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Atomic absorption spectrometric determination of heavy metals in Indian system of medicine used against cold and fever

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ABSTRACT

Toxic (Pb, Cd, Hg, Pd and Cr) as well as essential (Zn, Mn, Ni, Co, Cu and Fe) heavy metals in different types of Allopathic (Paracetamol and Citrazine hydrochloride), Ayuurvedic (Mruthyunjaya Ras and Shwaskuthar Ras), Unani (Habb-e Mubarak, Qurs-e Nazla and Habe-Surfa) and Homeopathic (Bryonia-200 and Allium Cepa-200) medicine commonly used against cold and fever were determined by atomic absorption spectrometry (AAS). The contents of Cr, Hg, Pb and Fe in formulations procured from different market outlets were found beyond the WHO permissible limits. Ayurvedic formulations when compared to Unani, Homeopathic and Allopathy contain higher amounts of Hg and Pb. Unani and Ayurvedic formulations had higher quantities of Fe compared to Allopathic and Homeopathy.

KEYWORDS

Heavy metals;
Drug safety;
Ayurvedic;
Unani;
Allopathic;
Homeopathy;
AAS.

INTRODUCTION

The analytical activities concerning impurities are among the most important issues in modern pharmaceutical analysis for assurance of quality and safety of drugs. The inorganic impurities generally originate from various sources and phases, i.e., raw materials, reagents, solvents, electrodes, catalysts, reaction vessels, plumbing and other equipments used during the synthesis of pharmaceuticals and characteristic of the synthetic route of manufacturing processes[1]. Heavy metals, due to their presence and wide distribution in soil and environmental pollution assimilate in herbal drugs[2]. Traces of cadmium and lead were detected in many of the herbal drugs[3]. Monitoring of heavy metals in process intermediates and final drug substances is an important activity in pharmaceutical industry. It is not only because of their ability to catalyze decomposition but also potential for toxicity. Heavy metals like lead and cadmium in pharmaceuticals pose the risk of serious health hazards even at very low doses[4,5]. Longer occupational exposures to lead cause adverse effects on psychological and behavioral activities in living beings. An intake of 0.06 mg of Pb/day for a period of one month is enough for chronic poisoning. Its chronic toxicity causes kidney dysfunction, osteomalacia and obstructive lung diseases. Cadmium is another human carcinogen[6] associated with the risks of serious health hazards. It accumulates in the human body and has a biological half-life of 30 years. An intake of 1ppm of Hg/day leads to cause adverse effects on the renal and nervous systems[7]. Ni is a widely used heavy metal, which exert a potent toxic effect on peripheral tissues as well as on the reproductive system. Nickel causes dose-related decreases in bone marrow cellularity and in granulocyte macrophage and pluripotent stem cell proliferative

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TABLE 1 : Acceptable daily intake and disorders of heavy metals

S. No	Heavy metal	Acceptable daily intake (ADI) for adult (PPM)	Disorders
1	Hg	1	Adverse effects on the renal and nervous systems
2	Cd	0.3	nephrotoxic effects and bone damage ell
3	Co	8	Irritation of gastrointestinal tract, nausea, diarrhea, lung and heart diseases
4	Cr	1.54	Respiratory cancers
5	Cu	21.5	Vineyard sprayer's lung and Wilson disease
6	Fe	231	Vomiting, cardiac depression, metabolic acidosis and Hepatic cirrhosis.
7	Ni	1.85	Dermatitis, pulmonary fibrosis, reduces sperm count and nasopharyngeal tumors.
8	Zn	169	Anemia and neurological degeneration
9	Mn	69.2	Parkinson like syndrome, respiratory and Euro psychiatric disorders
10	Pb	10	Adverse effects on the renal and nervous systems encephalopathy, cognitive impairment, behavioral disturbances, anemia, and hypertension

