Fast method for determination of Cd, Cu, Pb, Se, and Zn in whole blood by DRC-ICP-MS using the simple dilution procedure

C. TĂNĂSELIA*, T. FRENȚIU[a], M. URSU, M. VLAD[b], M. CHINTOANU, E. CORDOȘ, L. DAVID[c], M. PAUL, D. GOMOIESCU

INCDO-INOE 2000, Research Institute for Analytical Instrumentation, Donath 67, Cluj-Napoca, Romania
[a]Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, A. Janos 11, Cluj-Napoca, Romania
[b]Iuliu Moldovan Institute of Public Health, Pasteur 6, Cluj-Napoca, Romania
[c]Babes-Bolyai University, Faculty of Physics, M. Kogălniceanu 1, Cluj-Napoca, Romania

A fast and very sensitive method based on inductively coupled plasma mass spectrometry and Dynamic Reaction Cell (DRC-ICP-MS) for determination of Cd, Cu, Pb, Se, and Zn in whole blood was developed by using of the reference certified materials. After a simple dilution of the samples (1 : 9) in a diluent containing 5 g l^{-1} NH_3, 0.5 g l^{-1} Triton-X, 0.5 g l^{-1} EDTA and 6 ml l^{-1} butan-1-ol, they were directly introduced by pneumatic nebulization into the plasma. Excellent performances were achieved in reducing the polyatomic interferences on Cd, Se, and Pb isotopes through charge-transfer mechanism by the use of both butan-1-ol in the sample and DRC technology operated with methane as reaction gas. In the same circumstances, the results were only reasonably good for Zn and Cu. Limits of detection in undiluted whole blood sample were in the range of 1.5 ng l^{-1} (for Cd and Pb) to 150 ng l^{-1} (for Zn). The recovery degrees compared to the certified contents for a 95 % confidence level were: 105 ± 5 % (Cd); 102 ± 5 % (Se); 97 ± 6 % (Pb); 100 ± 11 % (Zn) and 106 ± 23 % (Cu).

(Received January 30, 2008; accepted February 7, 2008)

Keywords: Mass spectrometry, Dynamic reaction cell, Toxic and essential elements, Whole blood, Certified materials

1. Introduction

Determination of trace elements in biological fluids, such as blood and urine, is performed in order to distinguish both concentrations of essential and toxic elements. The heavy metals known to have a toxic effect on the human body, such as Cd and Pb, often occur in blood and urine. Cadmium and lead can enter the body via contaminated water, food, air and as well as a result of occupational exposure. The cigarettes also contain elevated Cd levels. After ingestion, cadmium and lead are predominantly associated with hemoglobin, and for this reason they should be determined from the whole blood. In Europe, the maximum allowable concentration level for Pb in whole blood is 600 ng ml^{-1}. Increased concentration of lead is responsible for high blood pressure, kidney damage, and anemia [1]. Cadmium was classified as a carcinogenic element, and when a chronic exposure occurs it accumulates in kidney and liver and is responsible for brittle bones [2]. Therefore, biomonitoring of toxic and carcinogenic elements in biological fluids and tissues has been used as indicator for various diseases [3,4] and occupational or environmental exposure of humans [5-9]. Essential trace elements such as copper, zinc, and selenium occur in blood and urine and their monitoring is useful for certain diseases and nutritional studies [10-12]. Copper is incorporated into ceruloplasmin, and lower biliary excretion leads to its accumulation in liver and cirrhosis. On the other hand, its deficiency leads to inefficient metabolism of Fe and proteins. Zinc is an important factor involved in protein synthesis, reproduction, bone formation, and growth [13]. Lower Zn levels are responsible for a deficient immunological and reproductive function, while Zn in higher concentrations is genotoxic and carcinogenic [14]. Although Se is an essential trace nutritional element, it has an antagonistic influence in healthy humans. At a dietary intake higher than 800 mg day^{-1} the Se compounds are toxic and responsible for skin and nails diseases. The Se content in whole blood reflects the nutritional intake level and is useful as an indicator of its depletion. Selenium deficiency (below 12 μg day^{-1}) is associated with cancer and Keshan disease (a fatal cardiomyopathy). The last one can be eliminated by supplementation with sodium selenite [15]. Selenoprotein enzymes, present mainly in the erythrocytes, protect membranes from damage caused by lipid peroxidation. Therefore, the selenium ingested with supplemental nutrients decreases the incidence of prostate cancer at a dietary intake above 750 μg day^{-1} [16,17]. Also, Se increases the immune function and decreases the toxicity of transition metals. Therefore, studies regarding Se ability to inhibit HIV [18] and decrease the toxicity of Cd and Hg [19] have been performed. While selenium is present in the erythrocytes, its accurate determination consists in whole blood analysis.

The trace essential and toxic elements in the biological fluids have been determined by the use of very sensitive spectral methods, such as graphite furnace atomic absorption spectrometry (GFAAS) [8,9,20-23] and inductively coupled plasma mass spectrometry (ICP-MS) [3 - 7, 24 - 32]. A very recent review about atomic and mass spectrometry and trends in clinical analyses was published by Parsons and

Barbosa [33]. The most attractive figures of merit for ICP-MS in the analysis of clinical samples are especially the high power detection and the multi-element determination capability. The polyatomic interferences in the quadrupole ICP-MS which can result from sample matrix or argon adducts have been overcome by the use of high resolution of sector field-inductively coupled plasma mass spectrometry (SF-ICP-MS) [3,4,25,26,31] or the use of the Collision Reaction Cell quadrupole ICP-MS (CRC-ICP-MS) [6,24,27,31] or Dynamic Reaction Cell quadrupole ICP-MS (DRC-ICP-MS) [28,30]. The CRC-ICP-MS and DRC-ICP-MS are capable of measuring a wide range of element in a single analysis after a simple dilution of the clinical samples.

