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Fast and simple multi-element determination of essential and toxic metals in whole blood with quadrupole ICP-MS

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ABSTRACT

The development of reliable and sensitive methods for elements quantification in low-level concentration is a necessary requirement to detect increases or deficiencies considered unusual in human body. In this sense the sensitivity and selectivity of mass spectrometric methods are particularly appropriate to deal with this issue since many elements in biological material are at extremely low concentration levels and necessity to be monitored. Simultaneous determination of Be, Bi, Ba, Pb, Pd, Pt, Rh, Ni, V, Cr, Mn, As, Cd, Mo, Co, Hg, Tl, Sr, Sb, Se, Cu and Zn in whole blood by ICP-MS is described. Sample preparation consisted of a simple dilution (twenty-fold) with 0.05% Triton X-100 in 0.2% HNO3. The method proved to be accurate (accuracy ranged from 88.9% to 108%), precise (CV was below 5% for all elements) and sensitive (LOQ were below 1 µg L^{-1} for most of the studied elements). Use of DRC was only necessary for chromium and vanadium determination in blood. The proposed method uses low quantities of blood and this feature is essential when analyzes involve human biological samples. The obtained results show the applicability of the developed methodology for the rapid, sensitive and simultaneous determination of inorganic constituents in whole blood.

INTRODUCTION

Technological development has been reflected in the increase of xenobiotics in the environment. Therefore differences between occupational and environmental exposure are becoming significantly smaller (Paoliello *et al.,* 1997). This fact contributes and accentuates the necessity to monitore that aim to identify exposure sources and assess the exposure distribution in the general population (Kuno *et al.,* 2010; Heitland and Köster, 2004). Biomonitoring then becomes an important tool to estimate human exposure to contaminants and their concentrations in the blood is generally used as a biomarker (Barbosa Jr, 2005; Needham *et al.,* 2007; Palmer *et al.,* 2006; Apostoli, 2002). This implies in an increasing necessity of more sensitive and especially faster methods with minimal sample handling. In the past many determinations were correlated to monoelemental analyzes such as lead or cadmium in whole blood, but currently multielement methods in biological material became more common due to technological advances. In this sense inductively coupled plasma mass spectrometry (ICP-MS) a multi-element technique with appropriate sensitivity is progressively replacing conventional techniques as atomic absorption or electrochemical methods (Fong *et al.,* 2007; D'Ilio *et al.,* 2008; Goullé *et al.,* 2005; Zhou *et al.,* 2002). Although these techniques may achieve the required sensitivity to determine elements at trace levels, they are lacking in speed and ease to use. ICP-MS could be very useful for a wide range of clinical applications because the great sensitivity acquired in this technique with low detection limits enabling toxicologists to sufficiently estimate the toxic levels and environmental metal exposure (Parsons and Barbosa Jr, 2007; Ammann, 2007; D'Ilio *et al.,* 2006; Bárany *et al.,* 1997; Rivero Martino *et al.,* 2000; Sariego Muñiz *et al.,* 1999). Furthermore, this method offers new possibilities in several fields such as clinical chemistry, clinical toxicology, forensic toxicology as well as workplace testing or environmental exposure and also applicable in epidemiological studies (Bortoli *et al.,* 1992).The development of reliable and sensitive methods for elements quantification in low-level concentration is a necessary requirement to detect increases or deficiencies considered unusual in human body.

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In this sense the sensitivity and selectivity of mass spectrometric methods are particularly appropriate to deal with this issue since many elements in biological material are at extremely low concentration levels and necessity to be monitored.

Some of the elements addressed in this study exhibit an essential role in the biological system, while others may have toxic effects when present in high concentrations or even have no known biological function, but they might be related to some diseases or illness (Apostoli, 2002; Bazzi *et al.,* 2008).

In the case of Pt, Pd and Rh their occurrence in the environment is increasing, primarily due to its use in catalytic converters for motor vehicles (Ek *et al.,* 2004). Thus, the assessment of health risks posed by human exposure to these elements has gained interest.

