ORIGINAL PAPER

# Analysis of heavy metals by atomic absorption spectrophotometry in commercially available multivitamins

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**ABSTRACT** The concentration of heavy metals like nickel, manganese, zinc, chromium, copper and iron were examined in multivitamin drugs sampled from an indigenous market of a well reputed manufacturer employing atomic absorption spectrophotometer (AAS). Intake of nickel in excess results in different diseases like liver, stomach and kidney disorder. This research paper is focused to know the concentration of toxic metals in multivitamins tablets and syrups. Study of different tablets and syrups from different companies showed varying level of toxic metals. Variable concentration of nickel was found in tested products ranging from 0.8 – 92.28 µg/g. The high nickel concentration was found in Vidaylin-T (Tab) (92.28 µg/g) manufactured by Abbott laboratories Karachi Pakistan and low in Becotin syrup (0.8µg/g) manufactured by Life pharmaceutical company Multan Pakistan. The concentration of zinc in tested sample was 0.37 to 980.93µg/g which is within the permissible limit. Most of tested multivitamin samples contain cobalt concentration less than the recommended daily intake.

**Keywords:** AAS (Atomic Absorption Spectrophotometers), Indigenous market, Multivitamins, Toxic metals

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## INTRODUCTION

It is need of the day to develop analytical methods for precise determination of hazardous material in foodstuff and pharmaceutical products, particularly in nutritional supplement drugs, where national and international standards in terms of their potential and safety should be properly observed (Ventola, et al., 2010). Pharmaceutical drugs are under strict regulations as compared to nutritional supplement drugs. Nutritional supplement drugs have great potential having active ingredients like herbs, vitamins, minerals, enzyme metabolites etc. In USA, the Food and Drug Administration (FDA) has been regulating dietary supplements through the Dietary Supplement Health and Education Act (DSHEA) since 1994. According to this law, these drugs makers have to ensure that a nutritional supplement item is safe to use and medical advantages suggested for this drug are authenticated prior to its commercialization (Kathy et al., 2004)

There are several dietary supplement drugs in the market which are used to fulfill the demand of vitamins for human body and are known as multivitamin drugs. A vitamin is basically an organic compound which is compulsory as a nutrient in small quantities by an organism. In other words, those organic compounds are known as vitamins when they couldn't produce in required quantities by an organism, and must be taken in diet. The need of vitamins is specific for different organisms (David et al., 2006). For example, ascorbic acid (vitamin C) is a vitamin for humans, but not for most other animals, and biotin and vitamin D are mandatory in the human diet merely in certain conditions. By convention, the word vitamin doesn't comprise other necessary nutrients such as dietary minerals (which are inorganic), vital fatty acids or essential amino acids (which are required in larger amounts than vitamins), nor does it incorporate the bulky number of other nutrients that support health, but are otherwise requisite less often (Lieberman et al., 1990).

Like most other developing countries of the world, the use of multivitamin is common in Pakistan. Other than accessibility and affordability, the promoters of multivitamin products believed that these are safe and harmLess because of good synthesis practice (GMP), but excessive intake of multivitamin products due to lack of awareness about its harmful effect might be dangerous. This may causes intake of heavy metals up to toxic level. The intake of heavy metals like Pb, Cd, Cr, etc. is lethal for human beings even in small amount (Anthea et al., 1993). So, it is highly recommended that heavy metals in multivitamin drugs should be analyzed prior to their commercialization.

In this article we have reported the determination of heavy metals by Atomic Absorption Spectrophotometry (AAS) in commercially available multivitamins collected from the local market in Pakistan. To the best of our knowledge, this kind of exploration using AAS on the drugs, commercially available in Pakistan has never been reported so far.

## MATERIAL AND METHODS

### Chemicals

The chemicals used throughout this research project are perchloric acid ($HClO_4$, Riedel-oe-haen), Nitric acid (65%, E. Merck), sodium nitrate (99%, E. Merck), potassium nitrate (99%, E. Merck), calcium acetate dihydrate (99%, E. Merck), ferrous ammonium sulphate hexahydrate (99%, E. Merck), zinc acetate dihydrate (99%, E. Merck), cobalt acetate (99%, E. Merck), chromium nitrate(99%, E. Merck), copper acetate monohydrate (99%, E. Merck), nickel acetate tetrahydrate (99%, E. Merck) and manganese chloride (99%, E. Merck).

