
# Reid 2022

## Metallo protein quantification by LC-ICP-MSMS

Eric van der Horst, Director Method Development LC-MSMS

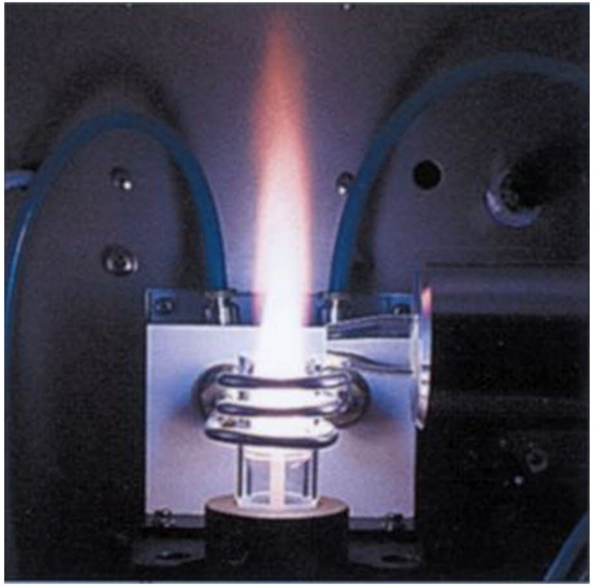
June 2022

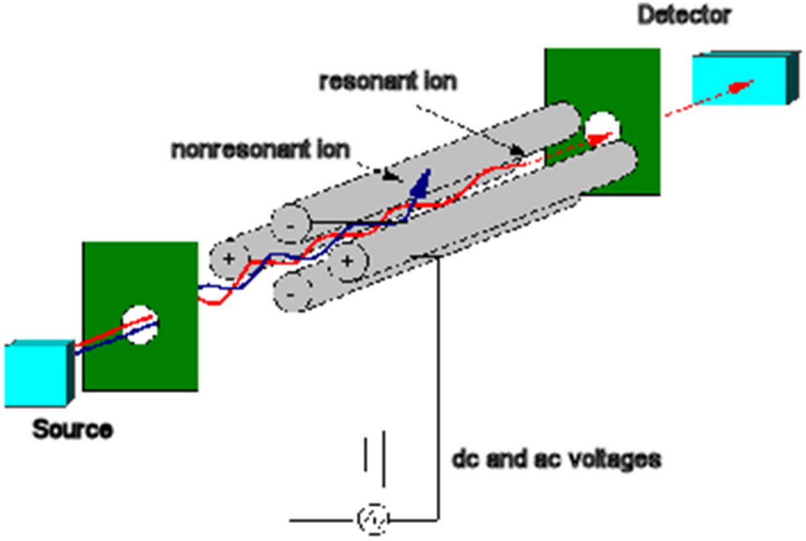
# Table of Contents

# ICP-MS

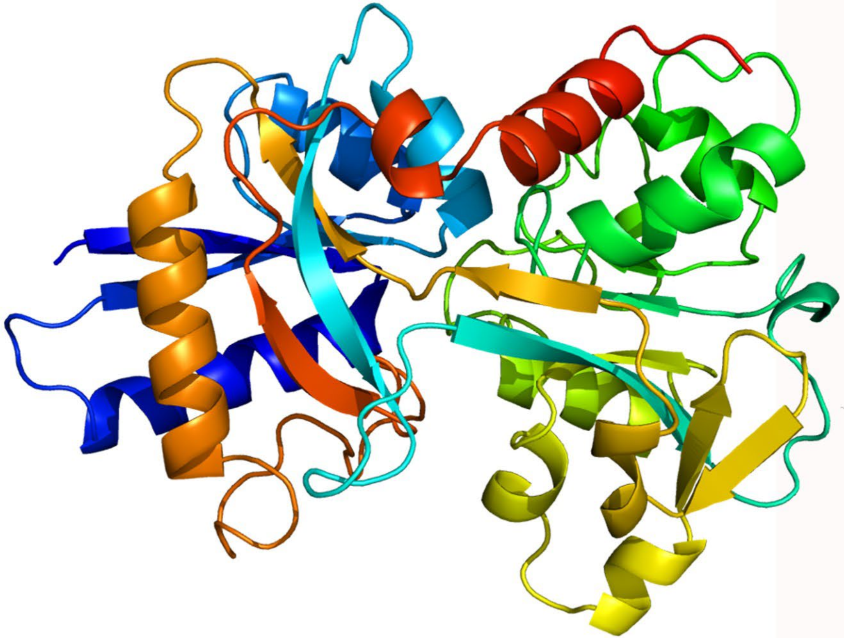
Plasma: Gas stream passes through plasma maintained by a strong RF field (1-2 kWatt, 27 - 41 MHz) and Argon