The suggested method has few steps of preparation, is fast, sensitive and allows the simultaneous determination of several elements in whole blood. Existing methods in the literature usually use Rh and Bi as internal standards. Thus we propose a method with other internal standards since these elements were targets to be determined.

The aim of this study is to propose a method for the simultaneous determination of nutrient and toxic elements in whole blood by ICP-MS.

MATERIAL AND METHODS

Material

The measurements were carried out using a Perkin Elmer DRC II mass spectrometer (Norwalk, CT, USA). The ICP mass spectrometer was equipped with an AS-93 plus auto sampler besides a quartz cyclonic spray chamber, quartz bore injector and a Meinhard concentric nebulizer for sample introduction. The main operating conditions are listed in Table 1.

Table. 1: ICP-MS operating conditions.

Parameter	Value
Plasma power (W)	1400
Nebulizer gas flow rate (L. min^{-1})	0.98
Auxiliary gas flow rate (L. min^{-1})	1.05
Plasma gas flow rate (L. min^{-1})	15
Integration	16 sweeps, 3 replicates
Scan mode	Peak hopping
Read delay (s)	15
Sample time (min)	2.37
Standard mode	^{9}Be, ^{55}Mn, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{68}Zn, ^{75}As, ^{77}Se, ^{78}Se, ^{82}Se, ^{88}Sr, ^{98}Mo, ^{103}Rh, ^{105}Pd, ^{106}Pd, ^{108}Pd, ^{111}Cd, ^{112}Cd, ^{114}Cd, ^{121}Sb, ^{123}Sb, ^{194}Pt, ^{195}Pt, ^{196}Pt, ^{202}Hg, ^{203}Tl, ^{206}Pb, ^{207}Pb, ^{208}Pb, ^{209}Bi, ^{138}Ba, ^{135}Ba, ^{137}Ba, ^{52}Cr, ^{51}V
DRC mode	^{51}V, ^{52}Cr
Reaction gas	Ammnonia (NH_3)
RPq	0.7
Cell gas A	0.8

Daily performance was assessed (Mg intensity > 6000 counts s^{-1}, In intensity > 30000 counts s^{-1}, and U intensity > 20000 counts s^{-1} for 1 µg L^{-1} concentration, with the CeO^+/Ce^+ and Ba^{2+}/Ba^+ ratios < 3%). A mixed solution of internal standards (5 µg L^{-1} Ge, Re, In, Sc in 0.2% v/v nitric acid) and a solution of 200 µg L^{-1} of gold (in 0.2% v/v nitric acid) were aspirated separately by a second channel of peristaltic pump allowing on-line addition to standard and sample solutions. Sample or standards and internal standards flows were joined and mixed together before the nebulizer using a connector. Six milliliters Vacuette® tubes with royal blue cap to trace elements were used for blood collection (NH Trace Elements Sodium Heparin).

Calibration standards from multi-element stock solution and sample dilutions were prepared in an ISO class 7 clean room equipped with laminar flow ISO class 5 (ISO, 1999). In order to minimize contamination risks, polymethylpentene (PMP) volumetric flasks, polyethylene tubes and beakers were soaked overnight in 20% (v/v) nitric acid followed by rinsing with demineralized water and dried.

Reagents

Water used (specific resistance 18.2 MΩ cm^{-1}) was supplied by a Milli- Q Academic purification system (Millipore, USA). Suprapur® nitric acid 65%, triton X-100 and stock standard solutions of Be, Mn, Ni, As, Se, Cd, Tl, Pb, Bi, Ba, Cr, Se and Au (1000 µg.mL^{-1}) were from Merck (Darmstadt, Germany). Stock standard solutions of Re, Cu, Zn, Sr, Mo, Rh, Pd, Sb, Pt, Hg and V (1000 µg.mL^{-1}) were obtained from Spex CertiPrep. Stock standard solutions of Ge and In (10 µg.mL^{-1}) were from Spex CertiPrep. Stock standard solution of Sc (1000 µg.mL^{-1}) was from Sigma Aldrich.