### Preparation of samples

In china dish 1 mL of sample (syrup of multivitamin weighed), 4 mL of $HNO_3$ and 1 mL of $HClO_4$ (perchloric acid) were added and mixture was kept overnight. Subsequently, it was heated for 20 min in heating mental to evaporate all the liquid. It was then cooled for 15 minutes. Again 4 mL of $HNO_3$ and 1 mL of $HClO_4$ were added into china dish. Then it was heated for about 45 min to evaporate all liquid then cooled for 15 min and this whole procedure was repeated thrice till the whole sample consumed and sample became colorless. Finally 1% $HNO_3$ was added into the sample, homogenized and was filtered with whattman 42 filter paper. Finally, total volume of 25 mL was obtained by diluting it with distilled water and was stored in sample bottles.

### Estimation of metals employing atomic absorption spectrometer

An atomic absorption spectrometer (Hitachi; Model A-1800) equipped with standard burner and acetylene flame has been employed. The radiation source employed for different metals was metal cathode lamp of that specific metal to be analyzed. Instrument was warmed about half an hour before absorbance measurements.

## RESULTS AND DISCUSSION

The concentration of heavy metals like nickel, manganese, zinc, chromium, copper and iron were examined in multivitamin products available in local market using atomic absorption spectrophotometer. The results are shown in Table 1 and 2. Daily intake of metals according to manufacturer dose is given in Table 3.

**Table 1** Metal Concentration (μg/g) in multivitamin tablet samples

| Multivitamin | Ni | Cu | Zn | Fe | Mn | Cr | Co |
|---|---|---|---|---|---|---|---|
| Daylets (Tab) | 23.89 | 453.953 | 308.42 | N/D* | 182.45 | N/D | N/D |
| Once a day(Tab) | 35.74 | N/D | 325.93 | N/D | 255.56 | N/D | 15.93 |
| Megacit(Tab) | 38.46 | N/D | 32.97 | N/D | 50.67 | N/D | 21.37 |
| Biovit-m(Tab) | 23.18 | 7.645 | 192.35 | N/D | 131.19 | N/D | N/D |
| Optilets-m(Tab) | 26.82 | 104.944 | 219.22 | 126.87 | 55.27 | N/D | 89.79 |
| Centural gold (Tab) | 39.17 | N/D | 37.20 | N/D | 62.00 | N/D | N/D |
| Nutrifer(Tab) | 23.78 | N/D | 8.86 | N/D | 27.97 | N/D | N/D |
| ADE-(Tab) | 36.46 | N/D | 21.92 | N/D | 41.54 | N/D | 33.46 |
| Diabetone cap | 13.83 | 161.674 | 518.43 | 1697.4 | 514.59 | N/D | N/D |
| Geritol cap | 8.06 | N/D | 30.61 | N/D | 51.56 | N/D | 10.31 |
| Divasas(Tab) | 18.90 | 33.401 | 316.43 | N/D | 58.45 | N/D | N/D |
| Xeebon(Tab) | 23.64 | N/D | 423.91 | N/D | 42.12 | N/D | N/D |
| Visionance cap | 43.49 | N/D | 186.92 | N/D | 661.40 | N/D | N/D |
| Theragram-M(Tab) | 25.87 | N/D | 1.36 | N/D | 43.57 | N/D | 9.53 |
| Vidaylin-T(Tab) | 92.28 | N/D | 187.74 | 293.81 | 158.04 | N/D | N/D |
| Prefectil-cap | 47.45 | 9.408 | 229.06 | N/D | 76.49 | N/D | N/D |
| Aiktab(Tab) | 24.44 | N/D | 258.48 | N/D | 62.04 | N/D | N/D |
| Engran(Tab) | 62.13 | N/D | 12.08 | N/D | 56.09 | N/D | N/D |
| FAZ(Tab) | 25.89 | N/D | 980.93 | N/D | 50.95 | N/D | N/D |
| Skinvite(Tab) | 56.13 | N/D | 25.00 | N/D | 45.36 | N/D | N/D |
| Theragram(Tab) | 31.39 | N/D | 25.00 | N/D | 64.06 | N/D | N/D |
| Sofvid(Tab) | 28.65 | N/D | 25.00 | N/D | 53.39 | N/D | N/D |

