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DEVELOPMENT AND VALIDATION OF A RAPID, SIMPLE AND COST EFFECTIVE ICP-MS METHOD FOR WHOLE BLOOD TRACE ELEMENT ANALYSIS

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ABSTRACT

Accurate trace metal analysis in whole blood by ICP-MS depends strongly on sample preparation. This study compares three practical pre-treatment strategies: simple acid dilution, non-digestion & microwave digestion, for simultaneous determination of arsenic, lead, cadmium, manganese, chromium, cobalt, and thallium in whole blood. The methods were assessed for analytical recovery, precision, matrix effect, carryover, turnaround time, reagent consumption, & per-sample processing cost. The simple HNO_3 dilution method demonstrated the best balance of accuracy, speed, and economy, showing comparable recoveries to digestion for lead (Pb), cadmium (Cd), Arsenic (As), Manganese (Mn), Cobalt (Co), Chromium (Cr) & Thallium(Tl), while significantly reducing sample preparation time and acid usage. The simple HNO_3 dilution method demonstrated the equally comparable result with non-digestion Triton X-100/NH_3 method & Microwave digestion method.

KEYWORDS: ICP-MS ICAP RQplus, blood, acid, dilution, Triton X-100, digestion cost comparison, trace metals.

INTRODUCTION

The determination of trace and toxic elements in whole blood by inductively coupled plasma mass spectrometry (ICP-MS) plays an essential role in clinical toxicology, biomonitoring, occupational exposure assessment and nutritional studies. Accurate quantification of elements such as arsenic (As), lead (Pb), cadmium (Cd), manganese (Mn), chromium (Cr), cobalt (Co) and thallium requires not only a sensitive instrumental platform but also an efficient sample preparation strategy capable of minimizing matrix effects associated with the complex blood matrix, whole blood contains high concentration of proteins, lipid, salts and cellular components that may cause signal suppression, polyatomic interferences, and sample introduction problems if not properly treated before sample analysis.

Several sample preparation approaches have been employed for blood ICP-MS analysis, among which simple acid dilution, direct non digestion surfactant-based dilution, and microwave digestion are the most widely practiced. Each approach offers distinct advantages and limitations in terms of matrix clean-up, analytical recovery, throughput, contamination risk, and operation cost.

The simple acid dilution method is one of the most straightforward sample preparation techniques and involve direct dilution of whole blood with dilute nitric acid (trace metal free). This application requires minimal sample handling, reduced turnaround time, lower reagent consumption.

The non-digestion alkaline dilution method using NH_3 and Triton X-100 is also emerged as an effective

alternative for high throughput ICP-MS laboratories. Comparative studies have shown that alkali dilution provides analytical agreement comparable to acid digestion for many blood elements, with better precision for several analytes and high throughput.

The microwave acid digestion method remains the conventional reference approach for blood elemental analysis. Complete mineralization of the organic matrix using concentrated nitric acid, with hydrogen peroxide, offers excellent matrix removal and improved accuracy for challenging analytes such as chromium & Iron. Despite its strong analytical performance, microwave digestion is associated higher reagent usage, longer preparation time, increased vessel-related contamination risk, and greater per-sample operational cost.

Clinical and toxicology laboratories increasingly require rapid, economical, and high-throughput workflows, a systematic comparison of these three sample preparation method is of significant analytical and practical relevance. The present study aims to comparatively evaluate simple acid dilution, tritox-100 based non digestion dilution, and microwave digestion methods for whole blood ICP-MS analysis with emphasis on analytical performance and cost effectiveness.

Here, based on all three method (Digestion and TritonX method already validated and working) a practical laboratory-level cost comparison presented for blood sample preparation method in ICP-MS.

