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# QUANTITATIVE AAS STIMATION OF HEAVY METALS AND TRACE ELEMENTS IN MARKETED AYURVEDIC CHURNA PREPARATIONS IN INDIA

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## ABSTRACT

*Churna* preparations are an important and widely used form of Ayurvedic herbal formulations in India. These are prepared by mixing powdered form of single or mixture of several crude drugs meant to be dispensed as such. Since the quality of raw material plays an important role in the overall quality of a herbal formulation due to common practice of collecting and processing medicinal plants from different geographical sources and the fact presence of certain trace elements and heavy metals have a great significance in this matter, the present study is based on the screening of 19 popular herbal *Churna* preparations sold in the Indian market for the quantitative analysis of essential trace and toxic heavy metals by atomic absorption spectrometry. Heavy metals like Pb, Cd and trace metals like Ca, Mg, Al, Cu, Zn were determined using flame atomic absorption spectrometer (FAAS) and heavy metals such as As and Hg were determined by hydride generation technique (cold vapour atomic absorption spectrometery). The results reveal that among the trace (micronutrients) metals Ca and Mg were found in highest amount. Sixteen samples for Hg content and eight for Pb content were exceeding the WHO permissible limits. Arsenic was found below the permissible limit while Cd was above the permissible limit in all the tested samples. In conclusion, the quality of herbal *Churna* preparations sold in India market is questionable and need to be regulated efficiently before launching in to the market. Besides, the present paper provides a simple, convenient and reliable AAS method for the quantitative analysis of trace and heavy metals in herbal products which can be utilized for industrial purpose.

**INTRODUCTION:** Safety and efficacy of herbal medicines are two main issues of a drug therapy to which, the source and quality of raw materials plays an important role [1]. There is wide awareness among the scientific community regarding the quality control of herbal drugs and formulations in the last decade. Associated factors such as the use of fresh plants, temperature, light exposure, water availability, nutrients, period and time of collection, method of collecting, drying, packing, storage and transportation of raw material, age and part of the plant collected, etc., can greatly affect the quality and consequently the therapeutic value of herbal medicines [2]. The World Health Organization, in a number of resolutions, has also emphasized the need to ensure the quality control of plant products by using modern techniques, suitable analytical methods and by applying suitable standards [3, 4].

The human body requires a number of trace elements like Ca, Mg, Al etc. in order to maintain good health. These trace elements, essential for human nutrition are accumulated in different parts of plants transferred from the environmental conditions during their normal growth pattern [5, 6]. In human beings, these elements are mostly required in amounts less than 100 milligrams per day and are present in specific tissues and fluids of body. They maintain the certain physio-chemical processes, structural components of tissues and as constituents of enzymes in many metabolic pathways [7]

On the other side, several scientific report have indicated that herbal medicines also contain the toxic heavy metals which can cause various toxic effects like cancer, liver dysfunction, lung disease, cerebral hemorrhage, alopecia etc. One of the major reasons of incorporation of toxic metals in medicinal plants is due to the increase in contamination of the general environment [8]. The sources of this environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium containing dung, organic mercury fungicides, and the insecticide lead arsenate [9]. According to the WHO (World Health Organization, 1991), lead, cadmium, chromium, and other heavy metals must definitely be controlled in medicines in order to assure their safety [10].

Various different types of herbal preparations are prepared from medicinal plants in which the most frequently used type of herbal preparation is *Churna. Churnas* are preparations comprising of fine powders of medicinal plants and may be single or in combination [11]. Several attempts have been made regarding the estimation of toxic heavy metals and trace elements in medicinal plants and formulations however no attempt has been made so for in *Churna* preparation despite of being the maximum probability of adulteration [12-15].

Therefore, it was imperative to screen the present state of herbal *Churna* preparations popular in Indian market in terms of heavy metals (Pb, Cd, As, Hg) and trace elements (Ca, Mg, Zn, Cu and Al).

**Experimental**

**Samples:** Nineteen samples of marketed herbal *Churna* preparations were collected from local market of Mansa, Punjab. The product name and indications printed on the label is presented in **table 1**.

