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Analysis of Human Serum and Whole Blood for Mineral Content by ICP-MS and ICP-OES: Development of a Mineralomics Method

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Abstract

Minerals are inorganic compounds that are essential to the support of a variety of biological functions. Understanding the range and variability of the content of these minerals in biological samples can provide insight into the relationships between mineral content and the health of individuals. In particular, abnormal mineral content may serve as an indicator of illness. The development of robust, reliable analytical methods for the determination of the mineral content of biological samples is essential to developing biological models for understanding the relationship between minerals and illnesses. This manuscript describes a method for the analysis of the mineral content of small volumes of serum and whole blood samples from healthy individuals. Interday and intraday precision for the mineral content of the blood (250 µl) and serum (250 µl) samples was measured for eight essential minerals, sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) by plasma spectrometric methods and ranged from 0.635 – 10.1% relative standard deviation (RSD) for serum and 0.348 – 5.98% for whole blood. A comparison of the determined ranges for ten serum samples and six whole blood samples provided good agreement with literature reference ranges. The results demonstrate that the digestion and analysis methods can be used to reliably measure the content of these minerals, and potentially to add other minerals.

Keywords

Mineralomics; reference ranges; metallomics; plasma spectroscopy; bioanalytical

Introduction

The human body makes use of a variety of essential inorganic compounds, including minerals, to support biological processes such as electron transfer reactions or the

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Ethical statement

This manuscript does not contain samples that were obtained from clinical studies and no personally identifiable patient data is included. Sample collection procedures followed the Helsinki Declaration guidelines regarding informed consent of human volunteers.

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transportation of gas molecules.[1] The levels of all of these minerals are constantly maintained through the processes of homeostasis to prevent an excess (toxicity) or shortage (deficiency) of the nutrients. There are a variety of illnesses that can be associated with elevated or lowered levels of essential minerals. For example, Wilson's Disease and hemochromatosis are a result of elevated copper and iron levels, respectively, in the body. [2,3] Therefore, an understanding of the interactions of minerals in the body can support the diagnosis and treatment of illnesses.

The field of metallomics has developed to encompass the study of all inorganic compounds, both toxic and essential, and their interactions in biological systems.[4–7] A subfield of metallomics has emerged that is referred to as mineralomics, which focuses on the essential minerals and their relationship to the health of individuals.[8] Mineralomic studies can be combined with traditional metabolomics studies to provide a comprehensive view of the chemicals contained in the body and their relationship to a variety of health states including heart disease and obesity.[9,10]

A number of studies using methods for measuring metals in biological samples have been reported, but to our knowledge, few studies use validated methods. [11–17] In addition, these studies analyzed whole organ tissue, but not the less invasive biological fluids, or provided measurements of only a few minerals. The study described here aims to provide a method for measuring a broad panel of essential minerals in relatively noninvasive biological samples. Intraday (same day) and interday (different day) precision, linear range, and other figures of merit were determined for eight minerals in both serum and blood.

The minerals chosen as the focus of this study are sodium (Na), potassium (K), calcium (Ca), zinc (Zn), magnesium (Mg), copper (Cu), iron (Fe), selenium (Se), molybdenum (Mo), cobalt (Co), chromium (Cr), and manganese (Mn). Inductively-coupled plasma mass spectrometry (ICP-MS) was used for quantification of analytes that are present in the ng/mL or low μg/mL concentration range in samples. For the more ubiquitous elements that were expected to be present in the high μg/mL concentration range, such as sodium and potassium, inductively-coupled plasma optical emission spectroscopy (ICP-OES) was used for quantification.

A desirable characteristic of an omics panel is that it makes use of a minimal volume or weight of sample, and that for human subject screening, the medium used be collected in a minimally invasive manner. These characteristics make the omics screening process less intrusive to patients, as well as providing the ability to obtain samples from biological sample repositories, where only limited amounts of samples may be available. The methods described in this study were developed for the analysis of serum and whole blood samples, and use 250 μl of sample. Previous reports have generally used sample volumes of at least 500 μL, so the method presented in this report provides an improvement in terms of sample availability and the invasiveness of collection.[17]

Methods

Samples and Materials

Ten serum samples and six whole blood samples with lithium heparin anticoagulant taken from different subjects were purchased from Bioreclamation IVT (Baltimore, MD). Samples were collected from healthy adult human volunteers (equal numbers of male and female volunteers) and were used as received for digestion. Sample collection procedures followed the Helsinki Declaration guidelines regarding informed consent of human volunteers. Basic demographic information of donors is provided in the Online Resource, Table S1. A subsample of each vial was taken and pooled in a separate acid-washed sample vessel by pipette. Acids and other chemicals were obtained from Fisher for use in the digestion process. National Institute of Standards and Technology (NIST)-traceable 10 mg/L and 1,000 mg/L elemental standards were obtained from High Purity Standards (Charleston, SC) for preparation of multielement calibration standards. Approximately 18 MΩ cm^{-1} water was used in all sample preparation and analysis steps.

Instrumentation

Samples were prepared in a Class 100 clean hood to prevent contamination by atmospheric particulates. Digestion was performed by microwave methods using the Discovery SPD (CEM, Matthews, NC). After digestion, the samples were analyzed for trace minerals using the Thermo (Bremen, Germany) X-Series 2 ICP-MS equipped with a concentric glass nebulizer and peltier-cooled glass spray chamber. Bulk mineral concentration was determined by ICP-OES analysis on the Thermo iCAP equipped with a polypropylene cyclonic spray chamber. Analysis was performed in both radial and axial mode depending on the element and matrix.

Digestion method

Prior to digestion, all samples were vortexed thoroughly to provide a homogeneous matrix for digestion. Samples were immediately pipetted to prevent settling prior to removing the sample. A sample volume of 250 μL of each serum, whole blood, pooled serum, and pooled whole blood sample was dispensed into an acid-washed glass microwave digestion vessel. Samples were prepared in triplicate for each analytical day. Three batches of samples were prepared on separate analytical days so that a total of nine replicates were prepared over three analytical days to allow for the analysis of interday (different day) and intraday (same day) precision. Reagent blanks were prepared by addition of deionized water in place of the samples. For each analytical day, seven reagent blanks were prepared to monitor background concentrations of all analytes.

