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RESEARCH PAPER

# Fast and accurate determination of K, Ca, and Mg in human serum by sector field ICP-MS

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**Abstract** Electrolytes in serum are important biomarkers for skeletal and cellular health. The levels of electrolytes are monitored by measuring the Ca, Mg, K, and Na in blood serum. Many reference methods have been developed for the determination of Ca, Mg, and K in clinical measurements; however, isotope dilution thermal ionization mass spectrometry (ID-TIMS) has traditionally been the primary reference method serving as an anchor for traceability and accuracy to these secondary reference methods. The sample matrix must be separated before ID-TIMS measurements, which is a slow and tedious process that hindered the adoption of the technique in routine clinical measurements. We have developed a fast and accurate method for the determination of Ca, Mg, and K in serum by taking advantage of the higher mass resolution capability of the modern sector field inductively coupled plasma mass spectrometry (SF-ICP-MS). Each serum sample was spiked with a mixture containing enriched $^{44}Ca$, $^{26}Mg$, and $^{41}K$, and the $^{42}Ca^{+}$:$^{44}Ca^{+}$, $^{24}Mg^{+}$:$^{26}Mg^{+}$, and $^{39}K^{+}$:$^{41}K^{+}$ ratios were measured. The Ca and Mg ratios were measured in medium resolution mode ($m/\Delta m \approx 4\,500$), and the K ratio in high resolution mode ($m/\Delta m \approx 10\,000$). Residual $^{40}Ar^{1}H^{+}$ interference was still observed but the deleterious effects of the interference were minimized by measuring the sample at K>100 ng $g^{-1}$. The interferences of $Sr^{++}$ at the two Ca isotopes were less than 0.25 % of the analyte signal, and they were corrected with the $^{88}Sr^{+}$ intensity by using the $Sr^{++}$:$Sr^{+}$ ratio. The sample preparation involved only simple dilutions, and the measurement using this sample preparation approach is known as dilution-and-shoot (DNS). The DNS approach was validated with samples prepared via the traditional acid digestion approach followed by ID-SF-ICP-MS measurement. DNS and digested samples of SRM 956c were measured with ID-SF-ICP-MS for quality assurance, and the results (mean ± expanded uncertainty in mg $dL^{-1}$ unit) for Ca (DNS=10.14±0.13, digested=10.11±0.10), Mg (DNS=2.093±0.008, digested=2.098±0.007), and K (DNS=15.48±0.11, digested=15.50±0.28) were in good agreement with the certified values (Ca=10.17±0.06, Mg=2.084±0.023, K=15.55±0.13). Major sources of uncertainty are sample measurement, spike calibration, and instrument factor including mass discrimination of the spectrometer and the detector deadtime.

**Keywords** Ca · Mg · K · Electrolyte · Serum · Isotope dilution · High resolution · Sector field · ICP-MS

## Introduction

Electrolytes play an essential role in the human body [1]. They are responsible for maintenance of precise osmotic gradient to regulate the hydration of the body, blood pH, and proper functioning of nerve and muscle [1]. Abnormal levels of electrolytes may either be the cause or the consequence of a variety of disorders, and the determination of electrolytes is one of the most important functions of the clinical laboratory [1]. The levels of electrolytes are monitored in blood serum [2]. Many methods have been developed for the determination of electrolytes. Among them are flame atomic emission spectrometry (FAES) for K [3], flame atomic absorption spectrometry (FAAS) for Ca and Mg [4, 5], ion selective electrode (ISE) for K [1], ion chromatography (IC) for Ca, K, Mg, and Na [6], and inductively coupled plasma optical emission spectrometry (ICP-OES) for Ca and K [7]. Although these are carefully validated reference

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methods for the determination of electrolytes serum, none of them is a primary method discussed below.

Accuracy and traceability of clinical measurements are particularly important to the public health and the nation's well-being. It was estimated that inaccuracy and reliability issues added tens of billions of dollars in cost for nondiagnostic clinical tests in the US alone [8]. The European Communities' Directive on in vitro diagnostic (IVD) medical devices codified the needs for traceability and accuracy in laboratory medicine [9]. In response to the IVD directive, the Joint Committee for Traceability in Laboratory Medicine (JCTLM) published a database that lists clinical methods that meet the standard for higher order reference measurement procedures [10].

