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DETERMINATION OF ELEMENTAL COMPOSITION IN DIETARY SUPPLEMENTS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Dietary supplements intake has grown in the last years because of their potential health benefits. This supplementation is very common among athletes, elderly population and consumers that want to increase the total daily nutrient intake. Consequently, elemental composition evaluation in these supplements is of great interest due to its increasingly high consumption and the brand variety offered in the market. This study aimed to evaluate the elemental composition in three types of dietary supplements acquired in a pharmacy and drugstore in São Paulo city. Concentrations of As, Br, Ca, Co, Cr, Cu, Fe, K, La, Na, Sb, Sc, Se and Zn were determined in these supplements by applying neutron activation analysis (NAA) followed by a gamma ray spectrometry. From the concentrations obtained in the dietary supplement analyses, the data obtained were compared to the values presented on the product label. These comparisons indicated in general, a good agreement of the data obtained and the values of the product label depending on the supplement. From the results obtained it can be concluded that NAA is an important tool for the analysis of this type of products due to its reliability of results and its multielemental character.

1. INTRODUCTION

With the knowledge of the chemical elements functions in the human organism and the improvement of the analytical techniques, studies on elemental composition in pharmaceutical products have become of great interest to evaluate the effects of certain elements in these products.

Among these pharmaceutical products, the dietary supplements have been highlighted. Dietary supplements intake has grown in the last years because of their potential health benefits. This supplementation is very common among athletes, elderly population and consumers that want to increase the total daily nutrient intake. These supplements are sold without any medical prescription. Thus, it is very important to verify the quality by analyzing the elemental composition to compare the values obtained in the analyses with those stated on the label of the products. Furthermore, the supplements may contain toxic elements.

There are varied conclusions and opinions concerning or encouraging the usage of dietary supplements. Chandra [1] who studied the dietary supplements effects in the cognitive functions in an elderly population showed that these products can improve cognitive functions by improving life quality and can delay the development of Alzheimer's disease.

Garcia-Rico et al [2] analyzed dietary supplement samples by atomic absorption spectrometry and verified that some supplements present Pb in quantities 10 % higher than the tolerable daily ingestion value.

Marrero et al [3] using inductively coupled plasma optical emission spectroscopy (ICP-OES) determined several elements in supplements acquired in Argentina and United States and compared with the declared values on the label. Toxic elements such as As, Cd, Hg and Pb were also determined. These researchers found that in general, the values obtained in the determinations are in agreement with the declared values on the labels. However, among different pills of the same package of the dietary supplement, they found significant differences, with a variation of up to 100 %.

Brazilian governmental agencies such as the Health Ministry and the *Agência Nacional de Vigilância Sanitária* (ANVISA) are trying to control and supervise all products related to human consumption. Therefore, it is very important to develop reliable methods for dietary supplement analysis, since these products can contain toxic elements or not present the concentration of the essential elements declared on the labels.

In this study, three samples of dietary supplements were analyzed, using the Neutron Activation Analysis (NAA) method, to compare with the declared values on the label of these products and to identify any toxic elements.

2. MATERIALS AND METHODS

2.1. Sample Acquisition

Dietary supplement samples were acquired in drugstores or supplement stores in São Paulo city. Two protein supplements and one multivitamin and mineral supplement were analyzed. These supplements were coded and the characteristics of the analyzed supplements are described in Table 1.

2.2. Sample Preparation

Using an agate mortar, the tablets of the coded supplement CTA were milled to a powder to obtain better homogeneity. The supplement coded as SW and M were already in powder form. This powder obtained was weighed in a Shimadzu balance with precision of ± 0.00001 g and packed in polyethylene bags previously demineralized using HNO_3 acid solution and purified water (Milli-Q).