Parameter	Simple acid Dilution Method	Triton X-100 Non-Digestion Method	Microwave Digestion Method
Principle	Blood diluted with dilute HNO_3	Blood diluted with TritonX-100	Completed digestion using concentrated acids under microwave heating
Typical reagent	0.5% HNO_3, Online IS	TritonX-100, Butanol/Ammonia, IS	Concentrated HNO_3 , H_2O_2,ultrapure water
Sample preparation Time	2-5min./sample	10-15 min/sample	>90 min/batch
Equipment Required	Basic pipettes/Vortex	Basic pipettes/Vortex	Microwave digestion system
Capital investment	Very low	low	Very high
Reagent cost/sample	30-50	100-150	300-500
Consumable cost	Minimal	Moderate	High (vessels, acids, digestion tubes)
Throughput	Very High	High	Moderate to low
Suitability for routine clinical screening	Excellent	Good	Leas Practical
Accuracy of ultra-trace analysis	Good	Good	Good
Recovery of complex material	Moderate	Moderate	High
Best use	High volume clinical labs	Multi –element biological screening	Research/reference labs

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

Ultrapure water (18.2 MΩ*cm) was used throughout the study. Ultrapure (Trace metal grade) nitric acid (HNO_3), Ammonia solution, Hydrogen peroxide (H_2O_2), Triton X-100, EDTA and N-Butanol were of trace metal grade. Multi- element calibration standards containing arsenic (As), lead (Pb), cadmium (Cd), manganese (Mn), chromium (Cr), Cobalt (Co), & thallium (Tl) were used for calibration and quality control. Rhodium (rh), Scandium (Sc), bismuth (Bi), germanium (Ge) were used as internal standard mostly.

Whole blood reference material and commercial control samples were used for validation and comparative recovery studies.

2.2 Sample collection

Whole blood samples were collected in trace-element certified K_2EDTA tubes and mixed thoroughly before aliquoting. All samples were stored at 2-8^{0}c until analysis.

2.3 Comparative Sample Preparation Methods

Three different pre-treatment procedures were evaluated.

2.3.1 Method A: Simple Acid Dilution

A direct acidic dilution procedure was prepared using.

- 0.5 % HNO_3
- Online addition of internal standard with 2% IPA

Procedure

- Whole blood collected in metal free tube is only accepted: 100 µl
- 4.9 ml 0.5 % HNO_3 (Diluent)
- Final dilution: 1:50

2.3.2 Method B: Triton X Based non Digestion

This method requires multiple reagents handling and high chances of error & contamination is possible.

A certain amount of butanol, Ammonia, EDTA and Triton X diluted into MQ water and used as diluent during sample preparation.

Procedure

- 50µl whole blood sample
- 4.95 ml non digestion diluent
- Vortex briefly

The mixture was vortexed thoroughly and allowed to stand for sedimentation of particles, if required centrifuge samples.

2.3.3 Method C: Microwave Acid digestion

For complete mineralization, microwave digestion was performed.

Digestion mixture

- Whole blood: 1ml
- Concentrated HNO_3: 4 ml
- H_2O_2: 1 ml

The sample were transferred into PTFE microwave vessels and digested under the program developed for blood digestion.

After the digestion, sample were cooled at RT and digested sample quantitatively diluted to 25 ml, with 25 times dilution with ultrapure water before ICP-MS analysis.

2.4 ICP-MS

Instrumentation

All prepared samples were analysed using thermos fisher single quadrupole ICP-MS, ICAP RQplus operated in helium collision mode.

Operating Conditions

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

ISC-65 Auto sampler parameters

Uptake time	30 s
Wash time	30 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

2.5 Comparative Evaluation Parameters

Microwave acid digestion method and the Triton X-100 based non-digestion dilution method were previously validated in our laboratory for whole blood ICP-MS analysis of trace and toxic elements. Validation parameters including Linearity, precision, accuracy, carryover, & limit of detection (LOD) were found to be within acceptable analytical limits for arsenic, lead, cadmium, manganese, chromium, cobalt, and thallium.

The digestion method demonstrated excellent matrix destruction and reliable recovery for all analytes, particularly in samples with high cellular content. In parallel, the Triton X method based on direct haemolysis and alkaline dilution, showed comparable analytical performance with superior turnaround time and lower reagent consumption.

