



The role of analytical techniques in the determination of metals and metalloids in dietary supplements: A review

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ABSTRACT

This review is focused to cover the application of different analytical techniques for the determination of metals and metalloids in different categories of dietary supplements. Atomic spectrometric methods based on flame atomic absorption spectrometry, electrothermal atomic absorption spectrometry, atomic fluorescence spectrometry, X-ray fluorescence spectrometry and plasma-based techniques such as inductively coupled plasma optical emission spectrometry and inductively coupled plasma–mass spectrometry are reviewed because a considerable amount of research is presently performed in this field. Even when much less reported in the literature, the application of neutron activation analysis, isotope dilution mass spectrometry and hyphenated methodologies for speciation studies based on the use of separative techniques in combination with specific detectors are also discussed. This survey contains 46 references and covers mainly the literature published over the last fifteen years.

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1. Introduction and general considerations

Dietary supplements (DS) are products that intend to supplement the diet, which contains vitamins, minerals, herbs, botanicals, biofortified yeasts, ayurvedic formulations, amino acids, or any combination of the above ingredients [1]. They are prepared through laboratory synthesis or from natural products including different part of plants and fish oil [2]. Dietary supplements can be found in the market in different forms such as gel, pills, caps, oils, capsules and tablets. We can say that DS are on a gray zone between drugs and nutrients.

The marketing and the current frantic pace are some of the reasons that have caused a significantly increase in DS consumption in the last 15 years, especially in countries with greater purchasing power. The consumers are attracted to these alternative products for a variety of reasons such as:

- (i). to compensate the lack of nutrients/compensate the diet,
- (ii). relatively low cost,
- (iii). easy access,
- (iv). supposed to be preventive of numerous diseases and disorders,
- (v). supposed to be natural, safe, without adverse effects,
- (vi). massive advertising campaigns,

- (vii). considered as alternative medicines without side effects,
- (viii). promise to be the elixir of youth, health and vitality.

Regrettably, many consumers have limited information on this topic and they are often not able to evaluate the health claims and possible health risks of these products. The situation is more worrying when they are consumed during extended periods of time without medical supervision or without proper control.

Medicinal plants products are very used as therapeutic medicines. In the same direction, the consumption of herbals supplements (HS) has expanded rapidly. According to Kowalski and Frankowski [3], it is estimated that 25% of the prescribed drugs are from plant origin with 121 active substances used in their formulation. They may content a single herb or a combination of herbs in an extract, powder, pill or tablet. The botanicals used in the manufacturer of these supplements use different parts of the plants from whole plant to fruits, roots, rhizomes, flowers, seeds, bark, stem and leaves. For this reason, these HS may contain a wide variety of chemical elements. Metals such as Al, Co, Cr, Cu, Fe, Mn, Ni and Zn are essential plant nutrients; however, they may become toxic at higher concentrations [4]. Contrary to chemically synthetic drugs, herbal medicines are classified as non-prescriptions and are not required to undergo strict approval procedures [5]. The Food and Drugs Administration (FDA) established in 2007 a rule to ensure the quality of HS and DS available to the public [6]. Respect to ayurvedic formulations, in USA they are regulated by the Dietary Supplements Health and Education Act but no proofs of safety are required [7]. In the

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European Union, ayurvedic medicines are market as DS, which are regulated under a specific legislation for traditional medicines [7,8].

Even when DS are, in many cases, assumed as beneficial for health and without side effects, many studies performed worldwide report the presence of heavy metals, bacteria, pesticides, etc. [9–13]. Respect to heavy metals, commercial supplements contains a variable type and amount of metals and metalloids that can be essential and non-essential elements. Some of them are introduced into the environment through industrial activities and vehicular traffic (especially those derived for plants). In addition, recognized toxic metals such as As, Cd, Ni, Pb, Sb can naturally occur in the environment, due to weathering, biological and volcanic activities.

In DS, pharmaceuticals and food products in general, especial attention should be paid to those that represent a health hazard such as As, Cd, Cr, Hg, Pb, etc. Metals and metalloids may be introduced in different ways, especially for botanical products, and depends on:

- (i). natural or anthropogenic environmental contamination,
- (ii). geochemical characteristics of the soil,
- (iii). growing conditions of the plants,
- (iv). ability of the plants to selectively accumulate specific elements,
- (v). purity of the raw materials,
- (vi). extraction process,
- (vii). formulation,
- (viii). manufacturing process,
- (ix). transport,
- (x). inappropriate storage.

The FDA published regulations hold supplements manufacturer or distributors for the content of the DS, which should only contain what is declared in the label and not any harmful or undesirable substances including pesticides and heavy metals [14]. This is a key point because researchers involved in the characterization of the elemental composition of these nutritional know that, in general terms, they contain much more elements that those declared in the labels and studies documenting this fact have been reported [11,15].

Consumers, especially those that take significant amounts of DS, may ingest high doses, receiving in this way overloads of metals especially when DS are consumed over long periods of time. Unfortunately, national and international directives governing the regulation of these supplements, in terms of their safety and efficacy, are in flux [16]. These supplements should contain only what is declared on the label and should not contain any harmful or undesirable substance as toxic metals [17]. The question is: Is it true? Researchers involved in the elemental characterization of DS know that it is not true and that much more elements, even toxic elements, can be found that are not declared in the label [18].

