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Evaluation of electrothermal vaporization for sample introduction aiming at Cu isotopic analysis *via* multicollector-inductively coupled plasma mass spectrometry

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Abstract

A new method for Cu isotopic analysis was developed using a commercially available electrothermal vaporization (ETV) device coupled to multicollector-inductively coupled plasma mass spectrometry (MC-ICP-MS).

The method demonstrated potential for the isotopic analysis of microsamples (*e.g.,* 5 µL) in a biological context. For example, Cu isotopic analysis of NIST 3114 (diluted to 1 mg L^{-1} Cu) using self-bracketing provided average δ^{65}Cu values of 0.00 ± 0.17‰ (2SD, n=10) and internal precision values of 712 ppm. In order to achieve this level of accuracy and precision, it is critical to properly deal with the short transient signals generated by the ETV-MC-ICP-MS, which implies using point by point calculations and time lag detector correction (TDC), as well as a criterion to reject potential outliers.

The results of this technique were compared with the results obtained *via* femtosecond-laser ablation-MC-ICP-MS using the same pre-treated serum samples. No significant differences were observed among the results obtained in both cases, while external precision was 0.26‰ for ETV-MC-ICP-MS and 0.24‰ for fs-LA-MC-ICP-MS, expressed as median value of 2SD (n=27), further proving the usefulness of the approach proposed in this context, as the use of ETV results in a more straightforward approach.

Keywords

ETV-MC-ICP-MS

Copper isotopic analysis

Transient signals

1.Introduction

Electrothermal vaporization (ETV) is a technique that can be used for sample introduction and treatment prior to measurement *via* different approaches. Depending on the design, the sample (liquid or solid) can be deposited onto a platform, which is subsequently introduced into the electrothermal device (such as a graphite furnace), or it can be directly introduced through a sample dosing port. Inside this electrothermal device, heating causes the sample release into the gas phase, which finally arrives to the system of detection. ETV was coupled to inductively coupled plasma optical emission spectrometry (ICP-OES) in 1974 [1,2]. In 1983, the first work on ETV coupled to inductively coupled plasma mass spectrometry (ICP-MS) was published by Gray and Date [3]. This coupling showed promising potential advantages, such as a high sample transport efficiency and thus low requirements in terms of sample volume/mass, the possibility to analyze solid and complex liquid matrixes directly, and even the possibility to separate the analyte from the matrix in some occasions, leading to a reduction of interferences (spectral and non-spectral) [4–9]. Due to these interesting features, ETV-ICP-MS became popular for many decades resulting in more than 416 publications up to the present date [10]. However, the lack of commercial support, the success of other sampling techniques (*e.g.,* laser ablation) and the level of experience required to achieve the full potential of the technique (which required both expertise in graphite furnace and in ICP-MS) have made this technique to be seldom used in the literature during the last decade (88 publications out of 416) [11,12].

Generally, ETV-ICP-MS can be used for monoelemental or multielemental analysis in different areas such as industrial, environmental, clinical and biological, food and beverages,

among others [9,13]. Also, it has been occasionally used for speciation studies [14–16]. Finally, isotopic ratio monitoring has been explored, but to a much lesser extent.

One of the reasons for this could be that the typical precision for elemental analysis obtained with an ETV-ICP-MS is around 3 - 5% RSD, and that is for liquid samples, while 1 - 2% values for isotopic dilution has been reported [17]. It has to be remembered that the ETV system provides very short transient signals from small sample volumes, which are not considered as ideal for precise isotopic ratio analysis, especially when coupled to sequential mass analyzers. Still, this level of precision could be enough for isotope dilution (ID) calibration [17,18] and, in some particular occasions, for clinical or archaeological applications [19–21], as long as the expected differences between the ratios are high enough.

Naturally, in order to deal with transient signals and improve the precision for isotopic ratios, ETV could be coupled to instrumentation with more potential for simultaneous monitoring, such as TOF-MS [22] or, for best precision in isotope analysis, multicollector (MC)-ICP-MS. However, the latter coupling has only been reported twice in the literature.

The first article was published in 2007 by Rowland *et al*. [23]. In that work, ETV-MC-ICP-MS is used for separating the signals originating from ^{87}Sr and ^{87}Rb in time, thus making it possible to measure Sr isotopic ratios directly without any sample preparation (e.g., chromatographic separation). This approach was used for Rb-Sr dating; the potassium feldspar reference material NIST SRM 607 was used for method validation. The precision reported was around 0.3% RSD on $^{87}Sr/^{86}Sr$ ratio, which is much better than the typical precision obtained with ETV-ICP-MS with a quadrupole analyzer, as stated before. However, this precision is still too high for the majority of geo-applications. It has to be mentioned that today, direct analysis

of Sr ratios can be performed by means of laser ablation with a precision between 0.02 – 0.05% RSD in wet plasma and ICP-MS/MS.[24,25].

The second work was published in 2014 by Okabayashi *et al.*[26] in which a micro-ETV (based on the use of a Re filament) coupled to MC-ICP-MS was evaluated for W isotopic analysis, introducing only a few nanograms of sample. The $^{182}W/^{183}W$ ratio was determined in 3 different iron meteorite samples, which had been previously analyzed by a desolvating nebulizer system [27]. The precision reported was around 0.005% expressed as 2RSD.

Nowadays, the interest in microvolume and/or microsample analysis is growing because, in many cases, the amount of sample available is limited and/or the sample is very valuable. These problems are very common in the field of clinical analysis where sometimes it is only possible to obtain a minimal amount of sample, such as studies with newborns or model rats, among other situations where just some microliters of sample can be obtained. To deal with these situations, ETV can still be useful as a sample introduction device, and a deeper insight into the possibilities of this technique for isotope ratio determination would be valuable.

In this regard, both aforementioned works were performed with home-made ETV devices. While developing simple ETV devices is certainly interesting, it makes it more difficult to replicate the methods developed in other labs. In this work, a method for Cu isotopic analysis in biological samples by ETV-MC-ICP-MS has been developed using a commercially available ETV vaporizer, with the aim to explore the potential of this technique to facilitate analysis of microvolumes under conditions easily replicable for laboratories. This application was chosen as Cu isotopic analysis has been reported to be of interest to help in the diagnosis and follow-up of some disorders (e.g., Wilson disease [28–30]), where analysis of

microvolumes is very interesting. Overall, isotopic analysis *via* MC-ICP-MS in a biomedical context has become a new topic of relevance during the last decade, in a field previously dominated by geo-applications [31–35].

To validate the method the NIST SRM 3114 reference material was used. Additionally, analysis of several serum samples was carried out and results were compared to those obtained in a previous work [30] for the same samples but using fs-LA-MC-ICP-MS, since this technique also enables analysis of microvolumes.

2. Experimental

2.1 Instrumentation

Cu total determination was carried out with an ELAN DRC II quadrupole ICP-MS (Perkin Elmer, Waltham, USA) using time resolved analysis (TRA), as described elsewhere [30].

Cu isotopic analysis in serum samples was carried out with a high-resolution multicollector inductively coupled plasma mass spectrometer Nu Plasma 1700 (Nu Instruments, Wrexham, UK) coupled to the electrothermal vaporization system (ETV), ETV - 4000c (Spectral Systems, Fürstenfeldbruck, Germany). Pyrolytic graphite furnace and platforms were acquired from the latter company.

For sample digestion and sample evaporation, an ULTRAWAVE microwave system (Milestone Inc., Shelton, USA) and an EVAPOCLEAN® unit (Analab, Bischheim, France) were used, respectively.