**TABLE 1: DETAILS OF HERBAL *CHURNA* PRODUCTS COLLECTED FOR STUDY**

| Product Name | Use |
|---|---|
| Gokshuradi Churna | Weakness, immunostimulent |
| Nimbadi churna | Skin problems |
| Divyarasayan Churna | Immunomodulator aphrodisiac |
| Kamdev churna | Immunomodulator |
| Lavanbhaskar Churna | Carminative, digestive |
| Avipattikar churna | Acidity, constipation |
| Triphala Churna | Constipation, Digestion |
| Hingwashtak Churna | Indigestion, flatulence |
| Panchsakar Churna | Constipation, intestinal disorders |
| Hingvashtaka Churna | Indigestion, colic and allied troubles |
| Agnimukh churna | Tasty, digestive |
| Pushyanug Churna | Gynecological disease |
| Triphala churna | Constipation, |
| Avipattikar churna | Acidity, constipation |
| Sitopladi Churna | Fever,cough, general debility |
| Lavanbhaskar Churna | Carminative, Digestive |
| Sitopaladi Churna | Fever, cough, general debility |
| Anwala churna | Acidity, constipation, blood purification |
| Kabja-har | Constipation |

**Instrument:** Atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS Element AS AAS4141) equipped with hydride generator was used for determination of trace elements and heavy metals. The hollow-cathode lamps for Al, Cu, Mg, Zn, Cd, Hg (ECIL) and Ca, As, Pb (Photron) were employed as radiation source. The fuel used was air/acetylene. Nitrogen was used as carrier gas.

**Chemicals:** Standards of Ca, Zn ,Cu, Mg, Cd,Pb (CPA chem), and standards of As, Hg were supplied with instrument. Sulpuric acid ($H_2So_4$), hydrogen peroxide ($H_2O_2$)- Loba chemie, Nitric acid($HNo_3$), hydrochloric acid(HCl)- Thomas baker, Sodium borohydride, Stannous chloride- E. Merck. Reverse osmosis water-Rions company.

**Sample preparation:** Samples were digested by wet digestion method. Briefly, 10 ml of nitric acid was added to 2 g of accurately weighed dried sample in a 100 ml beaker and was heated on a hot plate at 95°C for 15 min. The digest was cooled and 5 ml of concentrated nitric acid was added and heated for

additional 30 min at 95°C. The last step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 2 ml of deionized water and 3 ml of 30% hydrogen peroxide was added. With the beaker covered, the sample was heated gently to start the peroxide reaction. If effervescence becomes excessively vigorous, sample was removed from the hot plate and 30% hydrogen peroxide was added in 1 ml increments, followed by gentle heating until the effervescence was subsides. 5 ml of concentrated hydrochloric acid and 10 ml of deionized water was added and the sample was heated for additional 15 min without boiling. The sample was cooled and filtered through a Whatman No. 42 filter paper and diluted to 50 ml with deionized water.

**Sample analysis:** Digested samples were analyzed for Pb, Cd, Ca, Zn, Mg, Cu and Al using flame atomic absorption spectrophotometer and for As, Hg using hydride generation technique. Hg was analyzed by cold vapour atomic absorption spectrometery. Standard dilutions for each metal were prepared in five different concentrations from their respective stock solution (1000 ppm) to obtain calibration curve. All the measurements were run in triplicate for the samples and standard solutions. The instrumental conditions during the analysis of trace and heavy metals are listed in **Table 2**.

**TABLE 2: INSTRUMENTAL CONDITION FOR ANALYSIS**

| Parameter | Ca | Mg | Cu | Zn | Al | Cd | Pb | As | Hg |
|---|---|---|---|---|---|---|---|---|---|
| Wavelength (nm) | 422.7 | 285.2 | 324.7 | 213.9 | 309.3 | 228.8 | 217 | 193.7 | 253.6 |
| Slit width (nm) | 0.5 | 0.5 | 0.5 | 1.0 | 0.5 | 0.5 | 1.0 | 1.0 | 0.5 |
| Light source | HCL | HCL | HCL | HCL | HCL | HCL | HCL | HCL | HCL |
| Flame type | A | A | A | A | B | A | A | A | - |
| Current | 3.5 | 3.5 | 5 | 5 | 10 | 3.5 | 10 | EDL | EDL |
| AAS Technique | Flame | Flame | Flame | Flame | Flame | Flame | Flame | Hydride generator | Cold vapour |

HCL – Hollow cathode Lamp, Air/$C_2H_2$ – A, Air/$N_2O$/$C_2H_2$ – B.