N/D: Not detected

**Table 2** Concentration (μg/mL) of metals in multivitamin syrup samples

| Multivitamin | Ni | Cu | Zn | Fe | Mn | Cr | Co |
|---|---|---|---|---|---|---|---|
| Multibionta syrup | 1.12 | N/D | 0.79 | N/D | 1.47 | N/D | N/D |
| Potential syrup | 1.76 | N/D | 1.08 | N/D | 1.56 | N/D | N/D |
| Vidaylin-L syrup | 0.88 | N/D | 0.37 | N/D | 1.5 | N/D | N/D |
| Hemolif syrup | 1.42 | N/D | 0.79 | N/D | 1.58 | N/D | N/D |
| Lysolif-L syrup | 1.09 | N/D | 0.69 | N/D | 1.53 | N/D | N/D |
| Becotin syrup | 0.8 | N/D | 0.48 | N/D | 1.57 | N/D | N/D |

N/D: Not detected

**Table 3** Daily intake of metals (µg/day) according to manufacturer's dose

| Multivitamin | Ni | Cu | Zn | Fe | Mn | Cr | Co |
|---|---|---|---|---|---|---|---|
| Daylets (Tab) | 27.50 | 522.50 | 355.00 | N/D | 210.0 | N/D | N/D |
| Once a day(Tab) | 48.25 | N/D | 440.00 | N/D | 345.0 | N/D | 21.50 |
| Megacit(Tab) | 31.50 | N/D | 27.00 | N/D | 41.5 | N/D | 175.0 |
| Biovit-m(Tab) | 23.50 | 7.75 | 195.00 | N/D | 133.0 | N/D | N/D |
| Optilets-m(Tab) | 28.75 | 112.50 | 235.00 | 136.00 | 59.25 | N/D | 96.25 |
| Centural gold(Tab) | 34.75 | N/D | 33.00 | N/D | 55.00 | N/D | N/D |
| Nutrifer(Tab) | 25.50 | N/D | 9.50 | N/D | 30.00 | N/D | N/D |
| ADE-(Tab) | 39.50 | N/D | 23.75 | N/D | 45.00 | N/D | 36.25 |
| Diabetone cap | 9.00 | 105.25 | 337.50 | 1105.00 | 335.0 | N/D | N/D |
| Geritol cap | 6.25 | N/D | 23.75 | N/D | 40.00 | N/D | 8.00 |
| Divasas(Tab) | 21.50 | 38.00 | 360.00 | N/D | 66.50 | N/D | N/D |
| Xeebon(Tab) | 21.75 | N/D | 390.0 | N/D | 38.75 | N/D | N/D |
| Visionance cap | 30.25 | N/D | 130.00 | N/D | 460.0 | N/D | N/D |
| Theragram-M(Tab) | 23.75 | N/D | 1.25 | N/D | 40.00 | N/D | 8.75 |
| Vidaylin-T(Tab) | 21.75 | N/D | 44.25 | 69.25 | 37.25 | N/D | N/D |
| Prefectil-cap | 29.00 | 5.75 | 140.00 | N/D | 46.75 | N/D | N/D |
| Aiktab(Tab) | 26.00 | N/D | 275.00 | N/D | 66.00 | N/D | N/D |
| Engran(Tab) | 54.00 | N/D | 10.50 | N/D | 48.75 | N/D | N/D |
| FAZ(Tab) | 23.75 | N/D | 900.00 | N/D | 46.75 | N/D | N/D |
| Skinvite(Tab) | 49.50 | N/D | 22.04 | N/D | 40.00 | N/D | N/D |
| Theragram(Tab) | 24.50 | N/D | 19.513 | N/D | 50.00 | N/D | N/D |
| Sofvid(Tab) | 22.00 | N/D | 192.00 | N/D | 41.00 | N/D | N/D |
| Multibionta syrup | 8.00 | N/D | 7.900 | N/D | 14.7 | N/D | N/D |
| Potential syrup | 12.00 | N/D | 10.80 | N/D | 15.6 | N/D | N/D |
| Vidaylin-L syrup | 16.00 | N/D | 3.70 | N/D | 15.0 | N/D | N/D |
| Hemolif syrup | 80.00 | N/D | 7.90 | N/D | 210.0 | N/D | N/D |
| Lysolif-L syrup | 12.00 | N/D | 6.90 | N/D | 345.0 | N/D | N/D |
| Becotin syrup | 19.00 | N/D | 4.80 | N/D | 41.5 | N/D | N/D |