Primary methods, defined as "having the highest metrological qualities, whose operation can be completely described and understood, for which a complete uncertainty statement can be written down in terms of SI units", are highest on the metrological order [11]. Isotope dilution mass spectrometry (IDMS) is a technique based on the principle of isotope dilution, which is consistent with the scope of a primary ratio method that "measures the value of a ratio of an unknown to a standard of the same quantity; its operation must be completely described by a measurement equation" [11]. One of the historical IDMS methods, isotope dilution thermal ionization mass spectrometry (ID-TIMS) was used for many critical certification measurements at NIST, including Ca, K, and Mg in Standard Reference Material (SRM) 909b Human Serum [12] which is listed in the JCTLM database of higher order reference materials [10]. Despite its accuracy, ID-TIMS is seeing fewer applications recently with the development of isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS), due in part to the extended time and effort needed in sample preparation that requires a complete digestion followed by purification [12]. ID-ICP-MS was used for the certification measurement of Mg in frozen human serum European Reference Materials (ERM)-DA250 and ERM-DA251 [7]. Sera were spiked with $^{25}Mg$ enriched isotope, and the spiked samples were measured directly after a simple dilution [7], an approach known as dilute-and-shoot (DNS) that has been used in clinical measurements since the 1980s [13]. ID-ICP-MS determinations of Ca and K are more difficult as $^{38}Ar^{1}H^{+}$, $^{40}Ar^{+}$, and $^{40}Ar^{1}H^{+}$ molecular ions interfere with the measurements of $^{39}K^{+}$, $^{40}Ca^{+}$, $^{41}K^{+}$ at mass-to-charge ratios of 39, 40, and 41, respectively. ICP-MS operated in "cold" plasma condition was shown to greatly reduce the intensity of $^{38}Ar^{1}H^{+}$, $^{40}Ar^{+}$, and $^{40}Ar^{1}H^{+}$ molecular ions, which made measurements of $^{39}K$:$^{41}K$ and $^{40}Ca$:$^{42}Ca$ ratios possible [14]. However, interferences were still observed at masses 41 and 42, which necessitated matrix separation of the digested samples before isotope dilution measurements of Ca and K [14]. In contrast to the quadrupole ICP-MS with a unit mass resolution, high resolution sector field ICP-MS (SF-ICP-MS) is capable of mass resolution ($m/\Delta m$) as high as 10 000. Molecular ion interferences at Ca and K masses can be resolved by using sector field ICP-MS operating at resolutions higher than 4 890 with the exception of $^{40}Ar^{+}$ on $^{40}Ca^{+}$ [15]. SF-ICP-MS operating at mass resolution of 4 500 and octopole collision cell ICP-MS operating in hydrogen collision mode were successfully used to measure $^{44}Ca$:$^{42}Ca$ ratio for isotope dilution determination of Ca in serum after a simple dilution [16]. Despite our best efforts, no relevant literature reports were found for ID-SF-ICP-MS determinations of potassium in serum.

Accuracy and fast turn-around are two corner stones of clinical measurements. We report a fast and accurate method for the determination of Ca, K, and Mg in SRM 909c Human Serum by ID-SF-ICP-MS. The DNS approach was adopted for the measurement to maximize the throughput and to minimize the potential contamination from sample processing. We establish the accuracy of the DNS approach for ID-SF-ICP-MS determination of Ca, K, and Mg in serum by comparing the results of DNS samples with the results from acid digested samples.

## Experimental

*Instrumentation*[1] A Thermo Element model XR sector-field ICP-MS (Waltham, MA) was used. The SF-ICP-MS was equipped with a peltier cooled (4 °C) Stable Sample Introduction (SSI) system and a model ESI SC-E2 autosampler from Elemental Scientific (Omaha, NE). The SF-ICP-MS tuning and resolution optimization was performed with a standard 1 ng $g^{-1}$ multi-element tuning solution. A CEM model MARSXpress microwave systems equipped with 55 mL PFA microwave vessels (Matthews, NC) was used to digest the samples. A Mettler model AT 261 DeltaRange analytical balance (Columbus, OH) was used for weighing in the preparation of samples and standards. The balance is serviced and calibrated annually by Mettler, and the calibration was validated with weights traceable to International System of Units (SI).

*Reagents and standards* Optima grade nitric acid ($HNO_3$) from Fisher Scientific was used in the preparation of samples and standards. Locally prepared sub-boiling distilled water was used as a solvent to prepare all solutions. The enriched isotopic standards were obtained from Oak Ridge National Laboratory (ORNL, Oak Ridge, TN): $^{42}Ca$ isotopic standard, series LO batch 139693; $^{41}K$ isotopic standard, batch 149401; $^{26}Mg$ isotopic standard series RS batch 129A. Calibration standards from the National Institute of Standards and

[1] Disclaimer: Certain commercial items are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the equipment identified is necessarily the best for the purpose.

