Chapter - 6

Estimation of Trace and Heavy Metals by Atomic Absorption Spectrophotometer

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6.1. Introduction

Herbs used in formulation can present a health risk due to the contamination of toxic ingredients like heavy metals. Heavy metals are toxic depending upon their cations and are highly toxic when bonded to the short chains of carbon atoms (Hussain, 2006). Whereas, minerals and trace elements provided by herbs are essential to our bodies for numerous biological and physiological processes that are necessary for the maintenance of metabolic processes. Minerals are required in our diets in amounts greater than 100 milligrams per day and trace elements are required in amounts less than 100 milligrams per day. Plants may absorb these elements from soil, water or air during growing and processing. Concentration of these elements in plant is different for different species and is subjected to certain geochemical characteristics depending on the type of soil. Heavy metals are accumulated through atmospheric deposition including metalliferous mining, smelting and different industrial activities (Bin et al., 2001). For example, Ca, Mg and Zn have been reported to be essential for human health, whereas others such as Pb, Cd and As have been identified as toxic. The accumulation of heavy metals can have middle-term and long term health risks, and may result in illness to human fetus, abortion and preterm labor, and mental retardation to children. Adults also may experience high blood pressure, fatigue and kidney and brain troubles and strict periodic surveillance of these contaminants is therefore advisable (Cabrera et al., 1995). These trace metals in medicinal herbs play pivotal role as structural and functional components of metalloprotiens (MMPs) and enzymes in living cells (Ansari et al., 2004). Despite this, analytical methodology to determine heavy metals in medicinal plants has not received the same research effort as has been dedicated to the evaluation of phytotherapeutic properties (Marina et al., 2009). Thus, there is an urgent need to establish the identity, purity and quality assurance of herbal drugs in order to have full efficacy and safety of the herbal products (Mukherjee and Verpoorte, 2003).

Although herbal formulations were used worldwide since ancient time and there has been a renewed interest of herbal cosmetics, especially in the skin care segment with belief that chemical-based cosmetics are harmful and herbal cosmetics are safe being natural. Herbal skin care cosmetics consist of botanical ingredients/extracts enrich the skin with trace (nutrient) elements and other useful minerals to prevent infection and responsible for soothing effects over skin. But last few decades, it has been accounted that the absorption of toxic metals through skin is very significant and can cause deleterious effects over skin (Ayenimo et al., 2010; WHO, 1995). Thus the safety assessment of herbal ingredients becomes doubtful and need further attention of the scientific community and the regulatory agencies. It is essential to screen the level of trace and heavy metals contents in herbal cosmetic ingredients used before preparing the cosmetic formulation.
6.2. Review of literature

Traditionally the herbal drugs are well established for their therapeutic benefits. Depending upon their geographical sources sometimes the trace and heavy metals contain may differ and which may leads to severe toxicity. So, the toxicological and safety assessment of these herbal drugs is one of the major issues in recent days. The use of medicinal herbs to relieve and treat human diseases is an age old practice in Ayurveda. Many curative effects of medicinal herbs used in the phytotherapy are due to the presence of very minute quantities of trace elements. These elements are Fe, Cu, Co, Ni, Zn, Mg, Mn, Mo, Cr, V, Li, Se, F and I (Shirin et al., 2010). Plants readily assimilate such elements through roots, which are dissolved in water and remains in ionic forms. Other heavy metals like lead (Pb), cadmium (Cd), and mercury (Hg) are toxic at very lower concentration (LLobet et al., 2003). World Health organization (WHO, 1989) declares the maximum permissible levels in food and drug materials only for arsenic, cadmium, and lead which amount to 1.0, 0.3 and 10 mg/kg, respectively (Basgel and Erdemoglu, 2006). A high supplementation of Cu had been related with liver damage. Zn may produce adverse nutrient interactions with Cu and also, Zn reduces immune function and levels of high density lipoproteins (FDA, 2001). Pb is known to induce renal tumors, reduce cognitive development, and increase blood pressure and cardiovascular disease in adults. Cd induces kidney dysfunction, osteomalacia and reproductive deficiencies. Hg cause neurological disorders and has toxic effect on the kidney (Haider et al., 2004).

