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DEVELOPMENT AND COMPLETE VALIDATION OF A SENSITIVE ICP-MS METHOD FOR QUANTITATIVE DETERMINATION OF ALUMINIUM IN BIOLOGICAL SAMPLE

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ABSTRACT

Aluminium is one of the most abundant metals in the earth's crust and is increasingly recognized for its potential toxicological effects in patients with renal impairment, dialysis exposure, parenteral nutrition, and occupational exposure. Accurate determination of trace concentrations of aluminium in biological matrices is analytically challenging due to contamination risks, matrix effects, and spectral interferences associated with the mono-isotopic nature of aluminium (^{27}Al). This study describes the development and validation of an inductively coupled plasma mass spectrometry (ICP-MS-iCAP RQplus) method for aluminium quantification in biological samples using a simplified sample preparation approach. Sample preparation consisted of dilution of biological sample with ICPMS grade water to reduce matrix deposition and improve analyte stability. Analytical performance was evaluated according to laboratory internal QAD validation recommendations. The method demonstrated excellent linearity (r^2 > 0.999), acceptable precision and accuracy (<10% CV), satisfactory recovery (95–105%), and low limits of detection suitable for clinical applications. Optimization of plasma conditions and internal standardization minimized matrix-induced signal suppression and improved analytical robustness. The proposed method provides a rapid, sensitive, and cost-effective alternative for routine aluminium monitoring in clinical laboratories.

KEYWORDS: Aluminium, ICP-MS-iCAP RQplus, Trace Elements, Method Validation, QAD, Clinical Chemistry, Toxicology.

1. INTRODUCTION

Aluminium (Al) is the third most abundant element and the most abundant metal in the earth's crust. Although it has no known physiological role in humans, excessive exposure has been associated with neurotoxicity, osteomalacia, microcytic anemia, and dialysis encephalopathy. Human exposure occurs through food, drinking water, pharmaceuticals, dialysis fluids, and occupational environments. Aluminium toxicity is particularly relevant in patients with chronic kidney disease and transplant recipients due to impaired elimination mechanisms.[9]

Analytical determination of aluminium is challenging because of its low concentration in biological matrices and susceptibility to environmental contamination. Traditional techniques such as graphite furnace atomic absorption spectrometry (GF-AAS) have largely been replaced by ICP-MS due to superior sensitivity, lower detection limits, and multi-element capability.[10]

2. MATERIAL AND METHODS

2.1 Chemicals and Reagents

Ultrapure water (ICP-MS) (18.2 MΩ*cm) was used throughout the study. 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.

Serum/Plasma reference material and commercial control samples were used for validation and comparative recovery studies.

2.2 Sample collection

Serum to be collected only in (Trace element serum) tube. Ship refrigerated or frozen. Use powderless gloves during specimen collection. Patient should avoid taking antacids and aluminium containing medications prior to specimen collection. If Gadolinium or Iodine containing contrast media has been administered, the specimen should not be collected for 96 hours.

2.3 Sample Preparation

- 250µl serum sample
- Add 2.25 mL of ICP grade water as diluent
- Dilution 10 times
- Mode: KED
- Torch position: 7mm
- Internal standard: Online addition of IS to check matrix effect

2.4 Instrumentation

Principle of ICP-MS Analysis

ICP-MS combines an argon plasma ion source with mass spectrometric detection. Samples introduced through a nebulizer undergo desolvation, atomization, and ionization within plasma temperatures approaching 10,000 K. Aluminium is efficiently converted to its singly charged ion ($^{27}Al^{+}$), which is separated according to mass-to-charge ratio and quantified against calibration standards.

Since aluminium possesses only one stable isotope (^{27}Al), spectral overlap from polyatomic ions including $^{12}C^{15}N^{+}$ and $^{11}B^{16}O^{+}$ must be minimized through optimization of plasma conditions and collision/reaction cell technology. Prepared test samples were analysed using thermo fisher single quadrupole ICP-MS, iCAP RQplus operated in helium collision mode.

Operating Conditions 2.4 A

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: 2.4 B

Uptake time	30 s
Wash time	30 s

ASXpress parameters: 2.4 C

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 Experiment (Test method Validation Protocol)

1. Replication Experiment

The replication study is performed by analysing aluminium quality control samples or spiked samples repeatedly over a period of twenty consecutive days. The study assesses the consistency of the method when influenced by normal variations such as instrument condition, calibration preparation, reagent batches environmental conditions, and operator handling.

