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Sample preparation and calibration optimization for ICP-MS analysis of copper, zinc, selenium, rubidium, strontium, magnesium, iron, molybdenum and barium in human serum

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An optimized sample preparation and calibration approach for inductively coupled plasma mass spectrometry (ICP-MS) for multielement determination of Cu, Zn, Se, Rb, Sr, Mg, Fe, Mo, Ba in human serum is proposed. Four microwave-assisted digestion methods were tested. The chosen method (0.3 mL of sample mixed with 1.5 mL of HNO_3 and 1.5 mL of water, digested for 25 min at 190°C) provides complete digestion of the serum matrix with acceptable residual carbon content. Internal Standard (IS) Rh was introduced through an online IS kit. For introduction stability, the acid content in the IS and calibration standard solutions was equalized. It was observed that the high Na content in serum sample solutions causes two opposite effects on the analyte signals: suppression of ionization efficiency into the ICP and gradual enhancement of the ion transmission. For overcoming this matrix interference all calibration solutions and blanks were prepared in 15% v/v HNO_3 with 130 mg/L Na, as a matrix-match component. The proposed method for sample preparation and calibration strategy was found adequate for human serum ICP-MS analysis, with very good recoveries of the aforementioned elements, determined in two certified reference materials, *Seronorm Trace Elements Serum Level I and Level II, SERO AS, Norway*.

Keywords: ICP-MS, multielement analysis, trace elements, human serum, matrix-matched calibration, sensitivity drift

INTRODUCTION

Simultaneous determination of multiple elements in human biological materials is of growing interest, due to the accumulated scientific evidence of their pleiotropic effects on the human body, as well as their key importance for the environmental and occupational health. Multielemental quantification of elements in several orders of concentration magnitude (mg/L, μg/L, ng/L) is a challenging task, which requires robust, reliable, high-throughput analytical methods, with wide working range. Inductively coupled plasma mass spectrometry (ICP-MS) covers these requirements, therefore in the recent years it has been increasingly used for multielemental analysis in a large variety of biological samples, such as serum [1–8], plasma [4, 9, 10], whole blood [2, 3, 5, 9–12], urine [3, 9, 13], tissues [14, 15], hair [9, 10], etc. However, ICP-MS is not free from some drawbacks, as occurrence of spectral and non-spectral interferences [4, 16].

For reducing spectral interferences, it is of great importance to select the appropriate isotope for the analyte measurement [3, 14]. One of the most common approaches for coping with spectral overlap is using a collision cell. Working in kinetic energy discrimination mode (KED) overcomes polyatomic interferences, though it compromises sensitivity [16–18].

As opposed to spectral, the nonspectral interferences are most commonly caused by matrix effects (ME) of multiplicative kind [3, 4, 19]. An internal standard (IS) approach is effective for handling ME, if the interference effects over the signals of the analyte and IS are matched [3, 4, 19]. Multiple ISs are usually preferred for multielemental analysis [3, 4, 19]. However, previous studies [20, 21] demonstrate that if a mixture of NaCl and $CaCl_2$ was added to the calibration standards, any IS [Ge, Rh, Re, Ir] could be used for any analyte in blood samples.

There are different approaches for alignment of biological matrix diversity, described in literature, such as acid digestion or alkali dilution [1, 5, 9, 10,

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14, 15]. One of the most widely recommended methods is microwave assisted acid mineralization (MW) with different acid mixtures. After MW treatment some nonspectral interferences could still be observed, such as signal suppression or enhancement, as well as signal instability or drift [4, 20, 22, 23]. Owing to the interindividual diversity of the serum samples, ME occur to a greater extent if the so-called "dilute and shoot" approach for direct sample introduction is preferred [4, 7, 24]. According to several authors [4, 25, 26] the enhancement of the analyte signals is due to the presence of concomitant elements, such as Cl and S in the samples, or to the charge transfer from C^+ ions to elements with a FIP from 9 to 11 eV. Suppression of ionization efficiency is associated with changes in ion-atom equilibrium in the ICP, or the space charge effect, taking place beyond the cones. This phenomenon is often attributed to easily ionizable elements (EIE), i.e. macroelements K, Na, Mg, Ca, typical for human serum. Elevated levels of EIE in serum samples potentially cause bias in analytical results, in particular, in case of external calibration with aqueous standards [4, 20, 23, 27, 28]. An easy to use matrix-matching approach is adding Na, or mixed salts, containing EIE to the standard solutions [4].

