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Inter-comparison of stable iron, copper and zinc isotopic compositions in six reference materials of biological origin

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ABSTRACT

There is a lack of certified reference materials with an organic matrix for which metal isotope ratios have been certified. Here, we have determined the iron, copper and zinc stable isotopic compositions for six reference materials of biological origin with diverse matrices, i.e. BCR-380R (whole milk), BCR-383 (beans), ERM-CE464 (tuna fish), SRM-1577c (bovine liver), DORM-4 (fish protein) and TORT-3 (lobster hepatopancreas) in three different labs. The concentrations for six major and sixteen trace elements, spanning almost four orders of magnitude, were also measured and the results obtained show an excellent agreement with certified values, demonstrating that the dissolution step was quantitative for all the standards. By taking literature data into account, 39 possible pair-wise comparisons of mean iron, copper and zinc isotopic values (δ values) could be made. Results of Tukey multiple comparisons of means yielded 11 significantly different pairs. Most of these differences are of the same order of magnitude as the estimated mean expanded uncertainties (U, $k = 2$) (±0.10‰, ±0.05‰, and ±0.05‰ for the δ^{56}Fe, δ^{65}Cu and δ^{66}Zn values, respectively). The present inter-comparison study finally proposes nineteen new preferred values for the Cu, Zn and Fe isotopic compositions of six reference materials of biological origin.

1. Introduction

Involved in a wide range of enzymes and proteins, regulating metabolic pathways and physiological processes [e.g. 1, metals including copper (Cu), iron (Fe) and zinc (Zn), are vital to the organism and any imbalance can have adverse effects on human health [e.g 2. In recent times, in addition to the determination of concentrations, the measurement of stable isotope ratios or isotopic compositions is evolving into a new tool of choice to study the metabolism of essential mineral elements in living organisms, both of plant or and animal origin. By definition, isotope fractionation refers to changes in the relative abundance of naturally occurring stable isotopes of a particular element among coexisting reservoirs hosting this element [e.g. 3. Vibrational frequencies decrease with mass commanding heavy isotopes to be enriched in coordination with the stiffest bonds, in particular those involving the oxidized form and with ligands with the stronger electronegativity (O > N > S) [3]. So far, the transition metals, iron (Fe), copper (Cu) and zinc (Zn) have been the most studied for their isotopic composition in this context.

In plants, the Fe isotopic composition (δ^{56}Fe) varies according to the type of root uptake and shows differences among plant organs [4,5]. In animals, the Fe isotopic compositions are highly fractionated between organs [6–8] and provide information on the Fe intestinal absorption efficiency [7,9–11]. In healthy conditions, the blood Fe isotopic composition of human males is different from that of pre-menopausal females [6] due to menstrual losses [12,13] and the up-regulated Fe absorption to compensate for the losses. Varying Fe isotopic composition among blood compartments signals different redox processes in red blood cells hemoglobin and in serum transferrin [14–17]. For a still unknown reason, the body mass index seems correlated with the whole blood Fe isotopic composition [16,17]. Hepatic accumulation of Fe occurs due to hereditary hemochromatosis and is reflected in the isotopic

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composition of Fe in blood [18,19], while Fe dysregulations due to various etiologies resulting in anemia [20] can be scrutinized by means of the Fe isotopic compositions in whole blood and serum.

The Cu isotopes are fractionated during Cu uptake and translocation in plants [21,22]. In healthy animals, the Cu isotopes are processed slightly differently in the gut via the involvement of the microbiota [23] and Cu isotopic compositions are highly fractionated between organs [8, 24]. Similarly to Fe, the blood Cu isotopic composition is different between human males and premenopausal females due to menstrual losses and the corresponding reaction of the body to these losses [12,13]. The blood Cu isotopic composition has been shown to become lighter during ageing in a remote human Yakut population [25], and experiments on ageing *C. elegans* have confirmed this finding [26]. Copper isotopic compositions are highly sensible to disease conditions and have been investigated as a new biomarker for Wilson disease [27,28], cancer [29, 30], liver diseases [24,31] and neurodegenerative disorders [32–35].

Zinc isotopes in plants are fractionated between the organs, including roots [22,36]. Zinc isotopes are also fractionated between the organs of animals [37,38]. In humans, the blood and urine Zn isotopic composition have been suggested to reveal dietary habits [39] and more genrally, the Zn status in human [40]. The blood Zn isotopic composition has been shown to become heavier during ageing in a remote human Yakut population [25], but experiments on a worm model [26] and on a rodent model [41] did not confirm this finding. Larner et al. [42] have found different Zn isotopic compositions between breast tumors and adjacent healthy tissues, but the lack of a difference in the whole blood/serum Zn isotopic composition between breast cancer patients and healthy individuals restrains the interest in the Zn isotopic composition as a potential biomarker of cancer. Lobo et al. [30] did not reproduce the observed differences in Zn isotopic compositions between tumor and adjacent healthy tissues in oral squamous cell carcinoma. Recently, Schilling et al. [43], measured the urine Zn isotopic composition and found lower values in pancreatic ductal adenocarcinoma patients relative to healthy controls. Mouse models of neurodegenerative diseases have a brain Zn isotopic composition that is heavier than in normal wild type mice [41,44].

Determination of stable isotopic compositions of metals and the corresponding instrumentation was originally developed for the comprehension of geological problems. For quality control of the results obtained, geological reference materials were thus provided. The overall quality of the isotope ratio results depends not only on the measurement itself, but also profoundly on the sample preparation. The preparation of the sample involves the dissolution of the sample and the isolation of the metal of interest, typically by ion-exchange chromatography. Geological materials are inorganic samples with a silicate matrix containing high levels (>1%) of metals while the organic matrix of biological materials generally contains metals at trace levels only (<0.1%). The geological and biological matrices are therefore very different, and the preparation step (dissolution and target element isolation) must be adapted accordingly. As a consequence, geological reference materials cannot be used for the quality control of the processing and analysis of biological samples. To that end, the scientific community devoted to the study of metal isotopic compositions in living systems has reported isotopic values for reference biological materials. A list of the published values is given in Table S1 for Fe, Cu and Zn isotopic compositions [39,45–63]. Several observations can be drawn from that compilation. First, the Seronorm reference materials exhibit a significant isotopic heterogeneity between various lots for Fe and Cu. Second, the serum matrix seems to be, by itself, highly heterogeneous because the serum reference material BCR-639 has Cu and Zn isotopic compositions that are substantially different from those of the Seronorm materials. This holds for muscle too, because the two bovine muscle reference materials SRM-8414 and ERM-BB184 have Zn isotopic compositions differing by ~0.8‰. Third, to the best of our knowledge, there is no value published for the Fe and Cu isotopic compositions for reference materials of plant origin.

2. Experimental

The present study is an effort to fill some of the gaps that exist in the certification of isotopic compositions of biological reference materials. Here, we have determined the Fe, Cu and Zn stable isotopic compositions for five biological reference materials with a matrix of animal origin, i.e. BCR-380R (whole milk), ERM-CE464 (tuna fish), SRM-1577c (bovine liver), NRC-DORM-4 (fish protein) and NRC-TORT-3 (lobster hepatopancreas), and one biological reference material with a matrix of plant origin, BCR-383 (beans). We also have included an in-house fetal bovine serum (FBS) as a quality control sample. These isotopic compositions have been measured in three different labs, i.e. the Laboratoire de Géologie de Lyon (hereafter denoted LGL-TPE), Ecole Normale Supérieure de Lyon, France, the Atomic and Mass Spectrometry - A&MS research unit at the department of Chemistry (hereafter denoted A&MS), Ghent University, Belgium and Laboratoire G-TIME (hereafter denoted G-TIME), Université libre de Bruxelles, Belgium.

2.1. Sample description

The samples investigated consist of six biological reference materials. BCR-380R (whole milk), BCR-383 (beans) and ERM-CE464 (tuna fish) were purchased from the European Institute for Reference Materials and Measurements (IRMM), SRM-1577c (bovine liver) was purchased from the US National Institute of Standard and Technology (NIST), and DORM-4 (fish protein) and TORT-3 (lobster hepatopancreas) from the Canadian National Research Council (NRC). For all these reference materials, the homogeneity of the initial powder is warranted down to a sample size of ~100 mg by the institute in which it was prepared. Also included as a quality control sample, is a fetal bovine serum sample (FBS) sold by Sigma–Aldrich with the lot number 014M3399. The FBS material was freeze-dried and homogenized in an agate mortar. All materials were stored at the LGL-TPE. To ensure homogeneity preservation and consistent values between the different laboratories, all the reference materials were gently shaking before aliquots of at least 1g were collected and sent to the A&MS and G-TIME labs for analysis.

2.2. Sample digestion

For all sample digestions, and to avoid high measurement uncertainties due to heterogeneity of the reference material powder, a minimum sample size of 100 mg was weighed as recommended by the three institutes for reference materials.

2.2.1. LGL-TPE

All sample preparation procedures were carried out in clean laminar flow hoods using double-distilled acids to avoid any exogenous contaminations. Samples were first weighted and then dissolved in a mixture of HNO_3 (15 M) and H_2O_2 (30%) in Savillex® beakers at 120 °C for about 72 h. Attention was paid in the first hours because the mixture could be very reactive with the subsequent formation of nitric and carbon-based fumes that need to be regularly vented to avoid any overpressure. After complete dissolution, the samples were dried down and subsequently taken up with 5 mL of HNO_3 (0.5 M) from which a small aliquot was used for the quantitative determination of major and trace element concentrations.

2.2.2. A&MS

Sample preparation was performed in a class 10 clean lab. The acids used for digestion, HNO_3 (14 M) and HCl (12 M), were purified via sub-boiling distillation. Ultrapure water with a resistivity of ≥18.2 MΩ cm was obtained from an Element Milli-Q system and used for dilutions. Sample digestion was performed using HNO_3 (14 M) and H_2O_2 (30%) in closed Teflon Savillex® beakers at 110 °C for 16 h.

2.2.3. G-TIME

Sample preparation was carried out under a class-100 laminar flow hood in a class 1000 clean room. All the reagents used were purified by sub-boiling distillation and appropriate dilutions were made with 18.2 MΩ cm grade MilliQ water. To mineralize the sample, dry ashing of the sample placed in a pre-cleaned semi-opened ceramic crucible was carried out at 600 °C in a muffle furnace for 12 h. The powdered samples were transferred into a 15 mL Teflon vial (Savillex©) with 14 M HNO_3 and *suprapur* H_2O_2 at room temperature, followed by heating on a hot plate for 12–14 h. Samples were then dried down and dissolved using a 1:1 mixture of concentrated HCl and HNO_3.

2.3. Sample preparation and instrumentation

2.3.1. LGL-TPE

Iron was isolated from the concomitant matrix by ion-exchange chromatography, using a Bio-Rad column filled with 2 mL of AG 1-X8 (100–200 mesh) anionic resin. After elimination of the sample matrix with 8 mL of HCl (6 M) with traces of H_2O_2, Fe is eluted with 10 mL of HNO_3 (0.5 M). The procedure was repeated twice to ensure maximum iron purity for optimal isotope ratio measurements, leading to a total procedure blank of about 10 ng (n = 5), which generally represents 0.2 to 0.05%, and at worst 1% in the case of BCR380, of the amount of Fe present in the sample solutions prepared for isotopic analysis. After the purification, Zn remains in the iron fraction. To evaluate the effect of Zn on the measured iron isotopic compositions of the samples, we added various amounts of an elemental solution of Zn to the IRMM-014 Fe standard solution to obtain Zn/Fe ratios from 0 to 1. No deviation of the δ^{56}Fe values was observed within the analytical error (Fig. S1). The elution protocol is given in Table 1.

Copper and Zn were isolated by ion-exchange chromatography using a quartz column filled with 1.8 mL of AG MP-1 (100–200 mesh) anionic resin (Table 1). After elimination of the sample matrix with 10 mL of HCl (7 M) + H_2O_2 (0.001%), Cu and Zn were successively eluted with 20 mL of HCl (7 M) + H_2O_2 (0.001%) and 10 mL of HNO_3 (0.5 M) respectively, following the procedure described by Maréchal et al. [64]. The procedure was repeated twice leading to total procedural blanks that were on average 1.4 ng for Cu (n = 6) and 6.7 ng for Zn (n = 6), which represents in average 0.1% and 0.4% of the Cu and Zn amount, respectively, of element present in the sample solutions prepared for isotopic analysis.

The concentrations were measured following the method described in Garçon et al. [65] by ICP-OES (Thermo Scientific, iCap 6000 Radial) for major elements (Na, P, Mg, S, K and Ca) and by ICP-MS (Thermo Scientific, iCap-Q) for trace elements (Li, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Ba and Pb). Briefly, the concentrations were calculated using calibration curves based on multi-elemental solutions. These solutions were also used to monitor and correct for the instrumental drift over the analytical session. Matrix effects and instrumental drift were also corrected for using In and Sc as internal standards for trace and major elements, respectively.

Iron isotopic compositions were measured using a Thermo Scientific Neptune Plus MC-ICP-MS at LGL-TPE. The instrument settings are given in Table 2. On the day of the measurement session, Fe purified solutions were diluted to 1 mg/L and doped with Ni used as an internal standard to monitor and correct for instrumental mass discrimination. A 1 g/L of Ni elemental standard solution from Alfa-Aesar was diluted and added to the standard and sample solutions at a concentration matching the Fe concentration, namely 1 mg/L.

