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Measurement of the Trace Elements Cu, Zn, Fe, and Mg and the Ultratrace Elements Cd, Co, Mn, and Pb in Limited Quantity Human Plasma and Serum Samples by Inductively Coupled Plasma-Mass Spectrometry

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ABSTRACT

In public health studies limited volumes of blood are often collected and stored for future hypothesis testing. Archived samples are irreplaceable and therefore it is valuable to develop analytical techniques that require minimal sample volume. This work describes the measurement of trace elements Mg, Cu, Fe, Zn and ultratrace elements Cd, Co, Mn, Pb in limited quantity (150 μL) human serum or plasma samples. Samples were digested using a hotblock and analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The analytical method was evaluated using a quadrupole (Q) and sector field high resolution (SF) instrument to analyze trace elements in Seronorm® quality control serum material. The method was used to analyze 1888 blinded human plasma samples which were archived for the National Cancer Institute from the Nutrition Intervention Trial in Linxian China. The inductively coupled plasma method was capable of accurately analyzed limited quantity samples of human serum and plasma for the trace elements Mg, Cu, Fe Zn and the ultra trace elements Co, Mn and Pb. The concentration of Cd in human plasma samples was below the level of detection for 75% of the samples analyzed.

Keywords: Inductively Coupled Plasma Mass Spectrometry; Trace Element Measurements; Human Plasma; Serum

1. Introduction

Epidemiologists and scientists interested in studying nutritional, environmental, biologic and genetic factors that influence human health often collect and process blood samples into plasma or serum for analytical testing. Serum, plasma and blood samples are used to test hypotheses relating to trace element exposure/status, environmental pollutants, antibodies, proteins, DNA adducts and genotypes. Because of limited sample availability it is advantageous to develop analytical methods that require minimal sample volume.

Many studies that report trace element concentrations in serum and plasma samples require a minimum of 500 μL of sample [1-5]. In these publications, sample preparation is limited to a "dilute and shoot" procedure. The so called "dilute and shoot" methods rely on dilution of the sample, often with a mixture of ammonium hydroxide, triton X and EDTA, prior to analysis [6]. The advantages include minimum sample preparation which speeds up analysis and limits potential sources of contamination. However the resulting sample matrix requires a matched calibration curve for accurate quantification which must be constructed using standard additions to a sample. Furthermore, the presence of undigested proteins often results in buildup of residue on the nebulizer and sample introduction system and can cause instrument instability and clogging. Finally, the dilute and shoot methods require a nebulizer with sample flow rates of 1 mL/min or greater. This can be a challenge in small volume samples for ultra-trace elements such as Co, Pb and Cd which require a 1:10 to 1:20 sample dilution.

Digestion procedures have the advantage of eliminateing proteins which reduces matrix effects and instrument clogging. However, the digestion procedure can introduce random contamination and volatile elements such as Hg may be compromised. It is therefore important to analyze digestion blanks to determine if random contaminating of the sample has occurred [7].

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This paper examines the accuracy and precision of a digestion procedure for analysis of the trace elements Cu, Zn, Fe, Mg and the ultratrace elements Cd, Co, Mn and Pb in 150 µL of serum and plasma samples. The digestion method was evaluated by analyzing blank tubes and Seronorm® serum quality control material using high resolution sector field (SF) ICPMS and quadrupole (Q) ICP-MS. The method was then used to analyze 1888 blinded plasma samples for Mg, Cu, Fe, Zn, Cd, Co, Mn, Pb in a blinded study being conducted by the National Cancer Institute (NCI).

2. Experimental

2.1. Reagent and Standards

High purity water was obtained using a Millipore Millie-Q water purification system with a resistivity of 18.2 MΩ. Optima™ grade nitric acid (Fisher) and Trace Select grade hydrogen peroxide (Fluka) were used in all procedures. Calibration curves were constructed using commercial standards from Analytics. Analytical methodology was developed using in-house serum samples and Seronorm® trace elements serum level 1 (Accurate Chemical and Scientific Corporation). The Seronorm® serum was reconstituted following the manufacturer's directions.

2.2. Sample Digestion

A 150 µL aliquot of serum or plasma was transferred into a precleaned 15 mL polypro-pylene tube (Cen-Med). The sample mass was measured using a Mettler Toledo AT251 analytical balance. An addition of 150 µL of concentrated nitric acid and 100 µL of hydrogen peroxide were added to each sample tube. The tubes were loosely capped, centrifuged for 10 minutes at 4400 r/min and samples were placed on an environmental express hot-block digester at room temperature (Environmental Express). The hot-block heated samples to 96˚C for 90 minutes. If particulate was observed in the samples following 90 minutes at 96˚C they were placed back on the hot block digester for an additional 60 minutes.