2.2 Standards and reagents

For isotopic analysis, the NIST SRM 3114 (NIST, Gaithersburg, MD, USA) was deployed as a reference in the bracketing sequence. It shows a similar composition to the NIST SRM 976, which is out of stock [36]. It consists of an acidified aqueous solution (approximately 1.6 mol/L of HNO_3 in volume) prepared gravimetrically to contain a known mass fraction of copper.

A Pd monoelemental standard solution of 1000 mg L^{-1} (SCP SCIENCE, Villebon-sur-Yvette, France) was used as chemical modifier.

Instra grade HNO_3 70% was purchased from JT Baker (Phillipsburg, USA) and further purified by sub-boiling in a PFA system (DST 1000, Savillex, Eden Prairie, USA). HCl 35% ultratrace® was acquired from Scharlab (Barcelona, Spain). Ultrapure water (resistivity ≥ 18.2 MΩ cm) was obtained from a Direct-Q3 system (Millipore, Mollsheim, France).

For Cu isolation, a Cu specific resin (Triskem, Bruz, France) was used.

2.3 Samples and sample preparation

To evaluate the Cu isotopic analysis using the ETV system, we analyzed the same samples that had been analyzed in our group by fs-LA-MC-ICP-MS [30]. The serum samples were obtained from the Hospital Universitario Miguel Servet (Zaragoza, Spain) and from the Centre Hospitalier Universitaire d'Angers (France). The principles outlined in the declaration of Helsinki regarding all the experimental research involving humans or animals were followed.

In short, such samples were subjected to sample digestion with concentrated HNO_3 in a microwave oven, evaporation to almost dryness, redissolution in concentrated HCl, and chromatographic separation with Triskem Cu specific resins. The Cu fraction was finally evaporated and redissolved in 50 µL of 2% v v^{-1} HNO_3 in order to achieve the maximum preconcentration factor available working under these conditions, because in some cases only a small amount of sample was available and/or the Cu concentration was low. All the details on this procedure and also on the subsequent analysis of the samples for total Cu using ICP-MS and for isotopic analysis using fs-LA-MC-ICP-MS are provided elsewhere [30]. Briefly, for total Cu determination, 1 µL of the pretreated samples was directly injected into the peristaltic pump tubing of the ICP-MS using a micropipette.

For Cu isotopic analysis, and in order to avoid mass bias due to the variation of concentration between samples and standards (bracketing correction), Cu concentration was adjusted in the fractions used for analysis to 0.3, 0.5, 1 and 4 mg L^{-1} using 2% HNO_3 v v^{-1} for that purpose. Dilution factors depended on the original sample Cu concentration (ranging between 0.043 and 1.578 mg L^{-1}) which after preconcentration, varied between 0.3 and 9.7 mg L^{-1}.

2.4 Measurement protocol

Isotopic analysis was carried out with an ETV-MC-ICP-MS coupling. The optimization of the system was performed in liquid mode with pneumatic nebulization using a 200 µg L^{-1} Cu and Ni solution. For that purpose, the nebulizer and the cyclonic chamber were coupled to one of the entrances of a 2-inlet torch connected to the MC-ICP-MS, while the ETV was coupled to the other via a 10 cm long PTFE tube (6 od x 4 id mm). The ETV gas (Ar) was maintained at 0.55 L min^{-1} during the whole procedure (optimization and measurements). Considering that

samples were pretreated for Cu isolation, the mass spectrometer was operated in low resolution, in order to maximize sensitivity. Although this instrument houses 5 electron multipliers in addition to 16 Faraday cups, the signal was recorded on Faraday cups only in order to favor signal stability and isotopic measurement precision. The MC-ICP-MS was then optimized to achieve maximum signal intensity and stability. Conditions used for the isotopic analysis are shown in **Table 1.** For analysis of the samples 5 µL of sample plus 10 µL of 1000 mg L^{-1} Pd were deposited onto the platform, which was subsequently manually introduced into the graphite furnace with tongs. The ETV temperature program is also included in **Table 1.** Under these conditions, the signal peak duration was around 15 seconds with a pseudo gaussian profile. Five measurements were carried out per sample following the standard-sample-standard bracketing sequence using the NIST SRM 3114 as standard. Isotope ratio calculations were performed using the point by point method (PBP) [37]. This method is based on the calculation of the isotope ratio as the average of the ratios calculated for each point acquired (every 0.5 s, which was a parameter used in a previous work devoted to fs-LA introduction of the samples, also producing transient signals, and eventually enabling a fair comparison of results [30]) during a certain time interval.

Please notice that the measurements were carried out in continuous mode. Therefore, the time axis does not start at time zero. Regardless, every peak was processed individually considering the stable part of the isotope ratio.

When recording transient signal, isotope ratios measured by MC-ICP-MS generally shows a drift during signal acquisition attributed to the different time response of the Faraday pre-amplifiers [38]. As short transient signal were recorded in this study, a systematic correction of time lags between Faraday cups (TDC) was performed following the method described by

Claverie *et al.*[39], which is based on the synchronization of the two isotope signals obtained for ^{65}Cu and ^{63}Cu following the criterion that the coefficient of determination of the linear regression for the two isotopes would be as close as possible to 1. Finally, the results were expressed as delta values (δ) using the NIST SRM 3114 as standard, following the equation (1):

$$\delta(‰) = \frac{R_{sample} - R_{STD}}{R_{STD}} \times 1000 \quad (1)$$

where R_{sample} is the $^{65}Cu/^{63}Cu$ isotope ratio obtained for the sample and R_{STD} is the average $^{65}Cu/^{63}Cu$ isotope ratio determined for the NIST 3114 measured before and after that particular sample.

The TDC correction is described in detail elsewhere [39]. In short, the determination of the time lag was performed iteratively using a VBA macro. The isotopes signals were first interpolated (N=1000) and then shifted one over the other by one increment until the optimal value for the criterion was obtained. Once the optimal time lag was obtained, averages of data points were calculated, in order to obtain the exact same numbers of data points per second than before the interpolation.

3. Results

3.1 Optimization of the temperature program

The optimized temperature program used for analysis is included in **Table 1**. Optimization of this program does not follow exactly the same premises as for quantitative analysis with ETV-ICP-MS. In this case, three main factors were considered: (i) to avoid pre-vaporization of

Cu during drying and pyrolysis steps that could cause Cu isotopic fractionation, (ii) to achieve a signal profile providing the best stability for the isotopic ratios ($^{65}Cu/^{63}Cu$) and (iii) to minimize blanks and memory effects between analyses.

In our case, the pyrolysis step is not very critical in terms of matrix removal, as the samples were pre-treated for Cu isolation. Therefore, the pyrolysis temperature was selected in order to avoid a sudden change of temperature in the plasma, which might affect signal stability and the final precision for the ratios. The temperature chosen was 350 °C, a temperature roughly in between the drying and the vaporization temperatures.

For vaporization, different temperatures were tested. Use of high temperatures (such as 2500 °C) was discarded because at this temperature blanks were high while below 900°C the blanks were almost zero. This is probably due to recondensation of particles on colder parts of the system and revaporization when heating back at high temperatures, an issue that seems to be typical of this ETV device according to the authors' experience. Moreover, it was observed that Cu starts to vaporize around 500 °C, however, the signals were not reproducible at these low temperatures. In order to find the best vaporization temperature providing reproducible signals, different modifiers (Pd and Pt) and different vaporization temperatures between 400 °C and 900 °C were tested. Pd was chosen as modifier as it provided higher intensity and better reproducibility. As for the temperature, it was observed that between 600 °C and 700 °C the signals were more reproducible, and the peaks obtained showed a unimodal profile (see **Figure 1**). Since the profiles of both Cu isotopes followed the same behavior, in the next figures only ^{63}Cu signal and the ratio will be shown for the sake of simplicity. The vaporization temperature finally chosen was 600 °C, as it generally offered a higher stability for the isotope ratios, as shown in **Figure 1**. It can be observed in **Figure 1A**

that, at a vaporization temperature of 600 °C, the ratios obtained during the duration of the signal (corresponding to 5 µL of aqueous standard) are rather stable. There is a small increase in the ratios along the transient signal but this is mostly due to the Faraday detector time lag [38–40], as will be discussed in the next section. At 700 °C, on the other hand, **Figure 1B**, the ratios varied much more, and the profile is not constant among replicates. This behavior cannot be attributed to time lags, and it may obey to variations in the vaporization process.

