Analytica Chimica Acta 1025 (2018) 69–79

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Ultra-trace Cu isotope ratio measurements *via* multi-collector ICP-mass spectrometry using Ga as internal standard: an approach applicable to micro-samples

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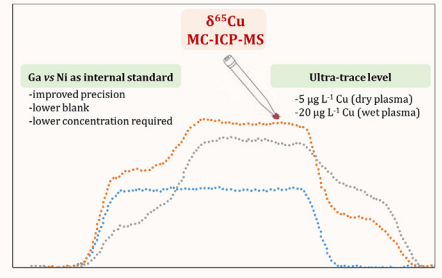
HIGHLIGHTS

- Cu isotopic analysis *via* MC-ICP-MS at a concentration level of 5 μg L^{-1}.
- Comparison of Ga and Ni as internal standard for mass bias correction.
- Minimum Cu, Ga and Ni concentrations required in wet and dry plasma conditions.
- Assessment of interference from Ar-based polyatomic ions affecting the $^{69}Ga^+$ and $^{71}Ga^+$ signals.
- Validation and application to micro-samples of serum and blood.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 January 2018
Received in revised form
3 May 2018
Accepted 8 May 2018
Available online 9 May 2018

Keywords:
MC-ICP-MS
Copper
Gallium
Isotope ratio
Interferences
Micro-samples

ABSTRACT

The capabilities of Cu isotope ratio measurements are often restricted by the small volumes of sample available and/or their low Cu concentration. In this work, an analytical approach was developed for performing Cu isotopic analysis *via* multi-collector ICP-mass spectrometry (MC-ICP-MS) at ultra-trace level using Ga as an internal standard for mass bias correction. The minimum concentration of Cu required for accurate and precise isotope ratio measurements was established to be 20 μg L^{-1} with wet plasma conditions and 5 μg L^{-1} with dry plasma conditions. The use of Ga as an internal standard for mass bias correction provided several advantages compared to Ni, *i.e.* improved internal precision on $\delta^{65}Cu$ values and lower blank levels. Ga can also be used at a 4-fold lower concentration level than Ni. However, in wet plasma conditions, the signals of $^{36}Ar^{16}O^1_2H^+$ and $^{40}Ar^{15}N^{16}O^+$ interfered with the signals of $^{69}Ga^+$ and $^{71}Ga^+$, respectively, while in dry plasma conditions, realized by the use of a desolvation unit, $^{69}Ga^+$ suffered from spectral interference from $^{40}Ar^{14}N^1_2H^+$. These interferences were resolved by using medium mass resolution. For validation purposes, the approach was applied to commercially available blood and serum samples. The $\delta^{65}Cu$ values for the samples measured at a concentration level of 5 μg L^{-1} Cu and 5 μg L^{-1} Ga using dry plasma conditions were in good agreement with those obtained for isotope ratio measurements at the "standard" concentration level of 200 μg L^{-1} Cu and 200 μg L^{-1} Ni using wet plasma conditions. In addition, the $\delta^{65}Cu$ values obtained for micro-samples of serum/blood (volume of 100 μL) were in good agreement with the corresponding ones obtained using the "standard" volume for isotopic analysis (500 μL).

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https://doi.org/10.1016/j.aca.2018.05.025

1. Introduction

Multi-collector ICP-mass spectrometry (MC-ICP-MS) allows accurate and highly precise isotope ratio measurements with a reasonable sample throughput [1]. However, for successfully exploiting the capabilities of high-precision isotopic analysis, isotope ratio measurements by MC-ICP-MS at ultra-trace level of the target element are sometimes required. Since the introduction of the technique in 1992 and until 2014, the concentrations at which isotope ratio measurements of transition metals, such as Cu, Fe, Zn or Ni, were typically carried out ranged between 0.5 and 5 mg L^{-1} [2,3].

Several innovations in MC-ICP-MS instrumentation were focused on an improvement of the sensitivity. The combination of a high-sensitivity interface (*e.g.,* the combination of a Jet-type sampler and an H-type skimmer in a Neptune MC-ICP-MS instrument) and enhanced pumping of the interface region resulted in a significant improvement in this context [4]. The concentrations generally used for isotope ratio measurements using the so-called high-sensitivity "jet interface" in wet plasma conditions are $\geq$0.1 mg L^{-1} for Cu [5,6] and even $\geq$ 0.2 mg L^{-1} for Fe, Ni and Zn [5–7]. The jet interface also provides an improved internal precision for these elements compared to the standard interface and gives rise to a smaller extent of mass discrimination owing to the increased ion transmission efficiency, but the requirements in terms of matrix- and concentration-matching of the sample and standard solutions become more stringent [5,8–10]. Further improvement in sensitivity can be obtained by enhancing the analyte introduction efficiency by using a desolvation system [4,11]. For improving the signal-to-noise ratio even further, Faraday cups with higher ohmic resistance (10^{12} or 10^{13} Ω) can be used [12,13]. Also electron multipliers provide higher sensitivity, but this type of detector is characterized by a dead time that needs to be corrected for when operated in pulse counting mode, while its linear dynamic range is more limited [14–16]. When using micro-flow systems, the sample consumption can be reduced to about 100 μL [17,18].

It is well known that instrumental mass discrimination compromises the accuracy of the isotope ratio data: the ions of the heavier of any two isotopes are transported more efficiently from the ion source to the detector, leading to biased isotope ratio measurement results. The extent of instrumental mass discrimination is affected by both the matrix composition and the target element concentration, thus necessitating chromatographic target element isolation and concentration-matching of the solutions investigated [1]. Several strategies for chromatographic isolation of the target element were developed, but nowadays, these are being revisited for processing of lower target element concentrations [11]. Although several approaches have been successfully applied for mass bias correction [19], the combination of internal normalization, based on a regression mass bias correction model with an admixed internal standard, and external correction in a sample-standard bracketing (SSB) approach is recommended. However, the selection of such an internal standard is not only limited by its physicochemical properties, but also by the detector configuration (Faraday cup array). In this context, the Cu isotope ratio is traditionally corrected for mass bias using Zn [3,11] or Ni [20,21], although also Ga has recently been suggested as internal standard [22].

To date, there are only a few works on high-precision isotopic analysis with Ga as the target isotopic system. Ga isotope ratio measurements by MC-ICP-MS have been carried out at low mass resolution and using dry plasma conditions combined with the jet interface to reach adequate sensitivity at low Ga concentrations [23]. Under these conditions, the minimum Ga concentration used for accurate and precise isotope ratio measurements was 20 μg L^{-1} [23]. Although Ga isotopic analysis has so far been used for geo- and cosmochemical applications only [24–27], its exploration in biomedicine could also show relevant information, as Ga, *e.g.*, binds to most ferric iron-binding proteins with an affinity similar to that of Fe [28]. However, as a non-essential element, Ga is typically present at extremely low concentrations only.