2.3. Sample Preparation for the VG Axiom High Resolution Sector Field ICP-MS Analysis

Digested samples were diluted (1:10) with 18.2 MΩ-cm H_2O and the internal standards In, Ga, Cs and Bi for analysis of the ultra-trace elements Mn, Co, Cd and Pb. A second dilution (1:1000) was prepared with the internal standards Cd and Y for analysis of the trace elements Mg, Fe, Cu and Zn. The diluted samples were centrifuged at 4400 r/min for 5 minutes prior to analysis.

2.4. Sample Preparation for NexION Quadrupole ICP-MS Analysis

Digested samples were diluted with 1:20 with 18.2 MΩ-cm H_2O and the internal standards Sc, Ga, Y, In and Bi. The samples were centrifuged at 4400 r/min for 5 minutes prior to analysis.

2.5. Instrument Parameters

Measurements were performed using a NexION 300 X quadruple (Q) ICP-MS (Perkin Elmer, Ma USA) and a VG Axiom high resolution sector field (SF) ICP-MS (VG Elemental, Winsford UK). The instrument parameters are summarized in **Table 1.** The (Q) ICP-MS was operated in standard mode for the analysis of the ultratrace elements Pb, Cd, Co and Mn and in collision mode for the analysis of Fe, Cu, Zn and Mg. The use of high purity He collision gas served the dual purpose of reducing polyatomic interferences and decreasing the instrument sensitivity so

Table 1. ICP-MS Instrument operational conditions and data acquisition parameters.

Conditions	VG Axiom HR-ICP-MS	NexION 300X ICP-QMS
ICP RF power (W)	1400	1600
Plasma gas flow (L/min)	16	18
Auxiliary gas flow (L/min)	1.2	1.2
Nebulizer gas flow (L/min)	1.15	0.98
Collision gas flow (L/min)	N/A	He 3.0
DRC settings (RPq Values)	N/A	0.25
Sample and skimmer cones	Nickel	Nickel/aluminum
Spray chamber	Jacketed cyclonic spray chamber	Jacketed cyclonic spray chamber
Nebulizer	Micro-flow PFA (100 µL/min)	200 µL/min Meinhard glass
Dwell time (ms)	30	50
Resolution	6000	300
Detector	Continuous dynode electron multiplier	Sequential dynode electron multiplier
Washout time	60	60

that Fe, Cu, Zn and Mg could be measured in the 1:20 dilution. The quadrupole analysis was checked by analyzing trace and ultra t race elements at a mass resolution of 6000 using a VG axiom SF ICP-MS. The (SF) ICP-MS was operated at a resolution of 6000 to eliminate mass interferences.

3. Results

The calibration range and linearity are reported in **Table 2**. Calibration curves were constructed using a zero point standard and a four point calibration series. The isotope, concentration range and linear response function of Q ICPMS instruments are given in **Table 2**. The R^2 value for the response function was greater than 0.998 for all calibration curves. The isotope ^{26}Mg was measured in the Q ICPMS method because ^{24}Mg, which has a higher abundance occasionally produced count rates outside the upper limits of the sequential dynode electron multiplier detector of the (Q) ICP-MS. The isotope ^{57}Fe was measured in the (Q) ICP-MS method to avoid interference from $^{40}Ar^{16}O^+$. The high resolution (SF) ICP-MS was capable of resolving the interfering $^{40}Ar^{16}O^+$ and ^{56}Fe.

The instrumental limit of detection (LOD) and method LOD for the measurement of Mg, Cu, Fe, Zn, Cd, Co, Mn, Pb and Mn by (Q) ICP-MS is presented in **Table 3**. The instrumental LOD was calculated as 3.3 times the standard deviation of 10 replicates of the instrument blank. The method LOD was determined by following the sample preparation and analysis procedures using 20 blank polypropylene tubes. The method LOD was calculated as 3.3 times the standards deviation of the 20 replicates and accounts for the dilution factor used in the experiments.

The accuracy and precision of the method was checked by analyzing replicates of 150 µL Seronorm® trace elements serum level the (SF) ICP-MS and the (Q) ICP-MS. The analysis was performed on 3 sets of 3 Seronorm® samples prepared and analyzed on 3 different days over a period of 15 days by 1 analyst. Results are presented in **Table 4**. A single element Mo standard was included to determine a correction factor for $^{95}Mo^{16}O$ which interferes with the measurement of ^{111}Cd. The isotope ^{111}Cd was chosen over the more abundant ^{114}Cd to avoid potential interference from ^{114}Sn.

Table 2. Linearity and range of the quadrupole (Q) ICP-MS calibration.

