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QUANTITATIVE DETERMINATION AND STANDARDIZATION OF TOXIC ELEMENTS BY USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY (ICPMS) FROM HEBAL MEDICINES

Raju Pundlik Suryawanshi[1]*, Kulakirti R. Suryawanshi[2]

[1]*Dept. of Chemistry, Government of Maharashtra Ismail Yusuf College, Jogeshwari, Mumbai - 400060, Maharashtra, India.

[2]Godavari Foundation's, Dr. Ulhas Patil Homoeopathic Medical College and Hospital, Jalgaon Kh. N.H. No.6 Jalgaon – Bhusawal Road, Jalgaon (Kh) - 425309, Jalgaon Maharashtra, India.

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***Corresponding Author**
Raju Pundlik Suryawanshi
Dept. of Chemistry,
Government of Maharashtra
Ismail Yusuf College,
Jogeshwari, Mumbai -
400060, Maharashtra, India.

ABSTRACT

Heavy metals are naturally occurring elements that have higher atomic weight and density greater than that of water. Those being widely used in domestic, agricultural and medical products have led to their wide distribution in the environment, raising concerns over their potential effects on human health. Their toxicity depends on several factors including those of their dose, route of exposure as well as the age, gender, genetics and nutritional status of exposed individuals. Because of their high degree of toxicity mercury, cadmium, lead and arsenic rank among the top priority of hazardous metals that are of public health significance. Use of herbal medicines is growing worldwide because of their minimal side effects. Herbal medicines require standardization with implementation and constant review of technical standards of production and effective quality control methods. It is necessary to promote this study in the view of importance of herbal medicines. From this perspective, the current submission on toxic elemental determination may pave the way for further clinical studies. These metals can bind to vital cellular components such as structural proteins, enzymes and nucleic acid and interfere with their functions. In human beings, these metals can cause severe physiological and health effects. Hence, it was thought to be of utmost importance to study their impact on human health. Some indispensable herbal medicines are to were scanned for toxic elements, like Hg

(Mercury), Cd (Cadmium), Pb (lead), As (Arsenic) etc. and these metals are then determined quantitatively by using modern technique of ICP-MS, which should be incorporated in routine quality control parameters.

KEYWORDS: Quantitative analysis, ICP-MS, herbal tablets, elemental analysis, toxic elements, heavy metals.

INTRODUCTION

Many synthetic drugs are derived from herbal preparations e.g. Aspirin (acetyl salicylic acid) and Reserpine (Sarpagandha). Due to increase in population, most of the people use herbal products which are now available in different forms like tablets, elixirs and powders.[1] Contamination or adulteration of herbal products with heavy metals such as lead, mercury, cadmium, arsenic, etc. is of major concern. However, some herbal products do contain heavy metals as essential ingredients. The poor quality control of these products causes health hazard as some products may present unusually high concentrations of toxic metals that could lead to fatality if consumed.[2] Therefore, it is thought necessary to study the levels of toxic elements being consumed by patient per tablet so that their repercussions can be evaluated. Ayurveda is based on the hypothesis that everything in the universe is composed of five basic elements viz. space, air, energy, liquid and solid (Panchamahabuta). They exists in the human body in combined form like vata (space and air), pitta (energy and liquid) and kapha (liquid and solid), they are together known as the three pillars of life in the ancient ayurvedic texts. In the preparation of herbal medicines, various parts of the plant such as the root, leave, bark, seed, flower, fruit and stem are used as raw materials, either individually or in combination. After passing through many processes, they are converted into finished herbal products. But patients are not aware about their contents and standards. World Health Organization gives some guidelines[3] for the preparation of herbal medicines and has listed some methods for the standardization of herbal medicines[4] it also gives maximum permissible limit of heavy metal[5] and quality controlled norms. It is important to follow the quality control norms to standardize the herbal medicines. Herbal medicinal products should be entirely free from mould, insects including their excreta, visible contaminant and microbial contaminants like bacteria, fungi and chemical residues. Animal matters such as insects and invisible microbial contaminants which can produce toxins are also among the potential contaminants of herbal medicines. Thin layer chromatography (TLC) is often needed to detect the contaminants. The main methods commonly used are Atomic Absorption Spectrophotometer (AAS), Inductively

Coupled Plasma (ICP) and Neutron Activation Analysis (NAA) for the quantitative analysis of toxic metals. Samples of herbal material are extracted by a standard procedure, impurities are removed by partition and/or adsorption and individual pesticides are measured by GC, MS or GC/MS.