In contrast, Cu is probably one of the most explored elements for isotopic analysis in a biomedical context [29], *i.e.* for diagnostic/prognostic purposes [30–32] and/or for enhancing the understanding of the diseases affecting the Cu metabolism and, thus, also the Cu isotope distribution across different body compartments [33,34]. However, the small amount of Cu typically available for analysis only jeopardizes the full exploitation of such approaches. To the best of the authors' knowledge, the lowest concentrations reported in previous works describing accurate and precise Cu isotope ratio measurements by MC-ICP-MS using a high-sensitivity jet interface and dry plasma conditions were about 50 μg L^{-1} [11,21]. This concentration is still not sufficiently low for addressing micro-samples, such as blood serum from mice (about 100 μL of sample available only) and from newborns [20], blood sampled using a micro-sampling device [7], cerebrospinal fluid, bone marrow and biopsies [30,35]. Also Cu isotopic analysis in cultured human cell models [18,36,37], explored for unraveling the sources of isotope fractionation, is facing such limitations. Moreover, next to the bulk serum/blood Cu isotopic composition, also the Cu isotopic composition of specific proteins can be valuable for providing insight into biological systems but, to date, this was only performed computationally [38].

The main goal of this work was the development of an analytical approach for measuring Cu isotope ratios at ultra-trace level using an MC-ICP-MS unit equipped with the high-sensitivity jet interface and using Ga as internal standard for mass bias correction. The minimum Cu concentration required for accurate and precise isotope ratio data was assessed using (i) wet plasma conditions and (ii) dry plasma conditions, obtained using an Aridus II desolvation system. Ga and Ni were compared as internal standards in both plasma conditions and their minimum concentration level required for adequate mass bias correction was evaluated. The study of Ar-based ions interfering with the signals of $^{69}Ga^{+}$ and $^{71}Ga^{+}$ was carried out under different measurement conditions (in terms of nitric acid concentration, gas flow rates and hot *vs*. cold plasma conditions). The minimum dilution factor required to avoid matrix effects precluding successful Cu isotope ratio measurements at ultra-trace level was evaluated using a serum reference material and using procedural blanks spiked with an in-house Cu isotopic standard. The approach was validated and subsequently applied to micro-samples of serum and blood.

2. Experimental

2.1. Reagents

Ultrapure water (resistivity > 18.2 MΩ cm at 25 °C) was obtained from a Milli-Q Element water purification system (Millipore, France). Nitric acid (14 M, PrimarPlus, trace analysis grade) was purchased from Fisher Scientific (UK) and further purified in-house by sub-boiling distillation. *Optima* grade hydrochloric acid (12 M) was acquired from Fisher Chemical (UK) and used as such. Ultrapure hydrogen peroxide (9.8 M) was purchased from Sigma-Aldrich (Belgium). Poly-Prep® columns and AG MP-1 resin (100–200 mesh, chloride form) were purchased from Bio-Rad (Belgium).

The Cu isotopic standard reference material NIST SRM 976 was acquired from the National Institute for Standards and Technology (NIST, USA). A standard solution of 1000 mg L^{-1} of Cu (Inorganic

Ventures, the Netherlands, lot C2-Cu02116), previously characterized for its isotopic composition [3], was used as an in-house isotopic standard for monitoring the quality of the isotope ratio measurements. Single-element standard solutions (1000 mg L^{-1}) acquired from Inorganic Ventures were used for (i) the preparation of standard solutions for quantification purposes (Cu and some major and minor elements) and (ii) mass bias correction (Ga or Ni admixed internal standard).

2.2. Samples and sample preparation

Blood and several commercially available serum materials were analyzed. Seronorm™ Trace Elements Whole Blood L-1 (lot 1406263) and Seronorm™ Trace Elements Serum L-1 (lot 1309438) reference materials were obtained from SERO AS (Norway). Goat serum (lot SLBR1636), sheep serum (lot SLBS0563), horse serum (lot SLBS7574), mouse serum (lot SLBR6772) and rabbit serum (lot SLBS5706) were purchased from Sigma Aldrich (Belgium). The samples were acid digested at 110 °C overnight (~18 h). Digestion of 500 μL of sample was carried out using a mixture of 2 mL of 14 M HNO_3 and 500 μL of 9.8 M H_2O_2, while digestion of smaller volumes of sample, such as 200 μL, 100 μL, 50 μL and 20 μL, was accomplished using a mixture of 1 mL of 14 M HNO_3 and 250 μL of 9.8 M H_2O_2. The sample digests thus obtained were evaporated to dryness at 90 °C and re-dissolved in 8 M HCl +0.001% H_2O_2 for subsequent Cu chromatographic isolation using AG MP-1 resin. The Cu isolation procedure was performed as described elsewhere [5,31,32]. Two column passes were carried out to assure proper elimination of Na, still present to some extent in the Cu fraction after one column pass. After two steps of drying/re-dissolving in concentrated nitric acid of the pure Cu fraction, the final residue was re-dissolved in 500 μL of 0.14 M HNO_3. In each batch of samples, procedural blanks were included and treated in the same way as the samples.

The complete sample preparation procedure was carried out in a laminar flow fume hood located in a class-10 clean lab to avoid contamination. Teflon Savillex® beakers used for sample handling and storage were pre-cleaned with 7 M HNO_3 and 6 M HCl in several steps and subsequently rinsed with Milli-Q water. The disposable polypropylene material was pre-cleaned by soaking in 1.2 M HCl for 24 h, followed by soaking in Milli-Q water for another 24 h and were subsequently dried.

2.3. Measurements

Isotope ratio measurements were accomplished using a Neptune MC-ICP-MS instrument (Thermo Scientific, Germany), equipped with the high-transmission jet interface, *i.e.* the combination of the jet interface (Jet-type Ni sampling cone and X-type Ni skimmer cone) and a large dry interface pump (130 m^3h^{-1} pumping speed). Sample introduction was accomplished using a 100 μL min^{-1} PFA concentric nebulizer mounted onto a dual spray chamber consisting of a cyclonic and Scott-type sub-unit (further referred to as conventional introduction system) or using an Aridus II desolvating nebulizer system (Teledyne CETAC Technologies Inc., USA), equipped with a 100 μL min^{-1} PFA C-type nebulizer and a heated spray chamber, combined with a PTFE membrane desolvator unit. All isotope ratio measurements were performed (i) at medium (pseudo) mass resolution, (ii) in static collection mode, involving 5 Faraday collectors connected to 10^{11} Ω amplifiers, (iii) on the interference-free plateaus at a position located ~0.038 u away from the peak centers and (iv) in hot plasma conditions. For further study of spectral interferences, some peak profiles were obtained in cold plasma conditions. The instrument settings and data acquisition parameters are provided in Table 1.

Depending on the instrument settings, the measurement solutions were diluted to final concentrations ranging between 5 μg L^{-1} and 200 μg L^{-1} in 0.14 M HNO_3. For mass bias correction, the solutions were doped with Ga or Ni as internal standard. The measurements were carried out in a sample-standard bracketing (SSB)

Table 1
Instrument settings and data acquisition parameters for the Neptune MC-ICP-MS instrument.

Neptune MC-ICP-MS instrument		
Sample and skimmer cone	Jet-type Ni sampling cone, 1.1 mm orifice diameter X-type Ni skimmer cone, 0.8 mm orifice diameter	
Cup configurations	L3:^{60}Ni; L1:^{61}Ni; C:^{62}Ni; H1:^{63}Cu; H3:^{65}Cu L4:^{63}Cu; L2:^{65}Cu; C:^{67}Zn; H2:^{69}Ga, H4:^{71}Ga	
Guard electrode	Connected	
Integration time (s)	4.194	
Number of cycles	45	
	Wet plasma (conventional introduction system)	
	Hot plasma conditions	Cold plasma conditions
RF power (W)	1265	800
Gas flow rates (L min^{-1})		
-Plasma	15	15
-Auxiliary	0.70	0.70
-Nebulizer	1.005	0.600
	Dry plasma (Teledyne CETAC Technologies Aridus II)	
	Hot plasma conditions	
RF power (W)	1265	
Gas flow rates (L min^{-1})		
-Plasma	15	
-Auxiliary	0.80	
-Nebulizer	0.805	
-Sweep	3.16	
-N_2	0.006	
Temperatures (°C)		
-Spray chamber	110	
-Membrane desolvator	160	

sequence with the NIST SRM 976 Cu isotopic reference material as external standard. An acid blank and procedural blanks were included in each measurement session. Baseline correction was performed before each measurement.