In the following study we aimed to propose a fast and effective acid digestion procedure for human serum, with limited sample volume, acceptable residual carbon content (RCC) and appropriate dilution factor. An optimized calibration for ICP-MS determination of Cu, Zn, Se, Rb, Sr, Mg, Fe, Mo, Ba is suggested, with online IS introduction and Na as a matrix-match component.

EXPERIMENTAL

Blood sampling

Thirty venous blood samples were collected from healthy adult human volunteers in the Central Laboratory of the University Hospital St. George in Plovdiv. Blood sampling was performed under fasting conditions, following the standard procedure for collection of blood specimens. Furthermore, the guidelines of Helsinki Declaration, regarding informed consent of human volunteers were duly met. The blood samples were let to clot for 20 min at room temperature. After centrifugation at 3000 rpm for 10 min, serum subsamples were separated and stored at -70°C until analysis. For collection and storage of the samples serum blood tubes (Kabe Labortechnik, Primavette V Serum, 2.6 mL) and cryotubes (Biosigma CL2ARBEPS CRYOGEN,1.8 mL) were used.

Chemicals and materials

All elemental standards used for preparation of the calibration standard solutions were NIST-traceable. Multielement standard (Etalon multi element ICP, 100 mg/L) was purchased from VWR Chemicals (Leuven, Belgium), ICP-MS monoelement Zn 100 mg/L, Cu 100 mg/L, Fe 100 mg/L, and Mg (CRM 6 components: Mg 60 mg/L, P 100 mg/L, Ca 300 mg/L, K 500 mg/L, Na 10000 mg/L) and NaCl (10161 mg/L) were from CPAchem Ltd. (Stara Zagora, Bulgaria). Rhodium (10 mg/L), was provided by Merck (Darmstadt, Germany). All solutions were prepared with ultra-pure water (18.2 MΩ cm) from ELGALabWater (PURELAB Chorus 2+) and nitric acid (suprapur) from Fisher Scientific UK Limited. The reference materials (Seronorm Trace Elements Serum Level I and Level II, SERO AS, Norway) were prepared according to the supplier's instructions.

External multielement aqueous standards were prepared in 15% HNO_3 at concentrations of 0.02, 0.1, 0.2, 1.0, 2.0, 4.0 and 6.0 µg/L for Se, Rb, Sr, Mo and Ba; 20.0, 40.0, 60.0, 80.0 µg/L for Cu, Zn and Fe; 0.5, 0.6 and 0.9 µg/L for Mg. A second group of calibration solutions was prepared in the same concentration ranges in 15% HNO_3, with a 130 mg/L Na content. Rh was used as IS in concentration of 2 µg/L.

All labware used for blood sampling, storage and analysis was previously tested for contamination with the elements of interest, following a procedure, previously described [29].

Digestion method

After thawing at room temperature, the serum samples were thoroughly vortexed for 10 min and immediately pipetted into a microwave digestion vessel. The MW was performed by Multiwave GO Microwave Digestion system with closed vessels (Anton Paar, Graz, Austria). Two levels of serum reference materials (Seronorm™, Norway) were digested and analyzed in four batches to test four digestion modes with variation of the sample and reagents quantity, the MW program and the dilution factor. The RCC in each digested sample was evaluated by means of MP-AES (Agilent 4500) at the spectral line 193.027 nm. Reagent blanks were prepared by addition of deionized water in the place of the sample. Mineralizates were cooled to room temperature and transferred to 15 mL polypropylene tubes. Deionized water was added to provide a final volume of 10 mL (dilution factor, DF=33.3).