Isotope	Concentration range, µg/L	Q equation
^{55}Mn	0.5 - 10	Y = 2.8E4·X − 7.4E2
^{59}Co	0.05 - 1.0	Y = 2.3E4·X + 6.8E1
^{111}Cd	0.05 - 1.0	Y = 1.6E3·X + 4.1E0
^{208}Pb	0.05 - 1.0	Y = 9.3E3·X + 6.6E1
^{26}Mg	200 - 1500	Y = 4.1E1·X − 9.5E1
^{57}Fe	10 - 150	Y = 3.2E1·X − 2.4E1
^{63}Cu	10 - 150	Y = 1.6E1·X + 5.7E2
^{66}Zn	10 - 150	Y = 1.7E2·X + 4.4E2

Table 3. Detection limits measured by quadrupole (Q) ICP-MS.

	Q ICPMS	Q ICPMS
	Instrument LOD µg/L	Method LOD µg/L
^{55}Mn	4.9E−03	1.1E−01
^{59}Co	9.6E−04	1.9E−02
^{111}Cd	1.9E−03	3.8E−02
^{208}Pb	2.2E−03	3.0E−02
^{26}Mg	2.9E−01	5.8E+00
^{57}Fe	4.3E−01	8.6E+00
^{63}Cu	1.6E−02	3.2E−01
^{66}Zn	4.3E−01	8.6E+00

Table 4. Seronorm® analysis performed by VG axiom high resolution sector field and NexION quadrupole ICPMS.

Element	n	SF ICPMS mean(SD)	Q ICPMS mean (SD)	Seronorm® certified value
Mn[a]	9	12.3 (0.1)	14.4 (0.14)	14.1 - 15.9
Co[a]	9	1.51 (0.06)	1.70 (0.07)	0.9 - 1.5
Cd[a]	9	0.11 (0.02)	0.12 (0.02)	0.126
Pb[a]	9	1.14 (0.09)	1.19 (0.07)	1.02
Mg[b]	9	20.0 (1.0)	18.7 (1.1)	18.8 - 21.4
Fe[b]	9	1.36 (0.04)	1.38 (0.03)	1.31 - 1.47
Cu[b]	9	1.69 (0.08)	1.49 (0.13)	1.607 - 1.775
Zn[b]	9	1.65 (0.07)	1.65 (0.01)	1.667 - 1.809

[a]µg/L; [b]mg/L.

The hotblock digestion method and the Nexion Q ICPMS were used to analyze 1888 plasma samples and 102 blinded quality control samples for Mn, Co, Cd, Pb, Mg, Fe, Cu and Zn for a study being conducted by the NCI. Samples were analyzed in batches of 60 to 90 samples with 3 digestion blanks, 3 Seronorm® serum quality control samples and 1 spiked Seronorm® serum quality control sample. A single element Mo standard was included in each batch to correct for the $^{95}Mo^{16}O$ interference on ^{111}Cd. In plasma samples the ^{111}Cd concentration was much lower than the Seronorm® serum and the $^{95}Mo^{16}O$ interference accounted for an average of 40% of the signal intensity measured at 111 amu. The measured concentrations in the quality control material and spike recoveries are summarized in **Table 5**. The Zn values were consistently less than the certified values. The spike recovery values were greater than 90% for all elements except Cu and Zn. The plasma samples and the Seronorm® serum were not corrected for spike recovery. The 66 digestion blanks on average contributed less than 5% of the plasma concentration for each the trace and ultra trace elements with the exception of Pb. The digestion blank on average contributed 17% of the Pb measured in the mean sample concentration and indicates that lead contamination occurred during sample digestion.

Unknown to the laboratory 102 quality control samples were prepared from a pooled batch of plasma and analyzed with the samples. One of the quality control samples was an outlier that contained high levels of Fe, Zn and Cu and low levels of Mn, Co and Pb and was removed from the analysis. Fourteen of the quality control samples had values less than the LOD for Cd. Of the 102 samples 97 were observed to be cloudy white by the analyst. The analysis of the blinded, pooled quality control material is valuable in that it provides a measure of the variance (CV) associated with the analysis of a 150 µL sample. The CVs for Mn, Co, Cd, Pb in the blinded quality control samples were 7.5%, 16%, 131% and 8.0%. The high CV for Co and Cd is likely the result of measuring these elements near the LOD and below the limit of quantification (3.3 × LOD). The CVs for Mg, Fe, Cu and Zn were 8.4%, 9.4%, 8.3% and 9.9%.

Plasma samples archived from the Linxian Nutrition Intervention Trial were analyzed using the Q ICP MS method. The 50th, 75th and 90th percentile of Mn, Co, Cd. Pb, Mg, Fe, Cu and Zn are reported in **Table 6** where N is the number of samples measured above the detection limit. For Cd, 75% of the samples were below the LOD of 0.06 µg/L. This LOD differs from the LOD in **Table 3** in that it is the maximum LOD observed over 4 months of data collection. Of the original 1888 samples, 9 were lost due to analyst error.

