Electronic Supplementary Material (ESI) for Journal of Analytical Atomic Spectrometry.


# SUPPLEMENTARY INFORMATION

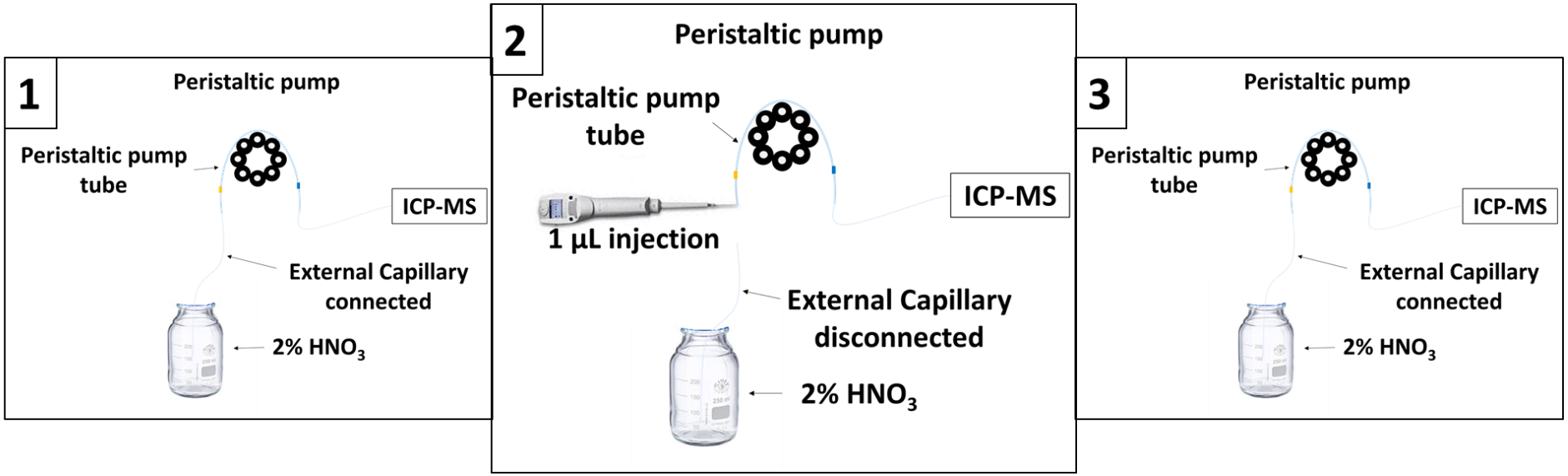



**Figure S1:** Schematic of the 1 µl injection process