Instrumentation

Multielement determination was carried out by Thermo Scientific iCAP Qc ICP-MS (Thermo Scientific, Germany). The mass spectrometer is equipped with a collision cell, working in Kinetic Energy Discrimination (KED) mode, with helium as collision gas. The iCAP Qc sample introduction system consists of a perfluoroalkoxy (PFA) nebulizer, quartz glass Peltier-cooled cyclonic spray chamber and quartz injector, nickel interface cones. The IS was added *via* a kit for online introduction (Thermo Scientific) to the sample solutions. For better precision relatively long dwell times were selected. Thermo Scientific QTegra Software was used for calculations of the analytical results. ICP-MS operating conditions are summarized in Table 1. Instrument settings for the analytes of interest and corresponding Instrumental detection limits (IDL) obtained are presented in Table 2.

Table 1. ICP-MS operating conditions

Plasma conditions	
RF-power	1550 W
Nebulizer gas flow	1.03 L min^{-1}
Auxilliary gas flow	0.80 L min^{-1}
Plasma gas flow	14.00 L min^{-1}
He gas flow	4.4 mL min^{-1}
Mass Spectrometer Settings	
Sweeps	15
Replicates	3
Survey run amu	22.39 – 245

Table 2. ICP-MS iCAP Qc instrument settings for target analytes and obtained instrumental detection limits (IDL).

Isotope	Dwell time, s	Resolution	IDL,µg/L
^{24}Mg	0.02	High	1.00
^{56}Fe	0.05	High	0.009
^{63}Cu	0.05	High	0.011
^{66}Zn	0.02	High	0.067
^{78}Se	0.2	Norm	0.022
^{85}Rb	0.05	Norm	0.004
^{88}Sr	0.05	Norm	0.013
^{95}Mo	0.1	Norm	0.001
^{137}Ba	0.5	Norm	0.007

Prior to each analytical run a performance check procedure for interferences and sensitivity levels was routinely accomplished ($^{140}Ce.^{16}O/^{140}Ce$ < 0.01; $^{59}Co/^{35}Cl.^{16}O$ > 17, ^{59}Co and ^{115}In > 30000 cps, ^{238}U > 80000 cps). All blanks, serum and QC samples were analyzed in duplicate. Continuing calibration verifications were performed after every ten serum samples. Continuing calibration blanks were inserted after calibration standards.

Table 3. Human serum acid mineralization – hot plate HCl and four microwave assisted methods (MW I-IV), final dilution factor (DF), residual carbon content (RCC, %) and internal standard shift.

	Hot HCl	MW I	MW II	MW III	MW IV
Serum, mL	0.5	0.5	0.5	0.3	0.3
HNO_3, mL	-	3.0	2.0	1.5	2.0
H_2O_2, mL	-	1.0	0	0	0
T, °C	-	180	160	190	180
DF	20	30	20	33.3	33.3
RCC, %	16	0.01	0.09	0.03	0.06
Rh IS shift	-	78 %	83 %	86 %	86%

RESULTS AND DISCUSSION

Digestion

Four methods (MW I-IV) for MW mineralization were tested to optimize digestion efficiency. Four batches of CRM samples (Seronorm Trace Elements Serum Level I and Level II) and blanks were prepared, with variable MW program temperature, DF and quantities of samples and reagents used. Every batch consisted of 2 blanks, prepared with deionized water and equal to the samples acid content, 5 serum CRM Level I and 5 serum CRM Level II. Thereafter the RCC in the digested samples was determined, as a relevant parameter for digestion efficiency. The lowest RCC was observed in the samples, digested at 180°C with 3 mL of HNO_3 and 1 mL of H_2O_2 (MW I). However, in the presence of H_2O_2 in ICP-MS measurement, the recorded blank signals for the elements of interest were significantly high. It was found inacceptable to work with the same HNO_3/H_2O_2 mixture at higher temperature, because of the risk of excessive pressure and explosion of the vessels. Digestion procedure MW III was found appropriate: 0.3 mL of sample, 1.5 mL of HNO_3 and 1.5 mL of deionized water, maximum temperature 190°C, ramp-time 15 min, hold-time 10 min. Compared to the other three programmes (MW I, II, IV) it provides complete digestion of the serum matrix, with minimum sample and acid volume, acceptable RCC and IS shift (Table 3). The results obtained from the ICP-MS analysis of the reference materials, mineralized by the aforementioned conditions, were in good agreement with the CRM values, as discussed later. The proposed method is characterized by minimized sample handling steps, requiring microvolumes of serum and only HNO_3 reagent for mineralization. The relatively high dilution factor provides improved nebulization, as well as reduced maintenance of the sample introduction system.

