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ESSENTIAL AND TOXIC ELEMENTS IN DIETARY SUPPLEMENTS DETERMINED BY ICP – MS

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ABSTRACT. Samples of widely used dietary supplements distributed on the Romanian market were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) in order to assess the levels of oligoelements Co, Cr, Cu, Mn, Ni, and Zn as well as potentially toxic heavy metals Cd and Pb, and compare them with the maximum allowable levels (MAL). The following concentration ranges were obtained (in $\mu g\ g^{-1}$): Cd 0.01-0.09; Co 0.03-0.6; Cr 1.1-11.9; Cu 5.5-14.7; Mn 1.8-39.1; Ni 0.4-4.7; Pb 0.8-3.1; Zn 18.8-12119.4. Several analyzed products had metals levels above the maximum allowable limits (Pb: one plant based product and one multivitamin product; Zn: two multivitamin multimineral products). The estimated cumulative daily intakes of only one formulation was higher than the oral permitted daily exposures set by the United States Pharmacopeia (USP) Advisory Panel on metal impurities (Pb: one plant based product). Such products present a significant additional source of metals in the human diet, and therefore could be harmful for human health.

Keywords: *dietary supplements, inductively couple plasma mass spectrometry, heavy metal levels, cumulative daily intakes*

INTRODUCTION

According to the Romanian legislation, dietary supplements are defined as food supplies which have the purpose to complete the normal diet and are concentrated sources of nutrients or other substances with nutritional or physiological effect, individually or in combinations [1].

The industry of dietary supplements has recorded a growth on a global level [2-5], mainly because of the increase in their consumption as alternative medicines, which is based on the consumers' belief that these products are natural, safe and without any adverse effects [2, 6-8]. According

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to the Office of Dietary Supplements (2005), in 2004, 55% of Unites States adults took a dietary supplement and 35% of these users took multivitamins and multiminerals supplements.

Essential trace elements, also known as oligoelements, have the specific and irreplaceable function of ensuring the optimal performance of the entire organism as well as activating the catalytic sites of enzymes. However, those elements can determine illness by their deficit, disequilibrium and poisoning if the limits are exceeded [9]. The clinical manifestations of metal poisoning have been characterized [10]. Heavy metal poisoning has decreased because of improved industrial hygiene and environmental controls so that the signs and the symptoms of such poisoning are likely to go unrecognized [11]. If metal poisoning is identified, the true source may be wrongly associated with environmental occupational exposures, not medicaments [12]. Failure to establish the true cause of exposure means that the subject continues taking the metal-containing product. Thus, the screening of traditional remedies and dietary supplements has been recommended to protect public health [13].

The objective of this study was to evaluate the metal levels in dietary supplements available on Romanian market, produced by both domestic and foreign manufacturers. The levels of oligolements, Co, Cr, Cu, Mn, Ni, and Zn, and as well of potentially toxic heavy metals Cd, Pb, were determined and compared with maximum allowable levels (MAL) [14]. Since metal bioaccumulation could cause toxic effects and ultimately result in health disorders or diseases if contaminated dietary supplements are used on a long term basis, a further objective of the present study was to estimate metal cumulative daily intakes (CDI) from analyzed dietary supplements. These were compared with the oral permitted daily exposures (PDE) for dosage forms, as stated by United States Pharmacopeia (USP) Advisory Panel on Metal Impurities [14], to deduce whether the analyzed products could present a potential threat to human health.

RESULTS AND DISCUSSION

Parallel to the metal determination in selected dietary supplements, the concentrations of metals were also determined in a certified reference material (CRM), INCT-MPH-2, for the quality control. This CRM represents a mixture of nine varieties of herbs grown in Poland, collected in a non-contaminated rural area and used for drugs production. The obtained results (Table 1) were in good agreement with the certified values, and the variation coefficient for the percent recovery means was below 5%.

The obtained levels of metals found in the analyzed samples are summarized in Table 2. It must be mentioned that the obtained results were compared to the MALs proposed by the experts in the USP Advisory Panel on Inorganic Impurities and Heavy Metals included in the revised document "Draft Metals and Limits Table" [14].

