

# Double Spike-Standard Addition Technique and Its Application in Measuring Isotopes

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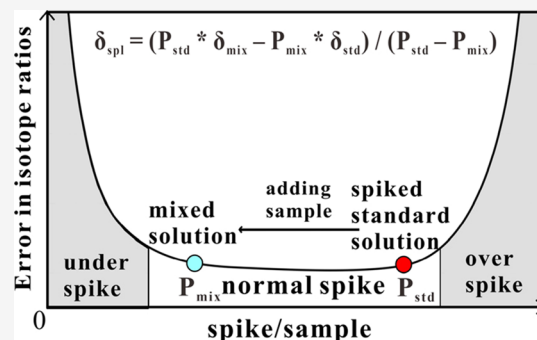


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**ABSTRACT:** Double spike (DS) method has been extensively used in determining stable isotope ratios of many elements. However, challenges remain in obtaining high-precision isotope data for ultra-trace elements owing to the limitations of instrumental signal-to-noise ratios and the systematics of precision of DS-based measurements. Here, the DS-standard addition (SA) (DSSA) technique is proposed to improve measurements of isotope compositions of ultra-trace elements in natural samples. According to the U-shaped relationship between DS measurement uncertainty and the spike/sample ratio, theoretical equations and an error propagation model (EPM) were constructed comprehensively. In our method, a spiked secondary standard solution with a high, precisely known spike/sample ratio is mixed with samples such that the mixtures have spike/sample ratios within the optimal range. The abundances of the samples relative to the added standards (sample fraction;  $f_{\text{spl}}$ ) and the samples' isotope ratios can then be obtained exactly using a standard DS data reduction routine and the isotope binary mixing model. The accuracy and precision of the DSSA approach were verified by measurements of cadmium and molybdenum isotopes at as low as 5 ng levels. Compared with traditional DS measurements, the sample size for isotope analysis is reduced to 1/6–1/5 of the original with no loss of measurement precision. The optimal mixing range  $f_{\text{spl}} = 0.15\text{--}0.5$  is recommended. The DSSA method can be extended to isotope measurement of more than 33 elements where the DS method is applicable, especially for the ultra-trace elements such as platinum group and rare earth element isotopes.



## INTRODUCTION

With advances in multiple-collector inductively coupled plasma mass spectrometry (MC-ICP-MS) and thermal ionization mass spectrometry (TIMS), a routine framework of isotope ratio determinations has been established over the decades.<sup>1–3</sup> High-precision isotope measurement using MC-ICP-MS requires a correction for instrumental mass discrimination, which can be determined by three methods: sample-standard bracketing (SSB),<sup>4,5</sup> element doping (ED),<sup>6–8</sup> and double spike (DS).<sup>1,9–14</sup> The DS approach was first proposed by Dodson<sup>9</sup> and then developed by the following researchers. Compared with SSB and ED, DS has better matrix tolerance and a lower requirement for full sample recovery during sample preparation, and it has become a routine method in determining isotopes.<sup>1,13–18</sup> Currently, stable isotope analysis faces two bottlenecks for those intending to obtain precise measurements using very small masses of the analyte: the minimum analyte mass for precise instrumental measurement and the minimum loading sample mass for chemical purification. The former can be further reduced by using high-sensitivity instrument hardware such as Faraday cups connected with high-gain amplifiers (e.g.,  $10^{13} \Omega$  resistor) or Daly detectors. The latter depends on the chemical procedure blank level, which is influenced by the laboratory cleanliness, reagent purity, and the chosen purification scheme. Sample

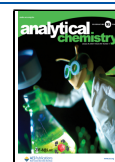
loading mass should be more than 100 times the total procedural blank to eliminate the blank effect, and isotope analysis of elements present in very low concentrations in samples requires relatively large sample sizes to be prepared for analysis. Moreover, these kinds of samples tend to have stronger matrix effects and greater analyte losses when using a given purification scheme and loading the same mass of the analyte element compared to normal samples with higher concentrations. In this case, the DS method is often a superior choice because of its high tolerance of matrix and relatively low-yield requirement.<sup>13,14,17</sup>

Standard addition (SA) is a broadly employed method to determine low concentrations of elements.<sup>19</sup> This method refers to the addition of high-purity standard material with known concentration to the sample, which can effectively reduce the blank and matrix effect.<sup>20–22</sup> Measurements calibrated by SA have greatly improved accuracy and

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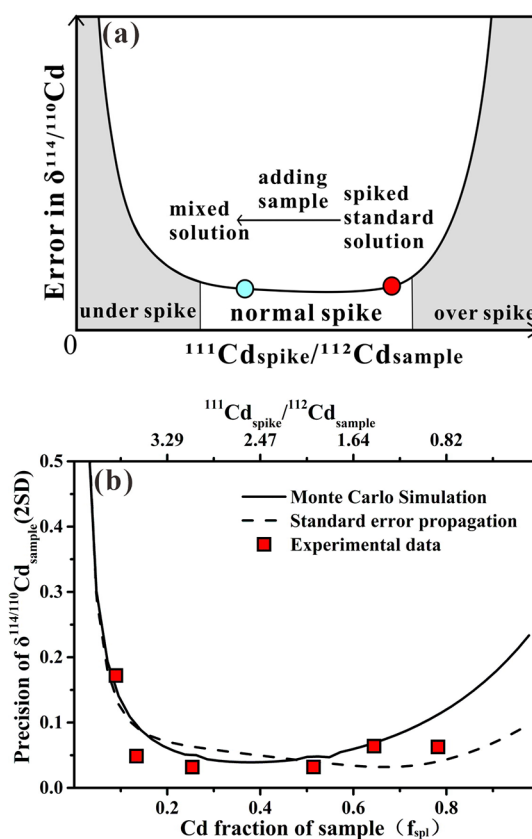


precision.<sup>22</sup> In recent years, attempts have also introduced SA to isotope measurement.<sup>23–26</sup> The SA method is primarily applied to check whether the isotope ratios are influenced by matrix effects, spectral interferences, and/or isotope fractionation during column chemistry.<sup>23–25</sup> At this stage, SA is still only evaluated to verify the accuracy and precision of isotope data. Recently, Huang et al.<sup>26</sup> combined SA and SSB to analyze Hg isotopic compositions of natural samples. The uncertainty of their method mainly comes from two parameters: the isotopic composition of the sample-standard mixture and the relative proportions of sample and standard. Since the proportion of sample and standard is difficult to measure precisely, the data quality did not exceed that of direct SSB. For example, the actual precision (2-standard deviation (2SD)) of the sample fraction is 2.5–3% using direct SSB.<sup>24,26</sup> In that way, the estimated uncertainty of Hg isotopes should be commonly greater than 0.14‰.<sup>26</sup> Although multipoint correction is carried out by applying the isotope binary mixing model,<sup>26</sup> the precision does not exceed that of traditional analytical methods, which restricts the application of the SA method in isotope analysis.

