Simultaneous analysis of 25 trace elements in micro volume of human serum by inductively coupled plasma mass spectrometry (ICP-MS)

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ABSTRACT

Introduction: In recent years, trace elements have gained importance as biomarkers in many chronic diseases. Unfortunately, the requirement for sample volume increases with the extent of investigation either for diagnosis or elucidating the mechanism of the disease. Here, we describe the method development and validation for simultaneous determination of 25 trace elements (lithium [Li], beryllium [Be], magnesium [Mg], aluminium [Al], vanadium [V], chromium [Cr], manganese [Mn], iron [Fe], cobalt [Co], nickel [Ni], copper [Cu], zinc [Zn], gallium [Ga], arsenic [As], selenium [Se], rubidium [Rb], strontium [Sr], silver [Ag], cadmium [Cd], caesium [Cs], barium [Ba], mercury [Hg], thallium [Tl], lead [Pb], uranium [U]) using only 20 μL of human serum.
Methods: Serum samples were digested with nitric acid and hydrochloric acid (ratio 1:1, v/v) and analysed by inductively coupled plasma–mass spectrometry (ICP-MS). Seronorm®, a human-derived serum control material was used as quality control samples.
Results: The coefficient of variations for both intra- and inter-day precisions were consistently <15% for all elements. The validated method was later tested on 30 human serum samples to evaluate its applicability.
Conclusion: We have successfully developed and validated a precise and accurate analytical method for determining 25 trace elements requiring very low volume of human serum.

1. Introduction

Trace elements are essential components in the biological structures of cells and have been reported to play important roles in human metabolic and physiological processes [1]. Significant differences in the levels of trace elements have been observed in many diseases such as breast cancer [2], acute leukemia [3], diabetes [4] and Parkinson's disease [5]. It has been postulated that the pathological events associated to trace elements were driven by activation in pathways closely linked to genotoxicity [6], endocrine modifications

[7], receptor mediation [8], immunosuppression [9], tumour promotion [10], tissue-specific toxicity and inflammatory responses [11].

Previously, trace elements levels have been analysed in various human biological samples such as hair [12], nails [13], tissues [14], and body fluids (cerebrospinal fluid [15], urine [16], saliva [17], blood [18]) for mapping the toxicological profiles [19] and measuring the occupational exposures [20]. Samples from tissues and body fluids have been demonstrated to provide the most reliable assessment of trace element status in the human body [21]. Nevertheless, the preferred approach for trace elements' assessment is by non- or less-invasive techniques where sample collection is concerned. As such, testing for circulating levels of trace elements in the blood has been commonly opted in most studies [22–25].

Advances in modern instrumentation, particularly from the aspect of the system's performance and capability in acquiring high resolution data have been useful in gaining insights into disease-associated changes in the levels of trace elements in the human body [26]. At present, simultaneous detection of multiple trace elements is made possible with the use of inductively coupled plasma mass spectrometer (ICP-MS) [27]. Despite having the advantage of powerful instrumentation, the use of ICP-MS is constrained by the inherent lack of coverage for all elements in one single analysis. The existing methods using ICP-MS have generally assessed an only limited number of elements ranging from seven [28] to 20 [29]. This is predominantly attributable to the dynamic range of isotopes and abundance of different elements in the human body [30]. To fit a wide linear dynamic range into one single analytical method remains a massive challenge to the researchers, let alone with the limited sample volume provided. The volume of sample for trace elements analyses has varied between authors, instruments and number of trace elements of interest [28,29,31–33]. To date, the smallest volume of serum reported for use in multi–trace element analysis was 150 μL [28] whereas the highest volume was 2 mL [30,32].

Therefore, the aim of this study is to develop and validate a method for simultaneous determination of 25 trace elements (lithium [Li], beryllium [Be], magnesium [Mg], aluminium [Al], vanadium [V], chromium [Cr], Mn, iron [Fe], cobalt [Co], nickel [Ni], copper [Cu], zinc [Zn], gallium [Ga], arsenic [As], selenium [Se], rubidium [Rb], strontium [Sr], silver [Ag], cadmium [Cd], caesium [Cs], barium [Ba], mercury [Hg], thallium [Tl], lead [Pb], uranium [U]) using the minimum possible volume of human serum.