Table 1. Defined and measured concentration and recovery grade of metals in the INCT-MPH-2 certified reference material

Metal	Defined concentration [a] µg g^{-1}	Measured concentration [b] µg g^{-1}	Recovery grade [c] %
Cd	0.19 ± 0.02	0.2 ± 0.02	104 ± 3
Co	0.21 ± 0.03	0.21 ± 0.02	102 ± 2
Cr	1.69 ± 0.13	1.62 ± 0.18	96 ± 4
Cu	7.77 ± 0.53	7.67 ± 0.8	99 ± 1
Mn	191 ± 12	194.1 ± 7.1	102 ± 2
Ni	1.57 ± 0.16	1.53 ± 0.08	98 ± 2
Pb	2.16 ± 0.23	2.11 ± 0.12	98 ± 3
Zn	33.5 ± 2.1	33.4 ± 0.6	100 ± 1

[a] results as the average ± the expanded uncertainty (U) which is obtained by multiplying the combined standard uncertainty (u_c) by a coverage factor k = 2 corresponding to 95 % confidence level, where u_c is defined as square root of the sum of the quadrats of the standard deviation of the overall mean (u_i) and the standard uncertainty estimated from the long term stability studies (u_s).
[b] results as the average ± the expanded uncertainty (U), obtained by multiplying the standard deviation (s) by a coverage factor k = 2 corresponding to 95 % confidence level.
[c] ratios of measured to defined concentrations ± the variation coefficient.

Table 2 presents the results expressed as medians, with the minimum and maximum values given in the brackets. Number of samples above the maximum allowable level (>MAL) as well as the ratio between measured metal levels and MALs (median, minimum and maximum) are indicated in the table.

Table 2. Metal levels in the analyzed dietary supplements

Metal	Metal level µg g^{-1}	MAL µg g^{-1}	Metal level / MAL %	>MAL
Cd	0.02 (0.01 – 0.09)	2.5	0.7 (0.2 – 3.5)	0
Co	0.2 (0.03 – 0.6)	100	0.2 (0.03 – 0.6)	0
Cr	1.5 (1.1 – 11.9)	25	5.8 (4.6 – 47.7)	0
Cu	8.9 (5.5 – 14.7)	250	3.6 (2.2 – 5.9)	0
Mn	2.2 (1.8 – 39.1)	250	0.9 (0.7 – 15.6)	0
Ni	0.5 (0.4 – 4.7)	25	2.1 (1.4 – 18.9)	0
Pb	1.1 (0.8 – 3.1)	1	98.3 (79.1 – 312.1)	2
Zn	74.7 (18.8 – 12119.4)	1500	5 (1.3 – 807.9)	2

Four analyzed dietary supplements formulations marketed in Romania contained higher levels of some metals compared to the MALs. For samples 2 and 5 which include Zn on their list of active substances (Table 5) and therefore could not be considered contaminated, the measured levels of Zn reached 178.6% and 807.9% respectively, compared to MAL (Table 2). The found levels are lower than those reported by Garcia-Rico et al., 2007 [15] and similar to those reported by Obi et al., 2006 [11] and Tumir et al., 2010 [16].

Zinc poisoning manifestations include nausea, vomiting, diarrhea, fever and lethargy. Long-term exposure to high excess zinc intakes could interfere with the metabolism of other trace elements [17].

Above MAL levels for lead were found in two dietary supplements, samples 3 and 7 (Table 5). The measured Pb levels for the contaminated formulations reached 127.5% (sample 3) and 312.1% (sample 7) compared to MAL (Table 2). These levels are in concordance with those reported by Dolan et al., 2003 [18] and Timur et al., 2010 [16]. However, the found concentrations were lower than the results reported by Garcia-Rico et al., 2007 [15], Obi et al., 2006 [11] and Garvey et al., 2001 [19]. Lead interferes with a variety of body processes and is toxic to many organs and tissues including the heart, bones, intestines, kidneys, and reproductive and nervous systems [20].

The low levels of the other determined metals (Cd, Co, Cr, Cu, Mn, Ni), found in the analyzed formulations, none above de MALs, indicate that the exposure to these contaminants through the tested dietary supplements is not expected to affect human health.

An interesting fact is that the maximum measured levels for the determined metals, were found in the same dietary supplement, namely sample 7, a plant based supplement produced by a local company. Lower levels of metals have been reported in herbal teas with fewer ingredients than in polyherbal teas [20], this leads to a hypothesis that the large number, origin and type of ingredient may be the reason of the maximum levels for Cd, Co, Cr, Cu, Mn, Ni and Pb. However, this hypothesis should be further investigated.

