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Multi-Element Analysis of Some Toxic Metals in Urine using Quadrupole ICP-MS

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Abstract

Occupational and environmental metal contamination is a global concern due to the occurrence of long term health effects in many organ systems. Deficiency or excess of essential metals, depending on personal and/or population nutritional status are also a big public health concern, requiring regular evaluation for early diagnosis and eventual therapeutic intervention. The objective of this study was to validate an easy and rapid analytical method using ICP-MS for the determination of 17 metals (As, Sb - semimetals; Be, Cd, Li, Pb, Pd, Pt, Rh, Sr, Tl, Mn - metals; Mo, Ni, Cr, Cu, Se - essential metals[1]) of toxicological interest. The studied working range demonstrated evidence of linearity and the proposed method proved to be accurate, with good repeatability and intermediate precision. Obtained Limit of Detection (LOD) for the studied analytes were lower or equal to those obtained by CDC in urine.

Keywords: ICP-MS, Metals, Urine, Validation Method

1. Introduction

Recently, scientific areas of environmental and occupational health have been shown a rising interest on the impact of chemical contamination and human exposure; leading to an expanded need for biomonitoring potentially exposed populations in order identify new sources of exposure[2,3].

Those contamination sources refer to different classes of chemicals, like solvents, pesticides, essential and non-essential metals, and can involve great contingents of people by various ways of exposure, like inhaled air, drinking water, soil, and food.

Occupational and environmental metal contamination is a global concern due to the occurrence of long term health effects in many organ systems, like central and peripheral neural system, cardiovascular, gastrointestinal, and urinary systems, including cancer[4]. Deficiency or excess of essential metals, depending on personal and/or population nutritional status are also a big public health concern, requiring regular biomonitoring for early diagnosis and eventual therapeutic intervention[5–7]. Biomonitoring of chemical substances and toxic elements in exposed populations, as well as the nutritional status evaluation, are critical to risk assessment of these populations[3,8]. Furthermore, the regular biomonitoring of the population produce important data for diagnosis, management, and definition of specific public health policies[8].

Several studies have been published showing many possible sources of exposure to metals. Metals like Thallium (Tl)[9] and Nickel (Ni)[10], for example, are object

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of study of patients submitted to clinical treatments and orthodontic procedures. Rhodium (Rh)[11], Palladium (Pd)[11], Platinum (Pt)[11], Arsenic (As)[12], Lithium (Li)[13] and Chromium (Cr)[14] are elements of toxicological interest in medical and environmental epidemiological investigation. Cadmium (Cd)[15], Lead (Pb)[15], Manganese (Mn)[16], and Antimony (Sb)[17], are also important in occupational evaluations besides being critical metals in environmental contaminations. Beryllium (Be)[18] is important regarding medical-legal issues. Metals like Copper (Cu)[19], Molybdenum (Mo)[20], Selenium (Se)[21], and Strontium (Sr)[21] are equally subjects of study in biomonitoring of exposed populations.

The measurements of the levels of metals at the different sources require a great analytical research effort. It implies on the implementation of more sensitive, accurate, and fast techniques, including little manipulation of the biological samples. Considering the specificities of each population and each kind of exposure, there has been in the last decades a great deal of research using advanced technologies aiming at the definition of reference values of several chemical substances and elements at general environmental scenario and at occupational set[22–24].

Nowadays, Inductive Coupled Plasma Mass Spectrometry (ICP-MS) is one of the most utilized techniques in toxicology laboratories for its specificity and sensitivity, combined with multi-elementary analysis availability[25–31].

Therefore, the objective of this study was to validate an easy and rapid analytical method using ICP-MS technique for the determination of 17 chemical elements (As, Be, Cd, Cr, Cu, Li, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Sb, Se, Sr and Tl) of toxicologic interest. The urine samples will be analyzed by direct injection, according to the accepted international criteria, including selectivity, linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy and precision.