Since both methods had already met routine validation acceptance criteria, the present comparative study

primarily focused on evaluating workflow efficiency, throughput, and cost effectiveness with the simple acid dilution approach.

3. Validation Experiment (Simple Acid Dilution Method)

3.1 Replication

A replication study of the simple acid dilution method was performed to confirm the reproducibility and analytical suitability of this minimal-preparation workflow for whole blood ICP-MS analysis. The study was designed to evaluate whether direct acidic dilution could provide consistent analytical performance under the same instrumental condition used for the previously validated digestion and triton X-100 non digestion methods.

Whole blood samples, quality control materials were processed using dilute nitric acid based direct dilution, followed by replicate analysis under identical calibration, internal standardization. And collision-cell conditions. The replication experiment focused on precision, recovery, signal stability, matrix related effects for arsenic, cadmium, manganese, chromium, cobalt and thallium.

The results of the replication study demonstrated acceptable repeatability and recovery for routine toxic metal screening, supporting the use of simple acid dilution as a rapid comparative approach, with no matrix influence and signal drift were observed during extended batch analysis.

Table 01: Replication Date Review (QAD), Dr Lal Path Labs Ltd.

Analyte	Inter Assay		Total Precision		Allowable CV%	Comment
	L1	L2	L1	L2		
Chromium	3.3	2.8	11.5	8.5	15.0	Acceptable
Manganese	3.5	2.0	6.3	8.2	15.0	Acceptable
Cobalt	2.6	1.9	5.8	5.7	15.0	Acceptable
Arsenic	4.8	3.4	5.9	7.7	15.0	Acceptable
Cadmium	3.7	3.1	5.7	7.4	15.0	Acceptable
Thalium	2.9	1.4	8.1	5.9	15.0	Acceptable
Lead	1.8	1.5	7.6	7.6	15.0	Acceptable

3.2 Carryover

Carryover is assessed by analysing high-concentration (H1, H2) sample in duplicate followed by triplicate measurement of a low sample (L1, L2, and L3).

Carryover (%) = (L2-L3)/ (H2-L3)*100

The simple acid dilution method demonstrated negligible carryover for all analytes, with low Cal, concentration remaining within acceptable limits (<1%), confirming suitability of the method for routine ICP-MS analysis of trace metals in blood.

Table 02: Carry Over Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	Carry Over %	Carry Over Limit %	Comment
Lead	1.2	15.0	Acceptable
Chromium	3.4	15.0	Acceptable
Manganese	No carry over	15.0	Acceptable
Cobalt	0.3	15.0	Acceptable
Arsenic	2.5	15.0	Acceptable
Cadmium	1.9	15.0	Acceptable
Thalium	2.0	15.0	Acceptable

3.3 Reportable Range

Patient specimens with known or assigned values are analysed in duplicates at five linearly related levels, to assess the reportable range. Assign values by running the specimen of high pool and low pool in triplicates on the same instrument before starting the experiment.

- Label the low pool “Pool 1” and the high pool “Pool 5.”
- Prepare Mixture 2 “Pool 2” (75/25) as a mixture of 3 parts Pool 1 plus 1 part Pool 5.
- Prepare Mixture 3 “Pool 3” (50/50) as a mixture of 2 parts Pool 1 plus 2 parts Pool 5.
- Prepare Mixture 4 “Pool 4” (25/75) as a mixture of 1 part Pool 1 plus 3 parts Pool 5.

Samples in “Pool 1” should have values near lower LOQ and samples in “Pool 5” have values near the upper limit of AMR.

Dilute the specimens with water or saline (as indicated by the manufacturer in the package insert).

Alternately, a high pool can be collected and serially diluted to form other pools spanning the whole AMR. Artificial samples (standards, QC materials and spiked QC materials) are given lower preference for carrying out this experiment since they may not account for matrix effects seen in biological samples. This experiment is done within a run.