responses, dermatitis, pulmonary fibrosis, reduces sperm count and nasopharyngeal tumors[8]. High content of chromium intake leads to the respiratory cancers in human beings[9,10]. Heavy metals, even in trace levels are considered to be harmful to human beings. Disorders, as well as the Acceptable Daily Intake (ADI), of heavy metals are given in TABLE 1. The permissible levels of heavy metals in pharmaceuticals are usually defined by the regulatory agencies and controlled by limit tests. These tests ensure that no inorganic-based contaminants are introduced into the drugs at any of the steps during the manufacturing process. European Pharmacopoeia (EP) has proposed a limit of 20μg g^{-1} of Pt in calcium folinate[11]. The United States pharmacopoeia (USP), British Pharmacopoeia (BP), European Pharmacopoeia (EP) and Japanese pharmacopoeia (JP) propose collective monitoring of total metal content in pharmaceutical products. The methods involve the precipitation of metal sulfides from an aqueous solution and visual comparison of the color to that of a simultaneously and similarly treated standard solution of lead. These methods are non-specific, less sensitive, time-consuming and less accurate. Thus there is a great need for development of highly sensitive and selective techniques for determination trace metals in pharmaceutical substances not only to meet the stringent specifications

TABLE 2 : Operating parameters of flame atomic absorption perkin elmer aanalyst300, with gast air compressor

Conditions	Hg	Fe	Zn	Cu	Mn	Ni	Co	Cr	Pb	Cd
Slit width (mm)	0.7	0.2	0.7	0.7	0.2	0.2	0.2	0.7	0.7	0.7
Cathode lamp current (mA)	6	30	15	15	20	25	30	25	10	4
Relative Noise	1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Resonance line (nm)	253.7	248.8	213.9	324.7	279 .5	232	240.7	357.9	217.0	228.9
Air flow Liter. min^{-1}	10	10	12	10	10	10	10	10	10	10
Acetylene flow Liter. min^{-1}	3	1	2	1	3	3	1	3	3	1
Read Time	5	2	2	2	5	3	3	2	2	2
Read Delay	1	1	1	1	1	1	1	1	1	1

but also ensure the safety and efficacy of drugs for human consumption.

Several attempts were made on estimation of heavy metal contamination of herbal drugs and pharmaceutical products. Rao et al.[12] reviewed the applications of ICP-MS in determination of trace level inorganic impurities in drugs and pharmaceuticals. ICP-MS coupled with LC, GC and CE was used for speciation of heavy metals in pharmaceutical products. The review covered the period from 1995 to 2005 during which the technique was applied not only for determination of metallic impurities but also the assay of various trace elements in pharmaceuticals. Murthy et al.[13,14] reported the levels of Pb in haematopoietic drugs using FAAS. Anca-Iulia Stoica et al.[15] have determined the content of cobalt in pharmaceutical products of B_{12} vitamin by spectrometric (FAAS, GFAAS and ICP-AES) and electrometric (AdSV) techniques. The matrix effects were overcome by using standard addition technique. Nancy Lewen[16] developed a rapid ICP-MS screen for heavy metals in pharmaceutical compounds as an alternative to the wet chemical heavy metals test prescribed by the United States Pharmacopoeia (USP), British Pharmacopoeia (BP), Japanese Pharmacopoeia (JP) and European Pharmacopoeia (EP).

Vanessa Steenkamp et al.[17] determined metal concentrations in plants and urine from patients treated with traditional remedies. A number of traditional South African herbal remedies associated with morbidity and mortality were analyzed for selenium, manganese, copper, lead, zinc and mercury contents. Few showed high levels of toxic metals, but the concentrations were sufficiently high as to cause concern since there was no quality control on the production of such remedies. Urine

TABLE 3 : Heavy metals concentration (ppm) in the different types of antipyretic drugs B.D = Below detection limit: Detection limits: Cd-0.0015ppm: Cr-0.006ppm: Ni-0.01ppm: Co-0.005ppm: Zn-0.001ppm: Mn-0.002ppm

