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Original Research Article

Determination of lead in dietary supplements by high-resolution continuum-source graphite furnace atomic absorption spectrometry with direct solid sampling

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ABSTRACT

A method has been developed for the determination of lead (Pb) in dietary supplements using direct solid sampling and high-resolution continuum source graphite furnace atomic absorption spectrometry. The lead concentration was determined in 74 food supplements. The zirconium (Zr) coated platform, and chemical modifiers [$Pd(NO_3)_2$ and $Mg(NO_3)_2$] were applied. Mass aliquots between 0.8 and 1.0 mg were weighed and directly inserted into the graphite furnace. The precision (expressed as RSD) was lower than 5%, and a detection limit of 2.16 pg was obtained at the 283.306 nm resonance line. The limit of quantification was 7.2 pg. The Pb recuperation in the standard reference material, Bitter Orange-Containing Solid Oral Form NIST SRM 3260, was 116% of the certified value. The contents of Pb in the dietary supplement samples varied in the 0.04–25.3 μg/day range. Two samples had results above that allowed by American Pharmacopoeia for inorganic analysis in food supplements.

1. Introduction

The global market for dietary supplements has shown a marked tendency to rise. According to the FDA, dietary supplements are products that may contain multiple ingredients, including vitamins, minerals, herbs, amino acids and dietary substances to supplement the diet. Dietary supplements are generally used by most people on a voluntary basis and without strict supervision and knowledge of their health/risk factor (Brown, 2016).

The use of dietary supplements is widespread in Brazil, but little has been discussed about the quality of the formulations. Contamination of food supplements by different elements may occur during the industrialisation process; this may be caused by a contaminated environment, anthropogenic pollutants or inadequate storage. Thus, it is necessary to control the amount of some of the elements that even at low concentrations can cause toxic effects to the consumer due to the daily intake (Amster et al., 2007; Avula et al., 2011; Szok et al., 2015).

There are few studies that analysed the presence of inorganic contaminants in dietary supplements. Szok et al. (2015) analysed Pb in dietary supplements through ICP-MS, one of the samples had 9.27 μg/g of Pb, a result higher than that allowed by the European Commission for these products (3.00 μg/g). Krawczyk (2014) determined trace elements in multivitamin supplements using HR-CS GFAAS, the samples were analysed in paste form, after being digested with nitric acid. The lead concentrations of the analysed samples were 0.004 to 0.008 mg. Tumir et al. (2010) analysed 30 samples of vitamins and herbaceous preparations widely used and sold in Croatia. The Pb concentration range reported was 0.25–3.86 μg/g. In various formulations, the levels of metal were above the maximum limits. In addition, the estimated cumulative daily intake was higher than the daily exposure allowed by the US Pharmacopeia Convention – Advisory Panel on Metal Impurities.

Considering the Pb toxicity to humans, certain countries set limits on this inorganic contaminant for dietary supplements. In 2017, the American Pharmacopoeia (USP) in chapter 2232 defined new limits for elemental contaminants in finished dietary supplement dosage forms (USP, 2017a). The Permitted Daily Exposure (PDE) was derived from the Provisional Tolerable Weekly Intake (PTWI) that is recommended by the FAO/WHO by subtracting the daily exposure (μg/day) to each

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elemental contaminant from air, food and drinking water. The lead PDE of 10 μg/day was established for a body weight of 50 kg. Currently, there is no Brazilian legislation about the limits of inorganic contaminants specifically for these products. As described in the ANVISA regulation RDC n° 243/2018, in the absence of specific Brazilian legislation, the Compendium of Food Supplements of the American Pharmacopoeia can be followed (Brazil, 2018).

The USP Chapter 233 determines inductively coupled plasma atomic emission Spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) techniques for the elemental evaluation of dietary supplements (USP, 2017c). Nevertheless, high-resolution continuous-source atomic absorption spectrometry coupled to a graphite furnace atomiser (HR-CS GFAAS) is highly sensitive and specific equipment for the analysis of inorganic components. This study reports on the development of a simple, fast and reliable method for the determination of Pb in dietary supplement samples by HR-CS-GF-AAS using direct solid sampling. This technique is attractive because it has the possibility of calibration with aqueous standards for direct solid analysis, besides it presents low detection limits and background correction by the least-squares algorithm.

2. Experimental

2.1. Instrumentation

Measurements were carried out using a Model ContrAA 700 high-resolution continuum-source atomic absorption spectrometer (HR-CS-AAS; Analytik Jena AG, Jena, Germany), equipped with a transversely heated graphite atomiser and an MPE 60z autosampler. Argon 99.996% (White Martins, São Paulo, Brazil) was used as the purging gas. Atomisation was carried out on pyrolytic-coated graphite tubes (with integrated platforms) from Analytik Jena AG. The samples were weighed directly onto the graphite platform using a Sartorius M2P micro-balance (Göttingen, Germany) with a precision of 0.001 mg. Instrumental parameters, operational conditions and furnace temperature programs are shown in Table 1. For the Zr tube covering, the tube was treated with $ZrCl_4$ by applying 20 μL of a 1 g/L Zr solution onto the furnace and submitting the tube to a specific temperature program, which is also shown in Table 1. This procedure was repeated 25 times to obtain a deposit of 500 μg of Zr as a permanent modifier.