N/D: Not detected

**Nickel**

Long lasting exposure of nickel may result to allergic disease like skin rashes which appear on skin surface. Data obtained from animal shows that large amount of nickel intake cause disorder of kidney, blood, stomach etc. (*R. Singh, et al* 2011). Dietary intake of nickel is 25-35 µg (*B. Zambelli et al* 2016).Variable concentration of nickel was found in tested products ranging from 0.8– 92.28 µg/g as shown in Table 1 and Table 2. The high nickel concentration was found in Vidaylin-T (Tab) (92.28 µg/g) manufactured by Abbott laboratories Karachi, Pakistan and low in Becotin syrup (0.8 µg/g) manufactured by Life pharmaceutical company Multan Pakistan. There is no significant data about the toxic level of nickel. The Table 3 shows the daily intake of nickel according to manufacturer dose. Among the tested sample, it has been found that five of the samples exceeded the recommended daily intake and these are once a day (tab) manufactured by CCL pharmaceuticals Kot Lakhpat, Pakistan, ADE (tab) manufactured by Image phorma Lahore Pakistan, Engran (tab) manufactured by Bristol Myers Squibb Karachi Pakistan, Skinvite (tab) manufactured by Zeb. Laboratories (pvt) Ltd Lahore Pakistan and Hemolif syrup manufactured by Life pharmaceutical company Multan Pakistan. Therefore, there is possibility of toxicity by using them.

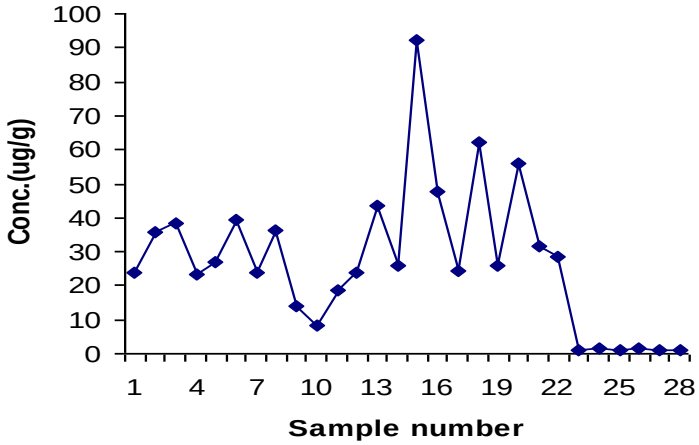


**Fig. 1** Conc. (µg/g) of Nickel in various multivitamin samples

**Copper**

For human metabolic system copper is vital element. It helps to perform numerous biological reactions within the body like production of energy, development of connective tissues, manufacturing of neuro transmitters etc (Calnan et al., 1956; Anke et al., 1995). High concentration of copper results in vormiting, nausea, diaherra, kidney and liver damage (Linder et al., 1996). Recommended daily dosage for children is 340-440 µg/day and for adults 890-900 µg/day (Olafsdottir et al., 2017). Copper concentration (µg/g) found in multivitamin tablets and syrups is given in Table 1 and 2. The daily intake of copper, according to manufacturer dose is given in Table 3. Only in Daylets (tab) manufactured by Abbott laboratories Karachi Pakistan, copper daily intake was found to be 522.5µg/day which exceeds the permissible limit for children but not for the adults. As, this concentration is usually not prescribed for the children but for the adults hence not harmful for them.

