Pakistan Journal of Pharmaceutical Sciences
Vol. 18, No.2, April 2005, pp.40-43

TRACE METALS ANALYSIS IN SELECTED PHARMACEUTICAL MULTIMINERAL FORMULATIONS

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Twelve pharmaceutical multimineral formulations, marketed in Pakistan by various multinational and national pharmaceutical manufacturers were analyzed for calcium, copper, iron, magnesium, manganese, potassium and zinc contents using atomic absorption spectrophotometric (AAS) technique. Generally, the Ca, Fe, K and Mg contents were normal to higher range compared with the labeled quantity. The Cu, Mn and Zn contents were low in most of the formulations when compared with the labeled amounts. Potassium was quantified in some formulations where it was not labeled this may be due to the excepients that may have been used in the formulations. The high contents like iron, calcium may also be due to the contamination or excepients used in the preparation of these drugs.

INTRODUCTION

Both animal and vegetable life depend for their existence on appropriate amounts of various trace elements, albeit in very small amounts (Harry, 1989). The literature revealed that out of 106 known elements 81 have been reported in human body (Vohra, 1981) all of which play vital biochemical role (Lyenger, 1985). The deficiency, access or medicinal use of these 81 elements (Vohra, 1982) has been reported to affect various physiological functions of the human body. Studies indicate that there is a definite correlation between the level of trace elements in the human body and some diseases (Cevik *et al.,* 2003; Ahmad *et al.,*1979 and Nada *et al.,*1999). The role of metals in curing ailments was first realized in Ayurveda. Several metallic preparations with organic macromolecules, referred to as *bhasma(s)* in Ayurvedic literature, are widely used for curing a variety of disorders (Singh and Garg, 1997). Mineral products, formulated as single ingredients or compound, are used either as dietary supplements, as therapeutic agents for prevention and treatment of specific deficiency states or as pharmacological agents to treat specific diseases. As treatment is becoming more expensive, more complicated and less reliable the availability in Pakistan of irrational multimineral combinations, which are either valueless when they do not provide the consumers the recommended allowances or are a health hazard if they burden the human system by over dosage, requires re-assessment by the regulatory agencies/authorities.

MATERIALS AND METHODS

General Experimental Procedure

All chemicals used in the experiment were of analytical grade (E.Merk/BDH). Incineration of the samples was carried out in a muffle furnace (Thermo-Activated Ltd., Great Britain) at 5000C for *5-6* hours. Atomic Absorption Spectrophotometer (Hitachi Model 170-10 with an air and acetylene burner head) was used for the measurement of trace elements. All glass wares used in the elemental analysis were soaked overnight in 50% w/v nitric acid, rinsed several times with distilled water and dried in a hot air oven before use.

Selection

Of the total number of mineral formulations registered for manufacture and sale in Pakistan twelve were selected for analyzing the contents of Ca, Cu, Fe, K, Mg, Mn, and Zn. These commercial supplements, which were a combination of minerals and vitamins, comprised seven brands of multinational manufacturers and five of the national manufacturers. The labeled contents of the elements contained in the selected pharmaceutical formulations are shown in table.

Procurement and sampling

Samples of the formulations investigated were procured directly from their manufacturers.

Sample pretreatment

Analysis was carried out according to the standard established procedures/methodology also adopted by the pharmaceutical industry in Pakistan for testing their multimineral-vitamin formulations.

Tablets

Ten tablets from each formulation were weighed and ground in a porcelain mortar and pestle. Powdered sample, in triplicate, each equivalent to one tablet, was transferred to pre-cleaned silica crucibles. These were first heated at low

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flame and then placed in a muffle furnace and heated at not more than 550°C for about 3 h or until carbon free. In case of those samples in which the ashing process was not completed within this time the contents were cooled to room temperature and moistened with a few drops of nitric acid. The crucibles were placed in a fume hood cupboard and the m ixture was heated to dryness on a hot plate. On completion o f reaction the crucibles were cooled and placed again in the furnace for 2 h to complete the ashing. On cooling, the ash contents were digested in 5 ml of 6N HC1 over gentle heat on a hot plate. The solution was diluted with about 5 ml distilled water and gently boiled further for 15-20 mm before filtering in to a 50 ml, volumetric flask. The volume was made up accurately with a sufficient quantity of distilled water. These sample solutions were preserved in washed and dried polyethylene bottles for assay of trace elements.

Hard gelatin capsules

For each sample formulation 10 capsules were opened and their contents weighed. Three samples of the powder equivalent to one capsule each were accurately weighed in thoroughly cleaned and dried porcelain crucibles and incinerated in a muffle furnace for 3 h at 550°C till the mass completely oxidized. The cooled contents of the crucible were, dissolved in 5 ml of 6N HC1 with gentle heating on a hot plate, diluted with distilled water and then made up to 50 ml volume as per procedure for tablets.