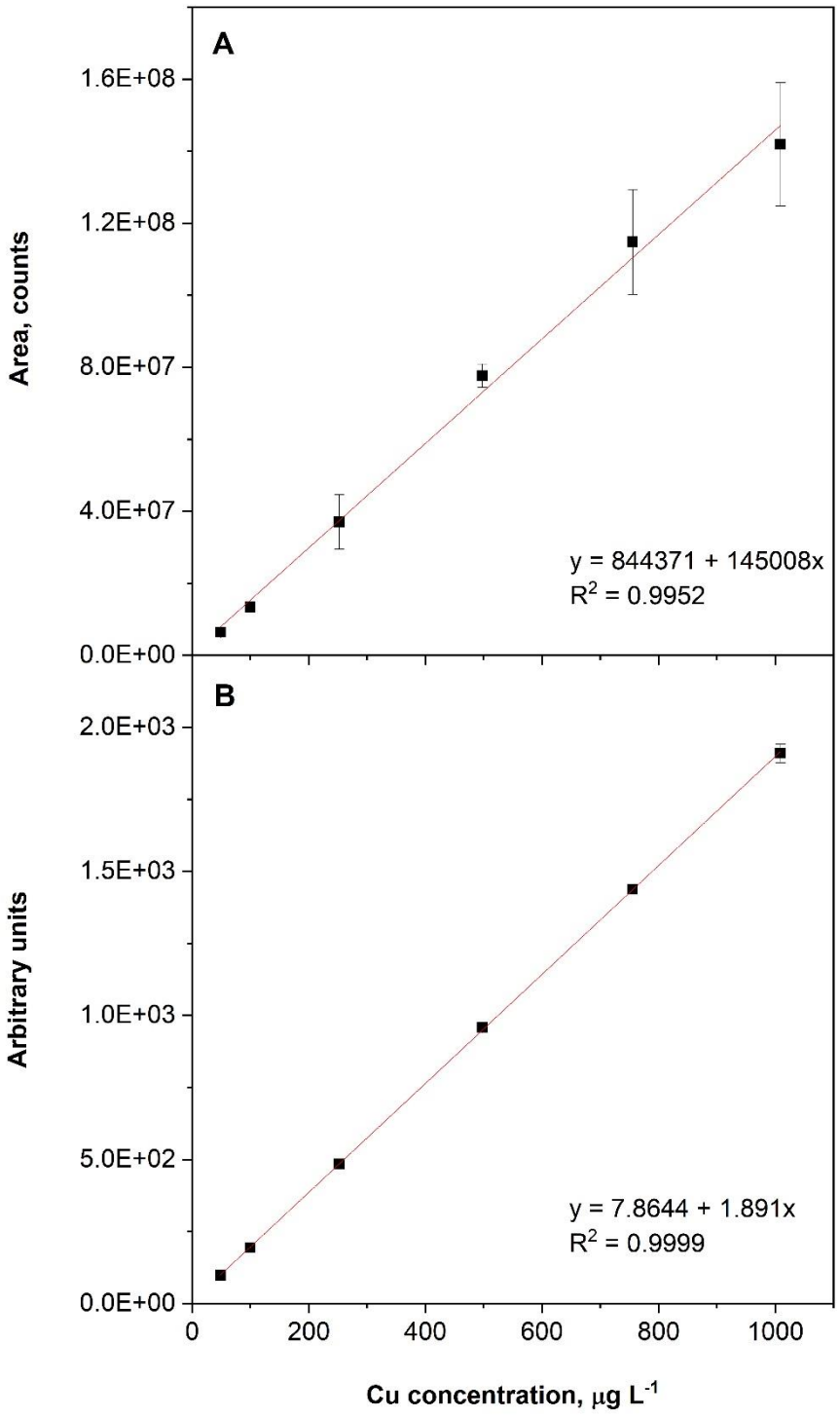
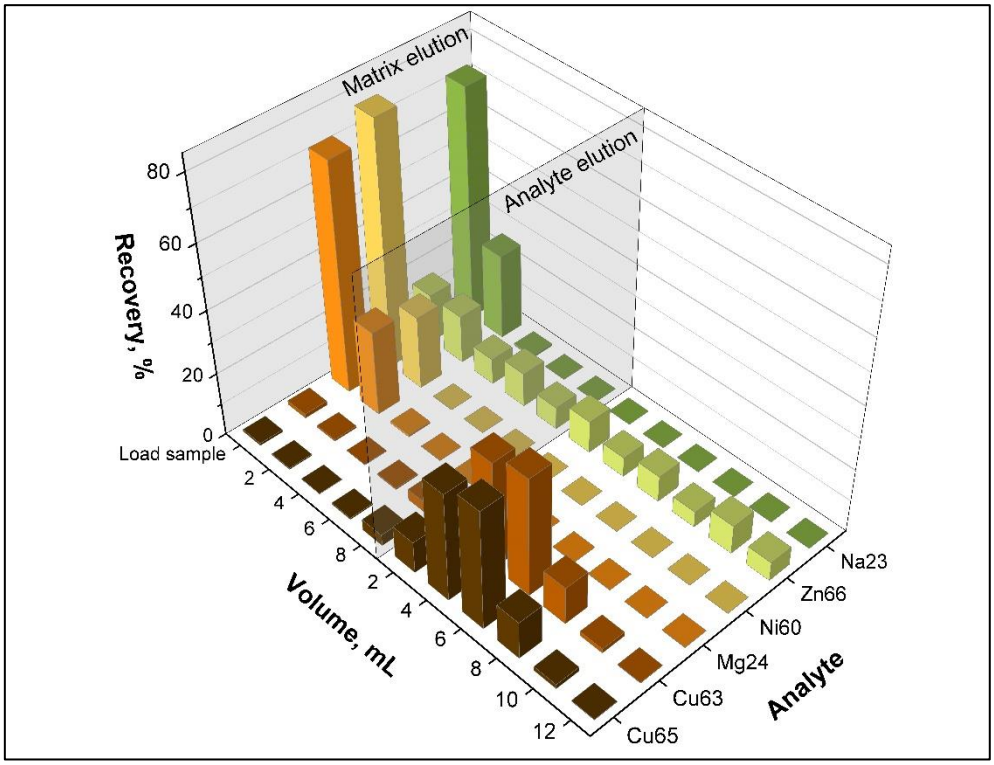



**Figure S2.** Cu calibration curves using the analytical protocol described in S1 A) without IS correction and B) with correction of Ni as IS.