Table 01: Tablet name with the Company name and pants as per label.

Sr. No.	Code.	Brand and Company Name.	Product Name.	Plants as per Label.
1	A	Baidyanath	Sarpagandha	Sarpagandha Powder
2	B	Saife life	Cardiaol vati	Suthi, Arjun ghan, Punarnava, Bringrajn, Abhrak bhasma, shuddha shiljit, Amalki ghan, Guduch ghan, Gokshur ghan, Akik pisti.
3	C	Saife life	Hemiplus vati	Amalaki, Haritaki, Bibhitaki, Sunthi, Pipali, marich, Vidang, Suvarna makshik bhasma, kasis bhasma.

Table 02: Sample Weight and dilution.

Sr. No	Code	Sampls	Weight	Dilution
1	A	Sarpagandha	0.37287	100 mL in1% HNO_3
2	B	Cardiaol vati	0.45548	100 mL in1% HNO_3
3	C	Hemiplus vati	0.2527	100 mL in1% HNO_3

Some simple procedures namely TLC, HPLC, GC, quantitative TLC (QTLC) and high-performance TLC (HPTLC) can determine the homogeneity of a plant extract. Over-pressured layer chromatography (OPLC), infrared and UV-VIS spectrometry, MS, GC, liquid chromatography (LC) used alone, or in combinations such as GC/MS, LC/MS and nuclear magnetic resonance (NMR), electrophoretic techniques, especially by hyphenated chromatography are powerful tools often used for standardization and to control the quality of both the raw material and the finished product. Validation and investigations must include studies on specificity, linearity, accuracy, precision, range, detection and quantitative limits depending on whether the analytical method used is qualitative or quantitative. TLC and HPLC are the main analytical techniques commonly used in cases when active ingredients are not known or too complex. Quality control for efficacy and safety of herbal products is of

utmost importance. The assurance of safety of herbal drug requires monitoring of quality of the finished product as well as the quality of the consumer information on the herbal product. It is important to make sure that the consumer is made aware of the interactions the herbs might have with other drugs they are taking. Unfortunately this information is not available with herbal medicines.[6] Standardized herbal medicines with maintained quality containing well defined constituents are required for reliable maximum beneficial therapeutic effects.

Most of the herbal medicinal products are not labeled appropriately according to their elemental contents. Keeping the above points in view, the determination of various metals in the herbal medicines was done by ICP-MS method which has high degree of sensitivity and specificity.

MATERIALS AND METHODS

Chemicals

Yttrium as internal standard, deionized water solution of 0.5% nitric acid and 2 ppm gold (Thermo- fisher ICP-MS icap model).

Sampling

In the present study, the marketed herbal tablets Sarpagandha, Cardiol vati, Hemiplus vati, Medomine vati, Arthowin vati and B.P.C capsules are selected for the analysis. The brand names of the products, license number and the plants used as per company's label are included (Table 01).

Experimental design

Code numbers namely A to C was assigned for Sarpagandha, Cardiol vati and Hemiplus vati. By taking the weight of each tablet on digital balance, each tablet sample was gently ground to fine powder using mortar and pestle and packed in butter paper until analysis. The dilution is given in Table 02 and the general analytical conditions are shown in Table 03. Quantitative multi-elemental analysis by inductively coupled plasma (ICP) [Icap-Q] mass spectrometry depends on complete digestion of solid samples.

Table 03: General analytical Conditions.

Sr. No.	Parameter	Value
1	Spray chamber temperature	2.7
2	Cool flow	14
3	Sampling depth	5

4	Plasma power	1550
5	Auxiliary flow	0.8
6	Nebulizer flow	1.0079
7	Spray chamber temperature	2.7
8	Peristaltic pump speed	25

Table 04: Multi-Elemental standards and Mercury analysis.

Sr. No	Concentration	Yttriu (1ppm)	MES	MES+Hg (20ppb)	Final Volume(mL)
1	Std 0.05ppb	750µL	-	75µL	30
2	Std 0.5ppb	750µL	-	750µL	30
3	Std 1.0ppb	750µL	-	1500µL	30
4	Std 2.0ppb	750µL	-	3000µL	30
5	Std 5.0ppb	750µL	150µL	-	30
6	Std 20ppb	750µL	600µL	-	30
7	Std 50ppb	750µL	1500µL	-	30
8	Std 100ppb	750µL	3000µL	-	30
9	Std 200ppb	750µL	6000µL	-	30

Table 05: Accuracy of Toxic Elemental Concentration ppm by ICP-MS.

