Microchemical Journal 108 (2013) 81–86

Contents lists available at SciVerse ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Inductively coupled plasma optical emission spectrometric determination of fifteen elements in dietary supplements: Are the concentrations declared in the labels accurate?

Julieta Marrero [a], Raúl Jiménez Rebagliati [b], Emanuel Leiva [a], Agustín Londonio [b], Patricia Smichowski [b,c,*]

[a] Comisión Nacional de Energía Atómica, Gerencia Ciclo de Combustible Nuclear, Av. Gral Paz 1499-B1650KNA, Pcia de Buenos Aires, Argentina
[b] Comisión Nacional de Energía Atómica, Gerencia Química, Av. Gral Paz 1499-B1650KNA, Pcia de Buenos Aires, Argentina
[c] Consejo de Investigaciones Científicas y Técnicas (CONICET), Av. Rivadavia 1917-B1033AAJ, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 13 December 2012
Received in revised form 26 December 2012
Accepted 28 December 2012
Available online 8 January 2013

Keywords:
Dietary supplements
ICP OES
Metals and metalloids
Variations among tablets

ABSTRACT

A study was carried out to establish a reliable procedure for determining 15 elements (As, Bi, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, V and Zn) in different brands of dietary supplements purchased in Argentina and USA. Supplements were digested with HNO_3 and H_2O_2 using an optimized microwave procedure. Inductively coupled plasma optical emission spectrometry (ICP OES) was selected for total element determination. The overall approach was tested in tablets of: (i) Se supplement, (ii) two multimineral supplements, (iii) cholesterol control tablets, (iv) multivitamins for men, and (v) a multivitamin + multimineral supplement. Arsenic, Cd and Pb concentrations were in all the analyzed samples below the detection limits for these elements (As, 1.2 μg g^{-1}; Cd, 0.09 μg g^{-1} and Pb, 1.5 μg g^{-1}). Elemental concentrations of the other elements investigated showed a great variability depending on the trade mark analyzed. Measured metal concentration ranged from 0.78 ± 0.19 μg g^{-1} (Ni) to 13.5 ± 0.7% (Ca). Most abundant elements, detected as percentage were Ca, Mg and Fe. In general terms, the study evidenced that metal content reported by the manufacturer in labels of dietary supplements agree with found values. On the other hand, significant differences in metal concentration were found among tablets of the same bottle.

1. Introduction

Many metals and metalloids have been known to be a necessary component in the human diet for a long time and its occurrence and function in biological systems has been well-documented [1–3]. In this connection, clinical studies showed that some metals and metalloids, despite their reported toxicity, may play an important role in therapeutic effects of the herbs containing these metals [4]. In general terms, dietary supplements can be considered as a potential source of metals and metalloids contamination, especially when they content toxic or potentially toxic elements constituting a risk for consumers.

In the last years, especially in the developed countries, the consumption of dietary supplements has increased. These compounds are considered by the consumers as drug alternatives for a variety of reasons, including their relatively low cost and their perceived safety and effectiveness [5]. They are defined as a product that intends to supplement the diet, which contains vitamins, minerals, herbs, or other botanicals, amino acids, or any combination of the above ingredients [6–9].

Another important issue to consider is that these products are regulated as food, and producers are not required to register these supplements before sale [6,10]. It is difficult to determine the quality of a dietary supplement product from concentrations declared in the labels. The degree of quality control depends on the manufacturer, the supplier, and others related to the production process. The first question to answer is: are the concentrations declared in the labels accurate? Tablets from the same bottle have the same elemental composition.

Numerous analytical techniques and instrumental approaches have been proposed for dietary supplement analysis. Valiente et al. [11] reported a comparative study on the determination of Se in tablets of vitamins–minerals–aminoacids, nutritional supplements and Se-enriched yeast by electrothermal atomic absorption spectrometry (ETAAS) and hydride generation-atomic absorption spectrometry (HG-AAS). The study evidenced that Se content reported on the labels was often inaccurate. In addition, significant differences were found among bottles of the same trade mark. García-Rico et al. [12] determined five metals namely, Cd, Cu, Hg, Pb, and Zn by AAS in 24 dietary supplements purchased in Mexico. According to their findings, some products presented more than 10% of the tolerable daily intake of

* Corresponding author at: Comisión Nacional de Energía Atómica, Gerencia Química, Av. Gral Paz 1499-B1650KNA, Pcia de Buenos Aires, Argentina. Tel.: +54 11 6772 7873; fax: +54 11 6772 7886.
E-mail address: smichows@cnea.gov.ar (P. Smichowski).

