

## Supplemental Information

### LC-ICP-MS Method for the Determination of “Extractable Copper” in Serum

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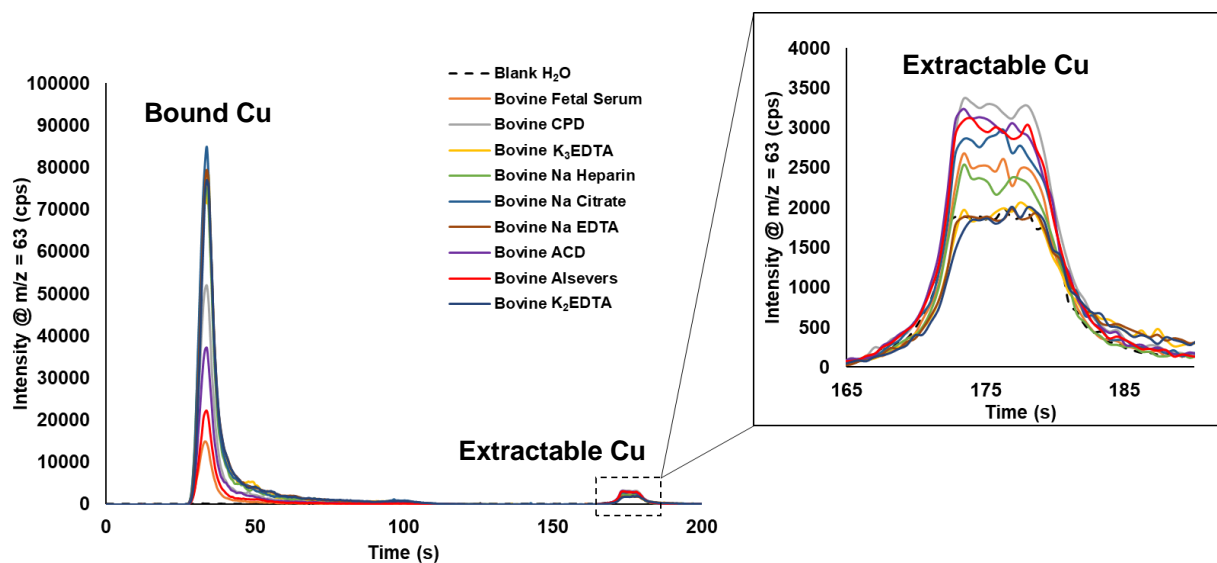
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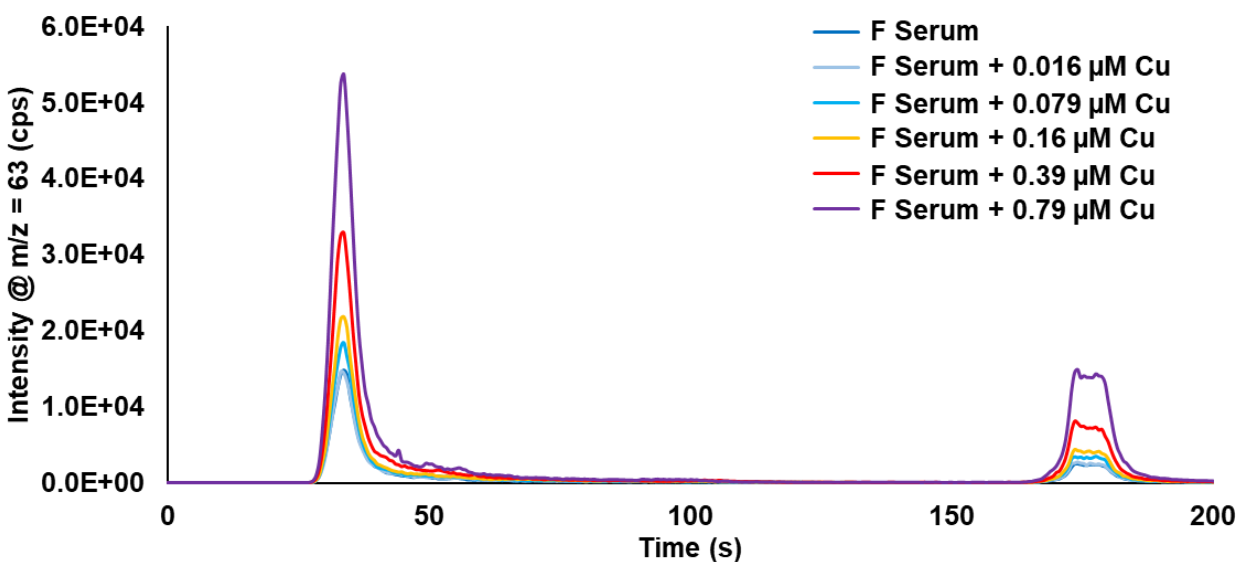
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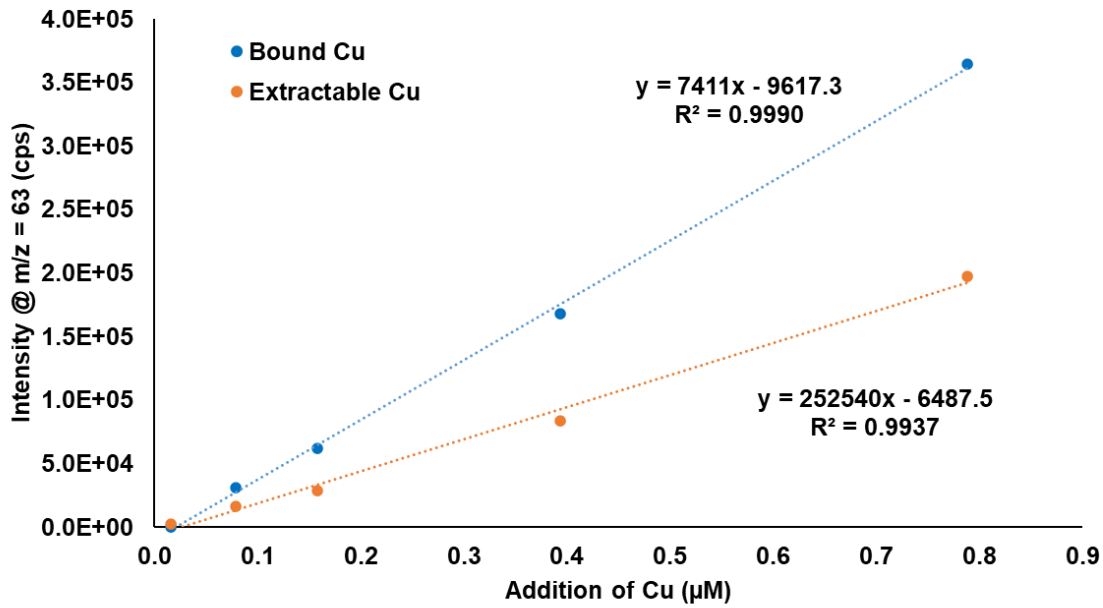
<sup>5</sup>Technical University Munich, School of Medicine, Institute of Toxicology  
and Environmental Hygiene, Biedersteiner Strasse 29, 80802 Munich,  
Germany.