2. Material and Methods

2.1 Material

All the validation essays for metal determination in urine were performed using an Inductive Coupled Plasma Mass Spectrometer (ICP-MS), model Elan DRC II, equipped with an auto-sampler model AS-93 Plus, Perkin Elmer (Norwalk, CT, EUA), operated with ultrapure argon gas (99.999%, Air Liquide, Brazil), in standard mode. A Meinhard® nebulizer (Golden, CO, USA) coupled to a glass cyclonic spray chamber was used. A standard quartz injector (2.0 mm) and platinum cones with 1.10 mm and 0.9 mm orifices diameters, sampler and skimmer cones, respectively, were used. Ammonium gas (99.999%, White Martins, Brazil) was used in the DRC (Dynamic Reaction Cell) mode for chromium determination. The selected ICP-MS operation conditions, as well as the isotopes monitored for the validation of the analytical method are shown in Table 1.

Table 1. ICP-MS operating conditions

Parameters	Conditions
Radio frequency potency (W)	1400
Nebulizer gas flow (L.min^{-1})	0.95-0.98
Plasma gas flow (L.min^{-1})	15
Auxilary gas flow (L.min^{-1})	1.2
Detector mode	Dual
Ammonia gas flow (L.min^{-1})	0.8
RPq (Rejection Parameter q)	0.7
Sweeps/reading	16
Replicates	3
Dwell time (ms)	50.0
Integration time (ms)	800
Ionic lens (V)	6.00-6.75
Monitored isotopes – standard mode	^{7}Li, ^{9}Be, ^{55}Mn, ^{60}Ni, ^{65}Cu, ^{75}As, ^{78}Se, ^{82}Se, ^{88}Sr, ^{98}Mo, ^{103}Rh, ^{105}Pd, ^{106}Pd, ^{111}Cd,^{114}Cd, ^{121}Sb, ^{195}Pt, ^{205}Tl, ^{206}Pb, ^{207}Pb, ^{208}Pb
Monitored isotope – DRC* mode	^{52}Cr
Internal satandards	^{45}Sc, ^{69}Ga, ^{74}Ge, ^{115}In, ^{187}Re

*Dynamic Reaction Cell
The following correction equations were adopted: ^{74}Ge (-0.116645*^{77}Se); ^{75}As (-3.127*(^{77}Se-0.784*^{82}Se)); ^{78}Se (-0.030461*^{83}Kr); ^{82}Se (-1.007833*^{83}Kr); ^{98}Mo (-0.109613*^{101}Ru); ^{105}Pd and ^{106}Pd (-0.097656*^{111}Cd); ^{114}Cd (-0.027250*^{118}Sn); ^{115}In (-0.014038*^{118}Sn) and ^{187}Re (-0.121362*^{189}Os).

2.2 Sample and Reagents

For minimizing the contamination risks of samples and reagents all plastic materials (volumetric flasks, polyethylene tubes and beakers) were chemically decontaminated by immersion in two consecutive baths of 20% nitric acid (HNO_3) for 24 hours in each bath, and then rinsed with

deionized water (resistivity of 18.2 MΩ.cm). Reagents and samples were manipulated in an ISO class 5 laminar air flow unit, installed in an ISO class 7 cleanroom. After the preparation of the reagents, all the solutions were stored into high density polyethylene flasks (Nalgene, EUA).

Nitric acid 65% (Suprapur®) and the standard solutions containing 1000 mg.L^{-1} of the elements under study (As, Be, Cd, Cr, Cu, Li, Mn, Mo, Ni, Pb, Pd, Pt, Rh, Sb, Se, Sr and Tl), were obtained from Merck (Merck Millipore, Darmstadt, Germany). These solutions were used for the preparation of a stock multi-element standard solution.

For correction of the variations of the signal intensity due to equipment instability and nonspectral interferences that occur during analysis, the following internal standards were selected: Germanium (Ge) and Scandium (Sc) 1000 µg.mL^{-1} (Inorganic Ventures, Lakewood, NJ, USA), indium (In) 1000 mg.mL^{-1} (Merck, Darmstadt, Germany), rhenium (Re) 1000 µg.mL^{-1} (High Purity Standard, Charleston, SC, USA) and Gallium (Ga) 1000 mg.mL^{-1} (Spex Certiprep, Metuchen, NJ, USA).