### 3.1.1. Cu isolation

In order to compare the efficiency of the two resins tested for Cu isolation (AG-MP1 and Triskem), elution profiles were determined for the Clincheck whole blood L-3 reference material and are shown in **Figure S3** (supplementary information). Elements included in the elution profiles comprised the analyte (Cu), matrix elements originating potential interferences (Mg and Na) and elements usually deployed as internal standard for Cu isotopic analysis with MC-ICP-MS (Ni and Zn)[31,40]. The elution profiles show a much broader elution peak for Cu when using AG-MP1 resin (7 mL) compared to the Triskem (1 mL). In both cases Ni, Na and Mg are eluted in the early matrix elution step (first 4 ml), although with higher efficiency for the Triskem resin. In fact, one of the clear advantages of the Cu-specific resin approach is that only one separation step seems to be required to minimize the amount of Na remaining in the eluate, while with the AG-MP1 resin, two consecutive separation steps are needed for this purpose.[41,42] On the other hand, while Zn is also eluted in the matrix elution step with the Triskem resin, it is continuously eluted with the AG-MP1.



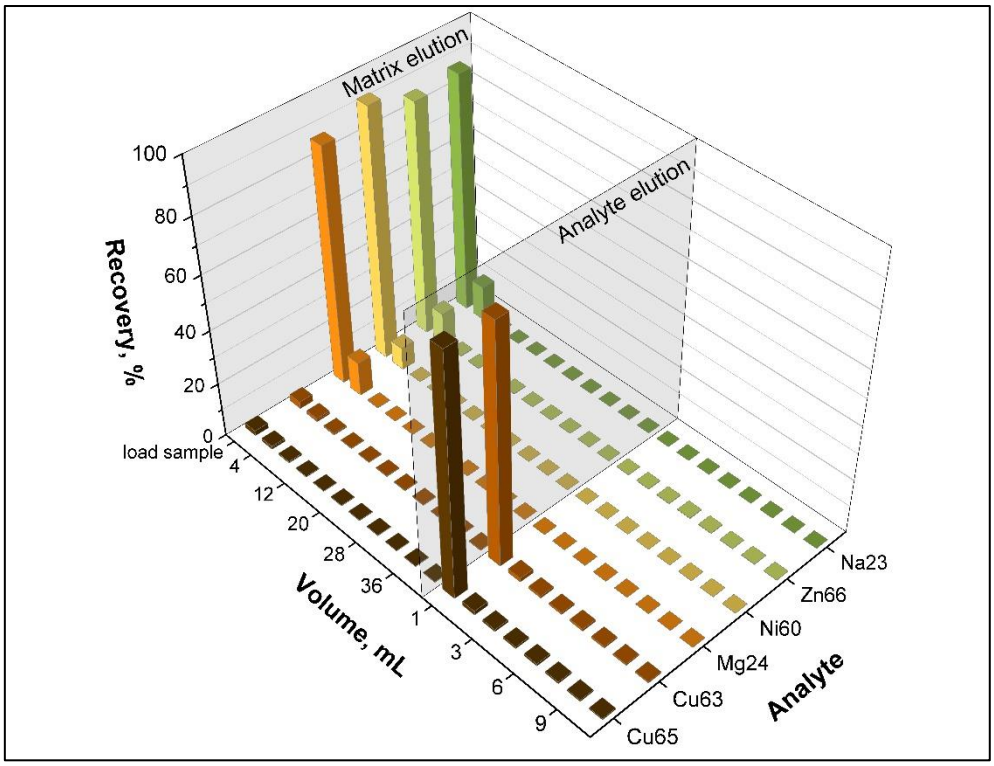



**Figure S3.** A) Elution profile for selected elements using AG-MP1 resin (Bio-Rad). B) Elution profile for selected elements using Cu-specific resin (Triskem). The Clincheck whole blood reference material L-3 (as described in section 2.3) was measured.

Cu isolation efficiency was then studied, with special attention to Na removal, and for three different reference materials: whole blood (Clinchek whole blood L-1), trace elements in tomato leaves (NIST 1573a) and trace elements in spinach leaves (NIST 1570a). Results on Cu recovery and Na elimination are shown in **Figure S4**. As seen from **Figure S4A** the percentage of Cu recovery is always higher with the Cu specific resin (for which only one separation procedure was performed) than with the AG-MP-1 resin using two consecutive separation procedures, reaching values close to 100%. Regarding the percentage of Na removal shown in **FigureS4B**, both protocols show a very good percentage of Na removal, always higher than 99.9 %. However, values obtained for the Cu specific resin are consistently better than those obtained with the AG-MP1 resin and with only one separation step needed, which is indeed a significant improvement.