S. No	Disease	Formulations	System type	Toxic Elements					Essential Elements				
				Cd	Cr	Hg	Ni	Pb	Cu	Fe	Co	Zn	Mn
1	Fever	Paracetamol	Allopathic	B.D	4.7±0.2	212±4.5	0.5±0.09	12.5±0.1	3.9±0.2	61.8±3.5	0.44±0.09	B.D	B.D
2		Mruthyunjaya Ras	Ayurvedic	B.D	10.9±0.5	6561±10.9	21.1±0.8	39.4±1.6	14±0.5	540.6±10.6	B.D	11.1±0.2	16.2±0.5
3		Habb-e Mubarak	Unani	B.D	B.D	B.D	0.24±0.01	20±0.4	5.5±0.3	158.4±2.5	0.44±0.05	11.7±1.5	9.5±0.4
4		Bryonia –200	Homeopathic	B.D	31.9±1.4	B.D	B.D	16.3±0.8	0.06±0.01	232.4±5.6	B.D	B.D	B.D
5	Cold	Citrazine hydrochloride	Allopathic	B.D	87.5±3.5	44.2±1.8	36.9±2.1	15.1±0.8	11.7±0.3	349.6±6.5	5.4±0.4	B.D	B.D
6		Shwaskuthar Ras	Ayurvedic	B.D	B.D	9752±35	2.5±0.3	63.5±4.5	14.4±0.4	317.6±5.5	1.9±0.02	10.6±0.3	46.1±2.1
7		Qurs-e Nazla	Unani	B.D	B.D	B.D	3.3±0.2	27.2±2.5	13.7±0.5	833±6.5	1.3±0.2	16.1±0.6	129.7±5.4
8		Habe-Surfa	Unani	B.D	B.D	B.D	4.3±0.3	20±0.4	13.7±0.6	719.4±5.4	3.02±0.09	17.8±0.1	99.3±2.9
9		Allium Cepa-200	Homeopathic	B.D	6.6±0.3	B.D	B.D	14.2±0.8	5.1±0.4	190.7±3.5	B.D	B.D	B.D

samples, obtained from 65 patients admitted to hospital following treatment with a traditional herbal remedies were also analyzed for metals. The data suggested that, in contrast to experience with traditional Chinese and Indian preparations, metal contamination from plants seems not high in traditional South African remedies. Analysis of Indian mint (*Mentha spicata*) for essential, trace and toxic elements and its antioxidant behavior was reported by Garg et al.[18]. Orisakwe et al.[19] assessed the heavy metal hazards of herbal remedies not regulated in Nigeria and reported the contents of cadmium, copper, iron, nickel, selenium, zinc, lead and mercury in random samples of Nigerian traditional products. Ready-to-use herbal products purchased from the open market were digested using HNO_3 and the heavy metal contents were determined by atomic absorption spectrometry. The results showed that 100% of the samples contained elevated amounts of heavy metals and alert the possibility of heavy metal toxicity from herbal products in Nigeria. Mascini et al.[20] proposed the application of disposable electrochemical sensors associated with electroanalytical instrumentation for the detection of heavy metals in herbal drugs. In particular samples of St. John's wort were analysed applying anodic stripping voltammetry. The content of Cd and Pb were evaluated. ICP spectroscopy was used as a reference method. Metal monitoring as a pattern recognition method is a promising tool in the characterization and/or standardization of phytomedicines. Gomez et al.[21] have determined the amounts of Ca, Cu, K, Li, Mg, Mn, Na, Ni, and Zn in phytopharmaceutical derivatives of *Hypericum perforatum* by flame atomic absorption spectrometry (FAAS) and electrothermal atomic absorption spectrometry (ETAAS). The Drug Control Authority (DCA), Malaysia[22] analyzed for the presence of heavy toxic metal, mercury, in tongkat Ali hitam herbal preparations by atomic absorption spectrophotometer, after performing a simple random sampling to enable each sample an equal chance of being selected in an unbiased manner. Results showed that 26% of these products possessed 0.53-2.35ppm of mercury, and therefore, do not comply with the quality requirement for traditional medicines in Malaysia. The quality requirement for traditional medicines in Malaysia not exceeds 0.5ppm for mercury. Fourteen mineral and trace elements (Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sr and Zn) were determined in the herbs and their infusions consumed for medical purposes in Turkey such as chamomile (*Matricaria chammomile* L.), fennel (*Foeniculum vulgare*), linden (*Tilia vulgaris*), nettle (*Urtica dioical*), rosehip (Fr.*Rosa* caninae), sage (*Salvia officinalis*) and senna tea (*Cassia anqustifolia*). Microwave digestion procedure was applied under optimized conditions for dissolution of medicinal herbs. Element concentrations in the medicinal herbs and their infusions were determined by FAAS and ICP-AES[23]. The accuracy and precision were verified against a GBW 07605 Poplar leaves and tea certified reference material. The mineral and trace element content of medicinal herbs and their infusions showed a wide variability. The determination of trace elements in Hypericum perforatum leaves and flowers, their teas, tinctures and tablets was carried out by ETAAS and ultrasonic nebulization system coupled