A pool of human whole blood was used as base blood for matrix-matching calibration. Blood samples, were collected into tubes (Vacuette) containing sodium heparinate, to prevent coagulation, then transferred into a 50 mL polyethylene tube to make a pool and kept at 4 ºC. The calibration curve preparation is illustrated in Table 2.

Table. 2: Calibration curve preparation.

	Multi-element standard solution* (mL)	Whole blood (mL)	Diluent solution (mL)	Water (mL)
Reagent blank	0	---	4.75	0.25
Sample blank	0	0.25	4.75	---
Standard 1	0.1	0.25	4.65	---
Standard 2	0.2	0.25	4.55	---
Standard 3	0.5	0.25	4.25	---
Standard 4	1.0	0.25	3.75	---
Standard 5	2.0	0.25	2.75	---

*Multi-element standard solution containing: Be, Mn, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Rh, Pd, Cd, Sb, Pt, Hg, Tl, Pb, Bi, Ba, Cr and V

Calibration standards were matrix-matched and were prepared in a diluent solution (0.05% (v/v) Triton X 100 in 0.2% (v/v) HNO_3) and whole blood (250 µL). Preliminary tests were carried out to establish the working range, based on the application of the method and the expected concentrations in the samples. Element concentrations in the calibration curve are showed in Table 3.

Table. 3: Concentrations of the elements in calibration curve (in ug L^{-1}).

Elements	Std 1 (ug L^{-1})	Std 2 (ug L^{-1})	Std 3 (ug L^{-1})	Std 4 (ug L^{-1})	Std 5 (ug L^{-1})
Be, Sr, Rh, Pd, Sb, Pt, Tl, Pb, Bi, Ba	1	2	5	10	20
Ni, V, Cr	2	4	10	20	40
Mn, Co, As, Mo, Cd	0.5	1	2.5	5	10
Se, Cu	10	20	50	100	200
Hg	0.2	0.4	1	2	4
Zn	50	100	250	500	1000

Sample preparation

Blood samples was left at room temperature and mixed well for a few minutes to homogenize with a vortex mixer. After homogenization, 0.25 mL of whole blood was accurately transferred into a 15 mL polyethylene tube with 4.55 mL of diluent solution (0.05% (v/v) Triton X-100 + 0.2% (v/v) HNO_3, mixed on a vortex mixer) following addition of 0.2 mL multi-element standard solution that corresponds to the second point of calibration curve. Samples were then thoroughly mixed.

RESULTS AND DISCUSSION

Selection of the internal standard

In ICP-MS the internal standard element should not be present in the samples and should be added to all blanks, standards and samples in equal concentrations. The behavior of the internal standard elements in the plasma is dependent on atomic mass and ionization efficiency. Internal standards are used to correct variations in signal intensity due to instrument instability and non-spectral interferences (suppression or enhancement of the signal caused by the matrix) (Vanhaecke *et al.,* 1992; Paquette *et al.,* 2010). Exisiting methods in literature for the determination of trace elements in biological materials have used rhodium or bismuth as internal standards. Selection of different internal standards was proposed in this study since Rh and Bi were one of the target elements to be analyzed. Internal standards were selected based on their performance regarding to precision and accuracy for elements determination in whole blood samples unspiked and spiked in concentrations equivalent to the first and second point of the calibration curve. Ge, Re, In and Sc were selected as internal standard. Concentrations of 5 µg L^{-1} and 40 µg L^{-1} of internal standards were tested and best results were obtained with concentration of 5 µg L^{-1}, which was chosen. Althoug ^{45}Sc has no mass number close to beryllium it worked as well as internal standard. In addition, ^{45}Sc has been used as internal standard element for the low-mass regions (mass range: 6-70) (De Boer *et al.,* 2004).