Selenium can be determined by ICP-MS by the use of two methods: hydride generation coupled to ICP-MS (HG-ICP-MS) and direct determination from serum or whole blood. Although Se has six isotopes, only $^{82}Se^{+}$ that has a low abundance (8.7%) can be determined by quadrupole ICP-MS without polyatomic interferences [34], but unfortunately with a low sensitivity. However, when HG-ICP-MS is used at the most abundant isotopes $^{80}Se^{+}$ (49.6%) and $^{78}Se^{+}$ (23.8 %), which the highest sensitivity can be obtained for, Se determination is not possible because these isotopes have polyatomic interferences with $^{40}Ar^{40}Ar^{+}$ and $^{40}Ar^{38}Ar^{+}$, resulted in the plasma. Moreover, in the hydride generation method the sample digestion is necessary, which is both time consuming and represents a possible contamination source. An accurate determination of Se is possible only by the use of SF-ICP-MS or charge-transfer mechanism of the argon adducts from plasma into carbides or nitrides when a quadrupole is used. With the last method, Se can be directly determined from the clinical sample by a simple dilution with different alcohols (methanol, ethanol or butan-1-ol) [35-37].

It was the aim of this paper to develop a fast and a very sensitive method for a simultaneous determination of Cd, Pb, Cu, Zn, and Se for isotopes with the highest abundance from whole blood. The method is based on a simple dilution of the whole blood with a diluent that contains butan-1-ol as chemical reagent in the Ar plasma, while applying the DRC technology in order to reduce the polyatomic interferences of the analyte isotopes with the Ar adducts. The fast, simple, and reliable method was in-house validated by analysis of the certified reference materials (CRMs) of whole blood. The figures of merit such as reduction of polyatomic interferences, limits of detection, and repeatability are also given.

2. Experimental

2.1. Instrumentation

A Perkin-Elmer SCIEX ICP-MS (model ELAN DRC II, Toronto, Canada) was used for the method development and applications. Parameter settings for ICP-MS operating are summarized in Table 1. The sample introduction system was a Meinhardt concentric nebulizer and a cyclonic desolation chamber made of quartz. The nebulizer gas flow, the lens, and the detector voltage were optimized for an approximate sensitivity of 1,000, 30,000, 50,000, and 40,000 pulses per second for $^{9}Be^{+}$, $^{59}Co^{+}$, $^{115}In^{+}$, $^{208}Pb^{+}$ respectively, and a concentration of 1 μg l^{-1} for each element.

Table 1. Parameter settings for DRC II ICP-MS.

Parameter	Value
Plasma	
Power / W	1250
Plasma gas flow / l min^{-1}	15.00
Auxiliary gas flow / l min^{-1}	1.10
Nebuliser gas flow / l min^{-1}	0.96
Sample uptake rate / ml min^{-1}	0.4
Quadrupole	
Quadruple rod offset (QRO) / V	0.00
Cell rod offset (CRO) / V	- 8.00
Cell path voltage (CPV) / V	- 20.00
Measurement mode	Peak hopping
Dwell time / ms	50
Integration time / ms	1000
Reading per point	20
Reading per replicate	1
Replicate measurements	3
DRC	
Reaction Gas	Methane
DRC mode QRO / V	- 6.00
DRC mode CRO / V	- 1.00
DRC mode CPV / V	- 15.00
Lens voltage start / V	4.00
Lens voltage end / V	11.00

Under such optimum conditions, the formation levels of the oxide ions ($^{140}Ce^{16}O^{+}/^{140}Ce^{+}$) and of the double charge ions ($^{138}Ba^{++}$) were found to be 1.4% and 3.4% respectively, for a solution with 10 μg l^{-1} of each element. The DRC parameters, Rpq (a coefficient derived from Mathieu equations, for trajectory stability, in quadrupole) and reaction gas flow (methane) were optimized before analysis, for lowest detection limit, for each isotope. The matrix effects of the easily ionized elements contained in the whole blood samples at high concentration on the plasma were compensated by the use of standard addition method. Data were acquired after a sample nebulization period of 120 s, which helps stabilizing the plasma. Additionally, in order to avoid the memory effect, the sample introduction system was rinsed with the diluent solution and Milli-Q water for 120 s between the samples. In these conditions, the relative standard deviation (RSD) of the background was below 8%,

for a background signal below 1.2 and 3.2 pulses per second at 220 and 8.5 m/z ratio.

