Research Article

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ESTIMATION OF HEAVY AND TRACE ELEMENTS IN AYURVEDIC DRUG (LOHA BHASMA) ALTERNATIVE MEDICINE FOR ANEMIA BY AAS AND ICP-OES

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Received on: 09/0617 Accepted on: 05/08/17

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DOI: 10.7897/2277-4343.085249

ABSTRACT

Nowadays, consumption of Ayurvedic medicines/ bhasmas/plants has been increasing due to increased demand and also these consumed medicines don't lead to abnormal and side effect on any organ or body part. These are prepared by different Ayurvedic practitioners. Ayurvedic herbo-mineral preparations were made by calcinations of purified metals, minerals, since it has been playing a vital role in various ailments due to its curative property by oral consumption. Thus, it is important to know quantitative elemental analysis of these bhasmas. In the present study Loha Bhasma an alternative medicine of ayurvedic drugs for anemia, is selected and is orally given for the anemia based patient treatment which in turn increases the RBC's level and reduces the malignant cells. The elemental analysis is carried out for Major and minor/trace concentration such as Mg, Al, K, Ca, Cr, Mn, Fe, Cu, Zn, As, Cd, Hg, Na, P, Pb, Sn, F, Cl and is carried out by Atomic Absorption Spectroscopy (AAS) and Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Hence measurement of the major, trace and heavy metal contents present in bhasmas manufacture by different brands is necessary from the health stand point of view. Commercially available Loha Bhasma is used for elemental analysis and purchased as branded names such as Patanjali loha Bhasma (PLB), Baidynath loha bhasma (BLB), Ayukalp loha bhasma (ALB) and Dhootapapeshwar loha bhasma (DLB). The result indicates that bhasmas prepared by different practitioners is not too different except few cases.

Keywords: Ayurvedic medicine (Loha Bhasma), AAS, ICP-OES, Iron deficiency anemia, quantity of elements, iron nanoparticle.

INTRODUCTION

According to the World Health Organization, two billion people, over 30 percent of the world's population are anemic. Although anemia is a symptom of many diseases, there are many instances where the cause cannot be determined. Iron deficiency anemia is one of the most common types of anemia and can be related to diet and poor intake of iron rich foods. Iron is important to the body for many reasons as it is required as a component in hundreds of enzymes and functions in the human body. Iron deficiency anemia can be improved with an iron enriched diet including animal and plant sources. Therefore, one needs several sources of dietary iron to make functioning of red blood cells. Iron deficiency anemia can be mild and may be undiagnosed until the symptoms intensify. The term cancer in general is a tumor that describes the cluster of cells undergoing uncontrolled growth in the body Tumors are abnormal growths in body. They can be either benign or malignant. Benign tumors aren't cancer. Malignant ones are. Benign tumors grow only in one place they cannot spread or invade other parts of body[1]. Iron nano particles can activate the immune system to attack cancer cells, according to a study led by researchers at the Stanford University School of Medicine[2].

In Ayurveda medicines are prepared by various metals, minerals, plants, alloys etc. for the treatment of different illness. The fine copper metal powder is used in preparation of Tamra bhasma (calx of Copper), tin metal powder is used in vanga bhasma (calx of Tin), zinc metal powder is used in yashad bhasma (calx of Zinc) and iron metal powder is used as primary element in loha bhasma (calx of Iron). Use of ayurvedic medicines is increasing because of its curative property and lesser or no side effects. Now a day, due to increased demand of ayurvedic medicines, various companies have started preparing medicines, bhasma is one of them. If bhasma is not prepared as per classical text, deposition of administered bhasma in vital organs may lead to serious consequences[3]. Survey of Literature showed that researchers made an attempt towards the standardization of bhasma[4] preparations. Also study of acute and sub-chronic toxicity of bhasma was carried out[5] by different laboratory. Ruby is restricted in therapeutic use because of its hardness property and due to lack of standardization, hence an attempt was made by researcher[6] to prepare and Standardize Manikya (Ruby) Bhasma (calx of ruby) to facilitate its use in Ayurvedic therapeutics. Similarly, Chemical analysis for inorganic constituents and also trace elements of Vanga Bhasma (calx of Tin) by XRF and ICP-MS method was carried out by researcher[7]. Tests were carried out for finding the phases of lauha(loha) bhasma (calx of Iron) at different puta (prepared at different temperatures or Quantum of heat) and also chemical characterization of the lauha(loha) bhasma (calx of Iron) at different steps[8]. Further, at different laboratories the study has been carried out about effect of particle size and elemental analysis for determining the

quality of Lauha Bhasma[9] (calx of Iron). Pharmaceutical and analytical studies were conducted for the chemical change of composition of loha bhasma (calx of Iron) during preparation process[10]. In this article an attempt was made to analyze Loha bhasma medicine (calx of Iron) as a chemical constituent present in as a majorand minor or trace amount through quantitative analysis manufactured by brands such as Patanjali, Baidyanath, Ayukalp, and Dhootapapeshwar.