Optimization of Dinamic Reaction Cell (DRC) operating conditions

Use of Dinamic Reaction Cell (DRC) was preliminary tested for chromium, vanadium, nickel, manganese, selenium, barium, copper and zinc. DRC operating conditions (optimization of NH_3 flow rate and Rejection Parameter q (RPq parameter) were determined by scanning the ion signal for various rejection parameter q (RPq) settings and cell gas in blood sample unspiked and blood sample spiked with 2 µg L^{-1} of chromium, vanadium and nickel, 0.5 µg L^{-1} of manganese, 10 µg L^{-1} of selenium, 1 µg L^{-1} of barium, 10 µg L^{-1} of copper and 50 µg L^{-1} of zinc. NH_3 flow rate was varied in the range of 0.1 – 1 mL min^{-1} in steps of 0.1 mL min^{-1}, and RPq was varied in the range of 0.45 - 0.8 in steps of 0.05. For Cu^{65}, Zn^{66} and Zn^{68} background equivalent concentrations (BEC) were higher than 5 µg L^{-1} and sensitivities were very low (intensity signal of 200 cps) in various RPq settings and ammonia flow rates as described above. Thus, the bandpass settings were not optimized for these isotopes. The lower background equivalent concentrations (BEC) were achieved for NH_3 flow rate of 0.8 mL min^{-1} for V, Cr and Ni and 0.6 mL min^{-1} for Mn, Se and Ba. The maximum ratio of the intensity of signal per background could be obtained with RPq values of 0.7 for V^{51}, Cr^{52}, Ba^{135} and Ba^{138}, 0.6 for Cr^{53}, 0.75 for Ni^{60}, Se^{78} and Se^{82}, 0.45 for Mn^{55}, 0.55 for Se^{77} and 0.65 for Ba^{137}. To evaluate the DRC performance for determination of V, Cr, Ba, Ni, Se and Mn, blood samples were fortified with 2 µg L^{-1} of chromium, vanadium and nickel, 0.5 µg L^{-1} of manganese, 10 µg L^{-1} of selenium and 1 µg L^{-1} of barium. In Table 4 the influence of DRC on the recoveries in the fortified blood samples is reported. Best recoveries for Ni, Mn, Se and Ba were achieved without the use of DRC-ICP-MS. On the other hand, the use of DRC improved the recoveries in blood samples for Cr and V using an RPq value of 0.7 and a NH_3 flow rate of 0.8 mL min^{-1}.

Table. 4: Recoveries obtained in blood samples with and without use of DRC conditions.

Analyte	Concentration added (ug L^{-1})	Without use of DRC conditions Recup* (%)	With use of DRC conditions Recup* (%)
Cr^{52}	2.0	90.3	101.5
Cr^{53}	2.0	106.8	102.7
V^{51}	2.0	103.9	91.6
Ni^{60}	2.0	99.8	96.4
Mn^{55}	0.5	104.8	109.6
Se^{77}	10.0	102.0	67.9
Se^{78}	10.0	102.1	58.5
Se^{82}	10.0	102.1	68.6
Ba^{135}	1.0	98.3	83.9
Ba^{137}	1.0	98.5	73.8
Ba^{138}	1.0	99.2	70.1

* n = 6 replicates

Validation

In order to assess the proposed method validation studies were performed. Following parameters were established with fortified samples: linearity, selectivity, accuracy, precision, detection limit and quantification limit. Due to unavailability of having a certified reference material containing all the elements studied the validation of the proposed method was performed by fortification means in whole blood sample. In addition, according to the commission of the European Communities (2002) when no CRM are available, it is acceptable that trueness of measurements is assessed through recovery of additions of known amounts of the analyte (s) to a matrix. Linearity was evaluated in the elements

working range (0,1,2,5,10 and 20 ug L^{-1} for Be, Sr, Rh, Pd, Sb, Pt, Tl, Pb, Bi and Ba; 0,2,4,10,20 and 40 ug L^{-1} for Ni , V and Cr; 0,0.5,1,2.5,5 and 10 ug L^{-1} for Mn, Co, As, Mo and Cd; 0,10,20,50,100 and 200 ug L^{-1} for Se and Cu; 0,0.2,0.4,1,2 and 4 ug L^{-1} for Hg; 0,50,100,250,500 and 1000 ug L^{-1} for Zn). Three independent replicates at each concentration level of the calibration curve were used to test the linearity. The verification of the outliers' absence was made by Grubbs test while data homocedasticity was checked by Cochran test. Results are presented in Table 5. As variances for all elements were considered equal along the tested curve it could be possible to apply the linear regression by the method of least squares. The linearity of the method was then verified by the linear regression equation determined and the residuals graph was plotted in order to verify experimental points deviation from the fitted line (not shown). It was observed an acceptable fit with a random pattern. Each element showed linearity from de first point of the calibration curve to the last point of the curve with correlation coefficient above 0.99 for all elements (Table 7). The results showed goodness of fit evidence and linearity was considered satisfactory. Calibration curve was matrix-matched with human whole blood.