2.2. Reagents, stock solutions, and whole blood certified reference materials

18 MΩ cm^{-1} Milli-Q DI water prepared in the laboratory using a Milli-Q system (Millipore, Watford, Hertfordshire, UK) and high purity grade reagents (AristaR or Specroscol): 25% NH_3 solution, EDTA, Triton-X, butan-1-ol and an ultra pure 16 mol l^{-1} HNO_3 solution (Merck, Darmstadt, Germany) for ICP-MS were used throughout this study. A certified multi-element solution by Merck for ICP-MS determination containing 10 mg l^{-1} Cd, Cu, Pb and 100 mg l^{-1} Se and Zn was used in order to prepare a stock by ten folds dilution with Milli-Q DI water. A diluent solution containing 5 g l^{-1} NH_3, 0.5 g l^{-1} Triton-X, 0.5 g l^{-1} EDTA and 6 ml l^{-1} butan-1-ol according to Perkin-Elmer procedure was prepared by dilution with Milli-Q DI water. A blank solution without butan-1-ol was also prepared in order to determine its influence on the polyatomic interferences and sensitivity. Control materials SERONORM 201605 Trace elements Whole Blood L1 lot nr. MR4206, SERONORM 201605 Trace elements Whole Blood L2 lot nr. 0503109, SERONORM 102405 Trace elements Whole Blood L3 lot nr. 0512627, SERONORM 201505 Trace elements Whole Blood L3 lot nr. OK0337 (SERO AS Norwegian), purchased from Promochem, Wesel, Germany, were used for quality assurance. The solutions were directly prepared in 10 ml polypropylene auto-sampler tubes. Pipettes with adjustable volumes (5 – 1000 µl and 1 – 5 ml, Eppendorf, Hamburg, Germany) were used as devices for dilution. The polypropylene tubes and the pipette tips were cleaned with a 10 % (v/v) HNO_3 solution for 24 h and further rinsed with Milli-Q DI water. After being dried in a clean air flow, all the devices were stored in closed polyethylene vessels. Argon 5.0 quality and methane 4.5 quality (LINDE Gaz – Cluj-Napoca, Romania) were used as plasma support and reaction gas in the DRC, respectively.

2.3. Sample preparation and analytical protocol

The control materials were prepared prior to analysis according to the manufacturer's recommendation. The samples were brought to room temperature for 30 min and then diluted with 5 ml of Milli-Q DI water. For calibration the standard addition procedure was performed by a simple ten-fold dilution with diluent solution prepared as above.

One ml aliquot volumes taken from freshly prepared control materials were fortified with 100, 200, and 500 µl of multi-element stock solution. All the solutions were filled up to 10 ml with the diluent solution. In the three calibration solutions the added concentrations for Cd, Cu, and Pb were: 10, 20, and 50 µg l^{-1} for each element. The added concentrations for the Se and Zn were 100, 200, and 500 µg l^{-1}. The samples were prepared as follows: an aliquot volume of 1 ml taken from the prepared control materials was diluted to 10 ml with the diluent solution. For background correction, 1 ml of DI water filled up to 10 ml with the diluent solution was used as blank. All the solutions were stored refrigerated at 4 °C until analysis.

3. Results and discussions

3.1. Isotopes and polyatomic interferences in ICP-MS

Polyatomic interferences in ICP-MS caused by the formation of polyatomic species in the plasma having numerous sources such as the sample matrix, reagents used for sample preparation, argon adducts themselves and in combination with other ions from the sample matrix or entrained atmospheric gases are well known. Polyatomic interferences cause systematic errors in the determination of elements by ICP-MS in biological samples that contain high amounts of Na, Ca, Mg, P, C etc. The selected isotopes for the determination of elements in the blood samples and the most prominent interfering ions are illustrated in Table 2 [38]. As shown in Table 2, the isotopes with the highest abundance, for which the highest sensitivity is achieved, can not be used as analytical species when a quadruple mass analyzer with a low resolution is used. Although the oxidation for Pt is less possible, the lead isotopes could have polyatomic interferences with the oxide ions of Pt. For this reason, the presence of these possible interferences as well as the opportunity for the use of the DRC technology will be studied. The mass spectra of the blank from m/z 70 to 90 recorded with the aid of the DRC II ICP-MS with and without the reaction cell using methane as reaction gas in the presence and the absence of butan-1-ol are presented in Fig. 1a-d. The mass spectrum for Se (10 µg l^{-1}) recorded with DRC II ICP-MS in the same m/z ratio in the presence and absence of butan-1-ol are also given in Figure 1 e,f. It can be seen from the background spectrum recorded in the standard operation without the reaction cell and in the absence of butan-1-ol (Fig. 1a) that the selenium can not be determined using a quadrupole ICP-MS, because all of the Se isotopes excepting the $^{82}Se^{+}$ are seriously affected by polyatomic interferences. Although the Se determination is possible at the ^{82}Se, this isotope provides a low sensitivity because its abundance is low (8.7 %) compared to the ^{78}Se and ^{80}Se isotopes. The polyatomic interferences on the analyte isotopes decrease, when butan-1-ol is added in the diluent solution, as a result of the charge-transfer mechanism in the argon plasma through which the concentration of polyatomic Ar species ions decreased (Fig. 1b). However, the polyatomic interferences are not completely eliminated and the determination of Se is inaccurate, as shown by this mass spectrum. The polyatomic interferences are almost completely eliminated when the DRC and methane is used as reaction gas, as the background spectrum was cleaned of argon adducts polyatomic species (Figure 1c,d). Also, the signal for the Se isotopes is higher in the presence of butan-1-ol, and hence its presence in the sample leads both to an

improved sensitivity for Se and a reduction of the matrix effects, respectively, when the determination is carried out from the whole blood by simple dilution (Fig. 1e,f). For this reason the presence of butan-1-ol in the diluent solution is useful, but the use of the DRC system is absolutely required.