In order to conclude whether the metal levels determined in this study could be considered harmful for human health, the cumulative daily intakes (CDI) of metals from dietary supplements, expressed as μg per day, were estimated based on the recommended daily doses (RDD) of dietary supplements. The CDIs for each metal tested in this study were then compared with oral permitted daily exposures for dosage forms, PDEs, as recommended by the USP [14]. Table 3 presents the CDIs of the determined metals as medians with minimum and maximum in the brackets, the number of samples for which the ingestion would lead to CDI of analyzed metals above the PDE for dosage forms, as well as the ratio between calculated CDIs and PDEs (median, minimum and maximum).

The CDIs for the products included in the study were lower than permitted and could not be considered harmful, except for sample 7 which had a CDI for Pb of 109.2% of the allowed PDE (Table 3). However, it should be taken into account that the use of the tested dietary supplements is just one of all possible yields that contribute to CDI, besides for instance, ingestion of food and water that can also contain metals, as well as inhalation of air [22].

Table 3. The cumulative daily intake (CDI) of metals for the analyzed dietary supplements

Metal	CDI μg/day	PDE μg/day	CDI / PDE %	>PDE
Cd	0.02 (0.01 – 0.31)	25	0.08 (0.03 – 1.2)	0
Co	0.2 (0.1 – 2.2)	1000	0.19 (0.01 – 0.2)	0
Cr	1.6 (1.3 – 41.7)	250	0.659 (0.5 – 16.7)	0
Cu	13.4 (6 – 85.4)	2500	0.54 (0.2 – 3.4)	0
Mn	3.7 (2 – 136.9)	2500	0.15 (0.1 – 5.5)	0
Ni	0.65 (0.5 – 16.6)	250	0.3 (0.2 – 6.6)	0
Pb	1.15 (0.89 – 10.92)	10	11.5 (8.9 – 109.2)	1
Zn	746.96 (24.38 – 12143.6)	15000	4.9 (0.2 – 80.9)	0

In most cases the levels of possible metal bioaccumulation were lower than permitted and therefore do not causes negative effects to human health.

CONCLUSIONS

From the seven dietary supplements chosen for analysis two were found exceeding the maximum level for Pb and two for Zn, even though they had Zn on their list of active substances and therefor it can't be considered contamination. The cumulative daily intake was exceeded for Pb in only one sample.

The present study emphasizes the need for an international regulatory framework to introduce the obligatory testing of metals in dietary supplements including the starting materials, the in-process and the finished product control, leading to the elimination of low quality products on the market and to assure a higher safety profile of dietary supplements.

EXPERIMENTAL SECTION

Samples

Samples of 7 dietary supplements marketed in Romania were selected for this study based on their popularity and frequent usage. The scope of their intended use was very broad (e.g. general improvement of health, cognitive enhancement and better brain blood supply, stress reduction, recovery after intense physical effort, immune system strengthening, support for the intestinal flora, cholesterol regulation, neutralization of free radicals, dietary vitamin and mineral supplementation, detoxification, body mass reduction). Detailed informations about the analyzed dietary supplements are presented in Table 5.

Sample preparation

Solid samples were homogenized by trituration, whereas the powder samples were used directly in the next step. Three replicates, for each sample and the CRM, were weighed (average sample weight: 0.304 ± 0.002 g) and put into the PTFE vessels for microwave digestion, followed by addition of 8 mL of Lunge mixture (2 mL HCl 37% and 6 mL HNO_3 65%). The digestion process took place in the closed PTFE containers of the microwave system according to the digestion program presented in Table 4. The procedure and settings are those recommended by the equipment producer for this type of samples.

Table 4. Operating conditions for the microwave digestion system

Parameter	Stage				
	1	2	3	4	5
Temperature / ºC	145	170	210	100	100
Pressure / bar	30	30	30	0	0
Time / min.	5	10	15	10	10
Slope / min.	2	2	2	1	1
Power* / %	80	80	80	0	0

** 100 % power refers to 1450 W*

Reagents and Standard Solutions

Calibration standards in the range of 0 – 100 μg L^{-1} were prepared by serial dilution of the Instrument Calibration Standard 3, from Perkin Elmer (Perkin Elmer, MA, USA) with 5% HNO_3 (v/v) and high purity water (18.2 MΩ cm-1) from a Milli Q system (Millipore, Milford, MA, USA).