2.3. Certified Reference Material

Accuracy and precision of the procedure of NAA, was verified by analyzing the certified reference materials (CRM) 1633b Constituent Elements in Coal Fly Ash and the 1632d Trace Elements in Coal (Bituminous), both provided by the National Institute of Standards & Technology (NIST). In order to present results on dry basis, the moisture content was determined in these products. Aliquots of 300 mg of each material were dried for 24 hours at

85º C in an oven. In this process, the CRM 1633b presented loss weight of 1.33 % and the CRM 1632d presented loss weight of 1.67 %.

Table 1: Characteristics of the analyzed supplements.

Supplement code	Characteristics	Form	Dose (g) per tablet or scoop	Humidity[a], (%)
CTA	Multivitamin and mineral supplement containing 13 microelements, made in Canada	Tablet	1.32	1.33
SW	Protein supplement for athletes containing 11 microelements, made in Brazil	Powder	20	5.53
M	Protein supplement for athletes containing 11 microelements, made in Brazil	Powder	25.8	5.18

a. Determined in this study

2.4. Preparation of Synthetic Elemental Standards

Synthetic elemental standards were used to quantify the element concentrations. These standards were prepared by pipetting aliquots of the standard solutions on sheets of Whatman N.40 filter paper. Synthetic standard solutions were obtained using certified standard solutions of elements from Spex CertiPrep, USA. The pipette used was from Eppendorff and its calibration was verified previously. The filter paper sheets were placed in a desiccator to dry the aliquots, and then packed in polyethylene bags.

2.5. Neutron Activation Analysis Procedure

Supplement samples, CRM and synthetic elemental standards were wrapped in aluminum foil and then placed into an aluminum irradiation device to be irradiated at the IEA-R1 nuclear research reactor of the Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN-SP). The irradiation time was 8 hours under a thermal neutron flux about 4.0×10^{12} n cm^{-2} s^{-1}.

After a 3 day decay period, samples and synthetic elemental standards were placed on stainless steel planchets to measure the induced gamma ray activities. These measurements were carried out using a high-purity Ge detector, GC19020 model coupled to a digital spectrum analyzer DSA 1000, both from Canberra. The system resolution used was 1.00 keV for the 121.97 keV peak of ^{57}Co and 1.80 keV for the 1332.49 keV peak of ^{60}Co. Genie 2000 version 3.1 software from Canberra was used for data acquisition and spectrum processing.

The radioisotopes were identified by their half-life and the gamma ray energies. Radioisotopes used in this study were ^{76}As, ^{82}Br, ^{47}Ca, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{59}Fe, ^{42}K, ^{140}La, ^{86}Rb, ^{124}Sb, ^{46}Sc, ^{75}Se and ^{65}Zn.

After identifying the radioisotopes, it was possible to quantify the elements present in the samples by comparative method applying equation 1 below [4].

$$C_a = \frac{m_p\, A_a\, e^{0{,}693\ (tda - tdp)/t_{1/2}}}{M_a\, A_p} \quad (1)$$

Where a and p refers to sample and standard, respectively:
M_a = Total sample mass; m_p = element mass in the standard; C_a = element concentration in the sample; $t_{1/2}$ = half-life of the radioisotope; td = decay time. The term $e^{0,692\ (tda-tdp)/t_{1/2}}$ allows to calculate the radioisotope activities in both sample and standard at the same decay time.

3. RESULTS AND DISCUSSION

3.1. Certified Reference Material Analysis

Results obtained in the analyses of CRM Coal Fly Ash and Coal Bituminous are shown in Tables 2 and 3 respectively. These Tables also present the values of the certificates and the standardized difference values or Z-score, calculated using Equation 2 [5].

$$Z_i - score = \frac{C_i - C_{ref,i}}{\sqrt{\sigma_i^2 + \sigma_{ref,i}^2}} \quad (2)$$

Where:
C_i = Concentration of element i obtained; $C_{ref,i}$ = concentration of the certified value or consensus value for element i; σ_i = combined uncertainty of the concentration for the obtained element i; $\sigma_{ref,i}$ = combined uncertainty of the certified value for the element i.