3.2. Limits of detection

The operation of the DRC was performed on the basis of the lowest detection limits criteria. Therefore, the methane flow rate and the RPq parameter were optimized for each isotope so that the lowest detection limits were achieved. The dependence between the limits of detection for $^{78}Se^{+}$ and $^{80}Se^{+}$ isotopes versus the methane flow rate and

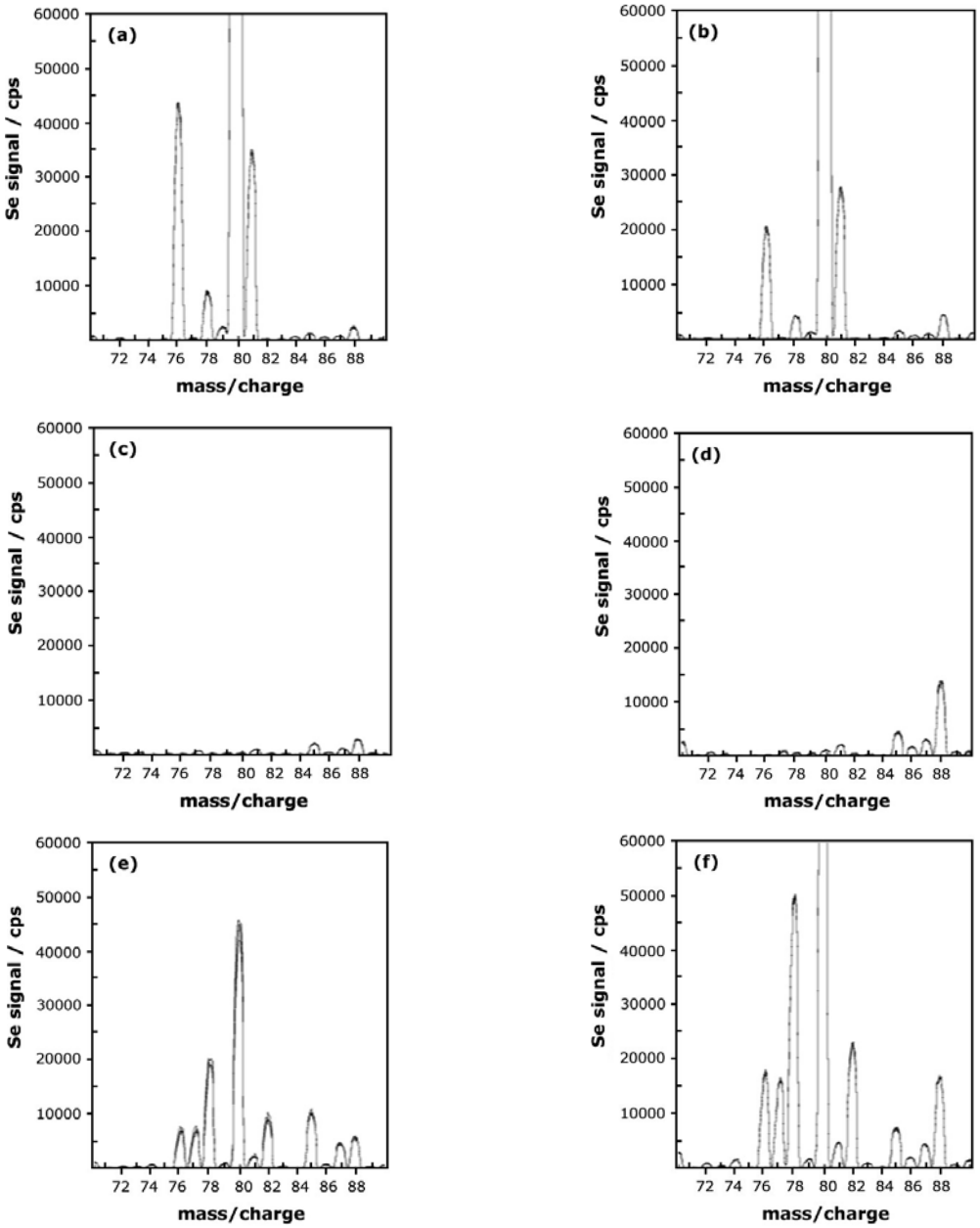

Fig. 1. Polyatomic interferences on selenium determination by ICP-MS. a. background spectrum in the absence of butan-1-ol and standard operation mode of ICP-MS without DRC system; b. background spectrum in the presence of butan-1-ol and standard operation mode of ICP-MS without DRC system; c. background spectrum in the absence of butan-1-ol and DRC mode using methane as reaction gas; d. background spectrum in the presence of butan-1-ol and DRC mode using methane as reaction gas; e. mass spectrum for 10 μg l^{-1} selenium in the absence of butan-1-ol using the DRC operation mode; f. mass spectrum for 10 μg l^{-1} selenium in the presence of butan-1-ol using the DRC operating mode.

Table 2. Polyatomic interferences on Cd, Cu, Pb, Se and Zn isotopes in ICP-MS [38].