6,000 K

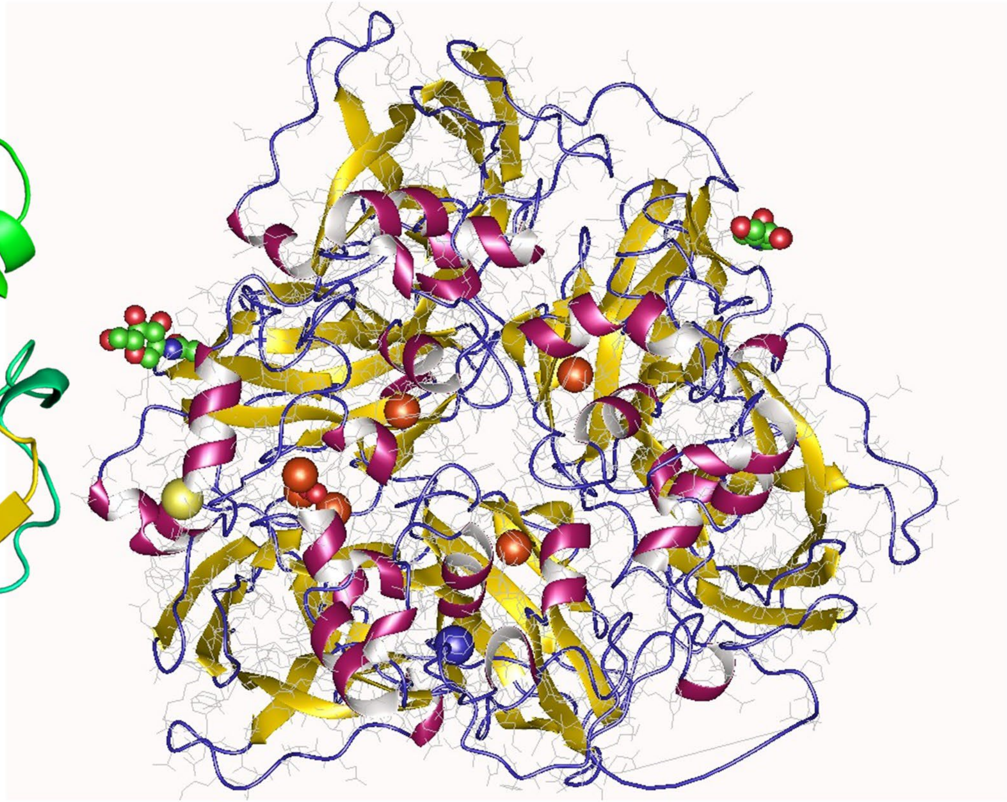





# Metallo proteins

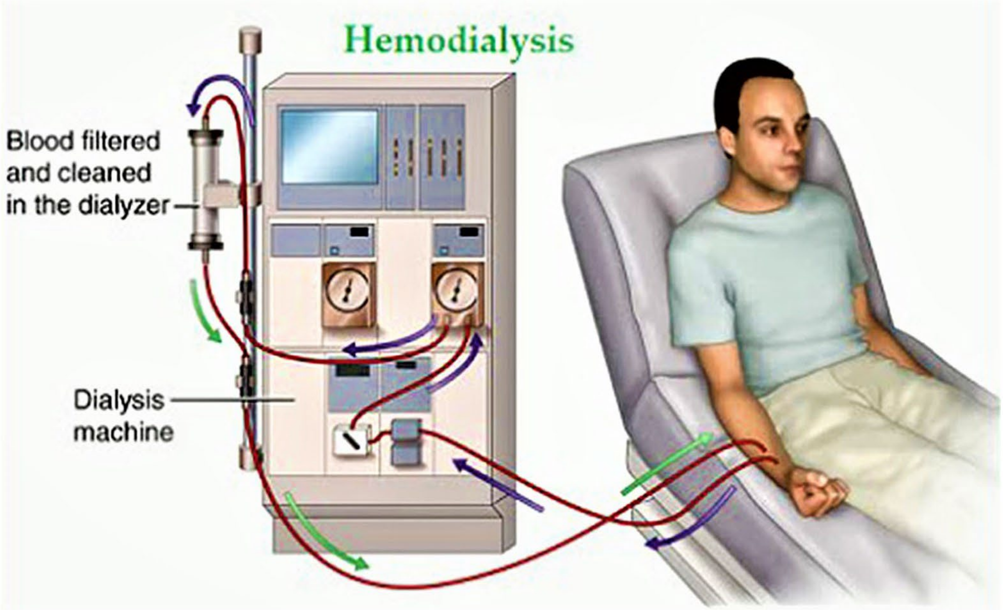


Transferrin (76kDa)



Ceruloplasmin (151 kDa)

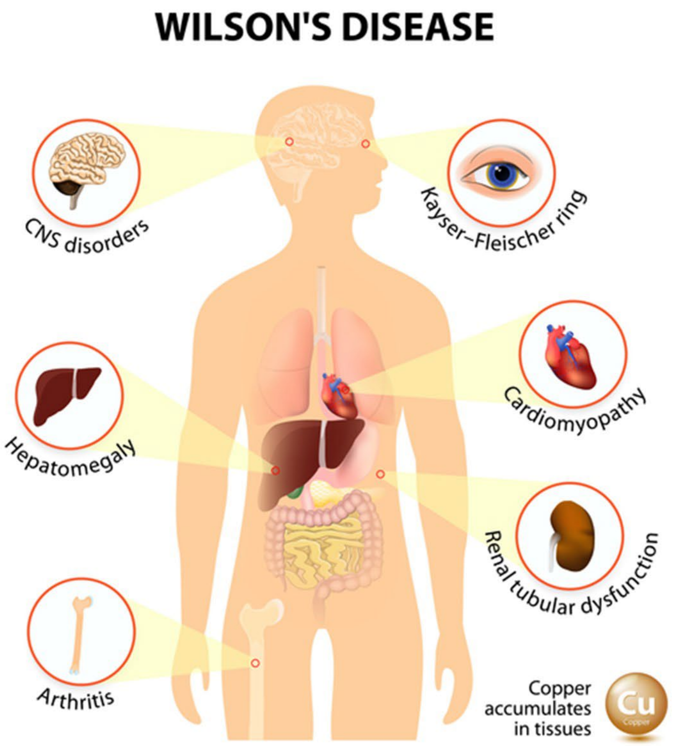
# Anaemia due to hemodialysis in Chronic Kidney Disease




## Key analytical parameters

- Total Iron (CI on ICP-MS)
- Transferrin bound iron (LC-ICP-MS)
- Total Iron binding capacity (LC-ICP-MS)

# Wilson disease (copper metabolism disorder)




## Key analytical parameters

- Total copper (FIA on ICP-MS)
- Ceruloplasmin bound copper (LC-ICP-MS)
- Free / echangeable copper (LC-ICP-MS)

# Total Iron / Copper by Continuous Infusion or Flow Injection Analysis

- "Dilute and shoot", no sample preparation, no chromatography
- Proxy matrices for the calibration curve for absolute quantitation
- Low level plasma/serum and standard addition for QC-levels (LLOQ often in proxy matrix)
- Fast
- Fe suffers from interference from Argon ($ArO^+$ is m/z 56, same as Fe) → remedy is addition of $H_2$

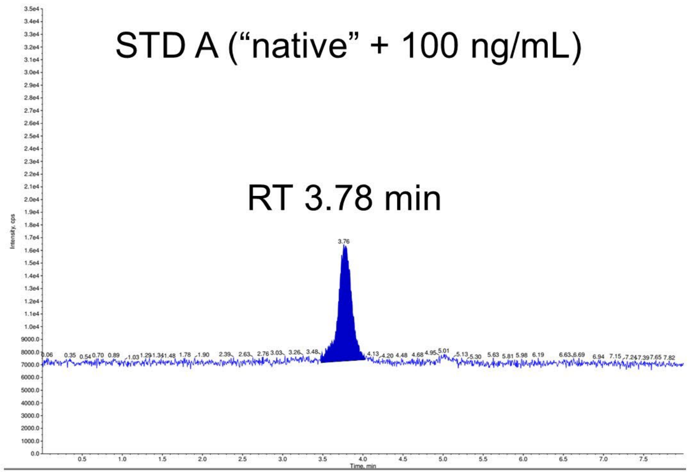
# Transferrin bound iron in heparin plasma