The Cu and Zn isotopic compositions were measured by Nu Plasma MC-ICP-MS (Nu Instruments, Nu Plasma LR) following the procedure described by Dinis et al. [61], which is summarized in Table 2. On the day of analysis, Cu and Zn purified solutions were diluted in a Zn-doped solution (Zn JMC 3–0749L, Johnson Matthey Royston, UK) and a Cu-doped solution (Cu SRM 976, National Institute of Standards and Technology, Gaithersburg, MD, USA), respectively, to match the concentration of the standard bracketing solution (usually 300 μg/L). Measurements were carried out in static multi-collection mode and one single measurement consisted of 1 block of 30 cycles with an integration time of 10 s.

2.3.2. A&MS

After digestion, the solution was evaporated to dryness and the residue was redissolved in a mixture of HCl (8 M) and H_2O_2 (0.001%) and allowed to stand for 1 h to make sure that all of the iron and copper were in their higher oxidation states. Copper, Fe and Zn were isolated from the sample matrix using 1 mL of AG-MP1 anion exchange resin, using a revised procedure from Ref [10,20,31] (Table 1). After the sample loading and the matrix elution using 3 mL of 8 M HCl +0.001% H_2O_2, Cu, Fe and Zn were sequentially eluted using 9 mL of 5 M HCl+0.001% H_2O_2, 7 mL of 0.6M HCl and 7 mL of 0.7M HNO_3, respectively. A second chromatographic separation was applied to the Cu fraction following the same protocol to ensure the complete removal of sodium. The final purified Cu, Fe and Zn fractions were evaporated and re-dissolved in 0.5 mL of 14M HNO_3 twice to remove residual chlorides. The final residue was re-dissolved in 0.5 mL of 0.28 M HNO_3. The overall procedure led to total procedural blanks (n = 4) of about 8 ng for Fe, 0.3 ng for Cu and 3.0 ng for Zn, which represents less than 1% of the amount of each element present in the measurement solutions.

A Thermo Scientific Neptune MC-ICP-MS instrument (Germany) was used for all isotope ratio measurements. Medium mass resolution was used for all isotope ratios to avoid spectral overlap. Measurements were performed in static collection mode, using Faraday collectors connected to 10^{11} Ω amplifiers. Instrument settings and data acquisition parameters are shown in Table 2. Fe purified solutions were diluted to 300 μg/L and doped with Ni (300 μg/L) as internal standard to monitor and correct for instrumental mass discrimination. Cu and Zn solutions were adjusted to 200 μg/L and doped with Ni and Cu (both at 200 μg/L), respectively. Baseline correction was performed for each measurement sequence. The in-house elemental standards A&MS-Cu, A&MS-Fe and A&MS-Zn, previously characterized isotopically [10,20,31], were included every 5 samples for quality assurance/quality control (QA/QC) of the isotope ratio measurements.

2.3.3. G-TIME

After complete dissolution, solutions were evaporated to dryness at 125 °C and HCl (6 M) was added to convert the metals into their chloride form prior to the chromatographic separation. Isolation of Zn was realized using a 2 mL Bio-Rad column loaded with pre-cleaned AG1-X8 100–200 mesh resin (analytical grade, chloride form) following a modified elution protocol from Maréchal et al. [64] (see details in

Table 1
Ion exchange protocols for the chromatographic separation of Cu, Fe and Zn.

Lab	LGL-TPE		A&MS	G-TIME
Element	Fe	Cu, Zn	Cu, Fe, Zn	Zn
Column	Bio Rad	Quartz	Bio Rad	Bio-Rad
Resin	2 mL AG 1-X8	1.8 mL AG MP-1	1 mL AG MP-1	2 mL AG 1-X8
Matrix	8 mL HCl (6M)	10 mL HCl (7 M) + H_2O_2 (0.001%)	3 mL HCl (8 M) + H_2O_2 (0.001%)	4 mL HCl (6M) + H_2O_2 (0.001%)
Cu elution		20 mL HCl (7 M) + H_2O_2 (0.001%)	9 mL HCl (5 M) + H_2O_2 (0.001%)	
Fe elution	10 mL HNO_3 (0.5 M)	13 mL HCl (2 M) + H_2O_2 (0.001%)	7 mL HCl (0.6 M)	
Zn elution		10 mL HNO_3 (0.5 M)	7 mL HNO_3 (0.7 M)	15 mL HNO_3 (1 M) + HBr (0.1 M)

Table 2
Instrument settings and data acquisition parameters for MC-ICP-MS analysis.

Lab	LGL-TPE		A&MS			G-TIME
Element (+ internal standard)	Fe (+Ni)	Cu (+Zn), Zn (+Cu)	Fe (+Ni)	Cu (+Ni)	Zn (+Cu)	Zn (+Cu)
MC-ICP-MS	Neptune	Nu Plasma	Neptune	Neptune	Neptune	Nu Plasma
RF power (W)	1200	1350	1200	1200	1200	1350
Plasma condition	wet, quartz cyclonic/scott double spray chamber	wet, cyclonic spray chamber	wet, cyclonic/scott double spray chamber	wet, cyclonic/scott double spray chamber	wet, cyclonic/scott double spray chamber	wet, cyclonic spray chamber
Sample uptake rate (mL min-1)	100	100	100	100	100	80–100
Coolant Ar flow (L min-1)	15	13.5	15	15	15	15
Auxiliary Ar flow (L min-1)	0.7–1.1	1.25	0.75–0.85	0.75–0.85	0.75–0.85	0.90
Nebulizer Ar flow (L min-1)	0.9–1.1	1	1.03–1.08	1.03–1.08	1.03–1.08	0.86
Mass resolution	4000 (or 10,000)	300	4000 (or 10,000)	4000	4000	300
Sampling cone	Ni Jet, ø = 1.1 mm	Ni	Ni Jet, ø = 1.1 mm	Ni Jet, ø = 1.1 mm	Ni Jet, ø = 1.1 mm	Ni
Skimmer cone	Ni H-type, ø = 0.8 mm	Ni H-type	Ni H-type, ø = 0.8 mm	Ni H-type, ø = 0.8 mm	Ni H-type, ø = 0.8 mm	Ni WA6
Cup configuration	H3: ^{62}Ni; H1: ^{60}Ni; L1: ^{57}Fe; L2: ^{56}Fe; L4: ^{54}Fe	H5: ^{69}Ga; H4: ^{68}Zn; H3: $^{67.5}$; H2: ^{67}Zn; Ax: ^{66}Zn; L1: $^{65.5}$; L2: ^{65}Cu; L3: ^{64}Zn; L4: ^{63}Cu; L5 ^{62}Ni	H3: ^{62}Ni; H1: ^{60}Ni; Ax: ^{58}(Fe + Ni); L1: ^{57}Fe; L2: ^{56}Fe; L4: ^{54}Fe	H3: ^{65}Cu; H1: ^{63}Cu; Ax: ^{62}Ni; L1: ^{61}Ni; L3: ^{60}Ni	H2: ^{68}Zn; H1: ^{67}Zn; Ax: ^{66}Zn; L1: ^{65}Cu; L2: ^{64}Zn; L3: ^{63}Cu	H6: ^{68}Zn; H4: ^{67}Zn; H2: ^{66}Zn; Ax: ^{65}Cu; L2: ^{64}Zn; L4: ^{63}Cu; L5: ^{62}Ni
Sensitivity	1 ppm ~ 12.5 V FeT	0.3 ppm ~ 7 V CuT ~ 5 V ZnT	0.3 ppm–15 V	0.2 ppm ~ 15 V	0.2 ppm–2.5 V	0.5 ppm ~ 7 V CuT
Blank signal (2% HNO_3)	^{56}Fe ~ 10 mV	^{63}Cu ~ 0.1 mV	<0.01%	<0.01%	<0.05%	^{64}Zn ~ 0.5 mV
Integration time (s)	10	10	4.194	4.194	4.194	10
Cycles	30	30	45	45	45	60

Ref. [66]). The sample is re-dissolved in 1 mL of 6 M HCl with 20 µl of 30% H_2O_2 before loading on the column. The matrix, Cu and Fe were discarded using HCl (8 M) followed by HCl (0.5 M), and Zn was eluted with 15 mL of 1 M HNO_3 + 0.1 M HBr (Table 2). Total procedural blanks (digestion and chromatography) were less than 10 ng of Zn. Dried Zn fractions were redissolved in 100 µL of concentrated HNO_3 and then diluted at 400 µg/L in 0.05M HNO_3 for isotope ratio measurements. Cu standard solution was systematically added to samples and standards as an internal standard with identical concentrations (400 µg/L). Zinc isotopes ratios were measured on a Nu Plasma II HR MC-ICP-MS (Nu Instruments) with the instrumental settings as given in Table 3. All Zn and Cu masses (^{64}Zn, ^{66}Zn, ^{67}Zn, ^{68}Zn, ^{70}Zn and ^{63}Cu, ^{65}Cu) were monitored, as well as ^{62}Ni to correct for interference between ^{64}Ni and ^{64}Zn. However, the ^{62}Ni beam intensity was systematically lower than the background signal ($\leq 1.10^{-4}$ V). The signals were measured by static multi-collection. A single measurement consisted of a measurement of 60 cycles, i.e. 3 blocks of 20 cycles (with an integration time of 10 s, each). On-peak baseline measurement with 30 s integration time was done prior to each analysis using a 0.05 M HNO_3 acid blank, and the value obtained is then subtracted on-line during the analytical sequence for all the samples/standards.

In all labs and for all isotope ratios measurements, instrumental mass discrimination and temporal drift were corrected with an exponential law using an admixed element as internal standard, combined with sample-standard bracketing, as recommended by Maréchal et al. [64]. The doping conditions are given in Table 2. All the results of isotopic ratios measurements are given in the delta annotation (expressed in ‰) and reported relative to the international isotopic standard solutions NIST IRMM-014 for Fe, SRM-976 for Cu and JMC 3–0749L for Zn using the following equations:

$$\delta^{56}Fe = \left[\frac{\left(^{56}Fe/^{54}Fe\right)_{sample}}{\left(^{56}Fe/^{54}Fe\right)_{standard}} - 1\right] \times 1000 \quad (1)$$

$$\delta^{65}Cu = \left[\frac{\left(^{65}Cu/^{63}Cu\right)_{sample}}{\left(^{65}Cu/^{63}Cu\right)_{standard}} - 1\right] \times 1000 \quad (2)$$

Table 3
Linear regression parameters for the observed mass fractionations. The theoretical slope (β) values are given for kinetically and thermodynamically controlled mass fractionations for comparison.

Lab				LGL-TPE	A&MS	G-TIME
$d^{57}Fe$ vs $d^{56}Fe$	Measured	Intercept		0.027 (±0.019)	−0.003 (±0.021)	
		Slope (b)		1.486 (±0.015)	1.422 (±0.018)	
	Theoretical b	Equilibrium	1.475			
		Kinetic	1.488			
$d^{67}Zn$ vs $d^{66}Zn$	Measured	Intercept		0.010 (±0.011)	0.013 (±0.008)	0.067 (±0.014)
		Slope (b)		1.515 (±0.023)	1.484 (±0.019)	1.427 (±0.031)
	Theoretical b	Equilibrium	1.479			
		Kinetic	1.490			
$d^{68}Zn$ vs $d^{66}Zn$	Measured	Intercept		0.018 (±0.006)	0.002 (±0.007)	−0.033 (±0.016)
		Slope (b)		1.967 (±0.012)	1.948 (±0.016)	1.985 (±0.034)
	Theoretical b	Equilibrium	1.942			
		Kinetic	1.971			

$$\delta^{66}Zn = \left[\frac{\left(^{66}Zn/^{64}Zn\right)_{sample}}{\left(^{66}Zn/^{64}Zn\right)_{standard}} - 1 \right] \times 1000 \quad (3)$$

All statistical analyses was performed using the R software [67].

3. Results

3.1. Uncertainty estimation for mass fractions

The uncertainties given in the present work are expanded uncertainties (denoted U), obtained by multiplying the combined standard uncertainty $u_c(y)$ of the estimate y by a coverage factor k such that $U = ku_c(y)$ with $k = 2$ corresponding to a level of confidence of about 95%. Following the recommendations of the Guide to the expression of uncertainty in measurement [68], identified sources of uncertainty (x) for the measurement of mass fractions (y) relate to instrumental (variable background stability and counting efficiency) and analytical (error in mass and volume measurements, contamination). The determination of the combined standard uncertainty can be thus considered as a linear combination of terms representing the variation of the output estimate y generated by the uncertainty of each input estimate x such that:

$$u_c^2(y) = \left[\sum_{i=1}^{N} c_i u(x_i) \right]^2 \quad (4)$$

where $u_c(y)$ is the combined uncertainty; c_i the sensitivity coefficient and $u(x_i)$ the standard uncertainty which can be estimated by using the standard deviation calculated form replicate measurements. Here, the sensitivity coefficients were the same for all the measurements and were not further considered.