Sr.No	Sample	Elements in ppm.			
		Hg	Cd	Pb	As
	Sarpagandha	ND	0.0003	0.00645	0.00068
2	Cardiaol vati	ND	0.00015	0.01261	0.00081
3	Hemiplus vati	ND	0.00028	0.02499	0.00298

However, fast and thorough sample digestion is a challenging analytical task in modern multi-elemental analysis. To determine each toxic metal concentrations, 0.125 mL internal standard and 4.675 mL of diluent was added in to 0.2 mL sample solution. Deionzed water solution of 0.5% nitric acid and 2 ppm gold was used as a diluent. Multi-elemental standards and mercury analysis is given in detail in Table 04. Statistical analysis: The obtained values were properly validated with standard deviation, standard error and coefficient variance. In addition to normal validation parameters, average weight equal to each tablet is analyzed from the crushed powder (3/4/5/6 tablets) as additional validation.

RESULTS AND DISCUSSION

Toxic elements namely Hg, Cd, Pb and As are of great importance for life in micro quantities and these metallic elements are considered as systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. Figure 1 indicates the graphical representation of each toxic element per sample. X-Axis indicates the sample number 1 to 3

and Y-axis indicate the detected values of toxic elements per sample in ppm. Figure 01. Graphical representation of toxic elements in each sample.

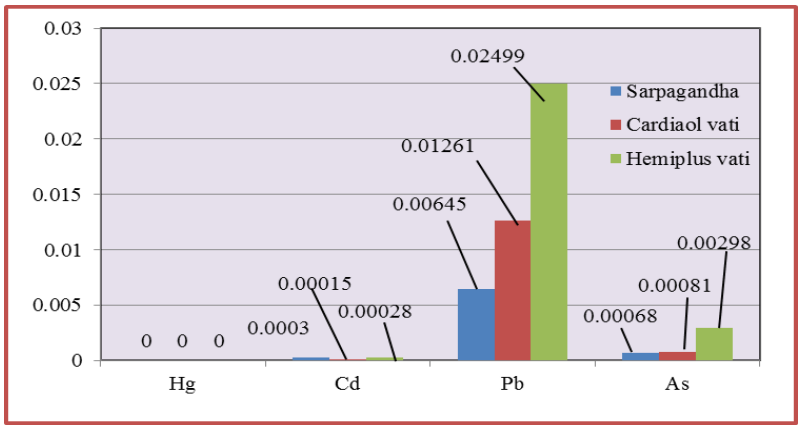

Figure 01: Graphical representation of Toxic Elements in each Sample.

As shown in Fig. 01, samples Sarpagandha (1), Cardiol vati (2), Hemiplus vati (3) detected the highest value of Pb-0.00645 ppm, 0.01261 ppm, 0.02499 ppm, respectively, second highest toxic element was As, Hg was not detected in all samples.

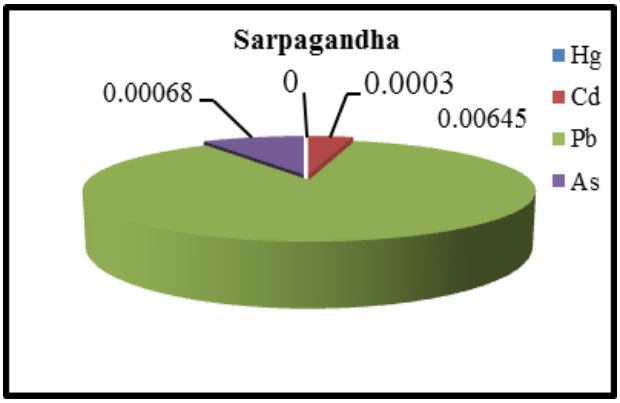

Figure 02: Sarpagandha.

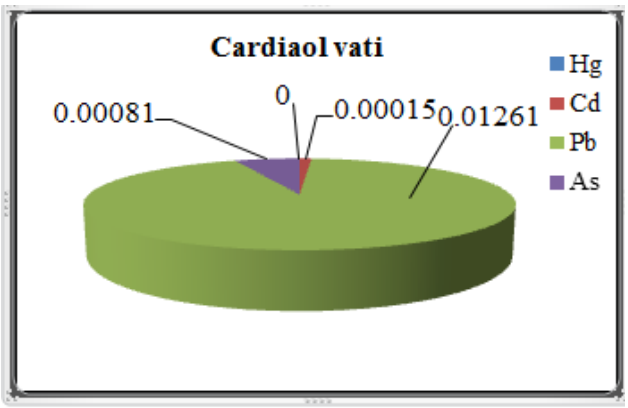

Figure 03: Cardiaol vati.