2. Methods

2.1. Instrumentation

Sample preparation was conducted in a fume hood to avoid atmospheric particulate contamination and a heated dry block was used for acidic sample digestion. Data acquisition was performed on the Agilent 7700x ICP-MS (Agilent Technologies, USA) with rotary pump and integrated sample introduction system for discrete sampling (ISIS-DS). The system was equipped with MassHunter Workstation Revision B.01.01 for instrument control and data handling software. In brief, the ICP-MS was operated in full quantitative mode with a Ni sampler and skimmer cones, MicroMist glass concentric nebulizer and quartz Scott-type spray chamber. Helium (He) collision mode was used for the multi-element analysis and the instrument was tuned to optimal conditions daily using tuning solutions (Agilent Technologies, USA) prior to analysis. The instrument's settings and operating parameters applied in the method are detailed in Table 1.

2.2. Reagents and standard solutions

Deionized (DI) water (18.2 MΩ, Siemens, USA) was used to prepare all aqueous solutions. Tuning solution, PA Tuning 1 and 2 solutions, Hg stock, mix stock standard solution (consisted of Li, Be, Mg, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Tl, Pb, U, calcium (Ca), potassium (K) and sodium (Na), each at 1000 mg/L) and internal standard solution (ISTD, containing scandium (Sc), germanium (Ge), indium (In), bismuth (Bi), rhodium (Rh), terbium (Tb) and lutetium (Lu), each at 1000 mg/L) were acquired from Agilent Technologies (USA), whereas nitric acid (65% HNO_3) and hydrochloric acid (30% HCl) were from Merck (Germany). The acids used were of purified grade with specification ≥99.999% trace metals basis. All laboratory wares, such as the micropipettor tips, tubes and vials, were cleaned by an overnight soak in 20% v/v HNO_3 and rinsed with DI water.

The analytical blank solution consisted of DI water spiked with 2000 μg/L ISTD. Six-point calibration standards at suitable ranges were generated for each element of interest. A series of 0, 0.2, 0.5, 1, 2 and 5 μg/L calibrators were used for determining Be, Cr, Mn, Co,

Table 1
Agilent 7700x ICP-MS operating parameters.

Parameter	Value
Plasma conditions	Forward power 1550 W
Plasma gas flow	15.0 L/min
Carrier gas flow	0.75 L/min
Dilution gas flow	1 L/min
He gas flow	4.5 mL min^{-1}
QP bias	-15 V
Oct bias	-18 V
Cell entrance	-40 V
Cell exit	60 V
Deflect	-0.8 V
Plate bias	-60 V
Nebulizer type	MicroMist
Sample uptake rate	1.5 mL/min

Ni, Ag, Tl and Pb; 0, 200, 500, 1000, 5000 and 10,000 μg/L were used for calibrating element Li and Mg; and 0, 10, 20, 50, 100 and 200 μg/L were used for calibrating the remaining elements.

2.3. Serum samples collection and preparation

Blood samples (3 mL) were collected in Vacutainer plain tubes (Becton-Dickinson, USA). The samples were allowed to stand for 10 min at room temperature prior to centrifugation at 4000 rpm, 4 °C for 10 min. The resultant supernatant layers were aliquoted into 1.5 mL tubes and stored at -80 °C until analysis. The serum samples were thawed overnight at 4 °C prior to digestion. On the day of analysis, the thawed samples were homogenised by gentle mixing using micropipette and 20 μL of each sample was transferred to its respective pre-cleaned 15 mL polypropylene tube, followed by 360 μL of concentrated HNO_3:HCl (1:1, v/v) and 2000 μg/L ISTD. The mixture was mixed vigorously and placed onto a heat block digester with set temperature at 90–95 °C for 2–3 h until the samples turned clear. After digestion, the samples were diluted with 2 mL of DI water, followed by further dilution into five-fold and 20-fold dilutions each prior to the analysis by ICP-MS.

2.4. Quality control materials and preparation

Seronorm® Trace Elements Serum Level 1 and 2 (Sero AS, Norway) were used as the reference and control samples during method development, validation and application. These human-derived multi-element serum control materials enabled quality assessment at two concentration levels (high and low) per element. Prior to analysis, the control materials were reconstituted according to the manufacturer's protocol and processed similarly as described for the serum samples.

