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# Measurement of Isotope Abundance Variations in Nature by Gravimetric Spiking Isotope Dilution Analysis (GS-IDA)

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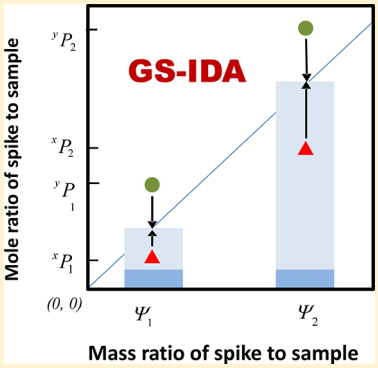
Supporting Information

**ABSTRACT:** Subtle variations in the isotopic composition of elements carry unique information about physical and chemical processes in nature and are now exploited widely in diverse areas of research. Reliable measurement of natural isotope abundance variations is among the biggest challenges in inorganic mass spectrometry as they are highly sensitive to methodological bias. For decades, double spiking of the sample with a mix of two stable isotopes has been considered the reference technique for measuring such variations both by multicollector-inductively coupled plasma mass spectrometry (MC-ICPMS) and multicollector-thermal ionization mass spectrometry (MC-TIMS). However, this technique can only be applied to elements having at least four stable isotopes. Here we present a novel approach that requires measurement of three isotope signals only and which is more robust than the conventional double spiking technique. This became possible by gravimetric mixing of the sample with an isotopic spike in different proportions and by applying principles of isotope dilution for data analysis (GS-IDA). The potential and principle use of the technique is demonstrated for Mg in human urine using MC-TIMS for isotopic analysis. Mg is an element inaccessible to double spiking methods as it consists of three stable isotopes only and shows great potential for metabolically induced isotope effects waiting to be explored.




With the exception of monoisotopic elements, the abundances of the stable isotopes of a chemical element are highly constant but not invariant in nature. Variations in the isotopic abundances of an element can arise both from radioactive decay as well as isotope fractionation processes. Reaction or transfer rates can differ between isotopes or isotopologues. Isotope effects are now exploited widely across scientific disciplines for tracing element transport and sample history. Isotope effects, however, can also be also induced during sample preparation or in the mass spectrometer. The latter makes the accurate measurement of isotope variations one of the biggest challenges in inorganic mass spectrometry. Natural isotope fractionation and instrumental fractionation follow mostly similar regularities.

Although still being the subject of scientific debate, it is widely agreed that the empirically derived "exponential law"[1] describes the mass dependency of isotope fractionation processes best as long as biasing factors in the measurement including uncorrected isobaric interferences, gain/amplifier instabilities, or peak shape are under control.[2] This law relates the measured/observed isotope ratio $R_m$ in a fractionated sample with its true/original isotope ratios $R_t$:

$$R_m = R_t \cdot M^f \quad (1)$$

where $R_m$ and $R_t$ refer to the mole ratio of the two isotopes $n_{(\text{isotope B})}/n_{(\text{isotope A})}$ or measured ion currents $I_{(\text{isotope B})}/I_{(\text{isotope A})}$, $M$ refers to the ratio of the nuclide masses $m_{(\text{isotope B})}/m_{(\text{isotope A})}$, and $f$ refers to the fractionation index which describes the degree of isotope fractionation. Equation 1 shows that fractionation is not determined by the absolute differences in the isotope's masses but their ratio. This entails that fractionation effects for elements of low atomic weight such as hydrogen, carbon, or oxygen are usually stronger. Isotope effects for the heavier elements have long been assumed to exist, but as they are much smaller they became routinely measurable more recently with the advent of new analytical techniques.[3]

Availability of multicollector-inductively coupled plasma mass spectrometry (MC-ICPMS) and, to a lesser extent, multicollector-thermal ionization mass spectrometry (MC-TIMS) turned exploration of isotope abundance variations of the heavier elements quickly into a research area of its own. Possible applications under scrutiny are now countless and reach from the earth and planetary sciences,[4] as traditional domains of stable isotope based research, to environmental[5] and even biomedical research.[6] In such measurements, instrumental mass bias is commonly corrected for by measuring intermittently a standard of known isotopic composition (standard sample bracketing) or by normalizing measurements to the known isotope ratios of a reference standard of another

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element spiked to the sample (external normalization).[7] These techniques, however, can only be used in MC-ICPMS which permits an easy switch between samples and shows a high but relatively constant mass bias during a measurement sequence.[3]

