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Analytical methodology

Development of high accuracy methods for the certification of calcium, iron, magnesium and potassium in human serum

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ABSTRACT

High accuracy methods were developed for the measurements of calcium, potassium, iron and magnesium in human serum using standard addition and isotope dilution mass spectrometry methods. The results were comparable to those obtained by other national metrology institutes and designated institutes as demonstrated in an inter-laboratory comparison. The methods were then adopted for the assignment of reference values in a human serum material and the uncertainties associated with the certified values were obtained by combining the uncertainty components from the characterisation, homogeneity and long-term stability of the materials. The certified values are traceable to the International System of Units and the material can be used by the clinical testing laboratories to validate their methods or as a quality control material to improve the accuracy of their measurements.

1. Introduction

The human adult body contains approximately 55–60% of water by weight, which is distributed as intracellular (two third) and extracellular (one third) fluids. These body fluids contain a plethora of ions, molecules and proteins to sustain life [1]. Calcium (Ca), iron (Fe), magnesium (Mg) and potassium (K) are some of the essential elements found in the human body [2]. The human body requires a delicate yet complex balance between the intracellular and extracellular environment [1,2]. Homoeostasis is achieved by various mechanisms such as osmosis, passive diffusion and active transport channels at the cellular level [1–3]. Abnormal levels of these elements are often an indication of underlying medical condition(s) or due to medication side effects [4–8]. In an emergency setting where patients may be unconscious, electrolyte (such as calcium, potassium and magnesium) imbalances require immediate medical attention as it could be life-threatening. Overall, the levels of these elements affect hydration of the body as well as blood pH, and are critical for proper nerve and muscle functions [1–3]. A daily intake of 800 mg of calcium is recommended for adults in order to maintain various cellular processes and bone strength [9]. Potassium plays an important role in maintaining the fluid balance of cells and heart rhythm [10]. Iron deficiency can result in anemia and other serious conditions [11], while magnesium regulates over 300 biochemical reactions in the body as enzyme co-factors [12]. In view of their biochemical importance and ease of accessibility with minimal discomfort to patients, the concentrations of elements in the human serum are frequently requested by physicians to aid in the assessment, diagnosis and management of patients [13–15].

Measurement of elements is routinely performed *via* blood testing or urinalysis with ion-selective electrodes [13]. Accurate measurements are important to provide global comparability in the diagnosis, treatment and monitoring of pathological disease [16,17]. This can be achieved through the use of quality control, preferably Certified Reference Materials (CRMs) [16,17]. It is also a requirement for routine testing laboratories complying with international standard such as ISO 15189 [18].

In this paper, the development of high accuracy methods for the determination of calcium, iron, magnesium and potassium in human serum are presented. The Health Sciences Authority (HSA, Singapore) participated in an inter-laboratory comparison (CCQM-K107) involving the analysis of the four elements in human serum to further validate the methods. Subsequently, a human serum CRM was developed in accordance with ISO Guides 34 and 35 [19,20].

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Table 1
Certified reference standards and isotope spikes used.

Element	Certified Reference Standard		Isotope Spike
	Code	Certified Value	(Isotopic Fraction)
Ca	SRM 3109a	10.025 ± 0.017 mg/g	NA
Fe	SRM 3126a	10.001 ± 0.023 mg/g	^{57}Fe (92.45%)
	SRM 937	99.90 ± 0.02%	
Mg	SRM 3131a	9.99 ± 0.02 mg/g	^{25}Mg (98.814%)
K	SRM 918b	52.4121 ± 0.0086%	^{41}K (99.17%)

2. Materials and methods

2.1. Materials

Nitric acid (69–71% HNO_3, Ultrapur-100, Tokyo, Japan), distilled twice using DST-1000 Sub-Boiling Distillation System (Savillex Corporation, Minnetonka, MN, USA), and Milli-Q Element (18.2 MΩ cm, Millipore Corporation, Bedford, MA, USA) water (Type 1 water) were used as solvent in sample preparations. All working solutions were diluted with 5% HNO_3 (v/v). The standard solutions and the spike solutions were prepared from certified reference standards obtained from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), and isotope spikes obtained from the Oak Ridge National Laboratory (Oak Ridge, USA) (Table 1).

The developed methods were validated using certified reference materials obtained from NIST and National Institute of Metrology (NIM, Beijing, China).

The human serum sample for the inter-laboratory comparison (CCQM-K107) was provided by LGC (London, England).