The established reportable range is suitable for clinical and toxicological assessment of trace metals in blood and supports reliable quantification across both low-level exposure and elevated concentration scenario.

Table 03: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Lead	2.2	7.5	6.5	2.4	0.8	15	Acceptable

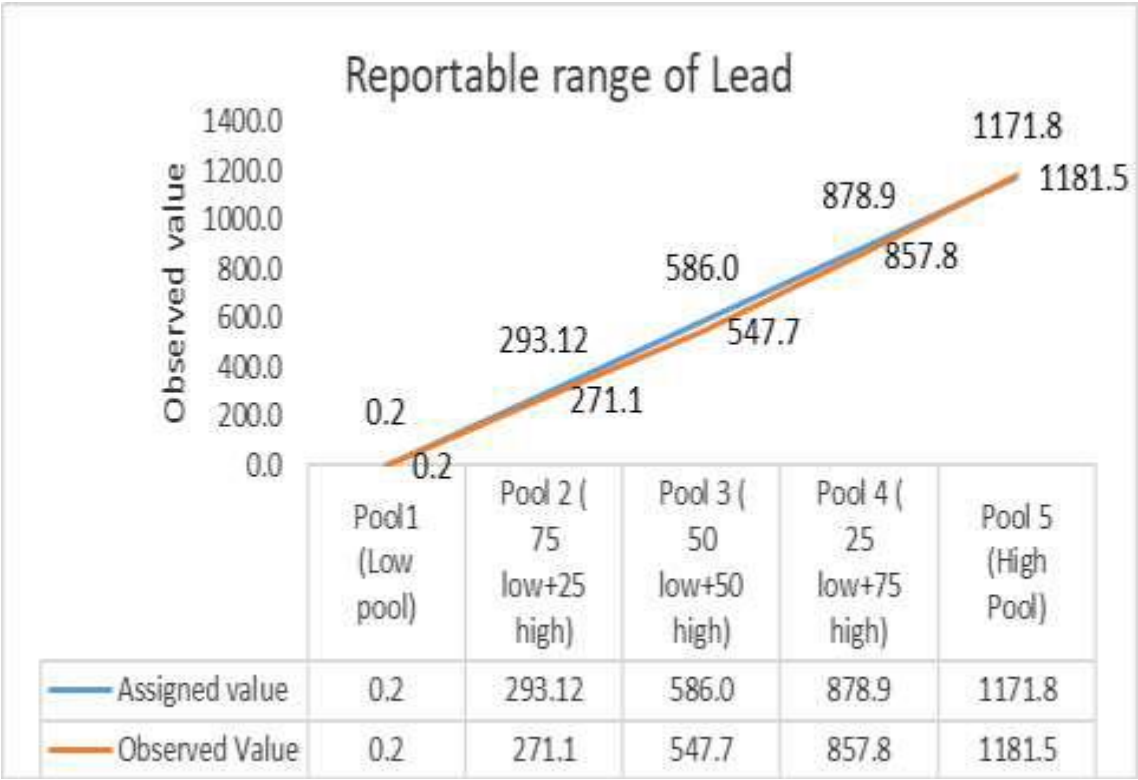


Table 04: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Arsenic	1.5	1.7	3.8	4.7	0.2	15	Acceptable

Table 05: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Cadmium	10.8	2.2	7.0	2.6	0.1	15	Acceptable