For checking method's accuracy and precision certified reference material (MRC) (NIST 2670 Toxic Metals in Freeze-Dried Urine – Elevated Level (Gaithersburg, Maryland, USA)), and control material (Lyphochek®) Urine Metals Control – Levels 1 and 2 (BIO-RAD, Irvine, CA, USA) were used.

2.3 Analytical Curve

For the construction of the analytical curve, an intermediary standard solution was prepared daily by diluting 80 times a multi-elemental stock solution containing 1.0 mg.L^{-1} of Li, Rh, Pd, Cd and Tl; 2.0 mg.L^{-1} of Be, Ni, Cu, As, Mo, Sb, Pt and Pb; 4.0 mg.L^{-1} of Cr, Mn and Se; and 10 mg.L^{-1} of Sr. The curve points were prepared using the standard addition technique in 0.2% HNO_3. The urine used in this preparation was collected daily from non-exposed donors. The preparation of the analytical curve is shown in Table 2.

Table 2. Analytical curve preparation

Analytical curve	Intermediary standard solution (mL)	Urine (mL)	0.2% HNO_3 (mL)	Water (mL)
Reagent blank	0	-	4.75	0.25
Sample blank	0	0.25	4.75	-
Standard 1	0.20	0.25	4.55	-
Standard 2	0.40	0.25	4.35	-
Standard 3	0.60	0.25	4.15	-
Standard 4	0.80	0.25	3.95	-
Standard 5	1.00	0.25	3.75	-

The solutions concentrations used for the analytical curve construction are represented in Table 3.

A multi-element internal standard solution with 5 µg.L^{-1} of each element was added on-line to the analyzed solutions with the help of a block mixer. Scandium was selected as an internal standard for the determinations of Li, Be, Mn, Ni and Cu; Germanium was selected for the analytes As, Se and Sr; Indium as internal standard for the analytes Mo, Rh, Pd, Cd and Sb; Rhenium for Pt, Tl and Pb, and Gallium for the analyte Cr.

2.4 Samples Preparation

For the validation of the analytical method, urine samples were diluted in a 1:20 rate in 0.2% HNO_3 in a 15 mL polyethylene tube. After homogenizing the samples were analyzed directly by ICP-MS.

2.5 Validation of the Analytical Method

The validation of the analytical method for the determination of metals in urine was conducted according to

Table 3. Concentrations of the elements used in the analytical curve for the determination of metals in urine

	Concentration (µg.L^{-1})				
Analytes	**Standard 1**	**Standard 2**	**Standard 3**	**Standard 4**	**Standard 5**
Li, Rh, Pd, Cd, Tl	0.5	1.0	1.5	2.0	2.5
Be, Ni, Cu, As, Mo, Sb, Pt, Pb	1.0	2.0	3.0	4.0	5.0
Mn, Se, Cr	2.0	4.0	6.0	8.0	10.0
Sr	5.0	10.0	15.0	20.0	25.0

the following parameters: selectivity, linearity, working range, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy and precision (reproducibility and intermediate precision), based in protocols described in Eurachem Guide[32] and INMETRO[33], under ABNT ISO/IEC 17025 criteria.

2.6 Estimation of Measurement Uncertainty

The estimation of the expanded measurement uncertainty (*Uc*) for the results obtained by the proposed method was calculated according to the described from EURACHEM/CITAC[34]. For this purpose, we considered data obtained from the validation procedure (precision and analytical curve), automated pipettes calibration and the effect of temperature on the volumetric measurement. The equation used to calculate *Uc* was obtained multiplying the coverage factor (*k*) by the combined standard uncertainty (u_c): $Uc = k.\ u_c$. The coverage factors were obtained from the Student's t-test distribution table with effective degrees of freedom (v_{eff}) given by the Welch-Satterthwaite formula.

3. Results and Discussion

3.1 Selectivity

The selectivity of a method can be demonstrated by its capacity to determine one analyte in the presence of possible interferences. The use of ICP-MS technique together with the direct introduction of high concentrations of organic matrix can result in physical interferences and/or spectral interferences of polyatomic ions.

