thermoscientific

Analysis of Whole Blood using ICP-MS: Effective, Productive and Accurate High Throughput Analysis

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ABSTRACT

The concentration of trace elements in biological samples can give valuable insights in research applications. ICP-MS can be a powerful tool for screening biological samples such as urine, blood or serum on a routine basis. Current generation instruments reliably remove all potentially occurring interferences and assure high throughput analysis with samples containing salt or organic materials.

This poster highlights the possibility for robust and accurate high throughput analysis of blood samples using ICP-MS. The proposed method was tested for linearity, accuracy and precision using certified reference standards. Long term analysis was simulated using porcine blood as a sample.

INTRODUCTION

Heavy metals such as cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), and thallium (Tl) are known to be highly toxic elements with potentially adverse health effects to humans. Other elements, such as copper (Cu), nickel (Ni), zinc (Zn) or selenium (Se) are known to be essential, vital for various metabolic activities or showing antioxidant properties. One of the objectives for clinical research is to investigate potential pathways of ingestion of toxic metals or to discover thresholds for adverse effects, and, consequently, the determination of applicable limits and guidelines. At the same time, it is important to not only monitor potentially harmful elements, but also to include essential elements into the analytical method to discover potential reciprocal effects.

To conduct a large biomonitoring survey requires a rapid, yet accurate and precise analytical methods. Inductively coupled plasma mass spectrometry (ICP-MS) is known as a highly sensitive and robust technique for the determination of a wide range of elements. Thus, an analytical method based on single quadrupole based ICP-MS enabling high throughput analysis in whole blood was developed for a variety of elements, including both toxic and essential. Blood is a complex matrix, which typically causes challenges during ICP based analysis, mainly because of the presence of salts and biomolecules, such as proteins or metabolites. In many cases, also the available amount of sample for analysis is very limited.

MATERIALS AND METHODS

A Thermo Scientific™ iCAP™ RQ ICP-MS was used for analysis in combination with a Teledyne Cetac ASX 560 autosampler and an ASXpress valve system. This setup allows to drastically reduce the time required for delivery of the sample to the plasma and rinsing before analysis of the next sample. At the same time, the contact between sample and the components of the sample introduction system is reduced, allowing to reduce matrix effects and the risk of carryover.

Table 1. Typical operating parameters iCAP RQ ICP-MS

iCAP RQ ICP-MS	
Nebuliser	MicroMist Nebuliser (400 µl/min)
Interface	Ni Sample and Skimmer cone, High Matrix Skimmer cone insert
Spray chamber	Cyclonic quartz
Injector	Quartz, 2.5 mm ID
Nebuliser Flow	1.10 L·min^{-1}
RF Power	1550 W
Number of Replicates	3
CRC Conditions	4. 8 mL·min^{-1} pure Helium, 3 V Energy barrier
Scan settings	0.05 s per analyte, 3 Main runs
Total Analysis time per sample	45 s

SAMPLE PREPARATION

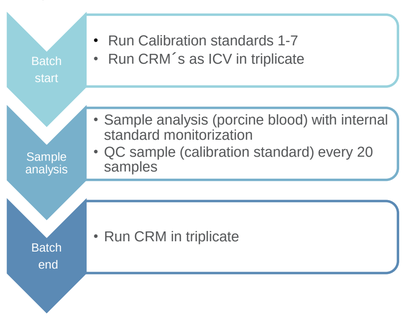
All blood samples were diluted manually to a final acid concentration of 0.5%. In brief, a 0.1 ml aliquot whole blood was transferred into pre-cleaned sample tubes followed by the addition of 4 ml of UPW, 50 µl of internal standard stock solution (containing 10 µg·g^{-1} of Be, 0.1 µg·g^{-1} of Ga, Y, Tb, and Ir). After addition of 25 µl of concentrated HNO_3 (OPTIMA™ grade, Fisher Scientific), the sample was made up to a total volume of 5 mL, mixed thoroughly using a vortex shaker and analysed.

For preliminary method validation, synthetic whole blood certified reference materials (Trace Elements in Whole Blood, L-1, L2 and L-3, Seronorm™, Sero AS, Norway) were reconstituted according to the manufacturer´s instructions and subjected to the dilution procedure before analysis. For simulation of long-term sample analysis, porcine blood samples were prepared following the same procedure. For quantitative assessment of all elements in a single method, external calibration was chosen and a calibration curve containing at least five different concentration levels per analyte was generated from multi-element stock solutions. Although in routine analysis of a large number of samples likely less standards would be used, this approach nicely demonstrates the linear range of the proposed method.
Table 2 contains a complete overview of all analytes and concentration ranges. As can be seen, the linearity of the method was demonstrated over at least three orders of magnitude for each analyte, including both trace levels (e.g. for As, Cd, Hg and Pb) as well as major constituents such as Mg, Ca or Zn.