For serum samples, a volume of 300 μL concentrated nitric acid (HNO_3) (Ultrex purity, Fisher), 200 μL concentrated hydrochloric acid (HCl) (Ultrex, Fisher), and 100 μL of non-stabilized 30% hydrogen peroxide (H_2O_2) solution (Ultrex, Fisher) was added to each vial. Deionized water was added to provide a final volume of 2.0 mL. An acid-washed stir-bar was added to each vial for the purpose of stirring during digestion. Samples were then sealed, placed in the microwave and digested following the program described in Table 1. For whole blood samples, 1.0 mL of HNO_3 was added to each vial and each sample was

allowed 30 minutes pre-reaction time in the clean hood. After completion of the pre-reaction time, 750 μL of non-stabilized 30% H_2O_2 solution was added to each vial. Once the addition of all reagents was complete, the caps were sealed and digested in the microwave following the program described in Table 1. At the end of digestion, all samples were removed from the microwave and allowed to cool to room temperature. In the clean hood, samples were quantitatively transferred to acid-washed 15 mL polypropylene sample tubes, spiked with a multielement internal standard to provide a final concentration of 10 ng/mL Indium, Scandium, and Praesodymium, and diluted to the final volume with deionized (DI) H_2O. Samples were stored in a monitored refrigerator at a nominal temperature of 8 °C until analysis.

Elemental Analysis

Bulk mineral calibration standards for ICP-OES analysis were prepared by dilution of NIST-traceable 1,000 mg/L Ca, Mg, Na, and K standards (as well as Fe for whole blood analysis). Samples were diluted 5-fold and 25-fold using an acid matrix-matched blank solution and analyzed at the wavelengths described in Table 2. Multiple wavelengths were generally monitored for each element to provide confirmation of the quantitative results, but only one wavelength is reported for each element. Calibration standards and samples for analysis of whole blood were monitored in radial mode at all wavelengths, while analysis of serum samples at all wavelengths was performed in axial mode. Continuing calibration check samples were analyzed at most after every ten samples and consisted of a blank measurement to monitor carryover, a high-range calibration standard, and a low-range calibration standard to assess instrument performance. The estimated limit of quantification (ELOQ) was taken as the lowest concentration calibration standard for each analyte and continuing calibration blanks were used to determine instrument detection limits (IDL) for each analyte element.

Trace mineral calibration standards for ICP-MS analysis were prepared using NIST-traceable 10 mg/L Co, Zn, Cu, Se, Mo, Cr, and Mn standards (as well as Fe for serum analyses). Two sets of calibration standards were prepared for elements that were anticipated to be low-level elements (Cr, Co, Mn, and Mo) and high-level elements (Zn, Cu, Fe, and Se). Samples were analyzed without dilution in the original storage containers to minimize the possibility of contamination. Elements were monitored in a multimode run. Se, Zn, Cr, Cu, Fe, and Co were analyzed in kinetic energy discrimination (KED) mode with 10% H_2/He gas to minimize polyatomic interferences, while Mo was analyzed in standard mode due to the lack of probable interferents in the system (FeAr is a potential interferent at ^{96}Mo and ^{98}Mo, but the low abundance of ^{58}Fe results in minimal impact at ^{98}Mo, especially in serum samples). Continuing calibration check samples were analyzed at most after every ten samples and consisted of a blank measurement to monitor carryover and mid-range calibration standards from both the low-concentration calibration curve and the high-concentration calibration curve. For the calibration, standards were accepted as passing if their back-calculated concentration was found to be within ±15% of the nominal concentration (±25% of the nominal concentration for the lowest concentration standard). Quality control checks were accepted as passing if the determined concentration for all elements was found to be within ±15% of the nominal concentration.

Results and Discussion

Digestion Method

The digestion method was found to be robust and sufficient for all samples. Each digestion was completed with no particulate matter remaining in the solution, suggesting complete digestion of the matrix. In particular with the whole blood samples, it is important that a sufficient amount of acid and hydrogen peroxide be used to allow complete breakup of the heavy organic matrix. An initial attempt was made to digest the whole blood samples with the same digestion matrix as the serum samples, but a flocculate remained in the container following microwave digestion. This observation suggests that the dilution of the acid and hydrogen peroxide upon addition of deionized water decreased the potency of the digestion matrix such that it was insufficient to completely digest the heavy cellular component of whole blood.

Previous reports of whole blood analyses have described a microwave digestion method where samples are prepared in a similar acid matrix as the method used here, but at lower microwave power levels and for a shorter length of time.[18,19] The description of the literature method includes a filtration step through Whatman filter paper to separate a residue from the sample solution. The strength of the method described here is the minimized sample handling steps, which helps to minimize the opportunities for contamination by more ubiquitous elements. Serum sample preparation methods have varied from simple dilution to microwave digestion at atmospheric pressure.[20,21] One benefit of the digestion method described here is the use of hydrochloric acid, which provides stabilization for some transition metals and could allow the adaptation of the digestion method to other elements. Further, the digestion described here allows for high throughput of a large number of samples by digestion of the samples in an automated microwave device that can hold as many as 72 samples to be individually digested with total control of the vessel pressure and digestion temperature. Analysis of organic matrix samples like serum without some form of digestion could result in significant carbon buildup on the cones of the instrument, making trace analysis difficult after a certain number of consecutive samples. Performing such a digestion helps to improve the overall throughput of the method, allowing analysis of a larger number of samples as in an omics study.