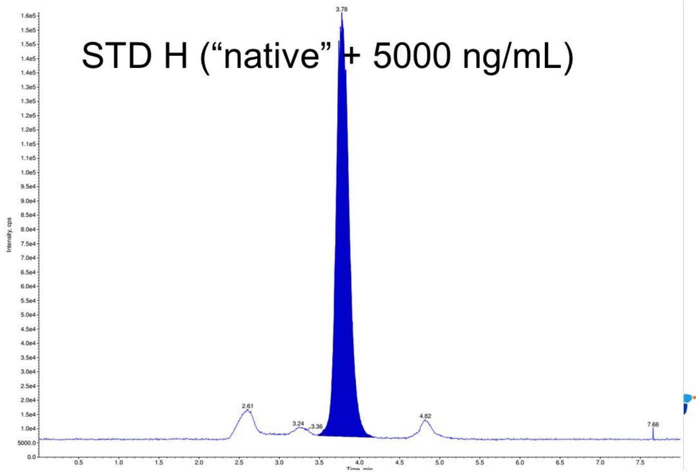
- Separation on a Waters BEH200Å SEC 7.8 x 150 mm column, isocratic elution

- Due to addition: study samples within normal reference ranges

- Proxy matrix for calibration samples in a PBS HSA / transferrin solution (Fe spiked)
  - Not homogenious at this scale -> native concentration based on standard addition of the calibration curve

- Low QC: low concentration matrix, higher QC's: standard addition of Fe solution



# Challenges for transferrin bound iron detection

- In general concentrations within (or above) normal reference ranges
- Lyophilyzed transferrin behaves simmilar to native transferrin
- Added Iron ($Fe^{2+}$) readily binds to transferrin, no equilibrium or volume effect
- Human serum albumin and lyophilyzed transferrin pose a small challenge
  - Material is not homogeneous in the scale (low mg) used
  - Different batches have different iron contents (same as different matrices)
- Solution: determine native concentration per calibration curve and for each batch of prepared QC's

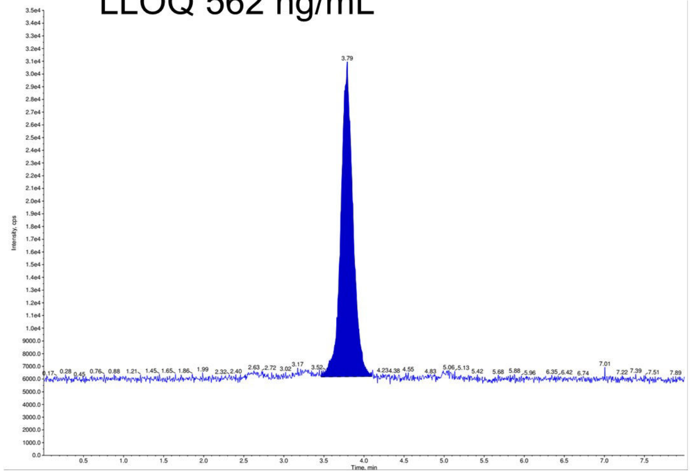
## Linearity demonstrated up to +5000 ng/mL

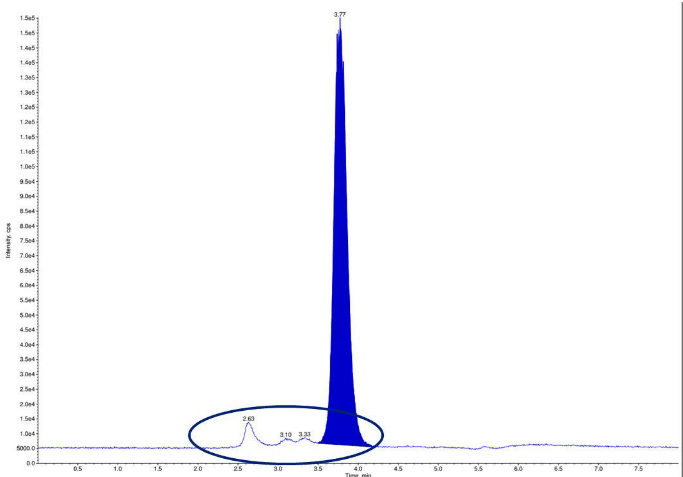
| Sample description | Nominal conc (ng/mL) | Ratio | (Y2-Y1) | (X2-X1) | d(x/y) | Native conc (ng/mL) | Corr nom. conc (ng/ml) |
|---|---|---|---|---|---|---|---|
| Blank_artificial_matrix_+_IS | 0.00 | 0.171 | N.Ap. | 0.00 | N.Ap. | 522 | N.Ap. |
| Blank_artificial_matrix_+_IS | 0.00 | 0.171 | N.Ap. | 0.00 | N.Ap. | | N.Ap. |
| STD_A_(Y+_100_ng/mL_07_Mar_2019) | 100 | 0.212 | 0.0405 | 100 | [illegible] | [illegible] | 622 |
| STD_B_(Y+_200_ng/mL_07_Mar_2019) | 200 | 0.242 | 0.0703 | 200 | 0.000352 | 488 | 722 |
| STD_C_(Y+_500_ng/mL_07_Mar_2019) | 500 | 0.341 | 0.170 | 500 | 0.000339 | 505 | 1022 |
| STD_D_(Y+_1000_ng/mL_07_Mar_2019) | 1000 | 0.499 | 0.327 | 1000 | 0.000327 | 524 | 1522 |
| STD_E_(Y+_2000_ng/mL_07_Mar_2019) | 2000 | 0.797 | 0.625 | 2000 | 0.000313 | 548 | 2522 |
| STD_F_(Y+_3000_ng/mL_07_Mar_2019) | 3000 | 1.06 | 0.892 | 3000 | 0.000297 | 577 | 3522 |
| STD_G_(Y+_4000_ng/mL_07_Mar_2019) | 4000 | 1.35 | 1.18 | 4000 | 0.000296 | 580 | 4522 |
| STD_H_(Y+_5000_ng/mL_07_Mar_2019) | 5000 | 1.65 | 1.48 | 5000 | 0.000295 | 580 | 5522 |
| STD_A_(Y+_100_ng/mL_07_Mar_2019) | 100 | 0.206 | 0.0344 | 100 | [illegible] | [illegible] | 622 |
| STD_B_(Y+_200_ng/mL_07_Mar_2019) | 200 | 0.250 | 0.0786 | 200 | 0.000393 | 436 | 722 |
| STD_C_(Y+_500_ng/mL_07_Mar_2019) | 500 | 0.339 | 0.168 | 500 | 0.000335 | 511 | 1022 |
| STD_D_(Y+_1000_ng/mL_07_Mar_2019) | 1000 | 0.524 | 0.352 | 1000 | 0.000352 | 486 | 1522 |
| STD_E_(Y+_2000_ng/mL_07_Mar_2019) | 2000 | 0.867 | 0.696 | 2000 | 0.000348 | 493 | 2522 |
| STD_F_(Y+_3000_ng/mL_07_Mar_2019) | 3000 | 1.14 | 0.974 | 3000 | 0.000325 | 528 | 3522 |
| STD_G_(Y+_4000_ng/mL_07_Mar_2019) | 4000 | 1.45 | 1.28 | 4000 | 0.000320 | 535 | 4522 |
| STD_H_(Y+_5000_ng/mL_07_Mar_2019) | 5000 | 1.81 | 1.64 | 5000 | 0.000328 | 523 | 5522 |