Table 1. Concentration of elements in linearity standard solutions (ng·mL^{-1})

Analytes	LL-1	LL-2	LL-3	LL-4	LL-5	LL-6	LL-7
Sb, As, Bi, Cd, Cr, Co, Hg, Ni, Tl, Mo, Sn, Mo, Sn, V	0.002	0.005	0.025	0.05	0.25	0.5	1
Pb, Mn, Se	0.02	0.05	0.25	0.5	2.5	5	10
Mg, Ca	3	7.5	37.5	75	375	750	1500
Cu	0.2	0.5	2.5	5	25	50	100
Zn	0.5	1.25	6.25	12.5	62.5	125	250

In total, two sequences were run on two independent days. Each contained a calibration curve generated by a series of standard solutions, followed by the analysis of all certified reference materials (L1, L2 and L3) in triplicate. After successful passing this initial quality control test, blocks of 20 unknown samples of porcine blood were analysed followed by a QC standard. In total, up to 400 unknown samples were analysed each day in slightly more than six hours, including all calibration and QC standards. After each batch, the certified reference materials were re-analyzed. The resulting sample volume of 5 mL (derived from 0.1 mL of whole blood) allowed to conduct at least two independent readings with each sample



RESULTS

The accuracy and precision of the analytical method has been assessed by analysing commercially available certified reference materials (CRMs) with different concentration levels. The exact concentrations in each level are summarized in Table 3.

Table 3. Certified concentrations and acceptable range for the different certified reference materials used in this study

Analytes	Unit	Certified Value L-1	Range	Certified Value L-2	Range	Certified Value L-3	Range
Antimony (Sb)	µg/L	3.3	2.6 – 4.0	22.3	17.8 – 26.8	21.9	17.5 – 26.3
Arsenic (As)	µg/L	2.1	1.7 – 2.5	12.2	9.8 – 14.7	27.3	21.8 – 32.7
Bismuth (Bi)	µg/L	< 0.005		4.9	3.9 – 5.9	47.0	37.5 – 56.4
Cadmium (Cd)	µg/L	0.28	0.23 – 0.34	5.1	4.1 – 6.1	9.9	7.9 – 11.9
Calcium (Ca)	mg/L	15.8	12.6 – 19.0	56	45 - 68	NA	NA
Chromium (Cr)	µg/L	0.77	0.61 – 0.92	10.0	8.0 – 12.0	35.5	28.4 – 42.6
Cobalt (Co)	µg/L	0.22	0.18 – 0.26	5.0	4.0 – 6.0	10.3	8.3 – 12.4
Copper (Cu)	mg/L	0.64	0.59 – 0.70	0.98	0.89 – 1.06	2.08	1.66 – 2.50
Lead (Pb)	µg/L	10.0	7.9 – 12.0	303	272 - 334	389	310 - 467
Magnesium (Mg)	mg/L	15.2	12.1 – 18.3	41.0	32.7 – 49.2	NA	NA
Manganese (Mn)	µg/L	19.7	18.1 – 21.3	24.2	22.2 – 26.1	33.3	26.6 – 39.9
Mercury (Hg)	µg/L	1.57	1.25 – 1.88	16.6	13.3 - 20.0	25.8	20.6 – 31.0
Molybdenum (Mo)	µg/L	0.37	0.30 – 0.45	4.5	3.6 – 5.4	6.2	4.9 – 7.4
Nickel (Ni)	µg/L	2.13	1.70 – 2.56	9.2	7.3 – 11.0	11.0	8.8 – 13.3
Selenium (Se)	µg/L	69	54 - 84	144	113 - 175	198	158 - 238
Thallium (Tl)	µg/L	0.007	0.005 - 0.008	10.1	8.1 – 12.1	25.2	20.1 – 30.2
Tin (Sn)	µg/L	0.21	0.17 – 0.25	4.7	3.7 – 5.6	9.9	7.9 – 11.9
Vanadium (V)	µg/L	0.26	0.21 – 0.31	3.1	2.4 – 3.7	4.4	3.5 – 5.3
Zinc (Zn)	mg/L	4.6	3.8 – 5.3	5.8	4.8 -6.8	9.42	7.52 – 11.31