Elements of Interest

The elements selected to be monitored were chosen based on their relative abundance and their requirement in essential biological processes. Na, K, and Ca are essential bulk metals for cellular signaling that are present in a range of biological processes and are tightly regulated through a series of ion channels and pumps. Mg is found in a variety of enzymes and cells such as muscle cells and has been shown to bind strongly to highly-charged molecules containing phosphates. Zn is also found in enzymes, where it contributes to catalysis of a variety of biological synthesis reactions. Fe is best known for its role in gas transportation when it is found in hemoglobin, but also plays a role in a variety of redox reactions in the cell. Mn is also heavily involved in redox reactions and catalysis of biological reactions due to its ability to be found in the II, III, or IV oxidation states. Cu plays a role in a number of oxidases and superoxide dismutase due to its distorted geometry

in the II oxidation state and its ability to redox cycle between I and II oxidation states. Co is found in a number of enzymes, generally at lower concentrations than Zn, Fe, and Cu, and is normally taken in as vitamin B_{12} or cobalamin. Mo and Cr are involved in a small number of enzymes and are usually present in very small concentrations, as evidenced by their low daily requirements (not characterized in the case of Cr). Se plays a role in a number of enzymes involved in metabolism and other redox processes.

Method figures of merit

The figures of merit for the analysis methods are shown in Table 3. The IDL was determined by multiplying the standard deviation of the quality control blanks by a factor of three and then accounting for dilution of samples during preparation. The ELOQ was conservatively taken as the concentration of the lowest acceptable calibration standard, which required a back-calculated concentration within 25% of the nominal concentration. This approach allows the concentrations determined in blood and serum samples to be compared to limits of detection and quantification on the same scale and provide additional confidence in the reported concentrations. The IDL values differ slightly between the two analytical methods for serum and whole blood, likely because of the different views that are used between the two methods (i.e. – axial vs radial). The detection limits measured here were found to be comparable to similar studies, even with the decreased volumes used here. [11–17] For the elements analyzed by ICP-OES, all of the measured concentrations were found to be significantly higher than the IDL and ELOQ due to the high concentrations of the elements in the biological samples. However, some of the more trace elements, notably Co, Cr, and Mn in serum, were not found to be present at levels greater than the IDL (as will be discussed later).

Serum elemental analysis results

The elemental composition of the digested serum samples and the inter-day analysis of the samples are shown in Table 4. The values shown in Table 4 are the average of triplicate preparations within a day and analyses (n=3). The final average concentration is an average concentration of the average values from each day. Determined concentrations from the intra-day analysis of all samples can be found in Tables S2–S4. The efficacy of the digestion and analysis methods was assessed by digesting three replicates on three separate days, providing a total of nine replicates for each serum sample. A suitable standard reference material (SRM) was not identified with certified concentrations for all of the elements of interest for human serum, with the closest being ERM-DA120a (European Reference Material, Human Serum), which is only certified for copper, zinc, and selenium, and NIST SRM 1598a (Inorganic Constituents in Bovine Serum). Therefore, for all of the study samples, the ability of the method to quantify the elements of interest was monitored by calculation of the precision of replicate analyses. Precision was taken as the relative standard deviation of the replicate preparations (n=3 on each of three days). Some sample replicates exhibited greatly elevated elemental concentrations, usually for Co and Cr, and these replicates were excluded based on a Q-test against the remaining replicate measurements at a 99% confidence interval. Analytes were taken as passing if the average concentrations were found to be above the established method limit of detection on all days and exhibited a RSD less than 15% for both intra- and inter-day analyses.

The results shown in Table 4 demonstrate that all of the elements measured by ICP-OES (Ca, K, Mg, and Na) passed acceptance criteria, and of the elements measured by ICP-MS, Cu, Zn, and Se were found to pass acceptance criteria. Some of the elements were found to be problematic for analysis by this method, namely Cr, Co, Fe, Mn, and Mo. In the case of Cr, Co, Mo, and Mn, the elements were expected to be present at a relatively low level in these samples. Published reference ranges for these elements in human serum are 0.12–2.1 ng/ml, 0.11–0.45 ng/mL, 0.55–3.00 ng/mL, and 0.54–1.76 ng/mL respectively.[22] The sample preparation described here dilutes the components of the serum by a factor of 40 prior to analysis, decreasing those concentrations well below the method limits of detection. It is possible that digestion of higher volumes of sample might allow for more reliable determination of some of these elements, but our approach is designed to support investigations where sample volume is very limited. Further, with the exception of Mo, all of these elements had to be measured in KED mode to remove potential polyatomic interferences. Chromium experiences a number of potential interferences from elements that are present at high concentrations in these samples, including ArC, CaC, and ArO. Cobalt is monoisotopic and experiences interferences resulting from the presence of ArOH, CaOH, and ArNa. Mn is also monoisotopic and experiences interferences resulting from the presence of KO, ArN, KN, and ArN. Iron experiences interferences, most notably, from ArO amongst others. These interferences make it essential to analyze those elements in KED mode to provide a constant background signal and exclude polyatomic interferences, especially those containing Ar. In the case of some interferences, the high concentration of their constituents makes it difficult to exclude it all, even in KED mode, making it more difficult to accurately quantify the analyte. Further, KED analysis generally leads to decreased sensitivity for most elements, which results in increased limits of detection. In such cases, analysis by sector-field mass spectrometry (SF-MS) at a higher resolution could potentially provide more accurate quantification.[23] The KED gas chosen for this method was a He/H_2 mix, a relatively inert gas that served to exclude interferences based on collisions as opposed to reactions. Alternative gases such as ammonia and hydrogen can chemically react with analytes and interferents as they travel through the reaction cell to change their molecular weight, excluding them from detection. This method is used frequently in the analysis of vanadium, where ammonia gas is used in the reaction cell. [24]

The analysis of selenium by this method is worth noting. The levels of Se present in the serum samples are low enough that ICP-OES lacks the sensitivity required to analyze the element. However, there is a significant issue of the polyatomic interference on most Se isotopes resulting from Ar dimers and CaAr, necessitating the analysis of Se in KED mode or higher resolution modes by SF-MS. Se exhibits a low ionization efficiency, resulting in low sensitivity for the element by ICP-MS. While the results presented here provided acceptable inter- and intra-day precision, suggesting accurate quantification, the signal for the analyte in all samples was very low. Targeted analysis for Se would allow one to tune the instrument settings to improve the sensitivity for Se, optimizing the analysis for that element, but doing so could decrease the sensitivity for other analytes.