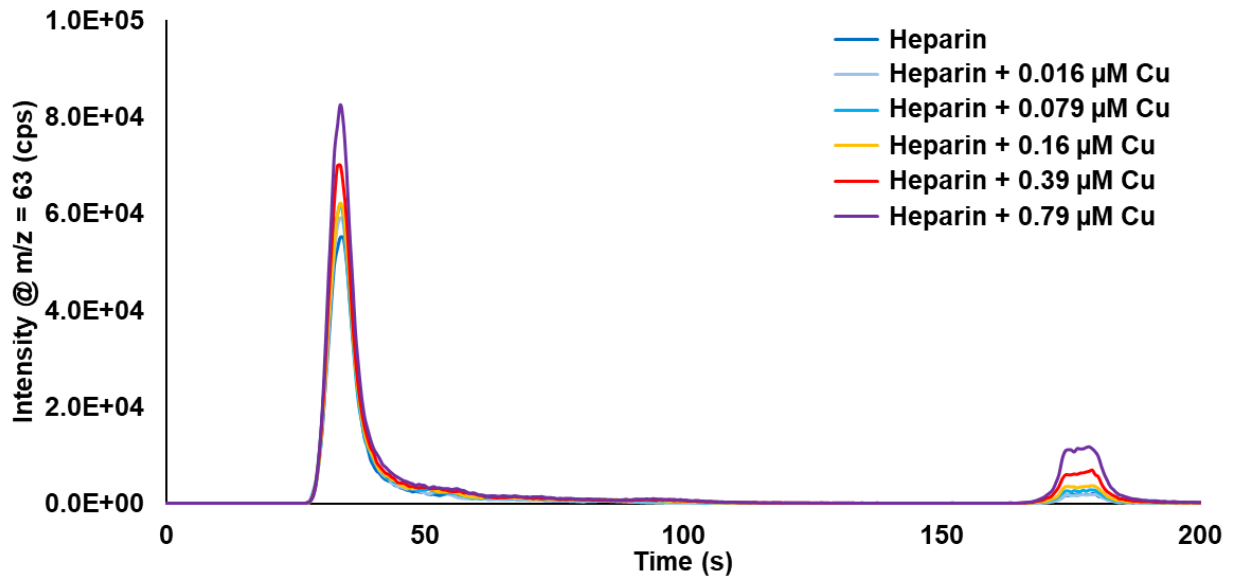
**Figure S1.** Chromatographic separation of bound and extractable Cu in bovine blood plasma and serum samples that were stored under different anti-coagulation agents/methods. Serum samples were analyzed using a 50x inline dilution factor. This set of samples was included to determine if there were any sample types that could not be analyzed with this separation method. CPD = citrate-phosphate-dextrose, ACD = acid-citrate-dextrose, Alsevers = saline solution with dextrose, sodium citrate, citric acid, and sodium chloride.