Physical interferences can occur, for example, because of differences in viscosity between matrix and analytical curve solutions, leading to different taxes of introduction in the plasma. Some matrix components can interfere in the ionization of the analyte in the plasma resulting in effects of suppression or increase of signal. Such interferences can be eliminated by the use of standard addition calibration and use of internal standard, among other methods.

The chosen internal standard for the determinations by ICP-MS must have a similar behavior of the element to be analyzed. Due to its utilization, instrumental instabilities secondary to the matrix are corrected[35]. Some of the criteria for its choice must be the proximity with the mass and the first ionization potential of the analyte, besides not being present in the analyzed matrix. The ^{45}Sc is employed as internal standard for elements of low atomic mass (up to 70 amu), while ^{72}Ge is widely used for elements with low and intermediate atomic masses (between 6 and 100 amu). ^{115}In is used for the intermediate masses and ^{185}Re for the higher ones (from 150 to 240 amu)[36–39].

The choice of the internal standards for the present study was also based in the preliminary results obtained for the accuracy and recuperation parameters. The elements cited above were used as internal standards for the analytes determined in the standard mode, as indicated in Table 1. However, for the determination of ^{52}Cr at DRC mode, ^{69}Ga was used as internal standard[40,41] (Table 1).

Isobaric interferences can be corrected by applying mathematical correction equations, by the selection when possible of an alternative isotope, or by the utilization of the DRC. The correction equations adopted in this study were described above (Table 1).

For the optimization of the DRC operation conditions, reaction gas (NH_3) flow and the rejection parameter (RPq), two solutions were used: one with diluted urine by the ratio 1:20 and another solution containing urine in the same dilution with the addition of a 6 $\mu g.L^{-1}$ Cr solution. The analyte signal was monitored in these solutions meanwhile gas flow and the RPq adjusting parameters were tested. The gas flow varied from 0.1 to 1.0 $mL.min^{-1}$, with increments of 0.1 $mL.min^{-1}$. The RPq parameter varied from 0.45 to 0.8 with 0.05 increments. The minimum Background Equivalent Concentration (BEC) signal for Cr was reached with a gas flow of 0.8 $mL.min^{-1}$ and an RPq parameter equal to 0.65.

3.2 Linearity

The linearity of the analytical curve was verified for the studied elements in the working range indicated in Table 3. Three independent replicates in each level of concentration were used for the test. For the verification of outliers, Grubbs' test was applied, and the homoscedasticity was demonstrated by the Cochran test. No outliers were detected throughout the working range. Therefore, variances for all elements were considered homogenous through the curve, with C_{critic} value of 0.7218, for a confidence level of 99% and the graphics of residues obtained presented a random distribution around the central line. The mathematical model applied (minimum squares)

showed to be adequate, with correlation coefficients higher or equal to 0.999 for all analytes. Also, the significance of the regression was tested, and p-value showed to be less than 0.05 for all studied elements. Therefore, the obtained results demonstrated evidence of linearity for the studied work range.

3.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and the Limit of Quantification (LOQ) for each analyte were calculated as three and 10 times the standard deviation of the concentration means from six independent preparations of a urine sample diluted 1:20, respectively. The obtained LOD and LOQ (Table 4) showed values equal or lower than those reported by Centers for Disease Control and Prevention[42], and lower than the first point of the analytical curve for all of the evaluated isotopes in urine matrix, respectively.