This survey intends to provide information on the techniques used to investigate the elemental composition of DS and briefly discuss if declared concentrations in the labels agree with levels found. Due to the diversity of DS investigated and characterized in terms of metals and metalloids content, it is impracticable to cover all of these in this review. For this reason, the discussion is restricted to key components and the most used instrumental analytical techniques that have been employed, alone or hyphenated, that have played a crucial role in the analysis of DS. The research done in the 15 years in the field of DS analysis using ion-exchange chromatography (IC), flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), atomic fluorescence spectrometry (AFS), X-ray fluorescence (XRF), neutron activation analysis and plasma-based techniques such as inductively coupled plasma optical emission spectrometry (ICP OES) and inductively coupled plasma-mass spectrometry (ICP-MS), is briefly described.

2. Sample preparation

In general terms, and according to the experience of the authors, the preparation of these samples for subsequent chemical analysis do not

pose a significant difficulty. For this reason, only some lines have been included on this topic. Nevertheless, in analytical chemistry sample preparation is usually a key step affecting analytical results. In the case of DS analysis it is necessary to take into account the great variations in the matrix composition of different supplements including herbs, plants, oils, gelatin, proteins, fat, etc. Special attention should be paid to avoid contamination from reagents, vessels, mortar and pestle, etc.

The normal operation of atomic spectrometric and plasma-based techniques requires that samples be necessarily put in solution before being presented to the technique of choice for measurement. Most of these techniques use a nebulization system to introduce the sample. To this end, microwave (MW)-assisted digestion was mainly used to mineralize DS samples. The most used procedure for solid samples (e.g., tablets) is the trituration with mortar and pestle to reach a homogeneous sample. For capsules and liquid samples, they can be introduced directly into the polytetrafluoroethylene MW oven vessels. Other alternative is to empty the liquid content into the MW oven vessel.

Oxidizing acids with high degree of purity such as HNO₃, and non-oxidizing acids such as HCl and HF, as well as H₂O₂, have been used to digest these samples. Several possibilities of acids and mixtures of reagents have been reported: (i) HNO₃, (ii) HNO₃ + HCl, (iii) HNO₃ + HF, (iv) HNO₃ + H₂O₂. Nitric acid and H₂O₂ was the mixture most commonly used [6,7,11,17,18]. Nitric acid is an almost universal digestion reagent and it is intended to destroy organic compounds into H₂O and CO₂ and to oxidize metals. In addition, HNO₃ forms water-soluble salts with most elements and consequently precipitation not are expected. When ICP-MS is used for quantification, HNO₃ is recommended to digest the samples because H, N and O are present in the plasma [4,12,13]. Nitric acid can also be used together H₂O₂ and HCl to improve the performance of digestion. Hydrochloric acid is also employed for DS digestion because chlorides are in general terms soluble, excepting for Ag, Hg and Pb [3,14].

An alternative method such as cryogenic grinding was reported and employed to composite soft samples such as oil filled capsules and candy-like products such as gummies and jelly beans [19]. This methodology acts cooling or chilling a material and then reducing it into a small particle size.

3. The role of analytical techniques in dietary supplement analysis

Numerous analytical techniques and experimental approaches have been proposed in the last 15 years to identify and measure metals and metalloids aimed at obtaining reliable results and correct evaluations in the field of DS analysis. In this context, researchers are benefiting from developments of innovative analytical methodologies where atomic spectrometric and plasma-based techniques are important tools for element determination. The outstanding developments undergone by these techniques over the last years have marked a parallel and irreversible progress in the exploitation of such techniques from both the viewpoint of research activities, routine analysis and regulatory tasks.

The selection of a technique for DS analysis will depend on the element/elements, number of elements to determine, concentration in the digested sample, detection capability, interferences (spectral, matrix), accuracy and precision required, linear dynamic range, multielemental capability, possibility of isotopic analysis, skill level required, instrument cost, and operating and maintenance costs of the instrument.

The widespread use of atomic spectrometric and plasma-based techniques in the field of DS analysis is well-reflected by the number of papers, reports, books, and other scientific publications that have become available in recent years.

3.1. Atomic spectrometric techniques

Atomic spectrometric methods based on FAAS, ETAAS and in less extent AFS have been used to determine metals and metalloids in DS especially when the focus was put in the determination of specific elements of toxicological relevance.

The continuous developments in atomic spectrometric techniques provide some of the most sophisticated and elegant methods for determination of trace, minor and major elements in DS. Important progress in these techniques has been achieved owing to improvements in instrumentation as well as the use of new methodologies for sample treatment.

3.1.1. Flame atomic absorption methods

In general terms, FAAS is a robust, simple to operate and consolidated technique. It has a relatively moderately priced instrumentation, good accuracy and an adequate precision (0.1–1%) for minor and major elements determination. In spite of this, FAAS presents the disadvantage of single element operation capability. Some examples of the applications of FAAS to DS analysis are briefly described in the following paragraphs.

Soriano et al. [20] developed a methodology for the determination of Cu, Fe, Mn and Zn in seven commercially available multivitamin/multimineral tablets by FAAS. Analytes were extracted with a diluted HCl solution and parameters influencing the extraction process (extraction solution: type and concentration, mixing mode including ultrasonic or magnetic stirring, extraction time and sample composition) were assessed. The study evidenced that in DS containing silicates, the time of contact between solid sample and extraction solution presented remarkable influence, being necessary up to 12 h to achieve a quantitative recovery.

