Clinical research

Analysis of whole blood samples using triple quadrupole inductivity coupled plasma mass spectrometry (ICP-MS)

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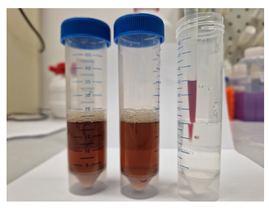
Abstract

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

Methods: A Thermo Scientific™ iCAP™ MTX ICP-MS, together with a Thermo Scientific™ iSC-65 Autosampler, was used for all analyses. Thermo Scientific™ Qtegra™ Intelligent Scientific Data Solution (ISDS) Software was used for instrument operation, data acquisition and subsequent calculations and reporting.

Results: The analytical method was rigorously tested for performance. Accurate method for the simultaneous analysis of major, essential and trace elements has been developed. The analysis results demonstrated excellent agreement with the certified values of the three certified reference materials (CRMs) of whole blood samples.

Introduction



Analysis of biological samples for essential and trace elements provides critical information for clinical research and forensic toxicology. Exposure to toxic metals is a risk factor for diseases, while other elements support biological functions when present at appropriate levels.

Element concentrations in blood can correlate with geographical area, lifestyle, and socio-demographic factors.

Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) is a highly sensitive technique that works with low sample volumes, enabling high throughput and robust analysis, even for demanding sample types. It can determine a wide range of elements, making it ideal for clinical research and occupational exposure analysis. However, analyzing samples with high levels of salts and biomolecules is challenging. The complex sample matrix can affect sensitivity, cause internal standard fluctuations, and increase system maintenance due to obstruction of the interface cones and adverse effects on the plasma conditions.

Developing a multi-element method is challenging due to the wide concentration ranges of essential and toxic elements and potential interferences. These challenges often make triple quadrupole ICP-MS instruments, the best choice for the analysis

Materials and methods

Sample preparation

- Whole blood samples were diluted 50-fold with a solution containing 0.01% Triton™ X-100, 0.05% NH_4OH, 0.02% EDTA, and 0.5% HNO_3.
- Calibration blanks, standards and quality control samples (Table 1), were prepared using the same diluent.
- Samples were spiked with an internal standard mix and gold solution was added to increase mercury stabilization.

Table 1. Details of the standard calibration, QC (CCV). All numbers are shown in µg·L^{-1}.

Standard group	STD-1	STD-2	STD-3	STD-4	STD-5	STD-6	STD-7	QC (CCV)
Al, As, Ag, Ba, Bi, Co, Cr, Ni, V, U, Pb, Mn, Ga, Tl, Se, Cd, In	0.002	0.025	0.05	0.2	1	10	50	0.2
Na, Mg, Fe, Si, P, K, Ca, S	200	1,000	10,000	50,000	-	-	-	1,000
Mo, Nb, Re, Ta, Ti, Ze, W	0.02	0.1	1	5	10	-	-	0.1
Sn, Sb, Pt	0.01	0.05	0.1	-	-	-	-	0.05
Hg, B, Li, Be	0.1	0.5	1	-	-	-	-	0.5
Cu, Zn, Rb, Sr	0.05	0.2	1	10	50	200	-	10

Test method(s)

An iCAP MTX ICP-MS equipped with iSC-65 Autosampler was used for all measurements. The details of instrument configuration is summarized in Table 1. To demonstrate the accuracy and precision of the analytical results, three different levels of whole blood certified materials (Seronorm™ Trace Elements in Whole blood L-1, L-2 and L-3, SERO, Norway) were analyzed along with set of unknown samples.

Figure 1. Thermo Scientific iCAP MTX ICP-MS

Table 2. Typical instrumental parameters used in this study

Parameter	Value (general analysis)	
Nebulizer	PFA concentric nebulizer, 400 µL·min^{-1}	
Peristaltic pump tubing	PVC orange-yellow tubing, 0.51 mm i.d.	
Peristaltic pump speed	40 RPM	
Humidifier	On	
Spray chamber	PFA cyclonic, cooled at 2.7 °C	
Torch	PLUS torch	
Injector	2.5 mm i.d., Quartz	
Interface	Nickel sampler and skimmer cone	
Plasma power	1,550 W	
Nebulizer gas	0.998 L·min^{-1}	
QCell setting	He KED	TQ-O_2
Qcell gas flow	100% He 4.2 mL·min^{-1}	100% O_2 0.32 mL·min^{-1}
CR bias	-21 V	-6.3 V
Q3 bias	-18 V	-12 V
Scan setting	0.1 s dwell time, 5 sweeps, 3 main runs	
Analyte time per sample	Total 3 min 14 s: including uptake (40 s) and wash out (30 s), **Step Ahead** (20 s) with **IMH**	