In order to obtain higher-quality isotope data of low-content elements in samples conveniently, we present the mathematical model of a DS-standard addition (DSSA) method scheme for the first time. In this method, a spiked secondary standard (std) solution with a precisely known spike (spk)/sample (spl) ratio greater than the optimal ratio is mixed with samples such that the mixtures have spk/spl ratios within the optimal range. The spk/spl ratio can be obtained precisely by DS data reduction calculations, and therefore, the precise abundance of the sample relative to that of the added standard (sample fraction;  $f_{\text{spl}}$ ) can be derived from the known spk/std ratio of the spiked standard and the measured spk/spl ratio of the mixed solution. This method can significantly improve data accuracy and precision relative to what can be obtained with standard methods on the same masses of the analyte isotopes. The effectiveness of the DSSA approach was verified by measuring Cd and Mo isotopes. Theoretically, this technique can be extended to more than 33 elements that contain three or more stable isotopes, which can be measured by DS methods.

## EXPERIMENTAL DETAILS

**The Theory of DSSA.** The DS is widely employed in determining stable isotope ratios.<sup>1,9–18</sup> DS works best when the spk/spl ratio is restricted to a limited range with measurement uncertainty minimized at a certain value but increasing nonlinearly as the actual ratio departs from this value, forming a U-shaped curve of uncertainty versus spk/spl ratio. In a range of spk/spl ratio close to the optimal ratio, the precision of the sample's isotope ratio extracted from the DS measurement and calculations is relatively insensitive to the spk/spl ratio. Samples with ratios in this range are said to be “normally spiked.”<sup>1,13,14,27,28</sup> Samples with ratios higher or lower than the normal range are considered “over spiked” or “under spiked.” For example, cadmium (Cd) has eight isotopes, which are <sup>106</sup>Cd, <sup>108</sup>Cd, <sup>110</sup>Cd, <sup>111</sup>Cd, <sup>112</sup>Cd, <sup>113</sup>Cd, <sup>114</sup>Cd, and <sup>116</sup>Cd. <sup>111</sup>Cd and <sup>113</sup>Cd were generally severed as DS.<sup>28–30</sup> The normal spike (<sup>111</sup>Cd<sub>spk</sub>/<sup>112</sup>Cd<sub>spl</sub>) range of the Cd DS is 0.8–6.0, and the optimal ratio is 2.0.<sup>28</sup> As shown in Figure 1a, DSSA means adding, to each sample, a spiked secondary standard solution with high and precisely known spk/spl ratio to produce a mixed solution with the normal spk/



**Figure 1.** (a) Schematic diagram of the double spike-standard addition technique. (b) Best mixing ratio of sample to standard solution by theoretical simulation (blank curves) and experimental measurement (red square). The sample and secondary standard solution used for the simulation curves and measured data points were NIST 2711a and Cd-Sp26 (Tables 1 and S4), respectively.

spl ratio. Ideally, the spk/spl ratios of the secondary standard solution and the mixed solution should be within the normal spike range. For Cd isotopes, we can use several pure Cd solutions like NIST 3108, Münster Cd, or Spex Cd-CUGB to serve as the secondary standard solution with a high <sup>111</sup>Cd<sub>spk</sub>/<sup>112</sup>Cd<sub>spl</sub> ratio (e.g., 4.00). When this solution is mixed with the sample, the <sup>111</sup>Cd<sub>spk</sub>/<sup>112</sup>Cd<sub>spl</sub> ratio should approach 2 or a slightly lower value (Figure 1a). After measurements are made on the mass spectrometer, the <sup>111</sup>Cd<sub>spk</sub>/<sup>112</sup>Cd<sub>spl</sub> ratio and  $\delta^{114/110}\text{Cd}$  value ( $\delta^{114/110}\text{Cd} = ((^{114}\text{Cd}/^{110}\text{Cd})_{\text{sample}} / (^{114}\text{Cd}/^{110}\text{Cd})_{\text{NIST 3108}} - 1) \times 1000\%$ ) of both the standard and the mixed solution can be precisely obtained by well-developed DS data reduction schemes.<sup>1,12,14,28,30</sup> Then, the actual sample fraction in the mixture ( $f_{\text{spl}}$ ) can be calculated according to the <sup>111</sup>Cd<sub>spk</sub>/<sup>112</sup>Cd<sub>spl</sub> and <sup>111</sup>Cd<sub>spk</sub>/<sup>112</sup>Cd<sub>std+spl</sub> values of standard and mixed solution. Finally, the sample's isotope composition is calculated using an isotope binary mixing model. The mathematical framework is as follows:

$$^{111}\text{Cd}_{\text{spk}}/^{112}\text{Cd}_{\text{std}} = P_{\text{std}} \quad (1)$$

$$^{111}\text{Cd}_{\text{spk}}/^{112}\text{Cd}_{\text{std+spl}} = P_{\text{mix}} \quad (2)$$

where  $P_{\text{std}}$  and  $P_{\text{mix}}$  represent spk/spl ratios of the secondary standard solution and the mixed solution, respectively. Subscripts “spk,” “std,” and “spl” refer to DS, standard solution, and sample, respectively.