| Run Date | Run Name | LLOQ 562 (ng/mL) | QC-blanc 916 (ng/mL) | QC-Low 762 (ng/mL) | QC-Med 1320 (ng/mL) | QC-High 2920 (ng/mL) |
|---|---|---|---|---|---|---|
| 23 Nov 2017 | FE-ICP2-001 | 556 | 693 [a] | 730 | 1300 | 2760 |
| | | 559 | 885 | 744 | 1290 | 2390 [a] |
| | | 570 | 976 | 767 | 1280 | 2980 |
| | | 571 | 908 | 739 | 1310 | 2680 |
| | | 558 | 913 | 769 | 1340 | 2780 |
| | | 555 | 900 | 706 | 1260 | 2780 |
| Intra-run Mean | | 562 | 879 | 743 | 1297 | 2728 |
| Intra-run SD | | 7 | 96 | 24 | 27 | 193 |
| Intra-run %CV | | 1.3 | 11.0 | 3.2 | 2.1 | 7.1 |
| Intra-run %RE | | -0.1 | -4.0 | -2.6 | -1.8 | -6.6 |
| n | | 6 | 6 | 6 | 6 | 6 |
| 28 Nov 2017 | FE-ICP2-002 | 615 | 1030 | 867 | 1360 | 3120 |
| | | 632 | 1030 | 827 | 1350 | 2890 |
| | | 653 | 983 | 850 | 1470 | 2980 |
| | | 634 | 966 | 817 | 1370 | 2900 |
| | | 654 | 994 | 851 | 1420 | 2970 |
| | | 635 | 1010 | 880 [a] | 1360 | 3000 |
| Intra-run Mean | | 637 | 1002 | 849 | 1388 | 2977 |
| Intra-run SD | | 15 | 26 | 24 | 47 | 83 |
| Intra-run %CV | | 2.3 | 2.6 | 2.8 | 3.4 | 2.8 |
| Intra-run %RE | | 13.4 | 9.4 | 11.4 | 5.2 | 1.9 |
| n | | 6 | 6 | 6 | 6 | 6 |
| 02 Dec 2017 | FE-ICP2-004 | 618 | 903 | 755 | 1270 | 2790 |
| | | 575 | 885 | 843 | 1260 | 2760 |
| | | 621 | 892 | 832 | 1250 | 2810 |
| | | 607 | 902 | 818 | 1230 | 2780 |
| | | 604 | 923 | 820 | 1280 | 2750 |
| | | 585 | 911 | 798 | 1270 | 2780 |
| Intra-run Mean | | 602 | 903 | 811 | 1260 | 2778 |
| Intra-run SD | | 18 | 13 | 31 | 18 | 21 |
| Intra-run %CV | | 3.0 | 1.5 | 3.9 | 1.4 | 0.8 |
| Intra-run %RE | | 7.1 | -1.5 | 6.4 | -4.5 | -4.9 |
| n | | 6 | 6 | 6 | 6 | 6 |
| Mean Concentration Found: (ng/mL) | | 600 | 928 | 801 | 1315 | 2828 |
| Inter-run SD | | 34 | 77 | 52 | 64 | 159 |
| Inter-run %CV | | 5.7 | 8.3 | 6.4 | 4.8 | 5.6 |
| Inter-run %RE | | 6.8 | 1.3 | 5.1 | -0.4 | -3.2 |
| n | | 18 | 18 | 18 | 18 | 18 |

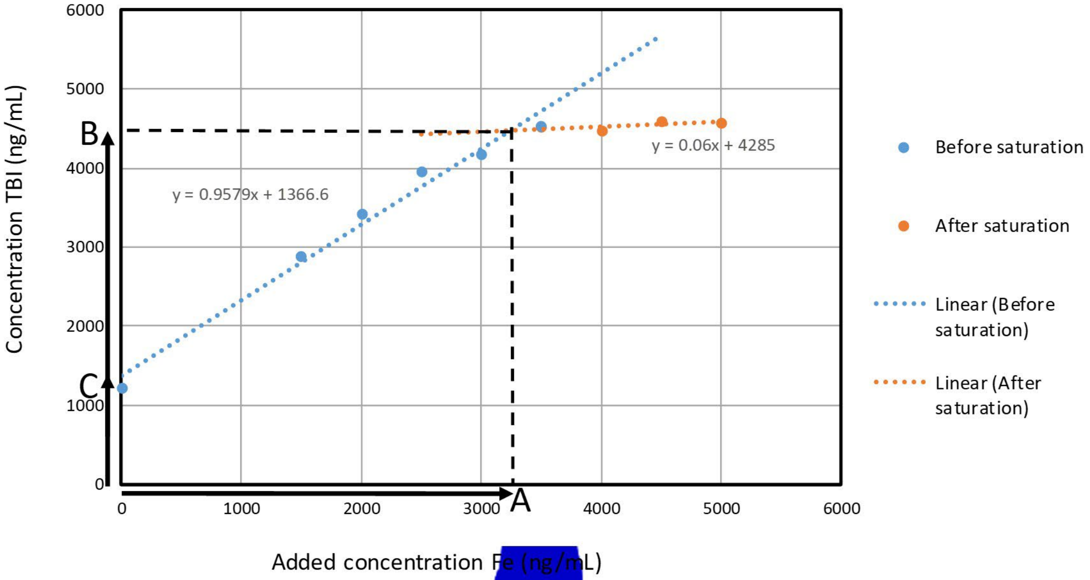
a) >15|%RE| from nominal (>20|%RE|at LLOQ); value used in statistical calculations