Recently it has been observed that herbal products contain a considerable amount of toxic heavy metals such as arsenic, cadmium, lead and mercury. Determination of Fe, Zn, Pb, Cd and Se content in medicinal plants by X-Ray Fluorescence analysis and Galvanostatic stripping chronopotentiometric analysis in 5 species Melissa officinalis, Agrimonia eupatoria, Hypericum perforatum, Salvia officinalis, and Achillea millefolium was carried out (Stroffekova et al., 2008). Some traditional herbal preparations used in Ayurveda, traditional Chinese medicine, traditional Tibetan medicine and other Asian traditional medicine systems were found to contain significant amounts of mercury, arsenic or lead causes encephalopathy (Martena et al., 2010). According to Harvard Medical School (2004), it was observed that 25-30% of herbal medicine products contained lead, mercury and/or arsenic could result in heavy metal intakes above regulatory standards. Several herbal ingredients could result in lead and arsenic intakes of 1,000 to 10,000 times greater than the regulatory standards. Vartika et al., (2001) has estimated the level of Pb, Cd, Cu and Zn in Alpinia galanga, Artemesia parviflora, Butea monosperrma, Coleus forskohlii, Curcuma amada, Euphorbia prostrate, Leucas aspera, Malaxis accuminata and Pueraria tuberose from Ayurveda. The concentration of Pb and Cd was found beyond the WHO permissible limits in most samples. US and Indian manufactured Ayurvedic medicines have been
tested for heavy metals and it was found that one-fifth of both US and Indian manufactured Ayurvedic medicines contained detectable lead, mercury or arsenic (Robert et al., 2004). Baranowska et al., (2002) has determined heavy metals in 27 samples of medicinal herbs by Flame AAS and Pulse polarography and stripping voltammetry and were digested by the wet method in a microwave oven. Different sample digestion methods have been performed to determine heavy metal content in traditional Chinese medicines produced by different manufacturers using ICP-MS and it was found that Pb and Cd differed widely with different manufacturers due to external contamination (Xiaro et al., 1999). Due to these reasons, increasing popularity of herbal medicines has also brought concern and fears because the quality, safety and batch to batch consistency of herbal formulations are not up to the mark, to meet the criteria needed to support its use worldwide. Thus, it becomes imperative that quality of these drugs must be maintained and it should be free from any type of contamination (Pakade et al., 2011). Thus, quantification of metals in plants especially medicinal herbs is part of quality control, which has been established their purity, safety and efficacy. In the present study, five plants species including *Clitoria ternatea*, *Piper betel*, and *Tagetes erecta* were selected from Ayurveda to determine the trace (Cu, Cr, Mn, Fe, Ni) and heavy (As, Pb, Hg) metals through atomic absorption spectrometry and thereby to assure the safer therapeutic application of these plants.

6.3. Determination of trace and heavy metals in the plant materials

6.3.1. Material

Leaf of *Clitoria ternatea* L. (Fabaceae), leaf of *Piper betel* L. (Piperaceae), and flower of *Tagetes erecta* L. (Asteraceae) were collected from the local cultivation land of West Bengal, India and authenticated. The samples were washed with Milli-Q water and dried at 40-60°C for 5-6 hours. After drying the sample were stored in air tight polyethylene container.

6.3.2. Reagent and chemicals

Milli-Q Water (Millipore, USA) was used throughout the analysis, HNO₃, HClO₄, HCl, H₂SO₄, were of analytical grade. Stock solution of 1000 ppm concentration for all the metals were procured from Merck (Darmstadt, Germany). All the working concentrations were prepared freshly on the day of analysis. All the glass wares used were treated with 2-4% HNO₃ overnight and washed with Milli-Q water.

6.3.3. Instrumentation

Atomic absorption spectroscopy (AAS) is a spectro-analytical instrument for the quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state (Figure 6.1). when a beam of optical radiation of a particular metal passess through gaseous metal atoms, that particular radiation is absorbed by the specific...
metal atom present in the gaseous state and the atom is transferred from ground to higher energy state and produces a characteristic radiation, which is recorded by the detector (Walsh, 1955). Metal ions in a solution are converted to gaseous atomic state by means of a flame. The technique of (FAAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800°C. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths. The characteristic wavelengths are element specific and accurate to 0.01-0.1nm. To provide element specific wavelengths, a light beam from a lamp whose cathode is made of the element being determined is passed through the flame. A device such as a photon multiplier can detect the amount of reduction of the light intensity due to absorption by the analyte, and this can be directly related to the amount of the element in the sample (Welz and Sperling, 1999).

