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AAS Estimation of Heavy Metals and Trace elements in Indian Herbal Cosmetic Preparations

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Abstract

In present study, heavy metals like Pb, Cd and trace (micronutrients) metals like Ca, Mg, Al, Cu and Zn were quantitatively estimated using flame atomic absorption spectrometry (FAAS). Also, heavy metals such as As and Hg were estimated by hydride generation technique (cold vapour atomic absorption spectrometry) using nitrogen as carrier gas in 21 herbal cosmetic preparations sold in Indian market. The results indicate that among the toxic heavy metals, two samples for Hg content and six for Pb content were exceeding the WHO permissible limits fixed for herbal preparations. Arsenic was found appreciably well below the permissible limit, but Cd was found above the permissible limit in the all samples. Trace elements like Ca and Mg were found in higher amount than Al, Cu, and Zn. Presence of trace elements can prove to be beneficial but presence of toxic heavy metals in such amounts surely has adverse effects on the consumer health who always take the herbal products in an impression of being safe because of the natural origin. In conclusion, enforcement of strict and separate regulatory guidelines and promotion of Good analytical practice (GAP), good manufacturing practices (GMP) and good agricultural and control practices (GACP) is suggested for herbal cosmetics by WHO and other regulatory agencies. This study presents the status of heavy metals and trace elements in marketed herbal cosmetic formulations and also provides a simple and convenient AAS method which can effectively be adopted at Industrial level for the quality control and standardization of herbal cosmetic preparations and other related products.

Keywords: Herbal cosmetics, trace elements, heavy metals, AAS.

Introduction

Herbal cosmetics are the valuable products consist of botanicals or their bioactive ingredients/extracts which enrich the skin with trace (nutrient) elements and other useful minerals, prevent from infection and hence responsible for their cosmetic effects. Although they are used worldwide since ancient time, but in the last decade, there has been a renewed craze of herbal cosmetics and personal care products, especially in the skin care segment with the growing belief that chemical-based cosmetics are harmful and herbal cosmetics are safe being natural[1,2]. But since, it has been reported that the absorption of toxic metals through skin is very insignificant and can cause deleterious effects on our body[3,4], there is an increase in scientific concern over the issue but unfortunately no sufficient data is available regarding the status of herbal cosmetics for the presence of heavy metals except a few preliminary studies[5,6]. Thus the safety of these herbal cosmetics becomes doubtful and need further attention of the scientific community and the regulatory agencies.

According to the World Health Organization (WHO), heavy metals concentration of herbal medicines must definitely be controlled[4]. But WHO is silent regarding the maximum permissible limits of heavy metals in herbal cosmetics. In this case, Health Canada has taken the initiative and implemented a few measures to control heavy metal concentration in cosmetics and determined the maximum acceptable limits i.e Lead (10ppm), Arsenic (3ppm), Mercury (3ppm), Cadmium (3ppm) and Antimony (5ppm)[7].

Considering the importance of trace elements and toxic effects of heavy metals in herbal formulations and the fact that most of the scientific studies are confined to the herbal products meant for oral consumption[2,8,9], it was imperative to screen the status of trace and heavy metals contents in herbal cosmetic products sold in Indian market. In the present study, 9 elements, Ca, Cu, Mg, Al, Zn, As, Hg, Pb and Cd were estimated quantitatively in 21 herbal cosmetic formulations by flame and hydride generation atomic absorption spectrometry followed by recovery studies as a part of method validation as per ICH guidelines.

Material and Methods

Samples: Twenty-one samples of marketed herbal cosmetic preparations were collected from local market of Delhi and Rohtak. The brand names were blinded and given the codes 1 to 21. The other details are presented in table-1.

Instrument: Atomic absorption spectrophotometer (EC Electronics Corporation of India Limited AAS Element AS AAS4141) equipped with a deuterium lamp for background correction 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. Hydride generator was used for hydride formation in As and Hg analysis. The flames used were air/acetylene and N_2O/acetylene. Nitrogen was used as carrier gas.

Chemicals: Nitric acid, hydrochloric acid, sulphuric acid, hydrogen peroxide, sodium borohydride and stannous chloride were of analytical grade (E. Merck). The water used in all experiment was ultrapure water obtained from Milli-Q-water purification system (Ranken Rion Ltd, India). The standard solutions were prepared in five different concentrations to obtain calibration curve by diluting stock solutions (CPA Ltd) of 1000 ppm of each element immediately before use.

Sample preparation: Samples were digested by the wet digestion method. 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 spectrometry. The 1000 ppm standard solutions of elements were diluted in five different concentrations to obtain calibration curve for quantative analysis. 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 giving details about parameters which are defined for respective metals.