2.5. Method validation

Quantification was performed by calculating the count ratio of each element of interest to the element in ISTD. System suitability of the method was assessed by the repeatability of the count obtained for all of the elements of interest and in ISTD. The limit of detection (LOD) was calculated as 3.3 times the standard deviation of 10 replicates of the instrument blank whereas the linearity was evaluated by analysing a series of standard concentrations (calibration curves) generated over the range of 0–5 μg/L, 0–200 μg/L or 0–10,000 μg/L. Recovery analysis on the extraction method was performed at three concentration levels for each respective calibration range (0.2, 2 and 5 μg/L; 10, 100 and 200 μg/L; 500, 5000 and 10000 μg/L) in three replicates. The percentages of recovery for all elements in the range were calculated by averaging the difference in extracted sample count over the non-extracted sample count and presented as mean recovery.

Intra-day and inter-day precision and accuracy were determined using three replicates of the QC samples. Results were expressed as percentage of coefficient of variation (CV %) for precision and percentage of bias for accuracy. The acceptance range for precision was set at 15% or lower for the calibrators and QC samples whereas 20% or lower for limit of quantitation (LOQ) concentration level. Similarly, the acceptance mean value for accuracy was set at <15% bias of the nominal value and <20% bias at the LOQ level.

Table 2
Linear range, ISTD, tuning mode, LOD, LOQ and regression correlation coefficient for 25 trace elements measured.

Mass number	Element	Linear range(μg/L)	ISTD	Tuning modes	LOD(μg/L)	R^2
7	Li	0-10,000	^{72}Ge	No gas	11.6500	0.996
9	Be	0–5	^{45}Sc	No gas	0.0109	0.990
24	Mg	0-10,000	^{72}Ge	No gas	0.9567	1.000
27	Al	0–200	^{45}Sc	No gas	0.9568	1.000
51	V	0–5	^{45}Sc	He	0.0807	0.999
52	Cr	0–5	^{45}Sc	He	0.2460	0.998
55	Mn	0–5	^{45}Sc	He	0.7321	0.998
56	Fe	0–200	^{45}Sc	He	2.3160	1.000
59	Co	0–5	^{45}Sc	He	0.0344	0.999
60	Ni	0–5	^{45}Sc	He	0.3380	1.000
63	Cu	0–200	^{72}Ge	He	1.5320	1.000
66	Zn	0–200	^{103}Rh	He	2.1520	0.999
69	Ga	0–5	^{45}Sc	He	0.0135	0.996
75	As	0–5	^{103}Rh	He	0.0065	0.996
78	Se	0–200	^{103}Rh	He	0.0858	0.998
85	Rb	0–5	^{159}Tb	He	0.2700	0.995
88	Sr	0–200	^{159}Tb	He	0.5976	1.000
107	Ag	0–5	^{103}Rh	He	0.1243	1.000
111	Cd	0–5	^{72}Ge	He	0.0067	1.000
133	Cs	0–5	^{159}Tb	He	0.0073	0.999
138	Ba	0–200	^{72}Ge	He	0.1192	1.000
202	Hg	0–200	^{175}Lu	He	0.0339	0.996
205	Tl	0–5	^{175}Lu	He	0.0308	1.000
208	Pb	0–5	^{159}Tb	He	0.0467	0.999
238	U	0–5	^{72}Ge	He	0.0015	1.000

2.6. Application on human serum samples

Upon successful development and validation on the analytical method, the method was further applied to 30 human adults' serum samples collected from volunteers aged 40–70 years. The volunteers were informed and consented to participate in the study. The ethics approval for this study was obtained from the Ethics and Research Committee of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) (FF-2015-380).

3. Results

3.1. System suitability

Each trace element was first tested at several fixed concentrations (50, 100 and 200 μL) to assess the system performance at the set operating parameters and run mode. Data obtained demonstrates mode-dependent high repeatability of the count ratio (data not shown). We observed consistent count ratio in low mass elements (Li, Be, Mg and Al) acquired in no gas mode whereas higher mass elements (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, Cs, Ba, Hg, Tl, Pb and U) in He gas mode. The sensitivity and linearity of each element were further assessed according to the acquisition mode determined at this phase.