Correction for mass discrimination was performed off-line by means of a combination of internal normalization (with Ga or Ni) according to the Baxter-revision [39] of the Russell equation and external correction in a SSB approach (further referred to as Baxter-SSB method). 2s-rejection of outliers was applied to the 45 cycles (Table 1). Finally, the Cu isotope ratio results were expressed as delta values (δ^{65}Cu, per mil, ‰) relative to the Cu isotopic reference material NIST SRM 976 [5,29].

Prior to isotope ratio measurements by MC-ICP-MS, elemental determinations were performed using an Element XR single-collector sector-field ICP-MS instrument (Thermo Scientific, Germany) operated at medium mass resolution (see Ref. [31]). Concentrations of Cu and some major elements that can give rise to spectral interference were determined before and after chromatographic isolation. The nuclides determined were ^{23}Na, ^{24}Mg, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{69}Ga, ^{72}Ge and ^{74}Ge. External calibration with Ge as internal standard was used for quantification purposes.

3. Results and discussion

3.1. Accuracy and precision of the Cu isotope ratio at different concentration levels

Isotope ratio measurements of the Cu in-house isotopic standard were carried out at different concentration levels for assessment of the minimum concentration of the target element required for accurate and precise Cu isotope ratios. Different concentrations were tested using the conventional introduction system and using the Aridus II desolvation system. The measurements were carried out in a SSB sequence with NIST SRM 976 as bracketing standard. All of these solutions were spiked with Ni or Ga as internal standard. The concentrations of Cu and the internal standard in the in-house standard were always adjusted to within ±3% of those in the bracketing NIST SRM 976 standard.

Table 2 compiles the slopes, intercepts and correlation coefficients (R^2) for the linear relationships for ln(^{65}Cu/^{63}Cu) *vs.* ln(^{62}Ni/^{60}Ni) and for ln(^{65}Cu/^{63}Cu) *vs.* ln(^{71}Ga/^{69}Ga) obtained for the NIST SRM 976 standard at several concentrations of the target element and spiked with different concentrations of Ni or Ga. Well-defined relationships ($R^2 > 0.90$) with a slope ~1 were obtained for the ln-ln regression lines for concentrations $\geq 20\,\mu g\,L^{-1}$ Cu and $\geq 100\,\mu g\,L^{-1}$ Ni or $\geq 20\,\mu g\,L^{-1}$ Ga in wet plasma conditions. For lower concentrations, there was no linear mass bias relationship between the analyte and the internal standard. The signal intensities for ^{63}Cu, ^{65}Cu, ^{69}Ga and ^{71}Ga were ~1.5 V, ~0.7 V, ~1.5 V and ~1.0 V, respectively, for a solution containing $20\,\mu g\,L^{-1}$ of both Cu and Ga. For $100\,\mu g\,L^{-1}$ Ni, the signal intensity was ~1.8 V for ^{60}Ni and ~0.3 V for ^{62}Ni. The low signal intensity for the less abundant ^{62}Ni isotope renders higher Ni concentrations mandatory for suitable mass bias correction. As can be seen in Table 2, the minimum concentration required for the isotope ratio measurements using the conventional introduction system was $20\,\mu g\,L^{-1}$ Cu and $100\,\mu g\,L^{-1}$ Ni or $20\,\mu g\,L^{-1}$ Ga as internal standard.

When using dry plasma conditions, well-defined relationships ($R^2 > 0.90$) with a slope ~1 for ln(^{65}Cu/^{63}Cu) *vs.* ln(^{62}Ni/^{60}Ni) and for ln(^{65}Cu/^{63}Cu) *vs.* ln(^{71}Ga/^{69}Ga) were obtained for concentrations $\geq 5\,\mu g\,L^{-1}$ Cu and $\geq 20\,\mu g\,L^{-1}$ Ni or $\geq 5\,\mu g\,L^{-1}$ Ga (Table 2). However, for lower concentrations these ln-ln relationships were deteriorated ($R^2 < 0.75$). The signal intensities for ^{63}Cu, ^{65}Cu, ^{69}Ga and ^{71}Ga were ~1.4 V, ~0.6 V, ~1.8 V and ~1.3 V, respectively, for $5\,\mu g\,L^{-1}$ of both Cu and Ga. At $20\,\mu g\,L^{-1}$ Ni, the signal intensity was ~1.9 V for ^{60}Ni and ~0.3 V for ^{62}Ni. The use of the desolvation system improved the instrumental sensitivity by a factor of ~4 compared to the conventional introduction system.

Table 2
Slope, intercept and correlation coefficient (R^2) for the linear relationship observed between ln(^{65}Cu/^{63}Cu) and ln(^{62}Ni/^{60}Ni) or between ln(^{65}Cu/^{63}Cu) and ln(^{71}Ga/^{69}Ga) for measurements performed at different concentrations of the target element and the internal standard Ni or Ga. The standard error on the slope and intercept is indicated by se.

Conventional introduction system – wet plasma conditions						
Cu concentration ($\mu g\,L^{-1}$)	Ni concentration ($\mu g\,L^{-1}$)	slope	intercept	R^2	se(slope)	se(intercept)
200	200	0.97	1.10	0.98	0.04	0.07
100	100	0.97	1.10	0.93	0.05	0.11
50	100	0.97	1.12	0.91	0.07	0.14
50	50	0.18	−0.41	0.07	0.14	0.28
20	100	0.93	1.04	0.95	0.05	0.10
10	100	−0.28	−1.30	0.07	0.20	0.39
Cu concentration ($\mu g\,L^{-1}$)	Ga concentration ($\mu g\,L^{-1}$)	slope	intercept	R^2	se(slope)	se(intercept)
200	200	0.96	−0.41	1.00	0.02	0.01
100	100	1.03	−0.38	0.97	0.07	0.03
50	50	1.08	−0.37	0.90	0.12	0.04
20	20	0.96	−0.41	0.90	0.08	0.03
10	10	−0.26	−0.86	0.06	0.22	0.08
Aridus II desolvation system – dry plasma conditions						
Cu concentration ($\mu g\,L^{-1}$)	Ni concentration ($\mu g\,L^{-1}$)	slope	intercept	R^2	se(slope)	se(intercept)
20	20	0.96	1.09	0.94	0.06	0.12
10	10	0.02	−0.73	0.01	0.04	0.08
10	20	0.94	1.05	0.91	0.10	0.18
5	20	0.95	1.07	0.94	0.08	0.15
2	20	0.67	0.53	0.73	0.12	0.23
Cu concentration ($\mu g\,L^{-1}$)	Ga concentration ($\mu g\,L^{-1}$)	slope	intercept	R^2	se(slope)	se(intercept)
20	20	0.99	−0.40	0.96	0.07	0.03
10	10	1.00	−0.40	0.98	0.04	0.01
5	5	0.96	−0.41	0.94	0.09	0.03
2	2	0.40	−0.62	0.42	0.12	0.04

Table 3
Isotopic composition of Cu, expressed as δ^{65}Cu (‰), relative to NIST SRM 976, obtained for pure in-house Cu isotopic standard solutions with different concentrations of the target element. The solutions were spiked with Ga or Ni as internal standard. The measurements were performed using wet and dry plasma conditions. n indicates the number of measurement replicates. The average internal precision on δ^{65}Cu after mass bias correction by means of the Baxter-SSB method is indicated by 2s.