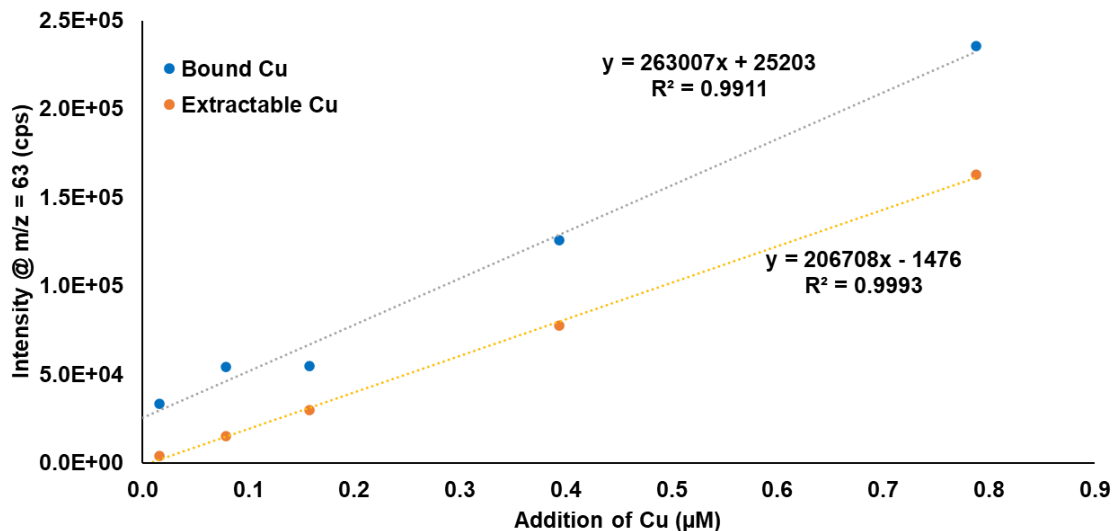
**Figure S2a.** Chromatograms of fetal serum (F Serum) with the addition of 0.016 - 0.79  $\mu\text{M}$  Cu (1 - 50  $\mu\text{g L}^{-1}$  Cu). Serum samples were analyzed using a 50x inline dilution factor.



**Figure S2b.** Bound and extractable Cu results from the fetal serum with the addition of 0.016 - 0.79 µM Cu (1 - 50 µg L<sup>-1</sup> Cu).