3.2. Sensitivity and linearity

As hypothesised, different linear concentration ranges were observed for all the measured elements. We further grouped the elements into three categories according to their linear ranges (0–5, 0–200 and 0–10,000 μg/L) and reported endogenous abundance in human serum [34], thereby the subsequent validation of the method is reported based on these categories (Table 2). The detection limit (LOD) varied between elements with the lowest detection at 0.001 μg/L for U whereas LOQ was set as the lowest concentration (non-zero) on the calibration curve measured for each element. The R^2 values for all calibration curves and elements were equal or greater than 0.990. From an array of the dilution factors being tested ranging from 5 to 200, we have found that factors of 5 and 20 were best suited for our established quantification ranges in this multi-element analysis.

3.3. Recovery

Recovery analysis was performed to assess sample loss during the extraction process. The recovery values achieved were all above 90 ($\pm$6.4) and within the acceptability range of 80–120%. The data obtained is summarized in Table 3. In the subsequent analysis, ISTD was applied to all calibrators, QCs and samples to correct for variations.

3.4. Determination of sample volume

After confirmation on the calibration range for each element, we proceeded with determination of serum sample volume required for the analysis. The aim was to select the lowest volume requirement with adequate coverage without compromising the sensitivity and linearity. We have performed analyses on several volumes of Seronorm Trace Elements Serum Level 1 and 2 (20, 25, 50 and 100 μL) and assessed the accuracy and precision. The obtained data reveals comparatively equivalent reproducibility for sample volume as low as 20 μL (Table 4).

3.5. Accuracy and precision

Next, we examined the accuracy and precision of the method by analysing the sample replicates of 20 μL Seronorm Trace Elements Serum Level 1 and 2 in three sets of triplicates each for intra- and inter-day assessment. Table 5 shows good precision was achieved for all the targeted elements with CV values within the acceptable range. However, only 15 elements were within the accuracy range (bias %) as per compared to the nominal value as provided by the Seronorm product. Slightly higher bias values were obtained for Al, Hg and

Table 3
Recovery of trace elements from 20 μL serum.

Linear Range (μg/L)	Nominal value (μg/L)	Mean recovery, % (CV %)[a]
0-5 (Be)	0.2	93.5 (7.55%)
	2	95.6(4.32%)
	5	101.4(6.10%)
0-200 (Cu)	10	90.7(3.42%)
	100	95.7(1.20%)
	200	97.2(1.12%)
0-10,000 (Li)	500	112.6(1.12%)
	5000	98.4(0.80%)
	10,000	92.8(0.60%)

[a] (Extracted sample count – Non-extracted sample count)/Non-extracted sample count.

Table 4
Precision and accuracy for various sample volume tested during method development.

Mass number	Element	Seronorm® certified value	Seronorm® certified value(μg/l)	Sample Volume							
				100uL		50uL		25uL		20uL	
				CV(%)	Bias(%)	CV(%)	Bias(%)	CV(%)	Bias(%)	CV(%)	Bias(%)
7	Li	L1	5261	0.78	-0.45	0.77	-0.78	0.75	-0.90	0.75	-0.90
9	Be	Ll	0.01	5.86	0.00	5.45	0.00	6.15	0.00	6.15	0.00
24	Mg	L1	16800	0.05	-6.70	0.05	-6.00	0.05	-7.00	0.05	-7.00
27	Al	L1	46.1	3.78	17.90	3.77	17.70	3.67	17.60	3.65	17.80
52	Cr	L2	5.7	3.56	-3.30	3.46	-3.50	3.80	-4.50	3.89	-4.40
55	Mn	L1	9.9	2.90	2.00	2.95	2.00	2.99	2.00	2.80	2.00
56	Fe	L2	2150	9.57	0.20	10.45	0.30	9.78	0.33	9.54	0.30
59	Co	L2	3.05	0.64	9.56	0.89	10.11	0.70	12.45	0.81	14.40
60	Ni	L1	5.64	4.28	-1.20	3.68	-1.20	4.99	-1.40	4.97	-1.60
63	Cu	L2	1850	1.67	3.72	1.90	4.56	1.89	4.70	1.87	4.10
66	Zn	L2	1617	4.55	1.30	3.89	1.30	4.66	1.30	4.33	1.30
78	Se	L2	138	1.08	-4.20	1.68	-4.50	1.09	-4.50	1.08	-4.70
88	Sr	L1	95	5.98	4.10	6.89	4.10	5.99	4.50	5.98	4.70
107	Ag	L2	0.22	7.93	0.20	8.91	0.20	12.87	0.20	12.86	0.00
138	Ba	L2	135	4.56	-10.40	4.88	-11.40	4.19	-12.60	4.18	-12.20
202	Hg	L2	2.05	3.78	15.60	3.79	16.50	5.89	16.90	7.37	17.10
208	Pb	L1	0.4	4.78	15.00	4.78	15.20	2.45	15.10	2.53	-15.10
238	U	L1	0.302	3.56	-6.40	3.78	-8.90	7.70	-8.90	10.88	-8.00