Wet plasma conditions		Ga as internal standard					Ni as internal standard				
Cu (μg L^{-1})	Ga or Ni (μg L^{-1})	δ^{65}Cu(‰) ± 2s			n	2s (internal)	δ^{65}Cu(‰) ± 2s			n	2s (internal)
200	200	0.23	±	0.04	35	0.013	0.23	±	0.04	23	0.019
100	100	0.23	±	0.04	20	0.017	0.22	±	0.03	26	0.021
50	100						0.22	±	0.04	23	0.022
50	50	0.23	±	0.06	20	0.032					
20	100						0.23	±	0.07	35	0.038
20	20	0.23	±	0.08	42	0.041					
Dry plasma conditions		**Ga as internal standard**					**Ni as internal standard**				
Cu (μg L^{-1})	Ga or Ni (μg L^{-1})	δ^{65}Cu(‰) ± 2s			n	2s (internal)	δ^{65}Cu(‰) ± 2s			n	2s (internal)
20	20	0.26	±	0.13	29	0.040	0.22	±	0.13	19	0.050
10	20						0.26	±	0.09	25	0.052
10	10	0.23	±	0.10	39	0.043					
5	20						0.22	±	0.11	40	0.050
5	5	0.21	±	0.12	48	0.050					

Thus, the minimum concentration level required using dry plasma conditions was 5 μg L^{-1} for Cu and 20 μg L^{-1} or 5 μg L^{-1} for the internal standards Ni and Ga, respectively.

Table 3 compiles the Cu isotope ratio results obtained for the in-house isotopic standard measured in wet and in dry plasma conditions, corrected for mass bias *via* the Baxter-SSB method using Ni or Ga as internal standard. The measurements were carried out at concentrations of Cu (as target element) and Ni or Ga (as internal standard) for which acceptable ln-ln regression lines were obtained (*vide supra*). The average δ^{65}Cu values obtained for the in-house isotopic standard were always in good agreement with the reference value, *i.e.* 0.22 ± 0.07 (2s) ‰ [3]. The internal precision (2s) accompanying δ^{65}Cu values obtained with wet plasma conditions for solutions containing 200 μg L^{-1} Cu was <0.02‰, while the internal precision (2s) on δ^{65}Cu values obtained with dry plasma conditions for solutions containing 5 μg L^{-1} of Cu was ~0.05‰. It is remarkable that the latter internal precision on δ^{65}Cu is similar to that obtained for solutions containing 200 μg L^{-1} Cu when using the standard interface and wet plasma conditions, *i.e.* ranging from 0.02 to 0.08‰ [5]. In Fig. 1, the internal precision on δ^{65}Cu obtained using wet and dry plasma conditions and corrected for mass bias using Ni or Ga as internal standard is plotted *versus* the concentration of Cu. A significant correlation was found between 2s on δ^{65}Cu and the Cu concentration (Spearman's correlation coefficient $\rho = -0.925$, $p = 0.000$, n = 14). As can be seen in Table 3, for solutions containing equal concentrations of Cu and with either Ni or Ga as internal standard, the use of Ga provided a slightly improved internal precision. This might be attributed to the higher signal intensities for ^{69}Ga and ^{71}Ga compared to the signal intensities for ^{60}Ni and ^{62}Ni (*vide supra*). Another advantage of the use of Ga as internal standard compared to Ni is related to the contribution from the blanks. For pure standard solutions containing 5 μg L^{-1} of both Cu and Ga, the contribution from acid blanks (0.14 M HNO_3) was 0.2% and 0.3%, respectively, while for solutions containing 20 μg L^{-1} of Ni, the contribution of the acid blank was 0.5%.

To the best of the authors' knowledge, only Hou et al. have

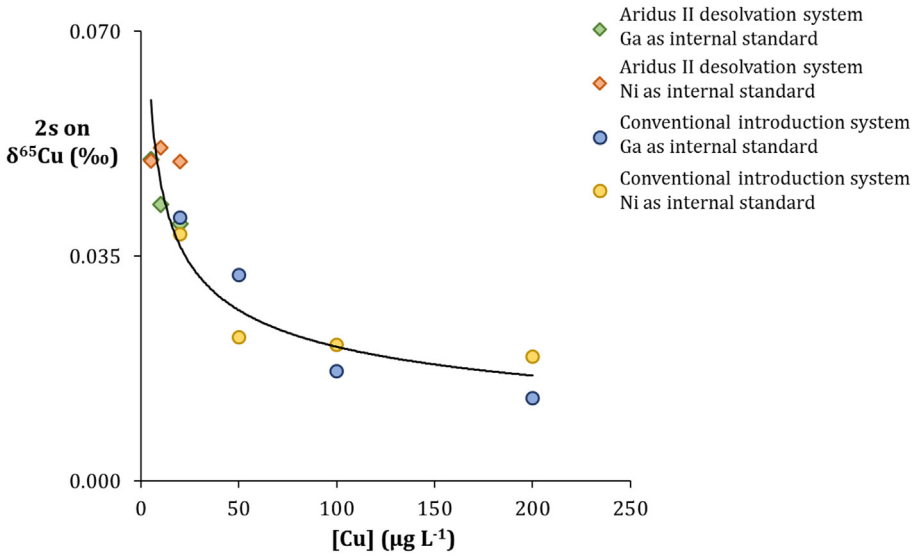

Fig. 1. Average internal precision 2s on δ^{65}Cu (‰), obtained after mass bias correction by means of the Baxter-SSB method using Ni or Ga as internal standard, *versus* the Cu concentration. The corresponding concentrations of Ga and Ni are indicated in Table 2. The diamonds and the circles represent the data obtained using dry and wet plasma conditions, respectively. The green and blue colours represent the data obtained using Ga as internal standard, while the yellow and orange colours represent the data obtained using Ni as internal standard. The concentration of Ga was always equal to that of Cu while the concentration of Ni using dry plasma condition was always 20 μg L^{-1} and using wet plasma conditions 100 μg L^{-1} or 200 μg L^{-1}. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