Soft gelatin capsules

Three samples of one capsule each were placed in porcelain crucibles and gently heated on a low flame to burn off the oil contents. The residue was ignited in a muffle furnace at 500°C for 1 h or for further period of time till it was free from carbon. Solutions of samples for trace elements analysis were prepared with the method similar to tablets.

Detection and measurement of trace elements by atomic absorption spectroscopy

Flame atomic absorption spectrometry was used as the analytical method for the measurement of calcium, copper, iron, magnesium, manganese, potassium and zinc in the plant sample solutions as well as in serum. Sample stock solutions were diluted to obtain appropriate concentrations that fall within the scope of analytical concentration of the standard solution of the trace elements. To eliminate the chances of ionization or interference by other elements specific buffer solutions were added to both the standard stock and unknown sample solutions while analyzing the following elements.

Calcium	Lanthanum chloride spectral buffer
Magnesium	Strontium chloride solution 0.25%
Potassium	Sodium chloride solution 0.13%
Sodium	Potassium chloride solution 0.12%

Calculations

The contents of the trace elements in the pharmaceutical multimineral formulations are expressed in mg/tablet or capsule. Data are presented as M ± SD and all the statistical calculations were carried out using Minitab™, a statistical package.

RESULTS AND DISCUSSION

The percent (± SD) contents of the Ca, Cu, Fe, Mg, Mn, K and Zn are presented in table 1 for triplicate analysis of trace metals in these formulations. Multi-mineral oral pharmaceutical formulations being marketed in Pakistan were selected were either in the form of hard/soft gelatin capsules or coated tablets.

The labeled calcium contents ranged from 11.15 mg to 164 mg/tablet or capsule in various formulations studied. Most of the formulations contain calcium as pantothenate or as dibasic phosphate salts. The concentration of the calcium in most of the formulations was in good agreement with labeled quantities, in formulation "D" the concentration was very high similarly, it was also high and in formulation E, J and K. The addition of unclaimed calcium carbonate or other calcium compounds as inert or inactive ingredients probably the cause of higher analytical values for calcium in comparison with those labeled on some multimineral formulations.

The copper content of the pharmaceutical multi-mineral formulations studied were in a wide range i.e. 0.04 to 3.00 mg/tablet or capsule (labeled contents). The analyses of these formulations showed the widespread quantity of copper i.e. 31% to 260% in these formulations results are shown in table 1. The formulation A only shows the higher concentration of the copper i.e. 260%, the formulation B, E and H were containing copper about 90% while other formulations contains less than 70% of copper. This could be attributed to manufacturing defects like inconstant mixing of the small proportion of copper salts in the huge batches of multi-mineral products.

The labeled contents of iron ranged between 10.00 mg to 65.74 mg elemental iron in the form of various salts. The iron contents were found to be in good agreement with the labeled quantities with exception of the formulation D and F, where the contents were high. The rationale for the availability of such widely varying doses of iron to the consumer, at exorbitant prices, has never been questioned by practitioners of modern medicines and also appears to have escaped the notice of the drug regulatory authorities of this country.

Potassium was labeled only in 9 formulations but the analysis shows that it is present in the entire preparations understudy. The labeled quantity of potassium in these formulations were ranged between 1.7 to 7.5 mg/tablets or capsule. The formulation A, C and H were not marked to

Table

Trace elements contents ($mg.ml^{-1}$) in various commercial preparations (Tablets and Capsules)