Table 1. Preparation of Spiked Secondary Standard Solutions and Their Isotopic Compositions

Cd solution	preparation	$\delta^{114/110}\text{Cd}$	2SD	$^{111}\text{Cd}_{\text{spk}}/^{112}\text{Cd}_{\text{spl}}$	2SD	$N^{\text{g}}$
Cd-Sp51 <sup>a</sup>	50.5%Sp <sup>b</sup> + 49.5%Nist <sup>c</sup> + Cd DS <sup>d</sup>	-1.068	0.031	4.108	$3.32 \times 10^{-4}$	6
Cd-Sp26	25.5%Sp + 74.5%Nist + Cd DS	-0.538	0.023	4.111	$3.05 \times 10^{-4}$	9
Cd-Sp11	10.9%Sp + 89.1%Nist + Cd DS	-0.230	0.024	4.035	$2.01 \times 10^{-4}$	6
Cd-Mu4	3.6%Mu <sup>e</sup> + 96.4%Nist + Cd DS	0.160	0.030	4.062	$1.45 \times 10^{-4}$	6
Cd-Mu10	10.4%Mu + 89.6%Nist + Cd DS	0.464	0.033	4.131	$6.43 \times 10^{-4}$	9
Cd-Mu20	20.3%Mu + 79.7%Nist + Cd DS	0.904	0.030	4.149	$1.66 \times 10^{-4}$	6
Mo solution	preparation	$\delta^{98/95}\text{Mo}$	2SD	$^{100}\text{Mo}_{\text{spk}}/^{98}\text{Mo}_{\text{spl}}$	2SD	$N^{\text{g}}$
Mo-NIST	100%NIST SRM 3134 + Mo DS <sup>f</sup>	-0.002	0.034	9.897	$3.80 \times 10^{-4}$	5
Mo-NOD	100%NOD-P-1 + Mo DS	-0.876	0.025	4.680	$1.21 \times 10^{-3}$	7
Mo-SD	100%SD1-5 + Mo DS	0.688	0.033	4.126	$6.33 \times 10^{-4}$	6

<sup>a</sup>The number at the end of the name means the percentage of Sp or Mu solution. <sup>b</sup>Sp, Spex Cd CUGB. <sup>c</sup>Nist, NIST SRM 3108. <sup>d</sup>Cd DS, <sup>111</sup>Cd-<sup>113</sup>Cd double spike. <sup>e</sup>Mu, Münster Cd. <sup>f</sup>Mo DS, <sup>97</sup>Mo-<sup>100</sup>Mo double spike. <sup>g</sup>Number of independent analyses.

Then, eqs 1 and 2 can be reorganized into eq 3:

$$^{112}\text{Cd}_{\text{std}}/^{112}\text{Cd}_{\text{std+spl}} = P_{\text{mix}}/P_{\text{std}} \quad (3)$$

The isotope binary mixing model can be expressed as the following equations:

$$f_{\text{spl}} + f_{\text{std}} = 1 \quad (4)$$

$$\delta_{\text{mix}} = f_{\text{spl}} \times \delta_{\text{spl}} + f_{\text{std}} \times \delta_{\text{std}} \quad (5)$$

where  $f_{\text{spl}}$  and  $f_{\text{std}}$  are the <sup>112</sup>Cd molar fractions of the sample and standard solution in the mixed solution, respectively. Due to the negligible offset between <sup>112</sup>Cd molar fraction and Cd molar fraction,  $f_{\text{spl}}$  and  $f_{\text{std}}$  directly represent the Cd fraction for later description.  $\delta_{\text{spl}}$ ,  $\delta_{\text{std}}$ , and  $\delta_{\text{mix}}$  are the  $\delta^{114/110}\text{Cd}$  values of sample, standard, and mixed solution, respectively. Rearranging eq 5 gives

$$\delta_{\text{spl}} = (\delta_{\text{mix}} - f_{\text{std}} \times \delta_{\text{std}})/f_{\text{spl}} \quad (6)$$

and  $f_{\text{spl}}$  and  $f_{\text{std}}$  can also be written as

$$f_{\text{std}} = ^{112}\text{Cd}_{\text{std}}/^{112}\text{Cd}_{\text{std+spl}} = P_{\text{mix}}/P_{\text{std}} \quad (7)$$

$$f_{\text{spl}} = 1 - f_{\text{std}} = 1 - P_{\text{mix}}/P_{\text{std}} \quad (8)$$

Combining eq 6 with eqs 7 and 8, we obtain

$$\delta_{\text{spl}} = (P_{\text{std}} \times \delta_{\text{mix}} - P_{\text{mix}} \times \delta_{\text{std}})/(P_{\text{std}} - P_{\text{mix}}) \quad (9)$$

**Error Propagation Model (EPM).** The uncertainty of calculated  $\delta_{\text{spl}}$  comes from the errors associated with the  $P$  and  $\delta$  values of the standard and mixed solutions. As described above, a U-shaped relationship exists between the uncertainty of the  $\delta$  value derived from measurements and  $P$ . That is, within the normal range of  $P$ , the uncertainty of the  $\delta$  value should be small and the  $\delta$  error increases nonlinearly when  $P$  is too large or too small.<sup>14,27,28,31</sup> Thus, we used Monte Carlo simulation and standard error propagation methods to estimate the uncertainty of the sample's  $\delta$  value derived from the DSSA method. Standard error propagation can be calculated through eq 9 (details listed in S1):

$$\sigma_{\delta\text{-spl}} = \frac{\sqrt{P_{\text{mix}}^2 \sigma_{\delta\text{-std}}^2 + P_{\text{std}}^2 \sigma_{\delta\text{-mix}}^2}}{P_{\text{std}} - P_{\text{mix}}} \quad (10)$$

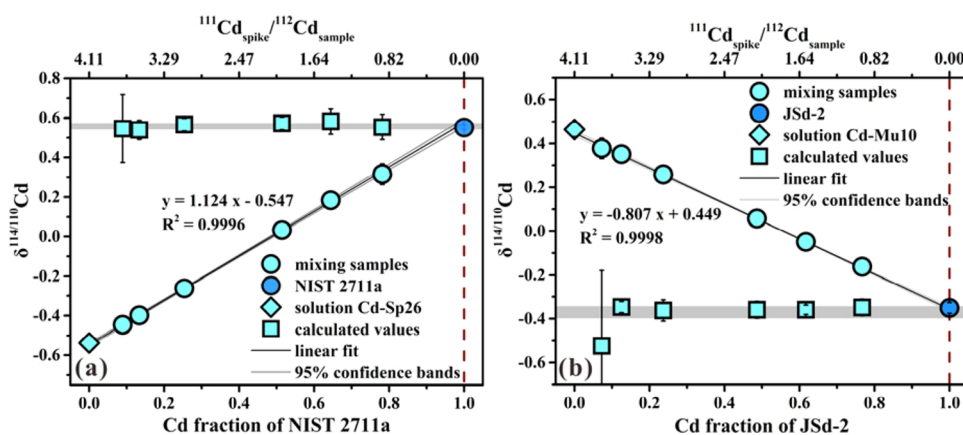
where  $\sigma_{\delta\text{-std}}$  and  $\sigma_{\delta\text{-mix}}$  are the 2SD values of the measured standard and mixed solutions.

