TECHNICAL NOTE 44453

Fast and accurate determination of essential and toxic elements in whole blood using ICP-MS for clinical research

Authors: Bhagyesh Surekar, Daniel Kutscher
Thermo Fisher Scientific, Bremen, Germany

Keywords: Essential elements, toxic elements, whole blood, iCAP RQ ICP-MS, quality control, biological samples

Goal

To demonstrate a simple, fast, robust and accurate analytical method for the determination of essential and toxic elements in a whole blood sample using single quadrupole ICP-MS.

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 for 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.

Some of the objectives for clinical research are 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 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 using 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, the available amount of sample for analysis is also very limited.

To prepare blood samples for analysis, they often are pre-treated with chelating agents such tetramethylammonium hydroxide (TMAH) or ethylenediaminetetraacetic acid (EDTA) to avoid coagulation of the blood. Alternatively, a closed vessel microwave digestion could also be applied to reduce the impact of the matrix. However, for large occupational health studies, it is important to have methods available that are fast (to allow for high throughput), yet robust (to allow for extended analysis time without maintenance being required), and able to provide accurate results from a limited amount of sample. Direct analysis of whole blood using ICP-MS after dilution is a very promising way. Combined with available accessories for discrete sampling, analysis times of less than 60 seconds for a full readout of both toxic and essential elements is possible.

This note highlights the possibility for robust and accurate high-throughput analysis of blood samples using ICP-MS. In total, the concentration for 19 elements (including toxic as well as essential elements) could be determined in 45 seconds per sample. Samples were diluted 50 times using nitric acid and were analyzed using external calibration. 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.

Experimental

Sample preparation and analysis

All blood samples were diluted manually to a final acid concentration of 0.5%. In brief, a 0.1 mL aliquot of whole blood was transferred into pre-cleaned sample tubes followed by the addition of 4 mL of UPW and 50 µL of internal standard stock solution (containing 10 $\mu g \cdot g^{-1}$ of Be, 0.1 $\mu g \cdot g^{-1}$ 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 analyzed.

For preliminary method validation, synthetic whole blood certified reference materials (Trace Elements in Whole Blood, L-1, L-2 and L-3, Seronorm™, Sero AS, Norway) were reconstituted according to the manufacturer´s instructions and subjected to the dilution procedure mentioned above before analysis. For simulation of long-term sample analysis, porcine blood samples were prepared following the same procedure. The blood was obtained from a local butcher.

All elements were analyzed using kinetic energy discrimination (KED) with pure helium as a collision gas. Because of the unique properties of the QCell collision/reaction cell (CRC), all elements regardless of mass can be analyzed at low levels, although lighter elements such as magnesium may suffer from a slight reduction in sensitivity as compared to standard mode (with the CRC acting as an ion guide). At the same time, the numerous (mostly polyatomic) interferences affecting key analytes such as vanadium, chromium or arsenic, are effectively removed.

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 fewer standards would likely be used, this approach nicely demonstrates the linear range of the proposed method.

Table 1 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 ($ng \cdot mL^{-1}$)

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

Instrumentation

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 drastic reduction in 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, reducing matrix effects and the risk of carryover. In brief, the sample is delivered into a sample loop of suitable volume using a vacuum pump. Once filled, the valve position of the six-port valve is switched to Inject and a carrier solution (0.5% HNO_3) pushes the sample to the plasma for analysis. At the same time, the autosampler probe and tubings can be rinsed again using the vacuum pump for analysis of the next sample. Tables 2 and 3 give an overview of the typical parameters for analysis.