Isotope	Abundance / %	Prominent interference
^{110}Cd	12.5	$^{39}K_2{}^{16}O^+$
^{111}Cd	12.8	$^{39}K_2{}^{16}O_2{}^1H^+$
^{112}Cd	24.1	$^{40}Ca_2{}^{16}O_2{}^+$, $^{40}Ar_2{}^{16}O_2{}^+$
^{113}Cd	12.22	$^{40}Ca_2{}^{16}O_2{}^1H^+$, $^{40}Ar_2{}^{16}O_2{}^1H^+$
^{114}Cd	28.7	$^{98}Mo^{16}O^+$, $^{98}Ru^{16}O^+$
^{63}Cu	69.1	$^{31}P^{16}O_2{}^+$, $^{40}Ar^{23}Na^+$, $^{23}Na^{40}Ca^+$, $^{46}Ca^{16}O^1H^+$, $^{36}Ar^{12}C^{14}N^1H^+$, $^{14}N^{12}C^{37}Cl^+$, $^{16}O^{12}C^{35}Cl^+$
^{65}Cu	30.9	$^{32}S^{16}O_2{}^1H^+$, $^{40}Ar^{25}Mg^+$, $^{46}Ca^{18}O^1H^+$, $^{36}Ar^{14}N_2{}^1H^+$, $^{32}S^{33}S^+$, $^{32}S^{16}O^{17}O^+$, $^{33}S^{16}O_2{}^+$, $^{12}C^{16}O^{37}Cl^+$, $^{12}C^{18}O^{35}Cl^+$
^{206}Pb	24.1	$^{190}Pt^{16}O^+$
^{207}Pb	22.1	$^{191}Ir^{16}O^+$
^{208}Pb	52.4	$^{192}Pt^{16}O^+$
^{78}Se	23.52	$^{40}Ar^{38}Ar^+$, $^{38}Ar^{40}Ca^+$
^{80}Se	49.82	$^{40}Ar_2{}^+$, $^{32}S^{16}O_3{}^+$
^{82}Se	9.19	$^{12}C^{35}Cl_2{}^+$, $^{34}S^{16}O_3{}^+$, $^{40}Ar_2{}^1H_2{}^+$
^{64}Zn	48.89	$^{32}S^{16}O_2{}^+$, $^{31}P^{16}O_2{}^1H^+$, $^{48}Ca_2{}^{16}O^+$, $^{31}P^{17}O^{16}O^+$, $^{34}S^{16}O_2{}^+$, $^{36}Ar^{14}N_2{}^+$
^{66}Zn	27.81	$^{50}Ti^{16}O^+$, $^{34}S^{16}O_2{}^+$, $^{33}S^{16}O_2{}^1H^+$, $^{32}S^{16}O^{18}O^+$, $^{32}S^{17}O_2{}^+$, $^{33}S^{16}O^{17}O^+$, $^{32}S^{34}S^+$, $^{33}S_2{}^+$
^{68}Zn	18.57	$^{36}S^{16}O_2{}^+$, $^{34}S^{16}O^{18}O^+$, $^{40}Ar^{14}N_2{}^+$, $^{35}Cl^{16}O^{18}O^+$, $^{34}S_2{}^+$, $^{36}Ar^{32}S^+$, $^{34}S^{17}O_2{}^+$, $^{33}S^{17}O^{18}O^+$, $^{32}S^{18}O_2{}^+$, $^{32}S^{36}S^+$

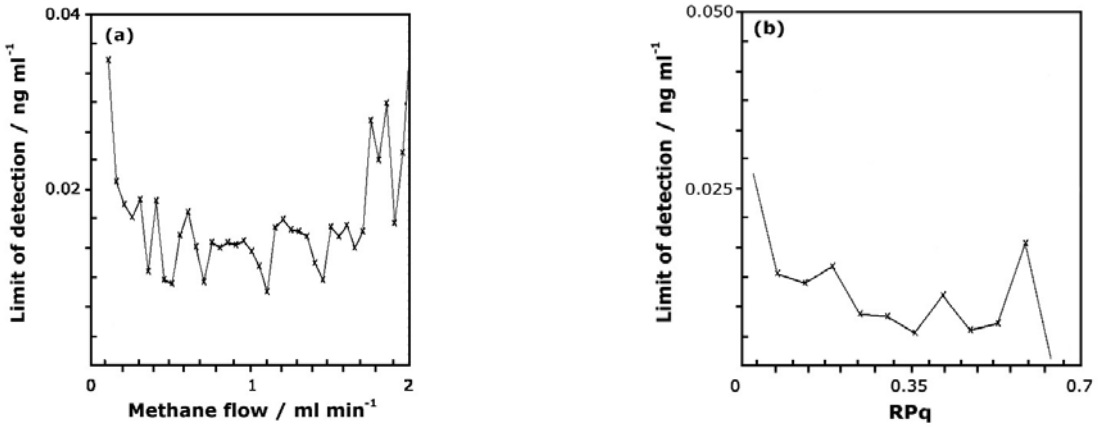

Fig. 2. Influence of methane flow rate (a) and RPq (b) on the limit of detection for $^{78}Se^+$ in the SERONORM CRM level 2 (10-fold diluted) in the DRC-ICP-MS mode.

RPq parameter obtained for the Se determination in the CRM of SERONORM whole blood (10-fold diluted) are presented in the Fig. 2a,b and Fig. 3a,b.

The improvement of the detection limits for these isotopes is evident as a result of the decreasing background when the methane flow rate and the RPq parameter were increased. Also, the signal for each isotope reaches the maximum level for the lowest detection limits. The same dependence was emphasized for the other isotopes. The limits of detection LOD (3σ criteria) in the optimum operating conditions (methane flow rate and RPq parameter) for each isotope calculated in the undiluted whole blood samples are specified in Table 3. The limits of quantification LOQ (five times LOD) are also presented.

As shown in Table 3, the limits of detection calculated to the undiluted whole blood are in the range 1.5 ng l^{-1} for Cd and Pb at $^{114}Cd^+$ and $^{208}Pb^+$, respectively to 150 ng l^{-1} for Zn at $^{68}Zn^+$. Therefore the elements characterized by toxic effects on the health humans can be accurately determined with a relative standard deviation (RSD) below 10 % at a concentration of around 10 ng l^{-1} Cd and Pb, significantly below the normal levels in whole blood of healthy humans. The limits of quantification for the studied elements are mainly limited by contamination during the sample preparation and analysis. Consequently, a careful control of impurities in all reagents and washing of the sample is necessary.