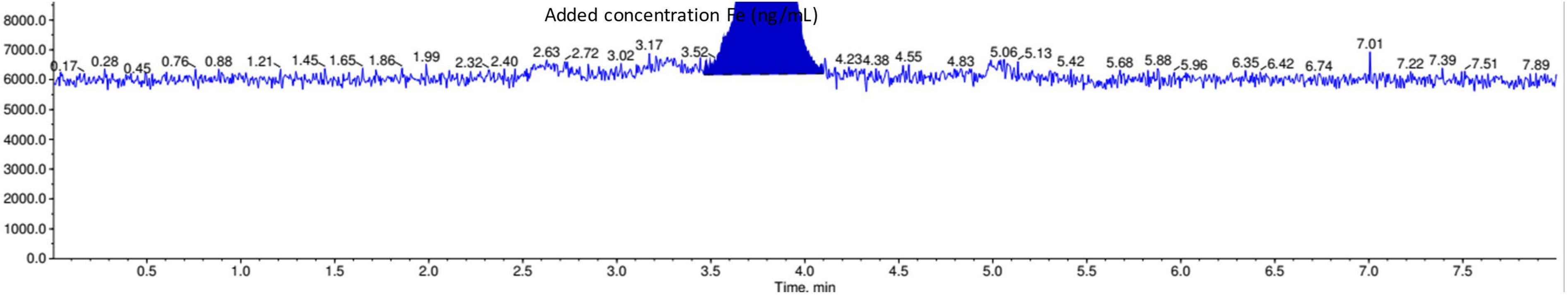
LLOQ 562 ng/mL



# Iron binding capacity (linearity truncated due to saturation)



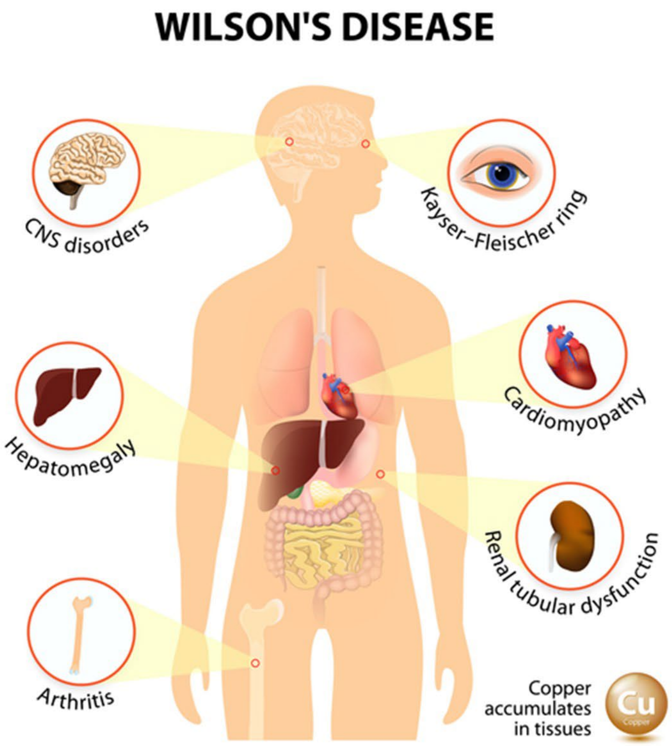
# Precision and accuracy

| Run Date | Run Name | Serum 1 (ng/mL) | Serum 2 (ng/mL) | Serum 3 (ng/mL) | Serum 4 (ng/mL) |
|---|---|---|---|---|---|
| … | … | 2898 | 3277 | 4286 | 3641 |
| | | 3060 | 3497 | 4552 | 3368 |
| | | 3346 | 3627 | 4378 | 3635 |
| Intra-run Mean | | 3101 | 3467 | 4405 | 3548 |
| Intra-run SD | | 227 | 177 | 135 | 156 |
| Intra-run %CV | | 7.3 | 5.1 | 3.1 | 4.4 |

- "Normal" precision and accuracy met
  - Method validation for Transferrin bound iron was validated already
- "Subjective" method, but differences between technicians are acceptable
- Normal stability program completed successfully

| TIBC MIN | TIBC MAX | MAX/MIN*100% |
|---|---|---|
| 2898 | 2898 | 100.0% |
| 3277 | 3235 | 98.7% |
| 4286 | 4286 | 100.0% |
| 3641 | 3641 | 100.0% |
| 3060 | 3201 | 104.6% |
| 3497 | 3938 | 112.6% |
| 4552 | 4586 | 100.8% |
| 3368 | 3481 | 103.3% |
| 3346 | 3281 | 98.1% |
| 3627 | 3047 | 84.0% |
| 4378 | 4833 | 110.4% |
| 3635 | 3765 | 103.6% |
| 4033 | 3933 | 97.5% |
| 5117 | 5425 | 106.0% |
| 3891 | 4340 | 111.5% |
| 4467 | 4174 | 93.4% |
| 3195 | 3302 | 103.3% |
| 3311 | 3344 | 101.0% |
| 4318 | 4305 | 99.7% |
| 4144 | 4427 | 106.8% |
| 3789 | 4274 | 112.8% |
| 4442 | 4257 | 95.8% |
| 4457 | 4387 | 98.4% |
| 4546 | 4243 | 93.3% |
| 3570 | 3570 | 100.0% |
| 4358 | 4492 | 103.1% |
| 4017 | 4205 | 104.7% |
| 4051 | 3860 | 95.3% |
| 4290 | 3966 | 92.5% |
| 3214 | 3361 | 104.6% |
| 2955 | 2874 | 97.2% |
| 3329 | 3153 | 94.7% |
| 4166 | 4089 | 98.1% |
| 3195 | 3344 | 104.7% |
| 4202 | 4173 | 99.3% |
| 3133 | 3345 | 106.8% |

# Ceruloplasmin bound copper




Important to realize

- All known patients are under treatment
- Typical treatment is administration of a chelating agent
- Resulting in low, to very low, copper concentrations (both total and ceruloplasmin bound)
- Control of NCC is key in the treatment

# Three approaches to determine NCC

- Total copper and available kits for ceruloplasmin content
  - The golden standard

- Ultra centrifugation after EDTA addition and total copper`measurement

- EDTA addition and ion exchange chromatography


# “Golden” standard: Substraction of Cu-Ceruloplasmin of total Copper

Cons

- Subtraction of two relatively large numbers with match uncertainties can lead to negative values for NCC
- Cumbersome: 2 analysis required
- Approximation on the number of Cu bound to each ceruloplasmin peptide
- Stability data between ceruloplasmin and other techniques does not match

Pro

- Esthablished method
- Characteristics are well known
- Technically least critical