Table 4. Limit of Detection (LOD) and Limit of Quantification (LOQ) for the analyzed isotopes, and values for limit of detection according to CDC (2015)

Isotope	Limit of detection ($\mu g.L^{-1}$)	Limit of quantification ($\mu g.L^{-1}$)	Limit of Detection ($\mu g.L^{-1}$), CDC[42]
^{7}Li	0.031	0.104	-
^{9}Be	0.034	0.112	0.072
^{52}Cr	0.006	0.019	-
^{55}Mn	0.006	0.021	0.08
^{60}Ni	0.029	0.098	-
^{65}Cu	0.082	0.274	-
^{75}As	0.078	0.259	1.25
^{78}Se	0.388	1.294	-
^{82}Se	0.340	1.132	-
^{88}Sr	0.376	1.252	2.5
^{98}Mo	0.184	0.613	0.99
^{103}Rh	0.001	0.004	-
^{105}Pd	0.004	0.015	-
^{106}Pd	0.002	0.006	-
^{111}Cd	0.008	0.027	0.056
^{114}Cd	0.005	0.015	0.056
^{121}Sb	0.049	0.164	0.041
^{195}Pt	0.006	0.020	0.009
^{205}Tl	0.021	0.069	0.02
^{206}Pb	0.020	0.066	0.08
^{207}Pb	0.016	0.052	0.08
^{208}Pb	0.021	0.069	0.08

3.4 Accuracy and Precision

Accuracy and precision of the analytical method were assessed with Certified Reference Materials (CRM) NIST 2670 Toxic Metals in Freeze-Dried Urine – Elevated Level for the analytes Be, Cr, Mn, Ni, Cu, As, Se, Cd, Pt and Pb. Due to unavailability of a CRM containing all studied elements, a fortified urine sample with concentrations equivalent to the first point in the analytical curve was also used.

Additionally, aiming at assuring the reliability of results, internal and external quality controls were executed. For the internal control it was used the Control Material Lyphochek® Urine Metals Control – Levels I and II for the elements Cr, Mn, Ni, Cu, As, Cd, Sb, Tl and Pb; for the external quality control our laboratory participated in the Programa Interlaboratorios de Controle de Calidad – PICC (Interlaboratory Program of Quality Control PICC) organized by the Instituto Nacional de Seguridad e Higiene en Trabajo, Ministerio de Empleo y Seguridad Social, Espanha (National Institute of Higiene and Work Safety of the Labor and Social Security Ministry, Spain), for the determination of chromium in urine.

To calculate the accuracy and precision of the method, six independent samples were prepared for the CRM and for the standard added solutions. For accuracy assessment the criteria included recovery means, and for the precision assessment (obtained under repeatability conditions) the Relative Standard Deviation (RSD), was estimated. For precision under intermediate precision conditions, CRM samples and the fortified urine samples were analyzed, and the results found in the period of one year were considered.

Accuracy and precision values obtained for the validation of the method for the studied analytes are presented in Table 5.

The results obtained for the recovery, when Certified Reference Materials (CRM) NIST was used for the elements Be, Cr, Mn, Ni, Cu, As, Se, Cd, Pt and Pb, stand in the interval between 88.7% and 109.7%, while for the urine fortified samples, recovery for all isotopes were in the interval between 85.8% e 105.1% (Table 5). These values were in accordance with the methods performance

Table 5. Results obtained for accuracy (recovery) and precision (relative standard deviation) parameters, for the CRM NIST 2670 and fortified urine samples