3.2 Data treatment

ETV sample introduction system provides transient signals that, if working conditions are well optimized, show a unimodal profile. This kind of signal is, in principle, optimal for applying the Linear Regression Slope (LRS) method for the isotope ratio calculation. The LRS method was proposed by Fietzke *et al.* [41,42], and it is based on the representation of the signal achieved for one isotope (^{65}Cu) *versus* the signal for the other (^{63}Cu), adjusting the data to a linear regression, where the slope corresponds to the ratio. This method takes into account the whole signal but, as in any linear regression model, the points with higher signal intensity ultimately show a higher influence in the calculated slope than those with lower signal intensities. During the experiments, it was possible to observe that sometimes, not very often, the ratio profile discussed in section 3.1 at 600 °C (**Figure 1A**), was altered and resembled the profile obtained at 700 °C (**Figure 1B**). This may reflect problems in the device used to always keep the desired temperature, which would not be surprising, among other reasons including the deterioration of consumables, namely the graphite parts. This effect is shown in **Figure 2**, where it can be seen that the deviation from the typical ratio profile

(**Figure 2A**) was not always the same, in some cases the drop was slight (**Figure 2B**) while for others it was far more pronounced (**Figure 2C**).

When performing the calculation with the LRS method, the precision obtained for the isotope ratios was poor. With this method, the drop in the ratio profile significantly affected the results even when it is very slight, because such deviation is produced in the area close to the maximum of the peak, and as discussed before, the points with higher intensities eventually influence more the slope value.

Thus, the point by point method (PBP)[37] was tested instead. For this method, setting of the data processing limits is an important parameter to optimize. As seen from **Figure 2,** the data processing limits (between red bars) was set considering only the most stable part of the signal, which includes most of the peak, excluding only the initial and final parts, where ratios are not reliable.

Results for the analyses showed that the influence of the variations in the isotope ratio are lower working with the PBP method than with the LRS method. However, if the drop is very pronounced, then the influence becomes significant. Thus, it is necessary to set a value for rejection. A practical criterion was developed for this purpose. This criterion was applied for rejection based on the difference in the isotope ratio between the negative peak apex (x_2) and the last point before the drop (*i.e.,* the left limit of the negative peak, x_1), as shown in **Figure 3**. Experimentally (using NIST SRM 3114), it was observed that if the difference between x_1 and x_2 (see **Figure 3**) was higher than 0.003, the ratio obtained was significantly lower than the typically expected ratio. Therefore, if the difference in the $^{65}Cu/^{63}Cu$ isotope ratio was lower than 0.003 the measurement was considered for further calculations with the PBP approach and time lag detector correction (TDC), but if the difference was higher

than 0.003 (isotopic ratio drops substantially), then the whole measurement was rejected. It must be stated that this is a criterion developed for this particular application and set-up, and it should not be directly applied to other situations without further studies. The overall percentage of signals rejected was between 5 and 10% depending on the day.

A quick look at **Figure 4A** and previous figures shows that the ratios trend up during the measurement. One explanation for that could be thermal fractionation during the vaporization process. This is a topic that, to the best of the authors' knowledge, has not been investigated before. However, this trend may also be an artifact due to time lag between detectors, which is a problem that has been detected before [38–40]. To verify the actual cause of this trend, TDC was systematically applied prior PBP isotope ratio calculation. **Figure 4** shows an example of the results before and after TDC correction. As can be observed in **Figure 4A**, before the TDC the ratios are going up along the peak, while after TDC this effect is practically eliminated (see **Figure 4B).** In this case in particular, the internal precision improves by a factor of 6 after TDC. That clearly indicates that this trend upwards in the ratio was produced by the time lag and not by any possible fractionation occurring during the vaporization of the analyte because, if this trend were due to the thermal fractionation, the TDC correction would not be able to correct it. This improvement in internal precision is not constant but varies for each peak: the precision improves by a factor between 1.5 and 7.0.

3.3 Precision and accuracy of the method

For precision and accuracy study of the method developed, Cu isotopic analysis of NIST SRM 3114 was carried out at different Cu concentration levels.

For analysis of NIST SRM 3114, 10 replicates of each concentration were measured, and the results were expressed as delta values applying equation (1) (*cf.,* section 2.3.) and using self-bracketing, which means that NIST SRM 3114 was treated as standard and as sample, except for the first and the last measurements that were only used as standards. Since the NIST 3114 is both the sample and the standard, the delta value expected would be 0. **Table 2** shows the results obtained. External precision for the delta values is expressed as two times the standard deviation (2SD) in ‰. Internal precision was calculated as the SD of the ratios obtained point by point (one ratio each 0.5 s), and it is expressed as 2SD in ppm.

As it is possible to appreciate in the **Table 2,** the results obtained show internal precision values within 685 ppm and 860 ppm and good accuracy, with δ^{65}Cu values very close to zero. These internal precision values are rather high, but this is not unusual for highly transient signals. It should be reminded that external precision values are the ones that provide a representative estimation of the uncertainty among replicates. These values ranged between 0.14 and 0.24‰. Also note that both external and internal precision seem to slightly improve with the Cu concentration likely as a results of better counting statics. However, statistically significant differences were not found at the 95% confidence level (*via* F-tests).

Next, real samples were analyzed with the ETV-MC-ICP-MS. Five replicates per sample were measured following the sequence standard-sample-standard bracketing and the results were expressed as delta value using the equation (1). These results were compared with the results obtained *via* fs-LA-MC-ICP-MS, which are published in ref. [30]. That is an alternative method in which 1 µL samples are deposited and dried in silicon wafers, in which micro-wells were previously produced. Results obtained for both analyses are graphed in **Figure 5.** The

correlation equation was obtained using the software Origin Pro2019b and it is y = (0.89194 ± 0.21196) x + (0.09455 ± 0.11821), which indicates that significant difference between both methods cannot be established at the 95% confidence level. The conclusions obtained with ETV-MC-ICP-MS in terms of the interpretation of the results would be analogous to those reported in ref. [30], so they will not be duplicated here. It will just be mentioned that the ratios obtained depend on the medical condition (Wilson disease or other disorders vs controls) and on the treatment. In short, delta values biased low can be attributed to the release of Cu release previously accumulated in the liver, which can be the result of medical treatment with a chelating agent after the diagnosis of WD disease, or else the result of an advanced stage of liver damage (cirrhosis due to WD or other diseases).

In terms of external precision, the median value for real samples *via* fs-LA-MC-ICP-MS would be 0.24‰ (range between 0.10 and 0.36‰), and 0.26‰ (range between 0.09 and 0.66‰) *via* ETV-MC-ICP-MS.

Overall, ETV seems like a viable alternative for analysis in which only sample microvolumes are finally available, which is not unusual in biomedical contexts.

Conclusions

This work examines the coupling of a commercially available ETV device to a MC-ICP-MS to monitor Cu isotope ratios for the first time. The results obtained demonstrate that this sample introduction strategy can provide information of similar quality in comparison with other more complex MC-ICP-MS approaches also relying on micro-analysis, which is furthermore of sufficient precision and accuracy to be of biomedical significance.