Excluding contamination as a significant source of uncertainty because the contribution of the procedural blanks was negligible and all samples were processed in a clean room, other analytical uncertainties can be considered insignificant compared to instrumental uncertainties associated with the ICP-MS or ICP-OES techniques, which are at best <5% (RSD). Comparison of expanded uncertainties for the determination of major elements (n = 18) and trace elements (n = 36) are of the same order of magnitude as those determined by NIST, IRMM and NRC (Fig. S2).

3.2. Determination of mass fractions

The results of the determination of mass fractions are given in Table S2. The number of measurements (n) correspond to the number of aliquots of digested reference materials measured one time. The table also includes the certified values (C_v) when available, allowing to calculate an accuracy (%) of the measurements (M_v) given the relationship:

$$\text{accuracy } \% = 100 - [100.(C_v \ M_v) / C_v] \quad (5)$$

For all the reference materials and all the elements, the calculation of the accuracy (n = 54) showed that the majority of the results have an accuracy > 90% (median = 93.0%), with two outliers, K in DORM-4 (69.5%) and Ni in SRM-1577c (47.8%), resulting in a mean accuracy value of 91.5% (Fig. 1). The overall comparison of the measured values and the certified values showed a very significant correlation ($p < 2e^{-16}$ ***, n = 54), spanning more than four orders of magnitude of mass fraction, a slope close to unity (0.993 ± 0.006) and a small offset of 0.383 ± 0.612 at origin (Fig. 2). The close agreement between measured and certified values whether for trace or major elements, demonstrates that the digestion step was quantitative at LGL-TPE. By extension to the other uncertified mass fractions, the present study allows to propose sixty-three new mass fraction values, i.e. sixteen for BCR-380R, thirteen for BCR-383, three for DORM-4, twenty for ERM-CE464, one for SRM-1577c and ten for TORT-3 (Table S2). These proposed new values are particularly of interest for the reference materials provided by IRMM (BCR-380R, BCR-383 and ERM-CE464) which are poorly characterized in terms of elemental mass fractions.

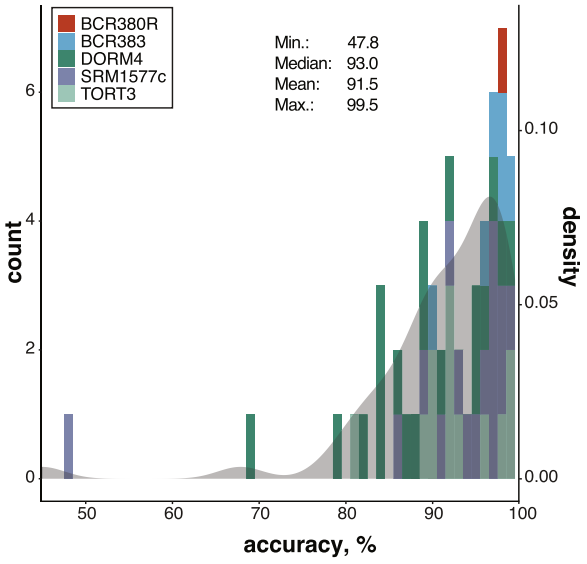

Fig. 1. Distribution of mass fraction values accuracy (%) in reference materials from this study. The summary of the distribution parameters is indicated. The grey shaded area corresponds to the density of the distribution of the accuracy of the results.

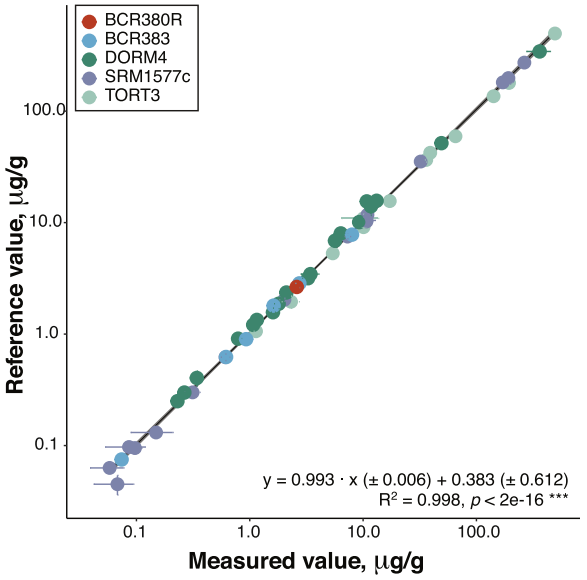

Fig. 2. Correlation between certified and measured mass fraction values (µg/g) in the reference materials analyzed in this study. The linear regression parameters are indicated along with the correlation coefficient and the associated p value. Error bars are U (k = 2).

3.3. Uncertainty estimation for isotopic compositions

The calculation of the combined standard uncertainty $u_c(y)$ for an isotope ratio can also be considered as a linear combination of terms of variations, but the calculation of the combined standard uncertainty of the delta value involves the uncertainties of the isotope ratios of the sample and those of the bracketing standard. A full demonstration of the calculation of the combined standard uncertainty of the delta value (δ) is provided by Sullivan et al. [51] and the final equation is given here for a

symbolic $r^{A/a}$ isotope ratio:

$$u^2(\delta) = \left(\frac{1}{r^{A/a}_{std}}\right)^2 \cdot u^2\left(r^{A/a}_{spl}\right) + \left(-\frac{r^{A/a}_{spl}}{r^{A/a^2}_{std}}\right)^2 \cdot u^2\left(r^{A/a}_{std}\right) \quad (6)$$

where the subscripts *std* and *spl* stand for the standard and the sample, respectively and u (r) is the standard uncertainty for the measured ratio, which can be estimated by using the standard deviation calculated from replicate measurements. We have calculated the expanded uncertainties U for the δ^{56}Fe, δ^{57}Fe, δ^{65}Cu, δ^{66}Zn, δ^{67}Zn and δ^{68}Zn values which are given in Tables S3–S9 along with the corresponding isotope ratios. The estimated mean expanded uncertainties were ±0.10‰ for the δ^{56}Fe value, and ±0.05‰ for the δ^{65}Cu and δ^{66}Zn values. The magnitude of the expanded uncertainties for the δ^{65}Cu value is close to that reported by Sullivan et al. [51], i.e. ± 0.07‰. For the Fe isotopic compositions, the higher U value is due to the fact that some reference materials (BCR-383, ERM-CE464 and one sample of FBS) at LGL-TPE exhibited enhanced instability. Reported error values in the present paper are therefore expanded uncertainty U with a coverage k factor of two, unless specified otherwise.

3.4. Determination of isotopic compositions

All results are given in table Tables S3–S9. One aspect of the quality of the isotopic results is assessed by the calculation of the exponent β relating the mass-dependent fractionation factors for two isotope ratios, which is different for kinetically and thermodynamically controlled fractionation [69]. Plots of δ^{57}Fe vs δ^{56}Fe (Fig. 3A), δ^{67}Zn vs δ^{66}Zn (Fig. 3B), and δ^{68}Zn vs δ^{66}Zn (Fig. 3C) yield the mass fractionation relationships in three-isotope spaces, allowing to calculate the β values, which correspond to the slopes of the respective best-fit linear regressions. Comparison with theoretical values (Table 3) show excellent agreement. In biological systems, mass fractionation is suspected to be under kinetic control, but the calculated β values do not show characteristics of kinetic mass fractionation (Table 3), probably because the fractionation per mass unit is too small, i.e. < 3‰ estimated by Young et al. [69] using magnesium isotope ratios.

Overall, we have considered as valid isotopic data sixty-four measurements of δ^{56}Fe values (LGL-TPE, $n = 30$, A&MS, $n = 34$), eighty-nine measurements of δ^{65}Cu values (LGL-TPE, $n = 57$, A&MS, $n = 32$) and one hundred measurements of δ^{66}Zn values (LGL-TPE, $n = 60$, A&MS, $n = 34$, G-TIME, $n = 6$).

4. Discussion

The results of Craddock and Dauphas [53] for the Fe isotopic composition of the SRM-1577c reference material ($n = 1$) and those of Sullivan et al. [51] for the Cu isotopic compositions of the DORM-4 ($n = 6$) and TORT-3 ($n = 6$) reference materials can be merged with the present results ($n = 253$). In order to determine preferred isotopic values, pair-wise comparisons of the mean isotope ratios have been performed using Tukey's HSD tests. A non-significant associated p value (>0.05) indicates that the means compared are not different, while an associated p value <0.05 indicates that the means compared are significantly different. All the results of the possible pair-wise comparisons are given in Table S10. Of thirty-nine comparisons, eleven means were significantly different, with a bias between them ranging from 0.06‰ to 0.25‰, with an average value of 0.09‰ (Table S10). However, most of the differences between the mean values are of the same order of magnitude as the calculated expanded uncertainties, i.e. ± 0.10‰, ±0.05‰, and ±0.05‰ for the δ^{56}Fe, δ^{65}Cu and δ^{66}Zn values, respectively. Taking the expanded uncertainties into account, only two differences remain significant, which are between LGL-TPE and A&MS labs for the δ^{65}Cu value of the BCR-380R reference material (0.25‰) and between LGL-TPE and G-TIME labs for the δ^{66}Zn value of the ERM-CE464 reference material (0.14‰). These discrepancies might be

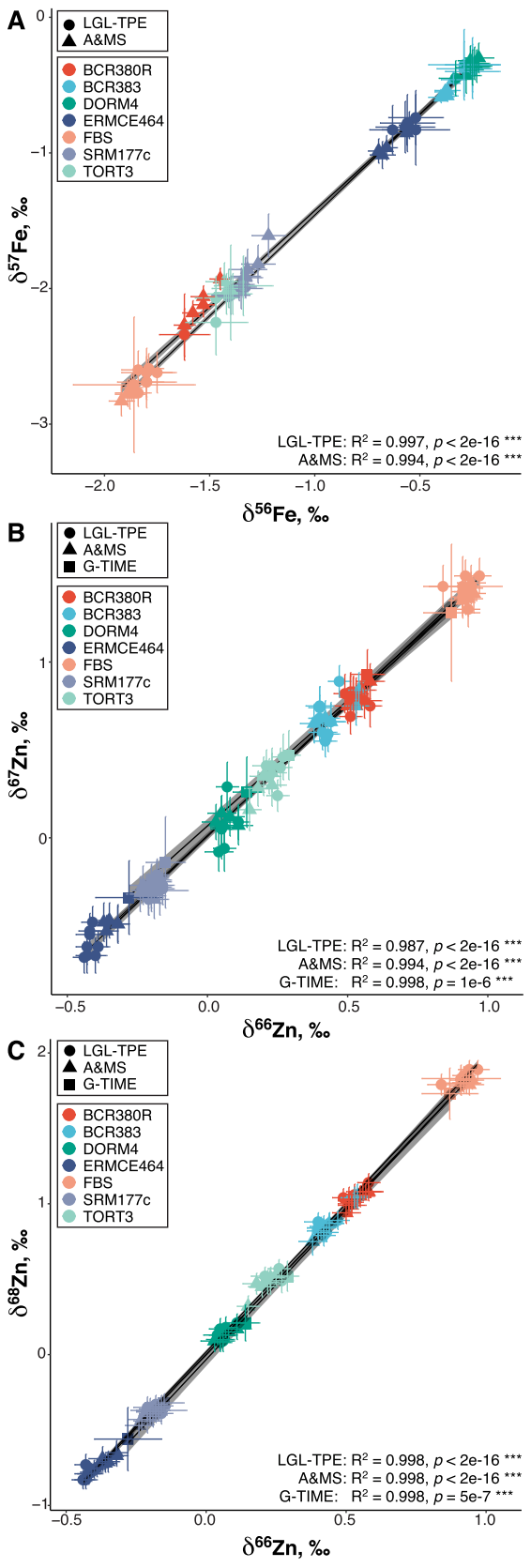

Fig. 3. Mass fractionation in three-isotope space for the reference materials analyzed in this study; A) δ^{57}Fe vs δ^{56}Fe; B) δ^{67}Zn vs δ^{66}Zn; C) δ^{68}Zn vs δ^{66}Zn. In all cases, the correlation coefficient and the associated p value are indicated. The linear regression parameters are given in Table 3. Error bars are U ($k = 2$).

explained by incomplete digestion of lipid compounds present in the samples, notably significant for the BCR-380R and ERM-CE464 reference materials which have a lipid content up to 27 wt%.

The final results are shown in Figs. 4–6 for the δ^{56}Fe, δ^{65}Cu and δ^{66}Zn values, respectively. The δ^{56}Fe values range from −1.92‰ to ~ −0.22‰, representing a span of variation of 0.85‰ per mass unit (Fig. 4). The range of variation is 0.75‰ per mass unit for the δ^{65}Cu values (min. = −0.15‰, max. = 1.32‰, Figs. 5) and 0.70‰ per mass unit for the δ^{66}Zn values (min. = −0.44‰, max. = 0.97‰, Fig. 6).

