

X Series ICP-MS Clinical Applications Note 2: Determination of Cu, Zn and Se in human serum samples

Key Words

- Copper
- Zinc
- Selenium
- Clinical analysis
- Serum

Introduction

Serum is the fluid that separates from clotted blood or blood plasma that is allowed to stand. It is similar in composition to plasma but lacks fibrinogen and other substances that are used in the coagulation process. Plasma itself is the straw coloured fluid in which blood cells are suspended. It consists of a solution of inorganic salts of sodium, potassium, calcium, etc., with a high concentration of protein (approximately 70 g/l) and a variety of trace substances. Serum is recognised as a useful medium in which to measure the body's copper, zinc and selenium levels, for the diagnosis and monitoring of certain diseases and for nutritional studies. Elevated copper levels in the body due to Wilson's disease can lead to liver, kidney and/or brain damage, as well as severe damage to the central nervous system. Copper deficiency can lead to inefficient utilization of iron and protein, and stunted growth. Zinc is implicated in the functioning of more than 200 enzymes, some of them associated with DNA and RNA synthesis, and is also involved with immune system functions. Selenium depletion (long term) has been suggested to be an increased risk factor for cancer and cardiovascular disease.

The matrix composition of serum is too heavy for it to be analysed directly by ICP-MS and usually there is insufficient sample to perform a direct measurement. So, most analysts dilute the samples typically 1:10 with either dilute acid or a customised diluent, appropriate for a broad range of clinical samples. The detection limits achievable with ICP-MS for Cu, Zn and Se are considerably below the levels found in serum, so dilution in this way does not compromise the analytical performance. Flame and graphite furnace AAS have generally been the techniques of choice for this application, but ICP-MS is becoming increasingly popular, mainly due to its fast, multi-element capability, ease of operation and simple method development. With the advent of collision cell technology, the well-known Ar-based interference problems that previously limited the detection limit capability of the technology for elements such as Se (Ar_2^+ interference) have been overcome. In addition, new developments in sample introduction technology have made it possible to efficiently aspirate clinical samples for long periods without causing nebuliser or torch injector blockage, leading to improved data

quality. This application note describes the performance of the X7 ICP-MS for serum analysis.

Sample preparation

All samples and standards were diluted 1:10 with a diluent containing the following reagents:

- 3% butan-1-ol, to match the carbon content of standards and samples, with the aim of ensuring that the ionisation efficiency of elements such as Se and As is the same in all solutions.
- 0.1% TAMA super cleaner (a high purity surfactant), to maintain a stable emulsion with the diluted sample.
- 0.05% HNO_3 , to ensure that trace elements are maintained in solution and to aid washout of these elements between samples. The acid concentration must be kept to a minimum, otherwise cellular components in blood samples in particular will precipitate or aggregate, thereby removing some analytes from solution.
- Ga, Rh and Re as low, mid and high mass internal standards respectively (only Ga was required for the application described here).

This customised diluent is also used for preparation of urine and blood samples, enabling a standard method to be used for all liquid clinical sample analyses.

Calibration solution preparation

External calibration solutions containing Cu (blank to 300 ng mL⁻¹), Zn (blank to 300 ng mL⁻¹) and Se (blank to 40 ng mL⁻¹) were prepared by serial dilution of parent 1000 µg mL⁻¹ stocks, using the same diluent used to dilute the samples.

Instrument configuration

The instrument was operated in standard and collision cell modes (using 8% (v/v) H_2 in He as the collision gas), with in-sample switching between the two modes. Standard (non-CCT) mode is the preferred method for acquiring data for ^{64}Zn , ^{65}Cu and ^{66}Zn and CCT mode is the better approach for measuring ^{63}Cu (eliminates the $^{40}\text{Ar}^{23}\text{Na}^+$ interference) and ^{80}Se (eliminates the $^{40}\text{Ar}_2^+$ interference) in a sodium-rich solution such as serum. The instrument was operated in X7 configuration (i.e. with a Peltier cooled impact bead spray chamber, single piece quartz torch (1.5 mm i.d. injector) and the enhanced sensitivity

PlasmaScreen Plus), together with Xi interface cones. A Burgener Miramist high solids parallel path nebuliser was used (see Figure 1) as this device does not block during aspiration of clinical samples and can be run at lower sample uptake rates (down to 0.4 ml min⁻¹) than conventional Burgener nebulisers.

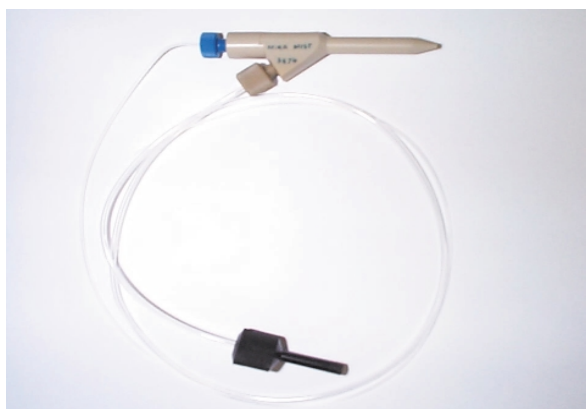


Figure 1. Burgener Miramist high solids parallel path nebuliser.