The method described here for sample preparation of serum allows for the analysis of a suite of analytes using a single sample preparation of a relatively small volume of liquid samples. For the elements that were found to pass acceptance criteria above, with the exception of Se,

the concentrations that were measured in the samples were found to be significantly higher than the IDL and ELOQ values. Therefore, it may be possible to decrease the sample volume to as little as 100 μL and still obtain acceptable sample precision. However, sample homogeneity and vortexing prior to digestion could become important factors in obtaining acceptable results with lower sample volumes.

Another element that would be of interest for analysis is nickel. However, the sampling cones that are used in the ICP-MS are commonly made of nickel metal, resulting in difficulty quantifying the element in samples. It is possible to use platinum cones to remove this barrier, but doing so could affect the figures of merit for the other elements of interest due to changes in sensitivity. Further, it is expected that the average nickel content of most biological samples would be relatively low, as the reference values for nickel are comparable to those of manganese in many biological fluids.[22]

Another measure of the validity of the analytical results was obtained by pooling the samples in equal volumes and then averaging the measured elemental concentrations. In the absence of any matrix-based chemistry, the concentration of each element measured in a sample pooled from equal volumes of the numbered samples (serum samples 1–10) should be the same as the average concentration of the element measured in the numbered samples.

$$C_{pool} = \frac{\sum_{1}^{n} C_{sample}}{n} \quad (1)$$

Elements were accepted as passing if the results differed by less than 10%. The results for this comparison are shown at the bottom of Table 4 and demonstrate that the elements Ca, K, Mg, Na, Cu, Zn, and Se passed this criterion. The other elements also failed the reproducibility test due to the low levels of the analytes or the potential for interferences on the measured mass-to-charge ratio.

An important point to consider is that the results obtained using this method provide concentrations of total metals present in the samples and do not take into account the species of metal present. This can be important for a number of the metals studied here including zinc, iron, and copper. The human body has evolved several biochemical mechanisms to sequester metals and minimize the potential toxicological impact posed by free metal ions. These mechanisms often act by binding the excess metals with proteins or small molecules to prevent chemical reactions from taking place. Some examples of this principle are transferrin and metallothioneins.[3,25] The speciation of metals in biological samples, including what they are bound to, could provide important information relating the metal concentration to health outcomes and illnesses but is not addressed by this method because the digestion method is harsh enough to break down most proteins and small molecules that would bind metals. Supplemental methods would be necessary to determine elemental speciation for relation to illnesses.

Whole blood elemental analysis results

The elemental composition of the digested whole blood samples and the inter- and intraday analysis of the samples are shown in Table 5. Determined concentrations from the intra-day analysis of all samples can be found in Tables S5–S7. The experimental design of the number of replicates and acceptance criteria were similar to those used in the serum analysis described above. The level of iron in whole blood is significantly higher than those in serum due to the presence of erythrocytes, necessitating the analysis of iron in whole blood by ICP-OES.

The results for the elemental analysis of all samples demonstrated strong reproducibility for all elements measured by ICP-OES (Na, K, Ca, Mg, Fe) and for Cu, Zn, and Se. Similarly to how Cr, Co, and Mo are too low to be measured accurately in serum with the sample volume used above, many of the samples were found to be below the limit of detection for these elements. The reference ranges for Cr and Co have not been established definitively, and these two elements were the most often contaminated elements in this study, making the determination difficult. The reference range for Mo is 0.8 – 3.3 μg/L (Table 6), which is close to the values measured here. The measured values for Mn are higher in whole blood than in serum (reference range of 8.0 – 18.7 μg/L, Table 6), but the determination of this element exhibited a higher relative standard deviation for two of the seven analyzed samples.[22] It may be possible to more accurately determine the concentration of Mn present if a greater volume of serum is used in the sample preparation. The spectral interferences that are present for most elements in the serum analysis are the same as those present in the whole blood analysis, necessitating the use of KED mode for most elements analyzed by ICP-MS with the exception of Mo.

The results obtained for the successfully analyzed elements in the whole blood samples upon comparison to the pooled blood sample demonstrated good agreement between the average concentration and that determined in the pooled sample. The variation between the average sample concentration and the pooled sample concentration for the passing elements ranged from 0.397 – 8.29 %, which are all below the predefined acceptance criteria of 10% difference.

Sample stability is particularly concerning in the case of whole blood samples as exposure to oxygen and elevation of sample temperatures when not in proper storage (such as when removing samples for digestion) can potentially result in clotting, which could alter the content and homogeneity of the blood samples, even with vortexing of samples. The samples analyzed here contained an anticoagulant in the form of lithium heparin, increasing the stability of the samples with regard to clotting. However, the first and third whole blood sample batches were prepared approximately two weeks apart from each other without storage under an inert atmosphere at recommended storage conditions, providing evidence of sample stability of at least two weeks. Minimal visual evidence of clotting was observed over the two weeks between sample preparations, suggesting that the anticoagulant was sufficient to promote sample stability. It could be useful to compare results for samples that were collected without the addition of anticoagulant to determine their stability, but such a discussion is beyond the scope of this study.

Mineral concentration reference ranges

Although a relatively small number of samples were analyzed for this study (n = 10 serum samples, n = 6 whole blood samples), the results obtained were compared to published reference ranges. The observed ranges and median values for sample mineral content are shown in Table 6. The ranges found in this study are also compared to those reported in the literature for the same elements, and demonstrate that the values obtained fall within previously recorded ranges for mineral content. The range of elemental concentrations measured in the serum samples is shown in Fig. 1 (ICP-OES) and Fig. 2 (ICP-MS). The passing element that demonstrated the highest degree of variability between subjects, as measured by the quotient of the determined concentration range over the median value, was magnesium in serum (53% variability) and copper in blood (66% variability).

