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A ROBUST ICP-MS METHOD FOR MULTI-ELEMENT TRACE METAL ANALYSIS IN HUMAN WHOLE BLOOD

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ABSTRACT

Monitoring trace and toxic elements in blood is essential for clinical toxicology, occupational exposure assessment and biomonitoring assessment, a rapid sensitive and robust method developed for the simultaneous quantification of Arsenic(As), Lead(Pb), Cadmium(Cd), Manganese (Mn), Chromium (Cr), Cobalt (Co), and Thallium (Tl) in human blood. Whole blood samples were prepared using a simple dilution protocol with trace metal free nitric acid, tubes and selected internal standard to minimize matrix effect and maintain analyte stability. Instrument parameters such as RF power, nebulizer gas flow, dwell time, and collision/reaction cell conditions were systematically optimized to enhance sensitivity and reduce spectral interferences. Quantification was performed using multi-point external calibration with internal standard correction. The method demonstrated excellent linearity for all analytes with correlation coefficients (r^2>0.99) across clinically relevant concentration range. The developed ICP-MS method is a reliable and high-throughput approach for simultaneous multi-element blood analysis. Its sensitivity, robustness and simplified sample preparation make it highly suitable for routine clinical diagnostics, toxic metal screening, and population biomonitoring studies.

KEYWORDS: ICP-MS, Trace element, CRM, Blood.

INTRODUCTION

The accurate determination of Metals at trace and ultra-trace concentration is of critical importance in clinical diagnostics, environmental monitoring, nutritional assessment, and toxicological studies.

Metals occurs in complex matrices such as biological fluids, tissues, food, water and environmental samples and determination of their adverse/good effect becomes a tedious process to follow analytically.

This analytical challenges necessitates technique that combines high sensitivity, and multi element capability while maintaining robustness against matrix-induced interference.

Inductively coupled plasma mass spectrometry (ICP-MS) has emerged as one of the most powerful analytical technique for elemental analysis due to its exceptional detection limits, wide linear dynamic range, and capability for simultaneous multi-element determination. By coupling efficient ionization in a high-temperature argon plasma with mass-based detection, ICP-MS is the most advanced and reliable technique for the assessment 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 salt and biomolecules, such as protein or metabolite, sample quantity for analysis is sometimes very less.

A rapid and accurate high throughput analysis of blood samples using ICPMS, including toxic as well as essential elements could be determined,. A simple HNO_3 dilution method established and analysed using external calibration. The Method was tested for Linearity, accuracy & precision using certified reference standard.

EXPERIMENTAL

Sample Preparation and analysis

Reagents & Standards: ICP-MS reagent purity directly controls ICP-MS sensitivity, accuracy and reliability.
ICP-MS preparation can easily be altered by presence of even negligible amount of metal impurities present in acids, water, or diluents can introduce background noise equal to or higher than the analyte concentration, leading to false positive results, poor precision, and elevated blank values.

It`s, strongly recommended to use only trace metal-grade reagents like nitric acid , ultrapure water and metal free plastic ware (PFA/PTFE tubes) shows low background equivalent concentration (BEC), better signal to noise ratio, accurate calibration and lower detection limit. This is especially critical for elements like Pb, Cd, As, Hg and trace essential metal in clinical environmental, & pharmaceutical testing.

Stocks & standards of high purity grade were purchased from reputed commercial sources. Standard solution must be traceable to national or international standard such as NIST.

1. Agilent Multi-element calibration standard 2 A
2. Internal standard (Chem Labs, USA)
3. Trace-Metal Grade Nitric acid (Chem Labs,USA)
4. ICP grade water
5. Tarson Tubes

All blood sample were diluted manually to a final acid concentration of 0.5%, 100µl blood sample was added to 4.9ml of 0.5 % HNO_3 and the total volume was made up to 5 ml. and mixed thoroughly using a vortex shaker, and analyzed.

For preliminary method validation, synthetic whole blood certified reference materials (Trace metal in whole blood Low level, High level, Bio-Rad, USA) were first reconstituted as per manufacturer`s instruction and prepared as per sample preparation described above.

All element were analysed using KED (Kinetic energy discrimination) mode, keeping torch position 7 mm with pure helium as a collision gas.

For quantitative assessment of all elements in a single analysis, external calibration was developed and linearity curve considering LOD of analyte from calibration Blank, 0.005,0.01,0.025,0.05,0.1,0.5,1,2.0,5.0,10.0,25 ppb.