**Reagents and Materials.** The optima-grade HCl, HNO<sub>3</sub>, and HF were purchased from the Beijing Institute of Chemical Reagents and distilled once for HF and twice for HCl and HNO<sub>3</sub> using sub-boiling stills (DST-4500, Saville, USA). Ultrapure water with a resistivity of 18.2 MΩ cm was obtained by Milli-Q Element system (Millipore, USA). H<sub>2</sub>O<sub>2</sub> (35%, wt./wt., guarantee reagent grade) was purchased from Alfa Aesar. Seven geological reference materials (GRMs), including basalts BHVO-2 and BCR-2, Fe-Mn nodules NOD-A-1 and NOD-P-1, dolomite Jdo-1, soil NIST 2711a, and stream sediment JSD-2, were analyzed in this study.

**Preparation of Spiked Secondary Standard Solutions.** High purity and concentrated solutions are preferred for preparing the secondary standard solutions; alternatively, solutions purified from natural samples with high element content are acceptable alternative choice. In this study, six Cd secondary standard solutions were prepared by mixing a <sup>111</sup>Cd-<sup>113</sup>Cd DS solution with NIST SRM 3108, Münster Cd, or Spex Cd-CUGB (Table 1). NIST SRM 3108 is the “delta-zero” standard for Cd isotopes.<sup>32</sup> The  $\delta^{114/110}\text{Cd}$  values of Münster Cd and Spex Cd-CUGB were  $4.458 \pm 0.041$  (2SD,  $n = 8$ ) and  $-2.115 \pm 0.038$  (2SD,  $n = 15$ ), respectively. The preparation of three spiked Mo secondary standard solutions was different from making Cd standard solutions. One of them was made directly by mixing NIST SRM 3134 (the “delta-zero” standard of Mo) with a <sup>97</sup>Mo-<sup>100</sup>Mo DS. The other two solutions were prepared by purifying high-Mo natural samples (NOD-P-1 or SD1-5), which were mixed with DS before chemical separation. NOD-P-1 is a widely used manganese nodule reference material.<sup>33,34</sup> SD1-5 is a black shale from Enshi, Hubei Province, China.<sup>35,36</sup>

**Sample Preparation and Mass Spectrometry.** Sample digestion followed procedures presented by Zhu et al.<sup>31</sup> The digested solutions were stored in 1 mL of 10% (v/v) HNO<sub>3</sub>. Then the sample aliquots containing 2.4–107 ng Cd or 1.5–60 ng Mo were mixed with the corresponding standard solutions. The  $^{111}\text{Cd}_{\text{spk}}/^{112}\text{Cd}_{\text{spl}}$  or  $^{100}\text{Mo}_{\text{spk}}/^{98}\text{Mo}_{\text{spl}}$  of mixed solutions were all within the normal spike range. The chemical purification schemes of Cd (Table S1) and Mo (Table S2) followed Tan et al.<sup>28</sup> and Zhu et al.,<sup>34</sup> respectively. The total procedural blanks of Cd and Mo were both below 0.06 ng.

The Cd and Mo isotopic compositions were measured on the Neptune Plus MC-ICP-MS (ThermoFisher Scientific) at the Isotope Geochemistry Laboratory, China University of Geosciences (Beijing). The instrumental and operating parameters are summarized in Table S3. The improved Aridus II with an ice chamber was adopted to enhance signal



**Figure 2.**  $\delta^{114/110}\text{Cd}$  values of mixing samples and calculated values. The error bar represents twice the standard deviation (2SD). The dark blue circles of NIST 2711a or JSd-2 are analyzed by normal DS method. The intersection of the fitting line and  $f_{\text{spl}} = 1$  (wine dash line) is a theoretical true value of a sample. (a) NIST 2711a was mixed with Cd-Sp26 solution in different fractions. The gray bar denotes the recommended value ( $0.557 \pm 0.032\%$ ) from Lu et al.<sup>40</sup> (b) JSd-2 was mixed with Cd-Mu10 solution in different fractions. The gray bar denotes the recommended value ( $-0.373 \pm 0.057\%$ ) from Lu et al.<sup>40</sup>

sensitivity and stability.<sup>37</sup> Due to the high-sensitivity instrumental settings (Table S3), only 1 mL of  $10 \mu\text{g L}^{-1}$  Cd or Mo was consumed in each analytical session. Accordingly, having  $\geq 30$  ng Cd or Mo was enough to measure each sample three times to achieve better accuracy and precision. The Mo isotope ratio is expressed as per mil ( $\%$ ) deviation relative to NIST SRM 3134:  $\delta^{98/95}\text{Mo} = ((^{98}\text{Mo}/^{95}\text{Mo})_{\text{sample}} / (^{98}\text{Mo}/^{95}\text{Mo})_{\text{NIST 3134}} - 1) \times 1000\%$ .

## RESULTS AND DISCUSSION

**Evaluating the Optimal Mixing Range.** According to the theory of DSSA and its EPM, sample fraction ( $f_{\text{spl}}$ ) is one of the critical factors affecting the calculated results. As shown in Figure 1b, the uncertainty of  $\delta_{\text{spl}}$  when plotted versus  $f_{\text{spl}}$  forms a U-shaped relationship similar to the U-shaped curve for the standard DS method provided in Figure 1a.<sup>14,17,38</sup> Therefore, the uncertainty shown in Figure 1b is likely attributed to the spk/spl ratios resulting from different sample-standard mixing ratios. When  $f_{\text{spl}}$  is  $\leq 10\%$  or  $> 80\%$ , the simulated propagation error and 2SD of the measured data are  $\geq 0.1\%$  (Figure 1b). Thus, the actual  $f_{\text{spl}}$  should be kept within the mixing range of 10–80% to obtain optimal precision. The measured precision of  $P_{\text{std}}$  or  $P_{\text{mix}}$  acquired from the DS data reduction is generally less than  $10^{-3}$  (Table 1). Following eqs 3, 7, and 8, the  $f_{\text{spl}}$  and  $f_{\text{std}}$  of mixed solution can be precisely obtained and error propagation to  $\delta_{\text{spl}}$  is then negligible (below  $0.001\%$ ) according to EPM (S1). Given that this method focuses on determining the elemental isotope compositions of ultra-low concentration samples, the sample size needs to be reduced as much as possible. The Monte Carlo numerical simulation recommends that  $f_{\text{spl}}$  in the range of 0.15–0.5 is ideal (Figure 1b). Consequently, compared to the SA coupled with SSB,<sup>24,26</sup> the DSSA can significantly reduce uncertainty contribution from the sample proportion in the sample-standard mixture and improve final data quality within the optimized mixing range.