Data analysis

Qtegra ISDS Software was used to optimize measurement modes using comprehensive autotune routines prior to the analysis. The other features offered by Qtegra ISDS Software including “Get Ready’ feature to facilitate automatic instrument readiness, the comprehensive Quality control toolset for on-going quality control check and automated calculations was also used during this study. The Quality control was ensured by monitoring internal standards response and periodic calibration checks during analytical sequence. The iCAP MTX ICP-MS demonstrated stability for prolonged measurements.

Results

Detection sensitivity and established linear range

Table 3 summarizes measurement mode, Q1 and Q3 quadrupole settings, internal standards and method detection limits (MDLs) calculated for each analyte. All coefficient of determination (R^2) for 43 elements were greater than 0.9995, indicating excellent linear response. All MDLs were calculated using a dilution factor of 50 (IDLs multiplied by 50) derived from the sample preparation process.

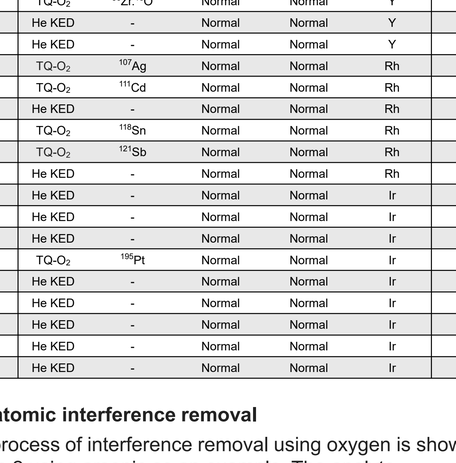
Table 3. Summary of analytes, measurement mode, and achieved MDLs . All numbers are shown in µg·L^{-1}.

	Mode	Q3 analyte	Q1	Q3	Internal standard	MDL (µg·L^{-1})
^{7}Li	TQ-O_2	^{7}Li	High	Normal	Sc	0.43
^{9}Be	TQ-O_2	^{9}Be	High	Normal	Sc	0.71
^{11}B	TQ-O_2	^{11}B	High	Normal	Sc	3.41
^{23}Na	He KED	-	Normal	Normal	Sc	31.8
^{24}Mg	He KED	-	Normal	Normal	Sc	2.43
^{27}Al	TQ-O_2	^{27}Al	Normal	Normal	Sc	0.81
^{28}Si	TQ-O_2	^{28}Si.^{16}O	High	High	Sc	8.65
^{31}P	TQ-O_2	^{31}P.^{16}O	High	High	Sc	13.5
^{33}S	TQ-O_2	^{33}S.^{16}O	High	High	Sc	361.2
^{39}K	TQ-O_2	^{39}K	Normal	Normal	Sc	5.10
^{44}Ca	He KED	-	Normal	Normal	Sc	79.6
^{49}Ti	TQ-O_2	^{49}Ti.^{16}O	Normal	Normal	Sc	0.52
^{51}V	TQ-O_2	^{51}V.^{16}O	High	Normal	Sc	0.04
^{52}Cr	TQ-O_2	^{52}Cr.^{16}O	High	Normal	Sc	0.66
^{55}Mn	TQ-O_2	^{55}Mn	Normal	Normal	Sc	0.20
^{57}Fe	He KED	-	Normal	Normal	Sc	118.0
^{59}Co	TQ-O_2	^{59}Co	Normal	Normal	Ge	0.03
^{60}Ni	He KED	-	Normal	Normal	Ge	0.32
^{63}Cu	TQ-O_2	^{63}Cu	Normal	Normal	Ge	0.47
^{66}Zn	TQ-O_2	^{66}Zn	Normal	Normal	Ge	1.76
^{71}Ga	He KED	-	Normal	Normal	Ge	0.24
^{75}As	TQ-O_2	^{75}As.^{16}O	High	Normal	Ge	0.48
^{80}Se	TQ-O_2	^{80}Se.^{16}O	Normal	Normal	Ge	0.50
^{85}Rb	TQ-O_2	^{85}Rb	Normal	Normal	Y	0.03
^{88}Sr	He KED	-	Normal	Normal	Y	0.14
^{90}Zr	TQ-O_2	^{90}Zr.^{16}O	Normal	Normal	Y	0.12
^{93}Nb	He KED	-	Normal	Normal	Y	0.02
^{95}Mo	He KED	-	Normal	Normal	Y	0.42
^{107}Ag	TQ-O_2	^{107}Ag	Normal	Normal	Rh	0.12
^{111}Cd	TQ-O_2	^{111}Cd	Normal	Normal	Rh	0.43
^{115}In	He KED	-	Normal	Normal	Rh	0.05
^{118}Sn	TQ-O_2	^{118}Sn	Normal	Normal	Rh	0.07
^{121}Sb	TQ-O_2	^{121}Sb	Normal	Normal	Rh	0.20
^{137}Ba	He KED	-	Normal	Normal	Rh	0.40
^{181}Ta	He KED	-	Normal	Normal	Ir	0.01
^{182}W	He KED	-	Normal	Normal	Ir	0.02
^{185}Re	He KED	-	Normal	Normal	Ir	0.02
^{195}Pt	TQ-O_2	^{195}Pt	Normal	Normal	Ir	0.12
^{202}Hg	He KED	-	Normal	Normal	Ir	0.12
^{205}Tl	He KED	-	Normal	Normal	Ir	0.04
^{208}Pb	He KED	-	Normal	Normal	Ir	0.05
^{209}Bi	He KED	-	Normal	Normal	Ir	0.03
^{238}U	He KED	-	Normal	Normal	Ir	0.02