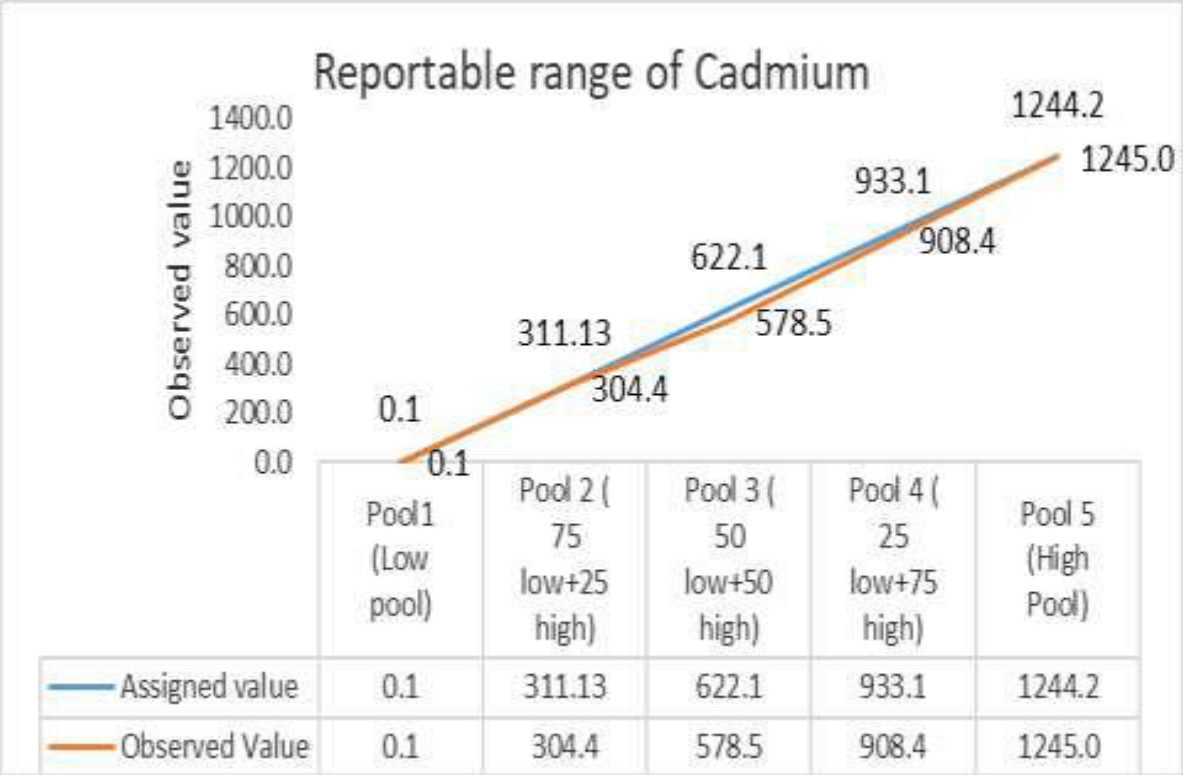


Table 06: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Cobalt	1.6	2.3	3.6	2.7	0.6	15	Acceptable