Technology (NIST) Standard Reference Materials were used for calibration: SRM 3109A Calcium Standard Solution, lot 050825; SRM 3141A Potassium Standard Solution, lot 051220; SRM 3131A Magnesium Standard Solution, lot 050302. All vessels used for sample preparation and storage were soaked with 10 % (all percentage concentration of a reagent is expressed as the volume fraction of the reagent in water) $HNO_3$ and rinsed with deionized water before use.

*Sample preparation* For measurements using the DNS approach, four ampoules of SRM 909c were thawed to room temperature. Serving as quality assurance for the measurement, one Level 2 ampoule of SRM 956c Electrolytes in Frozen Human Serum was processed similarly. The contents of each ampoule were homogenized by gently rotating and inverting the ampoule before opening. A sample was prepared by transferring 0.3 g serum from an ampoule into a 60 mL low density polyethylene (LDPE) bottle. Approximately 0.15 g Ca spike, 0.20 g K spike, and 0.32 g Mg spike containing 5 μg/g $^{42}Ca$, 267 μg/g $^{41}K$, and 39 μg/g $^{26}Mg$ respectively, were added to the bottle. Duplicate samples were prepared from each ampoule. Eight procedure blanks were prepared by transferring into each of the eight 60 mL LDPE bottles 10 % the amount of enriched isotopic spike added to the sample. The samples and blanks were diluted to 50 g with a diluent containing 0.1 % $NH_4OH$ and 1 % Triton X-100 by volume fraction in water.

For measurements using the digested approach, a separate set of four ampoules of SRM 909c and one ampoule of SRM 956c Level 2 were used. The same amount of sample and spike described above were transferred to pre-cleaned PFA microwave vessels instead of bottles to prepare the samples and eight procedure blanks. An aliquot of 9 mL $HNO_3$ was added to each vessel before it was capped for microwave digestion using the parameters in Table 1. The microwave vessels were allowed to cool to room temperature after the digestion. The contents of the vessels were transferred to 60 mL LDPE bottles and diluted to approximately 50 g with sub-boiling distilled water.

For the purpose of calibrating the isotopic spike solution with reverse IDMS measurements, two sets of calibration solutions (SpikeCal) were prepared. Each set consisted of duplicate spike calibration mixes prepared from the SRM 3100 series standard calibration solutions with independent serial dilutions. Each spike calibration mixture was prepared by transferring into a 60 mL LDPE bottle aliquots of 0.3 g $^{42}Ca$ spike, 0.2 g $^{41}K$ spike, 0.3 g $^{26}Mg$ spike, 0.3 g of 44 μg $g^{-1}$ Mg, 1 g of 45 μg $g^{-1}$ K, and 1 g of 60 μg $g^{-1}$ Ca, and then diluting the contents to 50 g with 2 % $HNO_3$. A mass bias correction solution was prepared by transferring into a 60 mL LDPE bottle aliquots of 0.3 g of 44 μg $g^{-1}$ Mg, 1 g of 45 μg $g^{-1}$ K, and 1 g of 60 μg $g^{-1}$ Ca, and then diluting the contents to 50 g with 2 % $HNO_3$.

*Sample analysis* All samples, blanks, and standards were diluted ten-fold in pre-cleaned 50 mL autosampler tubes with 2 % $HNO_3$ for measurement. The autolock mass feature was enabled to minimize any potential drift due to magnet hysteresis. The instrument parameters shown in Table 2 were used for the measurement of DNS and digested samples, though the DNS samples and the digested samples were measured on different days. Ca and Mg were measured together in medium resolution mode at masses of $^{42}Ca^+$, $^{44}Ca^+$, $^{24}Mg^+$, and $^{26}Mg^+$. A 30 ng $g^{-1}$ Sr solution was measured with the samples for correction of $^{84}Sr^{++}$ and $^{88}Sr^{++}$ at $^{42}Ca^+$ and $^{44}Ca^+$, respectively. K was measured separately in high resolution mode at masses of $^{39}K^+$ and $^{41}K^+$. The digested samples and the 30 ng $g^{-1}$ Sr were also measured in high resolution mode for Ca and Mg in an effort to validate Ca and Mg measurements at medium resolution mode. One of the spike calibration solution mixtures, designated B2, was measured every five samples throughout the measurement, and the measured isotope ratios for this solution were used to correct for instrument mass discrimination drift. This was done to minimize uncertainty in the ratio measurements because the isotopic composition of the spike calibration solution mixture was matched closely to that of the unknown sample. The average analyte isotope ion

**Table 1** Microwave settings for digestion of blood serum samples

| Step | Power (W) | Power setting (%) | Ramp time (min) | T (°C) | Hold time (min) |
|---|---|---|---|---|---|
| 1 | 800 | 100 | 25:00 | 140 | 20:00 |
| 2 | 0 | 0 | 0 | 0 | 20:00 |
| 3 | 800 | 100 | 25:00 | 195 | 20:00 |
| 4 | 0 | 0 | 0 | 0 | 25:00 |

