**SUPPLEMENTARY MATERIALS**

**Design of Experiment approach to optimize High Resolution ICP-OES method for biomonitoring of Zn level in human blood samples**

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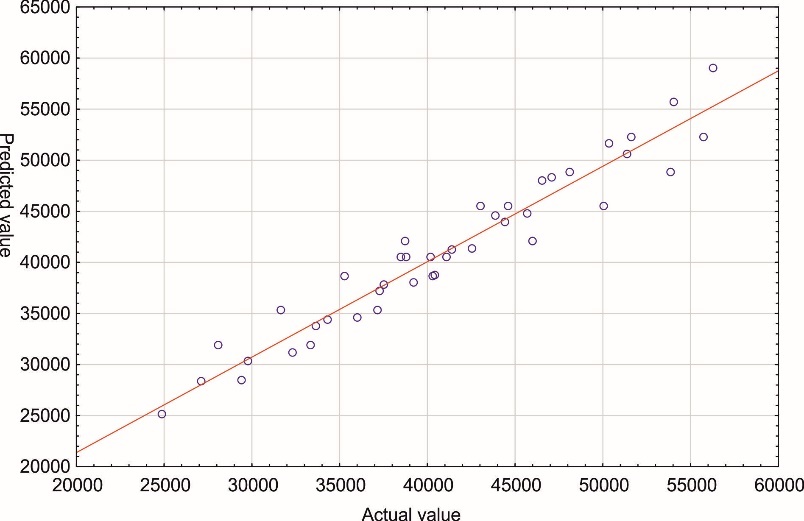
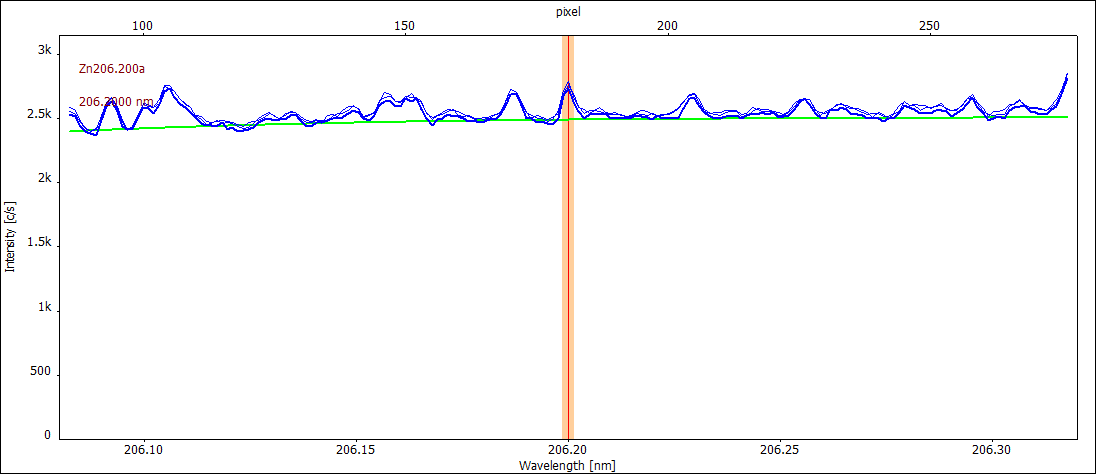
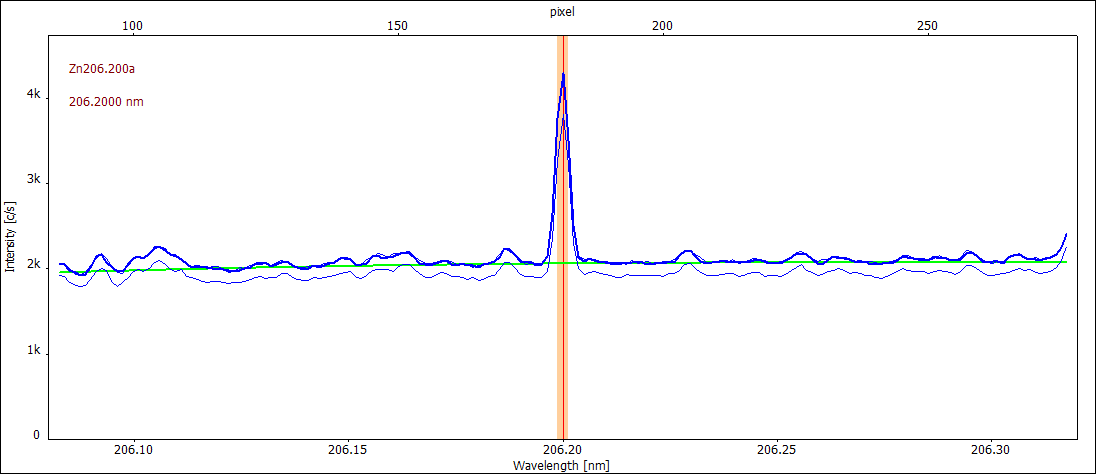


Fig. S1. Predicted vs. actual response

Fig. S2. Relationship between signal intensity and auxiliary gas flow - axial mode;



(a)



(b)

Fig. S3. Signal registered at wavelength 206.200 nm; (a) – blank oxidative mixture was heated in disposable vessel recommended for graphite block, (b) – blank oxidative mixture was heated using TOPwave system in reusable vessel previously subjected cleaning procedure recommended by manufacturer