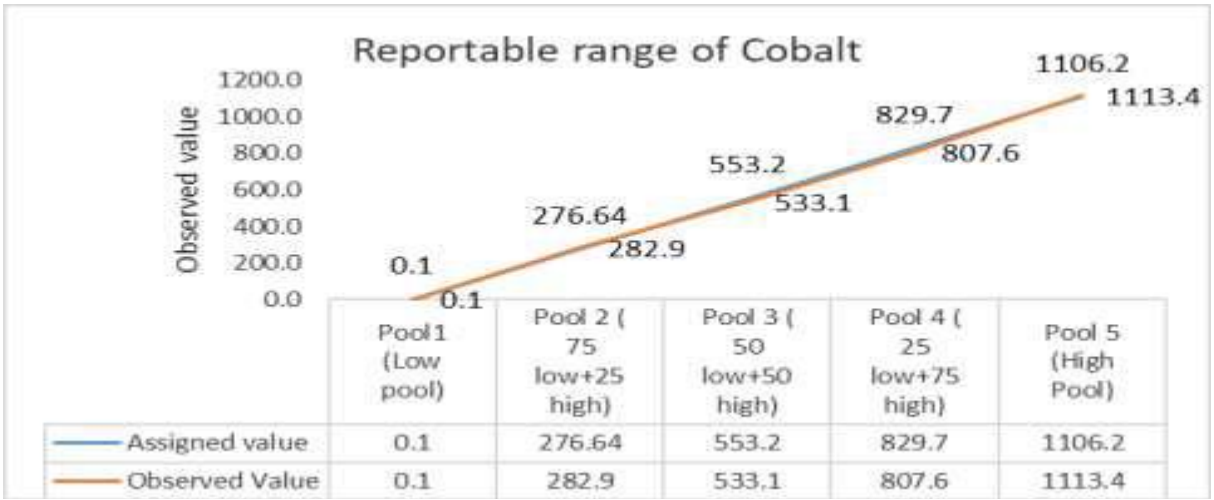


Table 07: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Chromium	10.8	9.9	3.4	4.1	0.5	15	Acceptable

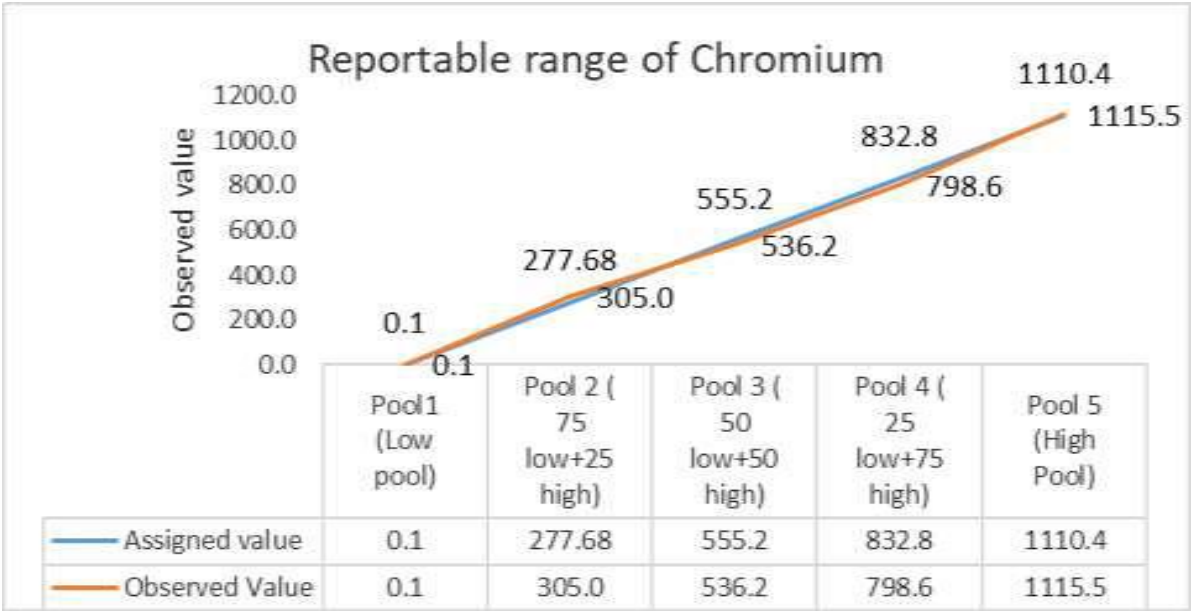


Table 08: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Thalium	0.1	1.8	4.8	8.6	2.2	15	Acceptable