Recovery studies: The method of standard addition which is considered as a validation method[10] was used to demonstrate the validity of our method. Hence, a recovery test was performed using method of standard addition. Standard solutions containing Ca, Al, Mg, Cu, Zn, Pb, Cd, As and Hg were prepared and spiked with digested samples, after dilution of sample to 50 ml.

Results and Discussion

The contents of trace elements in the screened preparations as a mean of triplicate determination are described in table-3. The calcium concentrations varied from 152 to 16591 ppm, eight samples having contents between 2626 and 5985 ppm. Sample 3 had the lowest calcium concentration and sample 16 had the highest. Calcium was also found below detectable limit in total seven samples. The copper concentrations varied from 2.8 to 49.1 ppm, most samples having contents between 9.4 and 24.3 ppm. Sample 20 had the lowest copper concentration and Sample4 had the highest. The concentrations of copper were comparable in sample 6 and 7 with a range of 20.4-20.5, in 8 and 9 with a range of 21.9-22.2.The magnesium concentrations varied from 112 to 10,681 ppm, 12 samples having contents between 4737 and 8350 ppm while three samples, 19, 20, and 21 had the equal magnesium concentration 112 ppm. Sample 9 had the highest magnesium concentration. The zinc concentrations varied from 4.8 to 56.57 ppm, most samples having contents between 31.4 and 56.57 ppm. Sample 20 had the lowest zinc concentration and sample 12 had the highest. The concentrations of zinc were comparable in sample 8 and 10 with a range of 50.46--50.49, the same being true for sample code 18 and 11 at 46 and 46.12. The aluminium concentrations level ranged from 126 to 5505 ppm, most samples having contents between 3900 and 5505 ppm. Sample 1 had the lowest aluminium concentration and sample 4 had the highest.

Among the heavy metals, data presented in table-4 reveals that the mercury concentrations varied from 0.041 to 2.183 ppm, most samples having contents between 0.041 and 0.309 ppm. Sample 5 had the lowest mercury concentration and sample 18 had the highest. But mercury was found below detectable limit in eight samples. The concentrations of mercury were comparable in samples 11 and 21 with a range of 0.072-0.076 ppm. According to the WHO, the permissible limit for mercury in herbal preparations is 1 ppm. In that way, two samples 9 (1.095 ppm) and 18 (2.183 ppm) were found to contain mercury concentration above permissible limit. The arsenic concentrations varied from 0.690 to 3.683 ppm, most samples having contents between 1.37 and 3.68 ppm. Sample 1had the lowest arsenic concentration and 16 had the highest. According to the WHO, the permissible limit for arsenic in herbal preparations is 10 ppm. All the herbal cosmetic products under investigation accumulated this metal at a level appreciably below the permissible limit.The cadmium concentrations varied from 0.625 to 1.875 ppm, most samples having contents between 0.625 and 1.09 ppm. Sample 19 had the lowest cadmium concentration and sample 14 had the highest. According to the WHO, the permissible limit for cadmium is 0.3 ppm in herbal preparations and unfortunately, all the herbal cosmetic products were found to contain cadmium concentration higher than the permissible limit.The lead concentrations level ranged from 1.470 to 33.1 ppm. Sample 4 had the lowest lead concentration and sample 21 had the highest. But lead was found below detectable limit in four samples. According to the WHO, the permissible limit for lead is 10 ppm. Six samples were contained Pb content above the permissible limit. The results of recovery study were within the acceptable range verifying the validity of proposed method for analysis (Table-5) and revealed that any small change in the drug concentration in the solution could be accurately determined by the proposed method.

In the present study, herbal cosmetic products were found to contain variable amounts of trace (nutrient) elements. The variation in concentration of these elements may be mainly due to compositional differences of products and environmental condition where constituent plant is grown, use of fertilizer, pesticides. But generally it is concluded that the studied products are rich source of essential elements Mg, Ca, Zn, Cu, and Al and hence might play an important role in the maintenance of the skin nutritional requirements[11,12].

Toxic heavy metals Pb, Cd, As and Hg were detected in all the investigated cosmetic products. The cosmetic products related regulations do not decide maximum permissible limit values for heavy metals content in cosmetic products except of 1 ppm for Hg (ACSB 2007). However, Cd is prohibited in any amount in cosmetics (Council Directive 76/768/EEC of 27 July 1976). The presence of heavy metals in cosmetics can cause serious problems to consumer as they can cause premature aging of the skin, skin allergies, and skin cancer. Further, toxic metals have a role to set up conditions that lead to inflammation in arteries and tissues, results in osteoporosis[13]. Thus, there is an urgent need for constant quality assessment of cosmetic products in the market in order to ensure the safety of consumers. To achieve this, regulatory bodies and the government sector should implement the stringent policies to regulate and monitor the standards of herbal products manufactured, advertized, sold, and used. At the same time, scientific community should develop simple and convenient analytical methods.