One of the advantages of this approach is the possibility to deploy commercially available ETV instrumentation, but also to develop cheap and simple home-made devices (as shown in reference [26]), thus providing new ways to obtain isotopic information from microsamples.

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Declarations of interest

The authors declare no conflict of interest.

References

[1] D.E. Nixon, V.A. Fassel, R.N. Kniseley, Inductively coupled plasma-optical emission analytical spectroscopy. Tantalum filament vaporization of microliter samples, Anal. Chem. 46 (1974) 210–213. https://doi.org/10.1021/ac60338a018.

[2] A. M. Gunn, D. L. Millard, G. F. Kirkbright, Optical Emission Spectrometry with an Inductively Coupled Radiofrequency Argon Plasma Source and Sample Introduction with a Graphite Rod Electrothermal Vaporisation Device, Analyst. 103 (1978) 1066–1073. https://doi.org/10.1039/AN9780301066.

[3] A. L. Gray, A. R. Date, Inductively Coupled Plasma Source Mass Spectrometry Using Continuum Flow Ion Extraction, Analyst. 108 (1983) 1033–1050. https://doi.org/10.1039/AN9830801033.

[4] E. Björn, W. Frech, E. Hoffmann, C. Lüdke, Investigation and quantification of spectroscopic interferences from polyatomic species in inductively coupled plasma mass spectrometry using electrothermal vaporization or pneumatic nebulization for sample introduction, Spectrochim. Acta Part B At. Spectrosc. 53 (1998) 1765–1776. https://doi.org/10.1016/S0584-8547(98)00215-8.

[5] R.E. Sturgeon, J.W. Lam, The ETV as a thermochemical reactor for ICP-MS sample introduction, J. Anal. At. Spectrom. 14 (1999) 785–791. https://doi.org/10.1039/A809460H.

[6] J.M. Carey, E.H. Evans, J.A. Caruso, W.-L. Shen, Evaluation of a modified commercial graphite furnace for reduction of isobaric interferences in argon inductively coupled plasma mass spectrometry, Spectrochim. Acta Part B At. Spectrosc. 46 (1991) 1711–1721. https://doi.org/10.1016/0584-8547(91)80175-3.

[7] F. Vanhaecke, S. Boonen, L. Moens, R. Dams, Solid sampling electrothermal vaporization inductively coupled plasma mass spectrometry for the determination of arsenic in standard reference materials of plant origin, J. Anal. At. Spectrom. 10 (1995) 81–87. https://doi.org/10.1039/JA9951000081.

[8] K. Grünke, H.-J. Stärk, R. Wennrich, H.M. Ortner, J.A.C. Broekaert, An investigation of different modifiers in electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS), Fresenius J. Anal. Chem. 359 (1997) 465–468. https://doi.org/doi.org/10.1007/s002160050613.
[9] M. Resano, F. Vanhaecke, M.T.C. de Loos-Vollebregt, Electrothermal vaporization for sample introduction in atomic absorption, atomic emission and plasma mass spectrometry—a critical review with focus on solid sampling and slurry analysis, J. Anal. At. Spectrom. 23 (2008) 1450–1475. https://doi.org/10.1039/b807756h.
[10] Web of Science. https://apps.webofknowledge.com. (Last accessed April 2021)
[11] J.S. Silva, A.S. Henn, V.L. Dressler, P.A. Mello, E.M.M. Flores, Feasibility of Rare Earth Element Determination in Low Concentration in Crude Oil: Direct Sampling Electrothermal Vaporization-Inductively Coupled Plasma Mass Spectrometry, Anal. Chem. 90 (2018) 7064–7071. https://doi.org/10.1021/acs.analchem.8b01460.
[12] J. Patočka, T. Černohorský, A. Krejčová, S. Slang, Carbon microparticles as a physical carrier for ETV-ICP-MS, Talanta. 204 (2019) 555–560. https://doi.org/10.1016/j.talanta.2019.06.045.
[13] M. Aramendía, M. Resano, F. Vanhaecke, Electrothermal vaporization–inductively coupled plasma-mass spectrometry: A versatile tool for tackling challenging samples, Anal. Chim. Acta. 648 (2009) 23–44. https://doi.org/10.1016/j.aca.2009.06.027.
[14] I. Gelaude, R. Dams, M. Resano, F. Vanhaecke, L. Moens, Direct Determination of Methylmercury and Inorganic Mercury in Biological Materials by Solid Sampling-Electrothermal Vaporization-Inductively Coupled Plasma-Isotope Dilution-Mass Spectrometry, Anal. Chem. 74 (2002) 3833–3842. https://doi.org/10.1021/ac020060i.
[15] H. Kataoka, Y. Okamoto, S. Tsukahara, T. Fujiwara, K. Ito, Separate vaporisation of boric acid and inorganic boron from tungsten sample cuvette–tungsten boat furnace followed by the detection of boron species by inductively coupled plasma mass spectrometry and atomic emission spectrometry (ICP-MS and ICP-AES), Anal. Chim. Acta. 610 (2008) 179–185. https://doi.org/10.1016/j.aca.2008.01.043.
[16] L. Xia, B. Hu, Z. Jiang, Y. Wu, R. Chen, L. Li, Hollow fiber liquid phase microextraction combined with electrothermal vaporization ICP-MS for the speciation of inorganic selenium in natural waters, J. Anal. At. Spectrom. 21 (2006) 362–365. https://doi.org/doi.org/10.1039/B514623B.
[17] M. Resano, M. Aramendía, F. Vanhaecke, Solid sampling-electrothermal vaporization-inductively coupled plasma mass spectrometry for the direct determination of traces of boron in biological materials using isotope dilution for calibration, J. Anal.At. Spectrom. 21 (2006) 1036–1044. https://doi.org/10.1039/B606118D.
[18] L.F. Dias, G.R. Miranda, T.D. Saint'Pierre, S.M. Maia, V.L.A. Frescura, A.J. Curtius, Method development for the determination of cadmium, copper, lead, selenium and thallium in sediments by slurry sampling electrothermal vaporization inductively coupled plasma mass spectrometry and isotopic dilution calibration, Spectrochim. Acta Part B At. Spectrosc. 60 (2005) 117–124. https://doi.org/10.1016/j.sab.2004.11.005.
[19] D.C. Grégoire, J. Lee, Determination of cadmium and zinc isotope ratios in sheep's blood and organ tissue by electrothermal vaporization inductively coupled plasma mass spectrometry. Invited lecture, J. Anal. At. Spectrom. 9 (1994) 393–397. https://doi.org/10.1039/JA9940900393.
[20] D.C. Gregoire, Sample introduction techniques for the determination of osmium isotope ratios by inductively coupled plasma mass spectrometry, Anal. Chem. 62 (1990) 141–146. https://doi.org/10.1021/ac00201a011.
[21] C.G. Lee, K. Iguchi, K. Esaka, M. Magara, F. Esaka, S. Sakurai, K. Watanabe, S. Usuda, Feasibility study of solid sampling electrothermal vaporization inductively coupled plasma mass spectrometry for the determination of particulate uranium in swipe samples from nuclear facilities, Anal. Chim. Acta. 517 (2004) 215–220. https://doi.org/10.1016/j.aca.2004.04.041.

