



## Estimation Of Copper, Zinc And Lead In Choornas By Flame Atomic Absorption Spectroscopy

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*Choornam is one of the most common dosage form used in Ayurveda and Siddha system of Medicine. A common misperception is that medicaments of natural substances may not cause toxicity. However, the safety of natural substances has been questioned recently due to the presence of heavy metals above the permissible range. In this study, an attempt was made to estimate the concentration of Cu, Zn and Pb in Choornas. Samples of four different types of Choornas were prepared as per IM-COPS standard procedure and digested by a Microwave-assisted method in closed vessels. The solvent used to digest the samples were HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> in the ratio of 4:6 with an irradiation power of 900W and time of 2.5 min. The digested samples were analyzed by Flame Atomic Absorption Spectroscopy. The linearity was plotted for each standard in concentration range of Cu (0.5-2.5 µg/ml), Pb (1.0-5.0 µg/ml) and Zn (5.0-25.0 µg/ml). The concentration of Cu, Zn and Pb in the four formulated Choornas were in the range of Cu (7.0-42.0 µg/gm), Zn (393-1604.4 µg/gm) and Pb (35.7 – 41.8 µg/gm). Thus the analysis of laboratory prepared authentic formulations revealed the presence of lead beyond the limits specified by WHO and FDA [10 ppm]. Copper and Zinc were found within the permissible limits. The contamination may be due to improper selection, identification, collection and variation in processing conditions.*

**Key words:** Ayurvedic choornas, Metal ions, AAS

### INTRODUCTION

The Indian system of medicine such as ayurvedic is an integral part of our national heritage. India has a treasure of various medicinal plants and these are used in ayurveda and also as extracts in modern system of medicine. The use of medicinal plants in both crude and prepared forms has greatly increased and although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effects. Considering the complexity of these drugs and their inherent biological variation, it becomes necessary to evaluate their efficacy, safety and quality [1]. Metallic elements are constituent plant compounds with biological activity as essential or toxic agents in metabolism. Thus the application of metal monitoring as a pattern recognition method in medicinal herbs is a promising tool for their characterization. The WHO (World Health Organization) estimates that 80% of the World population relies on these “Alternative” plant-based medicines as their primary medical intervention [2, 3]. However, the safety of natural substances use has been questioned recently due to the presence of heavy metals above the permissible range.

Choornam is one of the most common dosage form used in Ayurveda and Siddha system of Medicine. Choorna is a Sanskrit word meaning powder. Usually choornas are taken with milk, water, ghrita, honey, curd or buttermilk.

The last few years have seen a significant increase in the use of herbal medicine. Medical doctors are also prescribing herbal teas and herbal extracts as supplementary type of treatment in every day problems. The use of medicinal plants in both crude and prepared forms has greatly increased and although herbal remedies are often perceived as being natural and therefore safe, they are not free from adverse effects.

One of the major reasons to monitor levels of toxic metals in medicinal plants is that the contamination of the general environment has increased. The sources of these environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification of mud and agricultural expedients, such as use of fungicides and insecticides containing heavy metals.

Dhaniya choornam can be used for excessive menstrual bleeding, vitamin deficiency and night-discharge, for digestion, in jaundice, for sleep, in excessive thirst, in burning sensation, for belching, nasal bleeding and palpitation, in fever, in gout, in cough and breathing difficulty, as a coolant, in pregnancy (Giving coriander decoction to the pregnant woman from the fifth month onwards promotes flow of urine and prevents oedema or swelling of the feet, usually noticed from that month of pregnancy onwards). The dose of Dhaniya choornam is around 10 gm at a time once or twice a day. Navayasa choornam is used for jaundice, anaemia, vertigo, heart diseases, inflammations, piles etc. It is administered at a dose of 5 gm/day with honey or butter milk. Triphaladi choornam is used for eye diseases, skin diseases and constipation. It is taken with hot water or honey or ghee, 5 to 10 gm per day. Ashwagandhadi choornam is used for stress, nervine tonic, ensures sound sleep at a dosage level of 200 to 1000 mg per day [4, 5].

In general the most common method for the determination of heavy metals is atomic absorption spectroscopy. It measures the concentrations of metals in the samples in the concentration as low as ppb. In their elemental form, metals will absorb ultraviolet light when they are excited by heat. Each metal has a characteristic wavelength that will be absorbed. The AAS instrument looks for a particular metal by focusing a beam of UV light at a specific wavelength through a flame and into a detector. The sample of interest is aspirated into the flame. If that metal is present in the sample, it will absorb some of the light, thus reducing its intensity. The instrument measures the change in intensity. A computer data system converts the change in intensity into an absorbance.

The aim of the present investigation was therefore to determine the contents of heavy metals like lead, copper and zinc in laboratory made choornas. This paper also represents a simple and sensitive sample digestion procedure for the estimation of heavy metals by flame Atomic Absorption spectroscopy.

## **MATERIALS AND METHODS**

### **Reagents and Chemicals**

The water used was ultrapure water collected from milliQ system. Nitric acid and hydrogen peroxide were of analytical grade (Merck). Metal standard solutions [Sigma Aldrich] were prepared by appropriate dilutions of 1000 mg/L stock solutions.

### **Instrumentation**

The Atomic Absorption measurements were performed with a shimadzu model AA 6300 flame atomic absorption spectrometer (Tokyo, Japan) equipped with a deuterium background corrector. Hollow cathode lamps of specific metals were used as a radiation source.