The presence of Cd, Cu, Hg, Pb, and Zn in 24 dietary supplements purchased in Mexico with a great variety of components was reported by García-Rico and co-workers [1]. Microwave-digested samples were analyzed by FAAS and the most abundant elements resulted to be: Cu ($<0.19\text{--}137.85\ \mu\text{g g}^{-1}$) and Zn ($<2.83\text{--}4785.71\ \mu\text{g g}^{-1}$), followed by Pb ($<0.003\text{--}66.32\ \mu\text{g g}^{-1}$), Cd ($<0.001\text{--}2.90\ \mu\text{g g}^{-1}$), and Hg ($<0.24\text{--}0.85\ \mu\text{g g}^{-1}$). According to the authors, the estimated daily intakes of metals were below those recommended by WHO (World Health Organization) and the Institute of Medicine.

The importance and benefits introduced by vapor generation (VG) methods coupled to atomic techniques in the field of DS analysis have to be mentioned. Hydride generation (HG) has been employed for over a century for determining As by the Marsh reaction of the Gutzeit test. The introduction of AAS conducted to a massive application of HG to AAS and then to other analytical techniques due to the unquestionable advantages of this method. Nowadays, VG methods based on derivatization of acidified samples with sodium tetrahydroborate(III) have become, without any doubts, a useful method to determine typical hydride forming elements. One of the most studied elements in DS analysis is Se for being an interesting trace element in terms of human biology and health. Selenium can be essential as well as toxic for the human body and the borderline between these states is relatively sharp [21]. A wide range of DS containing Se (inorganic and/or organic forms) is available worldwide because many benefits for human health are attributed to their regular consumption. According to the labels and advertising, these Se-containing products help to protect cells against oxidative stress, maintain the normal functioning of the immune system, normal nails and hair, normal thyroid function and spermatogenesis and are also helpful in maintaining a healthy heart and blood vessels [22].

Recently, a UV-photochemical vapor generation (UV-PVG) system was optimized and applied to the determination of Se in selected DS [22]. The generation process was performed in continuous mode and combined with AAS, utilizing an externally heated quartz furnace as an atomizer. The efficiency of UV-PVG is strongly dependent on the presence of organic photochemical agents. In this case, formic acid was employed as the photochemical agent during the UV-assisted conversion of Se(IV) from the condensed to the gaseous phase and no further reagents were needed. This approach made possible to reach a very low limit of detection (LOD) of $40\ \text{ng L}^{-1}$. Commercially available DS containing Se as Se(IV) and Se(VI) were analyzed.

Other authors adopted the use of several techniques for DS characterization. A comparative study on the determination of Se in DS containing vitamins–minerals–amino acids and Se-enriched yeast by HG–AAS and ETAAS was reported [23]. Data obtained showed that the major shortcoming for HG–AAS was the presence of transition metals such as Cu, Mn and Zn affecting the formation and release of SeH_2 from the digested samples, with Cu causing the most serious problems. Selenium levels ranged from 15.9 to $81.3\ \mu\text{g Se}$ per tablet. As in other studies, the authors highlighted that the data obtained demonstrated that Se content reported on the labels were often inaccurate.

Tumir et al. [24] analyzed 30 samples of widely used vitamins and herbal preparations sold in Croatia. Samples were analyzed by several atomic spectrometric techniques in order to estimate levels of As, Cd, Cr, Hg, Ni, Pb and Zn. The following concentration ranges were reported (in $\mu\text{g g}^{-1}$): Pb 0.25–3.86; Cd 0.05–0.28; As 0.10–0.19; Hg 0.02–0.12; Cr 0.11–64.38; Ni 0.24–338.90; and Zn 1.00–95.3. The authors remarked that in several formulations metal levels resulted above the maximum allowable limits. Moreover, the estimated cumulative daily intakes of several metals were higher than the oral permitted daily exposures set by the US Pharmacopeial Convention – Advisory Panel on metal impurities.

3.1.2. Electrothermal atomic absorption methods

Electrothermal atomic absorption spectrometry is an important tool for the accurate determination of trace metal content in DS. In general terms, this technique is especially useful when a low mass or low volumes (5–40 mL) are available, owing to its low LOD ($<5\ \text{mg L}^{-1}$), precision (0.5–5%), accuracy and selectivity. It is a very sensitive technique and for this reason requires very clean reagents to avoid blank problems.

Lead has been one of the elements more studied in DS analysis because it is a toxic metal that affects the central nervous system and heme synthesis pathways. Initially, the presence of Pb in Ca-supplements was attributed to dolomite and bone-meal powders. However, an inter laboratory study showed that other salts such as Ca carbonate and Ca chelates may also contain substantial levels of Pb [25]. Even when the HG is not the technique of choice of Pb determination, a flow injection (FI)–HG–ETAAS method with in-atomizer trapping was proposed and applied to the determination of Pb in two Ca-based supplements, CaMgZn and Oyster shell calcium [26]. Lead hydride was generated from acid solution, containing potassium ferricyanide as an oxidizing agent, by the reaction with alkaline tetrahydroborate solution. Results were compared with those obtained by ETAAS. Concentrations $<0.7\ \mu\text{g g}^{-1}$ were reported for both DS.