0026-265X/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.microc.2012.12.013

Pb, indicating that production processes should be improved. Tumir and coworkers [13] reported the AAS determination As, Cd, Cr, Hg, Ni, Pb, and Zn in 30 samples of widely used vitamins and herbal preparations distributed on the Croatian market. The objective was to estimate contamination due to their potential toxicity if present above the maximum allowable levels (MAL). They found that several analyzed formulations had metal levels above the MAL such as: (i) Pb in one honey-based product and one medicinal herb-based product, (ii) Cr in one product containing vitamins, and (iii) Ni in two products containing vitamins and one product of animal origin.

The presence of Pb in dietary supplement samples (calcium carbonate, dolomite and oyster shell samples) and subsequent determination by graphite furnace atomic absorption spectrometry (GFAAS) was also investigated [14]. Lead concentrations in the ten commercial products analyzed varied from 0.21 to 1.34 μg g^{-1}. Recently, two techniques namely, laser ablation (LA)- and solution-based inductively coupled plasma mass spectrometry (ICP-MS) were used and compared to assess concentrations of 12 elements (Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, V and Zn) in six herbal supplements [5]. LA-ICP-MS was chosen to minimize sample preparation and to avoid the use of acids for sample digestion. There was generally good agreement (±15%) between concentrations determined by LA and ICP-MS and certified values.

The daily and continuous use of these products by adults became usual and makes necessary to ensure their quality. In this context, to assess the elemental concentration in these products with emphasis in those toxic or potentially toxic elements for human health is of prime importance.

The analysis of dietary supplement is a challenge because they have a complex matrix and contain many elements in a wide range of concentrations. Plasma-based techniques have gradually assumed prime importance in verifying whether foodstuffs and pharmaceutical products comply with health requirements and/or national or international regulations because they can be applied to all possible matrices and analytes and are characterized by extended dynamic concentration range (several orders of magnitude) and are multielemental in nature and possess high sensitivity and appropriate detection power.

The aim of this study was to investigate the presence of labeled and non-labeled metals and metalloids in different commercial brands of dietary supplements purchased in Argentina and USA. The supplements were selected based on their popularity and frequent usage. The main objective was to establish a reliable methodology for verification of elemental content as well as contribute with useful information to the assessment of product authenticity or adulteration. It is not the scope of this work neither to evaluate possible toxicological effects produced by the intake of these products nor to estimate tolerable daily intakes according to recommendations of health agencies.

2. Experimental

2.1. Instrumentation

A Horiba-Jobin Yvon Model Ultima 2 inductively coupled Ar plasma was used for major, minor and trace elements determination. Instrumental details and operating conditions are summarized in Table 1.

Welding Ar from Indura (Buenos Aires, Argentina) was used for ICP OES determinations. Deionized distilled water (DDW) was produced by a commercial mixed-bed ion-exchange system Barnstead (Dubuque. IA. USA) fed with distilled water.

Plastic bottles, auto-sampler tubes, and glassware were cleaned by rinsing with deionized water, soaking with a 10% (v/v) nitric acid solution for 24 h and then rinsing several times with deionized water. All samples and standards were stored in polyethylene bottles (50 mL) or Falcon® tubes.

Table 1
Instrumental characteristics and settings for ICP OES.

Instrument	Horiba-Jobin Yvon Ultima 2
Frequency of rf generator	40.68 MHz (radial view)
Coolant gas flow rate	12 L min^{-1}
Auxiliary gas flow rate	0.2 L min^{-1}
Sample gas flow rate	0.8 L min^{-1}
Solution delivery	1.0 mL min^{-1}
Automatic sampler	JY AS500
Nebulizer	Concentric gas nebulizer with cyclonic spray chamber
Polychromator	Monochromator use Czerney Turner optical system. wavelength range (nm): 120–450 focal length 1 meter
Detector	Photomultiplier tube
Measurement mode	Continuous nebulization
Wavelengths (nm)	As, 188.93; Ca 317.933; Cd, 228.802; Cr, 205.552; Cu, 327.396; Fe, 238.204; Hg, 253.652; Mg, 285.213; Mn, 257.610; Mo, 203.844; Ni, 221.647; Pb, 220.353; Sb, 206.833; Se, 196.026; V, 209.882; Zn, 206.191

An MLS-1200 (Millestone-FKW, Sorisole, Bergamo, Italy) MW apparatus equipped with ten Teflon-PFA (perfluoroalkoxy) vessels was used to digest the tablets/pills.

2.2. Reagents

Chemicals were of analytical reagent grade unless otherwise stated. Deionized water (Barnstead, Dubuque, IA, USA) was used throughout. All solutions were stored in high-density polypropylene bottles. Commercially available 1000 mg L^{-1} standard solutions (Merck, Darmstadt, Germany) of the elements under study were used. Diluted working solutions were prepared daily by serial dilutions of the stock solutions. Analytical reagent nitric acid (Merck) was used after additional purification by sub-boiling distillation in quartz still.

Nitric acid and H_2O_2 (Merck, Darmstadt, Germany) were used for sample treatment and preparation of the standards.