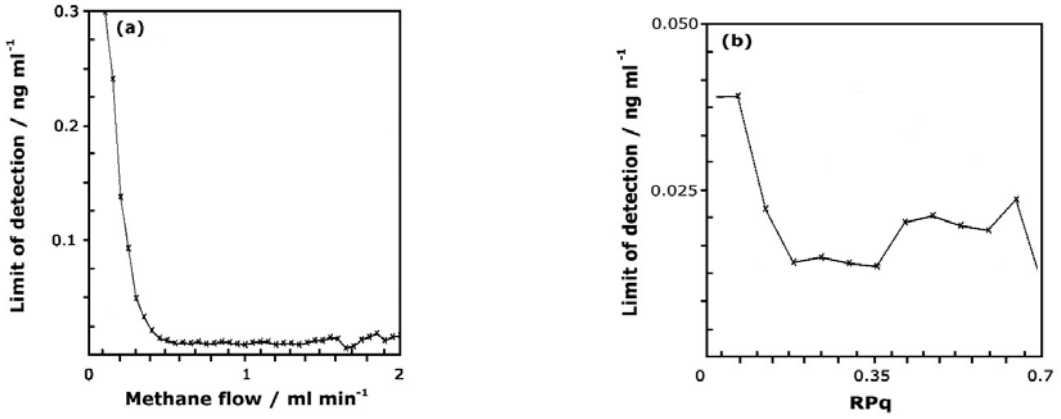

Fig. 3. Influence of methane flow rate (a) and RPq (b) on the limit of detection for $^{80}Se^{+}$ in the SERONORM CRM 10-fold diluted in the DRC-ICP-MS mode.

Table 3. Limits of detection (3σ criteria) ng l^{-1} and limits of quantification (LOQ) calculated in the undiluted whole blood samples and optimum operating conditions for DRC.

Isotope	Abundance / %	Operating conditions for DRC		LOD	LOQ
		Methane ml min^{-1}	RPq		
^{110}Cd	12.5	1.95	0.55	2.5	12.5
^{111}Cd	12.8	1.50	0.55	2.5	12.5
^{112}Cd	24.1	1.70	0.40	1.8	9
^{114}Cd	28.7	1.70	0.30	1.5	7.5
^{63}Cu	69.1	1.20	0.45	35	175
^{65}Cu	30.9	1.20	0.55	55	275
^{206}Pb	24.1	2.25	0.60	4	20
^{207}Pb	22.1	2.25	0.65	4	20
^{208}Pb	52.4	2.25	0.65	1.5	7.5
^{78}Se	23.52	1.20	0.50	18	80
^{80}Se	49.82	0.80	0.50	15	75
^{82}Se	9.19	1.20	0.50	80	400
^{64}Zn	48.89	1.50	0.65	110	550
^{68}Zn	18.57	1.50	0.50	150	750

The samples of whole blood were 1 : 9 diluted, in order to prevent the clogging of the concentric micronebulizer, the injector tube and the skimmer cones, while the sample introduction system was pre washed between samples with diluent and then Milli-Q water. In these conditions the memory effects and the samples contamination were avoided and the cleaning of the plasma torch and skimmer cones was not daily necessary.

3.3. Repeatability

The relative standard deviation of repeatability (s_r) was investigated by successive measuring (n = 10) of the mass signal for each isotope for a concentration of 5 μg l^{-1} for all elements. The values of s_r were between 0.5 – 3.2 %, that means a limit of repeatability (2.8 s_r) for a 95 % confidence level between 1.4 – 9.0 %.

3.4. Internal quality control

Precision and accuracy in internal quality control were assessed by analysis of four whole blood CRMs with different contents of metals. The average concentrations and the expanded uncertainty (U) for 95 % confidence level were calculated using the results achieved by different persons on 10 sub samples delivered by the supplier for each CRM. The found results compared to the certified ones are given in Table 4.

The data in Table 4 show a very good precision for Cd (1.6 – 4.3 %), Pb (3.8 – 5.7 %), and Se (2.1 – 3.0 %). A satisfactory precision was achieved for Zn (6.9 – 8.4 %), but smaller for Cu (1.8 – 14.3 %). A very good agreement was achieved between found and certified values for Cd, Pb, and Se.

Table 4. Comparison of found (n=10), certified and expanded uncertainty results (U) for 95% confidence level (k = 2) for the analysis of certified reference materials of whole blood obtained by DRC-ICP-MS.