The δ^{56}Fe value for the SRM-1577c reference material reported by Craddock and Dauphas [53] is −1.34 ± 0.03‰ (±2SD), in accordance with the values determined at LGL-TPE (−1.36 ± 0.06‰) and A&MS (−1.29 ± 0.08‰) (Table S8). The present Fe isotopic composition complements well the reduced variability already measured on whole blood reference materials, which permanently exhibit very low δ^{56}Fe values < −2‰ (Table S1). The DORM-4 reference material displays the highest δ^{56}Fe value determined here (−0.26 ± 0.07‰, Fig. 4). DORM-4 is a fish protein homogenate, but it is not specified whether it was prepared from a freshwater or a seawater fish. The Fe isotopic compositions of the tuna fish muscle ERM-CE464 reference material (−0.60 ± 0.09‰, Fig. 4) and the already measured shrimp and tuna muscles [6] exhibit slightly negative values, suggesting that the DORM-4 reference material was likely prepared from seawater fish. Note that the TORT-3 reference material, which is also produced from a seawater animal (lobster), has a negative δ^{56}Fe value (−1.41 ± 0.10‰), showing that the Fe isotope fractionation between organs is probably important in the entire animal kingdom. Regarding plants, the bean BCR-383 reference material also has a slightly negative δ^{56}Fe value (−0.33 ± 0.09‰, Fig. 4), in accordance with the values found by Walczyk and von Blanckenburg [6].

The δ^{65}Cu values for the DORM-4 reference material reported by Sullivan et al. [51] is 0.52 ± 0.06‰, in accordance with the values determined at LGL-TPE (0.47 ± 0.04‰) and A&MS (0.45 ± 0.04‰)

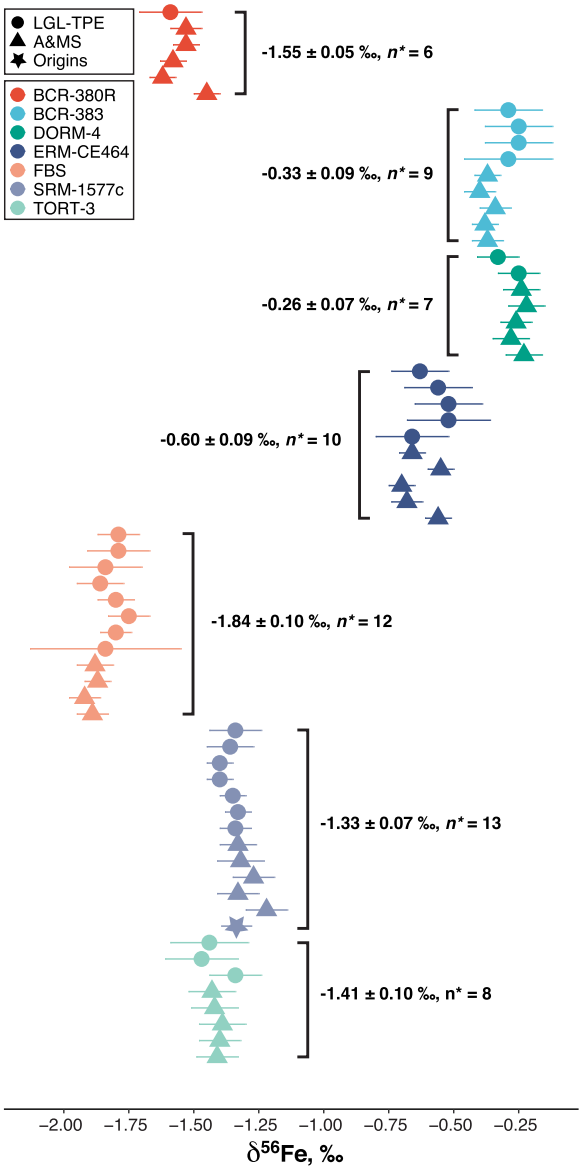

Fig. 4. Distribution of the δ^{56}Fe values in reference materials analyzed in this study. Preferred δ^{56}Fe values are indicated. Error associated to the preferred value is the mean of U (k = 2) for each aliquot digested and processed according to the chromatographic procedure. The n^* value corresponds to the number of these aliquots. Error bars are U (k = 2). Origins stands for the lab of Craddock and Dauphas [53].

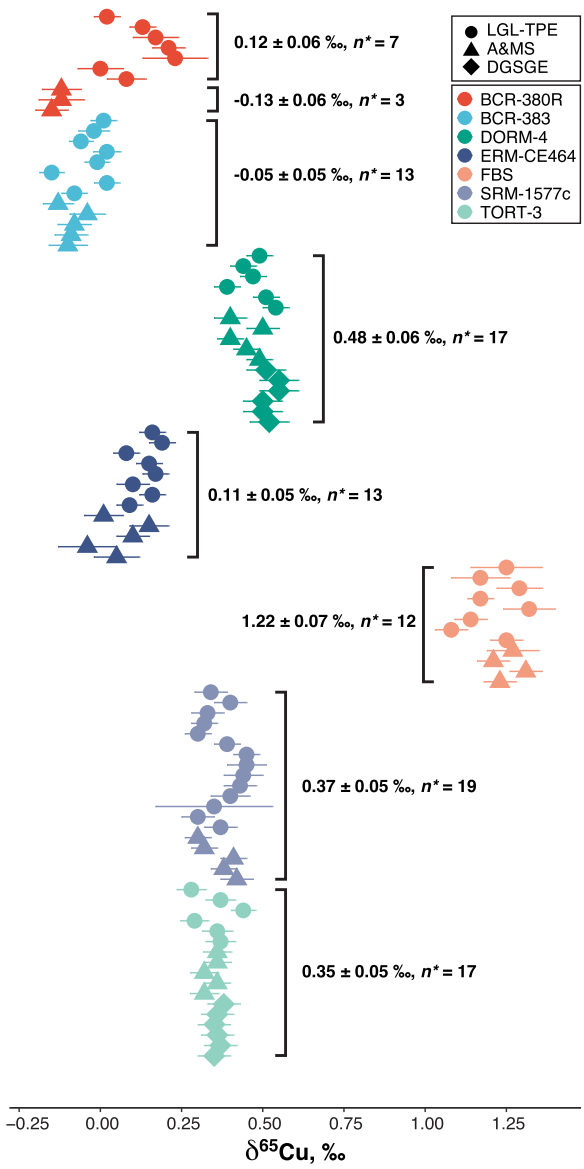

Fig. 5. Distribution of the δ^{65}Cu values in reference materials analyzed in this study. Preferred δ^{65}Cu values are indicated. Error associated to the preferred value is the mean of U (k = 2) for each aliquot digested and processed according to the chromatographic procedure. The n^* value corresponds to the number of these aliquots. Error bars are U (k = 2). DGSGE stands for the lab (Department of Geological Sciences and Geological Engineering) of Sullivan et al. [51].

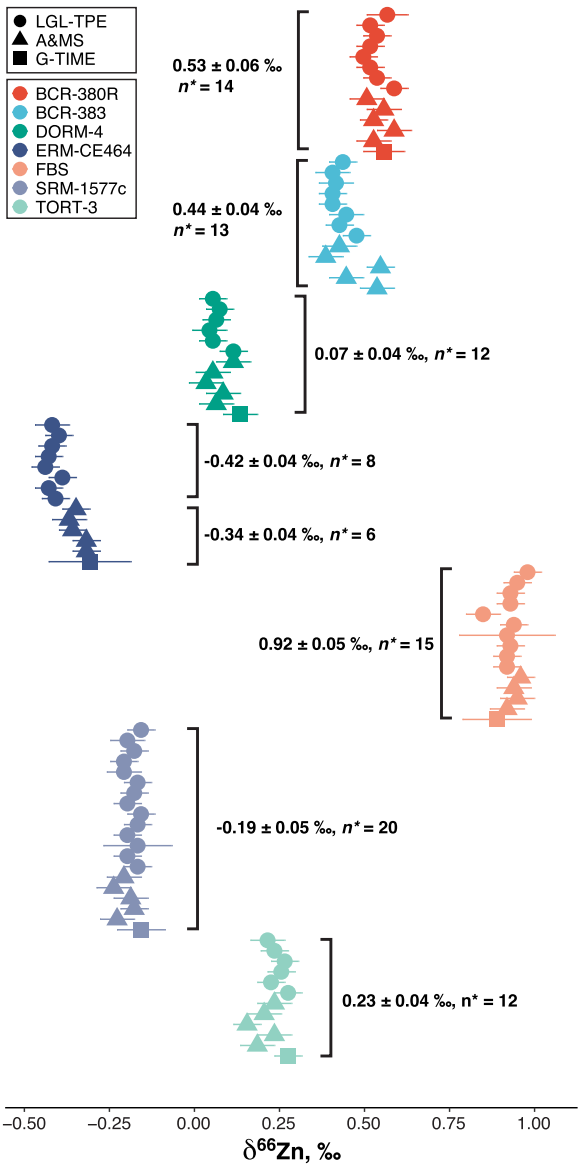

Fig. 6. Distribution of the δ^{66}Zn values in reference materials analyzed in this study. Preferred δ^{66}Zn values are indicated. Error associated to the preferred value is the mean of *U* (k = 2) for each aliquot digested and processed according to the chromatographic procedure. The *n** value corresponds to the number of these aliquots. Error bars are *U* (*k* = 2).

(Table S5). This also holds for the δ^{65}Cu value of the TORT-3 reference material which was measured at 0.35 ± 0.04‰ at LGL-TPE, 0.34 ± 0.04‰ at A&MS, and 0.36 ± 0.05‰ at DGSGE (Table S9). The δ^{65}Cu values obtained in the present study are within the range of those already reported for this reference material (Table S1). The FBS serum material, used as a quality control sample and not a reference material, shows a high δ^{65}Cu value (1.22 ± 0.07‰, Fig. 5). The fish protein DORM4 reference material has a δ^{65}Cu value of 0.48 ± 0.06‰, different from that of the dogfish liver DOLT-5 (−0.02 ± 0.08‰, ±2SD) (Table S1), which demonstrates that isotope fractionation among organs of lower vertebrates also takes place for Cu. Regarding plant, the δ^{65}Cu value of the bean BCR-383 reference material is close to 0‰ (−0.05 ± 0.05‰, Fig. 5).

The δ^{66}Zn values determined in the present study are situated within the range of the values already published for reference materials, i.e. between −0.4‰ and 1.1‰ (Table S1). BCR-639 exhibits a very negative value (~-3‰ [42,52], Table S1), which is unusual regarding the known Zn isotopic variability. The BCR-639 reference material is a "high-level" serum that has been doped with trace elements, and we think that it contains added Zn, probably highly purified Zn, with a negative δ^{66}Zn value (Rehkämper M., Pers. Comm.). As for Cu, the FBS serum material has a high δ^{66}Zn value (0.92 ± 0.05‰, Fig. 6). The other biological reference materials of animal origin show δ^{66}Zn values ranging from ~ −0.3‰ for the tuna fish muscle ERM-CE464 to ~ +0.5‰ for the whole milk BCR-380R, probably produced from cow milk (Fig. 6).

The results presented in this paper may be helpful for those researchers exploring high-precision isotopic analysis of Cu, Fe and/or Zn in biological and clinical sciences, providing them with a precious and sensitive tool for the quality control of their results.

Authors-credit

Lucie Sauzéat: investigation, resources, data curation, writing - review & editing; Marta Costas-Rodríguez: investigation, resources, data curation, writing - review & editing; Emmanuelle Albalat: investigation, resources, data curation, writing - review & editing; Nadine Mattielli: investigation, resources, data curation, writing - review & editing; Frank Vanhaecke: writing - review & editing, supervision; Vincent Balter: conceptualization, formal analysis, data curation, writing original draft, review and editing, supervision, project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121576.