2.3. Samples and sample handling

Five brands of different dietary supplements for adult's consumption were analyzed for evaluating total content of 15 elements. Dietary supplements were purchased in 2012 in different health food stores in Argentina and USA. Trade marks of dietary supplements were identified according to a code assigned in our lab (A, B, C, D and E). The variety of products and compounds described in the labels are as follows: A: multivitamin/mineral formula; B: vitamins and minerals (ginko + ginsen + guarana); C: selenium; D: cholesterol fighter and E: supplement for men. Content uniformity testing was performed by randomly selecting ten tablets from each bottle. Table 2 shows a detailed description of the composition of the dietary supplements analyzed according to the information reported by the manufacturer in the labels.

Ten tablets of each bottle were ground and mixed thoroughly by mortar and pestle. The grinding was carefully carried out to assure samples are not subjected to conditions that would alter their composition (e.g., heating). Three sub-samples of each mixture were accurately weighed and used for the subsequent microwave assisted digestion.

2.4. Sample treatment

Metals and metalloids determination by ICP OES requires preliminary digestion of the samples into liquid solutions. A ~0.5 g portion of each powdered sample was weighed into a PTFE beaker and 7 mL of nitric acid and 3 mL of H_2O_2 were added. The mixture was left overnight at room temperature. Nitric acid was used due to its

Table 2
Dietary supplements (DS) and tablets composition.

Sample identification	Label description
A	Vitamin A (as retinyl palmitate and β-carotene), Vitamin C (as ascorbic acid), Vitamin D (as cholecalciferol), Vitamin E (as d-α-tocopheryl acetate), thiamin hydrochloride (Vitamin B1), riboflavin (Vitamin B2), niacinamide, Vitamin B6 (as pyridoxine hydrochloride), folic acid, Vitamin B12 (as cyanocobelamin), biotin, d-Ca pantothenate, tri-calcium phosphate, di-calcium phosphate, ferrous fumarate, KI, MgO, $ZnSO_4$, selenium yeast, $CuSO_4$, $MnSO_4$, Cl_3Cr, $NaMoO_4$, KCl, *p*-aminobenzoic acid, inositol, choline bitartrate, Brewer yeast.
B	Vitamins B1, B2, B3, B5 (pantothenic acid), B6, B8 (biotin), B9 (folic acid), B12, C, E. Zn gluconte, Se yeast, MgO, $FeSO_4$, Ginkgo biloba, Ginseng extract, guarana.
C	Se yeast, dicalcium phosphate, Brewer yeast, vegetable stearic acid, vegetable Mg stearate
D	Niacin, pantothenic acid, $CaCO_3$, Cr picolinate, Cr aspartate, β-sitosterol complex, oat bran powder, lecithin, L-carnitine fumarate, stearic acid, Mg stearate.
E	Vitamins A (as β-carotene), C, (as ascorbic acid), D, E, K, B1 (thiamin), B2 (riboflavin), B6, B12, niacin, folic acid, biotin, pantotenic acid, $CaCO_3$, MgO, KCl, stearic acid, ZnO, MnSO4, Mg stearate, cholecalciferol, CuO, pyridoxine hydrochloride, lycopene, Na_2SeO_4, Cl_3Cr, cyanocobalamin.

excellent dissolving capabilities. Preliminary studies demonstrated that a maximum power last-stage was beneficial for better digestion [11]. This is attributed to the fact that the rate of digestion reaction of acid decomposition and oxidation power of the acids increases with higher power. The addition of 3 mL of H_2O_2 to the nitric acid was necessary to increase the oxidation efficiency. Microwave (MW) assisted digestion was used for sample dissolution following the procedure depicted in Table 3. After cooling, the digest was slowly evaporated to near dryness and then dissolved in ~10 mL of deionized water and no visible residual solid particles were detected. The solution was filtered, transferred into a 50 mL volumetric flask and diluted to the mark.

All the experiments were performed by triplicate. In all cases, a set of digestion blanks was prepared together with each digestion procedure tested. In order to decrease digestion blanks, purified nitric acid was used.

For checking the accuracy, aliquots of two certified reference materials namely, TORT-1, lobster hepatopancreas (NRCC, Ottawa, Québec, Canada) and MURST ISS-A2, Antarctic krill (EC-JRCIRMM) were subject to the same treatment that the samples and included in the over-all analytical process.

2.5. Analytical determinations

Arsenic, Bi, Cd, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, V and Zn were determined by ICP OES. The calibration curves were obtained with calibration standards prepared in the same acid medium as the samples. In spite of the complexity of the matrix analyzed, no standard addition calibration was necessary. The calibration was checked every 20 measurements.