To convert the ceruloplasmin activity in **mU/mL** to a concentration in **mg/L**:

[Ceruloplasmin] in **mg/L** = (Response in **mU/mL** / 3.33) * 10

To convert the copper concentration from ng/mL to µg/L:

[Copper] in **µg/L** = concentration in **ng/mL** x 1000 **ml/L** / 1000 **ng / µg/L**

To calculate the NCC concentration in **µg/L**:

[NCC] in **µg/L** = [Copper] in **µg/L** – 3 in **µg / mg** X [Ceruloplasmin] in **mg/**

# Ultra filtration

- Samples are treated with EDTA (3 g/L) and incubated for 60 minutes
- Centrifuged over a 10kDa MWCO filter (4000g, 60 minutes) with WIS in collection tube (Yttrium)
- Take an aliquot of the eluate and dilute in 1 ml nitric acid solution
- Analyse by continuous infusion in ICP-MS
- Calculate the actual NCC concentration
  - Internal standard response compared to standards: to caculate the filtrate volume
  - Response ratio internal standard and analyte: to calculate the concentration
  - Both: to calculate the amount of ”exchangeagble” copper in the filtration sample
  - Correct for the diluton by EDTA to come to the actual $Cu_{ex}$ concentration

# Results

- Total measurements, so proxy matrices can be used for copper response.
- Accuracy and precision very good (not in PBS, due to the instability in PBS)
- Fresh plasma/serum stored directly after collection at -80ºC yields lowest NCC results

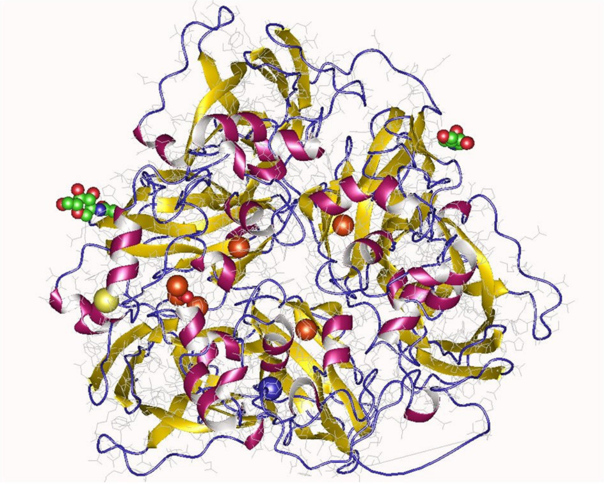
| Run Date | Run Name | LLOQ 25.0 (ng/mL) | QC-blanc 28.1 (ng/mL) | QC-Low 75.0 (ng/mL) | QC-Med 178 (ng/mL) | QC-High 628 (ng/mL) |
|---|---|---|---|---|---|---|
| 06 Apr 2017 | CU-ICP3-001 | 28.0 | 27.4 | 78.2 | 195 | 639 |
| | | 28.4 | 28.9 | 79.3 | 190 | 671 |
| | | 28.9 | 28.0 | 79.2 | 200 | 657 |
| | | 29.2 | 27.6 | 79.3 | 192 | 683 |
| | | 29.8 | 27.6 | 78.2 | 193 | 662 |
| | | 29.6 | 29.0 | 78.4 | 195 | 646 |
| Intra-run Mean | | 29.0 | 28.1 | 78.8 | 194 | 660 |
| Intra-run SD | | 0.7 | 0.7 | 0.6 | 3 | 16 |
| Intra-run %CV | | 2.4 | 2.5 | 0.7 | 1.8 | 2.4 |
| Intra-run %RE | | 15.9 | -0.1 | 5.0 | 9.1 | 5.0 |
| n | | 6 | 6 | 6 | 6 | 6 |
| 10 Apr 2017 | CU-ICP3-002 | 30.0 | 26.8 | 80.9 | 192 | 606 |
| | | 30.5 [a] | 26.5 | 78.3 | 195 | 595 |
| | | 30.6 [a] | 28.0 | 80.6 | 197 | 603 |
| | | 29.7 | 28.9 | 80.6 | 190 | 601 |
| | | 29.8 | 28.3 | 77.9 | 191 | 606 |
| | | 30.6 [a] | 30.5 | 82.0 | 194 | 605 |
| Intra-run Mean | | 30.2 | 28.2 | 80.1 | 193 | 603 |
| Intra-run SD | | 0.4 | 1.5 | 1.6 | 3 | 4 |
| Intra-run %CV | | 1.4 | 5.2 | 2.0 | 1.4 | 0.7 |
| Intra-run %RE | | 20.8 | 0.2 | 6.7 | 8.5 | -4.0 |
| n | | 6 | 6 | 6 | 6 | 6 |
| 11 Apr 2017 | CU-ICP3-003 | 32.7 [a] | 30.0 | 80.7 | 193 | 510 [a] |
| | | 32.2 [a] | 29.6 | 101 [a] | 192 | 620 |
| | | 32.4 [a] | 29.7 | 80.1 | 195 | 632 |
| | | 32.3 [a] | 28.3 | 68.7 | 188 | 611 |
| | | 31.3 [a] | 30.7 | 82.7 | 191 | 612 |
| | | 34.4 [a] | 30.1 | 79.8 | 247 [a] | 607 |
| Intra-run Mean | | 32.6 | 29.7 | 82.2 | 201 | 599 |
| Intra-run SD | | 1.0 | 0.8 | 10.6 | 23 | 44 |
| Intra-run %CV | | 3.1 | 2.7 | 12.9 | 11.3 | 7.4 |
| Intra-run %RE | | 30.2 | 5.8 | 9.6 | 12.9 | -4.7 |
| n | | 6 | 6 | 6 | 6 | 6 |
| Mean Concentration Found: (ng/mL) | | 30.6 | 28.7 | 80.4 | 196 | 620 |
| Inter-run SD | | 1.7 | 1.3 | 6.0 | 13 | 38 |
| Inter-run %CV | | 5.5 | 4.4 | 7.5 | 6.6 | 6.2 |
| Inter-run %RE | | 22.3 | 2.0 | 7.1 | 10.2 | -1.2 |
| n | | 18 | 18 | 18 | 18 | 18 |

a) >15|%RE| from nominal (>20|%RE|at LLOQ); value used in statistical calculations

# Very low stability (Days)

- Very low stability in plasma and serum  at -20ºC (ultrafiltrates are OK) in freeze/thaw and storage

- More exchangeable copper: released from proteins (albumin, ceruloplasmin etc...)