Pb but they remained below 20%. However, the measured values of V, Ga, As, Rb, Cd, Cs and Tl were off the acceptable range in this analysis. It is important to note that the Seronorm-certified values provided for V, Ga, As, Rb, Cd, Cs and Tl were not measured by ICP-MS, instead by either inductively coupled plasma optical emission spectrometry (ICP-OES), inductively coupled plasma sector field mass spectrometry (ICP-SFMS) or inductively coupled plasma atomic emission spectroscopy (ICP-AES).

3.6. Application on human serum samples

The validated method was successfully applied to the adults' serum samples. The values measured were within the quantification range and the representative box-plots of the elements measured were presented in Fig. 1.

4. Discussion

Several parameters were considered and evaluated during the phase of method development in this study. First, the acquisition mode of ICP-MS was established for the acquisition of each element of interest. This is a critical step prior to any testing as it determines the core setting for detection. We observed consistent results with no-gas mode for low mass elements below mass 40, in line with previous studies [35,36]. As for high-mass elements above 40, the introduction of helium (He) gas into the front-end improved the consistency of the results tremendously. High-mass elements were reported as more vulnerable to spectral interference; therefore, application of a non-reactive gas such as He can resolve this issue via the collision mode with kinetic energy discrimination (KED) [31].

The main objective of our method development was to attempt the use of one linear range for quantification of all elements of interest. However, due to the wide and dynamic ranges of the trace elements' abundance in the human body [34], the ideal one linear range for all elements was unachievable from the bioanalytical's point of view as we do not want the detection to be scattered at the extreme end of the calibration curve. Eventually, we decided on three linear concentration ranges for the analysis of 25 trace elements in accordance to their abundance in the serum. This strategy was previously adopted by Bravo et al. [32] and Liu et al. [37] in which the use of three to four linear concentration ranges were reported.

Inclusion of ISTD is imperative in trace element analysis, as ISTDs are used to correct for changes in analyte sensitivity caused by variations in the concentration and type of matrix components in the sample [27]. As a non-analyte isotope, ISTD was added to the blank solution, standards and samples before the analysis. Commonly, three or four internal elements are added to the samples for full coverage of an array of analytes [27]. In this work, we applied four elements as ISTDs to correct for 25 elements of interest. The ISTDs were selected close in mass number to the analyte elements. Adjustments in the eventual calculation were made by incorporating the count value of ISTD into the analytes' count from the unknown samples, QCs and calibrators. This approach will correct and improve the precision of the quantitative analysis in this work.

Nonetheless, the most critical aspect in a method development that is also possibly the largest source of errors is during the sample preparation process [38]. The sample itself comprised of a complex matrix that undoubtedly contributes to the disturbance of the background spectrum [39]. In addition, the major elements present in the solvents or acids used during sample preparation (e.g. nitrogen [N], sulfur [S], chloride [Cl]) may also participate in these reactions, thus directly affecting the true measurement. It is necessary to overcome the problem of matrix complexity but unfortunately, no single sample preparation technique would meet all analysis requirements, given the large number of analytes and variety of sample types. Among the sample preparation strategies that are used most often are acid digestion and dilution [40]. Numerous studies reported the use of acid digestion technique as the best pre-treatment

Table 5

ICP-MS method validation results for multi element in the serum reference material.

