

RESEARCH ARTICLE

VALIDATION OF AN ICP-OES METHOD FOR DETERMINATION OF HEAVY METALS IN LONG PEPPER (*PIPER LONGUM* L.) AND ASSESSMENT OF MARKET SAMPLES

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Abstract: The long pepper (*Piper longum* L.) is an essential medicinal spice commonly used in traditional healthcare. However, due to the growing threat of environmental pollution, the concentration of heavy metals in such medicinal plant materials poses a significant hazard. Therefore, an analytical technique based on ICP-OES which was developed for the quantitative determination of Cd, Cr, Pb, and Ni concentrations was validated in the long pepper samples. The method was tested for several validation parameters, which included linearity, LOD (limit of detection), LOQ (limit of quantification), accuracy, precision, and repeatability. Calibration curves obtained in the study showed excellent linearity with R^2 values ranging from 0.9988 to 0.9994. LOD and LOQ values determined in the analysis were found to fall within the ranges 0.05–0.20 $\mu\text{g/L}$ and 0.15–0.60 $\mu\text{g/L}$, respectively. Results obtained in recovery studies ranged between 97.8% and 99.2%, and RSD was not greater than 3%. Determination of cadmium, lead, chromium and nickel in both farm and market samples showed that Chromium and Nickel were within permissible limits, whereas Cadmium and lead could not be detected at all.

Keywords: *Piper longum*, ICP-OES, Heavy metals, Method validation, Medicinal plants

INTRODUCTION

Medicinal plants continue to play a significant role in traditional and modern healthcare systems. Among them, long pepper (*Piper longum* L.), a member of the family Piperaceae, is widely valued for its therapeutic properties, including digestive enhancement, respiratory support, and anti-inflammatory activity. Its fruits are extensively used in Ayurvedic formulations as well as in spice mixtures (Suresh *et al.*, 2011).

Despite medicinal importance, the safety of plant-derived materials is increasingly challenged by environmental contamination. Heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), and nickel (Ni) can enter plant systems through contaminated soils, irrigation water, agrochemicals, and industrial emissions. These elements tend to accumulate in plant tissues and may pose serious health risks upon consumption. (Sulaiman *et al.*, 2024).

In this context, reliable analytical methods are essential for monitoring heavy metal contamination in medicinal plants. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) is widely recognised for its capability in multi-element analysis due to its sensitivity, precision, and rapid detection (Senila, 2024).

However, the reliability of analytical data depends on proper method validation. Parameters such as linearity, detection limits, accuracy, precision, and reproducibility must be systematically evaluated to

ensure the suitability of the method (Ilieva *et al.*, 2018).

The present study was therefore undertaken to validate an ICP-OES-based method for the determination of Cd, Cr, Pb, and Ni in Long pepper and to assess their levels in selected farm and market samples of *Piper longum*.

Materials and methods**Instrumentation**

Heavy metal analysis was carried out using an ICP-OES system (Optima 8000, Perkin Elmer). The instrument was operated under optimised conditions to ensure accurate measurements following the procedure in the operation manual of the instrument.

Operating conditions:

- RF power: 1500 W
- Plasma gas flow: 12 L/min
- Nebulizer flow: 0.7 L/min
- Auxiliary gas flow: 0.2 L/min
- Sample flow rate: 1 mL/min

Analytical wavelengths:

- Cd: 228.802 nm
- Cr: 267.716 nm
- Ni: 231.604 nm
- Pb: 220.353 nm

Sample preparation

Long pepper samples (dried fruits) were collected from local herbal markets of Kerala and one sample collected from the experimental farm of Kerala Agricultural University, Vellanikkara. Samples were

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oven-dried at 50°C and ground into a fine powder using a stainless steel grinder. The powdered material was sieved through a 0.5 mm mesh to ensure uniformity of powdered sample prior to analysis.

Acid digestion

All glassware and plastic ware were thoroughly cleaned by soaking in 10% potassium permanganate followed by 10% nitric acid for 24 hours, and rinsed with ultrapure water to minimise contamination.

Each plant sample (0.5 g) was subjected to wet digestion using a mixture of nitric acid (HNO₃) and perchloric acid in a 9:4 ratio. The digestion process was continued until a clear solution was obtained,

filtered, then diluted to 50 mL with ultrapure deionised water.

Standard stock solutions (100 mg/L) of heavy metals Cadmium, Chromium, Lead and Nickel were diluted appropriately to prepare calibration standards. Blanks and reference standards and plant samples were analysed by feeding to the ICP OES instrument alongside the samples following the instrument manual guidance.

Prepared calibration curves using standard solutions. Known concentrations of Cd, Cr, Pb, and Ni added to long pepper samples to prepare spiked samples. Recovery % and % RSD calculated. One sample from KAU farm and 3 market samples were analysed for heavy metals using above procedure.