**Table 2** Instrument operating parameters

| Parameter | Ca | Mg | Sr | K |
|---|---|---|---|---|
| Rf power (W) | 1 360 | 1 360 | 1 360 | 1 360 |
| Plasma gas flow (L $min^{-1}$) | 16.00 | 16.00 | 16.00 | 16.00 |
| Auxilliary flow (L $min^{-1}$) | 0.80 | 0.80 | 0.80 | 0.80 |
| Nebilizer flow (L $min^{-1}$) | 1.375 | 1.375 | 1.375 | 1.375 |
| Sample uptake (mL $min^{-1}$) | 0.24 | 0.24 | 0.24 | 0.24 |
| Sampler/skimmer cone | Ni | Ni | Ni | Ni |
| Resolution ($m/\Delta m$) | 4 500 | 4 500 | 4 500 | 10 000 |
| Sample time (s) | 0.01 | 0.01 | 0.01 | 0.01 |
| Samples per peak | 50 | 50 | 50 | 50 |
| Runs | 10 | 10 | 10 | 10 |
| Passes | 5 | 5 | 5 | 10 |
| Integration window (%) | 40 | 40 | 40 | 40 |
| Detection mode | Triple | Triple | Triple | Triple |

count rates were downloaded as CSV files to a Microsoft Excel spreadsheet for calculation of analyte isotope ratios.

## Results and discussion

*Spectral interferences* The spectral interferences at the Ca, K, and Mg isotopes of interest for this work were investigated by taking into account the elemental composition of human serum. Table 3 summarizes the potential interferences [15–18] and the resolutions required to resolve them.

A minimum resolution of 2 687 and 2 735 is needed to resolve the spectral interferences from molecular ions at Ca and Mg isotopes, respectively. However, interference from $^{84}Sr^{++}$ and $^{88}Sr^{++}$ cannot be resolved from $^{42}Ca^{+}$ and $^{44}Ca^{+}$, respectively, even at the highest resolution (≈10 000) of the current commercial sector-field ICP-MS instruments. It was reported that the most significant interferences for measurements of $^{42}Ca^{+}$ and $^{44}Ca^{+}$ in serum were from molecular ions [16], although the extent of the $^{84}Sr^{++}$ and $^{88}Sr^{++}$ was not available from the literature [16]. Stürup et al. corrected the $^{84}Sr^{++}$ and $^{88}Sr^{++}$ interferences using the intensity of $^{87}Sr^{++}$ at 43.5 u for isotope ratio measurements of $^{44}Ca$:$^{43}Ca$ and $^{42}Ca$:$^{43}Ca$ in urine [17]. We used medium resolution (≈ 4 500) for the measurement of Ca and Mg isotopes. The intensity of $^{88}Sr^{+}$ was used to correct for the $^{84}Sr^{++}$ and $^{88}Sr^{++}$ interferences by using the $^{88}Sr^{++}$:$^{88}Sr^{+}$ of the 30 ng $g^{-1}$ Sr solution. For the serum samples in this work, the intensity of $3\times10^{5}$ c $s^{-1}$ for $^{88}Sr^{+}$ was much greater than the approximately 700 c $s^{-1}$ for $^{87}Sr^{++}$. The intensity of $^{88}Sr^{+}$ was used for the correction because of the higher intensity, despite the inconvenience of an indirect correction. It should be pointed out that the direct correction using the intensity of $^{87}Sr^{++}$ at 43.5 u is preferred when the intensity at the mass is higher (e.g. > 10000 c $s^{-1}$ to produce<1 % counting uncertainty for a 1 s integration) for the measurement, because this correction scheme is less susceptible to changes in the plasma conditions relative to the indirect correction scheme of this work. With a $^{88}Sr^{++}$:$^{88}Sr^{+}$ ratio of 2.8:100 under the experimental conditions, the $^{88}Sr^{++}$ accounted for approximately 0.25 % and 0.18 % of the ion counts at $^{44}Ca^{+}$ for SRM 909c and SRM 956c, respectively. The effect of $^{84}Sr^{++}$ on $^{42}Ca^{+}$ was at least two orders of magnitude less than $^{88}Sr^{++}$ on $^{44}Ca^{+}$, because the abundance of $^{84}Sr$ (0.56 %) is much lower than $^{88}Sr$ (82.58 %) while the intensity of $^{44}Ca^{+}$ is higher than $^{42}Ca^{+}$ due to the isotopic spike.