The most widely techniques to analyze trace and heavy metals are atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and X-ray fluorescence spectroscopy (XFS)[14]. However, the instrumental methods of ICP-MS, ICP-AES, and XFS are usually more costly, and their use is not as straightforward and convenient as AAS. 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.

Conclusion

21 herbal cosmetic preparations sold in Indian market found to contain some biologically important trace elements, which may be helpful to impart therapeutic efficiencies unfortunately, these products were also contained toxic heavy metal content above the permissible limits which may cause deleterious effects to the human health. In the present scenario, there is an urgent need to regulate them properly for the sustainable safety and efficacy.

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Table-1
Details of herbal cosmetic products collected

Sample code	Constituents	Use
1	*Prunus armeniaca, Glycyrrhiza glabra, Avena sativa.*	Remove dark circles and blemishes.
2	*Aloe barbadensis.*	Useful in skin darkness and dryness.
3	*Ocimum sanctum.*	Antiseptic, antifungal, cleansing pores.
4	*Carica Papaya.*	Heals damaged skin, control oil of skin.
5	*Fragaria vesca.*	Remove skin dead cells and impurities.
6	*Rosa, Trigonella foenum-graecum, Santalum album.*	Improve the complexion, skin pore cleansing and revitalizing.
7	*Citrus sinensis.*	Astringent, moisturizer, scars, acne, smoothness.
8	*Rosa, Santalum album.*	Antiaging, tone up the skin, tighten the pores.
9	*Citrullus lanatus.*	Moisturizer, refreshner, skin toner and tightener.
10	*Solanum xanthocarpum.*	Antiaging, skin cleansing.
11	*Citrus limon.*	Skin nourishment, cleansing and moisturizing.
12	*Citrus sinensis, Carica papaya, Malus domestic, Prunus serotina, Fragaria vesca, Citrus limon,* **Punica granatum**, *Prunus persica.*	Pimples, antiaging, skin cleansing and softening.
13	*Citrus limon, Crocus stivus, Curcuma longa, Santalum album.*	Depigmentation, skin cleansing and fairness.
14	*Azadiracta indica.*	Antiaging, skin softening.
15	*Rosa, Prunus amygdalus, Crocus stivus, Citrus limon, Curcuma longa, Santalum album.*	Fairness, soft skin.
16	*Embelia ribes, Rubia cordifolia, Sida cordifolia, Acacia catechu, Valeriana jatamansi, Swarna gairik, Acorus calamus, Psoralea corylifolia, Aloe barbadensis, Curcuma longa, Azadiracta indica, Santalum album*	For acne, pimples and blemishes.
17	*Eletteria cardamum, Valeriana jatamansi, Glycyrrhiza glabra, Vetiveria zizaniodes, Moringaoliefera, Cyperus scariosus, samudraphen, Brassica compestris, Valeriana wallichii, Saussurea lappa, Pavonia odorata, Cinnamomum glaucescens, Curcuma longa, Azadiracta indica, Santalum album*	Very efficacious in bringing new glows on the face.
18	*Symplocos Racemosa, Punica granatum, Mangifera indica, Azadiracta indica.*	Fairness
19	*Curcuma longa, Santalum album.*	To prevents and cures skin infection, inflammation and blemishes.
20	*Aloe barbadensis.*	Prevent ageing and dehydration of skin.
21	*Citrus limon, Triticum stivum, Glycyrrhiza glabra, Calendula officinalis, Aloe barbadensis, Curcuma longa, Azadiracta indica, Santalum album*	To detoxify and nourish the skin. Protect skin from sun burn and pollutants.

Table-2
Instrumental condition for analysis

Element	Current (mA)	Slit width (nm)	λ_{max} (nm)	Flame Color	Flame Type	AAS Technique
Ca	3.5	0.5	422.7	Orange	Air/C_2H_2	Flame
Mg	3.5	0.5	285.2	Orange	Air/C_2H_2	Flame
Cu	5	0.5	324.7	Blue	Air/C_2H_2	Flame
Zn	5	1.0	213.9	Blue	Air/ C_2H_2	Flame
Al	10	0.5	309.3	Red	Air/N_2O/C_2H_2	Flame
Cd	3.5	0.5	228.8	Blue	Air/C_2H_2	Flame
Pb	10	1.0	217	Blue	Air/C_2H_2	Flame
As	EDL	1	193.7	Blue	Air/C_2H_2	Hydride generation
Hg	EDL	0.5	253.6	-	-	Cold vapor

AAS= Atomic absorption spectrometry

Table-3
Trace metal content in herbal cosmetic products (ppm)