**Recovery study**: The method of AAS was validated by the method of standard addition [16,17]. In order to demonstrate the validity of our method, a recovery study was carried out. A synthetic solution containing Ca, Mg, Zn, Cu, Al, Cd, Pb, Hg and As were prepared for the performance of the recovery test. Portions samples were mixed with the synthetic solution, then the elements were determined after dilution of the samples to 50 ml.

**RESULTS AND DISCUSSION:** Concentrations of different trace elements, heavy metals and recovery studies are presented in **table 3, 4 and 5** respectively. Data reveals that the Calcium concentrations varied from 227 to 10934 ppm. Sample 19 had the lowest Calcium concentration and 9 had the highest.Calcium was found in detectable limit in all samples.The Copper concentrations varied from 0.09 to 50 ppm. Sample 6 had the lowest Copper concentration and 11 had the highest. The concentrations of Copper were comparable in 2 and 3 with a range of 14.24-14.29 ppm, in 10 and 12 with a range of 16-34-16.50ppm, in 14 and 16 with a range of 11.89-12.07 ppm. The Magnesium concentrations varied from 237.5 to3701 ppm. Sample 19 had the lowest Copper concentration and 12 had the highest. The concentrations of Magnesium were comparable in 4 and 5 with a range of 751-770 ppm, in 6 and 18 with a range of 1047-1057 ppm. The Zinc concentrations varied from 7.58 to 38.94 ppm. Sample 18 had the lowest Zinc concentration and 4 had the highest. The concentrations of Zinc were comparable in 3 and 9 with a range of 18.31-18.54 ppm, in 8 and 12 with a range of 24.28-24.89 ppm, the same being true for 13 and 19 at 13.79-13.94 ppm. The Aluminium concentrations level ranged from 286.35 to 828.33 ppm.

Sample 11 had the lowest aluminium concentration and 12 had the highest. The concentrations of aluminium were comparable in 4 and 6 with a range of 384.3-387 ppm, in 8 and 9 with a range of 310.3-311 ppm, the same being true for 13 and 16 at 367-369 ppm.

Calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of prothrombin to thrombin and also takes part in milk clotting.

**TABLE 3: TRACE ELEMENTS CONCENTRATION IN HERBAL CHURNA PRODUCTS (ppm)**

| Sample Code | Ca | Cu | Mg | Zn | Al |
|---|---|---|---|---|---|
| 1 | 6287.7±272.9 | 8.51±0.16 | 2312.67±1574.34 | 17.45±0.04 | 445.67±28.67 |
| 2 | 6691.7±315.1 | 14.29±0.41 | 1595.33±12.50 | 27.62±0.09 | 579.77±20.30 |
| 3 | 4294.3±188.6 | 14.24±0.34 | 1117.67±26.58 | 18.54±0.16 | 437.33±11.15 |
| 4 | 2116.0±77.1 | 5.07±0.18 | 751.67±15.07 | 38.94±0.54 | 387.00±9.85 |
| 5 | 2473.7±287.9 | 4.81±0.16 | 770.83±7.64 | 23.41±0.26 | 401.67±13.05 |
| 6 | 4873.7±115.7 | 0.09±0.03 | 1057.67±16.62 | 21.56±0.12 | 384.33±9.50 |
| 7 | 2046.0±131.6 | 1.53±0.26 | 821.67±5.20 | 10.95±0.16 | 357.67±9.61 |
| 8 | 5758.0±860.4 | 2.02±0.10 | 1701.00±29.46 | 25.89±0.10 | 311.00±9.85 |
| 9 | 10934.0±456.1 | 4.97±0.13 | 2970.00±193.71 | 18.31±0.21 | 310.33±9.45 |
| 10 | 5859.3±307.2 | 16.50±0.17 | 2413.33±70.24 | 27.92±0.30 | 318.33±10.02 |
| 11 | 3282.7±191.2 | 50.70±0.59 | 2179.33±52.17 | 15.42±0.07 | 286.33±11.59 |
| 12 | 8233.0±86.6 | 16.34±4.85 | 3701.67±538.92 | 24.28±0.13 | 828.33±6.43 |
| 13 | 3990.3±243.3 | 6.03±2.47 | 1984.33±191.72 | 13.79±0.14 | 369.33±6.43 |
| 14 | 5101.0±174.9 | 11.89±1.47 | 1728.00±60.62 | 14.03±0.13 | 457.00±8.54 |
| 15 | 571.7±38.2 | 9.46±0.18 | 415.83±14.22 | 28.10±0.22 | 360.33±10.02 |
| 16 | 2424.3±76.0 | 12.07±0.46 | 492.33±6.43 | 8.68±0.14 | 367.67±4.51 |
| 17 | 4307.0±152.4 | 3.89±0.86 | 1487.67±190.54 | 20.19±0.11 | 635.00±20.66 |
| 18 | 2028.3±28.9 | 41.39±0.47 | 1047.67±31.01 | 7.58±0.04 | 375.33±9.61 |
| 19 | 277.5±43.3 | 2.73±0.00 | 237.50±11.46 | 13.94±0.10 | 359.00±15.72 |