According to Konieczka and Namiesnik [5], the Z-score results are considered satisfactory if $|Z| \leq 2$. If $2 < |Z| < 3$, the result is considered uncertain, but if $|Z| \geq 3$, the result is considered unsatisfactory.

In Table 2, the Z-score values showed that the concentration of the elements on the CRM Coal Fly Ash, are in agreement with the obtained values for Ca, Fe, K, Na and Se. For As and Cr, the result was less satisfactory. However, in Table 3, the Z-score values for As and Cr of the CRM Coal Bituminous, were satisfactory. In Table 3, only Ca presented an uncertain result. However, in Table 2, the Z-score for Ca was satisfactory.

Table 2: Elemental concentrations of CRM Coal Fly Ash. Results in mg kg^{-1} unless otherwise indicated.

Elements	$\overline{X}$ ± SD[a]	Z-score[b]	Values of the certificate [6]
As	123.4 ± 5.6	2.2	136.2 ± 2.6
Br	2.98 ± 0.41		2.9[c]
Ca, %	1.43 ± 0.28	0.3	1.51 ± 0.06
Co	39.6 ± 2.6		50[c]
Cr	159.1 ± 9.7	3.9	198.2 ± 4.7
Cs	8.75 ± 0.82		11[c]
Fe, %	7.01 ± 0.42	1.8	7.78 ± 0.23
K, %	1.84 ± 0.09	1.1	1.95 ±0.03
La	79.5 ± 2.2		94[c]
Na, %	0.22 ± 0.01	1.6	0.201 ± 0.003
Rb	122.5 ± 2.8		140[c]
Sb	4.93 ± 0.14		6[c]
Sc	34.8 ± 1.6		41[c]
Se	10.49 ± 0.67	0.3	10.26 ± 0.17
Zn	10.35 ± 0.18		210[c]

a. Arithmetic mean and standard deviation of three determinations; b. Z-score value; c. Noncertified values.

Table 3: Elemental concentrations in CRM Coal Bituminous. Results in mg kg^{-1} unless otherwise indicated.

Elements	$\overline{X}$ ± SD[a]	Z-score[b]	Values of the certificate [7]
As	5.84 ± 0.14	1.5	6.1 ± 0.2
Ca, %	0.166 ± 0.008	2.7	0.144 ± 0.003
Co	3.19 ± 0.21	1.1	3.424 ± 0.048
Cr	13.72 ± 0.19	0.1	13.7 ± 0.1
Cs	0.605 ± 0.008	0.8	0.598 ± 0.006
Fe, %	0.79 ± 0.03	1.5	0.749 ± 0.016
K, %	0.1097 ± 0.0003	0.3	0.1094 ± 0.0026
La	5.88 ± 0.12		6[c]
Na	359.16 ± 29.75	2.1	296.9 ± 4.2
Rb	7.82 ± 0.78	0.6	7.36 ± 0.20
Sb	0.453 ± 0.002	1.1	0.445 ± 0.015
Sc	2.91 ± 0.11	0.2	2.89 ± 0.03
Se	1.36 ± 0.09	0.8	1.29 ± 1.2
Zn	11.59 ± 0.29	2.0	12.9± 1.2

a. Arithmetic mean and standard deviation of two determinations; b. Z-score value; c. Noncertified values.

3.2. Elemental Composition of Dietary Supplements

For the dietary supplement analyses, the results were calculated on dry weight basis and are shown in Table 4.

Table 4: Mean concentration and standard deviation on dry weight basis. Results in µg g^{-1} unless otherwise indicated.