### **Sample preparation and analysis:**

Samples of four different types of choornas were prepared as per IMPCOPS (The Indian Medical Practitioners Cooperative Pharmacy and Stores) procedure. The prepared samples were dried at 110° C for 2 hours. The dried samples were digested by a microwave assisted method. 0.250 gm of the samples were accurately weighed, 4 ml of conc. Nitric acid was added and subjected to microwave irradiation for 90 secs [900 W, 100°C]. The samples were cooled and 3 mL of 30% v/v hydrogen peroxide was added for further oxidation of the digested organic material and subjected to microwave irradiation for 90 secs [900 W, 100°C]. The samples were cooled and further 3 mL of 30% v/v hydrogen peroxide was added to ensure complete oxidation and irradiated for 90

secs [900 W, 100°C]. The digested samples were then cooled, filtered and made up to 25 mL with ultrapure water. These samples were subjected to analysis for the estimation of metals.

#### Recovery tests

Recovery tests were carried out by standard addition method. A standard solution containing copper, zinc and lead were prepared for the performance of the recovery test. The samples were spiked with the synthetic standards, digested as per the procedure developed and the concentrations of the metals were determined by the recommended procedure.

## RESULTS AND DISCUSSION:

### Determination of metal content:

AAS is the most common analytical tool adopted for measuring trace metals in biological and herbal materials. The operating conditions that provided the best sensitivity are tabulated in Table

1. Table 1: Instrument parameters for the determination of copper, zinc and lead

Instrument parameters	Lead	Copper	Zinc
Optical parameters			
Element	Pb	Cu	Zn
Lamp current (mA)	10	6	8
Wavelength (nm)	283.3	324.8	213.9
Slit width (nm)	0.7	0.7	0.7
Lamp mode	BGC – D <sub>2</sub>	BGC – D <sub>2</sub>	BGC – D <sub>2</sub>
Atomizer/gas flow rate setup			
Fuel gas flow rate (L/min)	2.0	1.8	2.0
Support gas flow rate (L/min)	15.0	15.0	15.0
Flame type	Air – C <sub>2</sub> H <sub>2</sub>	Air – C <sub>2</sub> H <sub>2</sub>	Air – C <sub>2</sub> H <sub>2</sub>
Burner height (mm)	7	7	7
Calibration mode	Absorbance		
Linearity range	1.0 – 5.0 ppm	0.5 – 2.5 ppm	5 – 25 ppm
y = mx ± c	Y = 0.0131x – 0.0035	y = 0.0527x + 0.0011	y = 0.0065x – 0.0246
r <sup>2</sup>	0.999	0.996	0.992

The linearity were plotted for each standard in concentration range of Copper (0.5-2.5 µg/ml), Lead (1.0-5.0 µg/ml) and Zinc (5.0-25.0 µg/ml), based on the detection limit and sensitivity of the instrument. The sample digestion procedure was optimized to ensure complete digestion of organic matters in terms of the amount of nitric acid and hydrogen peroxide added. The metal contents found in the samples under investigation are shown in

Table 2: Results of analysis and recovery studies

S. No.	Samples	Amount present [µg/g]*			% Recovery*		
		Lead	Copper	Zinc	Lead	Copper	Zinc
1	Dhaniya choornam	35.72 ± 0.97	42.16 ± 0.42	1604.41 ± 0.93	97.16 ± 0.77	98.96 ± 0.29	98.21 ± 0.59

2	Navayasa choornam	38.00 ± 0.46	24.25 ± 0.37	731.78 ± 0.19	97.78 ± 0.47	101.28 ± 0.61	100.81 ± 0.44
3	Triphaladi choornam	35.72 ± 0.85	9.14 ± 0.18	447.03 ± 0.48	97.95 ± 0.43	100.81 ± 0.67	101.09 ± 0.23
4	Ashwagandhadi choornam	41.81 ± 0.26	7.65 ± 0.92	618.49 ± 0.37	95.88 ± 0.61	101.19 ± 0.23	100.97 ± 0.36

\* Average and standard deviation of three determinations

The analytical results obtained for lead indicate that they are present at concentrations slightly above the acceptable daily intake recommended by the World Health Organization [10 ppm]. The concentration of Cu, Zn and Pb in the four formulated Choornas were in the range of Cu (7.0-42.0 µg/gm), Zn (393-1604.4 µg/gm) and Pb (35.7 – 41.8 µg/gm). The amount of metals consumed per dose of the choornam was also calculated and presented in

**Table 3: Amount of heavy metals present per dose**

S. No.	Samples	mg of metals consumed per dose		
		Lead	Copper	Zinc
1	Dhaniya choornam	0.3572	0.4216	16.0441
2	Navayasa choornam	0.1900	0.1213	3.6589
3	Triphaladi choornam	0.3572	0.0914	4.4703
4	Ashwagandhadi choornam	0.0084	0.0015	0.1237

### Recovery tests

Recovery tests were performed by standard addition method. A standard solution containing copper, zinc and lead were prepared for the performance of the recovery test. The samples were spiked with the synthetic standards and the concentrations of the metals were determined by the recommended procedure. The results were considered satisfactory; with percentage recoveries being in the range of 94.82 – 101.86 [Table 2].

### CONCLUSION

Based on the results obtained in the present work it may be concluded that the proposed method of digestion is suitable for the determination of heavy metals in the ayurvedic formulations. The digestion procedure is fast and simple, thus making the technique more attractive for its use in the quality control of ayurvedic formulations. The concentration of Cu, Zn and Pb in the seven formulated Choornas were in the range of Cu (7.0-42.0 µg/gm), Zn (393-1604.4 µg/gm) and Pb (35.7 – 41.8 µg/gm). Thus the present analysis of laboratory prepared authentic formulations reveals the presence of lead beyond the limits specified by WHO and FDA [10 ppm]. Copper and Zinc were found within the permissible limits. The contamination of heavy metals may be due to improper selection, identification, area of collection, variation in the weight of the drug added to the formulation and processing conditions.

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