Polyatomic interference removal

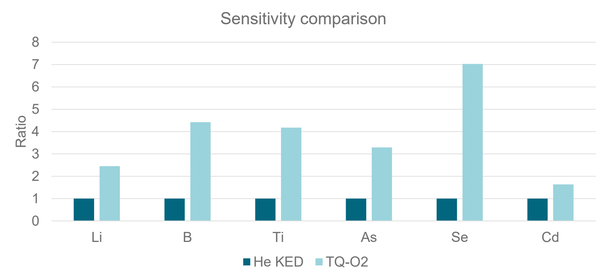
The process of interference removal using oxygen is shown in Figure 2 using arsenic as an example. The analyte of interest reacts with oxygen and forms a molecular ion with a new mass-to-charge ratio (referred to as a mass-shift reaction), and the previously isobaric (i.e., having the same nominal mass) interference does not react in a similar way and can therefore be eliminated using the third quadrupole of the system. The first quadrupole provides an additional mass filtration before the collision/reaction cell, so that unwanted side reactions with other components present in the ion beam are effectively suppressed.

Figure 2. Schematic showing the use of TQ-O_2 mode and a mass shift reaction for interference free detection of arsenic (As).



Achieving high sensitivity is crucial when analyzing toxic elements in diluted whole blood samples, where these elements are often present in ultra-trace amounts. Collision cell technology can reduce sensitivity in the low mass range, leading to the use of "No gas" or "Standard" mode for analytes like lithium or beryllium. However, reactive modes, unlike KED mode, apply a negative bias potential at the CRC outlet, overcoming some ion losses from collisions with gas molecules. Using TQ-O_2 mode typically does not negatively affect absolute sensitivity (Figure 3).

Figure 3. The sensitivity comparison between He KED and TQ-O_2 mode



Accuracy

Some essential elements are found in high concentrations, while metals like vanadium, chromium, cobalt, arsenic, and selenium are typically below 10 µg·L^{-1} in whole blood. Complex spectral interferences from sodium, magnesium, phosphorus, sulfur, potassium, calcium, and iron generate oxides, isobaric interferences, or peak tailing for some key analytes. The removal of these interferences is crucial and was achieved using TQ-O_2 mode during this experiment.

Table 4 shows results for three whole blood CRM samples, detailing concentrations of both toxic and essential elements. Some analytes showed significant variation between samples. ICP-MS's capability to provide fast and accurate multi-element analysis can significantly advance research, such as larger studies assessing biological variation or occupational health.