**Table 3** Resolution required to resolve the likely spectral interferences in electrolyte in serum measurements

| Isotope | Natural abundance (%) | Likely interferences | Resolution[a] |
|---|---|---|---|
| $^{42}Ca^{+}$ | 0.648 | $^{40}Ar^{2}H^{+}$ | 2 349 |
| | | $^{40}Ar^{1}H_{2}^{+}$ | 2 165 |
| | | $^{26}Mg^{16}O^{+}$ | 2 221 |
| | | $^{24}Mg^{18}O^{+}$ | 1 640 |
| | | $^{84}Sr^{++}$ | 21 968 |
| $^{44}Ca^{+}$ | 2.086 | $^{12}C^{16}O_{2}^{+}$ | 1 280 |
| | | $^{28}Si^{16}O^{+}$ | 2 687 |
| | | $^{88}Sr^{++}$ | 16 463 |
| $^{39}K^{+}$ | 93.258 | $^{38}Ar^{1}H^{+}$ | 5 688 |
| | | $^{23}Na^{16}O^{+}$ | 1 858 |
| $^{41}K^{+}$ | 6.730 | $^{40}Ar^{1}H^{+}$ | 4 888 |
| | | $^{25}Mg^{16}O^{+}$ | 2 165 |
| | | $^{23}Na^{18}O^{+}$ | 1 512 |
| $^{24}Mg^{+}$ | 78.993 | $^{12}C_{2}^{+}$ | 1 603 |
| | | $^{48}Ca^{++}$ | 2 735 |
| $^{26}Mg^{+}$ | 11.012 | $^{12}C^{14}N^{+}$ | 1 269 |

[a] The resolution, calculated as $m/\Delta m$, needed to separate the interferencing species from the isotope

A minimum resolution of 5 688 is needed to alleviate the $^{39}K^{+}$ and $^{41}K^{+}$ from $^{38}Ar^{1}H^{+}$ and $^{40}Ar^{1}H^{+}$ interferences, respectively; therefore, K was measured separately from Ca and Mg in high resolution mode (≈10 000). Although the resolution for this work was much higher than the 5 688 required to separate the $ArH^{+}$ interferences, the effect of the $ArH^{+}$ was still observed as slanted baselines for the $^{39}K^{+}$ and $^{41}K^{+}$ peaks. The baseline intensity for $^{41}K^{+}$ was more than three times greater than that for $^{39}K^{+}$, probably due to the fact that $^{40}Ar$ is three orders of magnitude more abundant than $^{38}Ar$. The elevated baseline at $^{41}K^{+}$ can be a challenge for the measurement of $^{41}K$:$^{39}K$ for K of a natural composition, as Becker et al. found the measured $^{41}K$:$^{39}K$ of a 100 μg $L^{-1}$ solution of SRM 985 Assay-Isotopic Standard for Potassium was higher than the certified value by 2.3 % and attributed this to incomplete separation of $^{40}Ar^{1}H^{+}$ [15]. For this work, the baseline intensity at $^{39}K^{+}$ and $^{41}K^{+}$ was frequently monitored and the measurement of the baseline accounted for 9 of the 40 total measurements. The baseline intensity was subtracted before the $^{41}K$:$^{39}K$ ratio was calculated. The serum samples for the measurement were over-spiked with $^{41}K^{+}$ to a target ratio of 1.3:1 for $^{41}K$:$^{39}K$, and each sample contained approximately 200 μg $L^{-1}$ of the analyte. The contents of $^{41}K$ in these samples were 17 times higher than Becker et al. used in their measurements [15]. The increased count rate for $^{41}K^{+}$, due to the higher mass fraction of $^{41}K$ in the sample, minimized the effect of the baseline at $^{41}K^{+}$, as evidenced by the agreement between the measured and the certified value of K in SRM 956c discussed below.

*Dilute and shoot measurements for serum* Accuracy and fast turn-around are the desired goals for all analytical measurements, and they are particularly true for clinical measurements as discussed before. The DNS approach, which minimizes the time for sample preparation and reduces the possibility of

**Table 4** Results of Ca, Mg, and K in SRMs 909c and 956c found with the DNS and the digested approach. All values are in mg/dL units unless noted otherwise