Analytical grade nitric acid 65% and hydrochloric acid 37% purchased from Merck (Darmastadt, Germany), were used for sample digestion. Argon (5.0 quality) from Linde Gas SRL Cluj – Napoca, Romania was used as working gas.

Instruments

During sample preparation a mortar grinder Restch RM 100 (Haan, Germany) and a closed PTFE vessel microwave digestion system, Berghof MWS-3+ with temperature and pressure control (Eningen, Germany), were used.

The quantification of metals was performed using an inductively coupled plasma mass spectrometer, ICP-MS without reaction chamber, (SCIEX – Perkin Elmer ELAN DRC II, ON, Canada). The operating parameters of the employed ICP-MS system are presented in Table 6.

Table 5. Code and label ingredients of dietary supplements used in this study

Code	Type of dietary supplement	Recommended daily dose [a] g/day	Principal label ingredients
1	Multivitamin; multiminerals for children	1.16	Inulin, vitamin A, vitamin B complex, vitamin C, vitamin D, vitamin E, Folic acid, Biotin, Iodine, Zinc, Iron, Selenium.
2	Multivitamin; multiminerals for children	1.13	Beta glucan, bioflavonoids complex, vitamin C, vitamin A, vitamin B complex, vitamin E, Zinc, Biotin, Folic acid, Iodine, Rosehips, Lysine.
3	Multivitamin	1.5	Vitamin A, vitamin B complex, vitamin C, vitamin D, vitamin E, Folic acid, Iodine.
4	Antioxidant; multivitamin with selenium	1.1	Vitamin A, vitamin C, vitamin E, Selenium.
5	Antioxidant; multivitamin and multiminerals	1	Vitamin A, vitamin B_6 (pyridoxine), vitamin C, vitamin E, Zinc, Selenium.
6	Hydro soluble multivitamins and multiminerals	10	Vitamin C, vitamin B complex, vitamin E, vitamin D, Potassium, Phosphorus, Calcium, Magnesium, Zinc, Iron, Chromium.
7	Plant based supplement for detoxifying and body mass reduction	3.5	Medicinal herbs powder (*Plantago*, *Cynara scolymus*, *Hypericum perforatum*, *Rosmarinus officinalis*, *Satureja hortensis*) blended with *Linum*, *Cuminum cyminum*, *Foeniculum vulgare* seed powder, *Hippophaë rhamnoides L.* flour, grey clay and lactic ferments of the Lactobacillus (*casei*, *plantarum*) and Bifidus genus (*longum*, *breve*). Excipients: isomalt and maltodextrin.

[a] the RDD was estimated by multiplying the dose weight with the number of doses recommended by the producer of the dietary supplement.

Table 6. Operating parameters for the ICP-MS system

Parameter	Value
Plasma	
Power / W	1250
Plasma gas flow / L min^{-1}	15.00
Auxiliary gas flow / L min^{-1}	1.10
Nebulizer gas flow / L min^{-1}	0.82
Sample introduction rate / mL min^{-1}	0.4
Quadrupole	
Quadrupole rod offset (QRO) / V	0.00
Chamber rod offset (CRO) / V	- 8.00
Cell path voltage (CPV) / V	- 20.00
Measuring mode	Peak hopping
Dwell time / ms	200
Detector dead time / ms	55
Readings per point	20
Readings per replicate	1
Measurements per replicate	5
DRC	
Module DRC QRO / V	- 6.00
Module DRC CRO / V	- 1.00
Module DRC CPV / V	- 15.00
Tension on start lens / V	4.00
Tension on end lens / V	12.50

Limits of detection

The limits of detection, LOD, were based on the 3σ criterion: standard deviations of ten consecutive metal measurements in the blank sample were multiplied by 3. The estimated LODs are given in Table 7.

Table 7. Limits of detection for the used method

Element	LOD μg g^{-1}
Cd	0.01
Co	0.01
Cr	0.13
Cu	0.2
Mn	0.37
Ni	0.01
Pb	0.01
Zn	0.42

The LODs for this method are more than 10 times lower than the maximum allowable limits, MAL, imposed by the USP [14], meaning that the used analysis method is suitable for the determination of metals from dietary supplements [22].

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