to Inductively Coupled Plasma Optical Emission Spectrometry (USN-ICP-OES)[24]. Caldas et al.[25] reported the concentration of Pb, Cd, Hg in brezilian herbal medicine of ginkgo biloba (Ginkgo biloba), celastraceae (Maytenus ilicifolia), cascara buckthorn (Rhamnus purshiana), eggplant (Solanum melongena), horse chestnut (Aesculus hippocastanum), Brazilian ginseng (Pffafia glomerata), centella asiatic (Hydrocotyle asiatica), guarana (Paullinia cupana), artichoke (Cynara scolymus) and chlorella (Chlorella pyrenoidosa) using AAS. Few showed high levels of Pb, and Hg, but the concentrations were sufficiently high as to cause concern since there was no quality control on the production of such remedies.

However, in the literature, a comparison of the heavy metal contents of Allopathic, Ayurevdic, Unani and Homeopathic was not reported. Thus, the present study has great potential and significance in comparing the heavy metal contents of different systems of medicine. Atomic spectroscopic techniques viz., AAS, ICP-AES and ICP-MS are widely used for analysis of trace metals. AAS is the most extensively used technique for determination of metals in deferent sample matrices. Generally flame-AAS (FAAS) and graphite furnace AAS (GFAAS) are used depending upon the concentration of the analytes to be determined. The present paper describes the estimation of trace levels of Pb, Ni, Hg, Cd, Zn, Mn, Co, Ni, Cu and Fe contaminations in different systems of Allopathic, Ayurvedic, Unani and Homeopathic drugs used against common colds and fever. The heavy metal contents of different systems of medicine were compared.

EXPERIMENTAL

Apparatus

Atomic absorption spectrophotometer (Perkin Elmer Aanalyst 300, USA), Hollow cathode lamp was used for detection of Lead, Cadmium, Copper, Nickel, Iron, Cobalt, Chromium, Manganese, Mercury and Zinc. The instrument was calibrated with standard solutions using the concentration mode and instrument conditions were given in the TABLE 2. The standard reference materials of all the metals (E. Merck, Germany) were used to provide calibration and quality assurance for each analytical batch. Replicate (n = 3) analyses were conducted to assess precision of the analytical techniques.

Reagents

All reagents were of analytical grade. Sub-boiled water and conc. HNO_3 (69%) (Merk, India) were used in the preparation of samples. Stock standard solutions of Zn, Cu, Fe, Mn, Pb, Ni, Cr, Co, Cd and Hg containing 1000ppm of each metal, were prepared. Calibration standards of each element were obtained by appropriate dilution of the stock solutions. All Allopathic, Homeopathic, Unani and Ayurvedic drug sample were procured from market outlets.