Table. 5: Comparison of variances by Cochran test applied to three independent replicates of each point of the calibration curve.

Cochran test			
Analyte	$C_{critical}$ (I=5, n=3)	$C_{calculated}$	Conclusion*
^{9}Be	0.6838	0.6326	+
^{55}Mn	0.6838	0.5872	+
^{59}Co	0.6838	0.6543	+
^{60}Ni	0.6838	0.6741	+
^{65}Cu	0.6838	0.3698	+
^{66}Zn	0.6838	0.5003	+
^{68}Zn	0.6838	0.5069	+
^{75}As	0.6838	0.6752	+
^{77}Se	0.6838	0.4993	+
^{78}Se	0.6838	0.4644	+
^{82}Se	0.6838	0.2129	+
^{88}Sr	0.6838	0.3167	+
^{98}Mo	0.6838	0.3854	+
^{103}Rh	0.6838	0.6293	+
^{105}Pd	0.6838	0.5338	+
^{106}Pd	0.6838	0.5471	+
^{108}Pd	0.6838	0.5659	+
^{111}Cd	0.6838	0.4933	+
^{112}Cd	0.6838	0.5617	+
^{114}Cd	0.6838	0.4830	+
^{121}Sb	0.6838	0.5769	+
^{123}Sb	0.6838	0.5951	+
^{194}Pt	0.6838	0.5948	+
^{195}Pt	0.6838	0.2098	+
^{196}Pt	0.6838	0.5901	+
^{202}Hg	0.6838	0.6051	+
^{203}Tl	0.6838	0.6640	+
^{206}Pb	0.6838	0.4221	+
^{207}Pb	0.6838	0.4028	+
^{208}Pb	0.6838	0.6313	+
^{209}Bi	0.6838	0.6206	+
^{138}Ba	0.6838	0.4826	+
^{135}Ba	0.6838	0.6450	+
^{137}Ba	0.6838	0.4885	+
^{52}Cr	0.6838	0.6245	+
^{51}V	0.6838	0.4450	+

*If C calculated < Ccritical : homocedastic (equal variances) +: homocedastic
I: number of points of the calibration curve n: number of replicates.

For each standard curve an unweighted least-squares linear regression of the response (counts/s) as a function of the nominal concentrations was applied (formula: $y = b + ax$, where x = concentration in µg L^{-1}, y = counts/s, a = slope and b = intercept). Typical calibration parameters (slope and intercept) were given in Table 7. Selectivity refers to the extent of the method to determine a certain analyte in the presence of other sample components (Codex, 2009). In ICP-MS technique spectral overlaps may be caused by polyatomic and isobaric interferences (D'Ilio *et al.,* 2011). These interferences can lead to overestimation of analyte concentration and the ways to overcome this problem would be choose an alternative isotope, use correction equations or use the dynamic reaction cell (DRC) technology. For Cr^{52} and V^{51} potential interferences were overcome by the use of DRC (see "optimization of DRC operating conditions"). To check the specificity of the method recoveries of fortified samples were measured. Observed concentrations and actual concentrations for all isotopes were quite consistent. Furthermore for the elements that have more than one isotope (Zn, Se, Pd, Cd, Sb, Pt, Pb and Ba) results obtained for these spiked samples were quite similar among the analyte different mass (see Table 6). As a consequence, the specificity of the method was considered acceptable. Accuracy was investigated by means of average recoveries for six replicates of pool blood sample fortified with elements in concentrations equivalent to the first point of the calibration curve. Obtained results were ranged from 88.9% to 108.0% for within-day accuracy while the range for between-day was 96.7% to 109.1% (Table 6). According to AOAC guidelines acceptable recovery ranged from 75% to 120% at the 1 ug/kg concentration (AOAC, 2002). Average recoveries for selenium and barium around 90% were lower than obtained recoveries for the other elements, but the results were in compliance with the specifications of AOAC criterion. The method precision was determined from replicate determinations of six independent preparations in whole blood fortified with concentrations of the elements equivalent to the first point of the calibration curve and assessed by coefficient of variation. Inter-assay accuracy and precision was estimated by carrying out twelve replicate assays on a sample of whole blood fortified for two weeks. The within (assays performed at the same day) and between-day accuracy and precision of the assay are summarized in Table 6. The observed values were within the acceptance criteria (AOAC, 2002). Detection limit (LD) was calculated as three deviation standard and quantification limit (LQ) was calculated as ten deviation standard from six independent replicates of pool human blood sample diluted twenty times and fortified with elements corresponding to the first point of the calibration curve. Quantification limits for copper and zinc were the highest compared to the others elements. The explanation for this is that Cu and Zn are major elements present in high concentration in blood. The results were showed in Table 7. Analysis of blood samples after twenty-fold dilution for the determination of 22 elements showed no deterioration in the quantification limits of the method since LQ for most elements were below 1 ug L^{-1}.

