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ORIGINAL ARTICLE

Quantification of metals in LECISAN® by inductively coupled plasma optical emission spectrometry

Cuantificación de metales en LECISAN®por espectrometría de emisión óptica con plasma acoplado inductivamente

Quantificação de metais em LECISAN® por espectrometria de emissão óptica de plasma acoplado indutivamente

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ABSTRACT

Introduction: the pollution resulting from industrialization has led to an increase in the concentration of metals in various environments and their incorporation into raw materials and finished products, which has a direct impact on human health. **Objective:** to quantify metals in the raw material used in the manufacture of the LECISAN® chewable tablet and in the tablet, to establish reference levels of: aluminum, calcium, cadmium, chromium, copper, iron, magnesium, lead, silicon, vanadium and zinc**. Method:** atomic optical emission spectrometry with inductively coupled plasma (ICP OES) was used and confidence intervals for the mean of the quantified values were established using statistical methods with a probability of 95%. **Results:** there are significant differences between the two presentation forms in terms of the amount of all metals, except aluminum ($p<0.05$). The variation coefficients were low, chromium obtained a value close to 10% in the raw material and lead was high (17.44%). The values are within the range reported as permissible according to Cuban standards (NC 493:2012) except for lead and copper. Higher concentrations of those elements with favorable health effects were observed. **Conclusions:** the results found should be considered as references for the evaluation of the impact that the daily administration regimen of the LECISAN® nutritional supplement would have on health and the analysis of factors related to the presence of metals in by-products of soybean oil refining for future research.

Keywords: heavy metals; soy lecithin; pollution; bioaccumulation; nutritional supplement; LECISAN®

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RESUMEN

Introducción: la contaminación resultante de la industrialización ha propiciado el aumento de la concentración de metales en diversos ambientes y su incorporación a materias primas y productos terminados, lo que repercute directamente en la salud humana. **Objetivo:**cuantificar metales en la materia prima empleada en la fabricación de la tableta masticable LECISAN® y en la tableta, paraestablecer niveles de referencia de: aluminio, calcio, cadmio, cromo, cobre, hierro, magnesio, plomo, silicio, vanadio y zinc.**Método:**se utilizó la espectrometría atómica de emisión óptica con plasma inductivamente acoplado(ICP OES) y se establecieron mediante métodos estadísticos los intervalos de confianza para la media de los valorescuantificados con una probabilidad del 95%.**Resultados:**existen diferencias significativas entre las dos formas de presentación en cuanto a la cantidad de todos los metales, excepto aluminio ($p<0,05$). Los coeficientes de variación fueron bajos, el cromo obtuvo un valor cercano al 10% en la materia prima y el plomo resultó elevado (17,44 %). Los valores se encuentran dentro del rango reportado como permisibles según norma cubana (NC 493:2012) excepto para plomo y cobre. Se observaron mayores concentraciones de aquellos elementos con efectos favorables para la salud. **Conclusiones:** los resultados encontrados deben ser considerados como referenciales para la evaluación del impacto que sobre la salud tendría el régimen de administración diaria del suplemento nutricionalLECISAN®yel análisis de factores relacionados con la presencia de metales en subproductos del refinado del aceite de soya para futura investigaciones.

Palabras clave: metales pesados; lecitina de soya;contaminación; bioacumulación; suplemento nutricional; LECISAN®

RESUMO

Introdução: a poluição decorrente da industrialização tem levado ao aumento da concentração de metais em diversos ambientes e à sua incorporação em matérias-primas e produtos acabados, o que tem impacto direto na saúde humana. **Objetivo:** quantificar metais na matéria-prima utilizada na fabricação do comprimido mastigável LECISAN® e no comprimido, estabelecer teores de referência de: alumínio, cálcio, cádmio, cromo, cobre, ferro, magnésio, chumbo, silício, vanádio e zinco. **Método:** foi utilizada espectrometria de emissão óptica atômica com plasma indutivamente acoplado (ICP OES) e os intervalos de confiança para a média dos valores quantificados foram estabelecidos por meio de métodos estatísticos com probabilidade de 95%. **Resultados:** existem diferenças significativas entre as duas formas de apresentação quanto à quantidade de todos os metais, exceto alumínio ($p<0,05$). Os coeficientes de variação foram baixos, o cromo obteve valor próximo a 10% na matéria-prima e o chumbo foi alto (17,44%). Os valores estão dentro da faixa informada como permitida segundo as normas cubanas (NC 493:2012) exceto para chumbo e cobre. Foram observadas concentrações mais elevadas desses elementos com efeitos favoráveis à saúde. **Conclusões:** os resultados encontrados devem ser considerados referências para a avaliação do impacto que o regime diário de administração do suplemento nutricional LECISAN® teria na saúde e a análise dos fatores relacionados à presença de metais em subprodutos do refino do óleo de soja para pesquisas futuras.