[22] P.P. Mahoney, S.J. Ray, G. Li, G.M. Hieftje, Preliminary Investigation of Electrothermal Vaporization Sample Introduction for Inductively Coupled Plasma Time-of-Flight Mass Spectrometry, Anal. Chem. 71 (1999) 1378–1383. https://doi.org/10.1021/ac9811625.
[23] A. Rowland, T.B. Housh, J.A. Holcombe, Use of electrothermal vaporization for volatility-based separation of Rb–Sr isobars for determination of isotopic ratios by ICP-MS, J. Anal. At. Spectrom. 23 (2008) 167–172. https://doi.org/10.1039/B709089G.
[24] E. Bolea-Fernandez, S.J.M. Van Malderen, L. Balcaen, M. Resano, F. Vanhaecke, Laser ablation-tandem ICP-mass spectrometry (LA-ICP-MS/MS) for direct Sr isotopic analysis of solid samples with high Rb/Sr ratios, J. Anal. At. Spectrom. 31 (2016) 464–472. https://doi.org/10.1039/C5JA00404G.
[25] D. Bevan, C.D. Coath, J. Lewis, J. Schwieters, N. Lloyd, G. Craig, H. Wehrs, T. Elliott, In situ Rb–Sr dating by collision cell, multicollection inductively-coupled plasma mass-spectrometry with pre-cell mass-filter,(CC-MC-ICPMS/MS), J. Anal. At. Spectrom. 36 (2021) 917–931. https://doi.org/10.1039/D1JA00006C.
[26] S. Okabayashi, S. Sakata, T. Hirata, Isotopic Analysis of tungsten using multiple collector-inductively coupled plasma-mass spectrometer coupled with electrothermal vaporization technique, Anal. Chim. Acta. 853 (2015) 469–476. https://doi.org/10.1016/j.aca.2014.11.002.
[27] A. Markowski, G. Quitte, A. Halliday, T. Kleine, Tungsten isotopic compositions of iron meteorites: Chronological constraints vs. cosmogenic effects, Earth Planet. Sci. Lett. 242 (2006) 1–15. https://doi.org/10.1016/j.epsl.2005.11.048.
[28] M. Aramendía, L. Rello, M. Resano, F. Vanhaecke, Isotopic analysis of Cu in serum samples for diagnosis of Wilson's disease: a pilot study, J. Anal. At. Spectrom. 28 (2013) 675–681. https://doi.org/10.1039/C3JA30349G.
[29] M. Resano, M. Aramendía, L. Rello, M.L. Calvo, S. Bérail, C. Pécheyran, Direct determination of Cu isotope ratios in dried urine spots by means of fs-LA-MC-ICPMS. Potential to diagnose Wilson's disease, J. Anal. At. Spectrom. 28 (2013) 98–106. https://doi.org/10.1039/C2JA30262D.
[30] M.C. García-Poyo, S. Bérail, A.L. Ronzani, L. Rello, E. García-González, B. Lelièvre, P. Cales, F.V. Nakadi, M. Aramendía, M. Resano, C. Pécheyran, Laser ablation of microdroplets for copper isotopic analysis *via* MC-ICP-MS. Analysis of serum microsamples for the diagnosis and follow-up treatment of Wilson's disease, J. Anal. At. Spectrom. 36 (2021) 968–980. https://doi.org/10.1039/D0JA00494D.
[31] M. Costas-Rodríguez, J. Delanghe, F. Vanhaecke, High-precision isotopic analysis of essential mineral elements in biomedicine: natural isotope ratio variations as potential diagnostic and/or prognostic markers, TrAC Trends Anal. Chem. 76 (2016) 182–193. https://doi.org/10.1016/j.trac.2015.10.008.
[32] F. Vanhaecke, P. Degryse, Isotopic Analysis: Fundamentals and Applications Using ICP-MS, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2012.
[33] T. Walczyk, F. von Blanckenburg, Natural Iron Isotope Variations, Science. 295 (2002) 2065–2066. https://doi.org/10.1126/science.1069389.
[34] F. Larner, M. Rehkämper, B.J. Coles, K. Kreissig, D.J. Weiss, B. Sampson, C. Unsworth, S. Strekopytov, A new separation procedure for Cu prior to stable isotope analysis by MC-ICP-MS, J. Anal. At. Spectrom. 26 (2011) 1627. https://doi.org/10.1039/c1ja10067j.
[35] F. Albarede, P. Télouk, V. Balter, V.P. Bondanese, E. Albalat, P. Oger, P. Bonaventura, P. Miossec, T. Fujii, Medical applications of Cu, Zn, and S isotope effects, Metallomics. 8 (2016) 1056–1070. https://doi.org/10.1039/C5MT00316D.
[36] Q. Hou, L. Zhou, S. Gao, T. Zhang, L. Feng, L. Yang, Use of Ga for mass bias correction for the accurate determination of copper isotope ratio in the NIST SRM 3114 Cu standard and geological samples by MC-ICPMS, J. Anal. At. Spectrom. 31 (2016) 280–287. https://doi.org/10.1039/C4JA00488D.
[37] V.N. Epov, P. Rodriguez-Gonzalez, J.E. Sonke, E. Tessier, D. Amouroux, L.M. Bourgoin, O.F.X. Donard, Simultaneous Determination of Species-Specific Isotopic Composition of Hg by Gas

Chromatography Coupled to Multicollector ICPMS, Anal. Chem. 80 (2008) 3530–3538. https://doi.org/10.1021/ac800384b.
[38] A. Gourgiotis, S. Bérail, P. Louvat, H. Isnard, J. Moureau, A. Nonell, G. Manhès, J.-L. Birck, J. Gaillardet, C. Pécheyran, F. Chartier, O.F.X. Donard, Method for isotope ratio drift correction by internal amplifier signal synchronization in MC-ICPMS transient signals, J. Anal. At. Spectrom. 29 (2014) 1607–1617. https://doi.org/10.1039/C4JA00118D.
[39] F. Claverie, A. Hubert, S. Berail, A. Donard, F. Pointurier, C. Pécheyran, Improving Precision and Accuracy of Isotope Ratios from Short Transient Laser Ablation-Multicollector-Inductively Coupled Plasma Mass Spectrometry Signals: Application to Micrometer-Size Uranium Particles, Anal. Chem. 88 (2016) 4375–4382. https://doi.org/10.1021/acs.analchem.5b04802.
[40] T. Hirata, Y. Hayano, T. Ohno, Improvements in precision of isotopic ratio measurements using laser ablation-multiple collector-ICP-mass spectrometry: reduction of changes in measured isotopic ratios, J. Anal. At. Spectrom. 18 (2003) 1283–1288. https://doi.org/10.1039/B305127G.
[41] J. Fietzke, V. Liebetrau, D. Günther, K. Gürs, K. Hametner, K. Zumholz, T.H. Hansteen, A. Eisenhauer, An alternative data acquisition and evaluation strategy for improved isotope ratio precision using LA-MC-ICP-MS applied to stable and radiogenic strontium isotopes in carbonates, J. Anal. At. Spectrom. 23 (2008) 955–961. https://doi.org/10.1039/b717706b.
[42] J. Fietzke, M. Frische, T.H. Hansteen, A. Eisenhauer, A simplified procedure for the determination of stable chlorine isotope ratios (δ37Cl) using LA-MC-ICP-MS, J. Anal. At. Spectrom. 23 (2008) 769–772. https://doi.org/10.1039/b718597a.