Instrument parameters

The instrument was operated using the following parameters:

RF power:	1340 W
Nebuliser gas flow:	0.78 L min ⁻¹
Auxiliary gas flow:	0.80 L min ⁻¹
Cool gas flow:	13.0 L min ⁻¹
CCT gas flow (8% H ₂ /He):	3 mL min ⁻¹
Isotopes measured:	⁶⁴ Zn, ⁶⁵ Cu, ⁶⁶ Zn, ⁷¹ Ga
Standard mode:	(internal standard) and ⁸² Se
CCT mode:	⁶³ Cu and ⁸⁰ Se
Dwell time per isotope:	20 ms
Sample uptake and wash time:	40 s
Sample acquisition time:	60 s per repeat (3 repeats per sample, 5 for the blank)

Data was obtained for Cu and Se in both standard and CCT modes, in order to compare the performance of both modes of operation.

Results and discussion

For the sample analysis, in-sample analysis mode-switching between standard and CCT mode was used. With the X Series ICP-MS, stable and reproducible automatic switching between different operation modes can be easily achieved, with a settle time of only 30 seconds to allow all the parameters to adjust to their required values and for the analyte signals to stabilise. Figure 2 below illustrates how reproducibly and rapidly the X Series can automatically switch from standard operation to CCT mode and back to standard again, for each sample in a run.

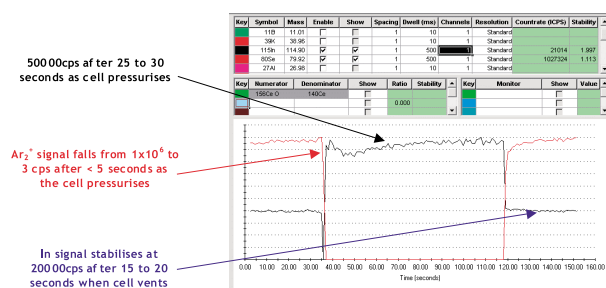


Figure 2. Reproducibility of in-sample switching.

Three well-characterised reference serum samples were supplied for analysis. The calibrations obtained for the target analytes are shown in Figures 3(a) to (f) below.

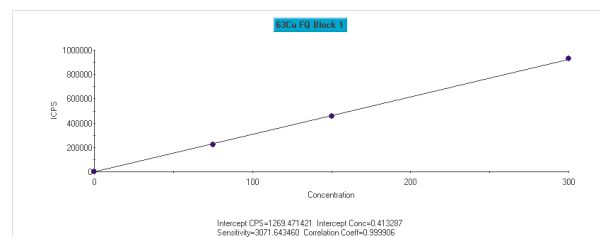


Figure 3(a). ⁶³Cu calibration (CCT mode), blank to 300 ng mL⁻¹.

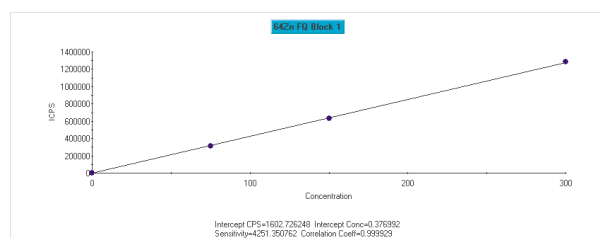


Figure 3(b). ⁶⁴Zn calibration (standard mode), blank to 300 ng mL⁻¹.

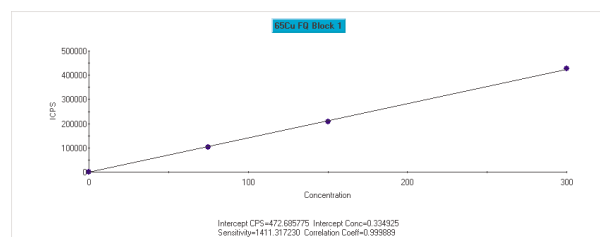


Figure 3(c). ⁶⁵Cu calibration (standard mode), blank to 300 ng mL⁻¹.

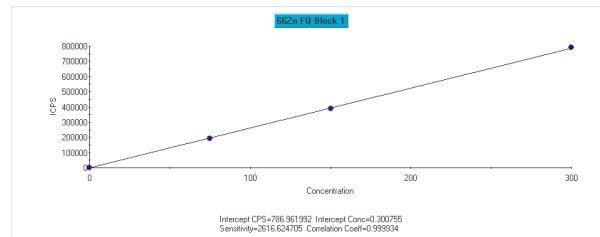


Figure 3(d). ⁶⁶Zn calibration (standard mode), blank to 300 ng mL⁻¹.