In Ayurveda Loha bhasma (calx of Iron) is usually suggested in treatment of anemia along with some other diseases. Anemia is caused due to lack of Red Blood Cells counts in blood or Hemoglobin content in blood which carries oxygen from lungs to rest of the body for functioning. If Blood is not replacing RBCs in time means there is chance of forming malignant cells in body[11] which in turn cause cancer, this can be caused by a stomach ulcer, stomach cancer, bowel cancer[12]. There are many things which may lead to a lack of iron in the body. If Immune system of the human is week in functioning than easily cells become cancerous. Weak immune system is one of reason for anemia. The primary treatment of anemia is iron rich diet which will supplement to body. From different laboratory studies it is c showed that iron rich nanoparticle triggers the immune system's ability to destroy tumor cells[1]. Hence here an attempt is made to determine the Iron content and other elements in Ayurvedic medicine Loha bhasma (calx of Iron).

In the preparation of herbal/ayurvedic medicines, various parts of the plant such as roots, stem, bark, leaves, flowers, seeds, buds, fruits etc. in dry form or in original form are used as a raw material as single or in combination[13]. The commercial brands like Patanjali, Baidyanath, Dhootapapeshwar, Ayukalp prepares the herbal medicines/bhasma after passing through purification, calcinations and levigation processes[14]and the product is ready for marketing purposes. These available marketing medicinal loha (calx of Iron) bhasmas are used for elemental analysis studies.

Most of the people use Ayurvedic/herbal medicines which are now available in different forms like tablets, elixirs, powders and bhasma. Since Ayurvedic traditional medicines have become more popular as alternative and supplementary medicines in recent years, so contamination of Ayurvedic drugs with trace elements is major concern. The poor quality of these medicines causes health hazards. The unusual presence of high concentrations of trace elements may lead to fatality, if consumed for a longer time. Hence it seems necessary to know the levels of trace and heavy elements present in the ayurvedic drugs (loha bhasma). Thus, in the present work an elemental analysis was carried out by Atomic Absorption Spectroscopy(AAS) and Inductively Coupled Plasma-Optical Emission Spectroscopy(ICP-OES).

MATERIALS AND METHODS

Commercially available Ayurvedic medicine loha bhasma of four brands were procured from ayurveda medicine stores and labeled as PLB, BLB, ALB, and DLB. The purchased bhasmas were used for elemental analysis. Elemental analysis was carried by AAS which was supplied by Thermo Scientific, with iCE 3000 Series[15].

Sample preparation for AAS analysis

As per the standard preparation of solution for the AAS[16], usually solution of 50 ml is prepared, in the proportion 1:25:25 ratio i.e., 1gm of sample is digested in 25 ml Conc. HCL and 25ml of Double distilled water and kept over a night and filtered the solution by Whatman filter paper, again this 50ml solution is distilled by adding 950ml of Double distilled water, finally 1000 ml solution was prepared which is used for the analysis purposes. Using the standards the calibration graph is plotted and the details of the lamps used for the different elemental analysis are given below in the Table 1.

Sample preparation for ICP-OES analysis

ICP-OES: Inductively Coupled Plasma -Optical Emission Spectrometer (ICP-OES) Make: Agilent Technologies Model: 725 series ICP-OES analysis was carried out at C-MET[17], using C-MET laboratory developed method used for the analysis.0.1 g of sample was taken in Teflon tubes and added 6.0 ml of Nitric acid and 2.0 ml of Hydrogen peroxide and allowed for 10 minutes in outside. Then samples are dissolved using Microwave Digestion System (Anton ParaMultiwave 3000). Microwave system parameters are as in following table 2.

Then sample solutions were made to 25.0 ml and filtered. These solutions were used for Elemental analysis using ICP-OES instrument (Agilent 725 series). Fluoride and Chloride analysis was carried out using IC Instrument (Metrohm). Chloride and Fluoride was extracted by water extraction method.