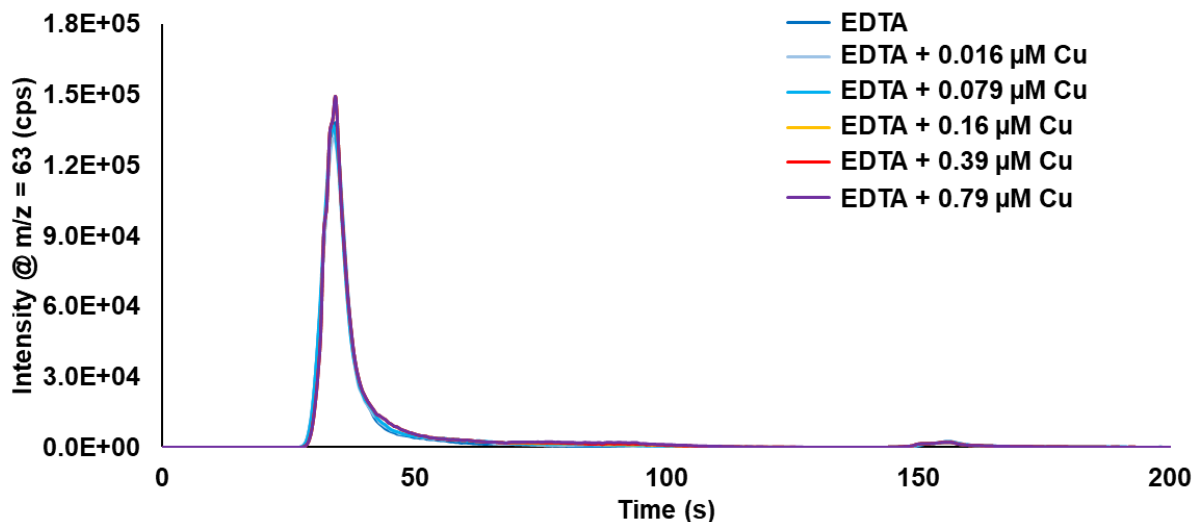


**Figure S2c.** Chromatograms of heparinized plasma with the addition of 0.016 - 0.79 µM Cu (1 - 50 µg L<sup>-1</sup> Cu). Plasma samples were analyzed using a 50x inline dilution factor.

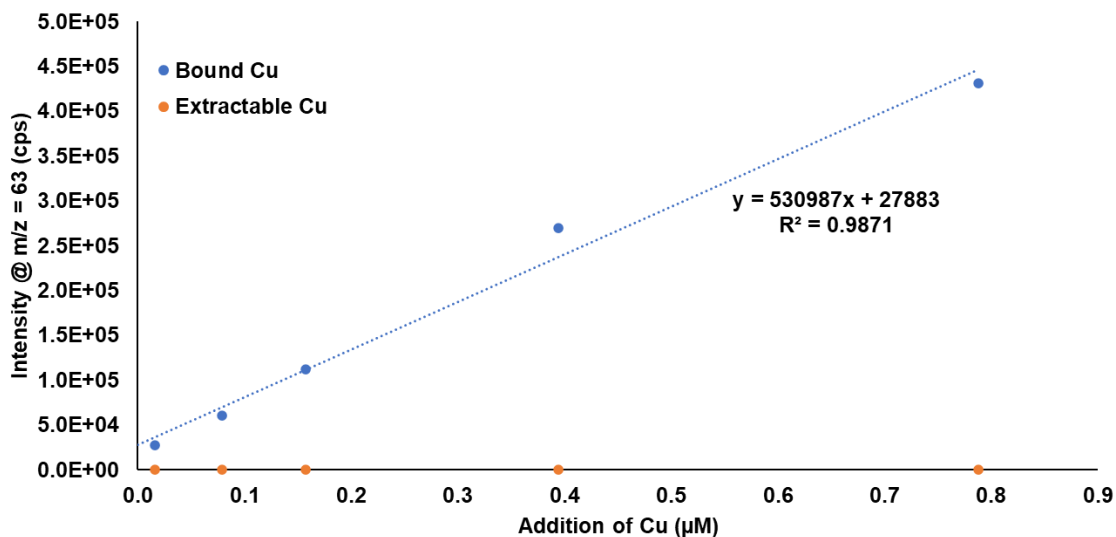


**Figure S2d.** Bound and extractable Cu results from the heparin samples with the addition of 0.016 - 0.79 µM Cu (1 - 50 µg L<sup>-1</sup> Cu).