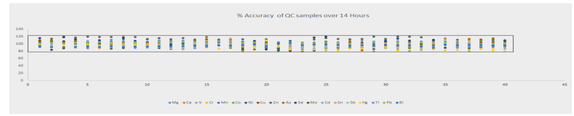
Each level of blood CRM sample was prepared in triplicate and analysed on each day the instrument was operated. The obtained results indicate that the determination of all analytes under study has been accomplished with outstanding accuracy at all concentration levels. The associated low values for relative standard deviation between different preparations indicates that the chosen sample preparation strategy based on simple 50 times dilution, in combination with the discrete sampling valve, helps to consistently overcome the challenges of the direct analysis of whole blood, not only within one batch of prepared samples, but also for different preparations on e.g. different days.

Table 3. Results obtained for all concentration levels

Analytes	Recovery L-1 [%]	RSD [%]	Recovery L-2 [%]	RSD [%]	Recovery L-3 [%]	RSD [%]
Antimony (Sb)	100.0	1.2	94.2	1.2	105.9	1.6
Arsenic (As)	95.2	1.9	102.5	1.9	114.7	2.1
Bismuth (Bi)			104.1	2.2	92.6	2.4
Cadmium (Cd)	107.1	1.2	98.0	1.2	108.1	0.5
Calcium (Ca)	99.4	0.5	105.4	0.5		
Chromium (Cr)	127.3	0.5	106.0	0.5	115.2	0.9
Cobalt (Co)	104.5	2.2	104.0	2.2	108.7	1.0
Copper (Cu)	103.1	0.6	105.1	0.6	110.6	2.5
Lead (Pb)	101.0	2.0	94.1	2.0	97.2	2.5
Magnesium (Mg)	102.0	0.4	104.6	0.4		
Manganese (Mn)	97.0	0.9	97.9	0.9	115.3	1.7
Mercury (Hg)	99.4	1.8	101.8	1.8	88.8	1.3
Molybdenum (Mo)	97.3	2.4	106.7	2.4	114.5	0.8
Nickel (Ni)	97.2	1.2	104.3	1.2	107.3	2.0
Selenium (Se)	92.8	5.2	92.4	5.2	113.1	1.8
Thallium (Tl)			102.0	2.6	114.3	1.6
Tin (Sn)	95.2	2.5	95.7	2.5	107.1	0.5
Vanadium (V)	103.8	3.8	96.8	3.8	106.8	1.2
Zinc (Zn)	100.0	0.9	105.2	0.9	98.7	1.2

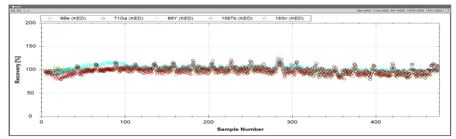
To enable rugged high throughput analysis of a large number of blood samples per batch, the analytical system must be extremely robust to avoid unwanted interruptions through drift or QC failures. The stability of the system has been verified during the analysis of in total 960 samples including 770 real blood samples over two days and a total of 14 hours. A summary of the results of all QC samples is given in figure 1. As can be seen, the average recovery for all analytes is closely within the expected range of ± 20%, indicating that no QC failures occurred during the runtime of the analysis.

Figure 7. Response for all QCs samples in both runs



The response of the internal standards is shown in figure 2, obtained on the second consecutive day of analysing blood samples. As with the response of the applicable QC tests, the response of the internal standards is well within the typically expected range and would not cause any failures or the need to re-run samples.

Figure 7. Response of the internal standards over a period of 7 hours (day 2, approximately 450 samples)



CONCLUSIONS

- An analytical method for the analysis of 19 elements (in a total runtime of only 45 seconds per sample) using single quadrupole ICP-MS has been developed and thoroughly tested for its performance
- It could be demonstrated that all elements could be analysed with high accuracy and precision as was demonstrated through the results obtained for certified reference materials
- The proposed sample preparation using direct dilution of whole blood by a factor of 50 using nitric acid has proven to be reliable and assuring good chemical stability for all analytes and effective elimination of potentially occurring matrix effects.

REFERENCES

1. Application Note 44453; Fast and accurate determination of essential and toxic elements in whole blood using the Thermo Scientific iCAP RQ ICP-MS for clinical research, Thermo Fisher Scientific

TRADEMARKS/LICENSING

PO44455-EN 0120S

ThermoFisher SCIENTIFIC