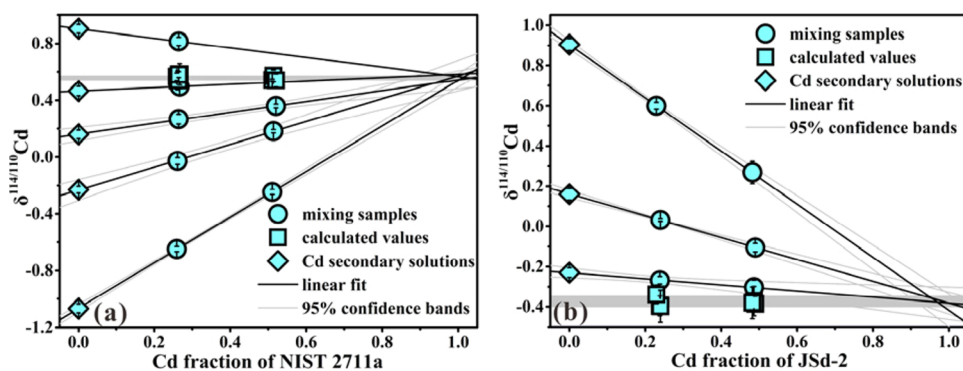
Taking Cd isotopes as an example, the  $^{111}\text{Cd}_{\text{spk}}/^{112}\text{Cd}_{\text{std}}$  ratios of spiked Cd standard solutions ( $P_{\text{std}}$ ) are around 4 (Table 1). When mixed with sample solutions, the lower the sample fraction ( $f_{\text{spl}}$ ) is, the closer  $P_{\text{mix}}$  is to  $P_{\text{std}}$  and *vice versa*. When the  $f_{\text{spl}}$  in the mixed solution increases to nearly 80% (e.g., 78.18%), the  $P_{\text{mix}}$  will decrease to 0.8 (e.g., 0.897, Table

S4). In this case, the 2SD of the final  $\delta^{114/110}\text{Cd}_{\text{mix}}$  derived from the DS data reduction calculations would be  $\geq 0.06\%$ .<sup>28</sup> Similarly, when  $f_{\text{spl}}$  is below 10%, the 2SD will reach  $\pm 0.172\%$  for NIST 2711a ( $f_{\text{spl}} = 8.96\%$ ) or  $0.345\%$  for JSd-2 ( $f_{\text{spl}} = 7.27\%$ , Table S4, and Figure 2), which is unacceptable for data quality requirements in many studies. However, for  $f_{\text{spl}}$  within the range of 10–80%, the calculated  $\delta^{114/110}\text{Cd}_{\text{spl}}$  values range from  $0.539 \pm 0.067$  to  $0.583 \pm 0.075\%$  for NIST 2711a and from  $-0.397 \pm 0.079$  to  $-0.339 \pm 0.047\%$  for JSd-2 (Table S4), consistent with published results.<sup>39,40</sup> Since the minimum sample size should be considered, we are more concerned about the data quality of low  $f_{\text{spl}}$ . The calculated  $\delta^{114/110}\text{Cd}$  values are  $-0.347 \pm 0.026$  and  $0.540 \pm 0.049\%$  for the  $f_{\text{spl}}$  as small as 12.54% (JSd-2) and 13.38% (NIST 2711a), respectively (Table S4 and Figure 2), with no deviation from the recommended values.<sup>40</sup>

Equally, the given mixing range is also validated by Mo isotopes. The  $\delta^{98/95}\text{Mo}$  value of NOD-P-1 acquired by DSSA is  $-0.854 \pm 0.029\%$  when its  $f_{\text{spl}}$  is 15.11% (Table S5), still in line with the reported value.<sup>34,41</sup> Considering both data robustness and the goal of minimizing sample size,  $f_{\text{spl}}$  should not be less than 15% nor more than 50% if the standards listed in Table 1 are used. Thus, 15–50% ( $P_{\text{mix}}/P_{\text{std}} = 0.85\text{--}0.5$ ) is the best recommended mixing range for  $f_{\text{spl}}$  before sample purification. This range is theoretically also applicable to isotope measurements of other elements for which DS methods can be used. It is noted that the  $P_{\text{std}}$  should be adjusted for each element according to the normal range of DS and  $P_{\text{mix}}$ .

**Validating the Accuracy and Precision.** Several selected GRMs with known isotope ratios were analyzed using normal DS. The  $\delta^{114/110}\text{Cd}$  values were  $0.552 \pm 0.013\%$  (2SD,  $n = 5$ ) for NIST 2711a and  $-0.351 \pm 0.025\%$  (2SD,  $n = 4$ ) for JSd-2, and the  $\delta^{98/95}\text{Mo}$  values were  $-0.654 \pm 0.039\%$  (2SD,  $n = 6$ ) for NOD-A-1 and  $-0.030 \pm 0.039\%$  (2SD,  $n = 3$ ) for BCR-2. These values are in good agreement with reported data<sup>30,34,39–42</sup> and will be utilized to compare with other data in the following sections.

According to the optimal mixing range mentioned above ( $f_{\text{spl}} = 15\text{--}50\%$ ), if the added Cd mass (excluding spike) of the secondary standard is fixed at 30 ng and the sample Cd mass is varied, or *vice versa*, we can make a series of mixed sample



**Figure 3.**  $\delta^{114/110}\text{Cd}$  values of samples mixed with different secondary solutions and their calculated values. The error bar represents twice the standard deviation (2SD). The details of secondary solutions are listed in Table 1. (a) Secondary solutions used include Cd-Sp51, Cd-Sp11, Cd-Mu4, Cd-Mu10, and Cd-Mu20. The gray bar denotes the recommended value ( $0.557 \pm 0.032\%$ ) of NIST 2711a.<sup>40</sup> (b) Secondary solutions used include Cd-Sp11, Cd-Mu4, and Cd-Mu20. The gray bar denotes the recommended value ( $-0.373 \pm 0.057\%$ ) of JSd-2.<sup>40</sup>

solutions, which contain 34.6–137.5 ng total Cd (sample Cd = 4.6–107.5 ng) and  $f_{\text{spl}}$  ranges from 13.38 to 78.18% (Table S4). These were done, and the precision and accuracy of DSSA-derived results were evaluated. As shown in Figure 2, when  $f_{\text{spl}}$  varies from 13.38 to 76.71%,  $\delta^{114/110}\text{Cd}$  values of NIST 2711a and JSd-2 calculated by DSSA agree well with the correspondingly published results,<sup>39,40</sup> and the precision is better than 0.079‰. Even if sample Cd is as low as 4.63 ng (NIST 2711a) or 4.31 ng (JSd-2), the  $\delta^{114/110}\text{Cd}$  values of NIST 2711a ( $0.540 \pm 0.049\%$ ) and JSd-2 ( $-0.347 \pm 0.026\%$ ) acquired by DSSA (Table S4) still match reported data<sup>39,40</sup> and the above results obtained by normal DS using higher amounts of analyte. Furthermore, basalt BHVO-2 with low Cd ( $0.09 \mu\text{g g}^{-1}$ ) and high X/Cd (X = interference and matrix elements) was analyzed to demonstrate the robustness of DSSA (Table S4). The four independent calculated results are  $-0.006 \pm 0.075$ ,  $0.000 \pm 0.024$ ,  $-0.031 \pm 0.075$ , and  $0.007 \pm 0.012\%$ , all consistent with recommended value  $0.010 \pm 0.073\%$ .<sup>40</sup>