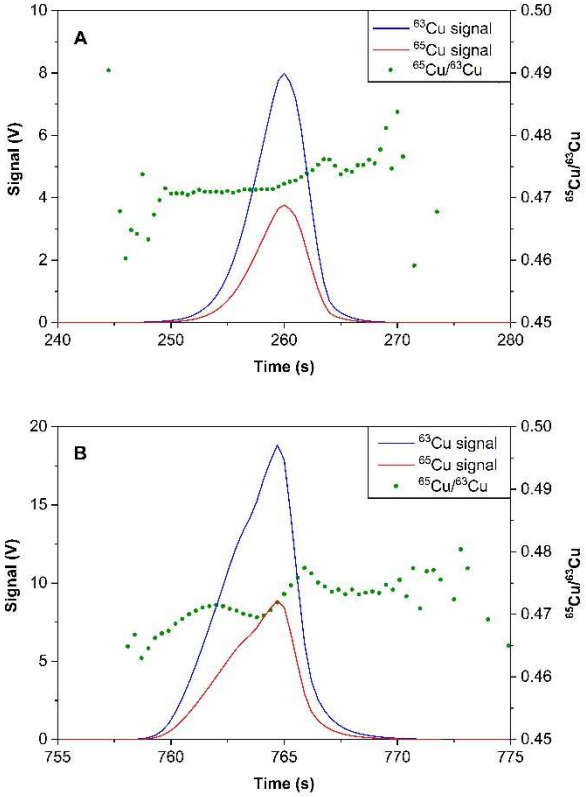
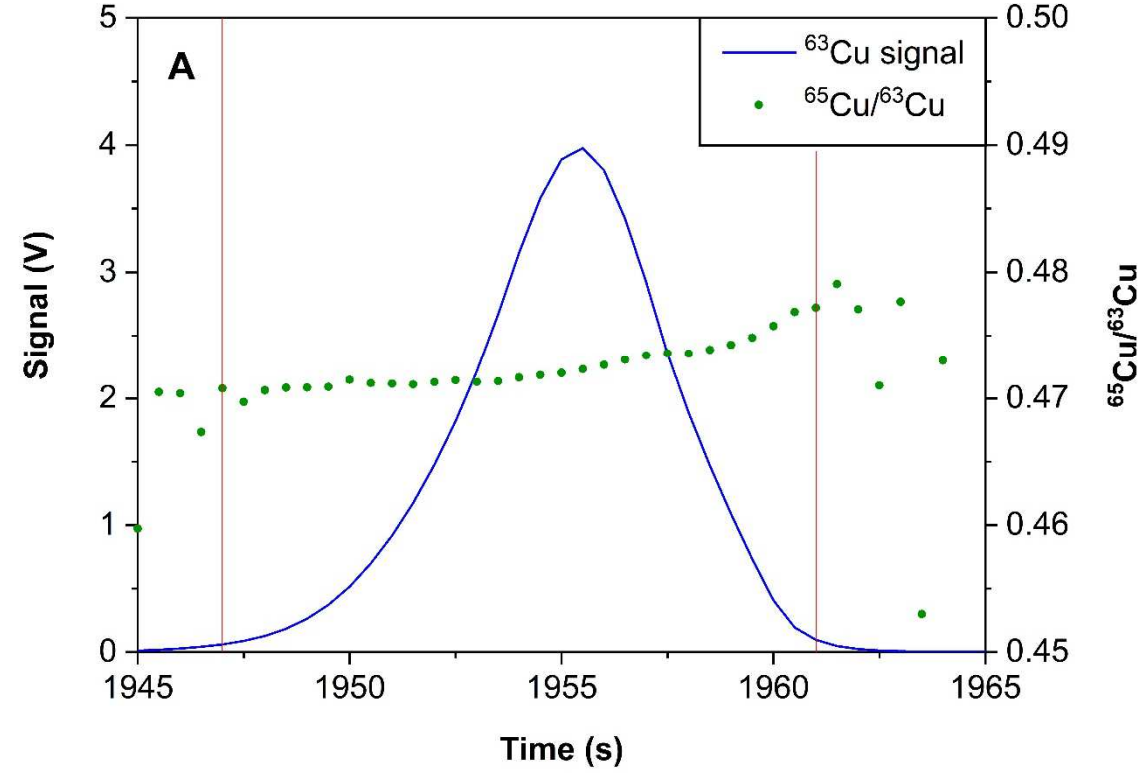
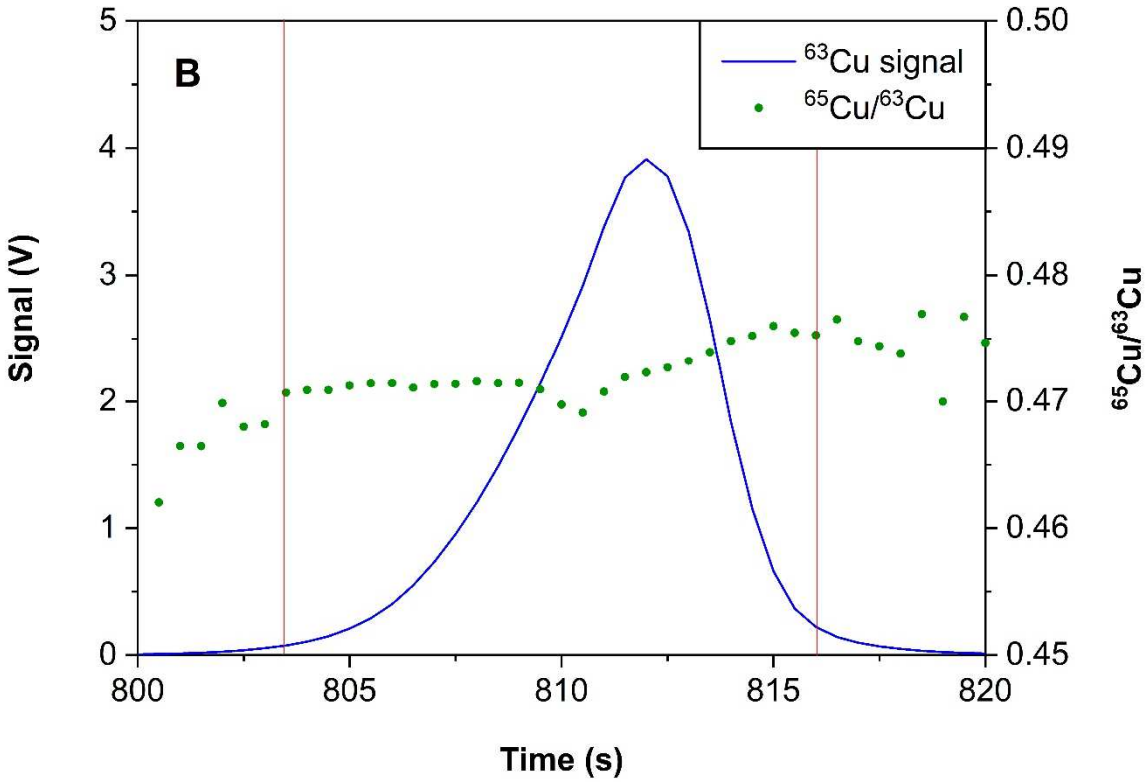


Figure 1. Example of transient signals of both ^{65}Cu (red) and ^{63}Cu (blue) isotopes obtained using 5 µL of a Cu standard of 1 mg L^{-1} (5 ng of Cu) and 10 µL of a Pd standard of 1 g L^{-1} carried out with a temperature program with $T_{pyrolisis}$ = 350 °C and A) $T_{vaporization}$ = 600 °C and B) $T_{vaporization}$ = 700 °C. No TDC has been applied at this stage.