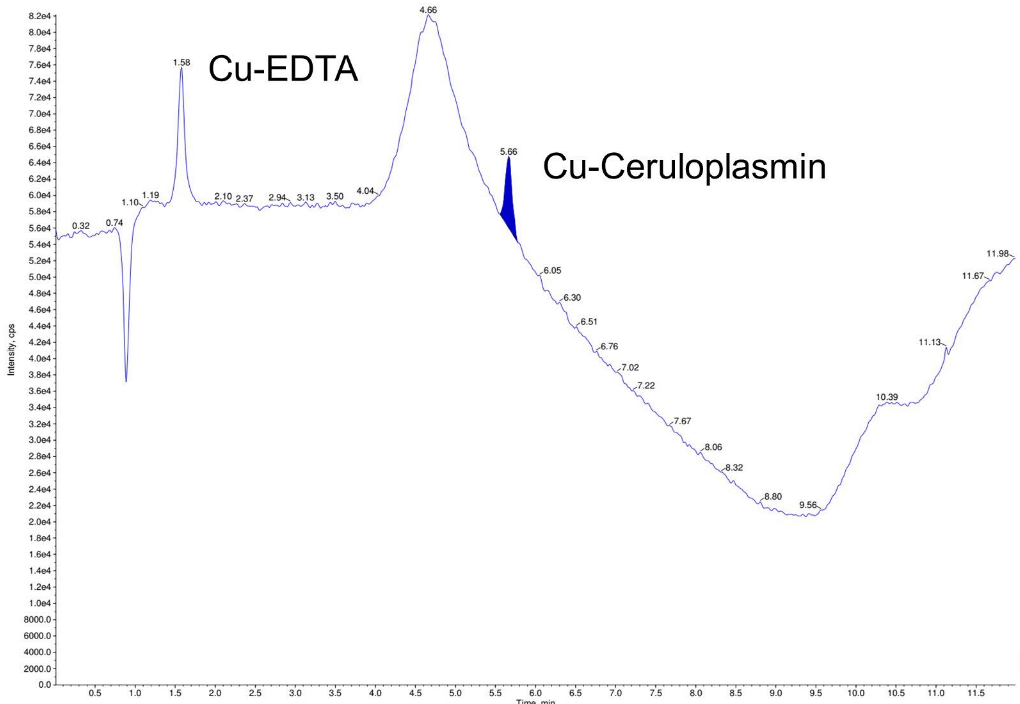


| Run Date | Run Name | QC-blanc 64.1 (ng/mL) | QC-Low 130 (ng/mL) | QC-Med 583 (ng/mL) | QC-High 856 (ng/mL) |
|---|---|---|---|---|---|
| 10 Apr 2017 | CU-ICP3-002 (FT1) | 69.3 | 123 | 480 [a] | 723 [a] |
| | | 65.6 | 120 | 524 | 774 |
| | | 66.9 | 123 | 560 | 774 |
| | | 64.1 | 121 | 512 | 788 |
| | | 64.2 | 133 | 548 | 794 |
| Intra-run Mean | | 66.0 | 124 | 525 | 771 |
| Intra-run SD | | 2.2 | 5 | 31 | 28 |
| Intra-run %CV | | 3.3 | 4.2 | 6.0 | 3.6 |
| Intra-run %RE | | 3.0 | -4.6 | -10.0 | -10.0 |
| n | | 5 | 5 | 5 | 5 |
| 20 Apr 2017 | CU-ICP3-006 (FT2) | 79.6 [a] | 148 | 546 | 837 |
| | | 87.1 [a] | 150 [a] | 550 | 789 |
| | | 88.7 [a] | 141 | 537 | 848 |
| | | 86.1 [a] | 155 [a] | 558 | 792 |
| | | 81.4 [a] | 126 | 538 | 848 |
| Intra-run Mean | | 84.6 | 144 | 546 | 823 |
| Intra-run SD | | 3.9 | 11 | 9 | 30 |
| Intra-run %CV | | 4.6 | 7.8 | 1.6 | 3.6 |
| Intra-run %RE | | 32.0 | 10.8 | -6.4 | -3.9 |
| n | | 5 | 5 | 5 | 5 |
| 20 Apr 2017 | CU-ICP3-006 (FT3) | 88.1 [a] | 139 | 518 | 872 |
| | | 82.4 [a] | 150 [a] | 539 | 851 |
| | | 88.9 [a] | 159 [a] | 576 | 880 |
| | | 89.8 [a] | 137 | 562 | 853 |
| | | 95.5 [a] | 151 [a] | 520 | 858 |
| Intra-run Mean | | 88.9 | 147 | 543 | 863 |
| Intra-run SD | | 4.7 | 9 | 26 | 13 |
| Intra-run %CV | | 5.3 | 6.2 | 4.7 | 1.5 |
| Intra-run %RE | | 38.8 | 13.2 | -6.9 | 0.8 |
| n | | 5 | 5 | 5 | 5 |

a) >15|%RE| from nominal (>20|%RE|at LLOQ); value used in statistical calculations

# Direct NCC (both Cu-Ceruloplasmin and Cu-EDTA in 1 assay)

- Plasma samples are treated with EDTA (3 g/L) incubate for three hours
- Analyze over a TSK-GEL Q-STAT 7 µm 4.6 mm x 10 cm Column (ammonium acetate gradient)



# Direct NCC (both Cu-Ceruloplasmin and Cu-EDTA in 1 assay)

Challenges

- Low concentrations in study samples due to medication, far outside of the range of healthy volunteers
- Ethical obstacle to obtain matrix from patients (+ medication) *or* expensive
- Lyopholized Cu-Ceruloplasmin shows different stability profile than “native” Cu-Ceruloplasmin
- “Depletion” of the matrix using selective Ceruloplasmin antibodies leads to a non-representative matrix
  - Efficiency was limited

# Solutions

- Use “low” and “High” native concentration plasma samples for the calibration curve and QC’s
- Prepare Cu-EDTA concentration bij addition of Cu to (diluted) plasma samples
- Prepare Cu-Ceruloplasmin concentrations by dilution of a high concentration plasma pool

- Things to consider with this approach:
  - Matrix effect
  - Effect of dilution on equilibrium values
  - No direct information on Cu-ceruloplasmin concentration
    - *Lyophilized Ceruloplasmin insufficiently homogeneous, stated concentration too wide to be of use*

- 1) Determine total copper
- 2) Calibration curve in proxy matrix to determine Cu-EDTA concentration (in the chromatography assay)
- 3) the substractions yields the starting Cu-Ceruloplasmin concentration