Similarly, DSSA was also applied to Mo isotope measurement. The  $\delta^{98/95}\text{Mo}$  values of Jdo-1, BCR-2, and NOD-A-1 obtained by DSSA were  $0.479 \pm 0.022$ ,  $-0.054 \pm 0.066$ , and  $-0.677 \pm 0.032\%$ , respectively (Table S5), in agreement with the results acquired by normal DS and published values using higher amounts of analyte.<sup>33,34,43</sup> The sample  $\delta^{98/95}\text{Mo}$  precision of DSSA was better than 0.066‰ when  $f_{\text{spl}}$  was within the optimal mixing range (15–50%). Overall, high accuracy and precision data of Cd and Mo isotopes can be achieved by DSSA while consuming as little as  $\sim 5$  ng Cd or Mo in the sample loaded for chemical purification.

**Comparison between Single- and Multistandard Measurement.** The isotope binary mixing model has demonstrated that the  $\delta_{\text{mix}}$  of mixed sample should fall on the line between the two endmembers in a  $f_{\text{spl}}-\delta$  plot (Figure 2). The isotopic ratio of one unknown endmember can be extrapolated via fitting of the linear equation if the other endmember and more than one mixed sample are known. A spiked secondary standard solution with certified  $\delta$  value can be mixed with a given sample in varying proportions ( $f_{\text{spl}}$ ); the  $\delta_{\text{mix}}$ ,  $\delta_{\text{std}}$ , and true  $\delta_{\text{spl}}$  should have good linearity (Figure 2), and the actual sample's  $\delta_{\text{spl}}$  can be calculated by eq 9, which should be on the same horizontal line with true  $\delta_{\text{spl}}$  (Figure 2).

Compared to single-standard measurement, the multistandard measurement (Figure 3) requires a sample to be mixed with more than two spiked secondary standard

solutions, which have distinctive isotope ratios. Each secondary standard solution and its mixed samples yield a fitting line with a specific slope. Theoretically, these fitting lines should have one intersection at  $f_{\text{spl}} = 1$ —the true  $\delta_{\text{spl}}$  (Figure 2). However, there are actually  $N(N - 1)/2$  intersections due to measurement uncertainty, where  $N$  is the number of standards used. Under ideal conditions, these intersections should be within the precision range (2SD) of the true  $\delta_{\text{spl}}$ . If not, this may indicate an analytical problem, and the analytical processes of those data should be checked, or the sample should be reanalyzed. Thus, we can get  $N$  values by single-standard calculation and  $N(N - 1)/2$  results by multistandard intersection analysis when mixing a sample with different  $N$  solutions individually. A total of  $N(N + 1)/2$  independent results can be obtained. As shown in Figure 3a, five secondary standard solutions and their mixed samples with NIST 2711a give five independent fitting lines and produce 10 intersections ( $N(N - 1)/2$ , the total should be  $N(N + 1)/2 = 15$ ). These intersections all agree with the single-standard results of 0.539–0.583‰. JSd-2 differs from NIST 2711a in that its accepted  $\delta^{114/110}\text{Cd}$  value is lower than the standard solutions, while that of NIST 2711a is higher, but its tests yielded similar results as NIST 2711a (Figure 3b): three intersections are still in accordance with its single-standard values of  $-0.397$  to  $-0.339\%$ . Generally, multistandard analysis of NIST 2711a, JSd-2, and BHVO-2 (Figure S1) show no significant difference in accuracy and precision with single-standard analysis (square points) and recommended values (gray bar). The  $\delta^{114/110}\text{Cd}$  difference of these three samples between single- and multistandard is within 0.057‰, which has no significant difference in accuracies ( $P < 0.05$ , Student's  $t$ -test). Moreover, the precision of calculated  $\delta_{\text{spl}}$  has no difference when secondary standard solutions used are more than two, which means six independent results can be acquired (as JSd-2 shown in Figure 3b). Therefore, two standard solutions are enough to acquire high-quality isotope data. Nevertheless, single-standard is an acceptable choice for commonly natural samples because of its simple experimental operation.

As  $\delta^{114/110}\text{Cd}$  values of secondary standard solutions vary from  $-1.068 \pm 0.031$  to  $0.904 \pm 0.030\%$ , the calculated results of NIST 2711a and JSd-2 have individual discrepancies less than 0.044 and 0.056‰ (Table S4), suggesting that the isotopic composition of the standard solution has no measurable effect for DSSA. Additionally, the Mo secondary standard solutions Mo-NOD and Mo-SD (Table 1) are

purified from actual samples, which is slightly different from Cd secondary standard solutions derived from the direct mixing of pure solutions. Still, the results show that DSSA works well when mixing these two solutions (Table S5), indicating that the selection of secondary standard solutions can be flexible.

**Applications in Measuring Isotopes.** The DSSA technique is applicable for isotopic measurement of more than 33 elements that DS can analyze. As the DS approach can be adopted for three-isotope elements like Si, Mg, and K,<sup>44</sup> our new approach could be extended to these three elements in the future. Additionally, DSSA is independent of the instrument and can also apply to TIMS. It provides a convenient choice for isotope measurement of trace or ultra-trace elements. Taking the widely studied UCC (upper continental crust) as an example, the mass fractions of 21 trace elements range from 0.022 ng g<sup>-1</sup> to 1.0 μg g<sup>-1</sup>.<sup>45</sup> The isotope analysis of these trace elements requires large sample masses for chromatographic purification. However, a large sample size often leads to high blank levels and strong matrix effects, forcing the purification procedures to be cumbersome. Minimizing the amount of element required for instrumental measurement and reducing the total procedure blank are critical to isotope analysis. Both requirements can be moderated to some extent using DSSA. The addition of secondary standard solutions increases the analyte mass and dilutes the matrix and interference elements in mixed samples, which are beneficial to chemical separation and instrumental determination. The amount of sample digestion can be reduced to 15% level of regular DS (Figure 1b), which contributes to less blank and matrix. Our measured Cd and Mo isotope results indicate that the isotopic compositions of samples with low element contents analyzed by DSSA can be as robust as those of higher content samples processed through the same purification procedure.