3. Results and discussion

3.1. Metals and metalloids concentrations in dietary supplements

The elements labeled on the commercial products were the ones chosen for this study. In addition, it was also our interest to monitor some toxic elements, including As, Cd, Cr, Hg, Pb, Sb and other elements, particularly Cr, Cu, Mn and Zn, which are considered essential elements but may also be toxic depending on dose. Table 4 summarizes mean concentrations of 15 elements measured in the five commercial available brands by ICP OES. Toxic elements such as As, Cd, Hg and Pb were in all cases bellow the respective detection limits as follows (in $\mu g\ g^{-1}$): As <1.2; Cd <0.09; Hg <1.3, and Pb <1.5. The most abundant elements (expressed as percentage) in all brands resulted to be: Ca (0.9%–13.5%), Mg (0.08%–10.8%) and Fe (57.7 μg^{-1}–3.9%). A brief description of each of the elements determined in this study is as follows:

Table 3
Operating conditions for the MW digestion of dietary supplements.

Sample intake	~0.5 g	
Microwave apparatus	Milestone, MLS-1200	
Reagents (Suprapur grade)	17 mL 65% (w/w) HNO_3 + 3 mL 30% (v/v) H_2O_2 Final volume: 50 mL	
Digestion cycle	Applied power (W)	Time (min)
Step 1	250	1
Step 2	0	1
Step 3	250	5
Step 4	400	5
Step 5	600	5

3.1.1. Antinony

The safety of the dietary supplement is dependent on the growing conditions of the raw material and its extraction, formulation, and manufacturing processes. For this reason, many potentially toxic elements such as Sb have been detected in botanical supplements containing Ginko, Echinacea, Ginsen, Grape seeds, Saw palmetto, etc. [15]. Antimony may produce adverse effects to humans and the environment and has no known physiological functions. In this study, Sb was only detected in samples A (multivitamins) and B (ginko + ginseng + guarana) with concentrations of 1.42 $\mu g\ g^{-1}$ and 1.53 $\mu g\ g^{-1}$, respectively.

3.1.2. Arsenic

Some kind of namely natural medicines can be contaminated with As and may produce symptoms of poisoning when consumed in large amounts or for extended periods of time. Arsenic is also contained in traditional Chinese medicine formulas. Dolan et al. [7] determined As in 95 different dietary supplements by high resolution (HR)-ICP-MS and reported As concentrations varying from 5–3770 $\mu g\ kg^{-1}$. According to their study, the highest concentration corresponded

Table 4
Metal and metalloids in dietary supplements. Concentrations (average of six tablets) are expressed in $\mu g\ g^{-1}$ except those indicated as percentage.

Element	Sample A	Sample B	Sample C	Sample D	Sample E
As	<1.2	<1.2	<1.2	<1.2	<1.2
Ca	3.79 ± 0.09%	0.09 ± 0.01%	7.49 ± 0.27%	12.8 ± 0.5%	13.5 ± 0.5%
Cd	<0.09	<0.09	<0.09	<0.09	<0.09
Cr	69.5 ± 3.1	14.7 ± 0.6	1.30 ± 0.07	43.5 ± 2.4	74.0 ± 3.8
Cu	1191 ± 42	6.8 ± 0.28	17.4 ± 0.8	1.70 ± 0.09	1200 ± 41
Fe	0.67 ± 0.02%	1.5 ± 0.067%	206 ± 8	249 ± 8	57.7 ± 3.5
Hg	<1.3	<1.3	<1.3	<1.3	<1.3
Mg	7.65 ± 0.35%	10.8 ± 0.4%	764 ± 28	0.22 ± 0.01%	7.79 ± 0.29%
Mn	3865 ± 112	246 ± 9	6.71 ± 0.34	18.5 ± 0.8	1137 ± 37
Ni	10.2 ± 0.4	<0.3	1.23 ± 0.07	<0.3	1.09 ± 0.07
Pb	<1.5	<1.5	<1.5	<1.5	<1.5
Sb	1.42 ± 0.07	1.53 ± 0.08	<0.5	<0.5	<0.5
Se	18.0 ± 0.6	21.4 ± 0.8	317 ± 12	<0.3	68.1 ± 2.7
V	1.41 ± 0.09	1.05 ± 0.07	1.21 ± 0.08	<0.3	3.07 ± 0.24
Zn	1.0 ± 0.04%	5133 ± 219	104 ± 4	5.46 ± 0.33	9307 ± 404

to: red clover blossoms, echinacea, licorice root, buckhorn bark, burdock root (plus 7 others). Arsenic was not detected in the present study. Arsenic concentrations resulted to be <1.2 μg g^{-1}.

3.1.3. Cadmium

By the aid of a powerful technique such as HR-ICP-MS, Dolan and coworkers [7] determined Cd in 95 supplement samples and concentrations varied from 10 to 368 μg kg^{-1}. The highest concentration was found in shark cartilage. In all the tablets analyzed in this study, Cd concentrations were <0.09 μg g^{-1}.