# Performance

| Run ID: | Analysis date: | Concentration (ng/mL) LLOQ 17.1 | | LQC 42.7 | MQC 427 | HQC 854 |
|---|---|---|---|---|---|---|
| QCB2111-00447 | 23 Nov 2021 | 18.9 | | 44.6 | 465 | 868 |
| | | 18.1 | | 44.2 | 478 | 880 |
| | | 17.4 | | 43.1 | 441 | 846 |
| | | 17.8 | | 42.6 | 448 | 893 |
| | | 18.0 | | 43.6 | 442 | 862 |
| | | 18.9 | | 44.1 | 443 | 811 |
| Intra-run Mean | | 18.2 | | 43.7 | 453 | 860 |
| Intra-run SD | | 0.6 | | 0.7 | 15 | 29 |
| Intra-run %RE | | 6.3 | | 2.3 | 6.0 | 0.7 |
| Intra-run %CV | | 3.3 | | 1.7 | 3.4 | 3.4 |
| n | | 6 | | 6 | 6 | 6 |
| QCB2111-00566 | 30 Nov 2021 | 17.7 | | 46.7 | 461 | 883 |
| | | 18.9 | | 44.6 | 440 | 817 |
| | | 17.5 | | 44.3 | 422 | 795 |
| | | 17.3 | | 44.6 | 419 | 845 |
| | | 19.3 | | 44.0 | 417 | 747 |
| | | 19.2 | | 44.1 | 423 | 765 |
| Intra-run Mean | | 18.3 | | 44.7 | 430 | 809 |
| Intra-run SD | | 0.9 | | 1.0 | 17 | 51 |
| Intra-run %RE | | 7.1 | | 4.7 | 0.8 | -5.3 |
| Intra-run %CV | | 5.0 | | 2.2 | 4.0 | 6.3 |
| n | | 6 | | 6 | 6 | 6 |
| QCB2201-00447 | 26 Jan 2022 | 20.5 | | 46.6 | 469 | 835 |
| | | 20.9 | a) | 46.5 | 448 | 827 |
| | | 20.9 | a) | 48.8 | 465 | 845 |
| | | 21.2 | a) | 46.8 | 456 | 844 |
| | | 20.5 | | 47.0 | 443 | 809 |
| | | 20.3 | | 46.9 | 437 | 815 |
| Intra-run Mean | | 20.7 | | 47.1 | 453 | 829 |
| Intra-run SD | | 0.3 | | 0.9 | 13 | 15 |
| Intra-run %RE | | 21.2 | | 10.3 | 6.1 | -2.9 |
| Intra-run %CV | | 1.6 | | 1.8 | 2.8 | 1.8 |
| n | | 6 | | 6 | 6 | 6 |
| Inter-run Mean | | 19.1 | | 45.2 | 445 | 833 |
| Inter-run SD | | 1.4 | | 1.7 | 18 | 39 |
| Inter-run %RE | | 11.5 | | 5.8 | 4.3 | -2.5 |
| Inter-run %CV | | 7.1 | | 3.7 | 4.0 | 4.7 |
| n | | 18 | | 18 | 18 | 18 |

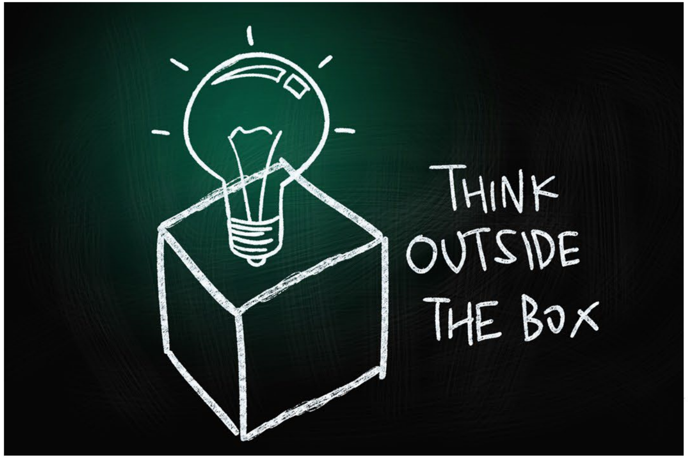
| Run ID: | Analysis date: | Concentration (ng/mL) LLOQ 21.0 | LQC 52.4 |
|---|---|---|---|
| QCB2111-00447 | 24 Nov 2021 | 23.1 | 51.1 |
| | | 20.3 | 54.6 |
| | | 22.5 | 53.0 |
| | | 22.1 | 53.3 |
| | | 21.6 | 55.3 |
| | | 23.1 | 54.9 |
| Intra-run Mean | | 22.1 | 53.7 |
| Intra-run SD | | 1.1 | 1.6 |
| Intra-run %RE | | 5.3 | 2.5 |
| Intra-run %CV | | 4.8 | 2.9 |
| n | | 6 | 6 |
| QCB2201-00447 | 26 Jan 2022 | 21.3 | 50.8 |
| | | 23.0 | 56.4 |
| | | 21.7 | 53.9 |
| | | 21.7 | 52.6 |
| | | 22.1 | 55.5 |
| | | 23.0 | 50.2 |
| Intra-run Mean | | 22.1 | 53.2 |
| Intra-run SD | | 0.7 | 2.5 |
| Intra-run %RE | | 5.4 | 1.6 |
| Intra-run %CV | | 3.2 | 4.7 |
| n | | 6 | 6 |
| QCB2204-00244 | 19 Apr 2022 | 22.6 | 49.8 |
| | | 21.6 | 50.0 |
| | | 23.6 | 51.6 |
| | | 23.9 | 55.4 |
| | | 24.2 | 56.7 |
| | | 24.1 | 53.5 |
| Intra-run Mean | | 23.3 | 52.8 |
| Intra-run SD | | 1.0 | 2.9 |
| Intra-run %RE | | 11.1 | 0.8 |
| Intra-run %CV | | 4.4 | 5.4 |
| n | | 6 | 6 |
| Inter-run Mean | | 22.5 | 53.3 |
| Inter-run SD | | 1.1 | 2.3 |
| Inter-run %RE | | 7.3 | 1.6 |
| Inter-run %CV | | 4.7 | 4.2 |
| n | | 18 | 18 |

# Take home messages

- ICP-MS can be a valuable tool to measure metallo-proteins
- Proxy matrices can be usefull, depending on the relative stability of the protein of interest in
- Working at – or above – normal references ranges is easier than below

- Be prepared to work outside of the guidelines: what is the goal, rather than trying to follow them

- With creativity most problems can be solved



# Thank You