The DSSA technique has potential in isotope determination for two situations. One is samples with the limited quantity or not readily available, such as rare meteorites, lunar samples, and biological tissues associated with element metabolism.<sup>46,47</sup> The other is ultra-trace elements in natural samples, like Cd and platinum group metals. Using the DSSA approach can ease the difficulty of analyzing such samples. For instance, preconcentration of large amounts (3–20 L) of seawater is necessary for traditional Cd isotope measurement, especially for lower Cd (Cd < 0.1 nmol kg<sup>-1</sup>) seawater.<sup>29,48</sup> The sample consumption of seawater would reduce to 1/5 of the conventional approach by DSSA (0.6–4 L). Moreover, Cd concentrations in common igneous rocks, such as peridotite, basalt, or granite, are mainly in the range of 0.02–0.1 μg g<sup>-1</sup>.<sup>49–51</sup> The average Cd concentrations of silicate earth and UCC are only 0.04 μg g<sup>-1</sup> and 0.09 μg g<sup>-1</sup>,<sup>45</sup> respectively. As at least 30 ng Cd is needed for three measurements to calculate a sample's SD, more than 3 g of samples must be digested. Like Cd, Pt isotope analysis needs at least 0.5–6.5 g of samples.<sup>53</sup> The finicky digestion and purification procedures caused by large sample size would lead to the increase of blank and less efficient removal of interfering elements. In this regard, the DSSA technique can directly reduce the sample size, simplifying experimental procedures and reducing blank.

Furthermore, the multistandard analysis can verify the isotopic composition of new GRMs or isotope reference solutions because of its convincing accuracy and precision. It is beneficial for cases where reference materials show isotope ratios that vary from lab to lab. Multistandard analysis of DSSA

is an appropriate choice to identify whether the variation is caused by sample heterogeneity or analytical deviation.

## CONCLUSIONS

This study puts forward a novel analytical technique coupling DSSA, which has advantages over SSB, ED, and traditional DS. The DSSA method can achieve high-precision isotope measurement of ultralow-content samples by adding spiked secondary standard solutions to allow high-precision measurements with reduced sample size. The high-precision and accuracy of Cd and Mo isotope measurements by DSSA obtained in this study verify the reliability of this new technique. The addition of a standard solution effectively dilutes the interference and matrix elements and increases the total element mass measured. The isotopic measurement of GRMs indicates that DSSA is powerful even if the sample size of Cd or Mo is as small as ~5 ng with a precision better than 0.079‰ (2SD). When considering both precision and sample size, a target range of 15–50% sample fraction is recommended. Using DSSA, the actual sample loading can decrease to nearly 1/6–1/5 of that needed for a conventional DS measurement with the same precision. The DSSA provides a powerful method for measuring isotope compositions of precious samples such as lunar rocks or meteorites. Meanwhile, this technique can further extend the application of isotopes in planetary, atmospheric, life, marine, environmental, agricultural, and other sciences.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.analchem.2c03802>.

Supporting information includes the detailed uncertainty calculation (S1), chemical purification procedures of Cd (Table S1) and Mo (Table S2), instrumental parameters (Table S3), measured results of Cd isotopes (Table S4) and Mo isotopes (Table S5), and comparison of Cd isotope data calibrated by single- and multiple standards (Figure S1) (PDF)

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## Notes

The authors declare no competing financial interest.