Palavras-chave: metais pesados; lecitina de soja; poluição; bioacumulação; suplemento nutricional; LECISAN®

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INTRODUCTION

LECISAN®, a product registered by Laboratorio Farmacéutico Oriente (LBF, BioCuba Farma, Santiago de Cuba, Cuba), stands out as a drug candidate due to its preventive and/or therapeutic potential.[1] Soy lecithin, the raw material used for its production, has multiple biological and pharmacological actions mainly attributed to its phospholipid constituents (phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol) and, theoretically, its properties are related to the contribution of polyunsaturated fatty acids, such as linoleic acid, isoflavones and phytosterols.[2]

In the manufacture of this chewable tablet from a by-product of the oil refining process, pharmaceutical quality excipients are used, as well as wet granulation and direct compression technology for its development. The registration of the product as a nutritional supplement was approved by the Cuban Regulatory Authority, the National Institute of Hygiene, Epidemiology and Microbiology (INHEM).[1]

The lecithin was characterized as a pharmaceutical raw material and the tablet developed complied with the established quality parameters, proven stability and adequate for a product of natural origin, being comparable to that of similar products available in the international market; however, the complex mixture of phosphatides appears combined with substances of other nature that are carried over from the crude vegetable oil source.[1]

The amount of pollutants facing mankind is growing rapidly, and current industrial production systems often use heavy metals for the extraction of a material or as an element in the refining of a product, which in principle results in their production at low production costs, but could lead to environmental problems due to their toxicity. However, this could lead to environmental problems due to their toxicity; therefore, methods for the treatment of these metals have been developed and their analysis is taken into consideration, especially in products intended for human consumption.[3]

Heavy metals stand out for their ability to interact with biological molecules, even though they are necessary in small amounts for several physiological processes, and tend to accumulate in the organism faster than they can be metabolized, a phenomenon called bioaccumulation.[4]

The objective of this research was to quantify the metals in the raw material used for the manufacture of the LECISAN® chewable tablet and the tablet as a pharmaceutical form, as well as to establish reference levels for: Aluminum (Al), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), Lead (Pb), Silicon (Si), Vanadium (V) and Zinc (Zn). This will provide a basis for the evaluation of the health impact of the daily administration regimen of the nutritional supplement studied.

METHOD

To carry out the experimental part of this work, reagents and equipment from the Laboratory of the EmpresaGeomineraOriente (UEB LaboratorioElioTrincado) and the Analytical Chemistry Laboratory, belonging to the Centro de Estudios de Biotecnología Industrial (CEBI), of the OrienteUniversity were used.

Reagents and equipment used

The pure quality nitric acid (HNO3) and hydrochloric acid (HCl) for analysis came from Merck, Germany. The certified solutions of 1000 ppm for each element for the standard solutions used in the preparation of the calibration curves were from BDH, England. The equipments used were: ICP-OCP-AES spectrometer with axial observation mode; (Spectro ARCOS, Analytical Instruments. Model: FHX, type: 76004553, Kleve, Germany); Torch (Vista Radial SOP, Germany); Muffle furnace (Barnstead ThermolyneFurnace 1300, DUBUQUE Model: FB1315M, Serial: 1256070665453, IOWA, U.S.A.);BOCHEM BUNSEN burner (Bunsen DIN burner model 30665, Usbeck, Germany)

Analytical Technique

Trace metal determinations in the raw material used for the manufacture of LECISAN® chewable tablet and in the finished dosage form were carried out according to the methods described in the United States Environmental Protection Agency (USEPA EPA- 600/4-79-020).(7) Measurements were performed with an ICP-OCP-AES spectrometer with axial observation mode. The operating parameters were: plasma power 1400 W, flow rate 30 rpm, coolant flow 12 L/min, auxiliary flow and nebulizer flow 1L/min, and vertical position of the torch.