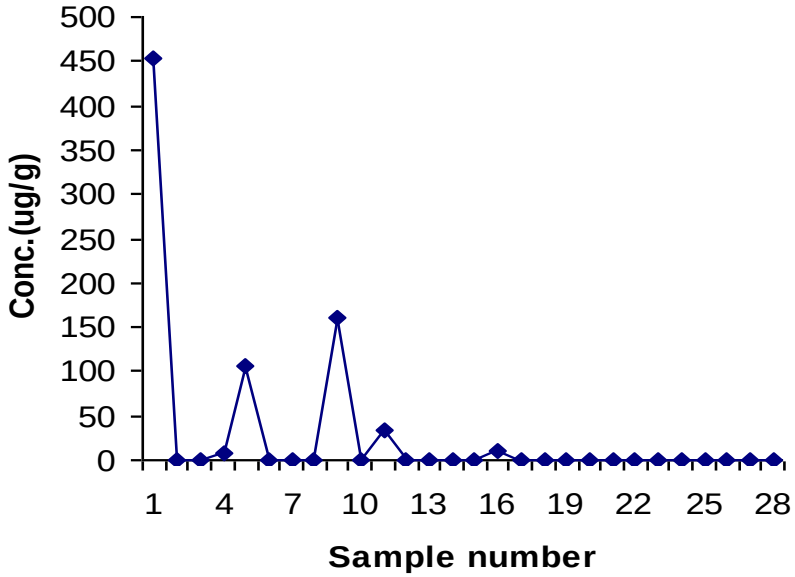


**Fig. 2** Conc. (µg/g) of Copper in various multivitamin samples

**Zinc**

Zinc is one of the essential trace elements for human and animals. The catalytic activity of approximately 100 enzymes is zinc dependent in our body. It also plays very important role in the structural stability of proteins and cell membrane. In addition, zinc is involved in cell signaling, release of hormones and in apoptosis (Wardle et al., 2000). Acute zinc toxicity (oral dose of 225-450 mg) causes abdominal pain, nausea, vomiting and diarrhea. Chronic exposure of zinc causes copper deficiency (Olafsdottir et al., 2017). The allowed dietary level for children is 4000-5000 µg/day, for man 13,000-19,000 µg/day and for woman 9000-13,000 µg/day (Truong-Tran et al., 2000). The concentration of zinc in tested sample was 0.37 to 980.93µg/g. The daily consumption of zinc was calculated according to the manufacturer recommended dose as shown in Table 3. Out of these samples, there was no product which exceeded the recommended permissible limit for children, men and woman.

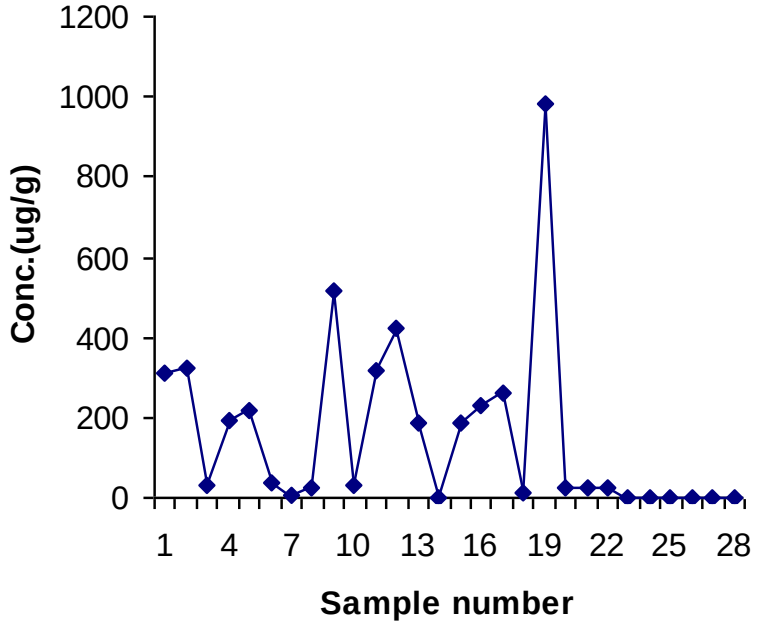


**Fig. 3** Conc. (µg/g) of Zinc in various multivitamin samples

**Iron**

The daily intake of iron, according to manufacturer dose is given in Table 3. The estimated concentration of iron in our samples was up to 1697.39 µg/g (Table 1 and 2). Iron concentration in most of the tested multivitamin samples was not detected at ppm level except Optilets-M (tab) containing 123.8 µg/g, Diabitone capsule containing 1697.4µg/g and Vidaylin-T (tab) containing 293.81µg/g of iron. The recommended dietary allowance is 7000-10,000µg/day for children, 8000 µg/day, for adults and 27000µg/day for women during pregnancy (Olafsdottir et al., 2017). Therefore, iron concentration in the tested sample was less than recommended dietary allowance.