Optimization of calibration

IS Rh was introduced through an online IS kit to calibrators and samples. The online IS merge system is crucial for eliminating of human error, associated with manual spiking of IS to each sample, standard and blank. It was experimentally proved that the precision is worsening if aqueous IS solution merges with samples containing 15% HNO_3. Merging of streams with the same acid media, prevents bubble formation in the capillaries of the inlet system. Therefore it was found beneficial if IS is also prepared in 15% HNO_3.

The IS recovery remained relatively stable, while measuring signals in blanks and calibration solutions in 15% HNO_3, with low sodium content (0.02-6.0 µg/L). But if standards with high sodium content (80-150 mg/L) are subsequently introduced, initial 15-20% drop of all signals (IS and analytes) is observed. Then in a series of 10 consecutive samples (Fig 1A) the drop is followed by a gradual enhancement of the signals, reflecting in a 15% positive shift of the IS recovery. Obviously, the ME was not effectively compensated by a single IS and leads to mass-dependent bias of CRM results (see Table 4). As previously commented by other authors [4, 20, 23, 27, 28] the sensitivity drifts could be explained by the presence of EIE, coexisting in significant levels in serum (Na, Mg, Ca and K). This presence provokes two opposite phenomena: i) loading plasma with EIE influences ion-atom equilibrium and causes suppression of the analyte signals; ii) saturation of mass spectrometer interface and vacuum system with EIE improves ion transport, due to a protective effect of EIE ions and reduction of mass-discrimination. Both effects are displayed in Fig 1A. For overcoming these MEs all calibration solutions and blanks were prepared in 15% HNO_3 with 130 mg/L Na as EIE. The Na content in the calibrators was calculated to match the serum sample mineralizates. Applying such pseudomatrix-matching calibration overcomes the ionization suppression of the IS (Fig.1B) and nine target analytes (Table 4). Our observations confirmed the opinion [4], that addition of Na to the calibration standards provides a successful matrix-match.

In case of a large set of human serum analyses a positive drift up to 10% of the IS was observed (Fig 1B). This sensitivity drift was effectively compensated by correction with IS (^{103}Rh). Continuing calibration verifications were performed after every ten serum samples, with a very good within-run precision. Continuing calibration blanks were inserted after calibration standards with insignificant carryover. Comparison of the analytical results of Seronorm Trace Elements Serum Level I and II is presented in Table 4. The corresponding recoveries were as follows: before optimization of the calibration recoveries (R) were in the range from 98 to 131% and after optimization recoveries (R*) were in the range from 98 to 113%.