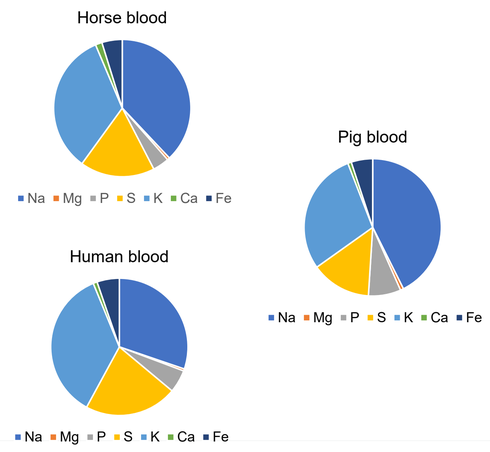
Table 4. Quantitative results obtained for the whole blood CRM samples. Analyte concentrations are reported as µg·L^{-1}. All numbers annotated with * are known reference values (expected values).

Analyte	L1 Reported values	L1 Measured (n=9)	L2 Reported values	L2 Measured (n=9)	L3 Reported values	L3 Measured (n=9)
^{7}Li	0.37	0.45	11.00	11.49	0.73	0.57
^{9}Be	<0.02	<MDL	5.50	5.49	10.10	10.84
^{11}B	-	87.39	-	44.11	-	321.50
^{23}Na	1,598,000	1,609,898	1,618,000	1,582,356	1,512,000	1,545,066
^{24}Mg	152,000	16,210	41,000	41,838	136,000	14,737
^{27}Al	10.40	13.76	57.00	52.72	88.00	89.53
^{28}Si	-	1,701	-	1841	-	2626
^{31}P	203,000	201,616	200,000	194,496	187,000	192,525
^{33}S	957,000	990,933	984,000	978,749	921,000	916,908
^{39}K	1,089,000	1,153,957	1,078,000	1,121,154	1,037,000	1,098,131
^{44}Ca	158,000	17,205	56,000	58,192	141,000	16,182
^{49}Ti	-	5.34	-	3.33	-	14.96
^{51}V	0.26	0.26	3.10	3.34	4.40	4.53
^{52}Cr	0.77	0.81	10.00	9.71	35.50	34.65
^{55}Mn	19.70	21.10	24.20	21.62	33.30	31.49
^{57}Fe	334,000	327,397	335,000	326,225	300,000	288,596
^{59}Co	0.22	0.31	5.00	4.87	10.30	10.22
^{60}Ni	2.13	3.58	9.20	8.50	11.00	11.48
^{63}Cu	640	601	940	842	2080	1938
^{66}Zn	4,600	4,307	5,800	5,202	8,360	7,519
^{71}Ga	0.05	<MDL	0.04	<MDL	0.04	<MDL
^{75}As	2.1	2.2	12.2	12.2	27.3	29.0
^{80}Se	69	67	144	126	198	223
^{85}Rb	1,420	1,443	1,460	1,406	1,180	1,275
^{88}Sr	41.00	39.66	75.00	68.82	37.00	39.13
^{90}Zr	0.41	0.34	-	0.17	0.28	0.33
^{93}Nb	0.03	0.01	0.03	0.02	0.04	0.04
^{95}Mo	0.37	0.38	4.50	4.46	6.90	6.59
^{107}Ag	0.10	0.42	9.70	10.26	0.08	0.50
^{111}Cd	0.28	0.26	5.10	4.63	9.90	9.81
^{115}In	-	<MDL	-	0.03	-	0.07
^{118}Sn	0.2	0.2	4.7	4.0	9.9	9.5
^{121}Sb	3.3	2.8	22.3	18.9	21.9	18.4
^{137}Ba	427	458	145	153	557	581
^{181}Ta	<0.001	0.02	<0.001	0.02	<0.001	0.02
^{182}W	-	<MDL	-	0.09	-	<MDL
^{185}Re	0.001	<MDL	0.00	<MDL	<0.001	<MDL
^{195}Pt	0.004	<MDL	0.00	<MDL	0.01	<MDL
^{202}Hg	1.57	1.57	16.60	14.79	25.80	<MDL
^{205}Tl	0.01	<MDL	10.10	8.04	25.20	21.57
^{208}Pb	10.00	8.99	303.00	272.95	389.00	320.88
^{209}Bi	0.01	<MDL	4.90	4.69	47.00	41.54
^{238}U	0.13	0.18	0.20	0.25	0.12	0.22