The above correction techniques have become the method of choice in MC-ICPMS for practical reasons and for reaching experimental limits of measurement repeatability. However, these advantages come with a not so insignificant risk of producing measurement artifacts. This makes implementation of stringent quality control protocols an indispensable requirement.[8] The alternative "Double Spiking" approach is certainly more challenging to use but less sensitive to bias as it is less sensitive to matrix effects. As such, it remains the undisputed reference technique in MC-ICPMS and the only way to measure isotope effects reliably by MC-TIMS.[8] Introduced merely 50 years ago[9] and widely used since then, this technique is limited to elements having at least four stable isotopes such as iron, calcium, or zinc. A less sophisticated single spiking approach has been suggested by Hirata et al. for osmium isotope ratio measurements.[10] Here we describe a new concept of using an isotopic spike for mass bias correction based on gravimetric spiking (GS) and isotope dilution analysis (IDA) of the blends (GS-IDA). This novel and fresh take on isotope spiking is applicable to elements with three stable isotopes only, which we demonstrate here for magnesium as a typical candidate element and by employing a refined MC-TIMS technique for isotopic analysis.

**Correction of Instrumental Mass Bias by Double Spiking.** Many excellent articles exist on double spiking theories and their application,[9,11−15] which allows us to describe classical double spiking concepts only briefly. The double spike is prepared by mixing two stable isotopes of the element of interest in highly enriched form. Choice of isotopes and mixing proportions are tailored to minimize measurement uncertainty. The resulting double spike is characterized for isotopic composition and added to the sample in a predetermined proportion to minimize impact of uncertainties in measured isotope ratios. As the isotope composition of the double spike is known, it can be used to monitor and correct for mass bias using iterative algorithms laid out elsewhere.[16−18]

Correction techniques aim at determination of the sample fractionation index $f_{sample}$

$$\frac{R_{sample}}{R_{ref}} = M^{f_{sample}} \tag{2}$$

with $R_{sample}$ and $R_{ref}$ being the same isotope ratio in the sample and a reference standard. While the fractionation index $f_{sample}$ is the same for all isotope ratios in the sample, the ratio $R_{sample}$ to $R_{ref}$ differs between isotope ratios and is dependent on $M$, based on the exponential law. Isotope abundance variations are commonly expressed on a relative $\delta$-scale using the isotope ratio of the reference sample $R_{ref}$ as the anchoring point ($\delta_{ref}$ = 0) in units of parts per thousand (per mil).

$$\delta = \frac{R_{sample} - R_{ref}}{R_{ref}} = \frac{R_{sample}}{R_{ref}} - 1 = M^{f_{sample}} - 1 \tag{3}$$

To determine $f_{sample}$ and thus its $\delta$-value by double spiking, the proportion $P$ of added spike to the amount of element in the sample (in moles) together with the methodological fractionation index $f_{method}$ must be known which is defined in accordance to the exponential law.

$$\frac{R_m}{R_t} = M^{f_{method}} \tag{4}$$

In conventional double spiking experiments, iterative procedures permit calculation of $f_{sample}$, $f_{method}$, and $P$ from the isotope ratios of the spiked sample, the isotope composition of the double spike, and the reference standard. As there are three unknowns, the technique requires measurement of three isotope ratios, which is only possible for elements having at least four stable isotopes.

**Measurement of Isotope Variations by Gravimetric Spiking and Isotope Dilution Mass Spectrometry (GS-IDA).** Isotope dilution mass spectrometry (IDMS) is widely used for measuring the amount of element in a sample by spiking the element with a highly enriched isotope of the same element.[19]

$$P = \frac{n_{spike}}{n_{sample}} = \frac{(R_{blend} - R_{sample})}{(R_{spike} - R_{blend})} \cdot \frac{\sum_i {}^iR_{spike} + 1}{\sum_i {}^iR_{sample} + 1} = A \cdot B \tag{5}$$

In eq 5, $n_{sample}$ and $n_{spike}$ are the number of moles of sample and spike in the blend, respectively, $R_{spike}$ is the isotope ratio of the pure spike, $R_{blend}$ is the isotope ratio of the blend (mix of spike and sample), and $R_{sample}$ is the original isotope ratio of the sample before spiking. Term $A$ in eq 5 is multiplied with term $B$, which is the sum of all isotope ratios in the sample and the pure spike. All isotope ratios in eq 5 have the same reference isotope in common.