Table 5. Results from analysis of 69 Seronorm® serum samples co-analyzed with 1888 plasma samples.

Element	n	Measured value	Seronorm® certified value	Spike recovery %
Mn[a]	66	15.9 (0.7)	14.1 - 15.9	100
Co[a]	66	1.45 (0.3)	0.9 - 1.5	97
Cd[a]	66	0.150 (0.04)	0.126	96
Pb[a]	66	1.20 (0.24)	1.02	92
Mg[b]	66	19.1 (1.0)	18.8 - 21.4	95
Fe[b]	66	1.33 (0.07)	1.31 - 1.47	93
Cu[b]	66	1.58 (0.08)	1.607 - 1.775	83
Zn[b]	66	1.27 (0.10)	1.667 - 1.809	76

[a]µg/L; [b]mg/L.

Table 6. Summary of the analysis of 1888 plasma samples by Q ICPMS.

Element	N	50%[a]	75%[a]	90%[a]
Mn	1879	3.83	4.25	4.96
Co	1879	0.40	0.47	0.60
Cd	469	<0.05	<0.05	0.06
Pb	1785	0.31	0.44	0.64
Mg	1879	24,186	27,855	30,998
Fe	1879	1221	1516	1856
Cu	1879	917	1034	1139
Zn	1879	921	1043	1173

[a]µg/L.

4. Discussion

The external calibration curves reported in **Table 2** were linear over the measured concentration range for each measurement technique with R^2 values of greater than 0.998. The quality control results presented in **Tables 4** and **5** and show that the (Q) ICP-MS and the (SF) ICP-MS methods both gave satisfactory results. This indicates that there were not significant mass interferences that the (Q) ICP-MS method was unable to handle using the collision gas. Based on this observation the (Q) ICP-MS method was used for all subsequent analysis.

Sample contamination may occur during sample analysis at the analytical laboratory. The presence of sample contamination that results from sample analysis was tracked using digestion blanks and quality control samples. The digestion blanks indicate that random Pb contamination of samples in the preparatory laboratory may occur using this method. However, these analytical blanks do not account for contamination that occurred prior to sample preparation and analysis. The Linxian Nutrition Intervention Trial collected whole blood and processed it into plasma samples which were archived for future analysis. The concentration of Mn and Co in samples could be compromised by the stainless steel needle stick used to collect blood [8]. A recent study by Penny and Overgaard in 2010 indicates that the needle stick may not signifycantly contribute Cr and potentially other metals if the first vial of blood is discarded and the second vial is used analysis [9]. Plasma samples are prepared by adding an anticoagulant to whole blood followed by centrifugation. In most cases the anticoagulant is heparin or an EDTA salt. Both of these chemicals may result in contamination of trace and ultratrace elements. In addition the sample collection tubes and rubber stoppers are potential sources of contamination. It has been this laboratories experience that rubber stoppers used to seal vials contain significant levels of Pb and Zn and their contact with the samples should be minimized.

The Linxian Nutrition Intervention Trial study samples were blinded to the analysis laboratory. Each sample was graded on appearance prior to digestion and analysis. In the 1888 samples we classified 104 as pink and an additional 48 as red which is indicative that hemolysis occurred when plasma samples were prepared from whole blood. We classified 92 samples as cloudy white which may indicate that the plasma is contaminated by a portion of the buffy coat. We compared the mean concentration value of samples classified as pink, red and cloudy white with normal samples using a 2-tailed independent samples t-test assuming equal variance (IBM SPSS 19). The mean Fe concentration in the pink and red samples and the Cu concentration in the red samples were significantly elevated ($p < 0.05$) when compared to normal samples. The mean Mg concentrations was significantly elevated

($p < 0.05$) and the Co concentration was significantly reduced ($p < 0.05$) in the cloudy white samples when compared to normal samples. Based on this analysis it is important for the analytical testing laboratory to provide qualitative information about the samples. At a minimum sample color should be noted.

5. Conclusion

We have demonstrated that a hotblock digestion method coupled with high resolution sector field and Q ICPMS is capable of accurately analyzing Mn, Co, Cd, Pb, Mg, Fe, Cu and Zn in 150 µL plasma and serum samples. The minimal sample volume required by the analysis is important because it preserves sample and allows researchers to couple trace element measurement with other analytical techniques to measure antibodies, proteins, DNA adducts and genotypes. To the best of our knowledge, there have been no other studies published in the literature that have quantified the trace and ultratrace elements with this low sample volume. The method was used to analyze 1888 plasma samples for Mn, Co, Cd, Pb, Mg, Fe, Cu and Zn.

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