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

Analyte	Inter Assay		Total Precision		Allowable CV	Comment
	L1	L2	L1	L2		
Aluminium	6.8	4.6	9.0	9.4	15	Acceptable

2. Carryover Study

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 water dilution method demonstrated negligible carryover Aluminium, with low Cal, concentration remaining within acceptable limits (<1%), confirming suitability of the method for routine ICP-MS analysis of trace metals Al in serum.

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

Analyte	Carry Over %	Carry Over Limit %	Comment
Aluminium	No carry over	15.0	Acceptable

3. Reportable Range Study

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.

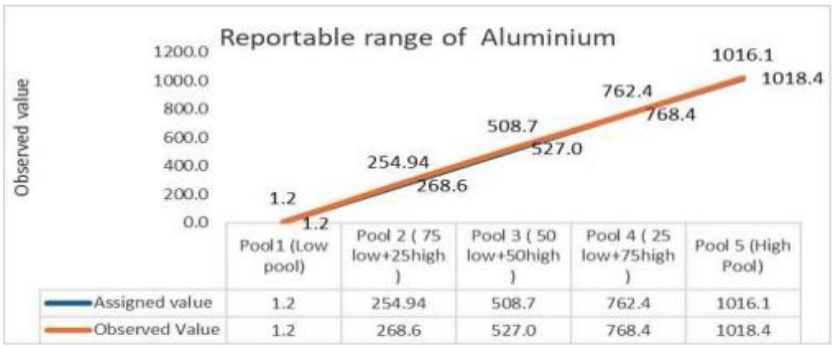
Dilute the specimens with water or saline (as indicated by the manufacturer in the package insert).

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
Aluminium	1.6	5.4	3.6	0.8	0.3	15	Acceptable



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

Analyte	Mean Bias %	Allowable Bias %	Comment
Aluminium	3.0	15	Acceptable

 # CAP proficiency testing samples also included in study

5. Limit of Detection/Limit of Quantification (LOB/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.

Limit of Blank (LoB)

LoB is the highest apparent analyte concentration expected when replicates of a blank sample containing no analyte ae tested.

Procedure

- Select a blank matrix free from the analyte (e.g., diluent, reagent blank, or analyte-free serum).
- Measure the blank samples at least 60 times
- Calculate: Mean of blank measurements (Mean_Blank), Standard deviation of blank measurements (SD_blank).

Calculation: $LoB=Mean_{blank}+1.645xSD_{blank}$

Limit of detection (LoD)

LoD is the lowest analyte concentration that can be reliably distinguished from the LoB and detected with acceptable confidence.

Procedure

- Prepare a low- level sample with analyte concentration near the expected detection limit
- Analyse the sample at least 60 times under routine conditions
- Calculate the deviation of the low concentration sample (SD-low)

Calculation

$LoD = LoB + 1.645 \times SD_{low}$

- LoB = previously established limit of Blank
- SD_loW = standard deviation of the low concentration sample.

Limit of Quantitation (LoQ)

LoQ is the lowest analyte concentration that can be measured quantitatively predefined accuracy and precision.

Procedure

- **Several low concertation levels around the expected LoQ were prepared and run as per QAD protocol.**
- Analyze each levels in replicates over a period of 20 days
- Calculate % CV and bias for each concentration.
- Defined acceptance criteria: CV ≤ 20% for trace element.

The lowest concentration meeting the predefined precision and accuracy criteria is designated as the LoQ.

Table 05: Limit of Detection/Limit of Quantification (LOD/LOQ) experiment Data Review (QAD), Dr Lal Path labs Ltd.

Analyte	Result	Comment
Aluminium	LOB is calculated to be- 0.003 LOD established -0.13 LOQ established- 0.52	Acceptable

CONCLUSION

The ICP-MS method for the determination of aluminium in human serum was successfully developed and validated according to established analytical validation guidelines. The method demonstrated excellent analytical performance with respect to linearity, precision, accuracy, recovery, and carryover. The established limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) confirmed the method's high sensitivity and its suitability for the reliable determination of trace concentrations of aluminium in serum samples. The validated assay exhibited robust analytical characteristics across the measuring range and provided accurate quantification at clinically relevant concentrations. Furthermore, the simplified sample preparation approach reduced reagent consumption and analysis time while maintaining analytical performance comparable to conventional digestion-based methods. Therefore, this method is suitable for routine clinical testing, biomonitoring studies, and the assessment of aluminium exposure in patient populations at risk of aluminium accumulation.

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