Sample preparation

To estimate the metals in the different types of drug samples of cold and fever, 1.0g powdered drug was taken in 100ml beaker, 5ml conc. HNO_3 were added and kept overnight (16 h). The solution was digested on a hotplate at 80°C for 10 min and allowed to cool at room temperature. 20ml of sub-boiled distilled water was added to the solution and filtered through Whatman filter paper No.42 into a standard flask. The final volume was made up to 100ml with sub-boiled distilled water. Necessary precautions were adopted to avoid possible contamination of the samples.

RESULTS AND DISCUSSION

Allopathic (Paracetamol and Citrazine hydrochloride), Ayurvedic (Mruthyunjaya Ras and Shwaskuthar Ras), Unani (Habb-e Mubarak, Qurs-e Nazla and Habe-Surfa) and Homeopathic (Bryonia –200 and Allium Cepa-200) used against cold and fever were selected. The present study was aimed at the determination of heavy metal content of Indian system of medicine viz: Ayurvedic, Unani and Homeopathy to compare not only amongst themselves but also vis-a-vis with Allopathic system of medicine. Heavy metals such as Pb, Cd, Hg, Pd, Cr, Zn, Mn, Ni, Co, Cu and Fe were estimated by AAS. The method was developed by varying slit width, cathode lamp current and resonance line width by analyzing standard reference materials supplied by E.Merck, Germany. The optimized instrumental parameters are given in TABLE 2. The method