A
63Cu signal
65Cu/63Cu
Signal (V)
65Cu/63Cu
Time (s)
0
1
2
3
4
5
0.45
0.46
0.47
0.48
0.49
0.50
1945
1950
1955
1960
1965


B
63Cu signal
65Cu/63Cu
Signal (V)
65Cu/63Cu
Time (s)
0
1
2
3
4
5
0.45
0.46
0.47
0.48
0.49
0.50
800
805
810
815
820

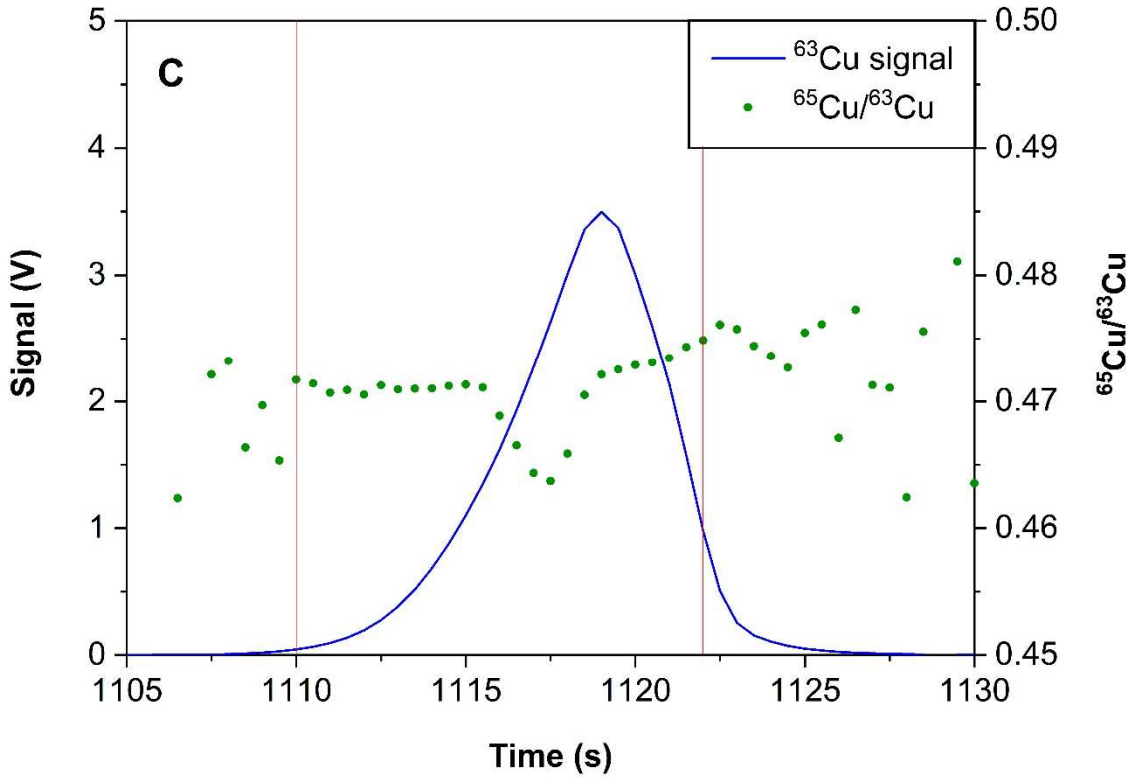


Figure 2. Examples of transient signals with their respective representations of the individual ratios calculated for each point (1 point every 0.5s) for 5 µL of a solution of 0.5 mg L^{-1} of Cu prepared with the NIST SRM 3114 + 10 µL of 1 g L^{-1} of Pd. In all cases, the conditions used were the same (Tpyrolysis = 350 °C and Tvaporization = 600 °C). No TDC has been applied at this stage. (A) Typical ratio profile; (B) ratio profile altered but with a slight drop; and (C) ratio profile altered with a more pronounced drop. The portion of the signal using for calculation isrepresented between the red bars.

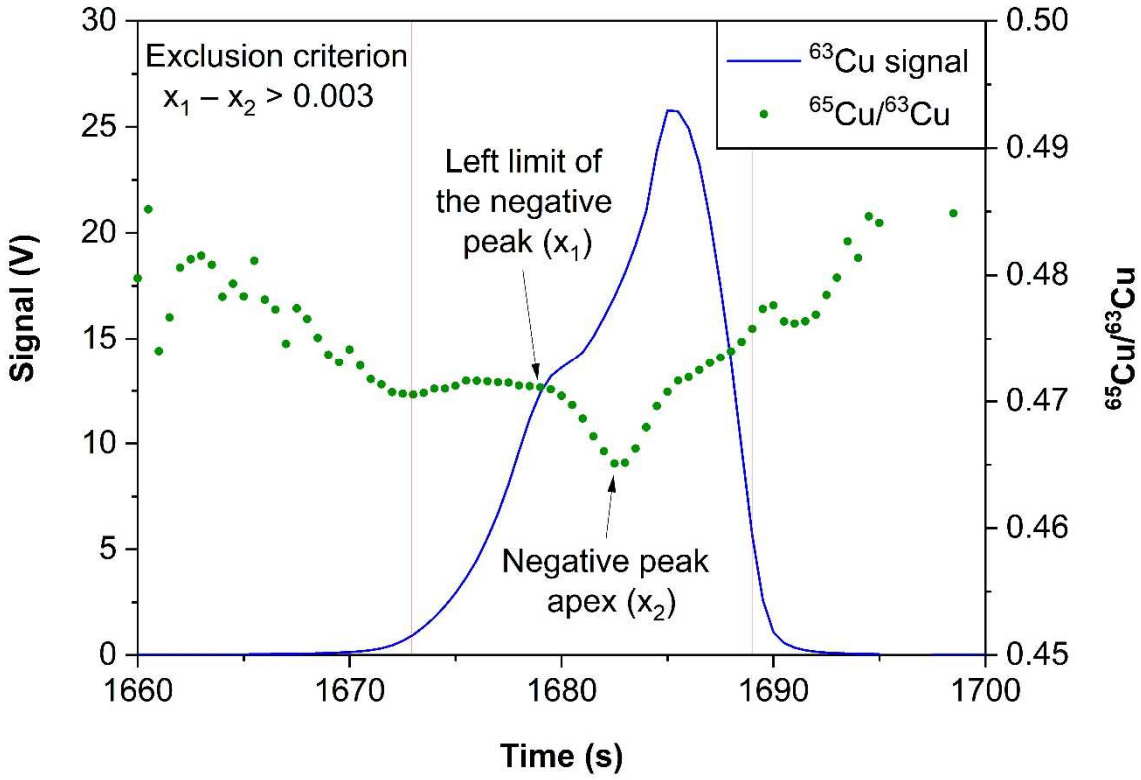


Figure 3. Example of application of the exclusion criterion.No TDC has been applied at this stage.