Working of AAS and ICP-OES

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements[18]. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (µg dm–3) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. Atoms of different elements absorb characteristic wavelengths of light. Analyzing sample to see if it contains a particular element means using light from that element. For example with lead, a lamp containing lead emits light from excited lead atoms that produce the right mix of wavelengths to be absorbed by any lead atoms from the sample. In AAS, the sample is atomized – i.e., converted into ground state free atoms in the vapor state – and a beam of electromagnetic radiation emitted from excited lead atoms is passed through the vaporized sample. Some

of the radiation is absorbed by the lead atoms in the sample. The greater the number of atoms there is in the vapor, the more radiation is absorbed. The amount of light absorbed is proportional to the number of lead atoms. A calibration curve is constructed by running several samples of known lead concentration under the same conditions as the unknown. The amount the standard absorbs is compared with the calibration curve and this enables the calculation of the lead concentration in the unknown sample.

ICP, abbreviation for Inductively Coupled Plasma, is one method of optical emission spectrometry[19]. When plasma energy is given to an analysis sample from outside, the component elements (atoms) is excited. When the excited atoms return to low energy position, emission rays (spectrum rays) are released and the emission rays that correspond to the photon wavelength are measured. The element type is determined based on the position of the photon rays, and the content of each element is determined based on the ray intensity. To generate plasma, first, argon gas is supplied to torch coil, and high frequency electric current is applied to the work coil at the tip of the torch tube. Using the electromagnetic field created in the torch tube by the high frequency current, argon gas is ionized and plasma is generated. This plasma has high electron density and temperature (10000K) and this energy is used in the excitation-emission of the sample. Solution samples are introduced into the plasma in an atomized state through the narrow tube in the center of the torch tube.

Table 1: AAS Instrument Analyzer Parameters

Element	Wavelength λ_{max} (nm)	Lamp current	Flame type	Slit width (nm)
Mg	285.2	75%	Air C2H2	0.5
Al	309.3	100%	N2O-C2H2	0.5
K	766.5	100%	Air C2H2	0.5
Ca	422.7	100%	N2O-C2H2	0.5
Cr	357.9	100%	N2O-C2H2	0.5
Mn	279.5	75%	Air-C2H2	0.2
Fe	248.3	75%	Air-C2H2	0.5
Cu	324.8	75%	Air-C2H2	0.5
Zn	213.9	75%	Air-C2H2	0.2

Table 2: Microwave system parameters

1	Pressure	50 bar
2	Time	90 min
3	Wattage	900
4	Temperature	240 ^{0}C

Table 3: Elemental analysis of Loha bhasma by AAS

Sample Code	Elemental Concentration in ppm								
	Mg	**Al**	**K**	**Ca**	**Cr**	**Mn**	**Fe**	**Cu**	**Zn**
ALB	286	439	2004	1114	1418	1453	21567	264	199
DLB	547	85	1137	1371	569	466	19561	2455	267
PLB	1810	ND	3414	5532	686	2024	20634	400	104
BLB	394	ND	122	810	331	1916	22733	781	144
Average value	760	262	1669	751	826	1465	21124	975	714
Mean	759.2	131.0	1669.2	2206.7	751.0	1464.7	21123.7	975.0	178.5
N	4	4	4	4	4	4	4	4	4
Std Dev	708.6	209.2	1394.4	2228.6	468.5	710.4	1350.0	1010	70.69

ND- not detected, ppm- part per million

Table 4: Elemental analysis of Loha bhasma by ICP-OES

Sample Code	Elemental Concentrations in ppm								
	As	**Cd**	**Hg**	**Na**	**P**	**Pb**	**Sn**	**F**	**Cl**
ALB	39.04	2.66	6.99	522.63	248.94	7.6	26.1	ND	3152
DLB	28.26	3.81	ND	205.69	281.21	27.7	32.3	186	414
PLB	ND	ND	ND	410.93	1647.1	21.74	26.92	83	180
BLB	46.82	3.21	ND	260.45	523.02	32.15	41.49	ND	554