A sample with a mass of approximately 0.8–1.0 mg was introduced into the atomisation compartment using a pair of tweezers from the Analytik Jena SSA 600 automated solid sampling accessory. All of the measurements were made in triplicate and based on the peak volume integrated absorbance equivalent to three pixels. Although the most sensitive analytical line for Pb is at 217.001 nm, all atomic absorption measurements were carried out at 283.306 nm (less sensitive or secondary line). This line is preferred because it has less interference from PO molecular structures. The absorbance values were normalised to 1.0 mg of sample.

Table 1
Linearity data for lead determination in dietary supplements using HR-CS GF AAS. T_{pir} = 1300 °C, T_{at} = 2200 °C, λ = 283.306 nm.

Parameters	Results
Analytic range (μg/L)	0 – 400
Slope ± standard deviation[a]	$0.0058 \pm 2.0 \times 10^{-4}$
Intercept ± standard deviation[a]	$0.0691 \pm 3.9 \times 10^{-2}$
Confidence limit of slope[b]	0.0002 to 0.0118
Confidence limit of intercept	0.0001 to 0.1381
Correlation coefficient (r)	0.9969
LOD (pg)	2.2
LOQ (pg)	7.2
m_0 (pg/1%A)	15.2
Analysis of variance	
Linear regression	3289.8 (4.6[c])
Linearity deviation	2.90 (2.96[c])

[a] Data obtained from three calibration curves.
[b] 95% confidence limit.
[c] Critical values for F at $p = 0.05$.

2.2. Reagents and samples

All reagents were of an analytical grade, and all the solutions were prepared with distilled and deionised water which was further purified by a Milli-Q high-purity water device (with an electrical resistivity of 18.0 MΩ cm; Millipore, Bedford, MA). All the laboratory material was immersed for at least 48 h in a 10% (v/v) nitric acid–ethanol solution and washed with Milli-Q purified water shortly before use. The reagents used in this study were supplied by Sigma-Aldrich (St. Louis, MO) and Merck (Darmstadt, Germany). Nitric acid was further sub-boiled and distilled in a Berghof Teflon apparatus (Eningen, Germany). Standard solutions were prepared from adequate dilutions of a 1000 mg/L Pb stock solution (NIST Standard Reference Material 3201a). The chemical modifiers used in this measurement were 1 g/L of $Pd(NO_3)_2$ (Fluka, Switzerland) and 1 g/L of $Mg(NO_3)_2$ (Merck).

The samples (n = 74) were obtained from Brazilian websites and stores specialising in dietary supplements. All products were stored at room temperature (18–30 °C) until analysis. No sample digestion procedure was performed. The tablets were ground and mixed before analysis, while the capsules were opened, and the content was directly analysed without any pre-treatment. The particle size influence was not evaluated in this study.

2.3. Method validation

The method was validated by the determination of the following operational characteristics: specificity, linearity, quantification limit, detection limit, precision and accuracy. The linearity was evaluated by ten-point calibration curves performed on three different days. The integrated absorbance values were obtained by injecting 20 μL of standard solutions containing 0, 10, 20, 30, 40, 50, 100, 200, 300 and 400 μg/L of Pb into the furnace. The linearity data were evaluated by an analysis of variance (ANOVA). The limits of detection (LOD, mg/L) were calculated from the equation LOD = 3.3 X Sa/b, where S*a* is the intercept standard deviation, and *b* is the slope. Three replicates were measured unless otherwise stated, and all of the measurements were based on the integrated absorbance values. The repeatability was expressed by the variation coefficients (expressed as RSD) of the results obtained in six samples prepared independently of the same batch, with the addition of 10 μg/L of standard Pb solution. The acceptance criteria for the relative standard deviation was NMT 20% (n = 6). The repeatability analysis was performed again on a different day for intermediate precision. The results were combined, so the total number of analyses was 12. The acceptance criteria for the relative standard deviation was NMT 25% (n = 12). The characteristic mass (ɱ0, pg) was calculated from the slope (*b*) of the calibration curve, using the equation ɱ0 = 0.0044 × 20/b for a sample volume of 20 μL. The accuracy was evaluated as a percentage of the recovery obtained from analysing samples spiked with known amounts of Pb standard at three fixed concentration levels. The accuracy was expressed by the recovery results obtained in triplicate for the following concentration levels: 10.0, 30.0 and 50.0 μg/L. Lead was also determined in Bitter Orange-Containing Solid Oral Form SRM 3260 from the National Institute of Standards and Technology (NIST, USA).

Statistical calculations and the construction of the boxplot graph for the analysis of the lead concentration in dietary supplements samples were performed with Prism 7 software (GraphPad, USA).