Paz de Mattos et al. [27] assessed the influence of citric acid as chemical modifier for Pb determination in Ca-based supplement (calcium carbonate, dolomite and oyster shell samples) by graphite furnace atomic absorption spectrometry (GFAAS). The efficiency of citric acid was compared to the use of a traditional modifier such as Pd. Three digestion procedures were investigated: MW-assisted digestion, high-pressure digestion with conventional heating, and dissolution at room temperature. No significant differences were detected among results obtained for the three procedures. Concentrations of Pb resulted relatively low in comparison with levels of Pb reported for many supplements. They were found at concentrations from 0.20 to $1.36\ \mu\text{g g}^{-1}$.

Selenium was determined in aqueous extract of medicinal plants used as HS for cancer patients and different diseases [28]. Generally, the aqueous extract of plant supplements are used as curing agent, and only the water extractable Se is available to humans. Selenium was put in solution by MW-assisted extraction and conventional extraction while total and residual Se was determined, prior to MW-assisted acid digestion. The aqueous extracts and digests were analyzed by ETAAS. Concentrations in aqueous extract were found in the range of $1.09\text{--}2.23\ \mu\text{g g}^{-1}$, equivalent to 21–33% of total Se content.

Graphite furnace atomic absorption spectrometry is an attractive technique for DS analysis especially because offers possibility of direct

solid sampling (DSS). Its benefits can be summarized as follow: (i) minimum possibilities of contamination and analyte loss, (ii) high sensitivity, (iii) reduced overall time of analysis, (iv) less consumption of hazardous reagents, (v) minimum waste generation, (vi) potentiality for simultaneous multi-element analysis. The coupling of DSS to high resolution-continuous source (HR-CS) GFAAS is an interesting alternative to other atomic spectrometric techniques and has been employed for elemental analyses of different matrices in the last years. In the field of DS analysis, it was employed for the determination of Pb in medicinal plants [29]. An assessment of chemical modifiers including Pd(NO₃)₂, Pd/Mg(NO₃)₂, NH₄H₂PO₄ and the W-coated platform demonstrated that Pd(NO₃)₂ resulted to be the best alternative. Ten medicinal plants were analyzed by DSS HR-CS GFAAS and line-source GFAAS using slurry sampling (SS) that was tested as comparative technique. Lead content ranged from 0.30 to 1.94 mg g⁻¹.

In a comprehensive study, three commercially available multivitamin dietary supplements were determined by HR-CS GFAAS with SS to assess the concentrations of As, Cd, Cr, Cu, Fe, Mn, Pb and Se. Results were compared with concentrations of metals and metalloids stated by producers [17]. The study included an investigation of the slurry preparation and the atomization into the graphite furnace and an evaluation of the usefulness of the proposed method in determining selected elements.

3.2. Plasma-based techniques

The continuous developments undergone by plasma-based techniques in the last decades have marked a considerable progress in the use of such techniques for environmental and food analysis purposes from the point of view of research activities and routine analysis. Even with these techniques are characterized for their multielemental capacity, many studies were focused to mono elemental determinations of a target element. Lead was one of the elements more studied because of its toxicity in humans. This metal can cause many harmful physiological effects even at low levels and deserves special attention.

A study was conducted to determine the content of Pb in 324 multivitamin-mineral products labeled for use by women and children [19]. After MW-assisted nitric acid digestion, Pb was determined in the resulting solution by ICP-MS. An alternative method such as cryogenic grinding was employed to composite soft samples such as oil filled capsules and candy-like products such as gummies and jelly beans. The authors reported data of estimated median and maximum Pb exposures that resulted to be 0.123 and 2.88 µg day⁻¹ for young children, 0.356 and 1.78 µg day⁻¹ for older children, 0.845 and 8.97 µg day⁻¹ for pregnant and lactating women and 0.842 and 4.92 µg day⁻¹ for adult women. The overall median value for Pb exposure was 0.576 µg day⁻¹. It is important to remark that 5 samples would have provided exposures that exceeded 4 µg Pb day⁻¹.

In other approach, a study was undertaken to determine Pb levels in 45 widely used pharmaceutical products by ICP-MS as well as to assess the risk of exposure from using these products [30]. The study evidenced that six products had Pb concentrations >100 ppb being the highest concentration 500 ppb. According to the authors, the average mass of Pb delivered to consumers by all the examined DS when taken as directed was 0.22 µg day⁻¹. It is expected that these values increase the levels of Pb in blood of an adult by <1%. In addition, five products were found to deliver >1 µg of Pb per day when used as directed. Current tolerable Pb limits in pharmaceutical substances vary widely. In spite of this, the examined products had Pb concentrations far below these levels.