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Biological reference material	Matrix	δ^{56}Fe, ‰ (vs IRMM014)	*n*	δ^{65}Cu, ‰ (vs SRM-976)	*n*	δ^{66}Zn, ‰ (vs JMC-Lyon)	*n*	Reference
Animal matrix								
Seronorm Trace elements whole blood L-3 (lot no. 1509408)	Whole blood	-2.21 ± 0.05	*1*	0.00 ± 0.03	*4*	0.11 ± 0.03	*1*	Yang *et al*. [44]
Seronorm Trace elements whole blood L-3 (lot no. 1112691)	Whole blood			0.36 ± 0.08	*8*			Miller *et al*. [32]
Seronorm Trace elements whole blood L-1 (lot no. OK0336)	Whole blood	-2.83 ± 0.15	*6*					Stenberg *et al*. [45]
Seronorm Trace elements whole blood L-1 (lot no. OK0336)	Whole blood					0.23 ± 0.02	*1*	Stenberg *et al*. [46]
Seronorm Trace elements whole blood L-1 (lot no. OK0336)	Whole blood	-2.30 ± 0.08	*2*			0.23 ± 0.06	*2*	Stenberg *et al*. [19]
Seronorm Trace elements whole blood L-1 (lot no. OK0337)	Whole blood	-2.45 ± 0.07	*2*	0.37 ± 0.11	*2*	0.33 ± 0.03	*2*	Van Heghe *et al*. [10]
Seronorm Trace elements whole blood L-1 (lot no. 404108)	Whole blood					0.22 ± 0.01	*1*	Stenberg *et al*. [46]
Seronorm Trace elements whole blood L-1 (lot no. 404108)	Whole blood	-2.37 ± 0.12	*2*			0.22 ± 0.06	*2*	Stenberg *et al*. [19]
Seronorm Trace elements whole blood L-1 (lot no. MR9067)	Whole blood					0.26 ± 0.01	*1*	Stenberg *et al*. [46]
Seronorm Trace elements whole blood L-1 (lot no. MR9067)	Whole blood	-2.34 ± 0.10	*2*			0.26 ± 0.04	*2*	Stenberg *et al*. [19]
Seronorm Trace elements whole blood L-1 (lot no. 1406263)	Whole blood			0.14 ± 0.05	*3*			Lauwens *et al*. [47]
Seronorm Trace elements whole blood L-1 (lot no. 1406263)	Whole blood	-2.37 ± 0.08	*3*					Anoshkina *et al*. [20]
Seronorm Trace elements serum L-1 (lot no. ?)	Serum			-0.09 ± 0.05	*1*			Costas-Rodriguez *et al*. [31]
Seronorm Trace elements serum L-1 (lot no. 0903106)	Serum			-0.24 ± 0.14	*4*			Lauwens *et al*. [48]
Seronorm Trace elements serum L-1 (lot no. 1309438)	Serum			-0.19 ± 0.05	*4*			Lauwens *et al*. [49]
Seronorm Trace elements serum L-1 (lot no. 1309438)	Serum			-0.20 ± 0.04	*3*			Lauwens *et al*. [47]
Seronorm Trace elements serum L-1 (lot no. 1309438)	Serum			-0.20 ± 0.03	*5*			Sullivan *et al*. [50]
BCR-639	Serum			-0.53 ± 0.06	*1*	-2.76 ± 0.2	*8*	Larner *et al*. [41]
BCR-639	Serum					-2.99 ± 0.12	*8*	Moore *et al*. [51]
Seronorm Trace elements Urine L-1	Urine					0.25 ± 0.03	*5*	Moore *et al*. [51]
SRM-8414	Bovine muscle					0.42 ± 0.03	*1*	Stenberg *et al*. [46]
ERM-BB184	Bovine muscle					-0.35 ± 0.13	*4*	Moore *et al*. [51]
BCR-184	Bovine muscle					0.02 ± 0.02	*1*	Costas-Rodriguez *et al*. [52]
BCR-189	Whole meal flour					0.61 ± 0.05	*1*	Costas-Rodriguez *et al*. [52]
BCR-063R	Skimmed milk					0.52 ± 0.04	*1*	Costas-Rodriguez *et al*. [52]
GBW-07601	Human hair					-0.01 ± 0.03	*1*	Stenberg *et al*. [46]
ERM-DB001	Human hair					-0.01 ± 0.04	*4*	Moore *et al*. [51]
SRM-1577a	Bovine liver					-0.04 ± 0.02	*1*	Stenberg *et al*. [46]
SRM-1577a	Bovine liver					0.24 ± 0.02	*1*	Costas-Rodriguez *et al*. [52]
SRM-1577c	Bovine liver	-1.34 ± 0.03	*3*					Craddock and Dauphas [53]
ERM-BB186	Pig kidney					0.00 ± 0.10	*5*	Moore *et al*. [51]
BCR-278	Mussel tissue			0.08 ± 0.04	*1*	0.82 ± 0.04	*1*	Maréchal *et al*. [54]
BCR-278	Mussel tissue					0.73 ± 0.01	*1*	Costas-Rodriguez *et al*. [52]
TORT-2	Lobster hepatopancreas					0.51 ± 0.04	*1*	Maréchal *et al*. [54]
TORT-3	Lobster hepatopancreas			0.36 ± 0.02	*6*			Sullivan *et al*. [50]
BCR-414	Plankton					0.42 ± 0.04	*1*	Maréchal *et al*. [54]
ERM-BB422	Fish muscle					-0.04 ± 0.04	*4*	Moore *et al*., [51]
DORM-4	Fish protein			0.52 ± 0.05	*6*			Sullivan *et al*. [50]
DOLT-5	Dogfish liver			-0.02 ± 0.08	*6*			Sullivan *et al*. [50]
Vegetal matrix								
SRM-1567a	Wheat flour					1.17 ± 0.08	*1*	Costas-Rodriguez *et al*., [52]
SRM-1568a	Rice flour					0.39 ± 0.01	*1*	Costas-Rodriguez *et al*., [52]
SRM-1570	Spinach					0.69 ± 0.03	*1*	Costas-Rodriguez *et al*., [52]
BCR-281	Ryegrass					0.40 ± 0.09	*7*	Arnold *et al*. [55]
BCR-281	Ryegrass					0.81 ± 0.10	*1*	Weiss *et al*. [56]
BCR-281	Ryegrass					0.38 ± 0.04	*9*	Smolders *et al*. [57]
BCR-281	Ryegrass					0.50 ± 0.10	*5*	Caldelas *et al*. [58]
BCR-482	Lichen					0.14 ± 0.03	*8*	Viers *et al*., [59]
BCR-482	Lichen					0.07 ± 0.10	*1*	Cloquet *et al*., [60]
BCR-482	Lichen					0.07 ± 0.11	*17*	Dinis *et al*., [61]
BCR-482	Lichen					0.09 ± 0.04	*6*	Sonke *et al*., [62]
V464	Oak leaves					-0.44 ± 0.02	*4*	Tang *et al*., [63]
BCR-62	Olive leaves					0.42 ± 0.02	*9*	Tang *et al*., [63]

Table S1

		Mass fraction, µg/g						Mass fraction, ng/g														
		Na	P	Mg	S	K	Ca	Li	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Cd	Ba	Pb
BCR-380R	**Measured value**	3260	7657	875	2598	13132	10863	25.1	<LD	n/a	222	1753	5.5	n/a	298	27740	4.8	n/a	16212	3097	n/a	752	6.6
	***U*, *k*=2**	106	126	39	59	410	215	2.6			6	132	0.2		31	1818	0.2		356	85		26	0.7
	n	*11*	*11*	*11*	*11*	*11*	*2*	*9*			*9*	*4*	*6*		*9*	*11*	*2*		*9*	*9*		*9*	*6*
	Certified value				2648																		
	***U*, *k*=2**																						
	Accuracy, %				98.1																		
BCR-383	**Measured value**	73.7	1623	929	618	7985	2739	44.9	n/a	813	13680	52220	37.4	1340	2162	10163	n/a	n/a	1303	4842	19.2	2957	95
	***U*, *k*=2**	2.8	31	43	16	338	80	4.0		147	446	1282	4.1	31.3	137	442			130	168	16	174	17
	n	*9*	*9*	*9*	*9*	*9*	*7*	*5*		*5*	*5*	*9*	*5*	*5*	*9*	*9*			*5*	*5*	*5*	*5*	*5*
	Certified value	75	1800	900	623	7800	2850																
	***U*, *k*=2**	7				400	230																
	Accuracy, %	98.2	90.2	96.7	99.3	97.6	96.1																
DORM-4	**Measured value**	11725	6355	789	7303	10771	2103	1072	1603	1798	3267	361348	230	1155	13158	49130	5650	3432	4829	9232	265	4566	341
	***U*, *k*=2**	530	234	28	401	476	89	16	29	72	162	34736	4	42	595	978	118	275	109	314	5	93	9
	n	*4*	*4*	*4*	*4*	*4*	*4*	*5*	*5*	*5*	*5*	*6*	*5*	*5*	*6*	*6*	*5*	*5*	*5*	*5*	*5*	*5*	*5*
	Certified value	14000	8000	910		15500	2360	1210	1570	1870	3170	343000	250	1340	15700	51600	6870	3450		10100	299		404
	***U*, *k*=2**	2400		80		1000	140		140	180	260	20000		140	460	2800	440	400		800	18		62
	Accuracy, %	83.7	79.4	86.7		69.5	89.1	88.6	97.9	96.1	96.9	94.7	91.9	86.2	83.8	95.2	82.2	99.5		91.4	88.5		84.4
ERM-CE464	**Measured value**	3797	8707	1100	7185	14055	313	41	28	286	1044	65545	n/a	283	1711	20265	4844	1482	5831	1557	527	477	n/a
	***U*, *k*=2**	104	180	14	155	368	7	5	3	47	83	3043		28	103	1884	283	86	108	75	28	82	
	n	*5*	*5*	*5*	*5*	*5*	*5*	*7*	*7*	*7*	*7*	*7*		*7*	*7*	*7*	*7*	*4*	*7*	*7*	*7*	*7*	
	Certified value																						
	***U*, *k*=2**																						
	Accuracy, %																						
FBS	**Measured value**	3021	102	29	495	436	132	7	7	n/a	71	1874	4	28	115	2659	n/a	n/a	1562	156	n/a	57	n/a
	***U*, *k*=2**	253	8	4	55	25	12	1	0		7	540	1		19	218			116	12		5	
	n	*9*	*9*	*9*	*9*	*9*	*9*	*5*	*6*		*7*	*9*	*4*	*1*	*7*	*9*			*7*	*7*		*7*	
SRM1577c	**Measured value**	2022	10927	610	7280	10692	149	n/a	n/a	n/a	10727	191484	313	68	264751	170966	n/a	n/a	32233	97	86	43	58
	***U*, *k*=2**	54	170	13.5	107	207	15.1				538	4503	14.4	15.1	5101	5122			634	7.0	9.1	13.3	10.8
	n	*14*	*15*	*16*	*15*	*15*	*14*				*14*	*16*	*11*	*3*	*18*	*18*			*13*	*4*	*13*	*4*	*3*
	Certified value	2033	11750	620	7490	10230	131			53	10460	197940	300	45	272500	181100	20	2031	35300	95	97		63
	***U*, *k*=2**	64	270	42	340	640	10			14	470	650	18	9	4600	1000	1	45	1100	4	1		1
	Accuracy, %	99.4	93.0	98.3	97.2	95.5	85.9				97.4	96.7	95.6	47.8	97.2	94.4			91.3	97.7	88.9		92.0
TORT-3	**Measured value**	19846	10857	1146	13842	14057	2454	315	10084	2312	17192	193811	1135	5400	493100	141626	65357	10043	5103	36075	39113	330	161
	***U*, *k*=2**	326	98	10.3	168	206	27.0	8.9	283	200	308	6239	27.1	248	9175	1653	3611	1787	31.6	501	194	67	7.1
	n	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*	*4*
	Certified value								9100	1950	15600	179000	1060	5300	497000	136000	59500	10900		36500	42300		
	***U*, *k*=2**								400	240	1000	8000	n/a	240	22000	6000	3800	1000		1600	1800		
	Accuracy, %								89.2	81.4	89.8	91.7	92.9	98.1	99.2	95.9	90.2	92.1		98.8	92.5		

Table S2

Lab	LGL-TPE								A&MS						G-TIME
Sample ID	BCR®-380R-1	BCR®-380R-2	BCR®-380R-3	BCR®-380R-4	BCR®-380R-5	BCR®-380R-6	BCR®-380R-7	BCR®-380R-8	BCR380R	BCR380R-1	BCR380R-2	BCR380R-3	BCR380R-4	BCR380R-5	380R #1
$R^{56/54}Fe$									15.69917	15.67459	15.67454	15.67385	15.67316	15.67581	
2se $_{R56/54}$									0.00060	0.00049	0.00037	0.00040	0.00034	0.00045	
$\delta^{56}Fe$ (‰)									-1.59	-1.53	-1.53	-1.58	-1.62	-1.45	
2se*									0.09	0.03	0.02	0.03	0.02	0.03	
U, k=2									0.12	0.06	0.05	0.05	0.05	0.05	
$R^{57/54}Fe$									0.36255	0.36183	0.36181	0.36179	0.63515	0.36187	
2se $_{R57/54}$									0.00002	0.00002	0.00002	0.00002	0.00005	0.00002	
$\delta^{57}Fe$ (‰)									-2.33	-2.06	-2.12	-2.18	-2.27	-1.93	
2se*									0.20	0.04	0.05	0.05	0.05	0.05	
U, k=2									0.185	0.08	0.09	0.09	0.23	0.08	
n									*2*	*2*	*2*	*2*	*2*	*2*	
$R^{65/63}Cu$	0.447086	0.447126	0.447141	0.447132	0.447141	0.447046		0.447263			0.445581	0.445581	0.445567		
2se $_{R65/63}$	0.000005	0.000006	0.000015	0.000009	0.000021	0.000014		0.000010			0.000013	0.000014	0.000009		
$\delta^{65}Cu$ (‰)	0.02	0.13	0.17	0.21	0.23	0.00		0.08			-0.12	-0.12	-0.15		
2se*	0.04	0.04	0.04	0.04	0.04	0.04		0.04			0.03	0.03	0.02		
U, k=2	0.04	0.04	0.07	0.05	0.10	0.07		0.06			0.06	0.07	0.05		
n	*1*	*1*	*1*	*1*	*1*	*1*		*1*			*2*	*2*	*2*		
$R^{66/64}Zn$	0.563613	0.563576	0.563579	0.563598	0.563586	0.563582	0.563296	0.563324		0.564082	0.564112	0.564093	0.564126	0.564096	0.562232
2se $_{R66/64}$	0.000015	0.000008	0.000008	0.000007	0.000007	0.000008	0.000007	0.000007		0.000009	0.000009	0.000008	0.000010	0.000009	0.000013
$\delta^{66}Zn$ (‰)	0.56	0.51	0.53	0.51	0.49	0.51	0.53	0.58		0.50	0.55	0.52	0.58	0.52	0.55
2se*	0.03	0.03	0.03	0.02	0.01	0.03	0.00	0.03		0.02	0.02	0.01	0.02	0.02	0.03
U, k=2	0.06	0.04	0.04	0.04	0.04	0.04	0.04	0.04		0.05	0.05	0.04	0.05	0.05	0.06
$R^{67/64}Zn$	0.081921	0.081922	0.081914	0.081930	0.081922	0.081926	0.081863	0.081857		0.082190	0.082198	0.082197	0.082202	0.082195	0.081609
2se $_{R67/64}$	0.000003	0.000003	0.000003	0.000004	0.000003	0.000002	0.000002	0.000004		0.000003	0.000003	0.000003	0.000002	0.000003	0.000005
$\delta^{67}Zn$ (‰)	0.78	0.83	0.78	0.80	0.82	0.69	0.81	0.75		0.75	0.84	0.82	0.89	0.81	0.93
2se*	0.005001	0.120000	0.120000	0.014555	0.014357	0.120000	0.002364	0.120000		0.04	0.04	0.03	0.03	0.03	0.02
U, k=2	0.10	0.11	0.10	0.12	0.11	0.10	0.10	0.12		0.10	0.11	0.10	0.10	0.10	0.14
$R^{68/64}Zn$	0.372582	0.372527	0.372543	0.372558	0.372549	0.372589	0.372192	0.372221		0.375317	0.375360	0.375345	0.375369	0.375335	0.370737
2se $_{R68/64}$	0.000007	0.000007	0.000008	0.000011	0.000007	0.000012	0.000006	0.000007		0.000009	0.000009	0.000009	0.000007	0.000009	0.000003
$\delta^{68}Zn$ (‰)	1.08	1.02	1.06	1.00	1.04	1.03	1.05	1.14		0.94	1.05	1.01	1.08	0.99	1.08
2se*	0.01	0.06	0.06	0.03	0.00	0.06	0.02	0.06		0.02	0.02	0.02	0.02	0.02	0.01
U, k=2	0.06	0.06	0.07	0.08	0.06	0.08	0.06	0.06		0.07	0.07	0.07	0.06	0.07	0.05
n	*2*	*1*	*1*	*2*	*2*	*1*	*2*	*1*		*2*	*2*	*2*	*2*	*2*	*3*