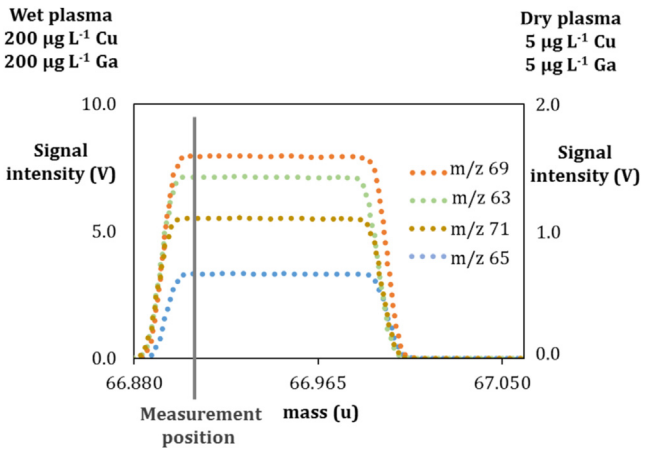

Fig. 2. Mass spectra (peak profiles) at m/z-values of 63, 65, 69 and 71 obtained upon scanning with the Neptune MC-ICP-MS instrument, equipped with the jet interface and operated at medium mass resolution. Values on the y-axis represent the signal intensity (V) obtained using (i) wet plasma conditions for a solution containing 200 µg L^{-1} Cu and 200 µg L^{-1} Ga (left y-axis) and using (ii) dry plasma conditions for a solution containing 5 µg L^{-1} Cu and 5 µg L^{-1} Ga (right y-axis). Values on the x-axis represent the mass (u).

previously used Ga as an internal standard for Cu isotopic analysis by MC-ICP-MS [22]. They performed the isotope ratio measurements at low mass resolution and at a concentration level of 200 µg L^{-1} Cu and 200 µg L^{-1} Ga [22]. MC-ICP-MS isotopic analysis of Ga as target element is still scarce and only applied to geological samples [23–27]. For this purpose, Cu was used as internal standard for mass bias correction [23,27]. A limited number of studies report the use of Ga as internal standard for Ge isotopic analysis [40–43]. Yuan et al. used 20 µg L^{-1} of Ga (as target element) and 40 µg L^{-1} of Cu (as internal standard) for Ga isotopic analysis *via* MC-ICP-MS at low mass resolution and using an Apex HF desolvator (without N_2 flow) as introduction system [23]. Thus, the concentrations of Cu and Ga established in the present study (both 5 µg L^{-1}) for Cu isotopic analysis by MC-ICP-MS are lower than those reported in previous works, in spite of the use of medium mass resolution, required to avoid spectral interferences.

3.2. Spectral interferences affecting the Ga^+ ion signals

Potential interferences affecting the Ga^+ ion signals mainly arise from argide, hydride and doubly charged ions, mostly derived from Zn, Fe, Ba and Ce [23]. However, after the chromatographic separation of Cu from the sample matrix, the presence of these elements in the pure Cu fractions is negligible. Although the contribution of major polyatomic argide ions can jeopardize accurate determination of the Ga isotope ratio, this was not assessed yet at ultra-trace level. Therefore, the presence of interfering ions at the mass-to-charge ratios of the Ga isotopes was investigated using standard solutions and acid blanks in dry and wet plasma conditions. While for the Ga isotopes the literature is scarce, ions interfering with the signals of the Cu and Ni nuclides are widely described in the literature and, thus, are not detailed in this work (*e.g.,* [44,45]).

3.2.1. Wet plasma conditions

In a pure standard solution containing 200 µg L^{-1} Cu and 200 µg L^{-1} Ga, the signals from the $^{69}Ga^+$ and $^{71}Ga^+$ isotopes do not show any observable spectral overlap (Fig. 2). However, the mass spectra (peak profiles) at m/z 69 and 71 of acid blanks (0.14 M HNO_3) obtained using wet plasma conditions, reveal the presence of $^{36}Ar^{16}O^1_2H^+$ and $^{40}Ar^{15}N^{16}O^+$, which would affect the signal intensities of the $^{69}Ga^+$ and $^{71}Ga^+$ isotopes, respectively, at low mass resolution (Fig. 3). The use of the medium resolution slit allows the interference-free measurement of the Ga isotopes by separation of their signals from those of the Ar-based polyatomic ions in a pseudo-high mass resolution approach [46] (Fig. 3A and B). As can be seen in Fig. 3(A) and (B), an increased HNO_3 concentration in the blank solution results in an increased signal intensity for $^{36}Ar^{16}O^1_2H^+$ and $^{40}Ar^{15}N^{16}O^+$. In order to confirm the presence of these major polyatomic argide ions in the acid blanks, some peak profiles were recorded in cold plasma conditions, obtained by reducing the plasma RF power (Table 1). As can be seen in Fig. 3(C) and (D), the levels of $^{36}Ar^{16}O^1_2H^+$ and $^{40}Ar^{15}N^{16}O^+$ were effectively suppressed in cold plasma conditions.

3.2.2. Dry plasma conditions

For the pure standard solutions containing 5 µg L^{-1} Cu and 5 µg L^{-1} Ga, at first sight, no interferences affecting the signals of $^{69}Ga^+$ and $^{71}Ga^+$ were observed in hot plasma conditions (Fig. 2). However the mass spectra (peak profiles) at m/z 69 and 71 for acid blanks recorded using dry plasma conditions, reveal a clear interference on $^{69}Ga^+$ (Fig. 2). The $^{36}Ar^{16}O^1_2H^+$ and $^{40}Ar^{15}N^{16}O^+$ signals observed in wet plasma conditions were successfully eliminated in dry plasma conditions. However, the signal from $^{69}Ga^+$ overlaps with that from the $^{40}Ar^{14}N^1_2H^+$ polyatomic ion at low mass resolution. The use of medium (pseudo) mass resolution, however, allows interference-free measurement of the signal of $^{69}Ga^+$ (Fig. 4A). As can be seen in Fig. 4B, increasing the N_2 gas flow rate, while keeping the Ar sweep gas flow rate constant, leads to substantially higher $^{40}Ar^{14}N^1_2H^+$ signal intensities. When no N_2 gas is used, the signal from $^{40}Ar^{14}N^1_2H^+$ seems to be eliminated, but the sensitivity for ^{63}Cu and ^{65}Cu is decreased by a factor of ~3 compared to the sensitivity obtained at the optimum N_2 gas flow rate. When the Ar sweep gas flow rate is increasing at a constant N_2 gas flow rate, the $^{40}Ar^{14}N^1_2H^+$ level is also increasing (Fig. 4C).

The use of the desolvation system provided a successful elimination of the solvent-based oxide and hydroxide polyatomic interferences $^{36}Ar^{16}O^1_2H^+$ and $^{40}Ar^{15}N^{16}O^+$, affecting the measurement of $^{69}Ga^+$ and $^{71}Ga^+$, respectively. This observation is in agreement with the results found in studies addressing other interfering ions (*e.g.,* $^{40}Ar^{16}O^+$ and $^{40}Ar^{16}O^1H^+$ affecting the signals of $^{56}Fe^+$ and $^{57}Fe^+$, respectively [47]). However, the use of the desolvation system leads to an interference from $^{40}Ar^{14}N^1_2H^+$ at the m/z of the $^{69}Ga^+$ signal, which is resolved in medium mass resolution.

3.3. Suitability of the Ga isotope ratio for mass bias correction

In order to evaluate the suitability of Ga as internal standard for Cu isotopic analysis, isotope ratio measurements were carried out in 7 commercially available blood and serum samples, *i.e.* Seronorm serum and Seronorm whole blood reference materials, goat serum, sheep serum, mouse serum, horse serum and rabbit serum. The Cu isotopic composition thus obtained was compared to that obtained using Ni as internal standard. For this purpose, 500 µL of the blood and serum samples were digested and subjected to the full sample preparation procedure. The Cu recovery for these samples after isolation was 102 ± 5%. The Na/Cu and Mg/Cu concentration ratios in the isolated Cu fractions were about 0.1 on average. The Ga/Cu and Ni/Cu concentration ratios in the Cu fractions were about 0.001 and 0.005 on average, respectively. Thus, the amount of Ga present in the purified Cu fractions is a factor of 5 lower than the amount of Ni. After the full sample preparation procedure, the samples were appropriate diluted and spiked with Ga or Ni for mass bias correction. The isotope ratio measurements were carried out at a concentration level of 200 µg L^{-1} of both Cu and the internal standard and with wet plasma conditions.