All elements measured here compare favorably to the reported literature ranges with the exception of sodium. This lack of agreement is a result of the background subtraction of measured sodium in the method blanks, which exhibited elevated levels. It is also unclear from the literature what degree of background subtraction was used in the original determinations of the reference ranges. In the case of Mn, Co, Fe, and Cr in serum, the highest concentration measured in this study was found to be significantly higher than the reported maximum concentration from the literature, most likely because of contamination of individual samples in this study at levels that were too low to be excluded by Q-test. It is also important to note that the reported reference concentrations for Cr, Mn, Co, and Mo in serum and Co and Mo in whole blood are lower than the ELOQ for these analyses, suggesting that the determined concentration of these elements may not have been accurate within 25%. The reproducibility of the measured concentrations here and the favorable comparisons of the concentration ranges to previously reported ranges suggests that the method of sample preparation and analysis provides acceptable results for the determination of Ca, K, Mg, Cu, Zn, and Se in serum and Ca, K, Mg, Cu, Zn, Fe, and Se in whole blood samples. Further, additional method optimization may allow a more accurate assessment of the sodium content of serum samples.

Conclusions

The determination of essential trace elements in biological samples is an essential tool in the -omics toolkit, providing information that can be related to a variety of health outcomes. Providing a method for the reliable quantitative analysis of samples allows for a higher degree of confidence in the results of these studies. Blood and serum are two widely-used matrices in -omics studies, and the methods described here allow for the quantitation of the minerals Na, K, Ca, Mg, Fe, Cu, Zn, and Se. Further investigation of the methods and development of methods for other matrices will allow for the principles described here to be applied to a broader range of studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

ICP-MS	Inductively Coupled Plasma-Mass spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
NIST	National Institute of Standards and Technology
ELOQ	Estimated limit of quantitation
LOD	Limit of Detection
KED	Kinetic Energy Discrimination
SRM	Standard reference material
SF-MS	Sector field mass spectrometry

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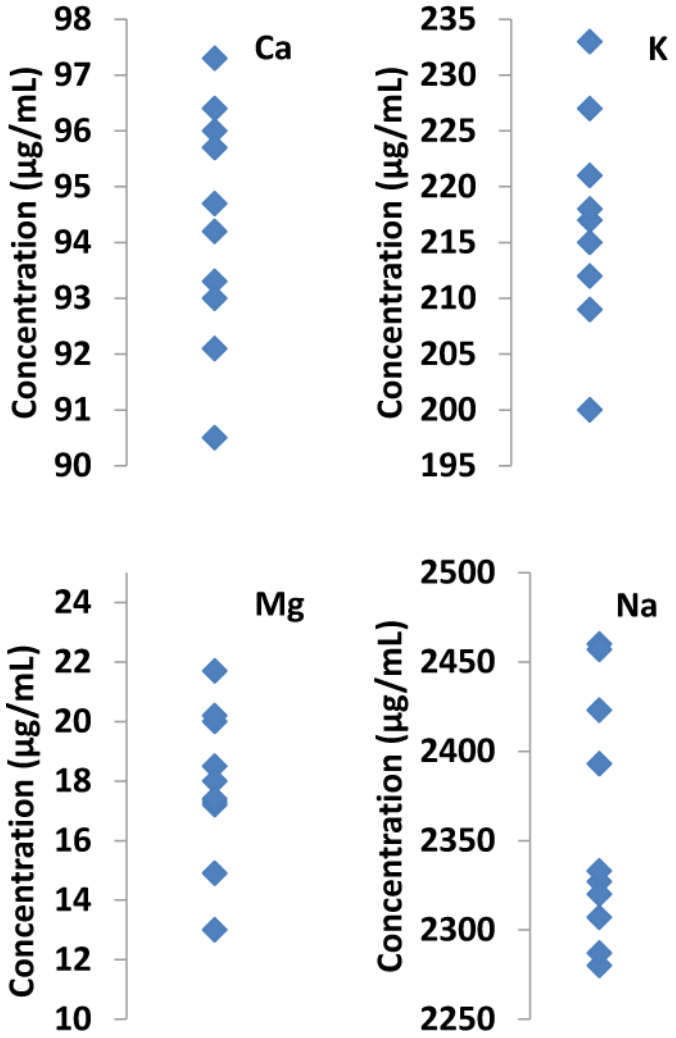

Fig. 1.

Distribution of elements measured in serum by ICP-OES that passed precision acceptance criteria

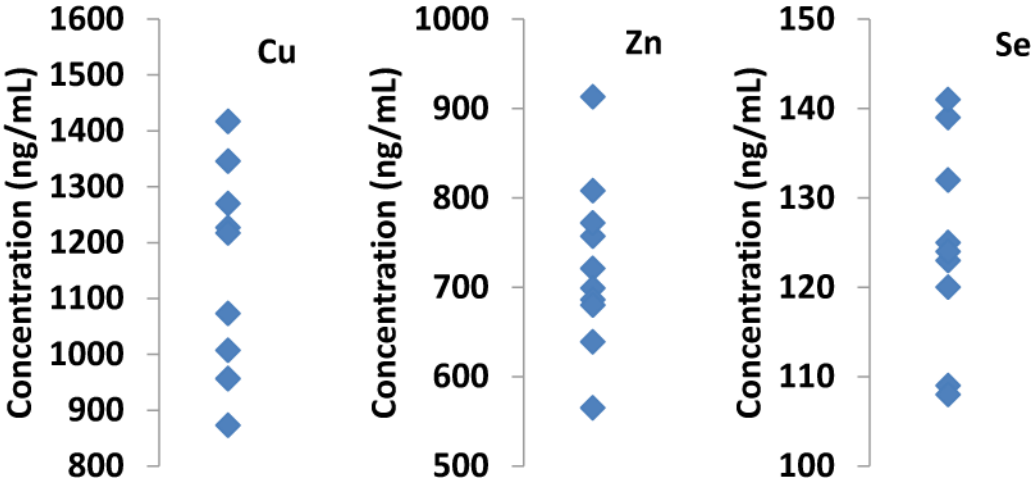

Fig. 2.

Distribution of elements measured in serum by ICP-MS that passed precision acceptance criteria

Table 1

Microwave digestion program for study samples.