Table S3

Lab	LGLT-TPE								A&MS				
Sample ID	BCR®-383-1	BCR®-383-2	BCR®-383-3	BCR®-383-4	BCR®-383-5	BCR®-383-6	BCR®-383-7	BCR®-383-8	Beans1	Beans2	Beans3	Beans4	BCR383-1	BCR383-2	BCR383-3	BCR383-4	BCR383-5
$R^{56/54}Fe$									15.72260	15.72315	15.72290	15.72109	15.69273	15.69235	15.69322	15.69270	15.69277
$2se_{R56/54}$									0.00066	0.00071	0.00070	0.00112	0.00026	0.00034	0.00034	0.00029	0.00031
$\delta^{56}Fe$ (‰)									-0.29	-0.25	-0.25	-0.29	-0.37	-0.40	-0.34	-0.38	-0.37
2se*									0.04	0.01			0.02	0.02	0.02	0.02	0.02
U, k=2									0.13	0.13	0.13	0.17	0.05	0.06	0.06	0.05	0.06
$R^{57/54}Fe$									0.36334	0.36336	0.36333	0.36329	0.36236	0.36236	0.36241	0.36237	0.36238
$2se_{R57/54}$									0.00003	0.00003	0.00003	0.00004	0.00001	0.00001	0.00001	0.00001	0.00001
$\delta^{57}Fe$ (‰)									-0.38	-0.35	-0.40	-0.35	-0.58	-0.59	-0.46	-0.55	-0.54
2se*									0.05	0.08			0.03	0.03	0.03	0.02	0.03
U, k=2									0.20	0.20	0.20	0.26	0.04	0.04	0.04	0.04	0.04
n									*2*	*2*	*1*	*1*	*2*	*2*	*2*	*2*	*2*
$R^{65/63}Cu$	0.446987	0.447036	0.447022	0.446953	0.446936	0.447000	0.447000	0.447000					0.4455727	0.4456133	0.4455966	0.4455914	0.4455890
$2se_{R65/63}$	0.000006	0.000008	0.000004	0.000006	0.000004	0.000004	0.000005	0.000005					0.0000075	0.0000098	0.0000089	0.0000087	0.0000110
$\delta^{65}Cu$ (‰)	0.01	-0.02	-0.06	0.02	-0.01	-0.15	0.02	-0.08					-0.13	-0.04	-0.08	-0.09	-0.10
2se*	0.00	0.00	0.04	0.04	0.04	0.04	0.04	0.01					0.02	0.02	0.02	0.02	0.02
U, k=2	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04					0.05	0.05	0.05	0.05	0.06
n	*2*	*2*	*1*	*1*	*1*	*1*	*1*	*2*					*2*	*2*	*2*	*2*	*2*
$R^{66/64}Zn$	0.563543	0.563516	0.563543	0.563517	0.563513	0.563246	0.563237	0.563269					0.564038	0.564014	0.564105	0.564047	0.564098
$2se_{R66/64}$	0.000007	0.000009	0.000009	0.000008	0.000006	0.000009	0.000006	0.000007					0.000010	0.000011	0.000009	0.000009	0.000009
$\delta^{66}Zn$ (‰)	0.43	0.40	0.41	0.40	0.40	0.44	0.42	0.47					0.42	0.38	0.54	0.44	0.53
2se*	0.019229	0.030000	0.030000	0.030000	0.030000	0.030000	0.005001	0.030000					0.02	0.02	0.02	0.02	0.02
U, k=2	0.04	0.05	0.05	0.04	0.04	0.05	0.04	0.04					0.05	0.05	0.04	0.05	0.05
$R^{67/64}Zn$	0.081909	0.081920	0.081917	0.081913	0.081912	0.081853	0.081841	0.081866					0.082185	0.082182	0.082198	0.082183	0.082191
$2se_{R67/64}$	0.000003	0.000003	0.000002	0.000004	0.000004	0.000002	0.000002	0.000002					0.000002	0.000003	0.000003	0.000003	0.000003
$\delta^{67}Zn$ (‰)	0.59	0.75	0.60	0.74	0.68	0.66	0.55	0.89					0.68	0.65	0.84	0.66	0.75
2se*	0.080407	0.120000	0.120000	0.120000	0.120000	0.120000	0.048273	0.120000					0.03	0.04	0.03	0.04	0.04
U, k=2	0.10	0.11	0.10	0.12	0.13	0.10	0.09	0.09					0.10	0.11	0.10	0.11	0.11
$R^{68/64}Zn$	0.372486	0.372475	0.372526	0.372469	0.372470	0.372144	0.372120	0.372151					0.375283	0.375247	0.375360	0.375293	0.375354
$2se_{R68/64}$	0.000006	0.000005	0.000009	0.000008	0.000007	0.000009	0.000008	0.000006					0.000008	0.000013	0.000011	0.000010	0.000008
$\delta^{68}Zn$ (‰)	0.81	0.88	0.84	0.84	0.82	0.87	0.79	0.89					0.85	0.75	1.05	0.87	1.04
2se*	0.00	0.06	0.06	0.06	0.06	0.06	0.05	0.06					0.02	0.03	0.03	0.03	0.02
U, k=2	0.06	0.06	0.07	0.07	0.06	0.07	0.07	0.06					0.07	0.09	0.08	0.07	0.07
n	*2*	*1*	*1*	*1*	*1*	*1*	*2*	*1*					*2*	*2*	*2*	*2*	*2*

Table S4

Lab	LGLT-TPE								A&MS					G-TIME
Sample ID	DORM4-1	DORM4-2	DORM4-3	DORM4-4	DORM4-5	DORM4-6	Fish Prot1	Fish Prot2	DORM4-1	DORM4-2	DORM4-3	DORM4-4	DORM4-5	DORM-4 #1
$R^{56/54}Fe$							15.73320	15.73385	15.69479	15.69508	15.69457	15.69419	15.69503	
2se $_{R56/54}$							0.00055	0.00050	0.00046	0.00041	0.00037	0.00047	0.00043	
$\delta^{56}Fe$ (‰)							-0.334	-0.251	-0.242	-0.223	-0.256	-0.280	-0.226	
2se*							0.053	0.084	0.029	0.026	0.024	0.030	0.028	
U, k=2							0.080	0.075	0.070	0.066	0.062	0.072	0.068	
$R^{57/54}Fe$							0.36365	0.36366	0.36245	0.36247	0.36246	0.36242	0.36245	
2se $_{R57/54}$							0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	
$\delta^{57}Fe$ (‰)							-0.45	-0.37	-0.34	-0.30	-0.32	-0.43	-0.36	
2se*							0.10	0.16	0.05	0.05	0.05	0.05	0.05	
U, k=2							0.13	0.13	0.12	0.11	0.11	0.13	0.12	
n							*3*	*3*	*2*	*2*	*2*	*2*	*2*	
$R^{65/63}Cu$	0.447000	0.447000	0.447000	0.447000	0.447368	0.447378			0.445809	0.445855	0.445812	0.445832	0.445852	
2se $_{R65/63}$	0.000006	0.000004	0.000005	0.000007	0.000006	0.000004			0.000007	0.000008	0.000006	0.000006	0.000006	
$\delta^{65}Cu$ (‰)	0.49	0.44	0.47	0.39	0.51	0.54			0.40	0.50	0.40	0.45	0.49	
2se*	0.02	0.04	0.04	0.04	0.01	0.04			0.02	0.02	0.01	0.01	0.01	
U, k=2	0.04	0.04	0.04	0.04	0.04	0.04			0.05	0.05	0.04	0.04	0.04	
n	*2*	*1*	*1*	*1*	*2*	*1*			*2*	*2*	*2*	*2*	*2*	
$R^{66/64}Zn$	0.563029	0.563045	0.563041	0.563022	0.563053	0.563078			0.563860	0.563829	0.563818	0.563847	0.563836	0.561988
2se $_{R66/64}$	0.000007	0.000009	0.000007	0.000010	0.000008	0.000008			0.000011	0.000011	0.000011	0.000010	0.000011	0.000010
$\delta^{66}Zn$ (‰)	0.05	0.07	0.06	0.04	0.05	0.11			0.11	0.05	0.03	0.08	0.06	0.13
2se*	0.03	0.03	0.03	0.03	0.03	0.02			0.02	0.02	0.02	0.02	0.02	0.03
U, k=2	0.04	0.04	0.04	0.05	0.04	0.04			0.05	0.05	0.05	0.05	0.05	0.05
$R^{67/64}Zn$	0.081800	0.081821	0.081794	0.081791	0.081805	0.081799			0.082134	0.082141	0.082136	0.082139	0.082135	0.081556
2se $_{R67/64}$	0.000003	0.000005	0.000004	0.000003	0.000007	0.000003			0.000003	0.000002	0.000003	0.000003	0.000003	0.000008
$\delta^{67}Zn$ (‰)	0.05	0.29	-0.06	-0.08	0.06	0.09			0.07	0.14	0.09	0.12	0.07	0.26
2se*	0.06	0.06	0.06	0.06	0.06	0.13			0.04	0.03	0.03	0.04	0.03	0.10
U, k=2	0.10	0.14	0.13	0.11	0.18	0.10			0.11	0.10	0.10	0.11	0.10	0.20
$R^{68/64}Zn$	0.371879	0.371891	0.371862	0.371871	0.371897	0.371922			0.375029	0.375004	0.374999	0.375021	0.375017	0.370432
2se $_{R68/64}$	0.000008	0.000009	0.000009	0.000007	0.000007	0.000006			0.000009	0.000010	0.000009	0.000008	0.000009	0.000021
$\delta^{68}Zn$ (‰)	0.17	0.18	0.09	0.13	0.08	0.21			0.17	0.10	0.09	0.15	0.13	0.21
2se*	0.12	0.12	0.12	0.12	0.12	0.00			0.02	0.03	0.02	0.02	0.02	0.08
U, k=2	0.07	0.07	0.07	0.06	0.06	0.06			0.07	0.07	0.07	0.06	0.07	0.12
n	*1*	*1*	*1*	*1*	*1*	*2*			*2*	*2*	*2*	*2*	*2*	*2*