RESULTS

Method validation

Table 1. Linearity (R²)

Element	Concentration range (µg/L)	R ²
Cadmium	0.5-100	0.9994
Chromium	1-100	0.9988
Lead	1-100	0.9992
Nickel	1-100	0.9989

Table 2. LOD and LOQ

Element	LOD (µg/L)	LOQ (µg/L)
Cadmium	0.05	0.15
Chromium	0.10	0.30
Lead	0.20	0.60
Nickel	0.11	0.33

Table 3. Accuracy and Precision

Element	Spike level (µg/L)	Recovery %	RSD %
Cadmium	10	98.5	2.5
Chromium	50	97.8	2.3
Lead	50	99.2	2.7
Nickel	50	98.7	2.6

Table 4. Repeatability and Reproducibility

Element	Mean concentration (µg/L)	RSD% (intra- day)	RDS% (inter-day)
Cadmium	9.85	2.4	3.1
Chromium	48.9	2.2	3.0
Lead	49.6	2.5	3.2
Nickel	49.4	2.3	3.1

Table 5. Real sample analysis:

Heavy metal content in Long pepper samples analysed

Parameter	FSSAI limit MRL (mg/kg)	Market Sample-1 (mg/kg)	Market Sample.2 (mg/kg)	Market Sample.3 (mg/kg)	Experimental Farm Sample.4 (mg/L)
Cadmium	1.5	BDL	BDL	BDL	BDL
Chromium	1.0	0.89	1.00	0.90	0.45
Nickel	1.5	0.90	1.50	0.80	0.55
Lead	2.0	BDL	BDL	BDL	BDL

BDL-Below detectable level, MRL-Maximum residue limit

Linearity

Calibration curves were prepared over appropriate concentration ranges for each element. A strong linear relationship was observed, with correlation coefficients (R^2) exceeding 0.998 for all elements, confirming excellent linearity (Table 1.)

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The method exhibited low detection and quantification limits, indicating high sensitivity suitable for trace-level analysis of heavy metals in plant samples (Table 2).

Accuracy and precision

Accuracy was assessed through spike recovery experiments. Recovery values ranged between 97.8% and 99.2%, demonstrating that the method provides reliable quantification. Precision, expressed as relative standard deviation (RSD), remained below 3%, indicating good repeatability of measurements (Table 3).

Repeatability and reproducibility

Both intra-day and inter-day variations were minimal, with RSD values below 5%. This confirms that the method is consistent and reproducible under routine laboratory conditions (Table.4).

Analysis of real samples

Four long pepper samples, including one farm sample and three market samples, were analysed. Cadmium and lead were found to be below detectable levels in all samples analysed. Chromium and nickel were detected in measurable quantities; however, their concentrations were generally within acceptable safety limits as per regulatory standards of FSSAI. (FSSAI,2011).

The results (Table.5) suggested that the analysed samples are largely safe with respect to heavy metal contamination, although continuous monitoring and more sample analysis are remains necessary.

DISCUSSION

The present study demonstrated that the ICP-OES method developed for the determination of Cd, Cr, Pb, and Ni is both reliable and analytically robust in Long pepper analysis. The high linearity ($R^2 > 0.998$), low detection limits, and satisfactory recovery values obtained in this study are in close agreement with earlier reports on ICP-OES-based heavy metal analysis in various plant matrices. (Fahad *et al.*, 2025).

For example, one study looking at plant products with ICP-OES also found similar straight-line results and could easily spot tiny amounts of metals after breaking down the samples with acid. This shows how good this method is for complicated plant materials. Likewise, when people analyzed many elements in medicinal plants, ICP-OES consistently

performed well for a bunch of them, including Cd, Cr, Ni, and Pb (Castro *et al.*, 2009).

Our recovery data (97.8–99.2%) were also in line with earlier tests, where adding known amounts of metals back into plant samples and then analysis, confirmed that the method is accurate and reliable.

Other similar studies have also pointed out that ICP-OES effectively detected very small amounts and is very sensitive when checking environmental and plant samples, making it a good choice for keeping an eye on trace metals (Parvathy *et al.*,2020).

In some studies toxic metals were found being too low to even detect. This could mean there's not much pollution around, or that farming practices are really good. In this study, we couldn't find any cadmium or lead in samples tested, which is a good sign with regard to safety. But then, some other studies on medicinal plants showed that how much heavy metal builds up can change a lot. It depends on where the plant grew, what kind of soil it was in, and what people were doing in that area.

In the tested samples chromium and nickel were present but their levels were within safe limits. There was a bit more of them in the market samples compared to the one from the farm. This difference might be because of how the plants were handled after harvest, how much they were exposed to the environment, or if they came from different growing areas. Even with this variation, all the levels we found were within safe limits, meaning the samples are safe to consume when it comes to heavy metal contamination.

CONCLUSION

The ICP-OES method validated in this study worked well for finding heavy metals in long pepper samples. It proved to have straight lines on graphs, could find very tiny amounts, recovered a lot of what was there, and was precise. Absence of cadmium or lead in the samples, is really encouraging for food safety. While chromium and nickel were present, their amounts stayed within safe limits.

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