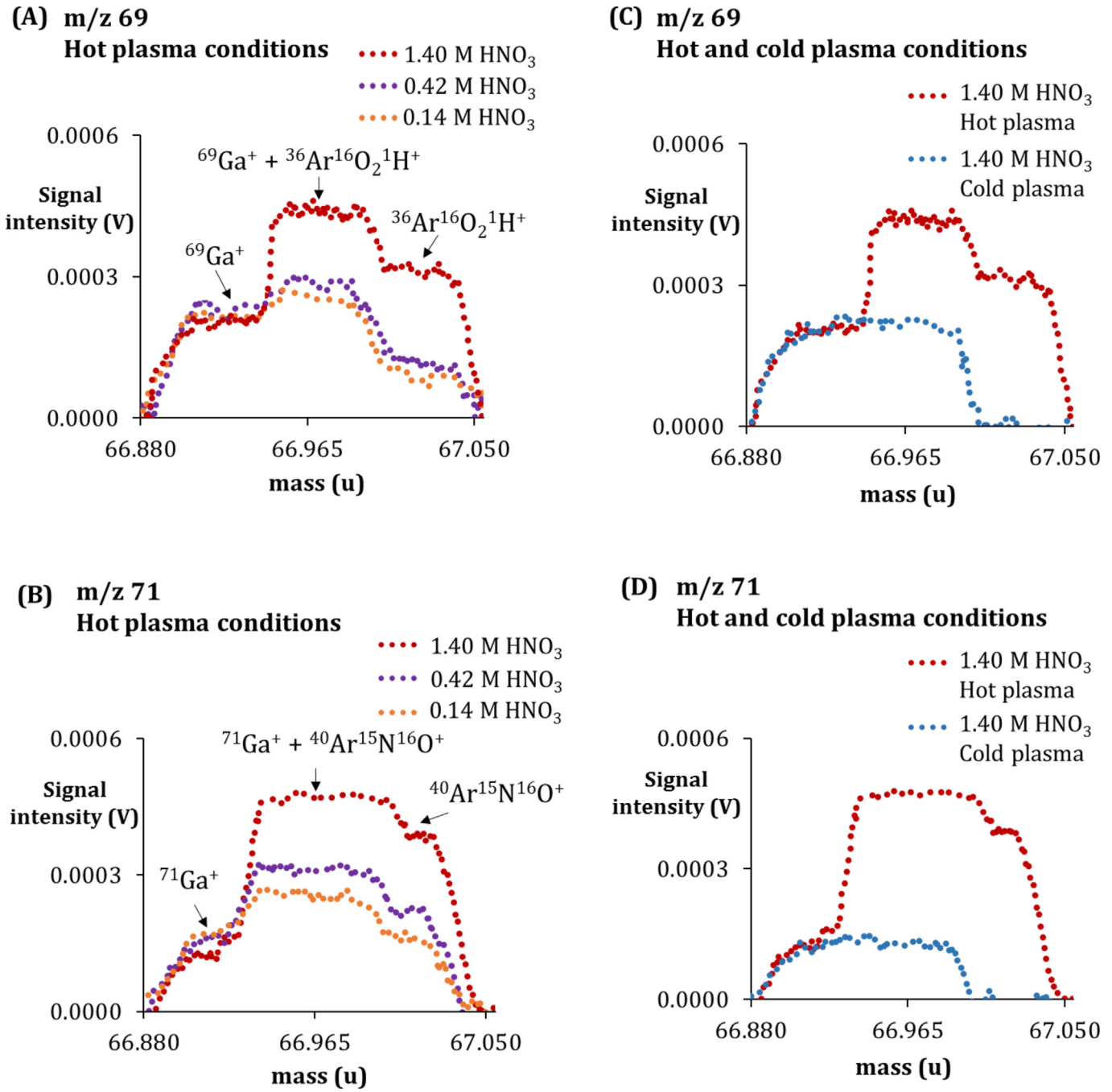

Fig. 3. Mass spectra (peak profiles) at m/z-values of 69 and 71 obtained upon scanning with the Neptune MC-ICP-MS instrument, equipped with the jet interface, using wet plasma conditions. The measurements were carried out at medium mass resolution. Values on the x-axis and the y-axis represent the mass (u) and the signal intensity (V), respectively. Mass spectra at m/z 69 (A) and 71 (B) obtained for blanks with different HNO_3 concentrations (0.14 M, 0.42 M and 1.40 M) using hot plasma conditions. Mass spectra at m/z 69 (C) and 71 (D) obtained for a blank (1.40 M HNO_3) using hot and cold plasma conditions.

The Cu isotopic composition, expressed as δ^{65}Cu value, of these samples is presented in Table 4. As can be seen, the Cu isotope ratios for commercially available blood and serum samples obtained using Ga as internal standard were in good agreement with those obtained using Ni as internal standard (paired *t*-test, $p > 0.05$). A maximum difference of 0.04‰ was found between the δ^{65}Cu values corrected with Ga and those corrected with Ni. The Cu isotopic composition of the Seronorm serum and whole blood reference materials were in good agreement with those reported in previous works [3,5,31,32]. The internal precision (2s) on the δ^{65}Cu values was 0.02‰ and 0.04‰ using Ga and Ni as internal standard, respectively (Table 4), and, thus, the use of Ga as an internal standard improved the internal precision by a factor of 2, as was also observed for the pure in-house standard solutions (*vide supra*). The contribution of the procedural blanks was negligible in all cases. The maximum bias observed between the results with and without blank correction was <0.01‰ using both internal standards.

3.4. Study of the minimum sample dilution factor

Since isotopic analysis at a level of 5 μg L^{-1} Cu and 5 μg L^{-1} Ga using dry plasma conditions is feasible, the minimum dilution