NIST 2670						
Isotope	**Certified value ($\mu g.L^{-1}$)**	**Obtained value ($\mu g.L^{-1}$)**	**Expanded uncertainty (%)**	**Recovery (%)**	**Repeatability (%)**	**Intermediate precision (%)**
^{9}Be	33	36.2	6.7	109.7	2.89	5.86
^{52}Cr	85	79.9	3.7	94.0	0.68	3.17
^{55}Mn	330	345.8	2.3	104.8	1.08	1.99
^{60}Ni	300	282.9	4.0	94.3	1.69	3.44
^{65}Cu	370	376.8	1.8	101.8	1.21	1.45
^{75}As	480	517.2	2.5	107.8	0.86	2.09
^{78}Se	460	422.5	5.3	91.8	1.49	4.54
^{82}Se	460	461.0	5.1	100.2	1.41	4.39
^{111}Cd	88	90,0	2.3	102.3	0.65	1.98
^{114}Cd	88	89.5	1.9	101.7	0.82	1.65
^{195}Pt	120	114.2	3.1	95.2	1.40	2.74
^{206}Pb	109	107.8	2.6	98.9	1.35	2.23
^{207}Pb	109	96.7	2.2	88.7	1.00	1.92
^{208}Pb	109	101.6	2.9	93.2	1.12	2.52
Fortified urine						
Isotope	**Added concentration($\mu g.L^{-1}$)**	**Obtained concentration($\mu g.L^{-1}$)**	**Expanded uncertainty (%)**	**Recovery(%)**	**Repeatability (%)**	**Intermediate precision (%)**
^{7}Li	0.5	0.51	6.2	101.2	1.42	1.39
^{9}Be	1.0	1.05	6.1	104.9	3.30	3.31
^{52}Cr	2.0	1.90	6.6	95.0	1.13	5.25
^{55}Mn	2.0	2.08	3,8	104.0	0.96	2.80
^{60}Ni	1.0	1.03	6.3	102.8	0.56	3.98
^{65}Cu	1.0	1.04	5.8	104.0	2.18	3.82
^{75}As	1.0	0,98	6.0	98.0	1.43	3.10
^{78}Se	2.0	2.10	10.8	105.1	8.49	8.84
^{82}Se	2.0	2.04	5.2	102.2	3.90	4.07
^{88}Sr	5.0	4.91	5.0	98.1	0.86	4.10
^{98}Mo	1.0	0.94	20.8	94.2	2.40	4.64
^{103}Rh	0.5	0.49	2.4	98.5	0.78	1.37
^{105}Pd	0.5	0.43	10.8	85.8	2.97	8.78
^{106}Pd	0.5	0.43	10.6	86.0	2.87	7.83
^{111}Cd	0.5	0.50	5.9	99.7	3.47	3.27
^{114}Cd	0.5	0.50	2.9	100.2	1.85	2.34
^{121}Sb	1.0	0.98	6.1	97.9	1.68	3.11
^{195}Pt	1.0	0.98	4.6	97.8	1.57	1.53
^{205}Tl	0.5	0.49	2.5	99.1	1.02	1.43
^{206}Pb	1.0	0.99	3.7	98.7	1.60	1.87
^{207}Pb	1.0	1.00	2.9	100.2	1.86	1.49
^{208}Pb	1.0	1.00	3.7	99.6	0.82	2.01

criteria recommended by AOAC[40] in which the expected recovery mean, related to the analyte concentration, must be between 80% and 110% for a concentration of 100 μg.L^{-1}. For concentration lower than 100 μg.L^{-1} the accepted interval is even broader, indicating the good accuracy of the studied method (Table 5)[43]. Obtained recovery results similar to the present study for the elements Be, Cd, Cu, Pb, Mn, Ni, Pt and Se, were also found[44]. Another research also found similar recovery values when compared with the recoveries from fortified urine samples, in the interval between 79% to 109% for the elements Be, Pt, Cu, Mn, Mo, Se, As, Cd, Ni, Pb, Tl and Sb[45].

The results of the internal quality controls showed good agreement with the declared values from the control material by BIORAD (in the two concentration levels), as indicated in Table 6. Regarding the Interlaboratory Quality Control Program (external quality control), the results obtained for chromium in three different concentrations levels showed z-score values, in module, equal or less than 2 ($|z| \leq 2$), indicating a satisfactory performance of the laboratory. These values demonstrate that the DRC cell was adequately optimized for the elimination of the polyatomic interference to which Cr might be subjected, as in the formation of the polyatomic ion $^{40}Ar^{12}C^{+}$.

Regarding the precision obtained with the proposed method, it was observed that for the measurements performed in conditions of repeatability, and even for the ones performed along the period of one year (condition of intermediate precision), all values of RSD were lower than 10%, considering the use of CRM, as well as for the fortified urine samples (Table 5). For the analyzed CRM, the biggest variation was observed for the analyte Be, which is the element with the lowest concentration in the sample (33 μg.L^{-1}), with obtained values of 2.89% and 5.86% for repeatability and intermediate precision, respectively. The precision values obtained for the fortified urine samples with concentrations between 0.5 μg.L^{-1} and 1.0 μg.L^{-1} varied between 0.56% to 3.47%, 1.37% and 8.78%, for repeatability and intermediate precision, respectively. For concentrations between 2.0 μg.L^{-1} and 5.0 μg.L^{-1}, this variation was 0.86% to 8.4%, for repeatability and intermediate precision, respectively. According to the AOAC criteria