Table 2. Typical operating parameters iCAP RQ ICP-MS

Operating parameters	
Nebulizer	MicroMist™ Nebulizer (400 µL/min)
Interface cones	Ni – tipped sample and skimmer
Skimmer cone insert	High matrix, 3.5 mm
Spray chamber	Cyclonic quartz
Injector	Quartz, 2.5 mm ID
Auxiliary flow	0.8 $L \cdot min^{-1}$
Cool gas flow	14 $L \cdot min^{-1}$
Nebulizer flow	1.10 $L \cdot min^{-1}$
RF power	1550 W
Number of replicates	3
Spray chamber temp.	2.7 °C
CRC gas	Helium
Helium flow	4.8 $mL \cdot min^{-1}$
Dwell time	0.05 s
Main runs	3
Total analysis time per sample	45 s

Table 3. Teledyne CETAC ASX-560 Autosampler and ASXpress fast sampling module parameters

CETAC ASX-560 Autosampler parameters	
Uptake time	15 s
Wash time	0 s
ASXpress parameters	
Extra loop rinse	True
Loop size	0.7 mL
Loop rinse delay	1 s
Loop evacuation delay	1 s
Loop load time	0.9 s
Equalization delay	1 s
Time to evacuate probe	1
Probe wash	5
Rinse station fill	10

The instrument was tuned daily using the autotune procedures provided in the Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution™ Software. A summary of the instrument´s performance was generated before starting an analysis sequence each day. 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 (L-1, L-2 and L-3) in triplicate. After this initial quality control test was successfully passed, blocks of 20 unknown samples of porcine blood were analyzed followed by a QC standard. In total, up to 400 unknown samples were analyzed 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 at least two independent readings to be conducted with each sample using the proposed setup.

The data acquisition and processing has been controlled using the Qtegra ISDS Software. The software features dedicated plug-ins for control of state-of-the-art sampling accessories such as autosamplers or discrete sampling valves, meaning that all steps in the above described procedure are fully automated and traceable inside the native data files created in Qtegra ISDS Software. The software also includes a comprehensive feature set for quality control testing, such as initial and continuous calibration verification (ICV and CCV) or recovery testing (quality control standards (QCS) or matrix spikes (MXS)).

Results and discussion

Initial method performance

Linearity is an extremely important parameter for trace level analysis using ICP-MS. As mentioned previously, at least five calibration standards were used to calibrate the instrument for analysis. At the same time, key parameters related to the analytical performance could be retrieved, such as the instrumental detection limit (IDL, indicating the minimum concentration of a given analyte that can be detected) and the blank equivalent concentration (BEC, indicating the cleanliness of the system or the degree of interference removal) (Table 4).

Accuracy and precision for the analysis of whole blood

The accuracy and precision of the analytical method has been assessed by analyzing commercially available certified reference materials (CRMs) with different concentration levels. The exact concentrations in each level are summarized in Tables 5, 6 and 7, respectively.

Table 4. Correlation coefficients and instrument detection limits (IDLs) of analytes obtained from the linearity study (calibration ranges - see Table 1)

Analytes	Correlation coefficient (R^2)	Instrumental detection limit (IDL) [ng·mL^{-1}]	Blank equivalent concentration (BEC) [ng·mL^{-1}]
Antimony (Sb)	>0.999	0.001	0.0008
Arsenic (As)	>0.998	0.004	0.0007
Bismuth (Bi)	>0.9999	0.0002	0.0004
Cadmium (Cd)	>0.999	0.0002	<0.0001
Calcium (Ca)	>0.9999	0.053	0.17
Chromium (Cr)	>0.9999	0.002	0.006
Cobalt (Co)	>0.9999	0.0002	0.0001
Copper (Cu)	>0.999	0.0004	0.004
Lead (Pb)	>0.999	0.0003	0.001
Magnesium (Mg)	>0.9999	0.089	0.18
Manganese (Mn)	>0.9999	0.0004	0.001
Mercury (Hg)	>0.9999	0.0009	0.002
Molybdenum (Mo)	>0.999	0.0007	0.0005
Nickel (Ni)	>0.9999	0.0008	0.004
Selenium (Se)	>0.9999	<0.0001	<0.0001
Thallium (Tl)	>0.9999	<0.0001	0.0001
Tin (Sn)	0.999	0.0008	0.002
Vanadium (V)	>0.9999	0.003	0.01
Zinc (Zn)	0.999	0.006	0.08

Table 5. Comparison of CRM values and experimental concentrations values obtained for CRM L-1 (N/A = not applicable. n.d. = not detected)