Sampling, sample and calibration solution preparation

Random sampling of the available batches was performed. The chewable tablet samples were crushed and homogenized with the use of an agate mortar, 10 g of this and the raw material were weighed and transferred separately to a previously treated platinum capsule. In both cases a filter paper, folded in the form of a cone, was placed in a platinum capsule with the base on the mass of the product, in such a way that it remained stable.

For the raw material, after the cone was impregnated with the sample, slow combustion was performed with a burner placed under the capsule, until black ash was obtained, ensuring that the flame remained uniform.

The platinum capsule with the cooled and dried carbonaceous residue was placed in a muffle furnace at 775±25 ℃ for 4 hours, until complete incineration indicated by the grayish white color of the ashes, which were passed into the platinum capsule and transferred to a 250 ml capacity beaker where 15 ml of 25% nitric acid and 1 ml of 1:1 hydrochloric acid were added.

Subsequently, it was heated very carefully in the beaker, avoiding to take it to dryness to pour the extract to a volumetric of 50 ml and it was made up to the mark with a solution of nitric acid at 10 %. Five standards between 0 and 50 ppm were prepared in 50 ml volumetric flasks in 10 % nitric acid, from certified solutions of 1 000 ppm for each element. From this solution, a multiple standard of 100 ppm was prepared with the elements and, from it, corresponding aliquots were taken for each point of the curve using a reagent blank.[5,6]

Measurement of metals in selected samples

The solutions thus prepared were ready for the determination of the concentrations of Aluminum (Al), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), Lead (Pb), Silicon (Si), Vanadium (V) and Zinc (Zn). The measurements of the emission line intensities according to references are shown in Table 1.[5,6]

Table 1 Emission lines used in the ICP-OES Spectro ARCOS ICP-OES

Metal	Lines (nm)
Al	308,215
Ca	393.366
Cd	228,802
Cr	284,325
Cu	324,754
Fe	259,941
Mg	279.553
Pb	220,353
Si	288.158
V	221.667
Zn	213,856

The general environmental conditions were maintained during the test: temperature between 15 and 30°C, and relative humidity between 20 and 80%. The equipment was configured to perform three instrumental replicates of each sample to calculate the mean, which was the final value used. The equipment should be started up some time before starting the sample analysis so that it can reach optimum operating conditions. The order of analysis of the samples in the equipment is: blank, proposed standards, tablet samples, raw material samples.

Adequacy of the method

For a confidence level of 95% and the t-statistic was equal to 1.96, the experimental detection limit (EDL) was calculated from the instrumental detection limit (IDL) using the following formula:

$$LDE = LDI \times k$$

The correction factor k being determined by the formula:

$$k = t / \sqrt{n}$$

Where n is the number of replicates used to determine the LOD. Three replicates were used to determine the LOD, resulting in k =1.96 / √3 = 1.13.

The results of the LDI and LDE calculation are shown below in Table 2.

Table 2 Results of method suitability parameters

Elements	LDI (3-σ)*	LDE
Al	0,0486000	0,0549180
Ca	0,0400000	0,0452000
Cd	0,0035900	0,0040560
Cr	0,0039100	0,00441830
Cu	0,0028600	0,00323180
Fe	0,0274000	0,0309620
Mg	0,0101000	0,0114130
Pb	0,0647000	0,0731110
Si	0,0500000	0,0565000
V	0,0072400	0,0081812
Zn	0,0768000	0,0867840

Quality control was performed by checking the calibration curve, verifying always that the regression coefficient was ≥0.99.

Statistical analysis of the results

The Minitab® statistical processor (64-bit© 2019, version 19.2) was used for statistical analysis. The Mann-Whitney U-test applied to two independent samples was used.

To establish the reference levels of the investigated analytes, the sample preparation process and measurements were made by the same analyst on the same day, to avoid biases. The mean value, standard deviation and coefficient of variation (CV) were determined for each sample in triplicate with a probability of 95 %.

This work is part of the sectorial project "Nutritional effects, pharmacology and preclinical toxicology of LECISAN®" (PS1089SC002), which aims to provide elements to support the product, currently registered as a nutritional supplement. It was approved by the ethics committee from the ethical, scientific and methodological point of view.