| SRM | DNS | | | Digested | | | Digested HR | |
|---|---|---|---|---|---|---|---|---|
| Resolution | MR Ca | MR Mg | HR K | MR Ca | MR Mg | HR K | HR Ca | HR Mg |
| 909c | | | | | | | | |
| Sample 1 | 10.17 | 2.205 | 16.29 | 10.06 | 2.163 | 16.22 | 10.14 | 2.190 |
| Sample 2 | 10.22 | 2.195 | 16.38 | 10.06 | 2.177 | 16.26 | 10.11 | 2.214 |
| Sample 3 | 10.15 | 2.197 | 16.24 | 10.06 | 2.184 | 16.24 | 10.10 | 2.208 |
| Sample 4 | 10.19 | 2.177 | 16.30 | 10.08 | 2.181 | 16.23 | 10.15 | 2.193 |
| Sample 5 | 10.15 | 2.202 | 16.40 | 10.19 | 2.186 | 16.30 | 10.09 | 2.197 |
| Sample 6 | 10.25 | 2.174 | 16.21 | 10.19 | 2.171 | 16.33 | 9.91 | 2.192 |
| Sample 7 | 10.31 | 2.175 | 16.26 | 10.10 | 2.173 | 16.31 | 10.01 | 2.179 |
| Sample 8 | 10.23 | 2.186 | 16.24 | 10.04 | 2.174 | 16.24 | 9.74 | 2.184 |
| **Average** | **10.21** | **2.189** | **16.29** | **10.10** | **2.176** | **16.27** | **10.03** | **2.189** |
| *s* | 0.059 | 0.013 | 0.067 | 0.058 | 0.0075 | 0.039 | 0.14 | 0.013 |
| RSD % | 0.58 % | 0.57 % | 0.41 % | 0.58 % | 0.35 % | 0.24 % | 1.4 % | 0.57 % |
| *U* | **0.10** | **0.021** | **0.13** | **0.11** | **0.016** | **0.27** | **0.15** | **0.016** |
| 956c | | | | | | | | |
| Measured | 10.14 | 2.093 | 15.46 | 10.11 | 2.098 | 15.50 | 10.12 | 2.094 |
| *U* | 0.13 | 0.019 | 0.12 | 0.10 | 0.016 | 0.24 | 0.13 | 0.017 |
| Certified | 10.17 | 2.084 | 15.55 | 10.17 | 2.084 | 15.55 | 10.17 | 2.084 |
| *U* | 0.06 | 0.023 | 0.13 | 0.06 | 0.023 | 0.13 | 0.06 | 0.023 |

contamination during sample preparation, has the potential to improve the measurement speed and accuracy for metals in biological fluids. The principle of isotope dilution measurements assumes that the analyte in the sample and the enriched isotopic spike reach isotopic equilibrium. Ca and Mg in serum are in a mixture of protein bound, small ligand bound, or ionized states [19, 20]. K is a major intracellular cation [1]. Since analytes in the isotopic spike are typically in the ionized state, the accuracy and traceability of the DNS approach for ID-SF-ICP-MS measurements of Ca, Mg, and K in serum are predicated on the equilibration of the analytes of the sample and the enriched isotopes of the spike. An assessment of the equilibration between the spiked and the endogenous analytes in the DNS sample may be difficult. Alternatively, the accuracy of the DNS approach can be evaluated by its commutability with the results of equilibrated samples, e.g., digested samples, discussed below.

*Results for the serum samples* Table 4 lists the results of Ca, Mg, and K in SRM 909c and SRM 956c. All expanded uncertainties (*U*) are expressed at a 95 % confidence level. Ca and Mg in the digested samples were measured in both the

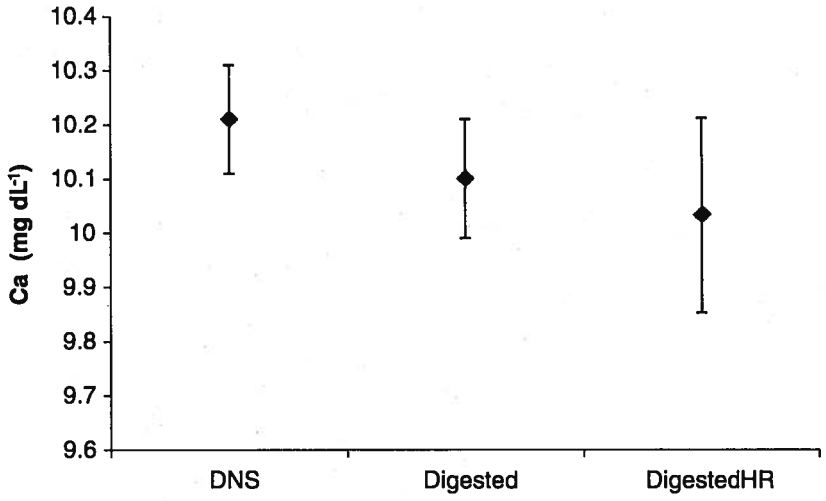


**Fig. 1** Ca found in SRM 909c with the DNS approach, digestion approach, and the digested samples measured in high resolution mode (DigestedHR). The *error bars* indicate the expanded uncertainty of the measurement

medium resolution (Digested) and the high resolution (DigestedHR) mode. The measured values of Ca, Mg, and K in SRM 956c quality assurance control samples agree with the certified values as indicated by the overlap between the measured values and the certified values, which assure the validity of the results of SRM 909c of same measurement. As mentioned before, the agreement between the measured and the certified value of K in SRM 956c suggests that the $^{38}Ar^{1}H^{+}$ and $^{40}Ar^{1}H^{+}$ interferences at $^{39}K^{+}$ and $^{41}K^{+}$, respectively, were appropriately corrected. Moreover, the fact that the magnitude of the expanded uncertainty of the measurement approximates that of the certified value suggests that the correction for the $ArH^{+}$ baseline did not unduly affect the uncertainty of the measurement.