Cadmium was quantified in NIST SRM 3280 (National Institute of Standards and Technology, USA) multivitamin/multielement tablets, using isotope dilution mass spectrometry (IDMS) at ng g⁻¹ levels [31]. Strategies of pre-concentration and matrix reduction can be used to minimize/reduce the impact of interferences and matrix suppression effects when Cd is determined at trace levels using ICP-MS. To this end,

the authors used various precipitation and solid-phase extraction separation approaches to isolate Cd, Mo and Sn present in the tablet matrix at µg g⁻¹ levels. This allowed measurement of ¹¹¹Cd/¹¹³Cd and ¹¹¹Cd/¹¹⁴Cd isotope ratios using both quadrupole collision cell technology ICP-MS and sector field ICP-MS equipped with a desolvating nebulizer system. This approach was followed to mitigate the MoO⁺ and MoOH⁺ molecular ion interferences that typically affect the envelope of Cd isotopes.

Moret et al. [32] reported levels of creatine, organic contaminants and heavy metals in creatine DS commercialized both, in their pure form or in formulation with other compounds. Arsenic, Cd, Hg and Pb were determined in the digested samples by ICP-MS. Creatinine was the most widespread organic component with amounts often exceeding recommended limits. Concentrations of As, Cd and Hg were in all cases lower than their respective LODs. Only Hg was detected when the instrument was coupled to a hydride generator, and in 14 samples Hg levels were over 0.10 mg kg⁻¹. In spite of this, the measured levels of Hg resulted below the limit of 1 mg kg⁻¹ defined for Hg [33].

Microwave-assisted digestion followed by collision/reaction cell ICP-MS, with an integrated octopole system, resulted a simple, fast and reliable method for the multi-element determination of 16 elements namely, As, Ca, Cd, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Se, V and Zn in 35 different commercially available multivitamin/mineral DS (tablet, capsule, liquid or powder form for children, women, men, young and adult) sold in USA [14]. The trueness of the method was checked by the analysis of standard reference materials (SRM 3280, SRM 1566b) and spiked samples. As other authors, emphasis was given to the deviation of calculated daily intake of each element from their corresponding label claim. Additionally, for toxic elements calculated daily intakes were compared with those of values established by regulatory guideline values (e.g., recommended dietary allowance). The results revealed that all analyzed products exhibited calculated daily intakes of As, Cd, Hg and Pb lower than those fixed in regulatory limits. The differences between the calculated and claimed daily intakes varied from moderately (20%) to significantly (>30%) for potentially toxic elements including Cr, Mn, Se and Zn.

Ayurvedic medicine, also called Ayurveda, is one of the world's oldest holistic (whole body) medical systems originated in India >3000 years ago and still remains one of the country's traditional health care systems. In spite of its widespread use, Ayurvedic medicine has been much less studied in comparison with traditional medicines. These products use herbal and metallic preparations, in which herbs are combined with specific metals, minerals and gems. For this reason, ayurvedic products have the potentiality to be toxic. With the objective to identify possible risks associated with the presence of toxic elements in ayurvedic formulations Giacomino and co-workers [7] determined 25 elements (Al, As, Ag, Au, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, Pd, Pt, Sb, Si, Sn and Zn) in 17 ayurvedic products manufactured in India and sold in through different distribution channels, including Internet. Metals and metalloids were determined by ICP OES in the digested samples. According to the findings of this investigation, in products purchased on Internet or in the Italian market the concentrations resulted lower than the safety limits fixed by the international authorities. On the other hand, five products purchased in India contained potentially dangerous concentrations of As, Cu, Hg and Pb. Surprisingly; these elements are intentionally added during the medicine preparation following the rasa shastra tradition. This comprehensive study also focused to assess the bioaccessibility of these products. It was observed that the amount of metals actually released during ingestion is lower than the total amount, but in some cases it is still higher than the maximum admissible level.

In the same direction, Filipiak-Szok [4] determined the content of metals and metalloids namely, Al, As, Ba, Cd, Ni, Pb and Sb by ICP-MS in Asiatic medicinal plants used in ayurveda or traditional Chinese medicine. Results indicated that the concentration (at µg g⁻¹ level) of metals

in medicinal plants differed significantly depending on the production area and confirmed that even within the same plant elemental concentrations may vary among parts of the plant.

As shown in previous pages, supplements are generally digested using acid mixtures followed by analysis by ICP–MS. In this general procedure, sample preparation is time consuming and can increase the possibility of contamination and loss of volatile elements. To avoid/minimize sample preparation and dissolution, other authors adopted the alternative of coupling laser ablation (LA) to ICP–MS (LA–ICP–MS). Briefly, LA introduces solid samples, as ablated particles and vapor, to an ICP–MS instrument where signal intensities from isotopes of elements can be measured and quantified. Laser ablation–ICP–MS and conventional closed–vessel digestion solution nebulization (SN)–ICP–MS were used and compared for the determination of 12 elements (Al, Ca, Cd, Co, Cu, Cr, Fe, Mg, Mn, Ni, V and Zn) in six herbal supplements. A single pressed leaf SRM was used for calibration. The optimization of the system showed that for LA–ICP–MS a low resolution ($m/\Delta m \approx 400$) yielded good recoveries for the SRM analyzed and results were comparable to those reached using SN–ICP–MS for most elements, except for Ca, which was better determined in medium resolution ($m/\Delta m \approx 4000$). With the exception for Zn that exhibited a relatively uniform concentration across samples, with an average value of 17.5 mg kg^{-1} , for the other elements their concentrations varied sometimes widely between the different types of supplements. This approach can be used as a screening method to rapidly gain information on the range of elements present in a DS.