Serum digestion microwave program					
Max Temp (°C)	**Ramp (min)**	**Hold (min)**	**Max Pressure (PSI)**	**Power (W)**	**Stir rate**
200	7:00	9:00	300	200	Medium
Whole blood digestion microwave program					
Max Temp (°C)	Ramp (min)	Hold (min)	Max Pressure (PSI)	Power (W)	Stir rate
200	7:00	9:00	400	200	Medium

Table 2

ICP-OES and ICP-MS analytes for each element.

ICP-OES Analytical Wavelengths[a]	
Element	Wavelengths (nm)
Ca	393.3, (396.8), 422.6
Fe[b]	238.2, 239.5, 240.4, (259.9)
K	(766.4)
Mg	279.5, (280.2), 285.2
Na	(588.9), 589.5
ICP-MS Analytical Masses[c]	
Cr	(52), 53
Fe[d]	54, (56)
Mn	(55)
Co	(59)
Cu	(63), 65
Zn	(64), 66
Se	77, (78), 80
Mo	95. 96, (98)

[a] All listed wavelengths were monitored for potential interferences, the reported wavelength is enclosed in parentheses.

[b] Iron was only measured by ICP-OES for whole blood samples.

[c] All listed masses were monitored for potential interferences, the reported wavelength is enclosed in parentheses.

[d] Iron was only measured by ICP-MS for serum samples.

Table 3

Figures of Merit, ng/mL of element

Serum Analysis Figures of Merit (ng/mL)												
Figure	**Ca**	**K**	**Mg**	**Na**	**Cr**	**Mn**	**Fe**	**Co**	**Cu**	**Zn**	**Se**	**Mo**
MLOD	62.2	223	42.4	597	1.45	0.109	2.53	0.0854	1.50	3.03	9.07	0.528
ELOQ	2000	2000	2000	10000	10.0	1.00	20.0	1.00	20.0	40.0	10.0	1.00

Whole Blood Analysis Figures of Merit (ng/mL)												
Figure	**Ca**	**K**	**Mg**	**Na**	**Cr**	**Mn**	**Fe**	**Co**	**Cu**	**Zn**	**Se**	**Mo**
MLOD	358	8690	335	8560	0.168	0.189	1460	0.0576	0.363	1.89	9.10	0.942
ELOQ	10000	20000	10000	20000	2.00	4.00	10000	1.00	10.0	40.0	40.0	1.00

Table 4

Serum Interday Analysis Results (ng element/mL sample)

Sample	Average	Ca[a]	K[a]	Mg[a]	Na[a]	Cr	Mn	Fe	Co	Cu	Zn	Se	Mo
1	Day 1	93.7	217	15.2	2430	ND[b]	1.14	74.1	0.238	1420	721	107	3.05
	Day 2	93.4	213	14.8	2070	3.06	2.07	424	0.329	1430	768	105	4.41
	Day 3	95.3	214	14.8	2340	4.27	2.38	615	0.160	1400	781	116	3.38
	Average	**94.2**	**215**	**14.9**	**2280**	**3.66**	**1.86**	**371**	**0.242**	**1417**	**757**	**109**	**3.61**
	%RSD	**0.872**	**0.729**	**1.37**	**6.71**	**23.4**	**28.2**	**60.4**	**28.4**	**0.882**	**3.43**	**4.63**	**16.0**
2	Day 1	92.9	206	17.4	2510	ND	2.02	242	0.198	985	724	114	1.04
	Day 2	92.0	202	17.5	2250	2.74	2.04	577	0.149	969	716	118	1.89
	Day 3	91.5	194	16.7	2220	1.75	ND	2330	0.120	917	723	128	0.714
	Average	**92.1**	**200**	**17.2**	**2327**	**2.24**	**2.03**	**1049**	**0.156**	**957**	**721**	**120**	**1.21**
	%RSD	**0.635**	**2.36**	**1.93**	**5.60**	**31.2**	**0.515**	**87.3**	**20.7**	**3.01**	**0.45**	**4.54**	**40.8**
3	Day 1	93.2	238	18.8	2570	ND	0.803	353	0.251	972	779	119	1.32
	Day 2	94.8	237	18.9	2350	1.98	1.08	298	0.469	970	776	118	2.88
	Day 3	92.1	223	17.7	2350	3.63	ND	16.9	ND	927	761	133	0.554
	Average	**93.3**	**233**	**18.5**	**2423**	**2.80**	**0.940**	**223**	**0.360**	**956**	**772**	**123**	**1.58**
	%RSD	**1.20**	**2.99**	**2.80**	**4.29**	**41.7**	**14.6**	**66.1**	**30.2**	**2.16**	**1.05**	**5.35**	**61.0**
4	Day 1	98.3	228	17.7	2440	ND	2.12	760	0.251	887	892	134	1.01
	Day 2	96.1	218	17.8	2320	3.78	3.44	1080	0.749	900	926	141	4.08
	Day 3	93.7	206	16.4	2240	ND	44.0	2230	0.480	834	921	142	1.15
	Average	**96.0**	**217**	**17.3**	**2333**	**3.78**	**16.5**	**1357**	**0.493**	**873**	**913**	**139**	**2.08**
	%RSD	**1.94**	**4.24**	**3.56**	**3.52**	**NA**	**118**	**46.5**	**41.2**	**3.26**	**1.63**	**2.68**	**67.9**
5	Day 1	97.4	231	22.1	2610	2.77	1.26	520	0.291	1090	705	123	0.918
	Day 2	95.8	221	21.9	2300	5.81	1.73	292	1.32	1100	680	123	2.34
	Day 3	96.0	211	21.2	2370	1.55	ND	448	ND	1030	713	129	ND
	Average	**96.4**	**221**	**21.7**	**2460**	**3.38**	**1.50**	**420**	**0.805**	**1073**	**699**	**125**	**1.63**