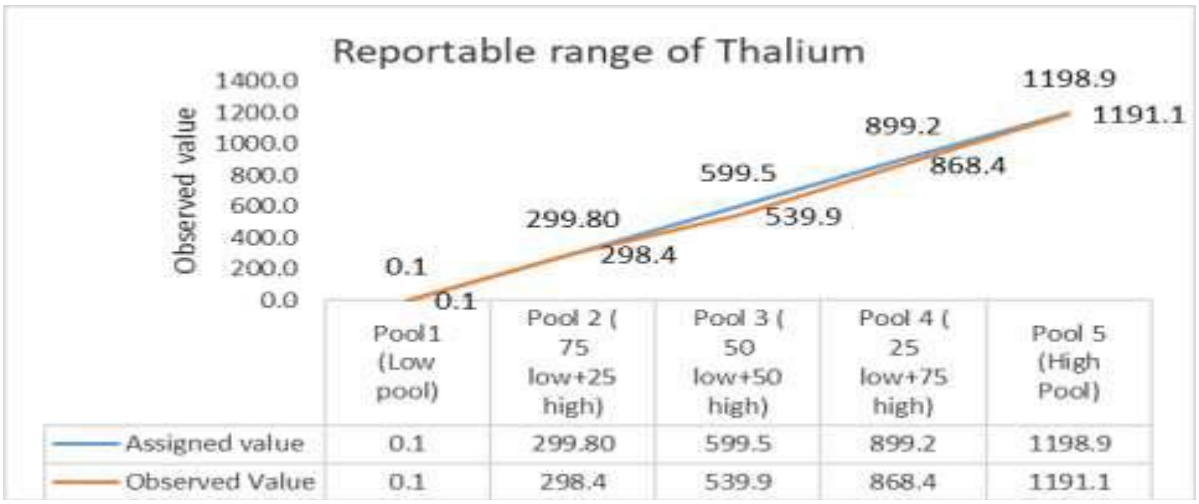
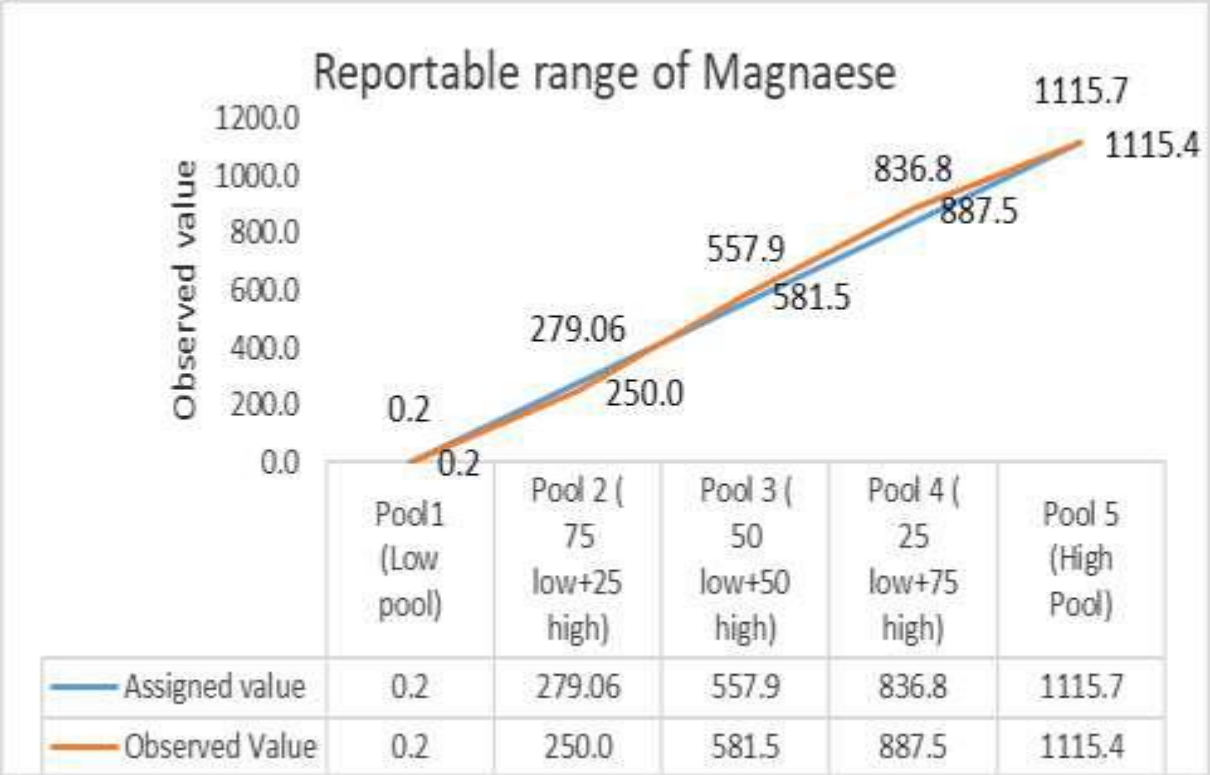


Table 09: Reportable Range Experiment Data Review (QAD), Dr Lal Path Labs Ltd.