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## REFERENCES

- (1) Johnson, T. M.; Herbel, M. J.; Bullen, T. D.; Zawislanski, P. T. *Geochim. Cosmochim. Acta* **1999**, *63*, 2775–2783.
- (2) Rehkämper, M.; Schönbächler, M.; Stirling, C. H. *Geostand. Geoanal. Res.* **2001**, *25*, 23–40.
- (3) Albarède, F.; Telouk, P.; Blichert-Toft, J.; Boyet, M.; Agraniér, A.; Nelson, B. *Geochim. Cosmochim. Acta* **2004**, *68*, 2725–2744.
- (4) Zhu, X. K.; O’Nions, R. K.; Guo, Y.; Belshaw, N. S.; Rickard, D. *Chem. Geol.* **2000**, *163*, 139–149.
- (5) Galy, A.; Belshaw, N. S.; Halicz, L.; O’Nions, R. K. *Int. J. Mass Spectrom.* **2001**, *208*, 89–98.
- (6) Walder, A. J.; Platzner, I.; Freedman, P. A. *J. Anal. At. Spectrom.* **1993**, *8*, 19–23.
- (7) Marechal, C. N.; Telouk, P.; Albarede, F. *Chem. Geol.* **1999**, *156*, 251–273.
- (8) White, W. M.; Albarede, F.; Telouk, P. *Chem. Geol.* **2000**, *167*, 257–270.
- (9) Dodson, M. H. *J. Sci. Instrum.* **1963**, *40*, 289–295.
- (10) Eugster, O.; Tera, F.; Wasserburg, G. J. *J. Geophys. Res.* **1969**, *74*, 3897–3908.
- (11) Russell, W. A.; Papanastassiou, D. A.; Tombrello, T. A. *Geochim. Cosmochim. Acta* **1978**, *42*, 1075–1090.
- (12) Siebert, C.; Nagler, T. F.; Kramers, J. D. *Geochem. Geophys. Geosyst.* **2001**, *2*, No. 2000GC000124.
- (13) Zhu, J.-M.; Johnson, T. M.; Clark, S. K.; Zhu, X.-K. *Chinese J. Anal. Chem.* **2008**, *36*, 1385–1390.
- (14) Rudge, J. F.; Reynolds, B. C.; Bourdon, B. *Chem. Geol.* **2009**, *265*, 420–431.
- (15) Gale, N. H. *Chem. Geol.* **1970**, *6*, 305–310.
- (16) Galer, S. *Chem. Geol.* **1999**, *157*, 255–274.
- (17) Klaver, M.; Coath, C. D. *Geostand. Geoanal. Res.* **2019**, *43*, 5–22.
- (18) Wang, X.; Johnson, T. M. *Anal. Chem.* **2021**, *93*, 7449–7455.
- (19) Bader, M. J. *Chem. Educ.* **1980**, *57*, 703.
- (20) Antler, M.; Maxwell, E. J.; Duford, D. A.; Salin, E. D. *Anal. Chem.* **2007**, *79*, 688–694.
- (21) Claverie, F.; Malherbe, J.; Bier, N.; Molloy, J. L.; Long, S. E. *Anal. Chem.* **2013**, *85*, 3584–3591.
- (22) Jones, W. B.; Donati, G. L.; Calloway, C. P., Jr.; Jones, B. T. *Anal. Chem.* **2015**, *87*, 2321–2327.
- (23) Nielsen, S. G.; Rehkämper, M.; Baker, J.; Halliday, A. N. *Chem. Geol.* **2004**, *204*, 109–124.
- (24) Tipper, E. T.; Louvat, P.; Capmas, F.; Galy, A.; Gaillardet, J. *Chem. Geol.* **2008**, *257*, 65–75.
- (25) Kurzawa, T.; König, S.; Labidi, J.; Yierpan, A.; Schoenberg, R. *Chem. Geol.* **2017**, *466*, 219–228.
- (26) Huang, S.; Song, Q.; Zhang, Y.; Yuan, D.; Sun, L.; Chen, Y.; Jiang, R.; Lin, H. *Anal. Chem.* **2019**, *91*, 7063–7069.
- (27) Wu, G.; Zhu, J.-M.; Wang, X.; Han, G.; Tan, D.; Wang, S.-J. *J. Anal. At. Spectrom.* **2019**, *34*, 1639–1651.
- (28) Tan, D.; Zhu, J.-M.; Wang, X.; Han, G.; Lu, Z.; Xu, W. *J. Anal. At. Spectrom.* **2020**, *35*, 713–727.
- (29) Xue, Z.; Rehkämper, M.; Schonbachler, M.; Statham, P. J.; Coles, B. *J. Anal. Bioanal. Chem.* **2012**, *402*, 883–893.
- (30) Liu, M. S.; Zhang, Q.; Zhang, Y.; Zhang, Z.; Huang, F.; Yu, H. *M. Geostand. Geoanal. Res.* **2019**, *44*, 169–182.
- (31) Zhu, J.-M.; Wu, G.; Wang, X.; Han, G.; Zhang, L. *J. Anal. At. Spectrom.* **2018**, *33*, 809–821.
- (32) Abouchami, W.; Galer, S. J. G.; Horner, T. J.; Rehkämper, M.; Wombacher, F.; Xue, Z.; Lambelet, M.; Gault-Ringold, M.; Stirling, C. H.; Schönbächler, M.; Shiel, A. E.; Weis, D.; Holdship, P. F. *Geostand. Geoanal. Res.* **2013**, *37*, 5–17.
- (33) Feng, L.; Zhou, L.; Hu, W.; Zhang, W.; Li, B.; Liu, Y.; Hu, Z.; Yang, L. *J. Anal. At. Spectrom.* **2020**, *35*, 145–154.
- (34) Zhu, H.-G.; Zhu, J.-M.; Tan, D.; Lin, X.; Lu, K.; Yang, W. *J. Anal. At. Spectrom.* **2022**, *37*, 1063–1075.
- (35) Zhu, J.-M.; Johnson, T. M.; Clark, S. K.; Zhu, X.-K.; Wang, X.-L. *Geochim. Cosmochim. Acta* **2014**, *126*, 228–249.
- (36) Xia, B.; Zhu, J.-M.; Wang, X.; Zhang, L.; Wu, G. *Sci. Total Environ.* **2021**, *783*, No. 147126.
- (37) Wu, G.; Zhu, J. M.; Wang, X.; Johnson, T. M.; Han, G. *Anal. Chem.* **2020**, *92*, 1463–1469.
- (38) John, S. G. *J. Anal. At. Spectrom.* **2012**, *27*, 2123–2131.
- (39) Borovicka, J.; Ackerman, L.; Rejsek, J. *Talanta* **2021**, *221*, No. 121389.
- (40) Lu, Z.; Zhu, J. M.; Tan, D.; Wang, X.; Zheng, Z. *Geostand. Geoanal. Res.* **2021**, *45*, 565–581.
- (41) Asael, D.; Tissot, F. L. H.; Reinhard, C. T.; Rouxel, O.; Dauphas, N.; Lyons, T. W.; Ponzevera, E.; Liorzou, C.; Chéron, S. *Chem. Geol.* **2013**, *362*, 193–210.
- (42) Skierszkan, E. K.; Amini, M.; Weis, D. *Anal. Bioanal. Chem.* **2015**, *407*, 1925–1935.
- (43) Liang, Y.-H.; Halliday, A. N.; Siebert, C.; Fitton, J. G.; Burton, K. W.; Wang, K.-L.; Harvey, J. *Geochim. Cosmochim. Acta* **2017**, *199*, 91–111.
- (44) Coath, C. D.; Elliott, T.; Hin, R. C. *Chem. Geol.* **2017**, *451*, 78–89.
- (45) Rudnick, R. L.; Gao, S. Composition of the continental crust. *In Treatise on Geochemistry* 2003, *1–64*, DOI: 10.1016/B0-08-043751-6/03016-4.
- (46) Montes-Bayon, M.; Bettmer, J. *Adv. Exp. Med. Biol.* **2018**, *1055*, 111–137.
- (47) Tanaka, Y. K.; Hirata, T. *Anal. Sci.* **2018**, *34*, 645–655.
- (48) Xie, R. C.; Galer, S. J. G.; Abouchami, W.; Frank, M. *Chem. Geol.* **2019**, *511*, 371–379.
- (49) Heinrichs, H.; Schulz-Dobrick, B.; Wedepohl, K. H. *Geochim. Cosmochim. Acta* **1980**, *44*, 1519–1533.
- (50) Taylor, S. R.; McLennan, S. M. *Rev. Geophys.* **1995**, *33*, 241–265.
- (51) Witt-Eickschen, G.; Palme, H.; O’Neill, H. S. C.; Allen, C. M. *Geochim. Cosmochim. Acta* **2009**, *73*, 1755–1778.
- (52) McDonough, W. F. Compositional model for the Earth’s core. *In Treatise on Geochemistry* 2003, *547–568*.
- (53) Creech, J. B.; Schaefer, B. F.; Turner, S. P. *Geostand. Geoanal. Res.* **2020**, *44*, 223–229.