High–resolution (HR)–ICP–MS was another technique used for DS analysis for its relevant advantages. This instrument allows the elimination of most polyatomic interferences by resorting to mass resolution ($m/\Delta m$) of 300, 3000 or 7500. Four relevant elements from their potential dangerous effects for human health namely, As, Cd, Hg and Pb were determined in 95 DS using MW–assisted digestion and HR–ICP–MS for quantification [13]. Precision and accuracy were demonstrated by element recovery from 17 DS and replicate measurements of eight reference materials. The authors reported concentration ranges for the four elements that exhibited a great variability: As, $<5\text{--}3770 \text{ } \mu\text{g kg}^{-1}$; Cd $<10\text{--}368 \text{ } \mu\text{g kg}^{-1}$; Hg, $<80\text{--}16,800 \text{ } \mu\text{g kg}^{-1}$; and Pb, $<20\text{--}48,600 \text{ } \mu\text{g kg}^{-1}$. Estimated exposures were assessed with respect to safe/tolerable exposure levels.

Other authors adopted the strategy of using ICP OES and ICP–MS for the multielemental determination of a variety of elements in supplements. Krejčová et al. [11] reported the application of both techniques for the determination 29 elements in multivitamin preparations and supplements. In addition, based on results obtained, samples were classified using multivariate statistical methods. This comprehensive investigation also focused to demonstrate the advantage of using time of flight ICP–MS in the analysis of samples with a complex matrix such as DS and to demonstrate the general advantage of the instrument in analysis of samples with high variability, and to map an elemental composition of nutritional preparations in respect to comparison with recommended intakes, producer's declarations, and content of contaminants presented. The elements measured were grouped as follows: macroelements (Ca, K, Mg, Na and P), microelements (Cu, Cr, Fe, Mn, Se and Zn), toxic elements (As, Cd, Ni, Pb and V) and Au, rare earth elements (REEs), Pd, Pt, Ti, etc. The concentrations of physiologically significant elements were (in mg kg^{-1}): Ca, 560–196,000; Cr, 0.486–26.6; Cu, 28–1460; Fe, 115–39,400; Mg, 308–70,300; K, from undetectable to 28,200; Mn, 28–1860; Na, 289–74,000; Mo, 0.0418–9.88; P, from undetectable to 217,000; Se, 0.395–161 and Zn 2.1–25,000. Some of the analyzed samples showed a possible health risk for Ni with concentrations $\sim 200 \text{ mg kg}^{-1}$. The authors provided useful data on the content of REEs and this is an important point to remark because of the lack of information on this group of elements in nutritional preparation. Respect to declared and found values, the study showed that in general, the determined average concentrations agreed with labels with few exceptions with reported concentrations varying from $\sim 10\%$ to 200% of declared values.

Even when much less used, the medium power radiofrequency capacitively coupled plasma atomic emission spectrometry can be mentioned among the different plasma–based techniques employed for metals and metalloids determination in DS. Using this approach, the multielemental determination of Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P and Zn in multiminerals/multivitamins was reported [34]. Determinations were carried out on commercially available tablets of DS and a SRM using the standard additions for calibration. The detection limits reached (expressed in mg g^{-1}) were in the range 0.003 (Na) to 1.5 (P) and were not depreciated by the non–spectral interference of mineral matrices of Ca, K, Mg and Na with the exception of Zn and P. Not significant discrepancies were observed between found and declared concentrations. Recoveries were in the range of 90–107% and repeatability between 1.0 and 13.0%. This technique could be an advantageous alternative to the more expensive ICP OES in the quality control of multiminerals/multivitamin preparations when the quantification of metals and metalloids at ultra–trace levels is not required.

3.3. Speciation studies

The possibility that certain concentrations of Se in food might protect against the development of cancer in humans has generated great interest in the development of methods devoted to the identification and quantification of Se in various chemical forms in nutritional preparations. Supplements usually contain inorganic selenite/selenate or selenium–enriched yeast in which Se organic species (selenomethionine; SeMet, methylselenocystine, etc.) are primarily included. Some researchers have put their efforts in the determination of total Se while others focused to develop analytical methodologies for the speciation analysis of Se in supplements.

The combination of high performance liquid chromatography (HPLC) with a plasma–based technique offers the advantage of a powerful fractionation technique characterized by a high degree of selectivity with simultaneous multielemental detection. Some studies reported in the literature are described in the following pages.

Grant and co–workers [35] reported the chemical association of several elements of nutritional and toxicological interest and their distribution among different molecular weight fractions of fulvic acid substances present in commercially available DS. The bioavailability of elements is one of the major selling points for these supplements; specifically by the fact that labels declare that metals are bound to the fulvic acid structure. As a consequence, they are more absorbable and more bioavailable. The detection of the different fractions was performed using an on–line sequential size exclusion chromatography UV–ICP–MS detection system. The human digestion system was mimicked in order to study the stability of the element–fulvic acid complexes and, therefore, the potential bioavailability of the elements in the human body. The instrumental coupling adopted offered highly sensitive and selective detection which made possible the determination of Ag, As, Ca, Co, Cr, Cu, Fe, Hg, I, Mg, Mn, Mo, Pb, Se and Zn. Elemental concentrations (in $\mu\text{g g}^{-1}$) ranged from 22 (Cu) to 11,669 (Ca). The results reached suggested that there was some association of the elements to the fulvic acids. It is important to remark that two–dimensional chromatography followed by electrospray mass spectrometry is a better alternative to further characterize these molecules.