Analytes	Certified value	Unit	Range	Mean	STDEV	RSD [%]
Antimony (Sb)	3.3	$\mu g \cdot L^{-1}$	2.6–4.0	3.3	0.06	1.8
Arsenic (As)	2.1	$\mu g \cdot L^{-1}$	1.7–2.5	2.0	0.06	2.8
Bismuth (Bi)	< 0.005	$\mu g \cdot L^{-1}$	N/A	n. d.	N/A	N/A
Cadmium (Cd)	0.28	$\mu g \cdot L^{-1}$	0.23–0.34	0.30	0.02	7.0
Calcium (Ca)	15.8	$mg \cdot L^{-1}$	12.6–19.0	15.7	0.35	2.2
Chromium (Cr)	0.77	$\mu g \cdot L^{-1}$	0.61–0.92	0.98	0.09	8.8
Cobalt (Co)	0.22	$\mu g \cdot L^{-1}$	0.18–0.26	0.23	0.02	8.6
Copper (Cu)	0.64	$mg \cdot L^{-1}$	0.59–0.70	0.66	0.01	1.5
Lead (Pb)	10.0	$\mu g \cdot L^{-1}$	7.9–12.0	10.1	0.25	2.5
Magnesium (Mg)	15.2	$mg \cdot L^{-1}$	12.1–18.3	15.5	0.27	1.7
Manganese (Mn)	19.7	$\mu g \cdot L^{-1}$	18.1–21.3	19.1	0.12	0.6
Mercury (Hg)	1.57	$\mu g \cdot L^{-1}$	1.25–1.88	1.56	0.08	5.4
Molybdenum (Mo)	0.37	$\mu g \cdot L^{-1}$	0.30–0.45	0.36	0.01	3.2
Nickel (Ni)	2.13	$\mu g \cdot L^{-1}$	1.70–2.56	2.07	0.06	2.8
Selenium (Se)	69	$\mu g \cdot L^{-1}$	54–84	64	0.58	0.9
Thallium (Tl)	0.007	$\mu g \cdot L^{-1}$	5–8	n.d.	N/A	N/A
Tin (Sn)	0.21	$\mu g \cdot L^{-1}$	0.17–0.25	0.20	0.01	2.8
Vanadium (V)	0.26	$\mu g \cdot L^{-1}$	0.21–0.31	0.27	0.04	15.7
Zinc (Zn)	4.6	$mg \cdot L^{-1}$	3.8–5.3	4.6	0.10	2.2

Table 6. Comparison of CRM values and Experimental concentrations values obtained for CRM L-2

Analytes	Certified value	Unit	Range	Mean	STDEV	RSD [%]
Antimony (Sb)	22.3	$\mu g \cdot L^{-1}$	17.8–26.8	21.0	0.25	1.2
Arsenic (As)	12.2	$\mu g \cdot L^{-1}$	9.8–14.7	12.5	0.23	1.9
Bismuth (Bi)	4.9	$\mu g \cdot L^{-1}$	3.9–5.9	5.1	0.12	2.2
Cadmium (Cd)	5.1	$\mu g \cdot L^{-1}$	4.1–6.1	5.0	0.06	1.2
Calcium (Ca)	56	$mg \cdot L^{-1}$	45–68	59	0.32	0.5
Chromium (Cr)	10.0	$\mu g \cdot L^{-1}$	8.0–12.0	10.6	0.06	0.5
Cobalt (Co)	5.0	$\mu g \cdot L^{-1}$	4.0–6.0	5.2	0.12	2.2
Copper (Cu)	0.98	$mg \cdot L^{-1}$	0.89–1.06	1.03	0.01	0.6
Lead (Pb)	303	$\mu g \cdot L^{-1}$	272–334	285	5.77	2.0
Magnesium (Mg)	41.0	$mg \cdot L^{-1}$	32.7–49.2	42.9	0.17	0.4
Manganese (Mn)	24.2	$\mu g \cdot L^{-1}$	22.2–26.1	23.7	0.21	0.9
Mercury (Hg)	16.6	$\mu g \cdot L^{-1}$	13.3–20.0	16.9	0.30	1.8
Molybdenum (Mo)	4.5	$\mu g \cdot L^{-1}$	3.6–5.4	4.8	0.12	2.4
Nickel (Ni)	9.2	$\mu g \cdot L^{-1}$	7.3–11.0	9.6	0.12	1.2
Selenium (Se)	144	$\mu g \cdot L^{-1}$	113–175	133	6.93	5.2
Thallium (Tl)	10.1	$\mu g \cdot L^{-1}$	8.1–12.1	10.3	0.26	2.6
Tin (Sn)	4.7	$\mu g \cdot L^{-1}$	3.7–5.6	4.5	0.12	2.5
Vanadium (V)	3.1	$\mu g \cdot L^{-1}$	2.4–3.7	3.0	0.12	3.8
Zinc (Zn)	5.8	$mg \cdot L^{-1}$	4.8–6.8	6.1	0.06	0.9