![Figure 6.1. Principle of atomic absorption spectrophotometer](image)

<table>
<thead>
<tr>
<th>Elements</th>
<th>Cu (nm)</th>
<th>Cr (nm)</th>
<th>Mn (nm)</th>
<th>Fe (nm)</th>
<th>Ni (nm)</th>
<th>As (nm)</th>
<th>Pb (nm)</th>
<th>Hg (nm)</th>
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<td>279.5</td>
<td>248.3</td>
<td>232.0</td>
<td>193.7</td>
<td>217.0</td>
<td>253.7</td>
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<td>5.0</td>
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<td>9.0</td>
<td>12.0</td>
<td>9.0</td>
<td>3.0</td>
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<tr>
<td>Flame</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
<td>AA</td>
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<tr>
<td>Fuel (L/min)</td>
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<td>2.90</td>
<td>2.95</td>
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<td>2.40</td>
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<td>Slit width (nm)</td>
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<td>0.5</td>
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<tr>
<td>Working range (ppm)</td>
<td>1-5</td>
<td>2-8</td>
<td>1-5</td>
<td>2-10</td>
<td>3-10</td>
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<td>Read time (Sec.)</td>
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<td>Wash time (Sec.)</td>
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Table 6.1. Instrumental condition for trace and heavy metal analysis by AAS
The atomic absorption measurements were performed using Thermofisher AA 303 atomic absorption spectro photometer with hollow cathode lamp (HCL) light source. For analysis of all the metals, oxy-acetylene flame was used. For the determination of arsenic and mercury, hydride generator was used and mercury was determined using cold vapor analysis. The standard instrumental configuration and experimental condition maintained for the analysis of Cu, Cr, Mn, Fe, Ni, As, Pb, Hg are given in table 6.1.

**6.3.4. Determination of arsenic and mercury**
Arsenic and mercury was determined using hydride generator, where these metals were converted in to their volatile hydride forms using sodium borohydride and concentrated HCl. The vapour of hydride generated in the system was sent to the optical cell using peristaltic pump. Determination of mercury was carried out by the way of cold vapor analysis.

**6.3.5. Sample preparation**
For the analysis samples were grinded to a fine powder and dried at 55-70°C for 6-8 hours in a controlled environment, to remove moisture. Immediately after drying accurately weighed sample, 3.0 g, was placed in a flask and treated with 3 mL of concentrated HNO₃ for 4-5 hours. A mixture of HNO₃ and HClO₄ in a ratio of 2:1 (3 mL per gram of sample) was added. The mixture was heated at 120-130°C for 5-6 hours, until fumes stops and resulting solution is clear. Then 10 mL of Milli-Q water was added and boiled again for 10-15 min and volum was reduced to the half, cooled to room temperature and filtered using whatman filter paper no. 42. The entire filtrate was mixed and made the volume upto 50 mL with Milli-Q water. Blank was also prepared for every sample in the same way. Each sample was aspirate twice and the experiment was repeated for five times.

**6.4. Statistical analysis**
Statistical analysis was performed using the GraphPad Prism Version 5.0. The results were represented as the mean ± SEM.