Element	CTA Supplement		M Supplement		SW Supplement	
	C ± SD[a]	RSD[b] (%)	C ± SD	RSD (%)	C ± SD	RSD (%)
Br	3.698 ± 0.073	2.0	10.29 ± 0.09	0.86	0.801 ± 0.043	5.4
Ca, %	15.63 ± 0.38	5.6	0.252 ± 0.014	5.7	0.808 ± 0.038	4.7
Co	0.174 ± 0.024	13.7	0.0244 ± 0.0025	10.1	0.116 ± 0.027	23.6
Cr	14.42 ± 0.47	3.3	0.155 ± 0.028	18.3	0.232 ± 0.067	28.3
Cu	432.49 ± 53.88	12.5	ND	-	ND	-
Fe	6039.24 ± 90.78	1.5	38.26 ± 1.71	4.5	113.1 ± 3.3	2.9
K	ND[c]	-	3703.3 ± 122.6	3.3	5349.0 ± 86.2	1.6
La	0.67 ± 0.10	14.6	0.0165 ± 0.0030	18.4	0.053 ± 0.009	17.4
Na	1403.14 ± 2.91	0.21	3058.6 ± 7.7	0.25	1760.7 ± 4.3	0.25
Rb	ND	-	3.26 ± 0.12	3.6	5.16 ± 0.19	3.6
Sb	0.046 ± 0.011	24.0	ND	-	ND	-
Sc	0.049 ± 0.004	7.3	0.0012 ± 0.0003	23.6	0.115 ± 0.001	1.2
Se	14.00 ± 0.51	3.6	0.109 ± 0.031	28.6	0.999 ± 0.074	7.4
Zn	4694.30 ± 47.53	1.0	21.04 ± 0.28	1.3	11.16 ± 0.21	1.9

a. Mean concentration and standard deviation. Number of determinations of supplement CTA, M and SW were 3, 2 and 2 respectively; b. Relative standard deviation; c. Not detected

Under the experimental conditions used, no toxic elements such as As, Cd and Hg were detected. However, Sb was detected in the CTA supplement, though in low concentration.

From the mean concentration obtained for these supplements (Table 4), it was possible to calculate the data to be used to compare with product label values. The data used for this comparison are presented in Table 5.

Daily intake of these supplements was calculated as per consumption instructions stated on the product labels.

According to the technical regulation of nutritional labeling of foods and dietary supplements in Brazil, there is a legal tolerance of ± 20% of the declared nutrient quantities on the label [8]. Adopting this criterion, in the CTA supplement, the quantities of the determined elements are in agreement with the label values.

In the M supplement, in 7 essential elements determined, only Na is in agreement with the label. The other elements are out of the range of the Brazilian regulation, especially Cr, in which is 170% over the label value. It was not possible to detect Cu in this supplement, although it is declared on the label.

In the SW supplement, in 5 essential elements determined, only Ca presented a good agreement with the label value. The element Se presented 6% of the declared value.

Table 5: Mass of the elements per tablet or dose in the analyzed dietary supplements, compared to the described values on the label. Results are given in µg unless otherwise indicated.

Sample	Elements	Mass of the element per tablet or dose[a]	Product label values
CTA	Ca, mg	221 ± 12	250
	Cr	18.60 ± 0.62	18
	Cu	558 ± 70	450
	Fe, mg	7.79 ± 0.13	8.1
	Se	18.06 ± 0.67	20
	Zn, mg	6.056 ± 0.077	7.0
M	Ca, mg	307.92 ± 0.14	187
	Cr	18.947 ± 0.028	7
	Cu	ND[b]	180
	Fe, mg	4.680 ± 0.002	2.8
	Na, mg	374.130 ± 0.008	377
	Se	13.351 ± 0.031	6.8
	Zn, mg	2.5730 ± 0.0003	1.4
SW	Ca, mg	305.389 ± 0.383	318
	Cr	8.786 ± 0.066	6.7
	Fe, mg	4.275 ± 0.003	2.5
	Na, mg	66.538 ± 0.004	81
	Se, mg	0.0377 ± 0.0001	0.60

a. Dose for M supplement is 5 scoops, or 129 g and dose for the SW supplement is 2 scoops, or 40 g; b. Not detected.

4. CONCLUSIONS

From the results obtained in the CRM, it is possible to conclude that the NAA is a good tool to analyze this type of samples, providing reliable results.

From the results obtained in the analysis of the dietary supplements, not all the supplements contain the stated values on the product labels. Beside this, no toxic elements were detected.

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