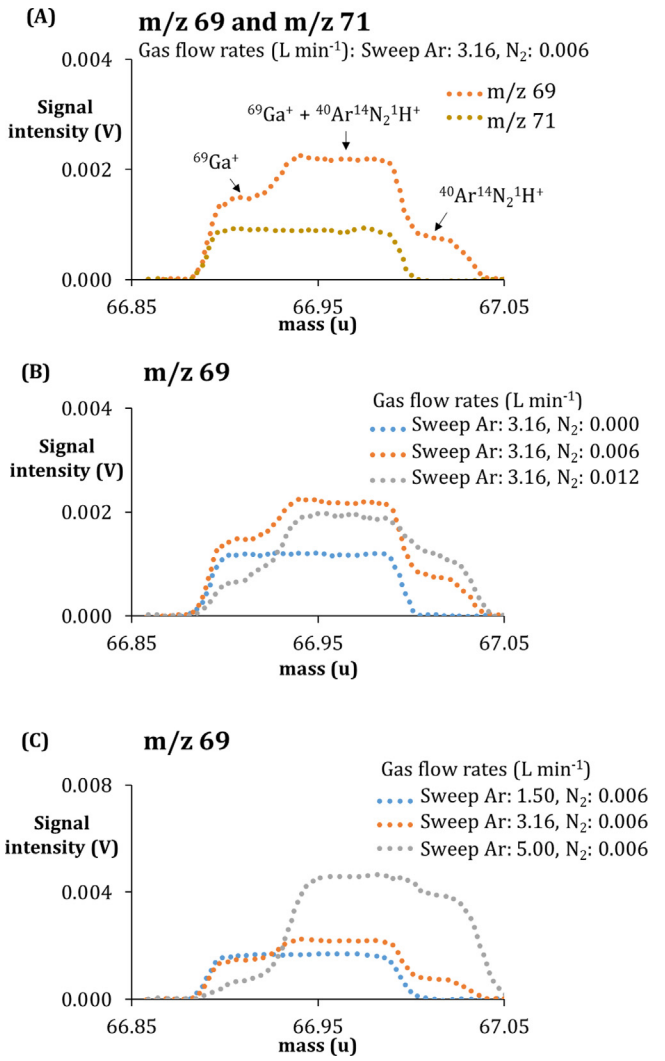

Fig. 4. Mass spectra (peak profiles) at m/z-values 69 and 71 obtained for a blank (0.14 M HNO_3) upon scanning with the Neptune MC-ICP-MS instrument, equipped with the jet interface, using hot and dry plasma conditions. The measurements were carried out at medium mass resolution. Values on the x-axis and the y-axis represent the mass (u) and the signal intensity (V), respectively. (A) Mass spectra at m/z 69 and 71. (B) Mass spectra at m/z 69 for scans with increasing N_2 gas flow rates (0.000 L min^{-1}, 0.006 L min^{-1} and 0.012 L min^{-1}) and at constant Ar sweep gas flow rate (3.16 L min^{-1}). (C) Mass spectra at m/z 69 for scans at constant N_2 gas flow rate (0.006 L min^{-1}) and increasing Ar sweep gas flow rates (1.50 L min^{-1}, 3.16 L min^{-1} and 5.00 L min^{-1}).

factor required to avoid matrix effects in the samples was evaluated. For this, different volumes (20 μL, 50 μL, 100 μL and 200 μL) of the Seronorm serum reference material were digested and subjected to the anion exchange chromatographic separation. The Cu recovery after isolation from these samples was 100 ± 5%. After the entire sample preparation procedure, the samples were diluted to a concentration of 5 μg L^{-1}. The measurement of these samples was carried out in a SSB sequence with a procedural NIST SRM 976 isotopic reference material (*i.e.* digested and isolated in the same way as the samples) as the bracketing standard. The concentrations of Cu and Ga in the sample measurement solutions were always adjusted to within ±10% of those of the bracketing NIST SRM 976 standard. The δ^{65}Cu values obtained for these samples are presented in Table 5.

The δ^{65}Cu values obtained for the Seronorm serum reference material for which 200 μL, 100 μL or 50 μL of reconstituted material were digested were in good agreement with the values reported in our previous works for which 500 μL of sample was digested, *i.e.* about −0.18‰ [5,31,32]. However, a shift in the Cu isotopic composition of about 0.20‰ towards lower δ^{65}Cu values was observed when using 20 μL of reconstituted Seronorm serum reference material for digestion only. The limited dilution factor of the latter sample possibly gave rise to a more pronounced matrix effect, probably caused by residual concomitant matrix components derived from the sample preparation procedure.

For further assessment of the minimum dilution factor necessary to avoid matrix effects, a similar experiment was performed using procedural blanks. The procedural blanks (prepared following the same sample preparation as the samples) were diluted with different dilution factors and doped with the Cu in-house standard and Ga for a final concentration in the measurement solution of 5 μg L^{-1}. As can be seen in Table 5, the δ^{65}Cu value obtained for the procedural blank diluted by a factor of 20 and spiked with the in-house isotopic standard was in good agreement with the reference value, *i.e.* 0.22 ± 0.07‰ [3]. However, the Cu isotopic composition of the procedural blank diluted by a factor of 10 and spiked with the in-house isotopic standard showed a shift of 0.25‰ towards lower δ^{65}Cu values, similar to the shift observed for the serum sample. This substantiates the statement above that the matrix effect causing this shift is due to matrix components resulting from the sample preparation procedure. A similar observation, related to the need for an appropriate sample dilution, was reported by Paredes et al. [18] Possible matrix components induced during the sample preparation procedure might be derived from resin-derived organics (disintegrating resin beads) introduced during the anion-exchange chromatographic procedure [48].

Hence, the minimum volume required for performing accurate Cu isotopic analysis of the Seronorm serum reference material (containing ~1000 μg L^{-1} Cu) at a level of 5 μg L^{-1} is 50 μL. This indicates that for performing isotopic analysis of serum Cu at this low level, a minimum amount of ~50 ng Cu needs to be present in the sample. However, the proposed approach is deemed feasible for samples containing a lower amount of Cu, on condition that the sample preparation procedure is revised and/or miniaturized for processing smaller sample volumes and/or when using a microflow system. It is therefore recommended to re-evaluate the minimum dilution factor required to avoid matrix effects affecting the Cu isotope ratio for each (i) sample type at hand, (ii) sample preparation procedure applied, (iii) Cu concentration present in the original sample and in the final measurement solution (iv) set of measurement conditions (*e.g.,* wet or dry), and/or (v) instrument settings (*e.g.,* gas flow rates).

3.5. Ultra-trace Cu isotopic analysis of serum and blood micro-samples

Cu isotope ratio measurements were carried out at ultra-trace level by applying the approach developed to micro-samples of commercially available serum and blood for validation purposes. Hence, the Cu isotope ratios were measured at the lowest suitable concentrations, *i.e.* at a concentration level of 20 μg L^{-1} Cu and Ga in wet plasma conditions and of 5 μg L^{-1} Cu and Ga in dry plasma conditions.

As the concentration in human or animal serum is often lower than that of the Seronorm reference material, about 100 μL of the blood and serum samples were digested and subjected to the full sample preparation procedure. The Cu recovery for these samples after isolation was 104 ± 6%. The Na/Cu and Mg/Cu concentration ratios in these purified Cu fractions were about 0.05 on average. The samples were appropriately diluted and spiked with Ga as internal standard. The measurements of these samples were carried out in a

Table 4
Cu isotopic composition, expressed as δ^{65}Cu (‰) relative to NIST SRM 976, of commercially available blood and serum samples, obtained *via* MC-ICP-MS using different measurement conditions. 2s indicates the external precision on n replicates (different digestion, isolation and measurements). The average internal precision (2s) and the effect of a procedural blank correction on δ^{65}Cu is also indicated.