**6.5. Results and discussion**
Each element has its individual impact in the structural and functional integrity of the living cells and organisms. Results shown in table 6.2 verify the presence of variable amounts of these metals in the medicinal plant samples. In the present work, concentration of eight metals, including heavy metals, were determined for some commonly used herbs from Indian origin. The quantitative determinations were carried out using standard calibration curve obtained by standard solution of metals having optimal detectable concentration ranges. The concentration of the metals obtained in plant material was expressed in terms of ppm (Table 6.2). The levels of heavy metal quantified in all the plant samples were within prescribed limits (WHO, 1999).
Copper is one of the most abundant trace elements present in the body with vital effect in the human physiological system. The total amount of the copper present in the body is assumed to be 50-120 mg and involved in lot of biochemical processes with presence in more than 13 enzymes viz. cytochrome oxidase, tyrosinase, monoamine oxidase etc., which are important for the preventing cell damage, anaemia and maintenance of connective tissue, nail, hair etc. The concentration of Cu determined in all the plant materials were found to be significant with highest concentration in P. betel with 19.61 ± 0.31 ppm and lowest in T. erecta with 9.90 ± 0.58 ppm. So this relatively high concentration of the Cu may be related to its medicinal values. Iron is an essential element for growth and required in the human body for the circulation of oxygen in the blood. Its deficiency can hinder metabolism. The concentration of the iron present in all the plants studied is high enough to justify its use in human circulation system, with highest value in C. ternatea (1566.690 ± 63.528 ppm) and lowest in T. erecta (458.599 ± 27.090 ppm). Manganese is one of the major minerals, which is related to the carbohydrate and fat metabolism and required for many enzymatic activities. The concentration quantified in analyzed samples varies from 871.884 ± 45.803 ppm in P. betel. The concentration of Ni in the plant sample was found to vary with minimum 3.736 ± 0.210 ppm in T. erecta to 22.153 ± 0.833 ppm in P. betel. It is believed that nickel is necessary for good health, but people with certain liver and kidney diseases are known to have low levels of nickel in their bodies. Also, excess nickel in the body is associated with a high incidence of heart disease, thyroid disease and cancer. Another trace metal Cr was quantified with minimum of 2.189 ± 0.083 ppm in C. ternatea. Most significantly, Cr is a vital component for insulin to stabilize blood sugar levels and helps our bodies to absorb energy from the food and stabilizes the level of energy. The problems that are associated with chromium involve skin rashes, stomach ulcer, kidney, liver damages, lungs cancer and ultimate death.

Heavy metals such as Pb, Hg and As were found within permissible limit as shown in table 6.2. Heavy metal toxicity results in damage CNS functions reduce energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Long-term exposure may result in slowly progressing physical, muscular, and neurological degenerative processes that mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis. Inhalation is the most frequent cause of exposure to mercury. The organic form is readily absorbed in the gastrointestinal tract (90-100%) and inorganic mercury is absorbed in the gastrointestinal tract (7-15%). Target organs are the brain and kidneys. Arsenic is the most common cause of acute heavy metal poisoning in adults blood, kidneys, and central nervous, digestive, and skin. Arsenic is thought to be necessary for the functioning of the nervous system and growth. It is present in food, water, and also in human body. Deficiency of this element in humans has apparently never been observed. An arsenic trioxide has been approved by the FDA to treat a rare and deadly
form of leukemia called acute promyelocytic leukemia. Pb has toxic impact on metabolic processes essential to growth and development, including DNA synthesis and mitotic activity. Pb poisoning forms complexes with oxo-groups in enzymes to affect virtually all steps in the processes of haemoglobin synthesis and porphyrin metabolism. Toxic level is associated with encephalopathy seizures and mental retardation (Ademorati, 1996; Schumann, 1990). Thus estimation of heavy metal contamination is an urgent need for constant quality assessment of herbal products to ensure the safety of consumers. Regulatory bodies have implemented stringent policies and the scientific community has developed convenient analytical methods to regulate and monitor the standards of herbal products manufactured, advertised, sold, and used.

In the present study, different plant materials were found to contain variable amounts of trace (nutrient) elements and the content of heavy metals were found within permissible limit according to WHO & FDA. The concentration variation of these elements may be due to environmental condition and geographical origin, use of fertilizer, pesticides etc. In this study, a simple, reliable, sensitive and convenient AAS method has been developed for quantitative estimation of trace metals and heavy metals which can conveniently be utilized for the quality control of herbal cosmetic preparations at industrial level. Based on the results it can be concluded that the proposed method of digestion is suitable, fast and simple, thus making the technique more attractive for its use in the quality control of herbs. The content of heavy metal was found to be in the prescribed limit. Other trace metals were detected and were found to present in the significant amounts in all the plants material. It can be concluded that the plant materials collected from specific region are safe and may not produce any toxic effect upon their therapeutic application.

<table>
<thead>
<tr>
<th>Plants</th>
<th>Trace elements</th>
<th>Heavy metals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cu</td>
<td>Cr</td>
</tr>
<tr>
<td>C. ternatea</td>
<td>9.57±0.40</td>
<td>2.18±0.08</td>
</tr>
<tr>
<td>P. betel</td>
<td>19.61±0.31</td>
<td>1.09±0.08</td>
</tr>
<tr>
<td>T. erecta</td>
<td>9.90±0.58</td>
<td>1.08±0.07</td>
</tr>
</tbody>
</table>

AA: Air: acetylene

Table 6.2. Metal content of some Indian medicinal plants detected by AAS (in ppm)

6.6. Publications