Figures 1, 2, and 3 show the measured values of Ca, Mg, and K in SRM 909c, respectively. Each figure displays the results for the DNS, Digested, and (for Ca and Mg only) DigestedHR approach. For Ca, Mg, and K, the DNS and the Digested approaches agree with each other as indicated by the overlap of the uncertainty intervals, indicating that the two approaches are commutable for the measurement of these electrolytes in SRM 909c and, as discussed above, SRM 956c serum samples. Since the analytes in the Digested samples were in equilibration, one might deduce that either the analytes in the DNS approach were also at equilibrium, or the ICP-MS was unaffected by the difference in the chemical form of the analytes of interest. In either case, the DNS is validated to be as accurate as the approach that requires sample digestion. Most likely, however, the analytes in a DNS sample were at equilibrium because the analytes of interest in serum are either as free ion (K) or in various states that are in dynamic equilibrium with the free ions (Ca and Mg) [1, 19, 20]. The addition of free ions from the isotopic spike simply shifts the equilibrium in the serum sample. Figures 1 and 2 also include the results of Ca and Mg measured in high resolution mode. The agreement between the medium resolution and high resolution results suggests that the medium resolution is sufficient to resolve molecular ion interferences for Ca and Mg measurements. The slightly larger expanded uncertainty at high resolution for both analytes, probably as a result of a larger drift and greater counting uncertainty in the high resolution mode than in the medium resolution mode, shows that the medium resolution mode is superior to the high resolution mode for Ca and Mg measurements.

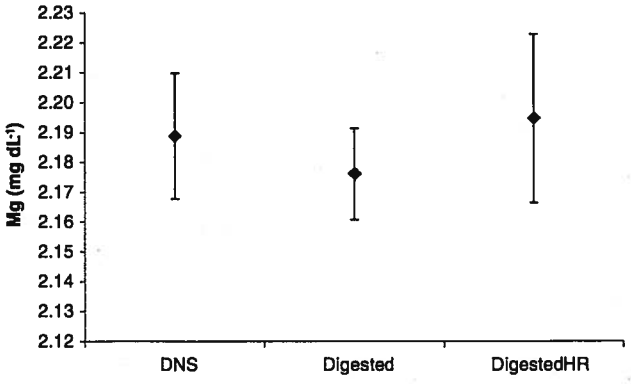


**Fig. 2** Mg found in SRM 909c with the DNS approach, digestion approach, and the digested samples measured in high resolution mode (DigestedHR). The *error bars* indicate the expanded uncertainty of the measurement

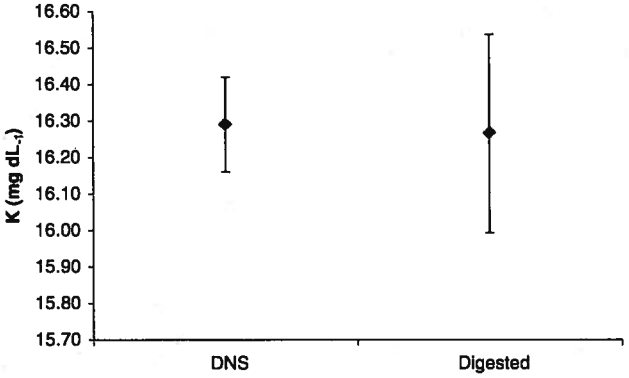


**Fig. 3** K found in SRM 909c with the DNS approach and the digestion approach. The *error bars* indicate the expanded uncertainty of the measurement