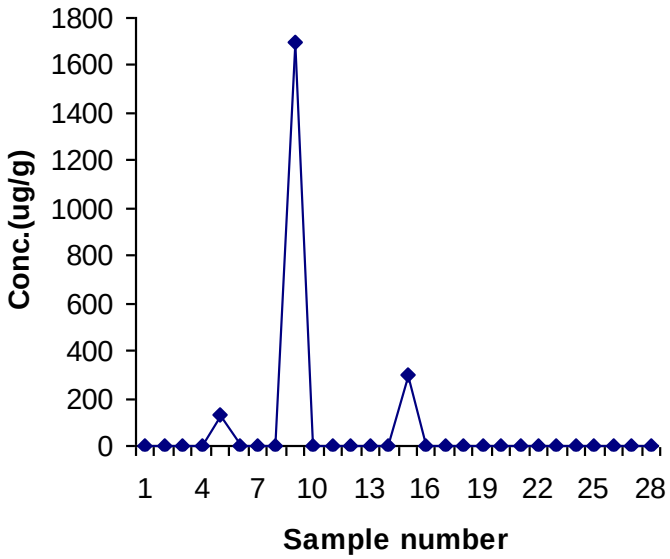


**Fig. 4** Conc. (µg/g) of Iron in various multivitamin samples

**Manganese**

For proper human health Mn is essential element. The daily intake of Mn, according to manufacturer dose is given in Table 3. It was found in all the tested samples; its concentration was from 1.47 to 661.40 µg/g as shown in Table 1 and 2. The highest concentration (661.40 µg/g) was found in Visionance capsule manufactured by Vitabiotics Ltd. London England and lowest (1.47 µg/g) in Multibionta syrup manufactured by Merck (pvt) Ltd Queta, Pakistan. The allowed dietary level for children is 1200-1500 µg/day, for man 2300 µg/day, and for woman 1800-2000 µg/day (Olafsdottir et al., 2017). From the above data, it is clear that all the tested multivitamin samples contain manganese concentration less than the recommended dietary allowance for children, men and women.