Analytes	**LL-00**	**LL-1**	**LL-2**	**LL-3**	**LL-4**	**LL-5**	**LL-6**	**LL-7**	**LL-8**	**LL-11**	**LL-12**	**LL-13**
Cd,Pb,As,Cr,Th,Co,Mn	Cal Blank	0.005	0.01	0.025	0.05	0.1	0.5	1	2	5	10	25

Instrumentation

A Thermo Scientific iCAP RQplus ICP-MS equipped with iSC 65 auto sampler and a sample introduction system, in which all sample introduction components are positioned for easy access for the user, simplifying and removal a re-fitting of each component during routine maintenance. ICP-MS is a high performance quadrupole inductively coupled Plasma Mass Spectrometry. The instrument is capable of analysing routine, robust, and highly sensitive trace elemental across a wide range of sample matrices. The instrument is having advanced Argon Gas dilution technology, which enables direct analysis of complex and high salt matrices without extensive manual dilution, the easyclick automatically tensioned peristaltic pump ensures reproducible sample introduction an minimizing the day to day variability.

The Qcell collision cell with helium KED mode effectively reduces common polyatomic interferences, improving analytical accuracy for difficult elements such as arsenic (As), selenium (Se), iron (Fe) and Chromium (Cr).

The instrument is working through an advanced Qtegra ISDS software that offers simplified method development, automated QC workflows, trend monitoring and enhance laboratory productivity.

Prepared sample, kept on auto sampler deck, the sample 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 auto sampler probe and tubings can be rinsed again using the vacuum pump for analysis of the next sample. Table 1 & 2 giving an overview of the typical parameters for analysis.

Table 1: Typical operating iCAP RQ plus ICP-MS. Operating parameters.

Nebulizer	**Micro Mist Nebulizer (400µL/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-Min^{-1}
Cool gas flow	14 L-Min^{-1}
RF power	1550 W
Number of replicates	3
Spray chamber temp.	2.7^{0}C
CRC gas	Helium
Helium flow	4.58 mL-Min^{-1}
Dwell time	0.05 s
Main runs	3
Total analysis time/sample	4 min./sample

Table 2: iSC-65 Auto sampler and ASXpress fast sampling module parameters.

iSC-65 Auto sampler parameters : 2 A

Uptake time	30 s
Wash time	30 s

ASXpress parameters: 2 B

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

Validation Studies as per protocol

An extensive validation protocol for 20 days followed to verify the performance of analytical method.

Instrument Set up

Before, performing any sample analysis make instrument ready as per application. The instrument tuning performed using the auto tune procedure provided in the Thermo Scientific Qtegra Intelligent Scientific Data Solution Software. Instrument performance also generated before starting an analysis sequence every day.

The study continued to 20 days, each day a series of standard solution run to generate calibration curve, followed by the analysis of all certified material(Low Qc, High QC) in triplicates, this experiment continues to check precision of the analytical method. The data acquisition and processing was controlled using Qtegra ISDS Software. The software's features all batch files, method parameters and quantified result were fully automated and completely Traceable inside the native data files created in Qtegra ISDS Software.

The performance of standards and quality controls reviewed continuous for 20 days.

RESULT AND DISCUSSION

Initial method performance

Linearity is an extremely important parameter for trace level analysis using ICP-MS. In the current established method more than 10 levels of standards were run considering LOD of analytes. The calibration standards were used to calibrate the instrument for analysis. At the same time, key parameters related to the analytical performance also checked 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 3).

Table 3: Correlation coefficients and instrument detection limit (IDLs) of analysis obtained from the linearity study (calibration ranges-Table1).

Analytes	**Correlation coefficient (R^2)**	**Instrumental detection Limit (IDL) PPb**	**Blank equivalent concentration (BEC) (PPb)**
Arsenic (As)	>0.9999	0.006	0.011
Cadmium (Cd)	>0.9999	0.002	0.002
Chromium (Cr)	>0.9999	0.007	0.066
Cobalt (Co)	>0.9999	0.001	0.004
Lead (Pb)	>0.9999	0.002	0.029
Manganese (Mn)	>0.9999	0.018	0.047
Thallium (Tl)	**>0.9999**	**0.001**	**0.001**

Accuracy and precision for the analysis of whole blood

The accuracy and precision of the analytical method has been assessed by analysing commercially available certified reference material s (CRMs) with different concertation levels. The exact concentration in each levels ae summarized in table 4, 5 and 6, respectively.

Table 4: Comparison of CRM values and experimental concentrations values obtained for CRM Low Level over the period of 20 Dys.