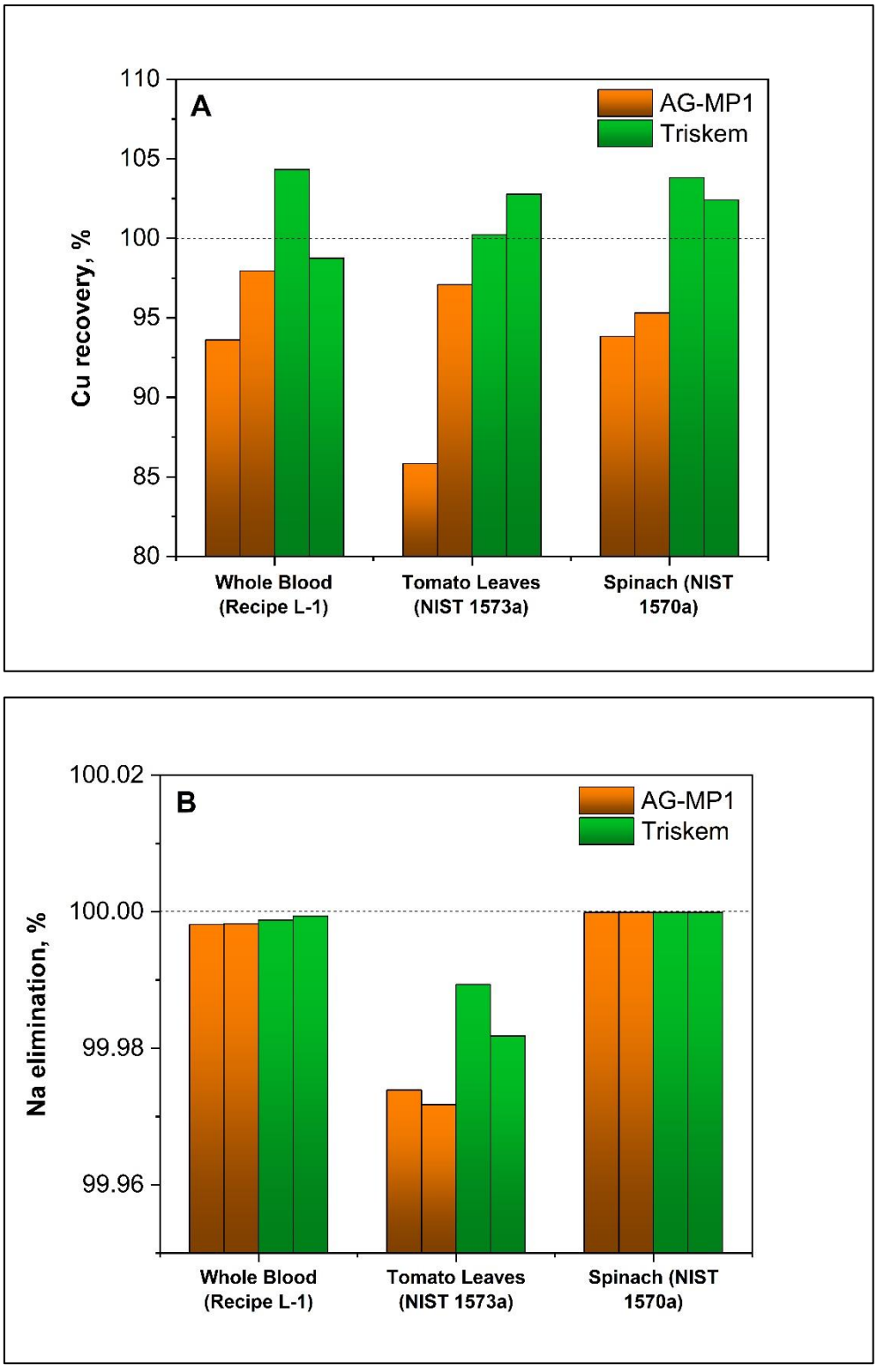



**Figure S4.** A) Cu recovery in 3 different reference materials after 2 consecutive separations using AG-MP1 (orange bars) and only one separation step employing the Cu-specific resin (Triskem) (green bars). B) Na removal in 3 different reference materials after 2 consecutive separations using AG-MP1 (orange bars) and only one separation step employing the Cu-specific resin (Triskem) (green bars). Two replicates were carried out per sample.