ND- not detected, ppm- part per million

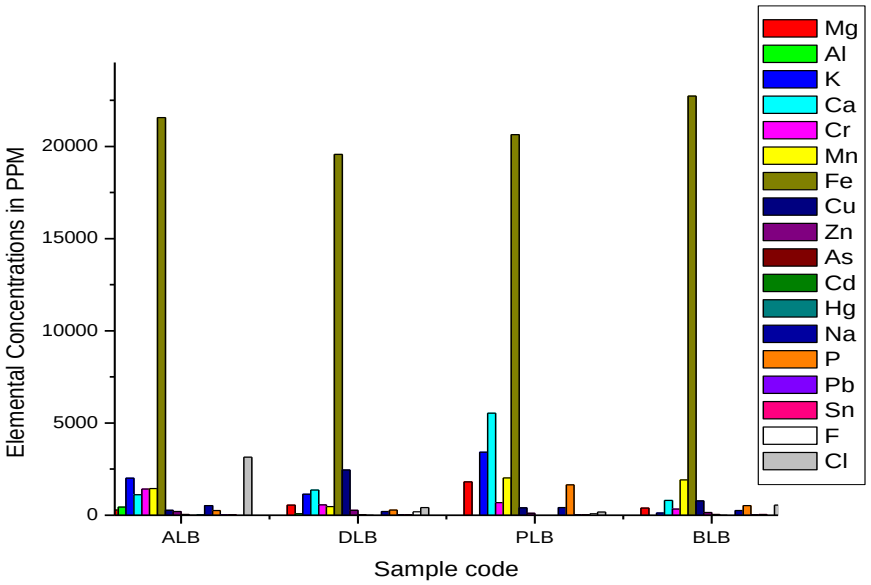

Figure 1: Elemental concentrations of loha bhasma in four brands.

RESULT AND DISCUSSION

The detected accuracy of essential elemental concentration in ppm level in loha bhasma (calx of Iron) of four brands by AAS method is presented in Table 3. Total of nine elements were found in loha bhasma in various proportions viz., Mg, Al, K, Ca, Cr, Mn, Cu and Zn along with Fe element. The respective elemental concentrations were presented from columns 2 to 9 respectively. Except Iron element in all bhasmas all other elements are available in trace amount and found to vary from 5 to 62 % with their respective mean values except few cases viz., for the elemental concentration of Mg, K, Ca and Mn from Patanjali, and Cu from Dhootapapeshwar.

From Figure 1 and also from Tables 3 the observed major content in the loha bhasma of four brands is found to be Iron (Fe). Following the iron other elements are also found present in the loha bhasma in trace form which is also observable from Figure. The variation level of concentrations of elements of the same drug by different brand may be due to the method of preparations or the secondary ingredients such as herbs and its parts like fruits juice, leaves juice, or decoction of plants etc., which were used while processing the mineral. Manufacturer of loha bhasma were not mentioning the percentage of content of elements as prescribed by the International or national standard bodies on the packet or boxes with specific bhasma or drug. Hence the data observed by the AAS method in this work may be useful in quality assurance and also the levels of trace elements being consumed by patient in the form of powder or bhasma is studied since patients are not aware about their contents and standards.

As per the WHO's guidelines the tolerable daily minimum requirement of iron consumption may be ranged from about 10 to 50 mg/ day for a person weigh in the range from 25 to 75 kg and as presented in Table 3 the iron (Fe) level found below the range mentioned[20] in loha bhasma by AAS method. Similarly, we also observed the heavy elements in the bhasma such as As, Cd, Hg, Pb and along with Fluorine, Chlorine, Sodium, Phosphorus elements in loha bhasma which are determined through ICP-OES analysis as given in the Table 4. This work is done due to non availability of the standard concentrations of AAS the data elemental concentrations were carried through ICP-OES.

By ICP-OES analysis the heavy metals such as As, Cd, Hg, and Pb are found in ALB. Whereas, Pb is found only in PLB loha bhasma but other elements such as As, Cd, Hg not detected. Similarly, in DLB and BLB all other elements are found except mercury which is not detected. Like high atomic numbers similarly low atomic number such as Fluorine (F) is found in two brands DLB and PLB only whereas Sodium, Potassium, Tin and Chlorine are observed in all the four brands. This shows that the loha bhasma manufactured by four manufactures might have adopted different process or the raw materials used by them may contain some percentage of high atomic number elements or they might be added during the manufacturing processes. The poor quality control of these medicines may cause health hazards. If the presence of unusual high concentrations of toxic minerals found, then it may lead to fatality if consumed for a longer period of time, from this point of view the present study is carried out by using AAS and ICP-OES methods.

CONCLUSION

The content of elements present in the loha bhasma are not indicated on the label by the different manufactures, but using AAS and ICP-OES are the techniques useful in determining matrix of multi elemental concentrations studies. These techniques give concentrations of minerals in major and trace amount contain in the ayurvedic drugs or bhasma, which is not noticed by any of the previous reports of the researchers. It may be helpful in quality test of the drugs in safety concern as suggested by the International and National Bodies.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to UGC BSR providing the financial support to carry out this work. The authors are also thankful to the Ayurveda medicine brands Patanjali, Baidyanath, Dhootapapeshwar and Ayukalp.

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Cite this article as:

Ashwini A and B. R. Kerur. Estimation of heavy and trace elements in Ayurvedic drug (Loha bhasma) alternative medicine for anemia by AAS and ICP-OES. Int. J. Res. Ayurveda Pharm. 2017;8(5):81-85 http://dx.doi.org/10.7897/2277-4343.085249

Source of support: Nil, Conflict of interest: None Declared