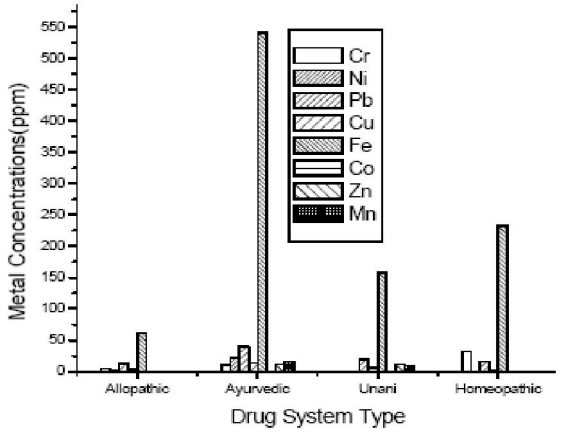

Figure 1a : Metal concentration in fever drugs

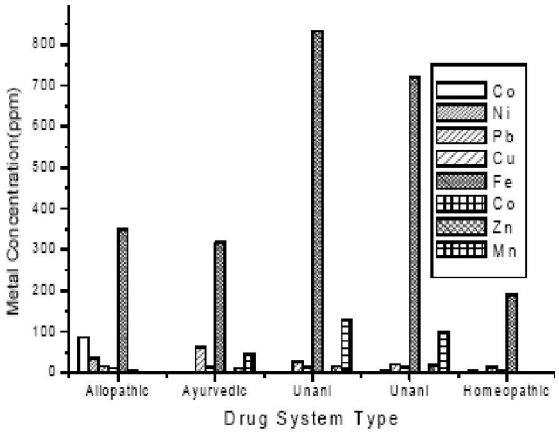

Figure 1b : Metal concentration in cold drugs

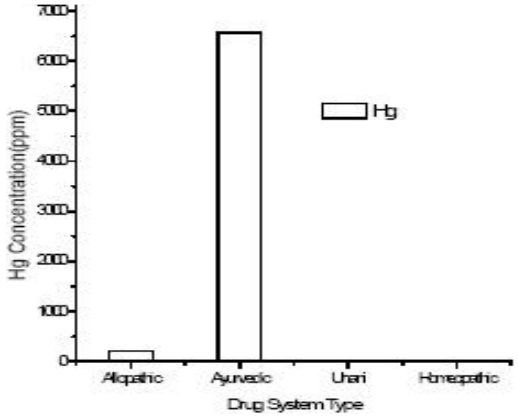

Figure 2a : Hg concentration in fever drugs

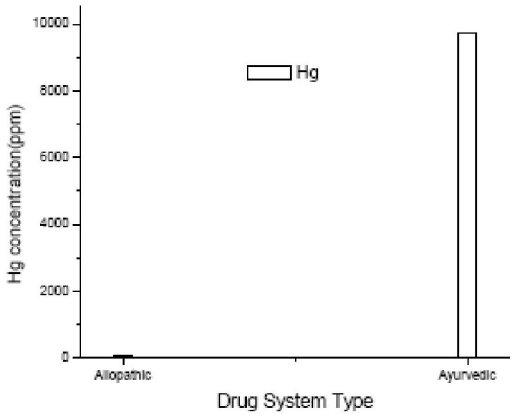

Figure 2b : Hg concentration in cold drug

was validated in terms of accuracy, precision, linearity and range, the linearity of detector response was estimated by each metal standard solution of 1.0,2.0,5.0,10.0µg/ml. By plotting absorbance verses metal concentration, a linear relationship was obtained. The results were found to be in agreement with RSD<4% (average of three determinations). The RSD values in all measurements of drug samples were <5%.

Toxic metals

Paracetamol contained minimum concentration of Pb while it was maximum in Shwaskuthar Ras (Figure 1). According to the WHO[26], a permissible limit of Pb in human beings and the acceptable daily intake (ADI) is 10 ppm (TABLE 1)[9,10]. But, the drugs examined contain beyond the permissible limits i.e. in ranges of 12.5 to 63.5ppm (TABLE 3). The highest total Pb concentration was found in the Ayurvedic drugs followed by Unani drugs, Homeopathic drugs and Allopathic drugs. This could be due to the fact that the preparation of Ayurvedic and Unani formulations involves the usage of Pb. All cold preparations were having the high amount of toxic and essential metal concentrations than the drugs used against fever.

The maximum Cr concentration was observed in the Citrazine Hydrochloride followed by Homeopathic and Unani drugs while Shwaskuthar Ras contain Cr the below the detection limit (Figure 1). The highest content of Cr was found in the Allopathic drugs followed by Homeopathic and Ayurvedic drugs. All Unani drugs were having the Cr content below detection limit. The Allopathic, Homeopathic and Mruthyunjaya Ras contained Cr in the range of 6.66ppm to 87.52ppm (TABLE 3) higher than the permissible limit 1.54ppm (TABLE 1). The possible sources of Cr in Allopathic drugs could be CrO_3, H_2CrO_4 and $H_2Cr_2O_7$ used as regents and catalysts.

Permissible limit of Ni is 1.85 ppb (TABLE 1)[9,10]. The maximum concentration of Ni was found in Ayurvedic drugs followed by Unani, Homeopathic and Allopathic drugs (Figure 1). All Homeopathic drugs were having the Ni content below detection limit where

as remaining systems have shown higher concentrations. The highest total Ni concentration was found in Allopathic followed by Ayurvedic and Unani drugs (TABLE 3).

Interestingly, the drugs under investigation accumulated Cd at a level appreciably below the permissible level (0.30ppm) (TABLE 1). On the basis of these results it may be assumed that the drugs are safe with respect to Cd toxicity.

The Shwaskuthar Ras has shown highest Hg concentration followed by Mruthyunjaya Ras and Allopathic drugs. The Unani and Homeopathic drugs contain Hg concentrations below its detection limit (Figure 3). The highest total Hg concentration was also found in the Ayurvedic drugs followed by Allopathic drugs. Hg is the most toxic element as designated by WHO[27] and FDA. It's permissible limit is 1ppm. The results obtained in the present investigation show that the Ayurvedic and Allopathic drugs contain higher concentration of Hg (44 to 9752ppm) whereas Unani and Homeopathic systems having below detection limits (TABLE 3). This could be due to the fact that Hg is generally used in preparation of Ayurvedic formulations.