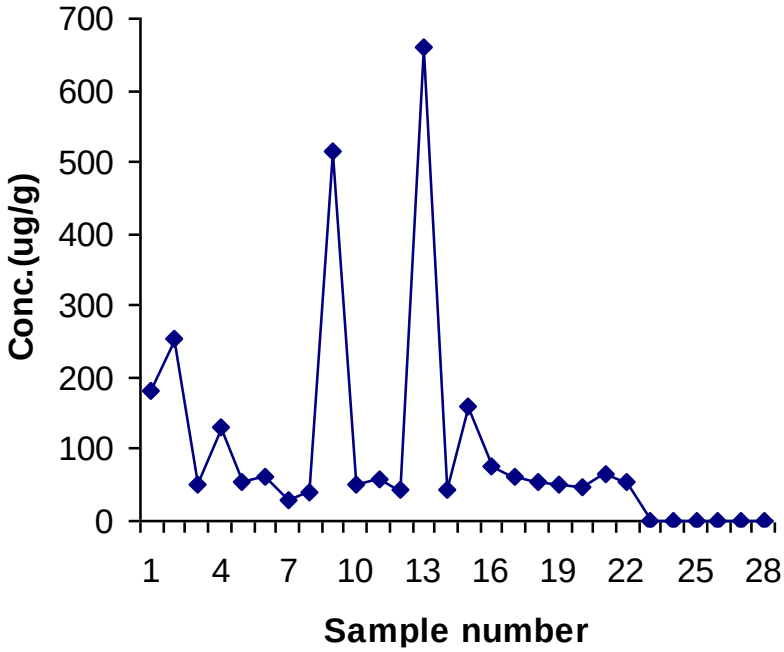


**Fig. 5** Conc. (µg/g) of Manganese in various multivitamin samples

**Cobalt**

Cobalt is a major component of cobalamin, also called as vitamin B-12. A lack of cobalt leads to anemia, a deadly disorder. The daily intake of cobalt, according to manufacturer dose is given in Table 3. The normal daily intake by ingestion is estimated at 20-40 µg. Cobalt was not detected in any of the multivitamin syrup sample but it was found in six samples out of 22 multivitamin tablet samples as shown in Table 1 and 2. Only one tablet sample (Optilet-M) contains cobalt concentration (89.79 µg) higher than the recommended daily intake which may be harmful for health. But most of tested multivitamin samples contain cobalt concentration less than the recommended daily intake.

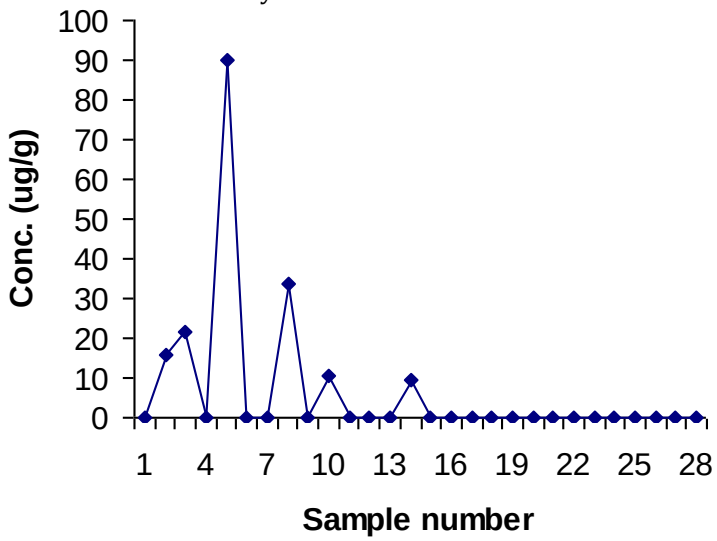


**Fig. 6** Conc. (µg/g) of Cobalt in various multivitamin samples

**Chromium**

Two ionic form of Cr is available i.e. trivalent and hexavalent. The trivalent chromium is utilized by human and is present in food. Glucose metabolism is regulated by Cr. There is no sufficient information available for minimum permissible limit of chromium. However, The Food and Nutrition Board established a satisfactory minimum level of chromium in ordinary diet i.e. for children 11-25 µg/day and for adults' 30-35 µg/day. Dermatitis and lungs cancer is caused by high level of Cr (Linder et al., 1996; Olafsdottir et al., 2017). Table 3 shows the daily intake of all the tested products on the basis of the recommended daily dose given on the product label. It has been found that chromium was not detected in any of the tested multivitamin product at ppm level.

**CONCLUSION**

The current investigation was carried out on twenty eight samples of commercially available multivitamin tablets and syrups from local market, which have been extensively prescribed by registered medical practitioners and doctors for the people suffering from deficiency of different vitamins and metals including, zinc, manganese, cobalt nickel copper, and iron. Above analysis has shown that concentration of the heavy metals analyzed is less than the toxic limit but some multivitamins contains higher concentration than the recommended value. For example, nickel in five and cobalt in one of the tested multivitamin products was found in higher concentration than the recommended value. All these metals proved to be very useful if they are taken in a recommended dose, but there is always a chance that there limits may be exceeded due to various factors i.e. plant sources used or in different process/solvents/ excipients in the manufacturing. This in turn may cause many chronic disorder and diseases rather to cure them. So it is recommended that

multivitamins may be used up to certain limit and must not be used as diet supplement for a very long period of time in order to avoid the accumulation of heavy metals in human body.

**Author Contribution Statement** Muhammad Saleem conceived the idea and wrote the manuscript. Jahangeer Abbas performed experimental studies. Imtiaz Ahmed and Muhammad Imran Tousif helped in manuscriopt writing. Muhammad Younas Khokhar supervised the overall study.

**Conflict of Interest** Authors declare that they have no conflict of interest.

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