3.1.4. Calcium

This element is important for optimal bone health and it is also needed for many other body functions, such as regulating heartbeats, conducting nerve impulses, making muscles contract, and helping blood clot. Although diet is the best way to get this element, Ca supplements are an option in case of diets poor on this element. In spite of this, it is necessary to know the pros and cons of these supplements, and which type of Ca should be chosen. Even when Ca supplements were not analyzed, Ca was a major element in all samples assessed in this study with concentrations varying from 0.09 to 13.5%.

3.1.5. Chromium

This metal is an essential trace element and plays an important role in processing carbohydrates and fats, and helping cells respond properly to insulin. For this reason this metal is very common in most supplements because it is supposed to help the body process of carbohydrates and fats. That is why, Cr is marketed as a weight loss aid for dieters and an ergogenic (muscle-building) aid for bodybuilders and athletes. All the samples under study contained Cr which concentrations varied from 1.30 to 74.0 μg g^{-1}.

3.1.6. Copper

Humans need Cu for normal growth and health. For patients who are unable to get enough Cu in their regular diet or who have a need for more Cu, specific supplements may be necessary. It is known that high intakes of Fe, Mn or Zn interfere with Cu absorption. This metal was detected in all tablets analyzed and it is important to highlight that Cu concentrations exhibited significant variations (6.8–1200 μg g^{-1}) depending on the commercial product analyzed.

3.1.7. Iron

In humans, Fe is an essential component of proteins involved in oxygen transport and its deficiency limits oxygen delivery to cells, resulting in fatigue and decreased immunity. In the tablets analyzed in this study, Fe exhibited a noticeable wide range of concentrations among the different brands (57.7 μg g^{-1}–1.5%). Higher levels corresponded to Sample B (vitamins and minerals + ginko + ginseng + guarana).

3.1.8. Lead

As regards Pb, several studies reported the presence of Pb in dietary supplements. According to Tyson and coworkers [16] one possible source of dietary Pb is that obtained from Ca supplements containing Ca carbonate that comes from a variety of naturally occurring sources. Dolomite and bone-meal powders as well as Ca carbonate and Ca chelates are substantial sources of Pb. Reported Pb concentrations detected in Ca supplements were: 0.55 and 0.66 μg g^{-1} [16]; 0.21–1.35 μg g^{-1} [14]. By using HR-ICP-MS, Pb was determined in 95 dietary supplements with concentrations in the range 20–48600 μg kg^{-1} [7]. Recently, Pb was determined in medicinal plants by high-resolution continuum source (HR-CS) GF AAS using direct solid sampling [17]. The authors reported agreement between their data and reference values at a 95% confidence level. Lead contents in medicinal plants ranged from 0.30 to 1.94 μg g^{-1}. Neither of the supplements analyzed in our work was a Ca supplement and Pb was not detected in any of the tablets analyzed (<1.5 μg g^{-1}).

3.1.9. Magnesium

This element is required for the proper growth and maintenance of bones. In addition, Mg is beneficial to maintain normal muscle and nerve function, keeps heart rhythm steady and supports a healthy immune system. It also helps to regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis. Magnesium is taken in dietary supplements to prevent its deficiency while athletes sometimes use Mg to increase energy and endurance. Magnesium and Ca are the elements that exhibited the highest concentration in comparison with the other analytes measured. The high levels of Mg found (764 μg g^{-1}–10.8%) were not surprising because a common ingredient in supplement capsules and tablets is Mg stearate which is added as a diluent (filler) and for lubrication purposes in the manufacturing process [5].

3.1.10. Manganese

Manganese is both nutritionally essential and potentially toxic. Even when this metal acts as an important co-factor for many enzymes and plays an essential role in the body functions, an overexposure could also lead to severe neurodegenerative damages [18]. For patients who are unable to get enough Mn in their regular diet, Mn containing supplements may be necessary. Manganese concentrations showed significant variability across the analyzed samples (6.71 μg g^{-1}–3865 μg g^{-1}).

3.1.11. Mercury

Mercury was not very often measured in supplements and only it was reported in a few studies. A HR-ICP-MS study revealed a wide range of Hg concentrations in 95 samples analyzed varying between 80 and 16800 μg kg^{-1} [7]. The highest level was detected in samples composed by *pinelliae rhizome*, *pericarpium citri chachiensis*, *poria*, *glycyrrhizae root*, and *zingiberis recens rhizome*. Mercury was not detected in any of the samples analyzed in this study (<1.3 μg g^{-1}).