**Table 5** Sources of uncertainty in the determination of Ca, Mg, and K in SRM 909c using the DNS approach. All uncertainty expressions are in relative terms (%) unless otherwise stated

| Sources of uncertainty | Ca | Mg | K | |
|---|---|---|---|---|
| Type A uncertainty | | | | DF |
| Sample measurement | 0.20 % | 0.20 % | 0.15 % | 7 |
| Spike calibration | 0.22 % | 0.26 % | 0.028 % | 3 |
| Blank measurement | 0.13 % | 0.10 % | 0.017 % | 7 |
| Combined type A uncertainty | 0.33 % | 0.34 % | 0.15 % | |
| Type B uncertainty | | | | DF |
| Calibrant uncertainty | 0.085 % | 0.082 % | 0.12 % | infinite |
| Instrument factor | 0.31 % | 0.31 % | 0.31 % | infinite |
| Spectral background | 0.11 % | 0.081 % | 0.16 % | infinite |
| $Sr^{++}$ correction | 0.014 % | – | – | infinite |
| Density correction | 0.004 4 % | 0.004 4 % | 0.004 4 % | infinite |
| Weighing measurements | 0.045 % | 0.022 % | 0.033 % | infinite |
| Combined type B uncertainty | 0.34 % | 0.33 % | 0.37 % | infinite |
| Summary uncertainty information | | | | |
| Combined uncertainty ($u_c$) | 0.48 % | 0.48 % | 0.40 % | |
| Degrees of freedom (Veff) | 46 | 30 | >60 | |
| Coverage factor (k) | 2.01 | 2.04 | 2.00 | |
| Expanded uncertainty (U) | 0.96 % | 0.97 % | 0.80 % | |

The uncertainty components for the ID-SF-ICP-MS measurements were analyzed and calculated in accordance with ISO guidelines [21]. The magnitude of the each uncertainty component is comparable for the DNS approach and the Digested approach. As an example, Table 5 lists the sources of uncertainty for the determination of Ca, Mg, and K in SRM 909c using the DNS approach. The contribution from the repeatability of eight samples, the repeatability of four spike calibrants in the reverse IDMS measurements, and repeatability of eight blanks were assessed with Type A evaluation of uncertainty [21]. The contributions from the primary calibrant, the instrument factors including mass discrimination of the spectrometer and the detector deadtime, spectral background fluctuation, $Sr^{++}$ correction (for Ca only), density measurements for conversion of units from mass fraction to mass concentration, and weighing for sample and standard preparation were assessed with Type B evaluation of uncertainty [21]. Sample measurements, spike calibration, and the instrument factors are the three major sources of uncertainty in the measurements of Ca, Mg, and K in serum. In comparison, the correction for spectral interferences for both $Sr^{++}$ on $Ca^{+}$ and $ArH^{+}$ on $K^{+}$ are minor contributors to the uncertainty of the measurement, suggesting that the measurement procedure described herein was optimized for accurate determination of Ca, Mg, and K in serum.

Table 4 shows that for all elements except K, a large expanded uncertainty corresponds to a large sample replication RSD %. This is because sample repeatability, which equals RSD % divided by the square root of the number of sample replicates, is a major source of uncertainty. For the K measurement using the Digested approach, however, one of the blanks was much higher than the rest. Despite contamination being a possibility, the blank was included in the calculations because there was no anomaly observed during the preparation of the blanks. Inclusion of the high blank resulted in a blank measurement uncertainty as high as 0.66 % relative to a typical 0.017 % shown in Table 5 for the DNS approach, and the blank measurement uncertainty became the largest source of uncertainty that determined the magnitude of the expanded uncertainty for the Digested approach. Relative to the complexity of microwave digestion for the Digested approach, the DNS approach requires only a simple dilution of the sample before measurements, which makes it more robust against the potential for contamination.

## Conclusion

With careful choice of isotopes and spiking levels, SF-ICP-MS is capable of resolving isobaric interferences in the isotope dilution measurements of Ca, Mg, and K in serum. Although interferences of $ArH^{+}$, especially $^{40}Ar^{1}H^{+}$ at $^{41}K^{+}$ cannot be completely eliminated due to its high intensity, the deleterious effects of the interferences can be minimized by measuring the $^{41}K^{+}$ at 100 ng $g^{-1}$ or higher mass fraction. The approach is effective for the determination of K in serum because the mass fraction of K in serum is well defined to range from 130 μg $g^{-1}$ to 240 μg $g^{-1}$ [1], which allows the dilution of serum to reach the target $^{41}K$ mass fraction after spiking for accurate isotope ratio measurement in high resolution mode. Potassium in SRM 909c was accurately determined under these conditions. Since the determining factor in $ArH^{+}$ intensity is the water loading in the plasma, a sample introduction device equipped with desolvation capability may prove effective in reducing $ArH^{+}$ interferences and improving the accuracy for measurement of $^{41}K^{+}$ at lower levels. The mass resolution power of the SF-ICP-MS obviates the separation step required in isotope dilution measurements of electrolytes in serum by ICP-MS. Combined with the DNS approach, ID-SF-ICP-MS is a fast and accurate method for the determination of Ca, Mg, and K in serum.

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