**Table S1**. Concentration of Cu, Na and Mg and percentage of Cu recovery and percentage of Na and Mg removal obtained after Cu separation step using Cu-specific resin (Triskem). Results are expressed as $\bar{x}$ ± U, where U= (t s)/√n; for a 95% confidence interval (n=5).

| | **Concentration after separation (µg $L^{-1}$)** | **Certified/ reference concentration (µg $L^{-1}$)** | | | |
|---|---|---|---|---|---|
| **$^{65}$Cu** | 738 ± 19 | 738 ± 110 | **%Recovery** | **$^{65}$Cu** | 100 ± 3 |
| **$^{63}$Cu** | 736 ± 25 | 738 ± 110 | | **$^{63}$Cu** | 100 ± 3 |
| **$^{23}$Na** | 14 ± 15 | 2438000* | **%Removal** | **$^{23}$Na** | 99.999 ± 0.001 |
| **$^{24}$Mg** | 14 ± 14 | 15900 ± 1600 | | **$^{24}$Mg** | 99.91 ±0.09 |

*The Na concentration determination was carried out with a DXC700 AU BECKMAN COULTER using the selective electrode technique (Na-Ag/AgCl) in the Hospital Miguel Servet.

### 3.3.1 Optimization of sample preparation for LA analysis

First, deposition of 1 µL of sample onto the Si wafer and drying at room temperature was tested. The diameter of the droplet at the moment of the deposition was around 3 mm, but it decreased down to approx. 0.3 mm until it was completely dry. The shape of the dried droplets was also highly variable. Ablation of these droplets would provide intense but very short transient signals with which it would not be possible to calculate the isotopic ratio in a stable and precise manner.

To avoid excessive droplet shrinking during drying, preheating of the Si wafer at 80 °C before deposition of the 1 µL droplet was tested next. With this strategy, the drying process was very fast, so that the diameter of the droplet (about 3 mm) did not vary significantly until it was completely dry. With this larger droplet area, longer signals could be obtained, although surface Cu concentration was not high enough to have good sensitivity with the samples of low concentration. Moreover, droplets often moved along the Si wafer surface before drying, so that mixing with other droplets. Due to this effect, it was also difficult to locate the position of the dried droplet with the laser camera.

For these reasons, before sample deposition, circular mini-wells of 1.8 mm diameter were engraved on the Si wafers, following the same ablation strategy used for the samples.

## 3.5 Analysis of real samples

| | Cu concentration (µg $L^{-1}$) | $\delta^{65}$Cu Raw (‰) sample | 2SD | |
|---|---|---|---|---|
| **ANGERS** | 163 | -1.28 | 0.33 | WD-C |
| | 153 | 0.38 | 0.26 | WD-C |
| | 45 | -0.8 | 0.24 | WD-C |
| | 999 | -1.49 | 0.37 | WD-C |
| | 932 | -2.29 | 0.3 | WD-C |
| | 186 | -0.31 | 0.21 | WD-C |
| | 51 | -0.48 | 0.1 | WD-C |
| | 1578 | -0.42 | 0.24 | HP |
| | 1077 | 0.09 | 0.13 | HP |
| | 734 | 0.01 | 0.29 | HP |
| | 989 | -0.24 | 0.17 | HP |
| | 1005 | -0.42 | 0.15 | HP |
| **MIGUEL SERVET** | 113 | 0.6 | 0.26 | WD |
| | 151 | 0.65 | 0.33 | WD-Zn |
| | 211 | 0.22 | 0.21 | WD-Zn |
| | 52 | 1.66 | 0.27 | WD-Zn |
| | 60 | -1.36 | 0.28 | WD-C + Zn |
| | 58 | 0.7 | 0.2 | WD-Zn |
| | 51 | 0.97 | 0.23 | WD-Zn |
| | 75 | -0.14 | 0.34 | WD-Zn |
| | 541 | -0.16 | 0.13 | NB |
| | 544 | 0.7 | 0.36 | NB |
| | 43 | 0.59 | 0.14 | NB |
| | 626 | 1.38 | 0.29 | NB |
| | 134 | 0.17 | 0.21 | NB |
| | 418 | 0.11 | 0.19 | NB |
| | 195 | 0.34 | 0.27 | NB |
| | 401 | -0.07 | 0.29 | NB |
| | 185 | 0.65 | 0.28 | NB |
| | 1021 | 0.17 | 0.2 | NB |
| | 195 | -0.28 | 0.22 | NB |