Values are expressed as Mean±SD, n=3, SD= standard deviation , n= no. of readings taken per sample

**TABLE 4: TRACE ELEMENTS CONCENTRATION IN HERBAL *CHURNA* PRODUCTS (ppm)**

| Sample Code | As | Hg | Cd | Pb |
|---|---|---|---|---|
| 1 | 0.918±0.018 | 0.319±0.002 | 1.950±0.651 | 11.033±3.014 |
| 2 | 1.174±0.037 | 0.388±0.010 | 1.322±0.179 | 11.037±1.137 |
| 3 | 0.446±0.045 | 1.418±0.017 | 1.370±0.361 | 5.567±2.971 |
| 4 | 0.326±0.023 | 0.473±0.004 | 2.858±0.590 | 3.458±1.703 |
| 5 | 0.757±0.029 | 10.349±0.274 | 1.908±0.250 | 11.367±3.960 |
| 6 | 0.538±0.027 | 8.626±0.102 | 1.575±0.563 | BDL |
| 7 | 0.357±0.024 | 8.214±0.198 | 0.625±0.000 | 11.367±3.960 |
| 8 | 1.181±0.158 | 11.455±0.304 | 0.692±0.115 | 7.077±2.861 |
| 9 | 0.417±0.019 | 13.372±0.329 | 0.625±0.000 | 5.100±3.177 |
| 10 | 0.560±0.028 | 12.416±0.425 | 0.725±0.173 | 14.653±0.577 |
| 11 | 0.856±0.037 | 16.138±0.411 | 1.583±0.240 | 14.990±2.491 |
| 12 | 0.634±0.037 | 20.147±0.422 | 0.758±0.231 | 10.043±1.158 |
| 13 | 0.935±0.032 | 25.450±0.474 | 0.925±0.436 | 2.800±1.514 |
| 14 | 0.667±0.028 | 4.622±0.440 | 0.625±0.000 | BDL |
| 15 | 0.747±0.041 | 3.332±0.472 | 0.792±0.289 | BDL |
| 16 | 2.727±0.334 | 20.470±0.408 | 0.692±0.115 | BDL |
| 17 | 1.428±0.239 | 27.692±0.405 | 1.158±0.379 | BDL |
| 18 | 1.158±0.143 | 27.479±0.427 | 0.625±0.000 | BDL |
| 19 | 0.716±0.024 | 19.340±0.507 | 0.625±0.000 | BDL |