Sample Code	Ca	Cu	Mg	Zn	Al
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
1	2626±52.37	9.4±0.3	2963±90	51.23±0.40	126±7.94
2	5985±273.33	10.4±0.3	5108±97	50.57±0.28	4823±37.72
3	152±10.52	10.0±0.3	6300±43	49.76±0.39	5178±48.40
4	BDL	49.1±0.8	7351±87	51.67±0.57	5505±31.64
5	782±243.53	48.5±0.4	6983±714	50.06±0.12	5168±37.86
6	BDL	20.4±0.1	4737±33	35.66±6.99	3901±81.17
7	189±115.77	20.5±0.7	5146±159	39.65±0.30	4460±40.53
8	3206±315.05	21.9±0.3	4929±97	50.46±2.51	4511±89.61
9	3661±315.05	22.2±0.5	10681±900	54.01±0.14	4917±109.8
10	BDL	24.3±0.5	8112±481	50.49±0.58	4268±36.83
11	BDL	19.5±0.1	8350±307	46.12±0.26	4825±77.03
12	3633±199.57	22.0±0.6	7917±196	56.57±0.57	5311±66.58
13	4419±189.67	19.9±0.2	5316±101	50.78±0.41	4779±82.56
14	1489±115.66	22.9±0.2	8317±83	49.26±0.26	582±12.86
15	12222±42.72	23.2±0.5	475±38	18.69±0.40	313±32.01
16	16591±473.6	20.0±0.2	2071±566	21.11±0.24	1122±87.40
17	4217±307.73	19.2±0.2	1358±14	31.4±0.34	806±90.27
18	4292±265.86	16.2±0.6	3508±115	46±0.14	3780±95.09
19	BDL	14.1±1.9	112±0	6.89±0.09	325±69.50
20	BDL	2.8±0.3	112±0	4.8±0.15	275±55.75
21	BDL	4.3±0.3	112±0	6.6±0.00	230±21.63

Values are expressed as arithmetic mean ±SD (n = 3). BDL = Below detectable limit.

Table-4
Heavy metal content in herbal cosmetic products (ppm)

Sample Code	As	Hg	Cd	Pb
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
1	0.690±0.026	BDL	0.725±0.100	8.450±0.050
2	1.370±0.036	BDL	0.792±0.208	6.750±2.091
3	1.467±0.025	0.118±0.004	1.235±0.115	12.850±0.577
4	2.147±0.035	0.168±0.007	0.792±0.289	1.470±0.852
5	1.843±0.035	0.041±0.007	0.892±0.252	7.400±1.975
6	1.637±0.029	BDL	0.992±0.321	10.040±1.158
7	2.013±0.006	BDL	0.785±0.115	6.750±0.563
8	2.263±0.021	0.311±0.012	0.825±0.100	11.300±1.988
9	2.927±0.047	1.095±0.031	0.858±0.343	8.400±1.302
10	3.077±0.191	0.065±0.010	0.792±0.231	6.430±1.975
11	2.637±0.038	0.072±0.014	0.725±0.173	9.350±2.963
12	3.213±0.040	BDL	0.758±0.153	9.390±3.438
13	2.927±0.047	BDL	1.258±0.551	12.030±3.995
14	1.557±0.042	BDL	1.875±0.499	6.750±2.479
15	2.237±0.038	BDL	1.092±0.252	BDL
16	3.683±0.181	0.309±0.021	1.158±0.208	BDL
17	2.923±0.045	0.157±0.021	0.692±0.115	BDL
18	3.423±0.045	2.183±0.026	0.992±0.321	BDL
19	1.887±0.021	0.278±0.011	0.625±0.000	16.630±3.992
20	1.430±0.035	0.083±0.010	0.658±0.058	20.580±3.746
21	1.563±0.038	0.076±0.010	0.625±0.000	33.100±1.975

Values are expressed as arithmetic mean ±SD ($n = 3$). BDL = Below detectable limit.

Table-5
Recovery studies for trace elements and heavy metals

Metal	Base Value (ppm)	Quantity Added (ppm)	Quantity Found^{a} (ppm)	Recovery(%)^{b}
Al	126±7.94	10.0	135.78	97.8
Ca	3661±67.72	8.0	3668.72	96.5
Cu	4.33±0.22	3.0	7.24	97
Mg	112±0.30	1.0	112.96	96
Zn	18.70±0.4	2.0	20.63	96.5
As	2.926±0.047	0.3	3.224	99.33
Hg	1.094±0.030	0.2	1.292	99.00
Cd	0.858±0.34	1.0	1.85	99.20
Pb	8.400±1.30	5.0	13.356	99.12

Recovery test, ^{a} Mean value ($n = 3$). ^{b} 100×[(found–base)/added].