The combination of liquid chromatography (LC) with HG–AFS was used for the selective determination of selenite, selenate, selenocystine and SeMet [36]. Inorganic and organic Se species were extracted from samples by a sequential technique that made possible to discriminate between water soluble and non–soluble Se fractions. The method was applied to the speciation analysis of Se in fortified foods (infant formulas and dietetic supplements), selenite was the only species detected in infant formulas while both, selenite and selenoaminoacids, were detected in DS. The concentration of inorganic species in the nutritional and Se–yeast based supplements varied as follows: Se(IV) between 34.4 and

730 $\mu\text{g g}^{-1}$ while Se(V) was not detected in any of the analyzed samples.

Recently, Niedzielski et al. [37] investigated the dissolution time under different conditions and solubility for the subsequent determination of Se species in 86 supplements by HPLC–HG–AAS. The study showed that the declared levels of total Se often differed from actual content. In addition, the authors pointed out that the label and information attached to the DS do not constitute a full or reliable source of information for consumers.

Other instrument used for nutritional supplements analysis is microwave plasma–atomic emission spectrometry (MP–AES). The main important advantages are as follows: (i) low running costs, (ii) laboratory safety (not flammable gases are required) and (iii) multi–element capabilities. This instrument can be considered as a suitable alternative to FAAS in terms of sensitivity. A relatively simple, cost effective and innovative procedure for the determination of SeMet and Se(IV) in biofortified yeast by ion–pair reversed phase LC–HG–MP–AES was reported [38]. After optimization of several chemical, physical and instrumental parameters, the conditions adopted allowed to reach limits of 11.9 mg Se g^{-1} and 104 mg Se g^{-1} for Se(IV) and SeMet, respectively. For comparative purposes, ICP–MS was used and the results obtained by both procedures resulted in good agreement.

Arsenic is an element that raises much concern from both the environmental and human health standpoints. Dietary supplements can be contaminated with this metalloid through the plants used in their formulation. Arsenic can be released into the environment through natural (volcanoes, weathering of minerals) and anthropogenic processes (ore smelting, pesticides, and coal combustion). Plants take up As from soil and water and have the ability to accumulate this metalloid in different parts, mainly as arsenate. A variety of analytical methods have been proposed to identify and measure As species in different matrices. The most used approach combined HPLC to ICP OES/ICP–MS. In this context, Wolle et al. [39] reported the speciation analysis of As in ten prenatal and children's DS prepared from a variety of plants using MW–enhanced extraction and HPLC–ICP–MS. The analyzed supplements were available in tablet, capsule or liquid forms. A multivitamin SRM from NIST (NIST SRM 3280) and a prenatal supplement sample were employed for method optimization. A comprehensive study of the appropriate mixture of reagents was carried out. The tested solutions were: water, methanol–water mixture, and aqueous solutions of α –amylase, α –amylase–protease mixture, HCl– H_2O_2 mixture, tetramethylammonium hydroxide, trifluoroacetic acid, ammonium oxalate and H_3PO_4 . A 0.3 mol L^{-1} H_3PO_4 solution was selected as the best alternative. Total As concentration varied from 59 to 531 ng g^{-1} and all DS contained arsenite and dimethylarsinic acid. Arsenate was found in only two samples. It is important to remark that an unknown specie of As was detected in one product.

3.4. Other techniques

X–ray spectrometric methods based on total reflection geometry have gained widespread strength in the past decade, principally because of their low detection power (LODs from 10^{-2} to 1 mg L^{-1}), multi–element capabilities, good selectivity and simplicity. On the other hand, screening methods based on XRF spectrometry are non–destructive and have demonstrated to be simple with a high sample throughput, and minimal sample preparation. In this context, they were used in screening studies focused to the detection of specific elements at ppm levels. Sanchez–Pomales and co–workers [40] reported a rapid and simple method for the determination of total Ag in nanobased commercially available liquid supplements using a portable XRF (pXRF) analyzer. Nanoscale Ag is an ingredient often found in nano–based DS due to its antimicrobial properties [41]. Results were compared with those obtained by ICP–MS. The authors remarked that the presence of additional ingredients in the DS such as proteins, stabilizers, Au, and SiO_2 did not affect the accuracy of Ag determination by the pXRF analyzer.

In other investigation, As was measured in foods and DS using instrumental neutron analysis (INAA) and radiochemical neutron analysis (RNAA) solvent extraction procedures [42]. The first technique was used to value–assign As mass fractions in DS while RNAA was employed to value assign As mass fractions in vitamin tablets and in foods. Arsenic was determined with good precision at levels lower than 100 g kg^{-1} in supplements by INAA, and lower than 10 g kg^{-1} in foods by RNAA.