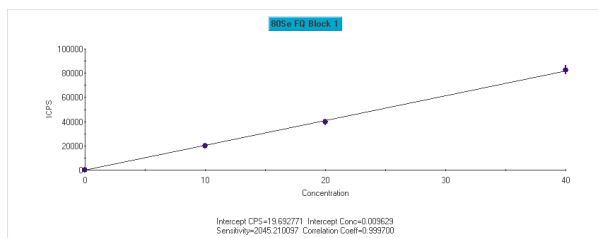


Figure 3(e). ⁸⁰Se calibration (CCT), blank to 40 ng mL⁻¹.

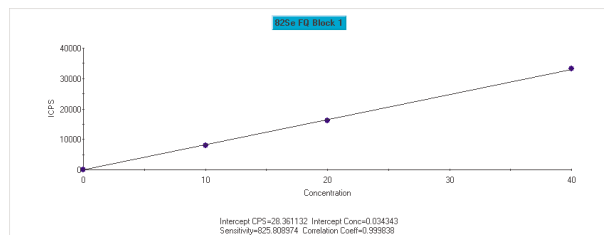


Figure 3(f). ⁸²Se calibration (standard mode), blank to 40 ng mL⁻¹.

The figures of merit for the above calibrations are presented below (Table 1).

PARAMETER	63Cu	65Cu	64Zn	66Zn	80Se	82Se
Sensitivity (cps/ ng mL ⁻¹)	3072	1402	4251	2617	2045	825
BEC* (ng mL ⁻¹)	0.41	0.34	0.38	0.30	0.01	0.03
3 σ detection limit (ng mL ⁻¹)	0.020	0.030	0.119	0.094	0.003	0.030

Table 1. Figures of merit for the calibrations.

* - blank equivalent concentration (= intercept counts per second / calibration slope)

The calibrations and the corresponding figures of merit show the excellent linearity, sensitivity and detection limit capability of the X Series ICP-MS when operated in both CCT and standard modes, using in-sample analysis mode-switching. The very low BEC achieved for ⁸⁰Se (0.01 ng mL⁻¹) demonstrates the efficiency of the collision cell for eliminating the ⁸⁰Ar₂⁺ interference. To put this in context, in standard mode this interference would lead to a BEC on ⁸⁰Se of between 200 and 250 ng mL⁻¹ i.e. up to 25000 x higher than achieved in CCT mode. The analysis results for each serum sample, together with the reference values, are presented below (Tables 2(a) and 2(b)).

SERUM SAMPLE 1						
ANALYTE	63Cu	65Cu	64Zn	66Zn	80Se	82Se
Measured value	341 ± 14	336 ± 2	673 ± 6	658 ± 6	56 ± 2	55 ± 1
Reference value	320	320	640	640	55	55
Relative accuracy (%)	107	105	105	103	102	100
Reported range	270 - 370		560 - 720		46 - 65	

Table 2(a). Analysis results (± 1 standard deviation) for serum sample 1, 1:10 dilution corrected (all concentration data in ng mL⁻¹).

SERUM SAMPLE 2						
ANALYTE	63Cu	65Cu	64Zn	66Zn	80Se	82Se
Measured value	1075 ± 12	1107 ± 9	1253 ± 22	1230 ± 14	127 ± 3	132 ± 3
Reference value	1062	1062	1232	1232	124	124
Relative accuracy (%)	101	104	102	100	102	106
Reported range	890 - 1110		1030 - 1260		109 - 136	

Table 2(b). Analysis results (± 1 standard deviation) for serum sample 2, 1:10 dilution corrected (all concentration data in ng mL⁻¹).

The measured results for all three analytes in both samples were found to be in excellent agreement with the reference values and in all cases, fell within the expected concentration range (based on measurements of these samples performed in other laboratories). Good agreement was also achieved between CCT and standard mode measurements, although, as noted in Table 1, lower detection limits could be achieved for Se using ⁸⁰Se, so this is the preferred isotope for routine measurement. All the measured values were far above the detection limit achievable with the X Series ICP-MS, even with the 10-fold dilution. Excellent relative accuracy results (i.e. relative to the reference values) were obtained for all the isotopes measured. For Se in particular these results again illustrate the extremely effective attenuation of the ⁴⁰Ar₂⁺ interference on ⁸⁰Se achieved with the X Series ICP-MS.

Conclusions

The X Series ICP-MS is an ideal instrument for trace element determination in clinical samples. It is a robust, easy to use bench-top instrument, well-adapted for incorporation into today's clinical laboratory. With its high sensitivity PlasmaScreen Plus, matrix tolerant Xi interface options and powerful polyatomic interference attenuation collision cell with in-sample analysis mode-switching capabilities, the X Series ICP-MS can easily achieve the required measurement accuracy and detection limits for all the elements that are currently of clinical interest, with only a simple dilution of the samples being required before analysis.

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