Isotope	SERONORM 201605 L1		SERONORM 201605 L2		SERONORM 102405 L3		SERONORM 201505 L3	
	Found ± U	Certified ± U	Found ± U	Certified ± U	Found ± U	Certified ± U	Found ± U	Certified ± U
^{110}Cd	0.85 ± 0.16	0.74 ± 0.04 in ICP-MS 0.70 ± 0.3 in GFAAS	5.9 ± 0.4	6.0 ± 0.4 in ICP-MS 5.1 ± 2.3 in GFAAS	12.2 ± 0.5	10.8 ± 0.5 in ICP-MS		11.3 ± 0.6 in ICP-MS 11.8 ± 0.8 in GFAAS
^{111}Cd	0.82 ± 0.18		5.7 ± 0.2		11.3 ± 0.4		11.9 ± 0.3	
^{114}Cd	0.75 ± 0.18		6.3 ± 0.4		11.4 ± 0.4		11.9 ± 0.4	
Average	**0.81 ± 0.18**		**6.0 ± 0.3**		**11.6 ± 0.4**		**12.0 ± 0.4**	
^{63}Cu	560 ± 76	564 ± 33 in ICP-MS	649 ± 25	666 ± 29 in ICP-MS 623 ± 21 in ICP-OES	2480 ± 725	1740 ± 151 in ICP-MS 1741 ± 148 in ICP-OES	599 ± 65	703 ± 30
^{65}Cu	559 ± 68		636 ± 22		2364 ± 660		599 ± 70	
Average	**560 ± 72**		**643 ± 24**		**2422 ± 693**		**599 ± 68**	
^{206}Pb	27.7 ± 3.3	27.6 ± 1.4 in ICP-MS 21 ± 4 in GFAAS	396 ± 22	393 ± 21 in ICP-MS 396 ± 100 in GFAAS	543 ± 45	503 ± 19 in ICP-MS	487 ± 45	641 ± 35 in ICP-MS 554 ± 22 in GFAAS
^{207}Pb	27.8 ± 3.0		390 ± 30		447 ± 36		484 ± 40	
^{208}Pb	28.9 ± 3.3		401 ± 35		480 ± 29		505 ± 56	
Average	**28.1 ± 3.2**		**396 ± 33**		**490 ± 37**		**492 ± 48**	
^{78}Se	82.9 ± 5.4	79.8 ± 5.4 in ICP-MS	121 ± 7	123 ± 10 in ICP-MS	164 ± 7	146 ± 10 in ICP-MS	170 ± 7	177 ± 11 in ICP-MS 160 ± 9 in GFAAS
^{80}Se	82.9 ± 3.6		122 ± 4		164 ± 10		176 ± 6	
^{82}Se	76.0 ± 5.5		115 ± 3		149 ± 8		152 ± 10	
Average	**80.6 ± 4.9**		**119 ± 5**		**159 ± 8**		**166 ± 8**	
^{64}Zn	5460 ± 460	5500 ± 300 in ICP-MS	4834 ± 422	5038 ± 369 in ICP-MS	9581 ± 860	8157 ± 372 in ICP-MS 8032 ± 512 in ICP-OES	4444 ± 305	4766 ± 282
^{68}Zn	5334 ± 260		5050 ± 380		9024 ± 684		3889 ± 390	
Average	**5397 ± 374**		**4942 ± 402**		**9303 ± 777**		**4167 ± 350**	

Therefore, the recovery degrees compared to the certified contents for a 95 % confidence level were: 105 ± 5 % (Cd); 102 ± 5 % (Se); 97 ± 6 % (Pb). The good results for these trace elements are supported by the fact that the DRC system operated with methane as reaction gas is very efficient for the decreasing of the polyatomic interferences in ICP-MS for $^{111}Cd^+$, $^{114}Cd^+$, $^{206}Pb^+$, $^{207}Pb^+$, $^{208}Pb^+$, $^{78}Se^+$ and $^{80}Se^+$ isotopes. The results were unsatisfactory for $^{113}Cd^+$ isotope, and for this reason it was not monitorized in this validation study. Also, the presence of butan-1-ol in the whole blood samples is a suitable reagent for the reduction of the concentration of $^{40}Ar^{40}Ar^+$ and $^{40}Ar^{38}Ar^+$ adducts direct in ICP, through charge-transfer mechanism, increasing the sensitivity and decreasing the matrix effects, respectively. Although the $^{82}Se^+$ isotope has no polyatomic interferences, it is not appropriate as an analytical species for the Se determination when DRC is used. This is mainly due to the two negative influences: a lower abundance of ^{82}Se and its possible loss in DRC. As shown in Table 4, the found results for Se are much lower for $^{82}Se^+$ isotope compared to the two other isotopes. Even if the skimmer cones are made of Pt, the operating of DRC with methane at a flow rate of 2.25 ml min^{-1} and an RPq of 0.60 – 0.65 is appropriate in order to reduce the polyatomic interferences of $^{190}Pt^{16}O^+$,$^{192}Pt^{16}O^+$ and $^{191}Ir^{16}O^+$ on all of the three Pb isotopes. Although the recovery degree was good for both Zn and Cu, their range is too large (100 ± 11 %) and (106 ± 23 %), respectively. This finding shows that the operating of DRC with methane ensures only a reasonably precise determination of Zn and Cu, as the polyatomic interferences can not be completely eliminated with methane. However, for routine determinations of Zn and Cu in whole blood, the DRC - ICP – MS operating mode with methane can be considered sufficiently valid. Ammonia is more suitable as reaction gas in DRC in order to reduce the polyatomic interferences on the $^{63}Cu^+$, $^{65}Cu^+$, $^{64}Zn^+$, and $^{68}Zn^+$ compared to methane. Such a study will be further developed in our laboratory.

4. Conclusions

A fast and very sensitive method for the determination of Cd, Cu, Pb, Se and Zn by DRC-ICP-MS after a simple dilution of the whole blood samples was in-house validated. The combined method for reducing the polyatomic interferences of the Ar adducts and sample matrix based on charge-transfer mechanism by the use of both butan-1-ol in the sample and methane as reaction gas in DRC offers very good results in terms of precision, accuracy, repeatability, and limits of determination for Cd, Pb, and Se. From the precision perspective, the results were only reasonably good for Zn and Cu, as methane is not a very suitable reaction gas in DRC technology, for reducing the polyatomic interferences for these elements. However, the approach of this fast and simple method is useful for a reliable determination of all the studied elements in both routine laboratories and toxicological studies. The method will be subsequently used by us in order to assess a possible relation between the contents of these elements in the whole blood and the prostate cancer risk.

Acknowledgements

The authors are grateful for the financial support provided by the Romanian Ministry of Education and Research – CEEX- project **Promarkdiater** no. 63/2005.