BDL= Below Detectable Limit

**TABLE 5: RECOVERY STUDY FOR TARCE ELEMENTS AND HEAVY METALS**

| Metal | Base value (ppm) | Quantity added (ppm) | Quantity found[a] (ppm) | Recovery (%)[b] |
|---|---|---|---|---|
| Ca | 2116.0±77.1 | 10.00 | 2125.45 | 94.50 |
| Al | 384.33±9.50 | 8.00 | 391.94 | 95.12 |
| Cu | 4.81±0.16 | 3.00 | 7.80 | 99.66 |
| Mg | 770.83±7.64 | 1.00 | 771.79 | 96 |
| Zn | 21.56±0.12 | 2.00 | 23.53 | 98.5 |
| As | 1.174±0.037 | 0.30 | 1.472 | 99.33 |
| Hg | 1.418±0.017 | 0.20 | 1.615 | 98.50 |
| Cd | 0.725±0.173 | 1.00 | 1.722 | 99.70 |
| Pb | 7.077±2.861 | 5.00 | 12.074 | 99.94 |

Recovery test. [a] Mean value (n=3). [b] 100×[(found-base)/added]

It plays a vital role in enzyme activation. Reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may cause spontaneous discharges of nerve impulses leading to tetany and convulsions [18-21]. Copper plays an important role in the metabolism of iron and as a cofactor in some enzymatic systems. Copper deficiency leads to impairment of iron absorption. In severe cases of copper deficiency, the development of anemia has been documented [22].

Magnesium is an active component of several enzyme systems in which thymine pyrophosphate is a cofactor. Oxidative phosphorylation is greatly reduced in the absence of magnesium. Mg is also an essential activator for the phosphate-transferring enzymes myokinase, diphophospyridinenucleotide kinase, and creatine kinase. It also activates pyruvic acid carboxylase, pyruvic acid oxidase, and the condensing enzyme for the reactions in the citric acid cycle. It is also a constituent of bones, teeth, enzyme cofactor, kinases, etc.

Zinc functions as a cofactor and is a constituent of many enzymes like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, DNA and RNA polymerase etc. Zn dependent enzymes are involved in macronutrient metabolism and cell replication [23]. The primary roles of zinc appear to be in cell replication and gene expression and in nucleic acid and amino acid metabolism. Vitamins A and E metabolism and bioavailability are dependent on zinc status [24]. It is necessary for optimum insulin action as zinc is an integral constituent of insulin. It is an important constituent of plasma. On the basis of results it can be said that studied products are rich source of Ca, Mg, Zn, Cu and Al.

Hence, the herbal churna products having the higher amount of trace elements are helpful in maintaining various functions of human body.

According to WHO the permissible limit of lead, arsenic, mercury and cadmium is 10 ppm, 10 ppm, 1 ppm and 0.3 ppm respectively. On that basis, out of nineteen samples sixteen samples for Hg content and eight for Pb content were exceed the WHO permissible limits. As was found appreciably well below the permissible limit while Cd was above the permissible limit. The presence of heavy metals in herbal medicens can cause serious diseases to the consumer. Lead cause adverse effects on psychological and behavioral activities in living beings. It is chronic toxicity causes kidney dysfunction, osteomalacia and obstructive lung diseases.

Cadmium is another human carcinogen associated with the risks of serious health hazards [25]. Liver and kidney are considered as the main target organs in acute and chronic Cd exposure [26, 27]. Arsenic inhibits antioxidant enzymes and this inhibition can lead to increased oxidative stress which may result in membrane damage and loss of membrane-bound enzymes like ATPases [28] .

The results from the present study emphasized that there is urgent need for quality control of herbal *Churna* preparations by implementing strict regulatory guidelines by the Government to ensure proper safety and efficacy. The safety of a drug is determined by its pharmacological-toxicological profile as well as the adverse effects caused by the impurities in bulk and dosage forms. So the toxic metal content should always maintain at minimal level. The proposed AAS protocol can be quite helpful for the quantitative estimations of heavy metals and trace elements concentration in herbal products and other phytopharmaceuticals.

**CONCLUSION:** Herbal *Churna* preparations sold in Indian market contain wide range of trace elements which are helpful in the prevention and control of several ailments. But these products were also found to contain toxic heavy metals above the WHO permissible limits which may cause various disorders. So, strict quality control guidelines need to be enforced for the safety and efficacy of herbal products.

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