2.2. Instrumentation

All the experimental work including sample preparation and analysis were conducted in a class 10,000 cleanroom (ISO Class 7). All standard and sample solutions were prepared gravimetrically on a microanalytical balance (Mettler Toledo XP205 or XP26, Greifensee, Switzerland).

The analysis of calcium by standard addition was carried out using Inductively-Coupled Plasma Optical Emission Spectrometer (ICP-OES, Shimadzu ICPE-9000, Japan). Determination of iron and magnesium were performed using Inductively-Coupled Plasma Mass Spectrometry (ICP-MS, Agilent Technologies, 7700x, Japan) equipped with an octopole collision cell capable of operating in the helium collision mode. Potassium was analysed using Finnigan Element 2 (Finnigan MAT GmbH, Bremen, Germany) Sector-Field ICP-MS (SF-ICP-MS) equipped with a self-aspirating PFA MicroFlow nebulizer.

All the instruments were optimized daily to achieve optimum sensitivity and stability according to manufacturer's recommendations. The typical operating conditions and data acquisition parameters are summarised in Table 2.

2.3. Methodologies – standard addition and isotope dilution mass spectrometry (IDMS)

Standard addition method offers the advantage of minimising matrix effects since different amounts of standard are spiked into the unknown sample to produce a multi-point calibration curve. Calcium analysis by standard addition was developed for food matrix in our previous work [21] and was adopted for the determination of Ca in human serum.

IDMS is a primary ratio method that is often used for the accurate determination of analyte concentration in complex matrix samples [21–24]. The "exact matching" IDMS technique was used for the analysis of iron, magnesium and potassium by preparing a standard solution that mimic the sample solution and this approach has been shown to minimise systematic errors [25].

Calculated amount of isotopic spike solutions were added into sample and standard solutions such that the isotope ratio of the blends were close to 1. The blends were also prepared such that the concentration of analytes and spike in both blends were closely matched [26,27].

Table 2
Operating conditions for ICP-OES, ICP-MS and SF-ICP-MS.

Operating Conditions	ICP-OES
Element	Ca
RF powder/W	1200
Plasma gas/L min^{-1}	10
Auxiliary gas/L min^{-1}	0.6
Carrier gas/L min^{-1}	0.7
View direction	Axial
Wavelength/nm	396.8
Operating Conditions	**ICP-MS**
Isotope Monitored	^{54}Fe, ^{56}Fe, ^{57}Fe; ^{24}Mg, ^{25}Mg,^{26}Mg
RF powder/W	1550
Ion Lens setting	Optimized daily
Helium gas flow/mL min^{-1}	5
Data acquisition integration time/s	2–5
Points per peak	3
Repetitions	3
Operating Conditions	**SF-ICP-MS**
Isotope Monitored	^{39}K, ^{41}K
RF power/W	1250
Scanning mode	E-scan
Setting time/ms	1
Resolution	10,000 (high)
Sample time/ms	200
Samples per peak	20
Runs	5
Passes	10
Mass window/%	100
Total time per sample/min	3.5
Detection mode	Both

2.4. Sample preparation and analysis procedure

For Calcium, appropriate amount of serum was weighed for each sub-sample and 50 mL of 5% HNO_3 was added. The resulting slurry was allowed to stand at room temperature for 12 h. The sample was then centrifuged and 5 sets of approximately 5g of the supernatant were weighed. Increasing amount of calcium standards were then spiked into 4 of the solutions. The solutions were further diluted with 5% HNO_3 to around 50 mL. The resulting solutions, comprising of 1 unspiked and 4 spiked solutions, were analysed by ICP-OES.

For Iron, magnesium and potassium, the calibration blend was prepared by weighing the standard solution (Fe, Mg or K) and appropriate amount of isotopic spike (^{57}Fe, ^{25}Mg or ^{41}K) and then diluting with 5% HNO_3 to around 50 mL. For sample blend, the serum sample and calculated amount of the isotopic spike were weighed. 2 mL of concentrated HNO_3 was then added to the sample and the mixture was allowed to stand for 12 h. Type I water was subsequently added to achieve 5% (v/v) HNO_3 and the sample was centrifuged. The sample blends (SB) and the calibration blends (CB) were further diluted volumetrically to roughly 20 μg/kg for magnesium or 2 μg/kg for iron before ICP-MS measurement. For potassium, the blends were further diluted volumetrically to roughly 140 μg/kg before analysis by SF-ICP-MS.

At least two reagent blanks were prepared by subjecting them to all sample preparation steps to evaluate possible blank contributions. Bracketing technique was adopted in all the analytical sequences to account for instrument drift and to improve precision of measurements. Instrument mass bias correction was performed