In the classical double-spike approach, $P$ must be known to obtain $f_{sample}$ and $f_{method}$. In GS-IDA, $P$ is eliminated by gravimetric mixing of the sample with different amounts of spike in solution. It can be shown that $P$ is linearly correlated with the mixing ratio $\Psi$ (see the Supporting Information for step-by-step derivation of the equations), which is proportional to the mass ratio of spike and sample solution and which can be determined at high accuracy and minimal uncertainty using a calibrated analytical balance. For elements with three stable isotopes, $P$ can then be calculated from two different isotope ratios ${}^xR_{blend}$ and ${}^yR_{blend}$. As fractionation affects isotope ratios differently, calculated values for ${}^xP$ and ${}^yP$ equal each other only in the absence of methodological bias (see Figure 1). Because $B$ in eq 5 is the same irrespective of which ratio is used for calculation of $P$, we can define this restriction as

$$\begin{aligned} {}^xA &= \frac{{}^xR_{blend,m} \cdot {}^xM^{-F_{method}} - {}^x\overline{R}_{ref,m} \cdot {}^xM^{f_{sample}}}{{}^x\overline{R}_{spike,m} \cdot {}^xM^{-F_{spike}} - {}^xR_{blend,m} \cdot {}^xM^{-F_{method}}} \\ &= \frac{{}^yR_{blend,m} \cdot {}^yM^{-F_{method}} - {}^y\overline{R}_{ref,m} \cdot {}^yM^{f_{sample}}}{{}^y\overline{R}_{spike,m} \cdot {}^yM^{-F_{spike}} - {}^yR_{blend,m} \cdot {}^yM^{-F_{method}}} = {}^yA \end{aligned} \tag{6}$$

with the true isotope ratios being expressed as measured isotope ratios following eqs 1 and 2. Only measured average isotope ratios of the reference standard $\overline{R}_{ref,m}$ and the spike $\overline{R}_{spike,m}$ and not their true values are needed when measurements are made relative to the reference standard on a $\delta$-scale using normalized fraction indices $F$. By definition, the $\delta$-value of the reference standard is zero absolute.

$$F_{spike} = f_{spike} - f_{ref} \tag{7}$$

$$F_{method} = f_{method} - f_{ref} \tag{8}$$


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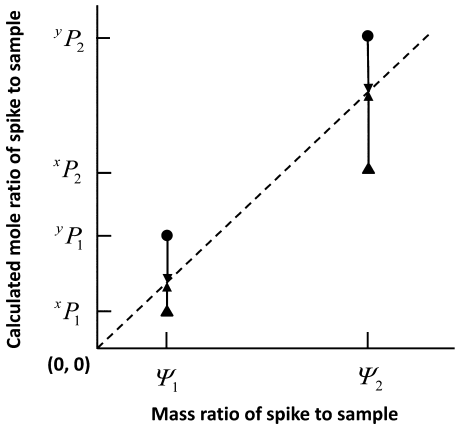


**Figure 1.** Plot of calculated mole ratio of spike to sample $P$ against mass ratio of spike to sample $\Psi$. In the absence of instrument mass bias, both are linearly correlated with the trend line (---) passing through the origin. When no spike is added to the sample, both $\Psi$ and $P$ equal zero. Instrumental mass bias affects the two isotope ratios in each blend differently. Accordingly, calculation of $P$ following isotope dilution principles yields different values when using the measured isotope ratio $^{x}R$ (▲) or the measured isotope ratio $^{y}R$ (●) of the blend. Instrument mass bias is corrected for when (a) calculations yield the same value for $P$ irrespective of the isotope ratio in the blend used for calculation and (b) both points fall on a straight line that passes through the origin.

When no spike is added to the sample, the trendline in Figure 1 must pass through the origin in absence of instrumental bias ($^{x}A_n = {}^{y}A_n = A_n$). This allows us to define an additional restriction for the intercept of the trendline

$$\sum_n A_n \cdot \sum_n \Psi_n^2 - \sum_n \Psi_n \cdot \left(\sum_n A_n \cdot \Psi_n\right) = 0 \quad (9)$$

Together with the two restriction for the blends, a definite solution for the three unknowns ($f_{sample}$, $F_{method,blend1}$, and $F_{method,blend2}$) can thus be obtained. Calibration of the spike against the reference standard by gravimetric spiking delivers $F_{spike}$. As can be seen from eqs 6 and 9, the GS-IDA approach requires measurement of two isotope ratios only ($^{x}R$ and $^{y}R$). For elements having more than three stable isotopes, all other isotope ratios can be neglected. Techniques for computing $f_{sample}$ using commercial software (Microsoft Excel, MATLAB) are accessible online (see the Supporting Information).

## EXPERIMENTAL SECTION

For experimental verification of GS-IDA we have chosen Mg, an element having three stable isotopes only ($^{24}$Mg, $^{25}$Mg, and $^{26}$Mg) which renders conventional double spiking techniques inapplicable. The same principles can be applied to elements having more than three stable isotopes for which any three isotopes can be selected for isotopic analysis while ignoring all other isotopes in the mass spectrum.