	Measurement conditions							
digested volume	500 µL		500 µL		100 µL		100 µL	
plasma conditions	wet		wet		wet		dry	
internal standard	Ni		Ga		Ga		Ga	
Cu and Ni or Ga concentrations	200 µg L^{-1}		200 µg L^{-1}		20 µg L^{-1}		5 µg L^{-1}	
	δ^{65}Cu (‰) ± 2s	n	δ^{65}Cu (‰) ± 2s	n	δ^{65}Cu (‰) ± 2s	n	δ^{65}Cu (‰) ± 2s	n
Seronorm serum	−0.20 ± 0.12	3	−0.18 ± 0.13	3	–		−0.22 ± 0.18	3
Seronorm whole blood	0.17 ± 0.06	3	0.13 ± 0.10	3	–		0.12 ± 0.03	3
goat serum	–		−0.64 ± 0.00	3	−0.66 ± 0.08	3	−0.62 ± 0.09	3
sheep serum	−0.14 ± 0.12	3	−0.17 ± 0.02	3	–		−0.22 ± 0.07	3
horse serum	−0.23 ± 0.10	3	−0.25 ± 0.00	2	−0.23 ± 0.05	3	−0.18 ± 0.01	3
mouse serum	−0.36 ± 0.07	3	−0.36 ± 0.06	3	−0.32 ± 0.09	3	−0.26 ± 0.17	3
rabbit serum	−0.31 ± 0.09	3	−0.33 ± 0.07	3	−0.29 ± 0.08	3	−0.31 ± 0.08	3
internal precision (2s)	0.04		0.02		0.04		0.06	
Effect of blank correction	<0.01		<0.01		<0.04		<0.05	

Table 5
Isotopic composition of Cu, expressed as δ^{65}Cu (‰) relative to NIST SRM 976, obtained for the Seronorm serum reference material and for procedural blanks spiked with the in-house Cu isotopic standard. The isotope ratio measurements were carried out at Cu and Ga concentrations of 5 µg L^{-1} using dry plasma conditions. s indicates the external precision on n replicates (different digestion, isolation and measurement), while * indicates the dilution factor considering a volume of sample/blank solution of 500 µL containing 5 µg L^{-1} of Cu after the full prepration procedure.

Seronorm serum reference material					
Digested volume serum	Dilution factor*	δ^{65}Cu (‰) ± s			n
200 µL	~80	−0.11	±	0.07	3
100 µL	~40	−0.22	±	0.09	3
50 µL	~20	−0.24	±	0.10	2
20 µL	~8	−0.43	±	0.03	2
Procedural blanks spiked with in-house isotopic standard solution					
Digested volume blank (MQ H_2O)	Dilution factor*	δ^{65}Cu (‰) ± s			n
500 µL	~20	0.22	±	0.03	2
500 µL	~10	−0.05	±	0.06	2

SSB sequence with a procedural NIST SRM 976 isotopic reference material as external standard. The concentrations of Cu and Ga in the sample solution were adjusted to within ±10% of those of the bracketing NIST SRM 976 solution.

The results obtained are summarized in Table 4. The Cu isotope ratios obtained for the 100 µL samples were in good agreement with those obtained for the 500 µL samples. By using wet plasma conditions and Ga as an internal standard, a maximum difference of 0.04‰ was found between the δ^{65}Cu values obtained at 200 µg L^{-1} and at 20 µg L^{-1} of Cu. The average internal precision on the δ^{65}Cu values at a level of 20 µg L^{-1} Cu was deteriorated by a factor of 2 compared to those obtained at the 200 µg L^{-1} Cu level only. Moreover, this precision is still similar to that obtained at 200 µg L^{-1} level using Ni as internal standard (Table 4).

The δ^{65}Cu values obtained at a concentration level of 5 µg L^{-1} in dry plasma conditions were also in good agreement with those obtained at a concentration level of 200 µg L^{-1} in wet plasma conditions (paired *t*-test, $p > 0.05$). The maximum difference in δ^{65}Cu between both conditions was 0.10‰. The average internal precision on the δ^{65}Cu values at 5 µg L^{-1} Cu and Ga was 0.06‰ and, thus, deteriorated by a factor of 3 compared to that obtained at 200 µg L^{-1} Cu and Ga (Table 5). However, this internal precision was still similar to that obtained at a level of 200 µg L^{-1} Cu using the standard interface [5].

The external precision (2s) on the δ^{65}Cu values, obtained for 3 replicate (digestion, isolation and measurement of the samples) isotope ratio measurements at the 5 µg L^{-1} level was always < 0.18‰. An external precision of 0.20‰ was reported for a quality control serum sample (n = 3) for isotope ratio measurements carried out at a level of 200 µg L^{-1} Cu using the standard interface and wet plasma conditions [20]. Thus, the external precision on the Cu isotopic composition at ultra-trace level was similar to that obtained at the "standard" concentration level used for isotopic analysis by MC-ICP-MS.

Although the blank contribution on the Cu isotope composition was more pronounced when smaller amounts of Cu were processed, this difference was still within 2s (Table 4). The maximum bias observed between the results with and without blank correction was <0.05‰.

4. Conclusions

The minimum Cu concentration required for accurate and precise Cu isotope data was 20 µg L^{-1} using wet plasma conditions and 5 µg L^{-1} using dry plasma conditions. Well-defined mass bias relationships were obtained between ln(^{65}Cu/^{63}Cu) and ln(^{62}Ni/^{60}Ni) and between ln(^{65}Cu/^{63}Cu) and ln(^{71}Ga/^{69}Ga) for concentrations ≥ 20 µg L^{-1} Cu and ≥100 µg L^{-1} Ni or ≥ 20 µg L^{-1} Ga using wet plasma conditions. In dry plasma conditions, well-defined linear relationships were obtained for concentrations ≥5 µg L^{-1} Cu and ≥20 µg L^{-1} Ni or ≥5 µg L^{-1} Ga. The use of Ga for mass bias correction improved the internal precision on δ^{65}Cu values and decreased the blank contribution compared to Ni.

Ar-based polyatomic ions $^{36}Ar^{16}O^{1}_{2}H^{+}$ and $^{40}Ar^{15}N^{16}O^{+}$ were observed to affect the signals of $^{69}Ga^{+}$ and $^{71}Ga^{+}$, respectively, using wet plasma conditions. The use of a desolvation system eliminated these solvent-based interferences, but led to a significant interference from $^{40}Ar^{14}N^{1}_{2}H^{+}$, affecting the signal intensity for $^{69}Ga^{+}$ at low mass resolution. Use of medium mass resolution allowed interference-free measurement of the $^{69}Ga^{+}$ and $^{71}Ga^{+}$ signal intensities.

The δ^{65}Cu values of several commercially available blood and serum samples obtained at a concentration level of 5 µg L^{-1} Cu and 5 µg L^{-1} Ga using dry plasma conditions were in good agreement with those obtained at the "standard" concentration level of 200 µg L^{-1} Cu and 200 µg L^{-1} Ni using wet plasma conditions. In addition, the isotopic composition obtained for samples for which

only 100 μL were digested were in good agreement with those for which 500 μL were digested. Cu isotopic analysis was successful for human and animal serum/blood samples containing ≥50 ng of Cu. Thus, the developed approach is suitable for ultra-trace Cu isotopic analysis micro-sample of serum, but it can also be valuable for other sample types, including cell cultures, bacteria, nanoparticles, biopsied material, cerebrospinal fluid, bone marrow, snow samples, rain water and seawater.

Conflicts of interest

Declarations of interest: none.

Acknowledgements

This work was carried out in the context of the EMPIR research project 15HLT02, ReMiND. This project has received funding from the EMPIR programme co-financed by the Participating States and from the European Union's Horizon 2020 research and innovation programme. SL thanks the Agency for Innovation by Science and Technology in Flanders (IWT-Vlaanderen) for her PhD grant. MCR thanks FWO-Vlaanderen for her post-doctoral grant.

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