Analyte	pool-1	pool-2	pool-3	pool-4	pool-5	Allowable Non Linearity %	Comment
Manganese	3.0	8.6	4.2	6.1	0.0	15	Acceptable



3.4 Method Comparison Experiment

A minimum of 40 patient specimens including both normal and abnormal covering the analytical measurement range of the analyte were analyzed by the new method (test method) and by an established method (comparison method). Test 4 - 8 specimens/day for 20 days; complete testing done within stability period.

Table 10: Method Comparison Experiment Data Review (QAD), Dr Lal Path labs Ltd.

Analyte	Verification Results	Comment
Lead	84/86 results (35-Normal, 49-Abnormal,) are correlating with expected results	Acceptable
Chromium	All 100 results (100-Normal, 00-Abnormal,) are correlating with expected results	Acceptable*#
Manganese	All 64 results (64-Normal, 00-Abnormal,) are correlating with expected results	Acceptable*
Cobalt	All 85 results (84-Normal, 01-Abnormal,) are correlating with expected results	Acceptable#
Arsenic	All 87 results (87-Normal, 00-Abnormal,) are correlating with expected results	Acceptable*#
Cadmium	All 74 results (69-Normal, 05-Abnormal,) are correlating with expected results	Acceptable#
Thalium	All 100 results (100-Normal, 00-Abnormal,) are correlating with expected results	Acceptable*#

***Abnormal samples not available**
 # CAP Proficiency testing sample included in study

3.5 Limit of Detection/Limit of Quantification (LOD/LOQ) experiment

Minimum of 60 blank measurements and 60 measurements of samples with a value equal to the LOD claim. A number of samples measured in replicate, rather than a single sample is preferred.

Table 11: Limit of Detection/Limit of Quantification (LOD/LOQ) experiment Data Review (QAD), Dr Lal Path labs Ltd.

Analyte	Result	Comment
Lead	LOB is calculated to be- 0.062 LOD established -0.102 LOQ established- 0.16	Acceptable
Cobalt	LOB is calculated to be- 0.005 LOD established -0.007 LOQ established- 0.02	Acceptable
Arsenic	LOB is calculated to be- 0.005 LOD established -0.02 LOQ established- 0.12	Acceptable
Cadmium	LOB is calculated to be- 0.003 LOD established -0.005 LOQ established- 0.02	Acceptable
Thalium	LOB is calculated to be- 0.001 LOD established -0.002 LOQ established- 0.005	Acceptable
Chromium	LOB is calculated to be- 0.08 LOD established -0.10 LOQ established- 0.3	Acceptable
Manganese	LOB is calculated to be- 0.06 LOD established -0.09 LOQ established- 0.2	Acceptable

CONCLUSION

The simple acid dilution method demonstrated a reliable, rapid, and highly cost-effective approach for trace elemental analysis in blood samples using ICP-MS. Compared with Triton X and conventional digestion-based procedures, the method significantly reduces sample preparation time, reagent consumption and operational cost while maintaining acceptable analytical performance. The procedure showed satisfactory accuracy, precision, linearity and reproducibility for routine clinical and toxicological applications.

Minimal sample handling also decreases the risk of contamination and analyte loss, improving overall workflow efficiency in high-throughput. The simplified preparation strategy enables faster turnaround time without compromising analytical reliability.

Therefore, the simple acid dilution method can be considered a practical and dependable alternative to traditional digestion method for routine ICP-MS analysis of blood matrices, particularly in laboratories seeking economical and scalable analytical solutions.

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