Sample	Average	Ca[a]	K[a]	Mg[a]	Na[a]	Cr	Mn	Fe	Co	Cu	Zn	Se	Mo
	%RSD	**0.736**	**3.70**	**1.85**	**5.40**	**53.1**	**15.6**	**22.7**	**63.8**	**2.87**	**2.00**	**2.28**	**61.84**
6	Day 1	98.3	225	20.6	2680	ND	2.14	857	0.291	1040	734	126	1.40
	Day 2	98.9	220	20.9	2220	2.77	6.22	460	0.311	1040	642	124	2.41
	Day 3	89.9	190	18.5	2280	4.23	9.44	738	0.300	941	682	126	1.61
	Average	**95.7**	**212**	**20.0**	**2393**	**3.50**	**5.93**	**685**	**0.301**	**1007**	**686**	**125**	**1.81**
	%RSD	**4.27**	**7.35**	**5.47**	**8.53**	**29.4**	**50.3**	**24.2**	**2.72**	**4.63**	**5.49**	**0.79**	**24.1**
7	Day 1	89.8	212	20.2	2540	ND	0.903	410	0.758	1330	558	125	1.19
	Day 2	90.4	209	20.6	2090	3.01	15.8	736	1.69	1230	580	124	1.32
	Day 3	91.4	205	20.0	2420	3.43	10.0	640	0.480	1250	558	124	1.37
	Average	**90.5**	**209**	**20.2**	**2320**	3.22	8.91	596	0.976	1270	565	124	1.29
	%RSD	**0.724**	**1.49**	**1.20**	**8.20**	9.16	68.9	23.0	53.0	3.40	1.85	0.44	5.73
8	Day 1	89.7	211	17.5	2440	3.39	0.743	317	0.131	1280	697	103	1.60
	Day 2	93.0	210	18.2	2020	ND	1.74	561	0.349	1160	659	110	1.33
	Day 3	96.4	216	18.5	2460	ND	8.46	357	0.200	1240	684	112	2.17
	Average	**93.0**	**212**	**18.0**	**2307**	3.39	3.65	412	0.227	1227	680	108	1.70
	%RSD	**2.92**	**1.12**	**2.37**	**8.80**	NA	94.0	25.9	40.0	4.07	2.31	3.33	20.7
9	Day 1	100	230	17.9	2700	ND	12.7	4040	0.131	1260	799	137	1.86
	Day 2	96.7	213	17.4	2220	2.86	1.84	1270	0.189	1210	810	137	1.61
	Day 3	95.5	211	16.8	2450	ND	ND	768	0.360	1180	815	149	2.23
	Average	**97.3**	**218**	**17.4**	**2457**	2.86	7.27	2026	0.227	1217	808	141	1.90
	%RSD	**1.89**	**3.91**	**2.49**	**7.98**	NA	74.6	71.0	42.8	2.71	0.83	3.90	13.5
10	Day 1	95.6	236	13.2	2510	14.2	3.66	546	0.318	1380	632	128	1.73
	Day 2	92.2	219	12.7	1970	1.50	1.74	815	0.709	1320	631	129	2.02
	Day 3	96.3	228	13.0	2380	ND	7.02	655	0.240	1340	653	139	1.46
	Average	**94.7**	**227**	**13.0**	**2287**	7.83	4.14	672	0.422	1346	639	132	1.74
	%RSD	**1.86**	**3.07**	**1.37**	**10.1**	114	52.71	16.4	48.5	1.84	1.58	3.99	13.2

Sample	Average	Ca[a]	K[a]	Mg[a]	Na[a]	Cr	Mn	Fe	Co	Cu	Zn	Se	Mo
Pool	Day 1	95.4	222	17.5	2500	3.17	1.98	1930	0.411	1170	740	127	1.54
	Day 2	96.3	220	18.5	2170	ND	45.8	1710	0.509	1190	757	127	2.01
	Day 3	97.5	229	18.1	2560	2.51	24.3	1410	1.54	1120	724	137	2.21
	Average	**96.4**	**224**	**18.0**	**2410**	2.84	24.0	1683	0.820	1160	740	130	1.92
	%RSD	**0.897**	**1.72**	**2.42**	**7.12**	16.5	74.4	12.7	62.3	2.53	1.79	3.49	14.5
Average		**94.3**	**217**	**17.8**	**2360**	**3.67**	**5.27**	**781**	**0.421**	**1134**	**724**	**125**	**1.86**
% Diff from pool		**−2.12**	**−3.25**	**−1.09**	**−2.06**	**29.1**	**−78.1**	**−53.6**	**−48.7**	**−2.20**	**−2.19**	**−4.30**	**−3.34**

[a] Values reported in units of μg/mL

[b] Values were not found to be greater than the IDL (Table 3)

Biol Trace Elem Res. Author manuscript; available in PMC 2015 July 01.

Table 5

Whole Blood Interday Analysis Results (ng element/mL sample)