Table S5

Lab	LGL-TPE												A&MS					G-TIME
Sample ID	ERM®-CE464-1	ERM®-CE464-2	ERM®-CE464-3	ERM®-CE464-4	ERM®-CE464-5	ERM®-CE464-6	ERM®-CE464-7	ERM®-CE464-8	Tuna1	Tuna2	Tuna3	Tuna4	Tuna5	ERMCE 464-1	ERMCE 464-2	ERMCE 464-3	ERMCE 464-4	ERMCE 464-5	CE464#1
$R^{56/54}Fe$									15.86519	15.71833	15.71892	15.71692	15.71634	15.68825	15.68995	15.68766	15.68793	15.68976	
2se $_{R56/54}$									0.00052	0.00073	0.00071	0.00106	0.00080	0.00028	0.00030	0.00031	0.00032	0.00027	
$\delta^{56}Fe$ (‰)									-0.63	-0.56	-0.52	-0.52	-0.66	-0.66	-0.55	-0.70	-0.68	-0.56	
2se*									0.01	0.07	0.05			0.02	0.02	0.02	0.02	0.02	
U, k=2									0.11	0.13	0.13	0.16	0.14	0.05	0.05	0.05	0.06	0.05	
$R^{57/54}Fe$									0.36829	0.36320	0.36322	0.36310	0.36314	0.36223	0.36229	0.36222	0.36221	0.36227	
2se $_{R57/54}$									0.00002	0.00003	0.00003	0.00004	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	
$\delta^{57}Fe$ (‰)									-0.83	-0.78	-0.74	-0.83	-0.89	-0.96	-0.80	-0.99	-1.02	-0.85	
2se*									0.03	0.10	0.13			0.03	0.03	0.03	0.04	0.03	
U, k=2									0.18	0.21	0.20	0.26	0.19	0.09	0.10	0.09	0.10	0.09	
n									*2*	*2*	*2*	*1*	*1*	*2*	*2*	*2*	*2*	*2*	
$R^{65/63}Cu$	0.447134	0.447146	0.447087	0.447114	0.447118	0.447221	0.447248	0.447223						0.445637	0.445698	0.445677	0.445613	0.445657	
2se $_{R65/63}$	0.000006	0.000006	0.000006	0.000005	0.000006	0.000008	0.000006	0.000006						0.000011	0.000010	0.000009	0.000019	0.000014	
$\delta^{65}Cu$ (‰)	0.16	0.19	0.08	0.15	0.17	0.10	0.16	0.09						0.01	0.15	0.10	-0.04	0.05	
2se*	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04						0.02	0.02	0.02	0.04	0.03	
U, k=2	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.04						0.06	0.06	0.05	0.09	0.07	
n	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*						*2*	*2*	*2*	*2*	*2*	
$R^{66/64}Zn$	0.563059	0.563076	0.563056	0.563056	0.563050	0.562789	0.562760	0.562762						0.563606	0.563593	0.563599	0.563619	0.563620	0.561735
2se $_{R66/64}$	0.000010	0.000008	0.000008	0.000008	0.000007	0.000007	0.000008	0.000006						0.000008	0.000009	0.000009	0.000008	0.000009	0.000032
$\delta^{66}Zn$ (‰)	-0.42	-0.40	-0.42	-0.43	-0.44	-0.39	-0.43	-0.41						-0.35	-0.37	-0.36	-0.32	-0.32	-0.31
2se*	0.01	0.01	0.03	0.03	0.03	0.03	0.03	0.03						0.01	0.02	0.02	0.01	0.02	0.10
U, k=2	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04						0.04	0.05	0.04	0.04	0.04	0.12
$R^{67/64}Zn$	0.081808	0.081805	0.081817	0.081812	0.081806	0.081743	0.081741	0.081757						0.082090	0.082089	0.082085	0.082089	0.082089	0.081500
2se $_{R67/64}$	0.000003	0.000002	0.000003	0.000002	0.000002	0.000002	0.000002	0.000002						0.000003	0.000003	0.000002	0.000003	0.000003	0.000008
$\delta^{67}Zn$ (‰)	-0.55	-0.67	-0.53	-0.62	-0.68	-0.62	-0.67	-0.48						-0.47	-0.48	-0.53	-0.49	-0.49	-0.34
2se*	0.29	0.04	0.06	0.06	0.06	0.06	0.06	0.06						0.04	0.04	0.03	0.03	0.04	0.10
U, k=2	0.10	0.10	0.10	0.10	0.09	0.09	0.10	0.09						0.10	0.11	0.09	0.10	0.11	0.21
$R^{68/64}Zn$	0.371888	0.371896	0.371881	0.371879	0.371882	0.371537	0.371534	0.371508						0.374700	0.374705	0.374694	0.374722	0.374717	0.370118
2se $_{R68/64}$	0.000009	0.000007	0.000007	0.000005	0.000007	0.000007	0.000008	0.000007						0.000009	0.000011	0.000007	0.000011	0.000010	0.000038
$\delta^{68}Zn$ (‰)	-0.79	-0.76	-0.79	-0.83	-0.83	-0.75	-0.73	-0.78						-0.71	-0.69	-0.72	-0.65	-0.67	-0.56
2se*	0.01	0.05	0.12	0.12	0.12	0.12	0.12	0.12						0.02	0.03	0.02	0.03	0.03	0.08
U, k=2	0.07	0.06	0.06	0.06	0.06	0.06	0.07	0.06						0.07	0.08	0.06	0.08	0.07	0.21
n	*2*	*2*	*1*	*1*	*1*	*1*	*1*	*1*						*2*	*2*	*2*	*2*	*2*	*2*

Table S6

Lab	LGL-TPE																		A&MS				G-TIME
Sample ID	FBS-1	FBS-2	FBS-3	FBS-4	FBS-5	FBS-6	FBS-7	FBS-8	FBS-9	FBS-10	FBS1	FBS2	FBS3	FBS4	FBS5	FBS6	FBS7	FBS8	FBS-1	FBS-2	FBS-3	FBS-4	FBS #1
$R^{56/54}Fe$											15.70675	15.69887	15.69814	15.69805	15.68132	15.71020	15.71074	15.91403	15.66908	15.66921	15.66848	15.66887	
$2se_{R56/54}$											0.00054	0.00090	0.00108	0.00067	0.00050	0.00056	0.00039	0.00223	0.00040	0.00025	0.00032	0.00031	
$\delta^{56}Fe$ (‰)											-1.79	-1.79	-1.84	-1.86	-1.80	-1.75	-1.80	-1.84	-1.88	-1.87	-1.92	-1.89	
2se*											0.03					0.07			0.03	0.02	0.02	0.02	
U, k=2											0.08	0.12	0.14	0.09	0.07	0.08	0.06	0.29	0.07	0.05	0.06	0.06	
$R^{57/54}Fe$											0.36275	0.36251	0.36246	0.36249	0.36180	0.36282	0.36285	0.36859	0.36157	0.36158	0.36155	0.36157	
$2se_{R57/54}$											0.00002	0.00003	0.00003	0.00002	0.00002	0.00002	0.00001	0.00009	0.00002	0.00001	0.00001	0.00001	
$\delta^{57}Fe$ (‰)											-2.63	-2.59	-2.77	-2.71	-2.62	-2.62	-2.69	-2.60	-2.78	-2.73	-2.83	-2.77	
2se*											0.03					0.05			0.04	0.03	0.04	0.03	
U, k=2											0.14	0.18	0.19	0.15	0.11	0.13	0.10	0.50	0.11	0.10	0.11	0.10	
n											*2*	*1*	*1*	*1*	*1*	*2*	*1*	*1*	*2*	*2*	*2*	*2*	
$R^{65/63}Cu$		0.447799	0.447757		0.447628	0.447575	0.447470	0.447734	0.447707	0.447769									0.446201	0.446170	0.446215	0.446183	
$2se_{R65/63}$		0.000024	0.000018		0.000014	0.000005	0.000017	0.000010	0.000008	0.000008									0.000017	0.000010	0.000008	0.000009	
$\delta^{65}Cu$ (‰)		1.25	1.17		1.29	1.17	1.32	1.14	1.08	1.25									1.27	1.21	1.31	1.23	
2se*		0.04	0.04		0.04	0.04	0.04	0.04	0.04	0.04									0.04	0.02	0.02	0.02	
U, k=2		0.11	0.09		0.07	0.04	0.08	0.05	0.05	0.05									0.08	0.05	0.05	0.05	
n		*1*	*1*		*1*	*1*	*1*	*1*	*1*	*1*									*2*	*2*	*2*	*2*	
$R^{66/64}Zn$	0.563479	0.563465	0.563499	0.563504	0.563761	0.563822	0.563828	0.563516	0.563514	0.563512									0.564335	0.564324	0.564332	0.564313	0.562403
$2se_{R66/64}$	0.000007	0.000007	0.000007	0.000006	0.000009	0.000008	0.000038	0.000007	0.000007	0.000006									0.000008	0.000009	0.000011	0.000010	0.000027
$\delta^{66}Zn$ (‰)	0.97	0.94	0.92	0.92	0.84	0.93	0.91	0.92	0.91	0.91									0.95	0.93	0.94	0.91	0.88
2se*	0.13	0.08	0.03	0.03	0.03	0.01	0.04	0.03	0.03	0.03									0.01	0.02	0.02	0.02	0.05
U, k=2	0.04	0.04	0.04	0.04	0.05	0.04	0.14	0.04	0.04	0.04									0.04	0.05	0.05	0.05	0.10
$R^{67/64}Zn$	0.081899	0.081897	0.081899	0.081902	0.081975	0.081968	0.082005	0.081917	0.081906	0.081909									0.082243	0.082241	0.082248	0.082240	0.081633
$2se_{R67/64}$	0.000002	0.000002	0.000002	0.000001	0.000004	0.000002	0.000006	0.000002	0.000002	0.000002									0.000003	0.000003	0.000003	0.000003	0.000016
$\delta^{67}Zn$ (‰)	1.49	1.42	1.36	1.40	1.43	1.30	1.43	1.49	1.37	1.40									1.39	1.36	1.45	1.35	1.28
2se*	0.16	0.07	0.06	0.06	0.06	0.07	0.13	0.06	0.06	0.06									0.04	0.03	0.03	0.03	0.18
U, k=2	0.10	0.09	0.09	0.09	0.12	0.10	0.17	0.09	0.09	0.10									0.11	0.10	0.10	0.10	0.39
$R^{68/64}Zn$	0.372362	0.372363	0.372389	0.372398	0.372811	0.372853	0.372938	0.372506	0.372486	0.372493									0.375647	0.375640	0.375638	0.375638	0.370992
$2se_{R68/64}$	0.000007	0.000006	0.000007	0.000006	0.000010	0.000007	0.000014	0.000005	0.000006	0.000007									0.000008	0.000008	0.000010	0.000011	0.000030
$\delta^{68}Zn$ (‰)	1.89	1.89	1.80	1.80	1.79	1.82	1.83	1.85	1.78	1.82									1.82	1.80	1.79	1.80	1.73
2se*	0.15	0.06	0.12	0.12	0.12	0.01	0.09	0.12	0.12	0.12									0.02	0.02	0.03	0.03	0.05
U, k=2	0.06	0.06	0.06	0.06	0.07	0.06	0.09	0.06	0.06	0.06									0.06	0.07	0.07	0.08	0.17
n	*2*	*2*	*1*	*1*	*1*	*2*	*2*	*1*	*1*	*1*									*2*	*2*	*2*	*2*	*2*