Table. 6: Summary of within and between-day accuracy and precision results for elements in fortified whole blood sample.

		Parameters					
		Within-day*			Between-day**		
Analyte	**Actual conc. (ug L^{-1})**	**Observed conc. (ug L^{-1})**	**Recup (%)**	**CV (%)**	**Observed conc. (ug L^{-1})**	**Recup (%)**	**CV (%)**
^{9}Be	1.0	1.04	104.0	4.0	1.06	107.8	4.7
^{55}Mn	0.5	0.51	102.7	1.6	0.53	105.2	4.2
^{59}Co	0.5	0.52	104.0	1.2	0.54	107.4	2.6
^{60}Ni	2.0	1.96	98.1	1.7	1.99	102.6	3.8
^{65}Cu	10.0	9.82	98.2	1.8	9.96	105.7	7.9
^{66}Zn	50.0	48.78	97.6	3.0	50.86	99.6	11.0
^{68}Zn	50.0	49.59	99.2	2.7	51.19	99.7	9.6
^{75}As	0.5	0.50	100.4	3.2	0.50	101.4	4.1
^{77}Se	10.0	9.86	98.6	2.0	9.87	100.1	2.7
^{78}Se	10.0	9.39	93.9	3.8	9.67	96.7	2.6
^{82}Se	10.0	9.79	97.9	2.1	9.98	102.6	3.9
^{88}Sr	1.0	0.95	94.6	3.2	0.97	101.7	6.6
^{98}Mo	0.5	0.48	95.3	2.4	0.48	98.4	3.1
^{103}Rh	1.0	1.02	101.8	0.9	1.05	104.4	2.8
^{105}Pd	1.0	1.01	101.5	2.1	1.03	104.6	3.4
^{106}Pd	1.0	1.02	101.6	3.1	1.02	104.7	5.4
^{108}Pd	1.0	1.01	101.3	1.4	1.04	104.1	3.8
^{111}Cd	0.5	0.51	102.3	1.7	0.51	102.6	1.8
^{112}Cd	0.5	0.50	100.8	2.0	0.52	102.9	4.9
^{114}Cd	0.5	0.51	102.9	2.0	0.53	104.0	5.4
^{121}Sb	1.0	1.00	100.0	1.1	1.01	101.1	3.5
^{123}Sb	1.0	0.99	99.2	0.7	1.01	100.7	3.5
^{194}Pt	1.0	1.01	100.6	1.5	1.01	103.2	1.5
^{195}Pt	1.0	1.01	100.9	1.0	1.03	103.6	2.9
^{196}Pt	1.0	1.01	100.6	2.2	1.02	102.8	1.9
^{202}Hg	0.2	0.20	101.0	1.4	0.2	107.0	4.4
^{203}Tl	1.0	1.03	102.7	1.5	1.05	104.9	2.3
206Pb	1.0	1.08	107.5	1.8	1.09	108.7	2.8
207Pb	1.0	1.07	107.4	2.5	1.08	107.6	3.1
208Pb	1.0	1.08	108.0	1.9	1.09	109.1	3.0
209Bi	1.0	1.00	99.6	1.4	1.00	100.4	2.2
138Ba	1.0	0.93	92.7	2.6	0.96	99.8	4.3
135Ba	1.0	0.93	92.6	3.7	0.96	99.9	5.1
137Ba	1.0	0.89	88.9	2.7	0.94	100.0	6.4
52Cr	2.0	1.97	98.7	2.8	2.04	106.0	3.8
51V	2.0	2.07	103.5	4.4	2.22	107.1	8.8