**Magnesium Isotopic Analysis.** All isotopic analysis was carried out with a thermal ionization mass spectrometer (Triton, Thermofisher Scientific, Bremen, Germany) using a rhenium double filament ion source. Tantalum oxide was used to enhance formation of positive $Mg^+$ ions which were analyzed using an array of nine Faraday cups for simultaneous ion beam collection after $m/z$ separation in a magnetic sector field. For technical details, the interested reader is referred to the Supporting Information. Measurement quality was assessed by plotting $^{25}$Mg/$^{24}$Mg and $^{26}$Mg/$^{24}$Mg isotope ratios of a measurement against each other after logarithmic transformation. A measurement was rejected when the coefficient of determination $r^2$ was less than 0.999.

External measurement precision (1 SD) for seven independent runs of a commercial Mg standard of natural isotopic composition (Titrisol, Merck, Germany) was 0.0018% for the $^{26}$Mg/$^{24}$Mg isotope ratio (0.139 436 6 ± 0.000 002 5) when normalized to the natural $^{25}$Mg/$^{24}$Mg isotope ratio[20] (0.126 63) and 0.000 91% for the $^{25}$Mg/$^{24}$Mg isotope ratio (0.126 575 9 ± 0.000 001 2) when normalized to the natural $^{26}$Mg/$^{24}$Mg isotope ratio[20] (0.139 32) using the exponential law.[21] Optimization of the MC-TIMS measurement technique allowed us to approach the instrumental limits of precision in Mg isotope ratio analysis, but this came clearly at the cost of losing analytical sensitivity. Sample loadings for Mg isotopic analysis using MC-TIMS and Faraday cups for ion detection are usually of the order of 0.5−2 μg using a mixture of silica gel and phosphoric acid to enhance formation of $Mg^+$ ions[22,23] or by making use of $MgF_2^-$ ions.[24] Reported precisions in Mg isotopic analysis ($^{26}$Mg/$^{24}$Mg, 1 SD) using these techniques are of the order of 0.01% to 0.05% for independent runs of the same sample after correction of instrumental mass bias by internal normalization. Such levels of repeatability are clearly inferior to the 0.0018% for normalized data sets that we are reporting here. This became possible by using tantalum oxide as the activator which permitted sample loadings of 30−40 μg of Mg per run, which yielded $Mg^+$ ion emissions that were sufficiently high and stable to generate data of the desired quality. This compares unfavorably to sample requirements of the order of <1 μg of Mg per analytical run using MC-ICPMS. It must be noted, however, that we have not attempted to improve measurement sensitivity in this study. At a Mg concentration of approximately 80 mg/L in human urine,[25] required amounts can be easily obtained from milliliter volumes of collected urine.

**Optimization of GS-IDA Method.** Spike composition and mass ratios $\Psi_1$ and $\Psi_2$ for the mixtures of spike and sample solution were optimized using an iterative procedure with minimization of combined standard uncertainty in the sample fractionation index $f_{sample}$ as the target. The combined standard uncertainty for a given set of variables was determined by Monte Carlo simulation as described earlier[26] and as laid out in the Supporting Information.

The fractionation indices, $F_{spike}$, $F_{method}$, and $f_{sample}$, were set to zero as their actual values do not affect the combined standard uncertainty of $f_{sample}$ after solving. Natural Mg isotope ratios as reported by Catanzaro et al. (1966) as the current best measurement recognized by IUPAC[27] were used for $^{x}\overline{R}_{ref,m}$ and $^{y}\overline{R}_{ref,m}$, while spike ratios $^{x}\overline{R}_{spike,m}$ and $^{y}\overline{R}_{spike,m}$ were set as variables for optimization. Known inhomogeneities in the reference material SRM980 used by Catanzaro et al.[28] did not affect our optimization process as we only needed an approximate isotopic composition of natural Mg in order to begin our optimization. Blend ratios were simulated based on the isotopic abundances of the reference and the spike for different mass ratios of spike to sample solution $\Psi_1$ and $\Psi_2$. Measurement uncertainty for the isotope ratios of reference and spike were set to 0.00091% and 0.0018% for $^{25}$Mg/$^{24}$Mg and $^{26}$Mg/$^{24}$Mg as experimental measurement precision for normalized data. Measurement uncertainty for the mass ratios $\Psi_1$ and $\Psi_2$ was set to zero as its contribution to the combined


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standard uncertainty is negligible when a calibrated, high precision analytical balance is used.

Results of the optimization are shown in Figure 2 for spike composition and in Figure 3 for the mass ratios $\Psi_1$ and $\Psi_2$ in the blend. There is no single isotopic composition but a wide range of optimal spike ratios that will give a low combined standard uncertainty of $f_{sample}$ (blue color region in Figure 2). The same is given for the optimal mass ratios of spike to sample solution which can range from 0.01 to 1 for $\Psi_1$ and 1 to 10 for $\Psi_2$ (see Figure 3). The latter demonstrates the robustness of the technique in terms of concentration matching of spike and sample solution when preparing the blends. Adjustments in $\Psi$ are unnecessary if concentrations of spike solution and sample solution are similar within 1 order of magnitude.