Different types of whole blood samples

Three different types of real whole blood samples were analyzed in the study. The samples were collected from a healthy human volunteer, a horse, and a pig. The analysis revealed clear differences in the amounts of major elements among the three blood types (Figure 4). Even when considering only the major elements, significant variation in the elemental composition was observed between the different sample sources. The capability of ICP-MS to provide fast and accurate data in a true multi-element analysis could significantly advance further research, for example, in larger studies to assess biological variation or occupational health.

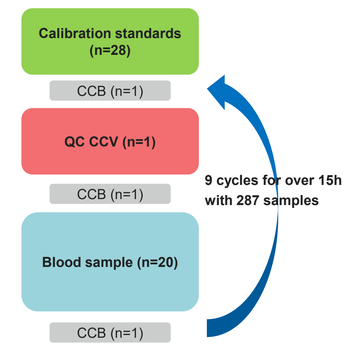
Figure 4. Analysis results of the major elements in three different types of whole blood.



Evaluation of long-term robustness

To simulate high-throughput analysis of a large number of samples and assess method robustness, a batch of samples containing the 50-fold diluted whole blood sample solutions previously analyzed were scheduled for the analysis (Figure 5).

Figure 5. Schematic overview of the batch analyzed for testing the long-term performance of the proposed method. Nine blocks, each containing 20 whole blood samples , were analyzed.



After generating calibration standard curves, the batch containing several blocks of the whole blood samples together with the required QC checks was analyzed. The total number of analyses was 287 (including 180 whole blood matrix samples and 28 calibrants and 54 QC checks), requiring a total analysis time of approximately 15 hours.

The iCAP MTX ICP-MS demonstrated robust, reliable long-term analysis for challenging matrices like whole blood. Internal standards (Figure 6) showed consistent recovery (75–112%) throughout the batch, indicating robust analytical performance.

Figure 6. Response of the internal standards in a batch covering about ~15 hours of uninterrupted analysis of 287 samples.

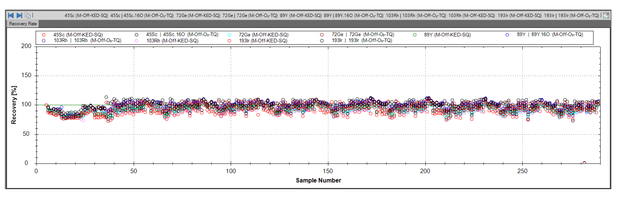
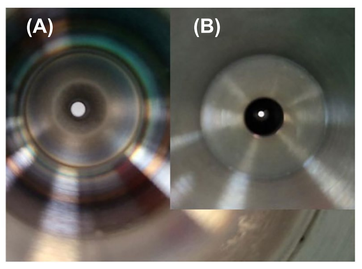


Figure 7 shows an image of the sample and skimmer cone after aspirating over 1,000 whole blood samples tested in this study. No deposition of material on the cone surface was observed; thus, this analytical set-up enables the analysis of whole blood solutions over longer period without need of reanalysis or instrument downtime for maintenance purpose.

Figure 7. Sample (A) and skimmer (B) cone condition after running more than 1,000 whole blood samples



Conclusions

- The combination of He KED and TQ-O_2 mode allows for highly sensitive analysis across the entire mass range (lithium to uranium). It effectively suppresses typical interferences.
- The method provides the required detection limits and a linear response for all analytes. It covers a concentration range of 8 orders of magnitude (from sub ng·L^{-1} to 50 mg·L^{-1}) and shows excellent agreement with three whole blood CRM values.
- The iSC-65 Autosampler, with the Step Ahead feature, reduced total analysis time by 9%. Combined with IMH and humidifier, it improved the method robustness and productivity of whole blood analysis with minimum downtime (maintenance of ICP-MS parts).
- Robust and stable analytical performance was demonstrated over 15 hours of continuous acquisition of 287 samples by the iCAP MTX ICP-MS with excellent quality control results including IS recovery and periodically analyzed QC sample.

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