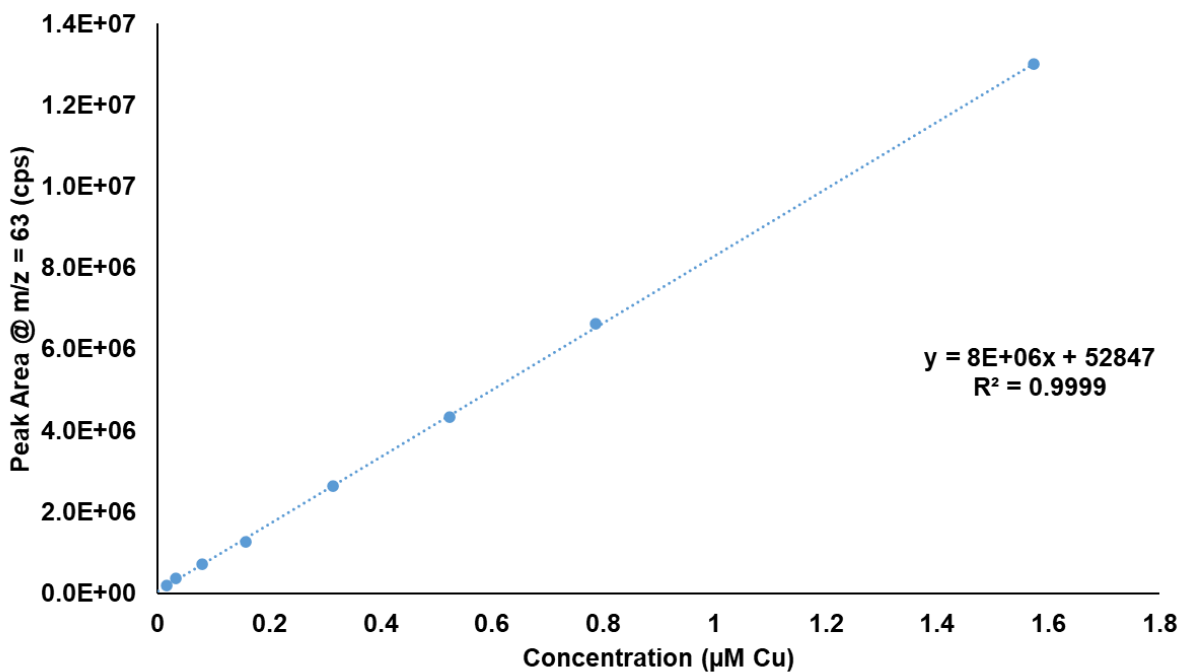
The addition of 0.016 µM Cu into heparin and EDTA bovine blood (Fig. S2f) resulted in a higher bound Cu value as compared to fetal serum. The amount of bound Cu in Fig S1 for fetal serum is much lower than heparin and EDTA, suggesting a lower protein level in the neat sample. Lower protein levels would result in less binding of extractable Cu when spiked in this study.



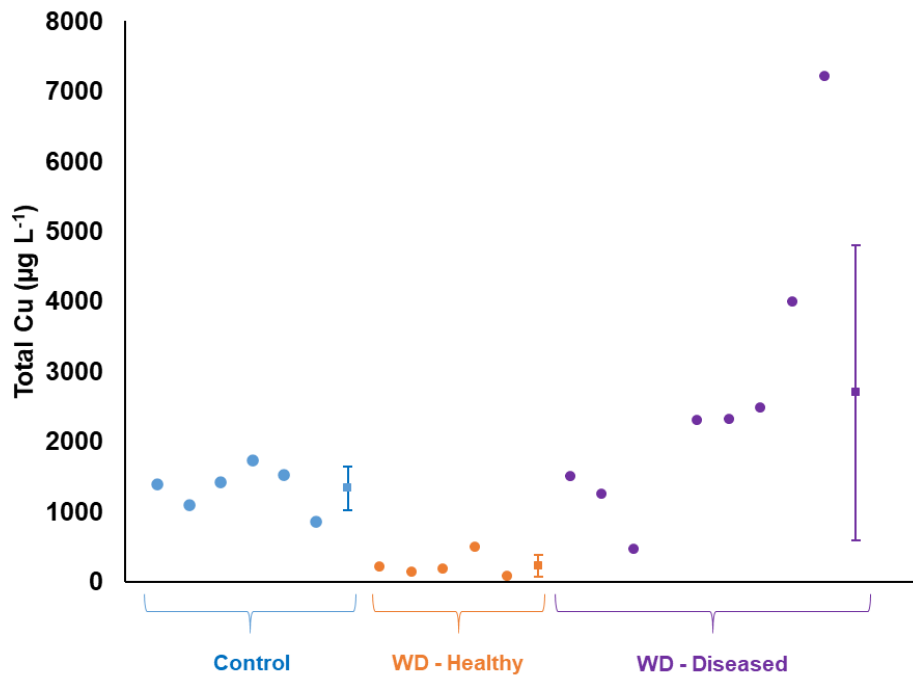
**Figure S2e.** Chromatograms of EDTA blood (analyzed as plasma) with the addition of 0.016 - 0.79 µM Cu (1 - 50 µg L<sup>-1</sup> Cu). Plasma samples were analyzed using a 50x inline dilution factor.



**Figure S2f.** Bound and extractable Cu results from the EDTA samples with the addition of 0.016 - 0.79 µM Cu (1 - 50 µg L<sup>-1</sup> Cu).



**Figure S3.** Typical calibration curve for extractable Cu ranging from 0.016 - 1.6 µM Cu (1 - 100 µg L<sup>-1</sup> Cu) using the chromatographic separation method for bound and extractable Cu.



**Figure S4.** Comparison of *Atp7b*<sup>+/-</sup> control, *Atp7b*<sup>-/-</sup> healthy (WD - Healthy), and *Atp7b*<sup>-/-</sup> Wilson diseased (WD - Diseased) rats for total Cu. The square data points with error bars represent the average ± standard deviation for each group.