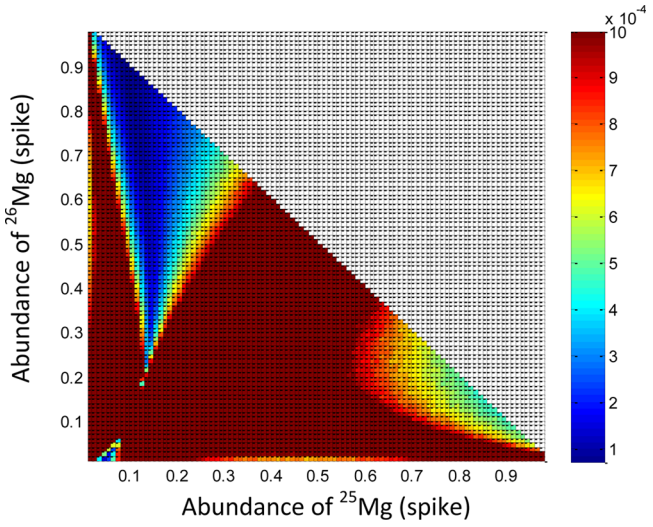


**Figure 2.** Pseudo color plot of the calculated combined standard uncertainty in $f_{sample}$ for various spike compositions as obtained by Monte Carlo simulation. Abundance of $^{24}$Mg is determined by the plotted abundances of $^{25}$Mg and $^{26}$Mg in the spike. Colors represent the magnitude of the combined standard uncertainty ($k$ = 1) on the given color scale. Combined standard uncertainties greater than $10^{-3}$ were truncated (dark red area) so as to better illustrate uncertainty distributions. Blue areas represent spike compositions which yield the lowest combined standard uncertainty in $f_{sample}$.

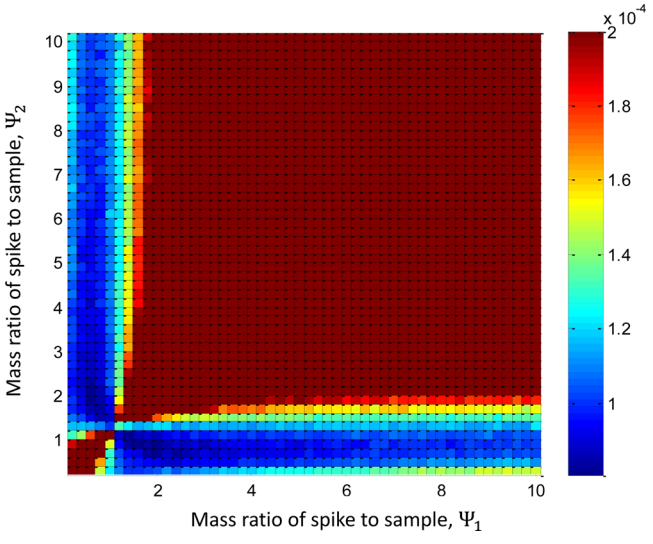


**Figure 3.** Pseudo color plot of the calculated combined standard uncertainty in $f_{sample}$ for different mass ratios of spike to sample $\Psi_1$ and $\Psi_2$ in the blend as obtained by Monte Carlo simulation. Simulations were based on a fixed spike composition (10% $^{24}$Mg, 10% $^{25}$Mg, and 80% $^{26}$Mg) in accordance with Figure 2. Combined standard uncertainties greater than $2 \times 10^{-4}$ were truncated (dark red area) so as to better illustrate uncertainty distributions. Blue areas represent values for $\Psi_1$ and $\Psi_2$ which yield the lowest combined standard uncertainty in $f_{sample}$.