* mean (n = 6) ** mean (n=12)

Table 7: Detection limits and Quantification limits of the method (µg L^{-1}), slope, intercept and correlation coefficient of the calibration curve (r^2).

Analyte	LD (µg L^{-1})*	LQ (µg L^{-1})*	Slope** (µg L^{-1})	Intercept** (µg L^{-1})	Correlation coefficient (r^2)
9Be	0.08	0.26	1.0119	0.0842	0.9992
55Mn	0.06	0.21	1.0205	-0.0133	0.9994
59Co	0.02	0.07	1.0198	-0.0097	0.9997
60Ni	0.11	0.37	1.0314	0.1324	0.9998
65Cu	2.83	9.43	1.0013	0.1751	0.9993
66Zn	19.9	66.3	1.0540	291.7248	0.9996
68Zn	18.2	60.7	1.0564	297.9272	0.9994
75As	0.07	0.25	1.0013	0.0427	0.9994
77Se	0.92	3.08	1.0104	0.0025	0.9998
78Se	1.43	4.75	1.0201	-0.6422	0.9999
82Se	0.96	3.20	1.0276	-0.6797	0.9996
88Sr	0.18	0.60	1.0313	-0.0402	0.9991
98Mo	0.04	0.12	1.0274	0.0286	0.9998
103Rh	0.03	0.09	1.0028	-0.0013	0.9998
105Pd	0.06	0.21	0.9927	0.0385	0.9996
106Pd	0.10	0.32	1.0021	0.0312	0.9998
108Pd	0.04	0.15	0.9991	0.0205	0.9997
111Cd	0.03	0.09	1.0023	0.0224	0.9997
112Cd	0.03	0.10	1.0163	0.0031	0.9995
114Cd	0.03	0.11	1.0083	0.0340	0.9996
121Sb	0.04	0.12	0.9941	0.1189	0.9998
123Sb	0.02	0.08	0.9806	0.1562	0.9996
194Pt	0.04	0.15	0.9969	0.0714	0.9994
195Pt	0.03	0.10	0.9994	0.0540	0.9997
196Pt	0.07	0.22	0.9918	0.0897	0.9993
202Hg	0.01	0.04	1.0091	0.1070	0.9986
203Tl	0.05	0.15	1.0022	0.0209	0.9997
206Pb	0.27	0.91	1.0050	0.0999	0.9997
207Pb	0.29	0.96	1.0056	0.0950	0.9996
208Pb	0.30	0.99	1.0017	0.0968	0.9998

209Bi	0.04	0.14	1.0006	0.0353	0.9998
138Ba	0.08	0.25	0.9997	0.0524	0.9997
135Ba	0.10	0.35	0.9952	0.0648	0.9998
137Ba	0.08	0.26	0.9972	0.0601	0.9999
52Cr	0.16	0.54	1.0056	0.0960	0.9998
51V	0.16	0.52	1.0085	0.0019	0.9999