Table S7

Lab	LGLT-TPE														A&MS											G-TIME
Sample ID	1577c-1	1577c-2	1577c-3	1577c-4	1577c-5	1577c-6	1577c-7	1577c-10	1577c-11	1577c-12	1577c-13	1577c-14	1577c-15	1577c-16	BovLiv1	BovLiv2	BovLiv3	BovLiv4	BovLiv5	BovLiv6	BovLiv7	SRM 1577c-1	SRM 1577c-2	SRM 1577c-3	SRM 1577c-4	SRM 1577c-5	1577c #1
$R^{56/54}Fe$															15.85479	15.85457	15.92705	15.99220	15.99361	15.84641	15.84245	15.67764	15.67792	15.67865	15.67763	15.67944	
2se $_{R56/54}$															0.00075	0.00066	0.00024	0.00021	0.00021	0.00029	0.00036	0.00037	0.00053	0.00044	0.00042	0.00047	
$\delta^{56}Fe$ (‰)															-1.34	-1.36	-1.40	-1.40	-1.35	-1.33	-1.34	-1.33	-1.32	-1.27	-1.33	-1.22	
2se*															0.09	0.04	0.04	0.02	0.02	0.07	0.03	0.02	0.03	0.03	0.03	0.03	
U, k=2															0.10	0.09	0.05	0.05	0.05	0.05	0.06	0.07	0.09	0.08	0.08	0.08	
$R^{57/54}Fe$															0.36790	0.36789	0.36911	0.37081	0.37086	0.36756	0.36742	0.36188	0.36190	0.36191	0.36188	0.36199	
2se $_{R57/54}$															0.00003	0.00002	0.00001	0.00001	0.00001	0.00001	0.00001	0.00002	0.00002	0.00002	0.00002	0.00002	
$\delta^{57}Fe$ (‰)															-1.98	-2.00	-2.05	-2.06	-2.00	-1.99	-1.97	-1.91	-1.86	-1.82	-1.92	-1.61	
2se*															0.12	0.05	0.03	0.05	0.04	0.07	0.12	0.05	0.06	0.04	0.04	0.06	
U, k=2															0.15	0.14	0.08	0.08	0.08	0.09	0.09	0.14	0.15	0.14	0.14	0.16	
n															*3*	*2*	*2*	*2*	*3*	*2*	*4*	*2*	*2*	*2*	*2*	*2*	
$R^{65/63}Cu$	0.447322	0.447346	0.447332	0.447343	0.447119	0.447158	0.447190	0.447295	0.447280	0.447275	0.447255	0.447289	0.447182	0.447287								0.445768	0.445775	0.445817	0.445801	0.445820	
2se $_{R65/63}$	0.000008	0.000008	0.000007	0.000004	0.000006	0.000005	0.000005	0.000012	0.000010	0.000008	0.000011	0.000039	0.000008	0.000009								0.000006	0.000006	0.000006	0.000005	0.000008	
$\delta^{65}Cu$ (‰)	0.34	0.40	0.33	0.32	0.30	0.39	0.45	0.45	0.44	0.43	0.40	0.35	0.30	0.37								0.30	0.32	0.41	0.38	0.42	
2se*	0.04	0.04	0.04	0.04	0.02	0.02	0.05	0.07	0.04	0.04	0.02	0.04	0.04	0.04								0.01	0.01	0.01	0.01	0.02	
U, k=2	0.05	0.05	0.05	0.04	0.04	0.04	0.04	0.06	0.06	0.05	0.06	0.18	0.05	0.05								0.04	0.04	0.04	0.04	0.05	
n	*1*	*1*	*1*	*1*	*2*	*2*	*2*	*3*	*2*	*4*	*3*	*5*	*10*	*6*								*2*	*2*	*2*	*2*	*2*	
$R^{66/64}Zn$	0.562848	0.562838	0.562840	0.562851	0.563178	0.563194	0.562889	0.563078	0.563110	0.563135	0.563124	0.563089	0.563136	0.563046								0.563683	0.563665	0.563694	0.563701	0.563673	0.561813
2se $_{R66/64}$	0.000005	0.000009	0.000007	0.000007	0.000009	0.000007	0.000007	0.000007	0.000006	0.000006	0.000007	0.000026	0.000006	0.000008								0.000010	0.000009	0.000011	0.000008	0.000011	0.000017
$\delta^{66}Zn$ (‰)	-0.16	-0.20	-0.18	-0.21	-0.21	-0.17	-0.18	-0.20	-0.16	-0.17	-0.20	-0.17	-0.20	-0.17								-0.21	-0.24	-0.19	-0.18	-0.23	-0.16
2se*	0.00	0.02	0.04	0.01	0.06	0.05	0.03	0.02	0.03	0.02	0.03	0.11	0.02	0.02								0.02	0.02	0.02	0.01	0.02	0.05
U, k=2	0.04	0.05	0.04	0.04	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.10	0.04	0.04								0.05	0.05	0.05	0.04	0.05	0.07
$R^{67/64}Zn$	0.081755	0.081755	0.081761	0.081755	0.081834	0.081839	0.081777	0.081826	0.081823	0.081832	0.081828	0.081832	0.081844	0.081822								0.082102	0.082102	0.082100	0.082110	0.082107	0.081514
2se $_{R67/64}$	0.000002	0.000002	0.000001	0.000002	0.000003	0.000002	0.000002	0.000002	0.000003	0.000001	0.000002	0.000005	0.000002	0.000002								0.000003	0.000003	0.000003	0.000003	0.000003	0.000010
$\delta^{67}Zn$ (‰)	-0.29	-0.33	-0.21	-0.35	-0.30	-0.25	-0.22	-0.24	-0.27	-0.24	-0.25	-0.30	-0.31	-0.28								-0.33	-0.32	-0.35	-0.23	-0.27	-0.14
2se*	0.00	0.03	0.04	0.18	0.10	0.16	0.06	0.04	0.07	0.03	0.05	0.20	0.04	0.04								0.04	0.03	0.04	0.04	0.04	0.15
U, k=2	0.09	0.09	0.08	0.09	0.10	0.10	0.09	0.09	0.11	0.08	0.09	0.14	0.09	0.10								0.11	0.10	0.11	0.11	0.11	0.26
$R^{68/64}Zn$	0.371547	0.371550	0.371548	0.371554	0.372047	0.372032	0.371669	0.371898	0.371916	0.371965	0.371946	0.371929	0.371939	0.371850								0.374799	0.374787	0.374813	0.374824	0.374803	0.370198
2se $_{R68/64}$	0.000008	0.000006	0.000006	0.000007	0.000010	0.000007	0.000006	0.000008	0.000010	0.000006	0.000006	0.000008	0.000007	0.000006								0.000012	0.000008	0.000010	0.000009	0.000010	0.000007
$\delta^{68}Zn$ (‰)	-0.39	-0.38	-0.33	-0.37	-0.35	-0.37	-0.36	-0.36	-0.34	-0.35	-0.41	-0.37	-0.39	-0.35								-0.44	-0.47	-0.40	-0.38	-0.43	-0.32
2se*	0.06	0.05	0.13	0.03	0.03	0.04	0.12	0.03	0.04	0.02	0.03	0.01	0.03	0.02								0.03	0.02	0.03	0.02	0.03	0.02
U, k=2	0.07	0.06	0.06	0.06	0.07	0.06	0.06	0.07	0.07	0.06	0.06	0.07	0.06	0.06								0.08	0.07	0.08	0.07	0.07	0.06
n	*2*	*2*	*2*	*2*	*2*	*2*	*1*	*10*	*6*	*2*	*2*	*2*	*10*	*3*								*2*	*2*	*2*	*2*	*2*	*3*

Table S8

Lab	LGL-TPE									A&MS					G-TIME
Sample ID	TORT3-1	TORT3-2	TORT3-3	TORT3-4	TORT3- 5	TORT3- 6	Lobster1	Lobster2	Lobster3	TORT3-1	TORT3-2	TORT3-3	TORT3-4	TORT3-5	TORT #1
$R^{56/54}Fe$							15.70470	15.70367	15.72291	15.67616	15.67627	15.67675	15.67656	15.67642	
2se $_{R56/54}$							0.00097	0.00083	0.00036	0.00047	0.00056	0.00055	0.00040	0.00040	
$\delta^{56}Fe$ (‰)							-1.44	-1.47	-1.34	-1.43	-1.42	-1.39	-1.40	-1.41	
2se*								0.03		0.03	0.04	0.04	0.03	0.03	
U, k=2							0.15	0.14	0.10	0.09	0.09	0.09	0.08	0.08	
$R^{57/54}Fe$							0.36272	0.36270	0.36327	0.36187	0.36184	0.36183	0.63540	0.36185	
2se $_{R57/54}$							0.00003	0.00003	0.00001	0.00002	0.00002	0.00002	0.00004	0.00002	
$\delta^{57}Fe$ (‰)							-2.09	-2.07	-1.98	-1.95	-2.04	-2.04	-2.03	-2.00	
2se*								0.02		0.05	0.06	0.06	0.04	0.05	
U, k=2							0.24	0.22	0.16	0.14	0.15	0.15	0.35	0.14	
n							*1*	*2*	*1*	*2*	*2*	*2*	*2*	*2*	
$R^{65/63}Cu$	0.44700	0.44700	0.44719	0.44700	0.44730	0.44731				0.44579	0.44579	0.44578	0.44579	0.44578	
2se $_{R65/63}$	0.00001	0.00001	0.00000	0.00001	0.00001	0.00001				0.00001	0.00001	0.00001	0.00001	0.00001	
$\delta^{65}Cu$ (‰)	0.28	0.37	0.44	0.29	0.36	0.37				0.36	0.36	0.32	0.36	0.32	
2se*	0.00	0.03	0.00	0.03	0.02	0.03				0.01	0.01	0.01	0.01	0.01	
U, k=2	0.04	0.05	0.04	0.04	0.05	0.05				0.04	0.04	0.04	0.04	0.04	
n	*2*	*1*	*2*	*1*	*2*	*1*				*2*	*2*	*2*	*2*	*2*	
$R^{66/64}Zn$	0.563122	0.563139	0.563143	0.563131	0.563160	0.563187				0.563928	0.563914	0.563885	0.563931	0.563901	0.562068
2se $_{R66/64}$	0.000011	0.000008	0.000008	0.000008	0.000007	0.000007				0.000011	0.000009	0.000009	0.000010	0.000011	0.000005
$\delta^{66}Zn$ (‰)	0.21	0.23	0.26	0.25	0.22	0.27				0.23	0.20	0.15	0.23	0.18	0.27
2se*	0.03	0.00	0.03	0.03	0.03	0.03				0.02	0.02	0.02	0.02	0.02	0.03
U, k=2	0.05	0.04	0.04	0.04	0.04	0.04				0.05	0.05	0.04	0.05	0.05	0.04
$R^{67/64}Zn$	0.081829	0.081828	0.081831	0.081822	0.081833	0.081838				0.082154	0.082158	0.082142	0.082159	0.082152	0.081574
2se $_{R67/64}$	0.000002	0.000003	0.000003	0.000002	0.000002	0.000004				0.000003	0.000003	0.000004	0.000003	0.000003	0.000002
$\delta^{67}Zn$ (‰)	0.41	0.41	0.40	0.24	0.35	0.46				0.30	0.35	0.16	0.37	0.28	0.47
2se*	0.06	0.17	0.06	0.06	0.06	0.24				0.04	0.04	0.04	0.04	0.03	0.05
U, k=2	0.09	0.10	0.11	0.09	0.10	0.13				0.12	0.11	0.12	0.11	0.10	0.14
$R^{68/64}Zn$	0.372010	0.372006	0.372029	0.372015	0.372060	0.372056				0.375141	0.375134	0.375084	0.375158	0.375141	0.370538
2se $_{R68/64}$	0.000006	0.000006	0.000006	0.000007	0.000010	0.000011				0.000009	0.000009	0.000009	0.000009	0.000010	0.000005
$\delta^{68}Zn$ (‰)	0.52	0.51	0.57	0.52	0.48	0.49				0.47	0.45	0.32	0.51	0.47	0.52
2se*	0.12	0.05	0.12	0.12	0.12	0.03				0.02	0.02	0.03	0.03	0.03	0.03
U, k=2	0.06	0.06	0.06	0.06	0.08	0.08				0.07	0.07	0.07	0.07	0.07	0.10
n	*1*	*2*	*1*	*1*	*1*	*2*				*2*	*2*	*2*	*2*	*2*	*3*

Table S9

	Pairwise comparison	Differences in mean levels			*p* value	
		Mean	Lower	Upper		
δ^{56}Fe						
BCR-380R	A&MS/LGL-TPE	0.05	0.24	-0.15	0.5299	NS
BCR-383	A&MS/LGL-TPE	-0.10	-0.07	-0.14	0.0002	***
DORM-4	A&MS/LGL-TPE	0.04	0.12	-0.03	0.1743	NS
ERM-CE464	A&MS/LGL-TPE	-0.05	0.05	-0.15	0.2557	NS
FBS	A&MS/LGL-TPE	-0.08	-0.04	-0.13	0.0020	**
	Origins/A&MS	-0.05	0.06	-0.16	0.4895	NS
SRM-1577c	Origins/LGL-TPE	0.02	0.1279	-0.09	0.9081	NS
	A&MS/LGL-TPE	0.07	0.13	0.01	0.0339	*
TORT-3	A&MS/LGL-TPE	0.01	0.08	-0.07	0.8327	NS
δ^{65}Cu						
BCR-380R	A&MS/LGL-TPE	-0.25	-0.38	-0.12	0.0017	**
BCR-383	A&MS/LGL-TPE	-0.05	-0.12	0.01	0.0912	NS
	A&MS/LGL-TPE	-0.03	-0.09	0.04	0.6052	NS
DORM-4	DGSGE/LGL-TPE	0.05	-0.02	0.11	0.1625	NS
	DGSGE/A&MS	0.07	0.01	0.14	0.0337	*
ERM-CE464	A&MS/LGL-TPE	-0.08	-0.15	-0.01	0.0233	*
FBS	A&MS/LGL-TPE	0.05	-0.05	0.15	0.3222	NS
SRM-1577c	A&MS/LGL-TPE	-0.01	-0.07	0.05	0.7156	NS
	A&MS/LGL-TPE	-0.01	-0.07	0.05	0.9405	NS
TORT-3	DGSGE/LGL-TPE	0.01	-0.05	0.07	0.8920	NS
	DGSGE/A&MS	0.02	-0.04	0.08	0.7267	NS
δ^{66}Zn						
	A&MS/LGL-TPE	0.01	-0.04	0.05	0.9252	NS
BCR-380R	G-TIME/LGL-TPE	0.04	-0.04	0.13	0.4116	NS
	G-TIME/A&MS	0.04	-0.05	0.13	0.5416	NS
BCR-383	A&MS/LGL-TPE	0.04	-0.02	0.10	0.1539	NS
	A&MS/LGL-TPE	0.00	-0.04	0.05	0.9861	NS
DORM-4	G-TIME/LGL-TPE	0.08	-0.01	0.16	0.0704	NS
	G-TIME/A&MS	0.07	-0.01	0.16	0.0853	NS
	A&MS/LGL-TPE	0.07	0.04	0.10	0.0001	***
ERM-CE464	G-TIME/LGL-TPE	0.14	0.08	0.19	0.0001	***
	G-TIME/A&MS	-0.03	-0.09	0.02	0.2809	NS
	A&MS/LGL-TPE	0.02	-0.03	0.06	0.6587	NS
FBS	G-TIME/LGL-TPE	-0.05	-0.13	0.04	0.3183	NS
	G-TIME/A&MS	-0.06	-0.15	0.03	0.1835	NS
	A&MS/LGL-TPE	-0.03	-0.05	0.00	0.0634	NS
SRM-1577c	G-TIME/LGL-TPE	0.03	-0.02	0.09	0.2570	NS
	G-TIME/A&MS	0.05	-0.04	0.14	0.2877	NS
	A&MS/LGL-TPE	-0.04	-0.09	0.01	0.0910	NS
TORT-3	G-TIME/LGL-TPE	0.05	-0.04	0.14	0.2923	NS
	G-TIME/A&MS	-0.07	-0.16	0.02	0.1104	NS

Table S10