**Spike Characterization.** A spike consisting of approximately 10% $^{24}$Mg, 10% $^{25}$Mg, and 80% $^{26}$Mg was prepared from isotopically enriched [$^{25}$Mg]-MgO, [$^{26}$Mg]-MgO (Chemgas, France), and elemental Mg (99.999% purity, Alfa Aesar). Materials were dissolved separately in 1 mL of 6 M HCl, and solutions were made up to 50 g with Milli-Q water. Aliquots were mixed gravimetrically to yield a spike of the desired composition at an approximate Mg concentration of 20 $\mu$g/g. Because we were refused a sample of DSM 3 as the common anchoring material for the Mg $\delta$-scale from its owner (Albert Galy, Cambridge), we decided to prepare our own anchoring material from a commercially available aqueous Mg standard (Titrisol, Merck, Germany). Both standard ($n$ = 7) and spike ($n$ = 5) were analyzed for Mg isotopic composition, and average Mg isotope ratios $\overline{R}_{ref,m}$ and $\overline{R}_{spike,m}$ were calculated without normalization or correction for instrumental mass bias. Measured isotope ratios of the reference standard (±1 SD) were 0.126 53 ± 0.000 21 ($^{25/24}\overline{R}_{ref,m}$) and 0.139 22 ± 0.000 44 ($^{26/24}\overline{R}_{ref,m}$). Measured isotope ratios of the spike (±1 SD) were 1.0173 ± 0.0046 ($^{25/24}\overline{R}_{spike,m}$) and 8.570 ± 0.076 ($^{26/24}\overline{R}_{spike,m}$), see the Supporting Information (Table S-1). The spike was calibrated by analyzing 5 sets of 2 mixtures of spiked and standard solutions each ($\Psi_1$ = 0.5, $\Psi_2$ = 5.0) with both being similar in Mg concentration. Mixing ratios but not element concentrations of spike and sample solution must be known accurately in GS-IDA, i.e., the spike does not have to be highly characterized or recalibrated when being diluted. Mixtures were prepared using a microanalytical balance (>0.5 g per weighing aliquot) at a measurement uncertainty of 0.0005 g ($k$ = 1) and dried down in Teflon cups in a Class 10 laminar flow hood in a Class 10 000 clean-room environment before filament loading. Measurements were repeated, in general, if they did not fulfill the following criteria: (1) ion signal intensity of the least abundant Mg isotope ≥1 V; (2) RSD of the ion signal intensity should be less than 10% among blocks; and (3) coefficient of determination ($r^2$) for the correlation between logarithmically transformed ratios (three-isotope plot) ≥ 0.999 for a single measurement. Removal of raw data showing a residual greater than $10^{-4}$ in correlation analysis usually improves the $r^2$ value to close to 0.9999. Measured isotope ratios for the blends were used to calculate the normalized spike fractionation index $F_{spike}$ (0.0160 ± 0.0015) following the procedures described in the Supporting Information.

**Method Validation.** As the only available certified isotopic reference material for Mg (SRM 980) was found to be isotopically inhomogeneous,[28] we decided to validate our technique by treating the referencing standard as a sample and analyze the referencing standard using the calibrated spike. From a metrological perspective, the GS-IDA technique can be considered validated if GS-IDA analysis of the reference standard (with an assigned $\delta$-value of zero) yields experimentally a $\delta$-value of zero when using the $F_{spike}$ value resulting from spike calibration (circular validation strategy). Cross


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validation against samples analyzed by MC-ICPMS were not considered as an alternative as standard sample bracketing techniques or external normalization are sensitive to artifacts and were considered secondary to our first principle approach. For method validation, we prepared five mixtures of referencing standard and spike at mass ratios of $\Psi_1 = 0.5$ and $\Psi_2 = 5.0$ as described earlier, analyzed the mixtures, and calculated the $\delta$-value of the reference material using the normalized spike fractionation index $F_{spike}$. The measured $\delta$-value of the reference standard for the $^{26}Mg/^{24}Mg$ isotope ratio was 0.00 ± 0.12 ‰ (1 SD), see the Supporting Information, Table S-2.

**Sample Analysis.** To demonstrate the applicability of the technique and evaluate performance characteristics, we analyzed a human urine sample for Mg isotopic composition. Urine and spike solution were mixed at mass ratios of $\Psi_1 = 0.5$ and $\Psi_2 = 5.0$ with both being similar in Mg concentration (~20 $\mu$g/g) followed by microwave acid digestion and ion-exchange separation using standard protocols (details in the Supporting Information). Mg recovery was 100.1 ± 0.8% (1 SD) at a total procedural blank of 5−11 ng as determined by processing aliquots of a pure $^{25}Mg$ spike solution. Modeling of data revealed that procedural blanks were low enough to have a negligible effect on measured isotope ratios within the repeatability of the measurement. Five aliquots of the same urine sample were analyzed independently using the described procedures. The analyzed $\delta$-value of the urine sample for the $^{26}Mg/^{24}Mg$ isotope ratio was 1.36 ± 0.10 ‰ (1 SD) relative to the used Mg standard (see the Supporting Information, Table S-2).

## DISCUSSION

**GS-IDA versus Double Spiking Technique.** Double spiking techniques have been used for decades as the undisputed reference technique for measuring natural isotope abundance variations. While GS-IDA also involves addition of an isotopically enriched spike to the sample, the conceptual approach we are presenting here is actually different. Double spiking requires preparation of a blend of two different spikes which permits, technically, normalization of measured isotope ratios to the isotopic composition of the added double spike. GS-IDA does not require a double spike. Because the technique follows IDMS concepts, a single 100% pure isotopic spike could also be used. This entails that optimization of spike composition is less crucial compared to double spiking experiments. Spike composition has some effect on measurement uncertainty, but the optimal range is rather wide (see Figure 2). This makes method optimization for different sets of spikes with different isotopic enrichments unnecessary once they have been determined for a given element. The method is also relatively robust when it comes to the choice of the optimal mixing ratios of spike to sample (see Figure 3). In addition, element concentration of spike and sample solution need not be known accurately as long as their mole ratio in the blend is of the same order as the mass ratio of the solutions. This can be ensured by semiquantitative analysis of the sample and spike solution.