3.1.12. Nickel

Nickel is an essential element because it is involved in some chemical processes in the body. It is a cofactor of the enzyme Urease. In spite of this, its precise function in the body is unknown. Nickel is used for increasing Fe absorption, preventing anemia, and treating osteoporosis. Nickel is safe for most adults in amounts no higher than 1 mg/day. In the dietary supplements analyzed Ni was detected at low concentrations in Sample A (10.2 ± 0.4 μg g^{-1}), Sample C (1.23 ± 0.07 μg g^{-1}) and Sample E (1.09 ± 0.07 μg g^{-1})

3.1.13. Selenium

Selenium is a trace essential and necessary element in the human diet. Deficiency of Se has been associated with an endemic form of cardiomyophathy. A variety of other diseases, including cancer, has been linked to a low Se status [19]. Individuals who have gastrointestinal disease or severe infection for depleted blood levels of Se may require Se supplementation. Selenium is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Other selenoproteins are involved in regulating thyroid function and play a role in the immune system. Viñas and coworkers [20] determined inorganic and organic Se species by liquid chromatography-hydride generation-atomic fluorescence spectroscometry (LC-HG-AFS). Three Se nutritional supplements and two selenized yeasts were analyzed. All samples contained Se mainly as Se(IV) at concentrations from 0.071 ± 0.002 μg g^{-1} to 0.112 ± 0.003 μg g^{-1}. In this study, Se concentrations varied from

<0.3 to 317 ± 12 μg g^{-1}. The higher concentration was detected in a specific supplement (Sample C: Natural Selenium).

3.1.14. Vanadium

It is an essential element. The use of supplements containing V became popular with bodybuilders because of it was supposed to increase cell volume and thus muscle mass. No research has proven this beneficial effect. Vanadium acts like insulin in the body and the main potential benefit is helping to control blood-sugar levels in people who have diabetes. Some trade marks declare that V containing supplements maintains a healthy blood sugar balance. Vanadium content was not reported in any of the commercial brands assessed. According to our findings, V was detected in almost all the supplements analyzed (except in Sample D) at concentrations between <0.3 and 3.07 μg g^{-1}.

3.1.15. Zinc

Zinc is an essential element that is naturally present in some foods, added to others, and available as a dietary supplement. Supplements contain several forms of Zn, including Zn gluconate/Zn sulfate/Zn acetate. Zinc gluconate may interfere with the absorption of antibiotics, so this combination may be unsafe. A daily intake of this metal is required to maintain a steady state because the body has no specialized Zn storage system. Zinc is involved in numerous aspects of cellular metabolism and it is required for the catalytic activity of approximately 100 enzymes. The ICP-MS determination of Zn in 35 dietary supplements showed a Zn content varying from 0.137 to 24.1 mg/serving size [21]. The content of Zn was determined in six herbal supplements by LA-ICP-MS [5]. A much more uniform concentration was observed among samples with Zn concentrations ranging from 6.33 ± 0.28 to 21.6 ± 1.0 μg g^{-1}. All the tablets analyzed in the present study contained Zn. A wide range of concentrations was observed among the five commercial brands (1.0 ± 0.04 to 9307 μg g^{-1}). García-Rico et al. [12] also detected significant variations among supplements purchased in Mexico: Zn (<2.83–4785.7 μg g^{-1}).

3.2. Measured vs. declared concentrations

As regards the elemental concentration claimed in the labels of the five commercial brands, the following analysis can be done: the concentrations of Mg, Ca, Cu, Fe, Se and Zn declared in brand A are in good agreement with those measured by ICP OES. Manganese showed a discrepancy of ~11%. According to the label, this complement contains 5 mg Mn per tablet (equivalent to 3476 μg g^{-1}) and the measured concentration (average of ten tablets) was 3865 μg g^{-1}.

For sample B, only concentrations of Mg, Fe, Se and Zn are informed by the manufacturers. For these four elements, we found good agreements between measured and labeled values.

For most elements, the lower elemental concentrations were measured in sample C (Selenium). It declares in the label a Se concentration of 0.2 mg per tablet (equivalent to 321 μg Se g^{-1}). According to our findings, the average concentration (ten tablets) of Se measured was 317 ± 2 μg g^{-1} which indicates a good agreement between declared and measured values.

The comparison between informed and found concentrations in dietary supplement C for Ca, Cu, Mg, Mn, Se and Zn exhibited a great concordance. For sample D, only concentrations of Ca and Cr are informed and also in this case exits concordance between measured and declared concentrations.

The study evidenced that in general terms no alarming results were obtained for the analyzed formulations because a good concordance was found between the levels reported by the manufacturer and found concentrations.