Essential elements

The concentration of Fe was found to be maximum in Habe-Surfa (cold Unani drugs) followed by Ayurvedic, Homeopathic and Allopathic drugs (Figure 1). The highest total Fe concentration was found in Unani followed by Ayurvedic, Homeopathic and Allopathic drugs. Fe is considered to be a micronutrient[28] with a permissible limit of 231ppm (TABLE 1). Both, Allopathic and Homeopathic drugs were having the permissible limits of Fe where as Ayurvedic and Unani drugs beyond the limits i.e. in the range of 317 to 833ppm (TABLE 3). This may be due to the fact that Fe is most abundant metal in the earth's crust and plants materials.

The maximum concentration of Zn was found in Unani drugs followed by Ayurvedic drugs. Allopathic and Homeopathic drugs were having the below detection limit of Zn (Figure 1). All drugs contain low concentration of Zn than the permissible limit (TABLE 3). On the basis of ADI it may be suggested that the drugs are safe as far as Zn toxicity is concerned.

Copper is relevant to human beings because it is both essential and toxic depending on the dose and duration of exposure. The maximum Cu concentration was found in Ayurvedic drugs followed by Unani, Homeopathic and Allopathic drugs (TABLE 3). The high concentration of Cu in the Ayurvedic and Unani drugs might be enrichment of Cu in herbal plants based on enzymatic participation in redox reactions[29], super oxide dismutase[30] and cytochrome oxidase. ADI for Cu is 21.5ppm (TABLE 1). All the drugs contained low Cu concentration than the permissible limit (Figure 1). On the basis of ADI it could be suggested that the drugs are safe in terms of Cu toxicity.

A maximum of 5.4 ppm of Co was found in cetrazine hydrochloride when compared to other drugs. The Homeopathic drugs were having lowest Co concentration. The highest total Co concentration was found in Allopathic drugs followed by Unani, Ayurvedic and Homeopathic drugs. The concentration of Co necessary per day for human body is 8µg (TABLE 1)[31]. Except Homeopathic drugs and Shwaskuthar Ras, the remaining drugs have shown excess content of Co i.e. in the range of 0.44ppm to 5.4ppm (TABLE 3). The high concentration of Co in Ayurvedic drugs might be due to the enrichment of Co in the plant materials.

Maximum concentrations of Mn were estimated in Habe-Surfa and Qurs-e Nazla formulations of Unani used against cold. Similarly the formulations of Unani against fever also showed maximum content of Mn (Figure 1). The highest total Mn concentration was found in Unani followed by Ayurvedic drugs (TABLE 3). Homeopathic and Allopathic drugs were having below the detection limit of Mn. ADI for the Mn is 69.2ppm (TABLE 1). The high concentration of Mn in Unani and Ayurvedic drugs might be due to the enrichment of Mn in the plant materials.

CONCLUSIONS

Heavy metal content of Indian system of medicine used against cold and fever was determined by AAS and compared with the modern western medicine. All cold preparations were having the high amount of toxic and essential metal concentrations than the drugs used against fever. Toxic metals like Pb, Ni and Hg, and essential metal Cu concentrations were found highest in the Ayurvedic drugs while Cr was least in the Ayurvedic drugs. The content of Cr and Co were highest in the

Allopathic drugs while Fe, Zn and Mn were in Unani followed by Ayurvedic drugs. Unani formulation contain below detection limits of toxic metals Cr and Hg. Ni, Co, Zn, Mn were found below detection limits in the homeopathic drugs. All the drugs were having the below detection of Cd. This investigation provides a status of heavy metal concentration in various drugs of Indian system of medicine sold in the market. There should be periodical assessment of heavy metal concentration in all drugs, in order to have quality assurance and safer use of drug products.

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