Table 7. Comparison of CRM values and Experimental concentrations values obtained for CRM L-3

Analytes	Certified value	Unit	Range	Mean	STDEV	RSD [%]
Antimony (Sb)	21.9	µg·L^{-1}	17.5–26.3	23.2	0.38	1.6
Arsenic (As)	27.3	µg·L^{-1}	21.8–32.7	31.3	0.67	2.1
Bismuth (Bi)	47.0	µg·L^{-1}	37.5–56.4	43.5	1.04	2.4
Cadmium (Cd)	9.9	µg·L^{-1}	7.9–11.9	10.7	0.06	0.5
Calcium (Ca)	N/A	mg·L^{-1}	N/A	N/A	N/A	N/A
Chromium (Cr)	35.5	µg·L^{-1}	28.4–42.6	40.9	0.38	0.9
Cobalt (Co)	10.3	µg·L^{-1}	8.3–12.4	11.2	0.12	1.0
Copper (Cu)	2.08	mg·L^{-1}	1.66–2.50	2.3	0.06	2.5
Lead (Pb)	389	µg·L^{-1}	310–467	378	9.50	2.5
Magnesium (Mg)	N/A	mg·L^{-1}	N/A	N/A	N/A	N/A
Manganese (Mn)	33.3	µg·L^{-1}	26.6–39.9	38.4	0.65	1.7
Mercury (Hg)	25.8	µg·L^{-1}	20.6–31.0	22.9	0.29	1.3
Molybdenum (Mo)	6.2	µg·L^{-1}	4.9–7.4	7.1	0.06	0.8
Nickel (Ni)	11.0	µg·L^{-1}	8.8–13.3	11.8	0.23	2.0
Selenium (Se)	198	µg·L^{-1}	158–238	224	4.00	1.8
Thallium (Tl)	25.2	µg·L^{-1}	20.1–30.2	28.8	0.47	1.6
Tin (Sn)	9.9	µg·L^{-1}	7.9–11.9	10.6	0.06	0.5
Vanadium (V)	4.4	µg·L^{-1}	3.5–5.3	4.7	0.06	1.2
Zinc (Zn)	9.42	mg·L^{-1}	7.52–11.31	9.3	0.12	1.2

Each level of blood CRM sample was prepared in triplicate and analyzed 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 a 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, for example, on different days.

Long term stability study

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 by monitoring the response of the internal standard during the analysis of in total 960 samples including 770 real blood samples over two days and a total of 14 hours. In addition, the recovery of the regularly interspersed QC standards (every 20 samples) has also been evaluated.

A summary of the results of all QC samples is given in Figure 1. 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.