Mass nu	Element	Seronorm® certified value	Seronorm® certified value(µg/l)	DF	Intraday		Interday		CV(%)		Bias(%)	
					Means	SD	Means	SD	Intraday	Interday	Intraday	Interday
7	Li	L1	5261	5x	5212.50	38.89	5230.67	41.79	0.75	0.80	-0.9	-0.6
9	Be	L1	0.01	20x	0.01	0.00	0.01	0.00	6.15	8.33	0.0	0.0
24	Mg	L1	16800	5x	15618.00	7.07	15834.33	374.73	0.05	2.37	-7.0	-5.7
27	Al	L1	46.1	20x	54.30	1.98	54.40	1.41	3.65	2.59	**17.8**	**18.0**
52	Cr	L2	5.70	5x	5.45	0.21	5.52	0.20	3.89	3.56	-4.4	-3.1
55	Mn	L1	9.9	5x	10.10	0.28	10.20	0.26	2.80	2.59	2.0	3.0
56	Fe	L2	2150.00	20x	2156.95	205.70	2158.00	145.47	9.54	6.74	0.3	0.4
59	Co	L2	3.05	20x	3.49	0.03	3.36	0.23	0.81	6.91	14.4	10.2
60	Ni	L1	5.64	20x	5.55	0.28	5.51	0.20	4.97	3.67	-1.6	-2.3
63	Cu	L2	1850.00	5x	1925.50	36.06	1873.33	93.88	1.87	5.01	4.1	1.3
66	Zn	L2	1617	20x	1638.10	70.97	1655.18	58.26	4.33	3.52	1.3	2.4
78	Se	L2	138	5x	131.50	1.41	131.00	1.32	1.08	1.01	-4.7	-5.1
88	Sr	L1	95	5x	99.51	5.95	96.34	6.92	5.98	7.18	4.7	1.4
107	Ag	L2	0.22	5x	0.22	0.03	0.23	0.02	12.86	10.19	0.0	4.5
138	Ba	L2	135	20x	118.50	4.95	122.33	7.51	4.18	6.14	-12.2	-9.4
202	Hg	L2	2.05	5x	2.40	0.18	2.45	0.14	7.37	5.67	**17.1**	**19.5**
208	Pb	L1	0.4	20x	0.56	0.01	0.55	0.02	2.53	2.76	**-15.1**	**-16.6**
238	U	L1	0.302	5x	0.33	0.04	0.33	0.03	10.88	7.70	-8.1	-8.0
Certified value provided by manufacturer and were measured by non ICP-MS												
51	V	L2	1.10	20x	4.12	0.16	4.09	0.12	3.78	2.97	274.5	271.8
69	Ga	L1	0.015	20x	0.24	0.02	0.23	0.02	9.03	7.53	1500.0	1433.3
75	As	L2	0.38	5x	0.19	0.01	0.18	0.02	3.82	8.65	-50.0	-52.6
85	Rb	L1	4.4	5x	6.15	0.07	6.00	0.26	1.15	4.41	-29.3	-31.0
111	Cd	L2	0.14	5x	0.25	0.03	0.24	0.03	11.31	12.91	78.6	71.4
133	Cs	L2	0.026	5x	0.84	0.01	0.84	0.01	0.85	1.19	3130.7	3130.7
205	Tl	L2	0.108	20x	0.25	0.01	0.24	0.01	2.89	4.17	131.4	122.2

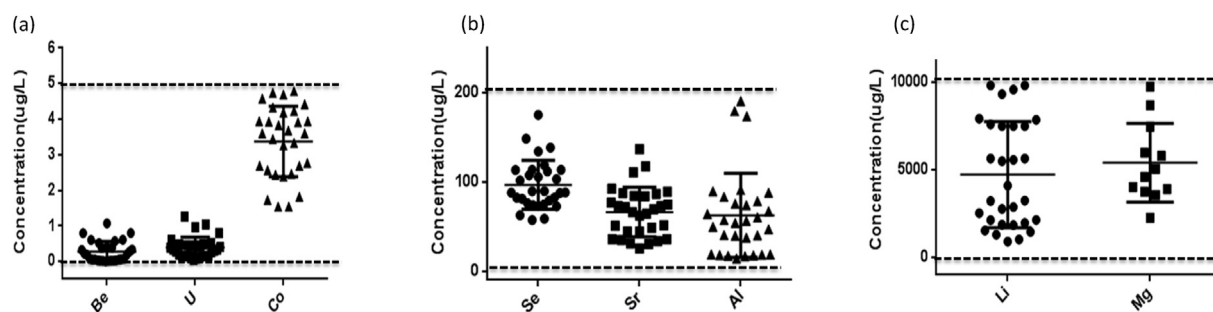Bold indicate value > 15%, DF Dilution factor, SD Standard deviation, CV Coefficient of variation.