*n = 6 LD (detection limit) LQ (quantification limit) **n = 3

Sample preparation

In the literature sample preparation procedures for analysis of blood by ICP-MS commonly involve digestion and dilution of the sample. The high organic matter content in whole blood can cause carbon deposition in the ICP-MS introduction system. This results in analytical signal suppression (Lemos and Carvalho, 2010). Furthermore direct analysis of samples with high organic matrix contents causes the plasma to be extinguished. Thus, it is important to apply one of the pretreatment of sample above mentioned. The digestion procedure consists in the destruction of organic matter of the sample using different acids or mixture of acids and hydrogen peroxide (Bolann *et al.,* 2007). This approach can reduce spectral and non-spectral interference problems, but has the risk of introducing contamination. Nowadays sample digestion using microwave is an interesting method (Kadar *et al.,* 2011; Pick *et al.,* 2010). Dilution is a way to reduce matrix effect of the sample and many studies report the use of different diluents applied to whole blood samples. Some of them include the use of diluted acid with Triton X-100 (Palmer *et al.,* 2006; Batista *et al.,* 2009), a non-ionic surfactant, which promotes the solubilization of proteins present in this matrix thereby preventing clogging on the nebulizer, sampler and skimmer cone (Mesko *et al.,* 2011; McShane *et al.,* 2008). On the other hand aqueous dilution of whole blood provokes precipitation of red cells (Subramanian, 1996). Methods in literature also report the use of phosphate and EDTA solutions (Bonefoy et al., 2002& 2005; Krachler et al., 2009) to improve the stability of the diluted blood solution preventing precipitation (Subramanian, 1996), but EDTA is hard to obtain in a pure form (Lemos and Carvalho, 2010). Direct dilution of whole blood can also be carried out with tetramethyl ammnonium hydroxide (TMAH) which also aid in protein solubility and stability of the sample solution (Subramanian, 1996; McShane *et al.,* 2008). However, the TMAH solubilization has the disadvantage of presenting limited time-stability of the sample solution (Subramanian, 1996). Another approach is the use of butanol (Bonnefoy *et al.,* 2002; 2005), ethanol or propanol to increase the analytical signal and reduce carbon buildup on the cones (Subramanian, 1996; McShane *et al.,* 2008). First we tried to digest the sample blood by dry ashing but the time spent was too long. Furthermore, it was difficult to solubilize the digested sample with low acid concentration. Thus, we chose to use the procedure for diluting the blood sample with Triton in acid medium and subsequent reading of the sample directly on ICP-MS. The use of Triton X-100 improved the washout of the sample after the analysis and no blockages were observed during the analysis. After measuring 250 to 375 blood samples, that took five days of measuring, the sample introduction system and torch were cleaned with diluted acid solution (10% (v/v) HNO_3) followed by rinsing with Milli-Q water. At the same time cones were cleaned with diluted acid solution (1% (v/v) HNO_3) and lenses were cleaned with Milli-Q water. Mercury memory effect is the major problem in mercury analysis by ICP-MS. Mercury adheres to the spray chamber wall (known as carryover problems or memory effects) and remains as vapor in the spray chamber and could be released during subsequent analyses (Fong *et al.,* 2007). In order to overcoming this problem gold, considered one of the most effective washout agents, was added to diluent to improve the washout for Hg. Use of gold prevents mercury volatilization and adsorption losses. When gold was removed from the diluent poorer precision was achieved. The suggested method involved simple dilution of the sample and proved to be fast since it enabled the simultaneous determination of 22 elements in just 4:22 minutes.

CONCLUSION

The proposed method uses low quantities of blood and this feature is essential when analyzes involve human biological samples. In conclusion, the proposed method for determining inorganic elements in human blood samples proved to be accurate, precise, sensitive, and for most elements limits of quantification were below 1 µg L^{-1}. The between-day percentage recovery ranged from 96.7% to 109.1%. The between-day precision did not exceed 11% for all analytes. Dynamic reaction cell (DRC) is an important tool in ICP-MS analysis for reduction or elimination of spectral interferences and its use was only necessary for chromium and vanadium determination in blood matrix. The proposed method requires minimal sample preparation involving a simple dilution. This fact contributes for its application in routine laboratory analysis for environmental and occupational monitoring studies.

The simultaneous inorganic elements determination in blood may be relevant since it may be useful as a quick and reliable research tool in public health and other areas.

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