Table 6. Internal and external quality control evaluation

Isotope	BIORAD			
	Level I		Level II	
	Obtained value (μg.L^{-1})	Acceptance range (μg.L^{-1})	Obtained value (μg.L^{-1})	Acceptance range (μg.L^{-1})
^{52}Cr	0.61	0.603-0.905	19.84	15.0-22.5
^{55}Mn	8.46	5.91-8.86	22.75	16.0-24.0
^{60}Ni	4.50	1.99-4.87	26.13	19.6-29.4
^{65}Cu	11.90	9.17-13.8	59.69	47.2-70.8
^{75}As	63.76	37.3-81.2	157.34	83.4-204
^{111}Cd	8.98	4.38-9.54	16.07	7.70-16.7
^{114}Cd	9.00	4.38-9.54	15.88	7.70-16.7
^{121}Sb	10.12	7.48-11.2	37.66	27.4-41.1
^{205}Tl	9.33	6.96-10.4	177.20	136-203
^{206}Pb	14.14	8.95-16.3	68.47	39.1-79.5
^{207}Pb	13.43	8.95-16.3	64.12	39.1-79.5
^{208}Pb	13.70	8.95-16.3	65.79	39.1-79.5
Interlabaoratory Quality Program – PICC				
Isotope	**Sample**	**Obtained results (μg.L^{-1})**	**Valid results[a] ± DP[b] (μg.L^{-1})**	**z-score**
^{52}Cr	344	55.2	60.9 ± 5.4	-1.1
	345	24.2	25.9 ± 4.1	-0.4
	346	7.3	6.4 ± 1.3	0.7

[a]Results included in interval $\overline{X} \pm 2s$, where $\overline{X}$ is the mean of the results from all of laboratories, excluding the results lower or higher than two standard deviations.
[b]Standard deviation.

for performance of methods, the expected precision for a concentration of 100 μg.L^{-1} is at maximum 15% (Appendix F, Table A4)[42]. Therefore, the results of RSD lower than 10% obtained in this study indicate that the proposed methodology shows good precision for both repeatability and intermediate precision conditions. The biggest variations, either in relation to the determinations performed in the same day, as in those performed in different periods, were observed for ^{78}Se. These variations corroborate with results from other studies in the literature[37,45].

3.5 Uncertainties of the Measurements

The uncertainties measurement evaluation was performed considering the contributions related to the intermediate precision, pipetted volume, and analytical curve. All the results found for relative uncertainties were lower than 11%, with the exception of Mo that was 20.8%. For this element, when the contribution of each parameter was evaluated, it was observed that the biggest contribution for the total uncertainty was related to the analytical curve (77%). This probably was due to the inadequate definition for added concentrations during the preparation of the analytical curve. According to the Fourth National Report on Human Exposure to Environmental Chemicals (2015), the geometric mean of Mo concentration in urine in the American population is 37.1 μg.L^{-1}, with maximum values reaching 160 μg.L^{-1} (95th percentile, upper limit of the 95% CI). Thus, considering that a urine sample has a relevant concentration of Mo, and that the calibration was performed by standard addition technique, we noticed that the additions used for the construction of the analytical curve (1.0 to 5.0 μg.L^{-1}) were insignificant when compared to the concentration of analyte in the sample, resulting in an analytical signal with small differentiation between the points.

Regarding the other studied analytes, the uncertainty results demonstrated that the proposed method was adequate for the determination of these metals in urine.

4. Conclusion

The proposed method allowed for the simultaneous determination of 17 elements in urine. It presented low detection and quantification limits, and good accuracy and precision. The method proved to be rapid, needing minimal pre-analytical processes, and small sample amount. The validated method will be of use in the determinations of metal concentrations, in assessment of human exposure in occupational or environmental conditions, and in the establishment of reference values.

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