Using speciated IDMS with mass balance, Martone et al. [43] proposed a detailed methodology to determine the health impact of inorganic Cr species in DS. Both species, Cr(III) and Cr(VI), were independently measured and verified with mass balance as both may be present in finished products. Since exists a difference in species stability (Cr(III) is stable in acidic conditions and Cr(VI) in alkaline ones), interconversions between species are expected in complex matrices and during the process of extraction. As a consequence, the difficulty of a reliable quantification is expected. To this end, speciated IDMS was implemented with IC–ICP–MS enabling tracking and correcting for the bidirectional Cr interspecies conversions that occur during extraction and sample handling prior to instrumental analysis. According to mass balance results of the off–the–shelf DS, the analyzed samples contained Cr(VI) ranging from <LOD to $122.4 \pm 13.0 \mu\text{g g}^{-1}$. The authors reported that the observed variations in the final products raised public health issues and pointed to a need of using a robust method that can accurately and reliably make species measurements including correcting for species conversions.

Mercury is a well–known toxic element for humans, plant and animals. It has mutagenic and teratogenic properties and as other heavy metals can be accumulated in the human body. Thus, to gain information on Hg levels in food, including DS, is of prime importance. Cold vapor in combination with AFS was applied to the determination of Hg in a variety of drugs and 33 DS available in the Polish market [3]. In the analyzed supplements the concentrations found for Hg ranged from 0.9 to 16.7 ng g^{-1} (median: 5.9 ng g^{-1}). According to the authors, analyzed samples did not pose a threat to the human health because Hg levels were far lower than the value recommended by the WHO as the provisional tolerable weekly intake of mercury.

Another approach was the study of the dissolution profile of DS. The combination of IC and chemometrics was tested to assess the dissolution profiles and kinetics models of Fe, Zn and Mn in four coated tablets of formulations containing vitamins and minerals [44].

In the last 15 years, only few studies based on the use of a spectrophotometric determination was used in the field of dietary supplements analysis. In this context, Pourreza et al. [45] reported the determination of Fe(II) in multivitamin tablets after solid phase extraction of its 2,2'–bipyridine complex on silica gel–polyethylene glycol. In other study, a method based on Hyper Rayleigh Scattering (HRS) and absorption spectral assays using surface–modified gold nanoparticles (AuNP) has been developed for the sensitive and selective detection of Cr(III), without interferences of other metal ions, including Cr(VI) [46]. To test the practical use of the HRS assay for real–life samples, a vitamin supplement containing Cr(III) was tested and no interference from other heavy metal ions were detected.

4. Conclusions

In order to arrive at reliable results and correct evaluations, numerous analytical methods have been proposed to measure the content of metals, metalloids as well as species in dietary supplements. In general terms, a great variability in elemental composition was observed in the majority of the studies included in this survey. Another point to remark is the necessity for specific certified reference materials for the analysis of these supplements.

Bioaccessibility studies, recommended values, maximum admissible levels, daily intakes, safety limits or risk of exposure of metals and metalloids in dietary supplements have not been discussed in this review.

In the opinion of the authors, all the components of these products should be declared in the label; even those found at concentrations

that do not pose risk for human health or that do not pose a significant risk for toxic metal overload. Information attached to the dietary and herbal supplements do not constitute a full or reliable source of information for consumers. In addition, a strict control of the variation in concentrations among tablets of the same bottle should be performed. These products should reach to the market without health risks for consumers. It only can be achieved through constant monitoring of product quality by independent scientific and public health institutions.

As a general remark, it is advisable to consume these products only under strict medical counsel and to purchase them from controlled commercial channels. Respect to manufacturers of nutritional preparations and dietary supplements, it is advisable that they control strictly the purity of reagents and products during all the stages of manufacturing as well as the final product which goes to the market.

It is the desire of the authors that this work contributes to a deeper discussion about the monitoring and quality control of these nutritional products. Without any doubt such activities necessitate the use of reliable and efficient analytical methods.

Abbreviations

| | |
|---------|--|
| AFS | atomic fluorescence spectrometry |
| AuNP | Gold nanoparticles |
| DS | Dietary supplements |
| DSHEA | Dietary Supplements Health and Education Act |
| DSS | direct solid sampling |
| ETAAS | electrothermal atomic absorption spectrometry |
| FAAS | flame atomic absorption spectrometry |
| FDA | Food and Drugs Administration |
| FI | Flow injection |
| GFAAS | Graphite furnace atomic absorption spectrometry |
| HG | Hydride generation |
| HPLC | High performance liquid chromatography |
| HR | High-resolution |
| HR-CS | high resolution-continuous source |
| HRS | Hyper Rayleigh Scattering |
| HS | herbals supplements |
| IC | ion-exchange chromatography |
| ICP-MS | Inductively coupled plasma mass spectrometry |
| ICP-OES | Inductively coupled plasma optical emission spectrometry |
| IDMS | Isotope dilution mass spectrometry |
| INAA | Instrumental neutron activation analysis |
| LA | Laser ablation |
| LC | Liquid chromatography |
| LOD | Limit of detection |
| MP-AES | Microwave plasma – atomic emission spectrometry |
| MW | Microwave |
| NIST | National Institute of Standards & Technology |
| pXRF | Portable X-ray fluorescence spectrometry |
| REE | Rare earth elements |
| RNAA | Radiochemical neutron activation analysis |
| SeMet | selenomethionine |
| SN | solution nebulization |
| SRM | Standard reference material |
| SS | Slurry sampling |
| UV-PVG | UV-Photochemical vapor generation |
| VG | vapor generation |
| WHO | World Health Organization |
| XRF | X-ray fluorescence spectrometry |

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