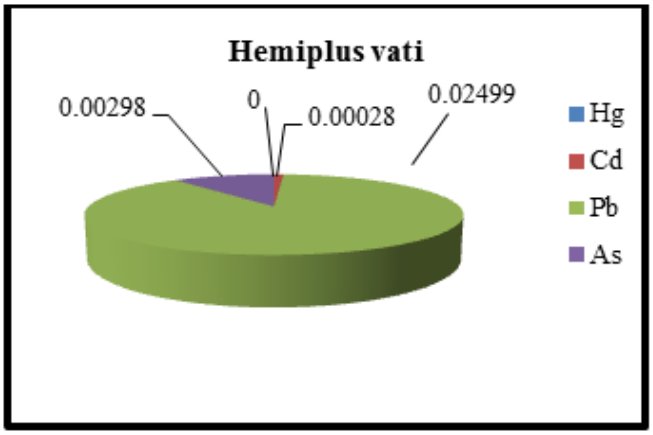

Figure 04: Hemiplus vati.

Table 06: LD50 of the elements (The Merck Index, 1989).

Sr. No	Elements	Compounds	LD50
1	Mercury	Mercumatilin sodium Mersalyl	238 mg/kg orally in rat 17.7.mg/kg iv in rat
2	Cadmium	Cadmium Chloride	88mg/kg orally in rat
3	Lead	Lead Acetate	200mg/kg iv in rat
4	Arsenic	Arcenic Acid	6mg/kg iv in rabbi

Table 07: JECFA Heavy Metal Limits.

Sr. No	Elements	Stated Limit (PTWI-Weekly)	Calculated daily limit (Adult, 70kg)	EU Status
1	Mercury	1.6 µg methylmercury/kg bw	16µg	Adopted 02/04/2004
2	Cadmium	07 µg Cadmium/kg bw	70 µg	Endorsed 06/02/1995
3	Lead	25 µg lead/kg bw	250 µg	Endorsed 06/09/1992
4	Arsenic	15µg inorganic arsenic/kg bw	150 µg	No Information Found

The detected level of toxic elemental concentration in selected samples by ICP-MS is given in Table 05. Recently published FDA regulations hold supplement manufacturers or distributors responsible for the contents of the dietary supplement which should only contain what they are labeled as and not any harmful or undesirable substances, including pesticides and heavy metals.[7] However, it should be made mandatory to include concentration of all elements present in the herbal preparations. Lead, Cadmium, Arsenic and Mercury are considered as toxic elements, therefore, their daily involuntary intake via herbal supplements has been regulated. Estimated exposures/intakes of As, Cd, Hg and Pb were assessed with

respect to safe/tolerable exposure levels described by various national and public health organizations.[7]

Sarpagandha

In Sarpagandha sample, most abundant element was Pb-0.00645 ppm, whereas, Cd was found in lowest concentration (0.0003 ppm) and Hg was not detected.

Cardiol vati

In Cardiol vati sample, most abundant element was Pb-0.01261 ppm, whereas, Cd was found in lowest concentration (0.00015 ppm) and Hg was not detected.

Hemiplus vati

In Hemiplus vati sample, most abundant element was Pb-0.02499 ppm whereas, Cd was found in lowest concentration (0.00028 ppm) and Hg was not detected.

CONCLUSION

Results obtained from ICP-MS analysis of tablet samples detected the accurate values of toxic elements concentration in ppm. All these values of toxic elements showed less toxicity in herbal medicines and detected below LD50 (Table 06) and Joint FAO/WHO Expert Committee on Food Additives (JECFA) values for heavy metal limits is shown in Table 07, these shows the herbal medicines are very safe for human consumption. The content of the toxic elements is not indicated on their label. Elemental analysis by ICP-MS is a recent technique which gives more accurate concentration of toxic elements contain in the samples which is not previously reported by researchers. Determination of metals is done by atomic absorption spectrophotometer in bhasma only, not in tablets. The concentration of the toxic elements is found below the hazardous levels. The tablets studied did not show concentration hazardous to humans.

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