A key part of this study was to verify the variation of concentrations among five tablets of the same bottle (the five trade marks were analyzed). To this end, coefficients of variation (% CV), defined as the ratio of the standard deviation to the mean value, were calculated and results are as follows:

Sample A: 6.4% (Sb) – 61% (Ni)
Sample B: 2.0% (Ni) – 29% (Mo)
Sample C: 2.5% (Mg) – 50% (Sb)
Sample D: 1.3% (Mg) – 15% (Cu)
Sample E: 0.1% (Sb) – 100% (Pb)

This study evidenced that it is of prime importance to assess not only the elemental concentrations (average of several tablets) but also the quantification of metals in individual tablets of the same bottle. It is clear evident from our data that variations as high as 100% were observed meaning that a consumer is not receiving the same dose in each intake.

Table 5
Accuracy check: analysis of the TORT-1 and MURST-ISS-A by ICP OES. Concentrations are expressed in mg kg^{-1} except those expressed as percentage.

Element	MURST-ISS-A2 (Antarctic krill)		TORT-2 (lobster Hepatopancreas)	
	Certified	Found	Certified	Found
As	5.02 ± 0.44	4.8 ± 0.3	21.6 ± 1.8	20.6 ± 1.0
Ca	1.45 ± 0.04%[a]	1.33 ± 0.05%	NR	0.84 ± 0.03%
Cd	0.73 ± 0.08	0.74 ± 0.06	26.7 ± 0.6	26.2 ± 0.5
Cr	0.73 ± 0.14[a]	0.79 ± 0.06	0.77 ± 0.15	0.93 ± 0.07
Cu	65.2 ± 3.4	59.5 ± 3.3	106 ± 10	112 ± 5
Fe	56.6 ± 2.8	53.6 ± 2.4	1 105 ± 13	98 ± 4
Hg	0.013 ± 0.003	<1.3	0.27 ± 0.06	0.33 ± 0.02
Mg	0.32 ± 0.01%[a]	0.32 ± 0.01%	NR	2094 ± 101
Mn	4.12 ± 0.16	3.8 ± 0.16	13.6 ± 1.2	11.0 ± 0.8
Mo	NR	<0.6	0.95 ± 0.10	0.88 ± 0.07
Ni	1.28 ± 0.13	1.56 ± 0.12	2.50 ± 0.19	2.48 ± 0.16
Pb	1.11 ± 0.11	1.27 ± 0.09	0.35 ± 0.13	0.42 ± 0.04
Sb	NR	<1.5	NR	<1.5
Se	7.37 ± 0.91	6.9 ± 0.5	5.63 ± 0.67	5.45 ± 0.4
V	1.21 ± 0.06[a]	0.43 ± 0.02	1.64 ± 0.19	1.66 ± 0.09
Zn	66.0 ± 3.1	60.8 ± 3.7	180 ± 6	181 ± 7

NR: non reported for the manufacturer.
[a] Informative concentration.

3.3. Reliability criteria and quality parameters

The result of the accuracy test was obtained through the analysis of two certified reference materials (CRMs) namely, TORT-1, Lobster hepatopancreas (NRCC, Canada) and MURST-ISS-A, Antarctic krill (EC-JRCIRMM). We selected two CRMs for checking accuracy with different elemental levels because, unfortunately, specific CRMs were not available in our laboratory. As shown in Table 5, the over-all picture turns to be satisfactory, especially if one considers that in certain instances (e.g., Ca, Fe, Mg) digestion solutions had to be diluted to reduce the analytical signal to acceptable levels. This might be in itself a potential source of errors, which in this case it was found to be under full control.

Using optimized conditions, analytical figures of merit including detection limit and precision of replicate measurements were established. Detection limits were calculated following the IUPAC rules on the basis of 3σ-criterion for ten replicate measurements of the blank signal. Limit of detection reached varied from 0.03 μg g^{-1} (Ca and Mg) to 1.5 μg g^{-1} (Pb and Sb). Relative standard deviations (RSDs) resulted in a more than satisfactory interval (3–11%), especially if one considers the complexity of the matrix under study.

4. Conclusions

Microwave assisted digestion followed by analysis by ICP OES has been a simple and reliable methodology for the determination of metals and metalloids in dietary supplements. The study of five

popular dietary supplements revealed that metal content strongly varied among trade marks indicating that no standard compositional exists. Toxic elements such as As, Cd, Hg and Pb were not detected in the tablets analyzed. In general terms, a good concordance between calculated concentrations and stated values on the labels was found with differences lower than 12%. The most important discrepancies were found among tablets of the same bottle with coefficients of variations as high as 100% (Pb in sample E, Supplement for men).

Dietary supplements are still used on a voluntary basis without strict supervision. Taking into account that their consumption can be on a long-term basis it is necessary to continue testing if the concentration of metals and metalloids declared in the labels is right. This kind of studies would contribute to the elimination of low quality products on the market and assure a higher safety profile of dietary supplements.

Information gained from analytical chemistry studies will contribute with other researchers devoted to study drug–dietary supplement interaction. Furthermore, all this information assures the public about the veracity or not metal concentrations of marketed products.