Fig. 1. Representative box-plot of trace elements levels in the serum samples of 30 individuals. The levels of trace elements measured were within the calibration range of (a) 0-5μg/L, (b) 0-200μg/L, (c) 0–10,000μg/L.

strategy for converting the matrix components in the serum sample into simple chemical form [33,41–44]. Therefore, we have tested different ratios of acids for our sample digestion and found that the ratio of 1:1 (v/v) concentrated HNO_3:HCl provided the best recovery result (data not shown). Our finding is in accordance to a previous report by Yebra et al. [45]. who supported the use of concentrated HNO_3 and HCl in multi-element analysis The use of different acids' ratio was previously reported for non-serum type of sample [46].

In this work, we report the use of DI water as the diluent for calibrators, QCs and samples despite suggestions of acids and alkalis by previous studies [31,32]. We recorded more precise and accurate concentrations close to the certified values by using only DI water as compared to several combinations of acids and alkalis (data not shown). This finding is in agreement with some of the previous works reported elsewhere [28,29,33] possibly due to the advantage of DI water as an analyte-free matrix compared to any acid or alkali mixtures [47]. Dilution with DI water not only aids in reducing the matrix effect at a higher rate [48], but also avoidsany unnecessary introduction of diluent background.

The overall accuracy in the method as assessed using the serum reference material (Seronorm) was satisfactory except for three elements (Al, Hg and Pb) which slightly exceeded the acceptable cut-off range of 15%. The setbacks may be due to spectral interference and matrix effect as were commonly reported. The detection of Al has been frequently implicated by interference with the other elements present either in the samples or the solutions used [49] thus, leading to inaccuracy in its determination. This issue has been highlighted as an intrinsic limitation of ICP-MS [50]. ICP-MS is also ill-favoured for the determination of Hg. Instead, a single element

Table 6
The measured concentrations of 17 selected serum trace elements in adults (n = 30) as compared to the values reported in the literature.

Element	Measured Value Mean(SD)(μg/l)	Malaysia		Western Region	
	n = 30	Reference value(μg/l)	Authors (year), Instrument	Reference value(μg/l)	Authors (year), Instrument
Li	6827.61(2306.07)	NA	NA	2776–5552	Severus et al. (2008) [63], ICPMS
Be	0.04(0.07)	NA	NA	0.04–0.3	Caroli et al. (1994) [64], AAS
Mg	26933.99(17043.47)	19800–23500	Ishak et al. (2005) [65], GFAAS	0.3–1.48, 14000-18900	Caroli et al. (1994) [64], AAS; Chinyere et al (2005) [66], GFAAS
Al	78.01(14.48)	NA	NA	0.5–8	Caroli et al. (1994) [64], AAS
Cr	6.43(4.79)	NA	NA	0.04–0.48, 6.48–6.75	Caroli et al. (1994) [64], AAS; Satija et al (2014) [17], AAS
Mn	14.23(6.72)	37.1–39.8	Ishak et al. (2005) [65], GFAAS	0.1–2.9	Caroli et al. (1994) [64], AAS
Fe	1826.74(606.19)	NA	NA	1100–1300	Caroli et al. (1994) [64], AAS
Co	3.87(1.63)	NA	NA	0.104–0.262, 0.3–5.1	Forrer et al. (2001) [31], ICPMS; Jantzen et al (2013) [67], NA
Ni	5.12(1.77)	NA	NA	8.26–8.85	Satija et al. (2014) [17], AAS
Cu	1621.73(711.29)	NA	NA	720–1800	Heitland & Koster (2006) [68], ICPMS
Zn	1478.86(582.91)	2662–2890	Ishak et al. (2005) [65], GFAAS	608.03–2013.70	Ghasemi et al. (2012) [69], GFAAS
Se	83.19(38.67)	23.85–33.65	Rejali et al. (2007) [70], AAS	112.7–145, 85-182	Forrer et al. (2001) [31], ICPMS; Heitland & Koster (2006) [68], ICPMS
		86.9–125.5	Ishak et al. (2005) [65], GFAAS		
Sr	65.65(29.35)	NA	NA	49.2–221.2	Forrer et al. (2001) [31], ICPMS
Ag	0.63(0.24)	NA	NA	<0.0017–0.4	Heitland & Koster (2006) [68], ICPMS
Ba	142.29(51.89)	NA	NA	0.17–1.9	Heitland & Koster (2006) [68], ICPMS
Pb	0.50(0.23)	NA	NA	0.12–0.51	Caroli et al. (1994) [64], AAS
U	0.48(0.34)	NA	NA	<0.003–0.006	Heitland & Koster (2006) [68], ICPMS