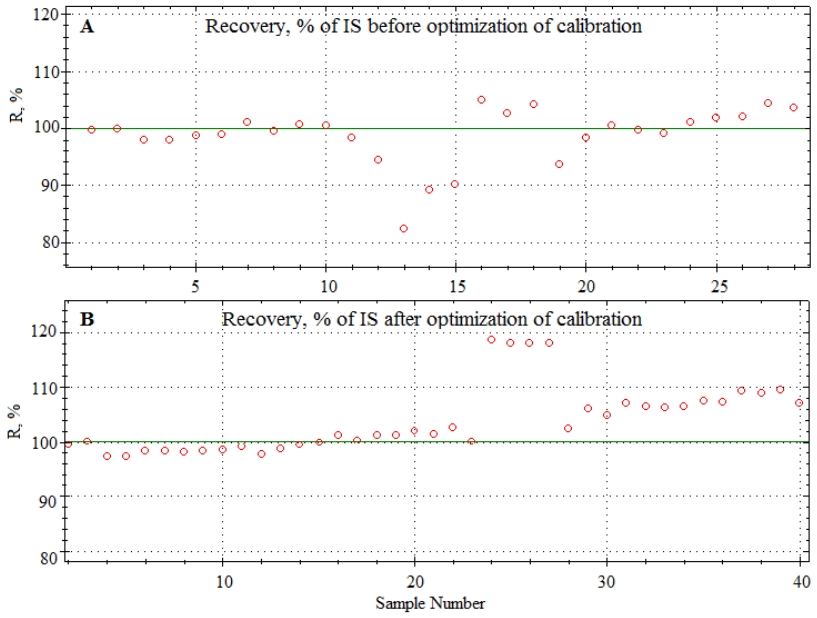

Fig. 1. Recovery of Internal standard Rh (2 µg/L) in consecutive measurements as follows: **A.** Sample numbers: (1-10) Calibration standards in 15% HNO_3 with low Na content; (11-15) Calibration standards in 15% HNO_3 with increasing Na content; (16-18) 15% HNO_3; (19-28) serum samples; **B.** Sample numbers: (2-23) Calibration standards in 15% HNO_3 with 130 mg/L Na content; (24-27) 15% HNO_3; (28-40) serum samples.

Table 4. Analytical results of certified reference materials SeronormTM -CRM Certified values (Cert/ref); ICP-MS measured values (Found), relative standard deviation (RSD, %), n=6.

	Mg, mg/mL	Fe, mg/mL	Cu, mg/mL	Zn, mg/mL	Se, µg/L	Rb, µg/L	Sr, µg/L	Mo, µg/L	Ba, µg/L
				Seronorm^{TM} Trace Elements Serum L-1					
Cert/ref	16.8	1.47	1.09	1.10	86	4.4	99	0.76	189
Found	21.1	1.85	1.34	1.35	106	4.9	117	0.82	204
RSD, %	10.7	0.20	5.0	5.7	3.6	15	5.3	13	4.6
R, %	126	126	126	128	123	111	118	108	108
Found*	17.6	1.49	1.04	1.20	87.1	5.1	99	0.80	186
RSD*, %	0.7	0.40	0.50	0.80	3.4	3.7	0.60	1.9	1.4
R*, %	105	101	98	113	101	116	100	106	98
				Seronorm^{TM} Trace Elements Serum L-2					
Cert/ref	33.9	2.15	1.93	1.53	136	8.7	110	1.21	139
Found	43.1	2.60	2.28	2.01	164	9.9	130	1.2	163
RSD, %	11.2	1.4	6.8	4.1	1.9	6.8	1.0	6.7	5.0
R, %	127	121	119	131	119	114	118	98	117
Found*	36.9	2.18	1.93	1.71	147	9.8	119	1.3	145
RSD*, %	2.0	1.6	2.8	0.70	2.2	2.4	0.90	1.7	0.70
R*, %	109	101	100	111	106	113	108	106	104

* Values obtained after applying the optimized pseudo-matrix matched calibration (see in the text).

CONCLUSIONS

The proposed approach for microwave-assisted acid mineralization provides fast and effective digestion with microvolumes of human serum required for multielement ICP-MS analysis. The following digestion method was found appropriate: 0.3 mL sample, 1.5 mL HNO_3, 1.5 mL H_2O maximum temperature 190°C, ramp-time 15 min, hold-time 10 min, DF (33.3). The proposed calibration strategy (calibrators in 15% HNO_3, 130 mg/L Na and Rh as IS in 15% HNO_3) provides a satisfactory matrix-match, assuring determinations of analytes with good analytical recoveries. We recommend this sample preparation and calibration as "fit for purpose" for ICP-MS determination of Cu, Zn, Se, Rb, Sr, Mg, Fe, Mo, Ba in human serum for medical and biomonitoring studies.

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