Drug formulation	Trace element concentration (mg/Tablet or Capsule)													
	Ca		Cu		Fe		K		Mg		Mn		Zn	
	Found (%)	Labeled	Found (%)	Labeled	Found (%)	Labeled	Found (%)	Labeled	Found (%)	Labeled	Found (%)	Labeled	Found (%)	Labeled
A (Capsules)	96.00 ± 43	11.152	260.0 ± 2.1	0.0398	88.4 ± 4.5	16.45	5.33 ± 0.471	-	111.1 ± 1.2	0.091	16.7 ± 1.0	0.0036	50.0 ± 2.3	0.041
B (Capsules)	120.0 ± 12.4	37.4	86.5 ± 3.1	1.0	89.1 ± 1.4	65.74	294.5 ± 4.5	1.7	172.5 ± 3.8	2.0	93.3 ± 1.1	0.03	0.014 ± 0.001	-
C (Capsules)	112.98 ± 1.0	14.0	ND	-	6.15 ± 0.011	6.57	4.50 ± 0.408	-	763.0 ± 7.5	0.6	108.6 ± 2.1	0.22	59.0 ± 2.1	1.61
D (Tablets)	264.7 ± 3.0	1.7	45.5 ± 2.8	1.0	1135.1 ± 1.1	10.0	100.0 ± 0.5	5.0	162.0 ± 3.1	5.00	44.9 ± 0.8	1.00	60.0 ± 0.7	1.5
E (Tablets)	135 ± 8.6	40.0	92.00 ± 1.3	2.0	92.2 ± 1.3	27.0	108.8 ± 2.4	7.5	95.4 ± 1.4	100.00	107.4 ± 1.0	5.00	74.3 ± 2.8	15.0
F (Capsules)	108.5 ± 4.1	103.7	56.0 ± 2.6	1.0	141.3 ± 2.3	10.0	103.4 ± 2.5	5.0	114.8 ± 3.9	6.00	49.6 ± 0.8	1.00	63.9 ± 2.4	1.2
F (Tablets)	109.9 ± 3.5	162.0	31.0 ± 2.1	3.0	92.6 ± 1.9	27.0	113.3 ± 1.7	7.5	91.8 ± 4.4	100.00	81.3 ± 1.1	7.50	68.2 ± 4.7	22.5
H (Tablets)	146.0 ± 1.3	50.0	94.4 ± 0.5	0.1	84.4 ± 2.1	65.7	6.00 ± 0.707	-	140.6 ± 2.4	10.00	286.0 ± 3.2	0.10	63.9 ± 2.4	1.2
I (Tablets)	117.9 ± 1.3	105.0	53.5 ± 1.6	1.0	96.9 ± 15.0	15.0	110.0 ± 0.7	5.0	208.1 ± 1.8	6.00	52.6 ± 1.1	1.00	56.7 ± 1.6	1.5
J (Capsules)	167.5 ± 5.3	50.0	60.8 ± 2.3	1.0	96.2 ± 4.7	15.0	123.4 ± 1.7	5.0	126.6 ± 4.9	6.00	50.8 ± 1.4	1.00	75.3 ± 4.7	1.5
K (Capsules)	136.8 ± 2.4	50.0	73.3 ± 3.2	1.0	96.7 ± 2.1	15.0	133.4 ± 3.2	5.0	128.3 ± 5.3	6.00	52.5 ± 2.7	1.00	73.3 ± 1.8	1.5
L (Capsules)	112.5 ± 3.1	50.0	67.7 ± 5.3	1.0	97.3 ± 1.7	15.0	160.0 ± 4.1	5.0	108.6 ± 2.2	6.00	53.5 ± 3.9	1.00	0455 ± 2.4	1.5

contain the potassium but the analysis showed that these formulations contain 5.33, 4.50 and 6.00 mg of potassium/tablet or capsule, respectively. The presence of potassium is probably may be due to the due to potassium salts of excepients or impurities from active and inactive natural ingredients like talc, gum acacia, gelatin, starch etc, which are employed in formulating the products. The same may be the reason for higher potassium contents for the formulations B, K and L.

The magnesium contents were found exceptionally very high (763.00%) in formulation C while in formulation B, D and I high that may also be due to the addition of various excepients like magnesium stearate which are commonly used as inactive or inert ingredients, in formulating tablets and capsules. Presence of minerals can also not be ruled out from gelatin, gum acacia, and starch that are inert binders necessary in formulating these products. While in other samples analyzed the magnesium contents were close to normal values or somewhat higher compared with the labeled contents, results are shown in table.

The manganese contents of twelve pharmaceutical multimineral formulations analyzed were significantly different from the labeled amounts. The labeled contents of manganese for most of the products was however 1 mg. The formulation H was found to contain the highest amount i.e. 286% of the manganese while formulation G contains the 81.3% and other formulations were found to have about 50% of the labeled quantity of this trace metal. It is possible that these differences in manganese contents may be related to manufacturing defects like improper mixing.

The contents of zinc listed on labels of different multiminerals pharmaceutical formulations varies from 0.1 mg (sulphate) equivalent to 0.041 mg elemental zinc, to 22.5 mg mg/capsule or tablet. Compared to the claimed contents the values of zinc obtained on the quantitative analysis of these products were to a great extent on the lower side. Improper mixing of the relatively minor quantities of zinc salts during the manufacturing process may be the likely cause of this variation. Product B was found to contain 0.0 14 mg/capsule whereas it was not mentioned on the actual formulation on the label. This could be due to some possible contamination during the manufacturing process.

This study indicates that trace metals in these multimineral formulations were found to be significantly different compared with labeled amount. This may be due to the use of various excepients in dispensing or may be due to the contamination during processing. The manganese and zinc contents were usually low that may be due to improper mixing or addition of inadequate amount of these metals. It can be concluded that more care is required in the preparations of these formulations to use the proper excepients, process cleaned containers and equipment to avoid contamination. The manufacturing process and methods of analysis should be validated before the commercial production.

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