Sample	Average	Ca[a]	K[a]	Mg[a]	Na[a]	Cr	Mn	Fe[a]	Co	Cu	Zn	Se	Mo
1	Day 1	60.1	1720	28.7	1590	ND[b]	15.6	500	0.507	950	7627	211	ND
	Day 2	59.8	1790	30.2	1730	2.33	18.2	491	0.543	953	8372	211	2.38
	Day 3	62.1	1650	28.5	1710	ND	12.4	493	0.523	976	8828	225	1.63
	Average	**60.7**	**1720**	**29.1**	**1677**	**2.33**	**15.4**	**495**	**0.525**	**959**	**8276**	**216**	**2.01**
	%RSD	**1.69**	**3.31**	**2.55**	**3.69**	**NA**	**15.4**	**0.805**	**4.88**	**1.22**	**5.98**	**3.07**	**26.7**
2	Day 1	66.1	2010	35.8	1530	1.70	27.8	494	0.700	1022	6493	204	1.09
	Day 2	58.6	2040	35.1	1580	1.41	61.3	476	0.823	1060	6574	204	1.74
	Day 3	61.3	1840	33.4	1460	ND	48.3	476	0.669	1010	6676	215	1.30
	Average	**62.0**	**1963**	**34.7**	**1523**	**1.56**	**38.0**	**482**	**0.761**	**1031**	**6581**	**208**	**1.52**
	%RSD	**4.99**	**4.49**	**2.86**	**3.22**	**13.1**	**44.4**	**1.77**	**11.4**	**2.08**	**1.14**	**2.56**	**20.7**
3	Day 1	65.9	1860	40.8	1680	ND	16.8	511	0.567	1039	5945	163	1.29
	Day 2	58.2	1850	39.0	1590	0.491	15.7	474	0.410	1005	6419	169	1.78
	Day 3	59.9	1690	36.3	1510	0.584	23.5	460	0.269	984	6310	164	ND
	Average	**61.3**	**1800**	**38.7**	**1593**	**0.538**	**19.6**	**482**	**0.415**	**1010**	**6225**	**165**	**1.54**
	%RSD	**5.42**	**4.33**	**4.75**	**4.36**	**12.2**	**20.0**	**4.40**	**29.3**	**2.25**	**3.26**	**1.73**	**22.9**
4	Day 1	66.9	1840	33.6	1640	0.420	12.7	492	0.513	1363	6454	208	1.71
	Day 2	63.3	1800	33.7	1520	9.09	17.0	474	0.530	1330	6704	204	2.98
	Day 3	66.1	1840	32.9	1680	ND	22.1	492	0.243	1376	6950	208	1.04
	Average	**65.4**	**1827**	**33.4**	**1613**	**4.76**	**17.3**	**486**	**0.429**	**1356**	**6703**	**207**	**1.91**
	%RSD	**2.32**	**1.03**	**1.06**	**4.21**	**129**	**22.1**	**1.77**	**30.7**	**1.42**	**3.02**	**0.842**	**51.7**
5	Day 1	58.7	1740	37.5	1600	ND	18.7	489	0.647	710	5662	194	1.65
	Day 2	57.0	1720	37.8	1520	0.911	19.0	478	0.436	706	6182	191	2.13
	Day 3	56.4	1680	35.1	1570	0.584	17.8	467	0.269	700	6258	189	5.16
	Average	**57.4**	**1713**	**36.8**	**2460**	**0.748**	**18.5**	**478**	**0.451**	**705**	**6034**	**191**	**2.98**

Sample	Average	Ca[a]	K[a]	Mg[a]	Na[a]	Cr	Mn	Fe[a]	Co	Cu	Zn	Se	Mo
	%RSD	**1.73**	**1.46**	**3.27**	**1.34**	**31.0**	**2.65**	**1.90**	**34.3**	**0.569**	**4.39**	**1.19**	**52.2**
6	Day 1	61.4	1690	37.1	1680	1.22	20.1	465	0.447	1136	6923	198	1.75
	Day 2	60.9	1610	37.7	1570	0.391	18.3	455	0.396	1117	7160	201	1.82
	Day 3	61.0	1660	36.0	1720	ND	27.5	455	0.243	1144	7312	194	1.92
	Average	**61.1**	**1653**	**36.9**	**1657**	**0.806**	**22.0**	**458**	**0.362**	**1132**	**7132**	**198**	**1.79**
	%RSD	**0.348**	**1.99**	**1.90**	**3.83**	**72.7**	**18.0**	**1.04**	**24.0**	**0.989**	**2.24**	**1.35**	**4.86**
Pool	Day 1	64.1	1730	34.9	1690	0.660	17.4	477	0.540	1016	6355	195	2.17
	Day 2	59.6	1690	35.5	1500	0.705	16.0	466	0.410	992	6416	194	2.06
	Day 3	64.7	1830	35.3	1750	3.74	23.0	492	1.22	1040	6136	179	ND
	Average	**62.8**	**1750**	**35.2**	**1647**	**1.70**	**18.8**	**478**	**0.722**	**1016**	**6302**	**189**	**2.12**
	%RSD	**3.62**	**3.36**	**0.73**	**6.47**	**84.8**	**16.0**	**2.20**	**49.0**	**1.93**	**1.91**	**3.88**	**3.41**
Average		**61.3**	**1779**	**35.0**	**1754**	**1.79**	**21.8**	**480**	**0.490**	**1032**	**6825**	**197**	**1.96**
% Diff from pool		**−2.40**	**1.68**	**−0.725**	**6.51**	**5.05**	**15.9**	**0.397**	**−32.1**	**1.58**	**8.29**	**4.49**	**−7.52**

[a] Values reported in units of μg/mL

[b] Values were not found to be greater than the IDL (Table 3)

Table 6

Elemental ranges for whole blood and serum

Serum Analysis Observed Concentration Range				
Element	**Max**	**Min**	**Median**	**Ref range[b]**
Ca[a]	99.9	89.7	94.4	91–106
K[a]	238	190	216	149–215
Mg[a]	22.1	12.7	17.7	17–22
Na[a]	2700	1970	2330	3103–3402
Cr	14.2	ND	3.38	0.12–2.1
Mn	44.0	ND	3.89	0.54–1.76
Fe	6410	ND	634	750–1500
Co	3.69	ND	0.330	0.11–0.45
Cu	1430	834	1145	800–1750
Zn	926	558	710	700–1200
Se	149	103	125	46–143
Mo	4.41	ND	1.72	0.55–3.00
Whole Blood Analysis Observed Concentration Range				
Element	**Max**	**Min**	**Median**	**Ref range[b]**
Ca[a]	66.9	56.4	61.2	44.4–65.3
K[a]	2040	1610	1760	1922–2108
Mg[a]	40.8	28.5	35.8	69.8–98.2
Na[a]	1730	1460	1635	1372–1568
Cr	9.09	ND	1.18	2.8–45
Mn	61.3	12.4	19.1	8.0–18.7
Fe[a]	521	445	482	309–521
Co	0.823	0.243	0.440	0.04–0.8
Cu	1376	700	1020	800–1300
Zn	8828	5662	6642	4400–8600
Se	225	163	202	58–234
Mo	5.16	ND	1.85	0.8–3.3

[a] Values reported in units of μg/mL

[b] Values taken from literature.[22,19,26–28]