Analytes	**Avg. Observed Value**	**unit**	**Target Range**	**Target Mean**	**Avg. Observed STDEV**	**Avg. Observed RSD (%)**
Arsenic (As)	5.42	µg/l	4.26—6.38	5.32	0.31	5.6
Cadmium (Cd)	3.53	µg/l	2.75-4.12	3.44	0.20	5.72
Chromium (Cr)	5.2	µg/l	4.18-6.96	5.57	0.17	3.31
Cobalt (Co)	6.3	µg/l	5.25-7.88	6.57	0.16	2.63
Lead (Pb)	82.7	µg/l	72.9-109	91.1	1.46	1.77
Manganese (Mn)	13.5	µg/l	11.8-17.6	14.7	0.47	3.50
Thallium (Tl)	3.7	µg/l	3.31-4.97	4.14	0.11	2.86

Table 5: Comparison of CRM values and experimental concentrations values obtained for CRM High Level over the period of 20 Dys.

High Level

Analytes	**Avg. Observed Value**	**unit**	**Target Range**	**Target Mean**	**Avg. Observed STDEV**	**Avg. Observed RSD (%)**
Arsenic (As)	10.12	µg/l	8.08-12.1	10.1	0.78	7.69
Cadmium (Cd)	6.83	µg/l	5.35-8.02	6.68	0.51	7.40
Chromium (Cr)	10.2	µg/l	8.47-12.7	10.6	0.29	2.84
Cobalt (Co)	11.8	µg/l	9.92-14.9	12.4	0.22	1.87
Lead (Pb)	232.1	µg/l	201-302	251	0.47	1.50
Manganese (Mn)	21.2	µg/l	17.4-26.1	21.8	0.44	2.04
Thallium (Tl)	7.5	µg/l	6.58-9.86	8.22	0.11	1.44

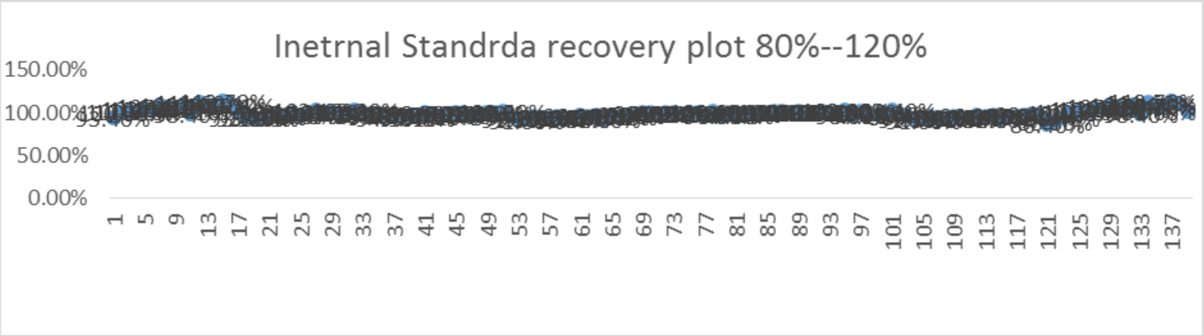
Each levels of blood CRM sample was prepared in triplicate and analyzed in triplicate and analysed on each day. The observed result indicates that the determination of all analytes under study has been accomplished with outstanding accuracy at all concentration levels. The associated low values of relative standard deviation between different preparations indicates that the chosen sample preparation strategy based on a 50 times dilation, 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 different days.

Method accuracy Experiment

A minimum 60 patient specimens including both normal and abnormal covering the analytical measurement range of the analyte were analysed by new method (test method) and by an established method (Comparison method). Three specimen/day for 20 days, the method comparison done with in the stability period. All value remain within 15% bias. Proficiency testing material CAP also included to check method accuracy and all sample came in acceptable limits.

The response of **Internal standard** also reviewed every day and the response of the internal standard, found well within the acceptable limits.

Table 6: Recovery of the internal standards monitored intraday replication, and Method accuracy



CONCLUSION

The developed ICP-MS analytical method for Arsenic, Cadmium, Chromium, lead, Manganese and Thallium in blood analysis demonstrated excellent sensitivity, selectivity, and robustness for the accurate quantification of trace and ultra-trace elements in whole blood samples.

The Method showed satisfactory performance in terms of Linearity, Precision, accuracy, recovery, carryover and LOD, making it suitable for routine clinical, toxicological and biomonitoring applications.

Efficient sample preparation and optimized instrumental conditions minimized matrix interferences blood analysis commonly associated with blood analysis, ensuring reliable and reproducible results. The use of internal standards and controlled calibration further improved analytical confidence and compensated for signal suppression or enhancement of effects.

Overall, the developed method is fit for purpose for trace metal estimation in blood, and can be effectively applied for clinical diagnostics, exposure assessment, and nutritional studies and toxic element monitoring.

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