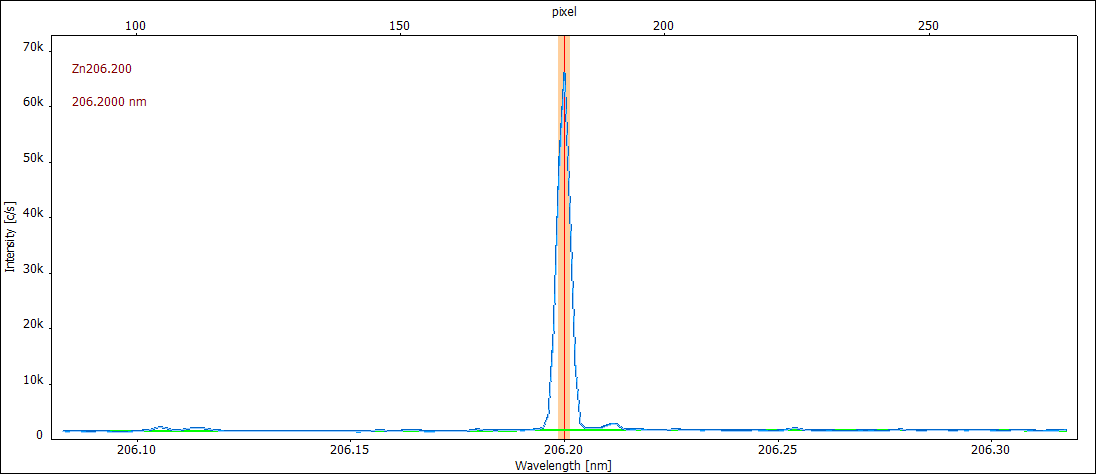


Fig. S4. Signal of Zn obtained in real blood samples after digestion

Table S1

Analysis of variance for quadratic model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Source | Sum of Squares | Mean Square | F-value | p-value |
| Model | 2.523E+09 | 3.154E+08 | 59.19 | < 0.0001 |
| RF Power (L) | 8.405E+08 | 8.405E+08 | 157.75 | < 0.0001 |
| Plasma gas flow (L) | 2.220E+07 | 2.220E+07 | 4.17 | 0.0493 |
| Nebulizer gas flow (L) | 1.156E+09 | 1.156E+09 | 217.03 | < 0.0001 |
| Sample flow rate (L) | 3.879E+08 | 3.879E+08 | 72.80 | < 0.0001 |
| RF Power (Q) | 1.084E+06 | 1.084E+06 | 0.2034 | 0.6549 |
| Plasma gas flow (Q) | 4.093E+07 | 4.093E+07 | 7.68 | 0.0091 |
| Nebulizer gas flow (Q) | 27343.19 | 27343.19 | 0.0051 | 0.9433 |
| Sample flow rate (Q) | 8.092E+07 | 8.092E+07 | 15.19 | 0.0005 |
| Residual | 1.758E+08 | 5.328E+06 | - | - |
| Lack of Fit | 1.714E+08 | 5.713E+06 | 3.87 | 0.1452 |
| Pure Error | 4.427E+06 | 1.476E+06 | - | - |
| Cor Total | 2.699E+09 | - | - | - |

Table S2

LOQ verification (n = 5)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| no matrix | | | | |
| Zn concentration  [μg L-1] | Concentration found  [μg L-1] | uimp  [%] | ubias  [%] | U\*  [%] |
| 0.50 | 0.32 | 15 | -36 | 39 | |
| 1.0 | 0.77 | 1.2 | -23 | 23 | |
| 2.0 | 1.9 | 1.6 | -5.0 | 5.2 | |
| 3.0 | 2.6 | 1.7 | -6.7 | 6.9 | |
| 4.0 | 4.0 | 1.5 | 0.0 | 1.5 | |
| 5.0 | 4.9 | 0.91 | -2.0 | 2.2 | |
| matrix matched | | | | |
| Zn concentration  [μg L-1] | Concentration found  [μg L-1] | uimp  [%] | ubias  [%] | U  [%] |
| 0.50 | 0.34 | 13 | -32 | 34 | |
| 1.0 | 0.78 | 4.4 | -22 | 22 | |
| 2.0 | 1.9 | 3.7 | -5.0 | 6.2 | |
| 3.0 | 2.8 | 3.6 | -6.7 | 7.6 | |
| 4.0 | 3.9 | 1.6 | -2.5 | 3.0 | |
| 5.0 | 4.8 | 2.1 | -4.0 | 4.5 | |

\*Combined standard uncertainty (U) was calculated from the formula:

|  |  |
| --- | --- |
|  |  |

uimp – precision expressed as residual standard deviation of measurements [%],

ubias - relative error [%]

Table S3

Regression analysis performed using GraphPad Prism 8.0 software.

|  |  |  |
| --- | --- | --- |
| Parameter | Matrix matched\* | No martix |
| Equation | y = 394.6x + 2032 | y = 418.3x-145.0 |
| R square | 0.99994 | 0.99995 |
| 95% Confidence Intervals slope | 385.5 – 403.6 | 409.3 – 427.3 |
| 95% Confidence Intervals  Y-intercept | -3042 - 7107 | -5184 - 4894 |
| 95% Confidence Intervals  X-intercept | -18,31 - 7,587 | -11,88 - 12,21 |
| Residual sum of squares | 1655 | 1643 |

\* matrix matched solution contains K 30 mg L-1, Na 640 mg L-1, Ca 20 mg L-1, Mg 4 mg L-1, Fe 100 mg L-1