References

[1] A. Whyte, G. Raumati Hook, G.E. Greening, E. Gibbs-Smith, J.P.A. Gardner, Human dietaryexposure to heavy metals via the consumption of greensheell mussels (*Perna canaliculus* Gmelin 1791) from the Bay of Islands, northern New Zeland, Sci. Total. Environ. 407 (2009) 4348–4355.
[2] R. Raghunath, R.M. Tripathi, B. Suseela, S. Bhalke, V.K. Shukla, V.D. Puranik, Dietary intake of metals by Mumbai adult population, Sci. Total. Environ. 356 (2006) 62–68.
[3] S. Caroli, G. Forte, A.L. Iamiceli, B. Galoppi, Determination of essential and potentially toxic trace elements in honey by inductively coupled plasma-based techniques, Talanta 50 (1999) 327–336.
[4] C.W. Huie, A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants, Anal. Bioanal. Chem. 373 (2002) 23–30.
[5] K. Bu, J.V. Cizdziel, L. Reidy, Analysis of herbal supplements for selected dietary minerals and trace elements by laser ablation- and solution-based ICPMS, Microchem. J. 106 (2013) 244–249.
[6] National Institute of Health, What's in the bottle? An Introduction to Dietary Supplements, NCCAM Publication Number D191, 2003. , (USA).
[7] S.P. Dolan, D.A. Nortrup, P.M. Bolger, S.G. Capar, Analysis of dietary supplements for arsenic, cadmium, mercury, and lead using inductively coupled plasma mass spectrometry, J. Agric. Food Chem. 51 (2003) 1307–1312.
[8] Office of Dietary Supplements, National Institutes of Health, Annual bibliography of significant advances in dietary supplement research, National Institutes of Health Publication Number 05-5312, 2005.
[9] L.C. Sander, K.E. Sharpless, S.A. Wise, Dietary supplement standard reference materials, Life Sci. 78 (2006) 2044–2048.
[10] I.A. Khan, J. Allgood, L.A. Walker, E.A. Abourashed, D. Schlenk, W.H. Benson, Determination of heavy metals and pesticides in ginseng products, J. AOAC Int. 84 (2001) 936–939.
[11] L. Valiente, M. Piccinna, E. Romero Ale, A. Grillo, P. Smichowski, Determination of selenium in dietary supplements by ETAAS and HG-AAS: a comparative study, At. Spectrosc. 23 (2002) 129–134.
[12] L. García-Rico, J. Leyva-Perez, M.E. Jara-Marini, Content and daily intake of copper, zinc, lead, cadmium and mercury from dietary supplements in Mexico, Food Chem. Toxicol. 45 (2007) 1599–1605.
[13] H. Tumir, J. Bošnir, I. Vedrina-Dragojević, Z. Dragun, S. Tomić, D. Puntarić, G. Jurak, Monitoring of metal and metalloid content in dietary supplements on the Croatian market, Food Control 21 (2010) 885–889.
[14] J.C.P. de Mattos, A. Medeiros Nunes, A.F. Martins, V.L. Dressler, E.M. de Moraes Flores, Influence of citric acid as chemical modifier for lead determination in dietary calcium supplement samples by graphite furnace atomic absorption spectrometry, Spectrochim. Acta B 60 (2005) 687–962.
[15] P. Raman, L.C. Patino, M.G. Nair, Evaluation of metal and microbial contamination in botanical supplements, J. Agric. Food Chem. 52 (2004) 7822–7827.
[16] J.F. Tyson, R.I. Ellis, G. Carnrick, F. Fernandez, Flow injection hydride generation electrothermal atomic absorption spectrometry with in-atomizer trapping for the determination of lead in calcium supplements, Talanta 52 (2000) 403–410.
[17] J.F. Rêgo, A. Virgilio, J.A. Nóbrega, J.A. Gomes Neto, Determination of lead in medicinal plants by high-resolution continuum source graphite furnace atomic absorption spectrometry using direct solid sampling, Talanta 100 (2012) 21–26.
[18] J. Crossgrove, W. Zheng, Manganese toxicity upon overexposure, NMR Biomed. 17 (2004) 544–553.
[19] L. Fishbein, Metals and Their Compounds in the Environment, second ed. VCH, Germany, 1991.
[20] P. Viñas, I. López-García, B. Merino-Moroño, N. Campillo, M. Hernández-Córdoba, Determination of selenium species in infant formulas and dietetic supplements using liquid cromatography-hydride generation atomic fluorescence spectrometry, Anal. Chim. Acta 535 (2005) 49–56.
[21] B. Avula, Y.H. Wang, N.S. Duzgoren-Aydin, I.A. Khan, Inorganic elemental compositions of commercial multivitamin/mineral dietary supplements: application of collision/reaction cell inductively coupled-mass spectroscopy, Food Chem. 127 (2011) 54–62.