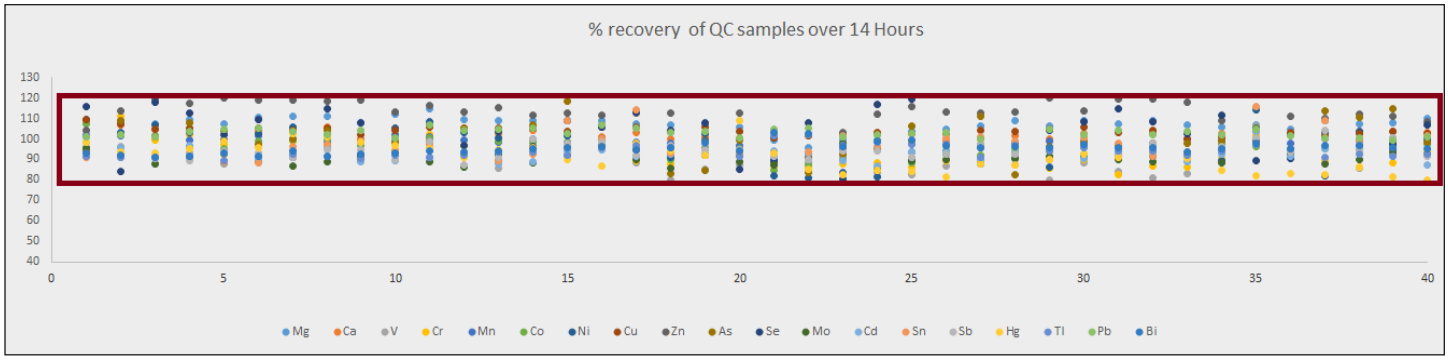

Figure 1. Percent recovery of all QC tests conducted during both runs (N=40) for all analytes

The response of the internal standards is shown in Figure 2, obtained on the second consecutive day of analyzing 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.

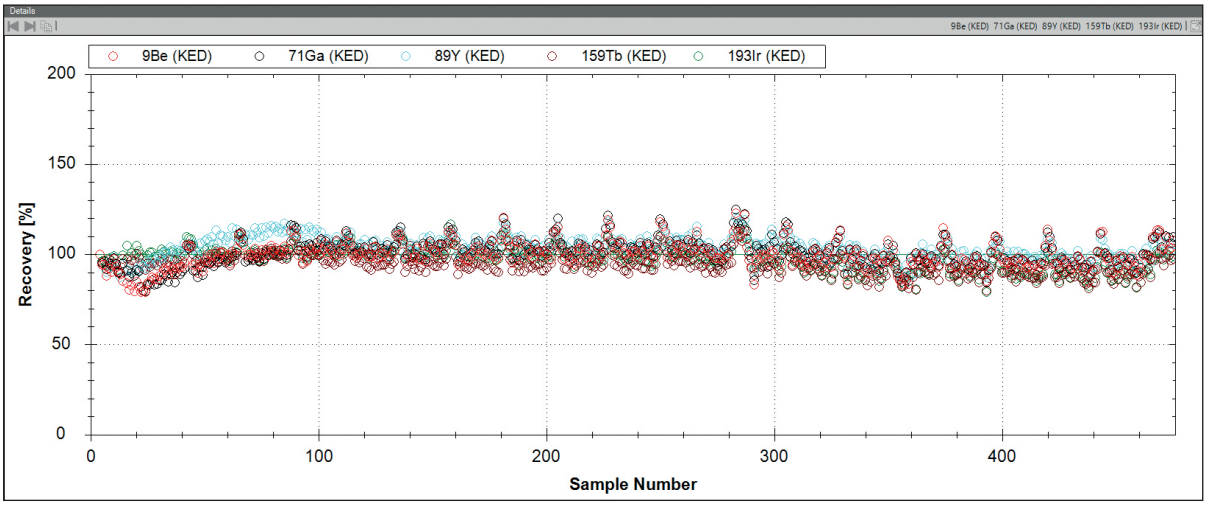

Figure 2. Recovery of the internal standards monitored over period of seven hours (Day 2)

Conclusion

An analytical method for the analysis of 19 elements using single quadrupole ICP-MS has been developed and thoroughly tested for its performance in, for example, a clinical research study involving a large number of samples. All elements could be analyzed with high accuracy and precision as demonstrated from the results obtained for certified reference materials. The proposed sample preparation with direct dilution of whole blood ensured chemical stability for all analytes and effective elimination of potential matrix effects. At the same time, the analytical method tested here allows for full trace elemental analysis in only 45 seconds per sample, enabling the high sample throughput and ensuring high laboratory productivity with limited instrument downtime.

Find out more at **thermofisher.com/ICP-MS**

ThermoFisher SCIENTIFIC