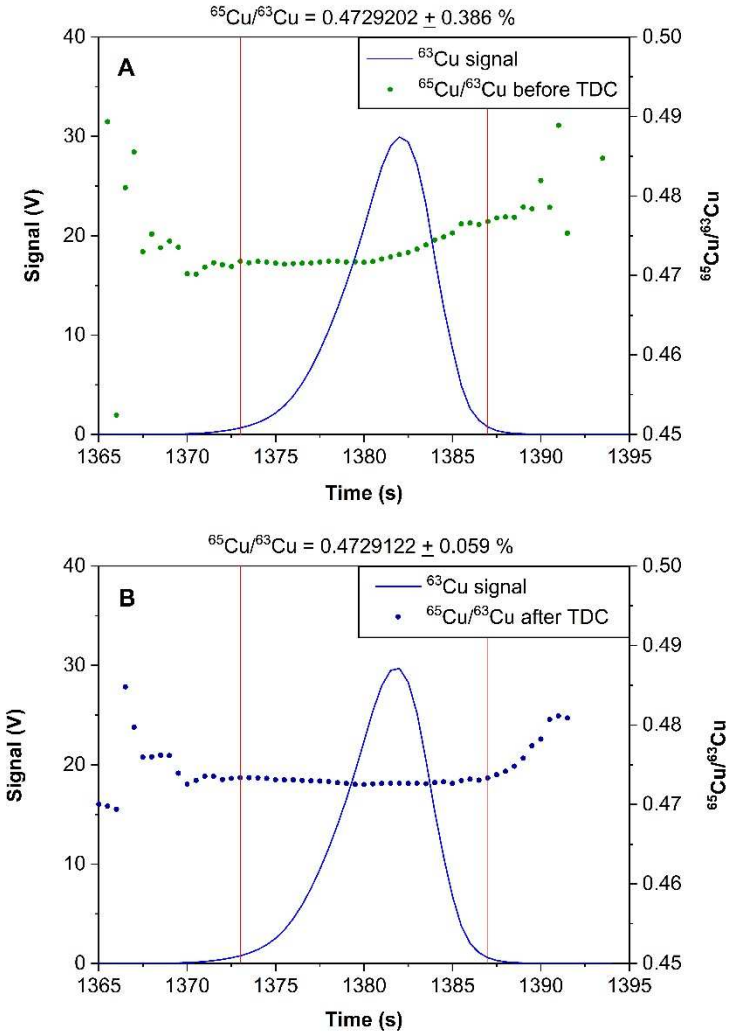


Figure 4. Example of transient signal obtained for the introduction with an ETV of 5 µL of a 4 mg L^{-1} (20 ng of Cu) Cu standard solution A) before time drift correction and B) after time drift correction. The signal profile is represented by the blue points, while the red bars represent the data processing limits. Green points (A) and navy-blue points (B) represent the individual ratios calculated point by point (1 point every 0.5 s) before and after time drift correction (TDC), respectively.

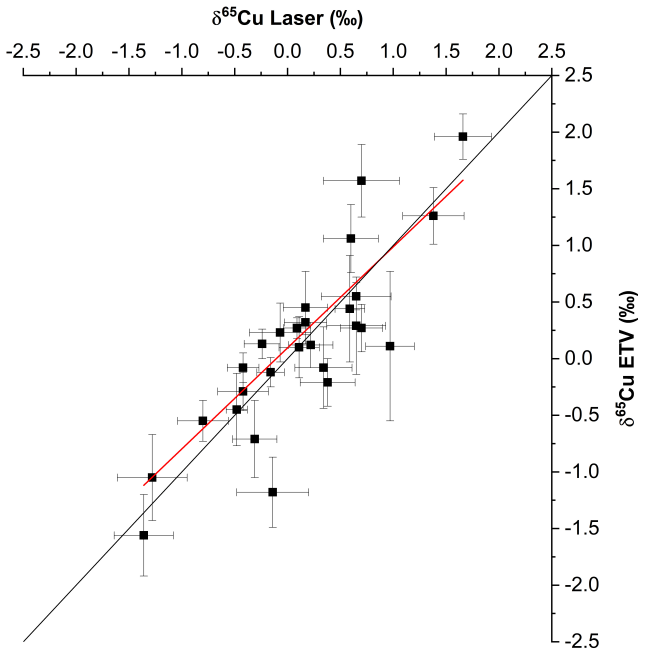


Figure 5. Correlation analysis for the δ^{65}Cu (‰) values obtained for the same samples analyzed by the ETV-MC-ICP-MS and *via* fs-laser-MC-ICP-MS. Error bars represent external precision as 2SD (n=5). The black line represents a perfect agreement (slope=1), while the red line represents the actual regression.

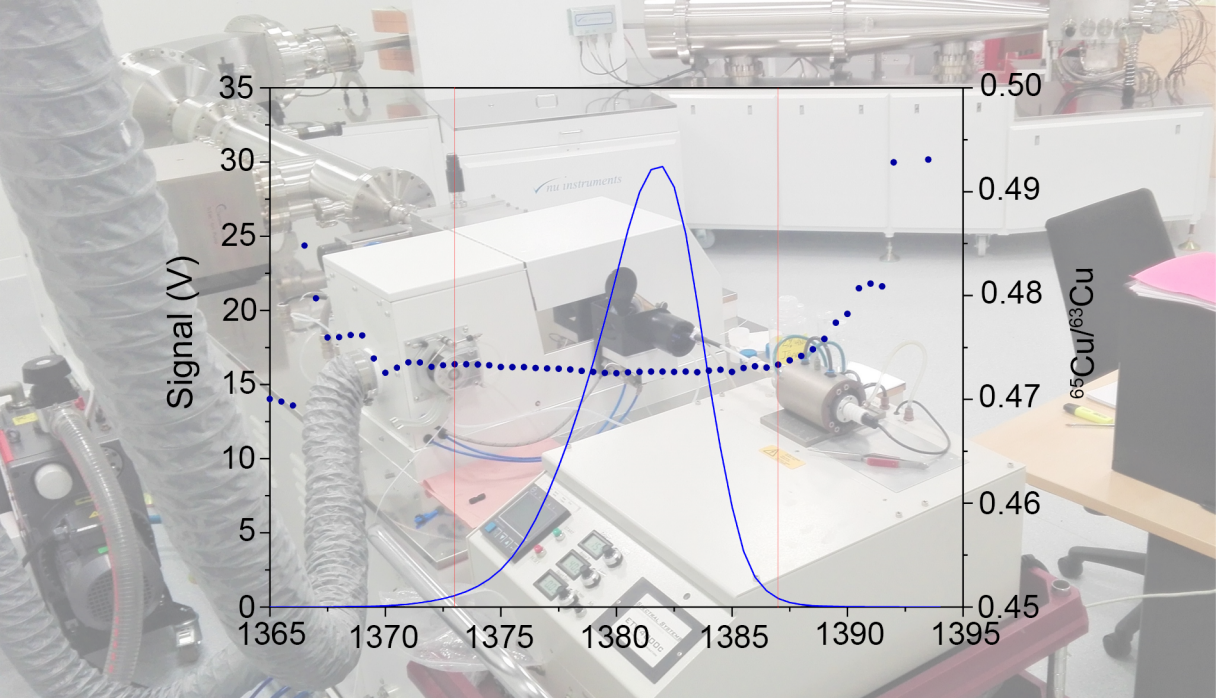
Table 1. ETV temperature program and instrumental conditions for Cu isotopic analysis of serum using ETV-MC-ICP-MS.

ETV temperature program																				
Temperature program	Temperature (°C)							Ramp (°C s^{-1})						Hold time (s)						
Drying step 1	80							2.7						30						
Drying step 2	110							1						30						
Pyrolysis step	350							24						30						
Vaporization step	600							12.5						20						
Cleaning step	2600							2000						4						
Nu MC-ICP-MS 1700																				
RF Power (W)											1300									
Instrument resolution											Low									
Acquisition Mode											Time Resolved Acquisition (TRA)									
Integration time (s)											0.5									
Nebulizer Pressure (Psi)											8.20 - 9.8[a]									
Auxiliary gas (L min^{-1})											1.10									
Plasma gas (L min^{-1})											13.00									
Faraday cup configuration																				
Collector	H9	H8	H7	H6	H5	H4	H3	H2	H1	Ax	L1	L2	L3	IC0	IC1	IC2	L4	IC3	L5	IC4	L6
m/z		67		66			65			64			63						61		60
a: Optimized daily																				

Table 2. Results of the analysis of the NIST SRM 3114 at different concentrations.

Volume (µL)	[Cu] (µg L^{-1})	Mass (as Cu in ng)	^{63}Cu Signal* (V.s)	^{65}Cu/^{63}Cu internal precision as 2SD (ppm)	δ^{65}Cu "self bracketing", ± 2SD (‰), (n=10)
5	300	1.5	11	808	0.03 ± 0.24
5	500	2.5	16	860	-0.05 ± 0.20
5	1000	5	47	712	0.00 ± 0.17
5	4000	20	156	685	-0.02 ± 0.14

*The average signal for each concentration level was calculated as the area below the signal considering between the data processing limits set for the calculation of the isotopic ratio.


Signal (V)
35
30
25
20
15
10
5
0
1365
1370
1375
1380
1385
1390
1395
0.50
0.49
0.48
0.47
0.46
0.45
$^{65}Cu/^{63}Cu$