NA Not available, SD Standard deviation, GFAAS Graphite furnace atomic absorption spectrometry, AAS Atomic absorption spectrometry, ICPMS Inductively Coupled Plasma Mass Spectrometry.

analysis with cold-vapour atomic absorption spectroscopy (CVAAS) was suggested for its determination [51,52] whereby this method had been legally adopted by the United States Environmental Protection Agency (EPA) as a standard guideline procedure [39] for any legal dispute. Nevertheless, there were reports on the assessment of Hg using ICP-MS but at a larger sample volume of at least 100 μL [53] and with the use of alkaline sample digestion [39]. The slightly higher value of Hg as assessed by our method is supported by the findings from Chan et al. who performed a comparative study between CVAAS and ICP-MS in measuring the Hg levels [54]. The group had reported a higher value of Hg for acquisition by ICP-MS. On the other hand, matrix effect has been commonly reported in Pb measurement [55–57]. To overcome this masking issue, researchers had suggested sample dilution at a higher fold [58]. Various dilution factors were applied for blood samples in elemental analysis [37,43,59] and this relied on the amount of starting material (sample volume) as well as the number of elements to be included in one single analysis. The challenge surfaced in a situation where sample volume is limited (in microliters) and more elements to be included. In this study, we developed the method for subsequent analysis in diseased patients' serum and targeting a volume of 50 μL of sample with mandatory duplicates in mind. Although we could not achieve a method with no flaws for simultaneous determination of 25 trace elements, we successfully developed a robust and reproducible method for this purpose given all the restrictions that we had to work with.

In this work, we report the successful application of the validated method to the adults' serum samples. The values measured using this method were in agreement with the findings from the literature (Table 6) except for that of Li, Mg, Al, Fe, Ag, Ba and U, in which we observed higher values. Additionally, we do observe some outliers from the volunteers' sample accounting for about 15–20%. This came as no surprise as the trace elements' levels are highly dependent on the environmental exposure [60] and this trend has been observed in previous human studies [28,59].

The limitation in this study is the use of only Seronorm as a single reference material in contrast to other studies that adopted more than one reference material in assessing the precision and accuracy of their method [31,37]. This shortfall had restricted a fair comparison for several elements that were included in our study due to the difference type of analyser used. It is important to highlight here that the same type of instrument used in establishing the reference value (of a reference material) should also be used in an analytical method to ensure that accurate comparison can be performed. As shown in our results, differences in values were clearly observed for elements measured by ICP-MS (by our method) as compared to non-ICP-MS (from the reference materials' information sheets), thus explaining the discrepancies. It is also noteworthy that stability parameters such as stock solution stability, freeze-thaw cycle stability, long-term stability, pre-extraction stability at room temperature and post-extraction stability in room temperature and autosampler were not determined in this work as the stability of trace elements in human serum and various environmental conditions are well-documented [61]. Trace elements have been reported as highly stable for years in storage at -20 °C [61] and during handling at room temperature [62].

5. Conclusion

We have successfully developed a reliable mass spectrometry method for quantifying 25 trace elements in human serum with extra low sample volume requirement. The method was validated with a commercial human-derived serum control and applied to human serum samples collected from volunteers. A good repeatability (high precision) was shown by all 25 trace elements confirmed the reliability of the method. We have also shown in this work that the levels of some elements may vary from the nominal values of reference material despite highly repeatable within- and between-days, and that the discrepancy is instrument-dependent. We suggest the use of this simultaneous multi-element detection method for assessing the level of trace elements in human diseases, particularly the chronic diseases. Our research group had adopted this analytical method for a case-control colorectal cancer (CRC) study for establishment of a prediction model for CRC using a machine-learning algorithm.

Author contributions

AMN wrote the first draft of the manuscript. SFC and RJ reviewed and edited the manuscript. All the authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no conflicts of interest related the contents of this article.

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