ± 2 x 0.065 mg/L = 0.1309 mg/L



Result: C_{metal} = 2.110 mg/L ± 0.1309

3. Results and discussion

To demonstrate that the method was validated important parameters such as the detection limit, linearity, precision and the limit of quantification were investigated. The first parameter investigated was linearity for different concentrations of metals. The range measured of each standard solution are cadmium 0.1, 0.2, 0.5, 1.0, 3 mg/L; copper of 0.1, 0.5, 1.0, 2, 5 mg/L, iron of 0.5, 1.0, 2, 3 mg/L, lead of 0.5, 1, 2.5, 5 mg/L and zinc of 0.05, 0.5, 1, 2 mg/L. The linear regression obtained has values of coefficient correlation of 0.998 (Cd), 0.999 (Cu), 0.9995 (Fe), 0.9959 (Pb) and 0.9946 (Zn). These results showed that the method was linear according to Eurachem [6].



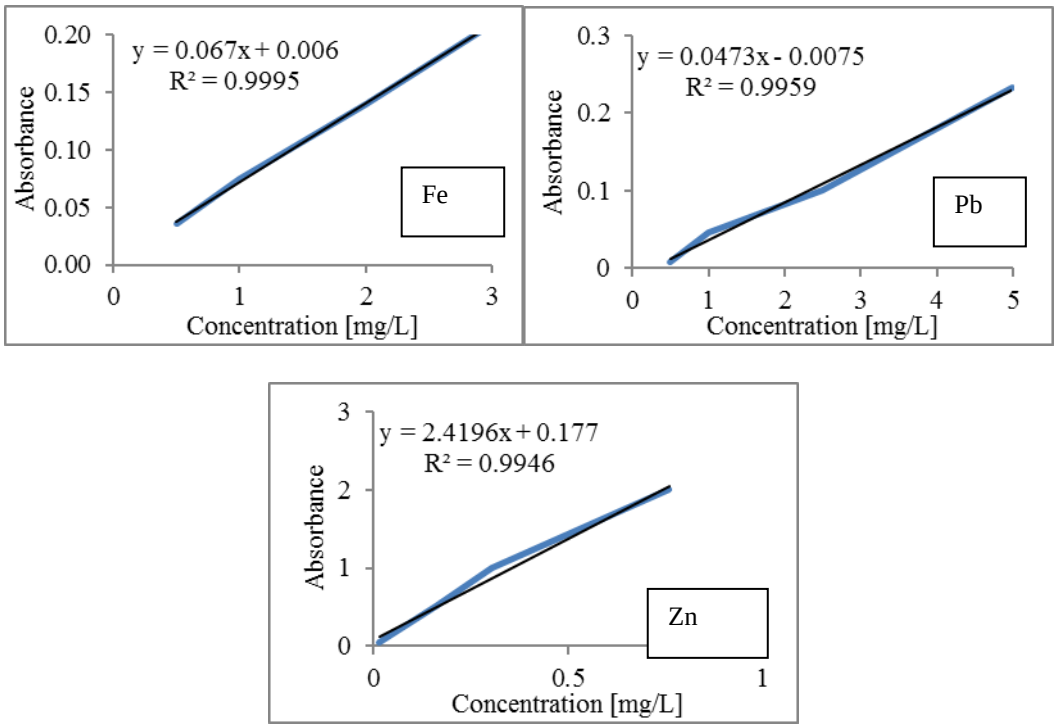


Fig. 4: The linear and nonlinear standard curves for cadmium, copper, iron, lead and zinc. Cd = cadmium; Cu = copper; Fe = iron, Pb = lead and Zn = zinc.

Table 3

Linear regression data for the curve of Cd, Cu, Fe, Pb and Zn

Parameters	***Cd***	***Cu***	***Fe***	***Pb***	***Zn***
Linearity range mg/L	0.1-3	0.1-5	0.5-3	0.5-5	0.05-2
R^2	0.998	0.999	0.9995	0.9959	0.9946
slope±SD	0.1983±0.008	0.1282±0,0008	0.067±0.002	0.04736±0.004	2.41962±0.25
Intercept±SD	0.0266±0,013	0.0107±0.002	0.006± 0.004	-0.0075±0.014	0.177040±0.12

The sensitivity is an important parameter which was analyzed for determining value of Limit of Detection (LoD) and Limit of Quantification (LoQ). According to Gonzales and Herrador, the lowest concentration of analyte which can be detected and not quantified is represented by LOD, while the lowest concentration which can be quantified with an acceptable level of precision and accuracy is represented by LoQ [1]. Ten samples were measured to obtain values of LoD and LoQ. The values of LoD and LoQ can be calculated using standard deviation (SD) of a series of the lowest concentrations using following equations [12]:

$$LoD=3 \text{ x } SD \qquad (17)$$

$$LoQ=10 \times SD \quad (18)$$

The results are presented in Table 2.

Table 4

The values of LoD and LoQ

	Cd [mg/L]	Cu [mg/L]	Fe [mg/L]	Pb [mg/L]	Zn [mg/L]
LoD	0.005	0.01	0.19	0.11	0.011
LoQ	0.01	0.06	0.66	0.37	0.039

Table 5

The value of uncertainties associated with each component

	Cd [mg/L]	Cu [mg/L]	Fe [mg/L]	Pb [mg/L]	Zn [mg/L]
$u_c(C_{Cd})$	0.002184	0.00722	0.0795	0.0350	0.0054
Ue	0.00460	0.0144	0.159138	0.07	0.0102
C_{Cd}	0.104 ± 0.0046	0.113 ± 0.0144	0.509 ± 0.159	0.521 ± 0.07	0.048 ± 0.0102

The precision can be represented by the following parameters: range, relative standard deviation (RSD), percentage of coefficient [13]. In this study, the precision was expressed by calculating a set of concentrations of the relative standard deviation. Under conditions of repeatability ten sample solution were analyzed and the result for each metal is: 0.1 mg/L Cd, 0.1 mg/L Cu, 0.5 mg/L Fe, 0.5 mg/L Pb and 0.05 mg/L Zn. The values of RSD obtained were 1.94% (Cd), 6.36% (Cu), 14.33% (Fe), 6.7% (Pb) and 7.45% (Zn). According to Gonzalez and Herrador the results of RSD are acceptable [1].

4. Conclusions

The results indicate that the applied method is linear for all the heavy metals in the range of Cd 0.1-3 mg/L, Cu 0.1-5 mg/L, Fe 0.5-3 mg/L, Pb 0.5-5 mg/L, Zn 0.05-2 mg/L, precise was represented by the value of RSD, limit of detection of Cd 0.005 mg/L, Cu 0.01 mg/L, Fe 0.19 mg/L, Pb 0.11 mg/L and Zn 0.011 mg/L, while limit of quantification of Cd o.01 mg/L, Cu 0.06 mg/L, Fe 0.66 mg/L, Pb 0.37 mg/L and Zn 0.039 mg/L. For uncertainty assessment, it could be observed that the highest influence as source is volume.

R E F E R E N C E S

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