A major advantage of the GS-IDA approach, and the key motivation for developing the GS-IDA technique, is the possibility to study natural isotope abundance variations by measuring only two isotope ratios of a given element as opposed to three isotope ratios for the conventional double-spiking technique. In this paper we demonstrated that isotope abundance variations can be measured by GS-IDA for elements having three stable isotopes only, such as Mg. For elements having more than three stable isotopes, isotope ratio measurements can be restricted to the three most abundant isotopes or those three isotopes for which measurements are least affected by isobaric interferences in the mass spectrum. This can be of particular advantage when adopting the GS-IDA technique for MC-ICPMS where isobaric interferences are much more common than in MC-TIMS. As shown earlier, all other isotopes can be safely ignored and do not have to be measured. This is of significant advantage for elements such as Ca for which all isotopes can be hardly measured simultaneously because of the limited mass dispersion of the multicollector of most instruments and the massive interference of $^{40}Ar^+$ ions with $^{40}Ca^+$ which renders measurement of the $^{40}Ca^+$ signal practically impossible. In addition, matrix effects are also minimized as in the conventional double spiking approach. In all mixtures, the spike amount varies while the amount of sample inclusive of all impurities is kept constant. Likewise, isotopic fractionation due to incomplete analyte recovery during sample purification is accounted for as the spike is added before sample digestion and separation.

**Measurement Accuracy.** Isotope abundance variations in nature are small for the heavier elements and rarely exceed a few parts per thousand (per mil) in a given isotope ratio. To decipher the information that is coded in these variations, measurements have not only to be extremely precise but also highly accurate to avoid misinterpretation or overinterpretation of data. This becomes a challenge when using MC-ICPMS for analysis. For iron, as an example, the range of natural isotope abundance variations in the $^{56}Fe/^{54}Fe$ ratio is of the order of 0.3% with the ratio being shifted further by 5−8% at the moment that the sample is transferred from the plasma to the mass analyzer.[29] In addition, iron isotope ratios of the sample can be altered during sample preparation.[30] Techniques employing standard-sample bracketing, external normalization, or a combination of both have been developed to correct for such adulterations and are now employed widely but they have their well-known limitations. They cannot correct for hidden isobaric interferences, differences in fractionation behavior between standard and sample because of matrix effects, or unexpected deviations in the fractionation behavior of the analyte element and the spiking element when using external normalization techniques. Control of these effects makes chemical separation of the analyte element indispensable for generation of meaningful data. Close to 100% recovery is also necessary to prevent isotope fractionation during ion-exchange separation,[31] thus ensuring high accuracy. Quality control protocols have been developed for MC-ICPMS[29] and are valuable to identify and reject measurement artifacts, but they are not perfect. Spiking techniques will therefore remain the reference method and benchmark for assessing measurement accuracy.

Similar to double-spiking, GS-IDA allows a better control of several sources of measurement bias. Spiking of the sample before isolation of the element for analysis automatically corrects for fractionation effects induced during sample preparation. Differences in the sample matrix between standard and sample are also of minor concern as the GS-IDA approach involves addition of a high purity spike to the sample. As for the standard addition method, blends to be analyzed differ only in the amount of analyte present and not in the matrix composition. At the same time, GS-IDA does not require knowledge of the true isotope ratios of the spike and the


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referencing standard. This becomes possible by calibration of the spike using a circular approach in which the referencing standard is used to determine the normalized spike fractionation index $F_{spike}$ using the very same algorithms that are used for sample analysis. For spike calibration, $F_{spike}$ is the unknown while the $\delta$-value of the referencing standard is known (zero by definition). In reverse, the $\delta$-value of the sample can be determined using the $F_{spike}$ value from spike calibration. In essence, the concept of calibrating the spike against the reference material in the GS-IDA approach closely follows the concept of reversed IDMS analysis in which the spike is characterized against a standard of known element concentration.[19] In both cases, the spike functions as the messenger that bridges quantitative information contained in the calibrant/reference with the quantitative information in the sample material.