References

[1] Second National Report on Human Exposure to Environment Chemicals, National Center for Environmental Health Division of Laboratory Sciences, Atlanta, GA, 2003.
[2] H. M. Crews, Spectrochim. Acta **53B**, 213 (1998).
[3] G. Forte, B. Bocca, O. Senofonte, F. Petrucci, L. Brusa, P. Stanzione, S. Zannino, N. Violante, A. Alimonti, G. Sancesario, J. Neur. Transmis. **111**, 1031 (2004).
[4] B. Bocca, A. Alimonti, F. Petrucci, N. Violante, G. Sancesario, G. Forte, O. Senofonte, Spectrochim. Acta **59B**, 559 (2004).
[5] E. Rainska, M. Biziuk, B. Jaremin, P. Glombiowski, P. Fodor, L. Bielawski, Intern. J. Environ. Health. Res. **17**, 113 (2007).
[6] P. Heitland, D. H. Koster, J. Trace Elem. in Med. and Biol. **20**, 253 (2006).
[7] A. Schutz, M. Olsson, A. Jensen, L. Gerhadsson, J. Borjesson, S. Mattsson, S. Skerfving, Intern. Arch. Occup. Environ. Health **78**, 35 (2005).
[8] M. Bulbul, P. Unak, R. A. Sisman, C. Coker, Frsenius Environ. Bull. **15**, 1477 (2006).
[9] I. H. Afridi, G. T. Kazi, K. M. Jamali, H. G. Kazi, B. M. Arain, S. Jalbani, Q. G. Shar, A. R. Sarfaraz, Toxicol. and Industr. Health **22**, 381 (2006).
[10] S. M. Awadallah, K. H. Abu-Elteen, A. Z. Elkarni, S. H. Qaraein, N. M. Salem, M. S. Mubarak, J. Trace Elem. Exp. Med **17**, 1 (2004).
[11] J. Virtamo, J. K. Huttunen, Trace Elements in Health and Disease, The Royal Society of Chemistry, Cambridge, 127 (1991).
[12] R. Cornelis, J. De Kimpe, X. Zhang, Spectrochim. Acta, **53B**, 187 (1998).
[13] G. D. Barcelaux, J. Toxicol. Clin. Toxicol. **37**, 279 (1999).
[14] C. T. Walsh, H. H. Sandstead, S. A. Prasad, M. P. Newberne, J. P. Fraker, Environ. Health Perspect., **102**, 5 (1994).
[15] J. Neve, F. Vertongen, L. Moll, Clin. Endocrinol. Metab. **14**, 629 (1985).
[16] Y. Yang, C. Zhou, J. Trace Elem. Electrolytes Health Dis. **3**, 129 (1989).
[17] L. DeFrancesco, M. Cimons, Nat. Med. **7**, 1076 (2001).
[18] N. Terry, A. M Zayed, M. P. DeSouza, A. N. Tarun, Ann. Rev. Plant. Phys. Mol. Biol. **51**, 401 (2000).
[19] C. Sasakura, K. T. Suzuki, J. Inorg. Biochem. **71**, 159 (1998).
[20] H. X. Chen, Y. X. Yu, Spectrosc. Snd. Spectral Anal.,

25, 477 (2005).
[21] M. Tuzen, Trace Elem. And Electrol. **19**, 202 (2002).
[22] A. Viksna, E. L. Selin, I. Kjellmer, J. Bursa, J. Trace and Microprobe Techn. **20**, 553 (2002).
[23] E. C. Berger, R. Kluger, R. Baron, L. Steffan, A. Engel, J. Trace Elem. in Exp. Med. **15**, 201 (2002).
[24] P Heitland, H. D. Koster, J. Anal. At. Spectrom. **19**, 1552 (2004).
[25] I. Rodushkin, F. Odman, R. Olofsson, M. D. Axelsson, J. Anal. At. Spectrom. **15**, 937 (2000).
[26] I. Rodushkin, F. Odman, J. Trace Elem. Med. Biol. **14**, 241 (2001).
[27] J. S. Christopher, D. R. Day, C. E. Bryan, C. G. Turk, J. Anal. At. Spectrom. **20**, 1035 (2005).
[28] C. Bonnefoy, A. Menudier, C. Moesch, G. Lachatre, M. J. Mermet, Anal. and Bioanal. Chem. **383**, 167 (2005).
[29] P-J. Goulle, L. Mahieu, J. Castermant, N. Neveu, L. Bonneau, G. Laine, D. Bouige, C. Lacroix, Forensic Sci. Intern. **153**, 39 (2005).
[30] D. E. Nixon, R. K. Neubauer, J. S. Eckdahl, A. J. Butz, F. M. Burritt, Spectrochim. Acta, **59B**, 1377 (2004).
[31] A. Sarmiento-Gonzales, M. J. Marchante-Gayon, M. J. Tejerina-Lobo, J. Paz-Jimenez, A. Sanz-Medel, Anal. and Bioanal. Chem. **382**, 1001 (2005).
[32] P. Heitland, H. D. Koster, Anal. Clin. Acta **365**, 310 (2006).
[33] J. P. Parsons, F. Barbosa Jr., Spectrochim. Acta, **62B**, 992 (2007).
[34] U. Tingi, T. Ginduzzo, R. Francis, D. Nicol, M. Shahin, P. Scheelings, J. Radioanal. Nucl. Chem. **259**, 469 (2004).
[35] C. E. Sieniawaska, R. Mesikov, T. Delves, J. Anal. At. Spectrom. **14**, 109 (1999).
[36] J. Goossens, F. Vanhacke, L. Moens, R. Dams, Anal. Chim. Acta, **280**, 137 (1993).
[37] R. M. Olivas, C. R. Quetel, O. F. X. Donard, J. Anal. At. Spectrom. **10**, 865 (1995).
[38] T. W. May, R.H. Wiedmeyer, At. Spectrosc. **19**, 150 (1998).

*Corresponding author: icia@icia.ro