**Restrictions of GS-IDA.** While the GS-IDA is robust in terms of chosen spike composition, blend composition, and matrix effects, measurement uncertainty is strongly influenced by the quality of the isotope ratio measurements. Similar to IDMS, uncertainties in isotope ratio measurements are magnified significantly when the amount ratio of spike to sample is calculated from measured isotope ratios. In the case of Mg, we found that the coefficient of determination ($r^2$) for the correlation between logarithmically transformed Mg isotope ratios (three-isotope plots) must be 0.999 or better for a given analysis to reach a target measurement uncertainty of ±0.1 in the $\delta$-value (1 SD). To reach this target, we identified criteria that measurements had to fulfill strictly for inclusion in data analysis such as the minimum required signal intensities, stability of the ion current during the measurement, and correlation analysis of measured data after logarithmic transformation. By applying these criteria strictly, external precision for independent measurements could be lowered to 0.0018% ($^{26}Mg/^{24}Mg$, 1 SD) for internally normalized data. Approximately two out of three loadings of sample or standard fulfilled all inclusion criteria. Even if analysis of two different blends of the sample may be considered as analytical replicates, although not strictly independent, sample analysis by GS-IDA is probably more time-consuming as compared to double spiking analysis, standard-sample bracketing, and external normalization, in descending order.

As for the double spiking technique, isotope fractionation in nature and during analysis must strictly follow a unifying fractionation law. For the GS-IDA approach presented here, we have chosen the exponential law but any law can be used, in principle, as long as it describes the mass dependence of the fractionation process both in nature and the ion source accurately.

On the basis of first principles, this disqualifies GS-IDA and double spiking techniques from analysis of samples affected by mass independent isotope fractionation (MIF) either in nature or during sample analysis. MIFs are known for the lighter elements and have recently been reported also for heavier elements.[32] However, on the basis of our current state of knowledge they appear to be rare in nature for the heavier elements and it still remains unclear to what extent they are actually measurement artifacts induced during isotopic analysis, namely, when using MC-ICPMS.[2,33] As an example, isotope fractionation becomes largely irreproducible and dependent on signal intensities when baseline signal intensities are measured incorrectly. Other sources of bias include over- or under-correction of isobaric interferences in the mass spectrum or offsets between detectors in the used multicollector array. This points to the undisputed strength of MC-TIMS for reliable measurement of isotope abundance variations. First, isobaric interferences are less common. Second, there are only a few exceptional cases for MC-TIMS in which a deviation from conventional mass dependent fractionation laws, namely, the common exponential law, was reported.[34−36] Finally, instrumental fractionation in MC-TIMS is much smaller but less stable as compared to MC-ICPMS. Progressive alteration of isotope composition of the sample during a MC-TIMS measurement caused by mass sensitive evaporation of the sample from the filaments results usually in a continuous drift of measured isotope ratios. This drift makes it easier to identify measurement artifacts using three-isotope-plots for technique optimization and to tightly control measurement quality when samples are being analyzed.

**Magnesium Isotope Variations in Urine.** We have applied the developed GS-IDA technique successfully to Mg isotopic analysis of a human urine sample after validating the technique by independent analysis of the calibration standard using a circular validation approach. The urine sample was found to be enriched in the heavier Mg isotopes relative to the referencing standard. Urine is a likely candidate for Mg isotope variations similar to Ca for which isotope variations have already been reported.[37,38] Both Ca and Mg are bone forming minerals, and Mg homeostasis is likewise controlled by tubular reabsorption in the kidneys with less than 5% of the filtered Mg load being excreted in urine.[39] Mg isotope variations in nature have been studied so far only by MC-ICPMS in the areas of geology and geochemistry.[31,40−49] We are the first to study Mg isotope variations in a human biological sample. Repeatability of ±0.1 ‰ for the presented GS-IDA technique using MC-TIMS compare well to reported measurement repeatabilities for MC-ICPMS (∼±0.1 ‰).

## CONCLUSIONS

By combining gravimetric spiking and standard addition principles, we were able to develop a fundamentally new concept for measurement of natural isotope variations of heavier elements. In contrast to established protocols for double spiking, the described GS-IDA approach requires measurement of three isotope signals only which makes it also applicable to elements such as Mg. Similar to the double spiking technique, GS-IDA is not restricted to a specific measurement platform and can be used both in MC-TIMS and MC-ICPMS. In MC-ICPMS, GS-IDA can become an alternative to standard-sample bracketing and external normalization which are more susceptible to measurement artifacts from first principles. While these techniques will remain the only choice for elements having two stable isotopes only, adoption of GS-IDA principles in MC-ICPMS as well as MC-TIMS opens up a new avenue to improve the accuracy of isotopic analysis in the measurement of natural isotope abundance variations in this rapidly developing area of interdisciplinary research.

## ASSOCIATED CONTENT

### Supporting Information

Step-by-step derivation of equations and detailed information on the optimization procedure and the calculation of $F_{spike}$ and $f_{sample}$ using the GS-IDA technique as well as technical details on how Mg isotopic analysis was carried out together with procedures for data analysis by robust simple regression and

data from all analytical runs. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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