RESULTS

Table 3 shows the comparative analysis between reference values of raw material and chewable tablet of LECISAN®, in which significant differences were observed between the two forms of presentation in terms of the amount of all metals, except Al (p<0,05). For all the parameters studied, the coefficients of variation (CV) were low. However, for Cr a value close to 10% was obtained in the raw material and for Pb it was high (17.44%), suggesting greater heterogeneity of its values.

Table 3 Statistical parameters for the comparative analysis between elemental composition values of raw material and chewable tablet of LECISAN®.

Metal	Concentration (ppm) Tablet			Concentration (ppm) Raw Material			Asymptotic significance (bilateral)
	Media	DE	CV%	Media	DE	CV%	
Al	3,83	0,02	0,58	3,91	0,09	2,44	0,246
Ca	359,54	11,19	3,11	1284,27	4,97	0,38	0,050
Mg	659,98	1,03	0,15	824,97	4,74	0,57	0,050
Si	43,32	0,61	1,41	21,93	0,18	0,81	0,050
Fe	22,33	0,38	1,69	39,47	0,94	2,39	0,050
Cu	1,26	0,01	0,79	1,35	0,01	0,56	0,046
V	0,89	0,00	0,16	0,46	0,00	0,62	0,034
Zn	1,68	0,07	3,97	7,48	0,08	1,04	0,050
Cr	3,98	0,19	4,80	0,52	0,04	9,43	0,046
Cd	3,27	0,00	0,08	1,65	0,00	0,06	0,034
Pb	1,68	0,29	17,44	3,96	0,32	8,16	0,050

Statistically significant p<0.05 value

Table 4 summarizes the elemental concentration in the raw material used to manufacture the LECISAN supplement and establishes comparisons with references in the country [8,9] for the maximum allowable intake levels and the values of the elemental concentrations.

Metal concentrations expressed in mg/L were in the following decreasing order [Ca] < [Mg] < [Fe] < [Si] < [Zn] < [Pb] < [Al] < [Cd] < [Cr] < [Cu] < [V].

For Cd and V, all samples showed similar values of 1.65 mg/kg and 0.46 mg/kg, respectively, and higher concentrations of those elements with favorable health effects are observed, except for the heavy metals Pb and Cu, which was higher and pose a risk due to bioaccumulation.

Table 4 Elemental composition determined in the raw material for the manufacture of LECISAN®.

Element	NC 493:2012[8]			Concentration (mg/L)				P-value
	IDA	ISTP	VDR[9]	Minimum	Intermediate	Maximum	X±S	
Al	ND	ND	ND	3,82	3,90	4,01	3,91±0,09	0,246
Ca	ND	ND	860,0	1281,27	1281,52	1290,01	1284,27±4,97	0,050*
Mg	ND	ND	325,0	820,55	824,39	829,98	824,97±4,74	0,050*
Si	ND	ND	ND	21,99	21,73	22,07	21,93±0,94	0,050*
Fe	0,8	ND	17,0-45,0	38,37	40,01	40,02	39,47±0,18	0,050*
Cu	0,50	ND	0,90	**1,34**	**1,35**	**1,35**	**1,35±0,02**	0,050*
V	ND	ND	ND	0,46	0,46	0,46	0,46±0,00	0,046*
Zn	1,00	12,0	0,80-14,0	7,39	7,55	7,50	7,48±0,01	0,034*
Cr	ND	ND	0,03	0,48	0,50	0,57	0,52±0,04	0,046*
Cd	0,1	0,007	ND	1,65	1,65	1,65	1,65±0,00	0,050*
Pb	0,2	0.025	ND	**3,68**	**389**	**4,32**	**3,96±0,08**	0,034*

X-mean, S-standard deviation *Statistically significant p<0.05 value
Legend:ADI-Admissible daily intake (mg/kg/day),PTWI-provisional tolerable weekly intake (mg/kg /week), DRV-dietary reference values (mg/day) estimated for an average adult weighing 70kg. ND Not determinable due to lack of data on adverse effects and/or lack of knowledge regarding possible lack of ability to handle excessive amounts.

DISCUSSION

Dietary supplements are defined as a substance or mixture of substances, intended to provide nutrients normally present in food, but may have an effect on normal organ function. They are regulated according to food standards and are exempt from the quality, effectiveness and safety verification tests developed by the FDA, which can take action only when it is proven that the supplement represents an imminent health hazard, and this guarantee is left solely in the hands of the manufacturers.[10,11]

The oral intake of trace elements and their homeostasis has become a major concern for the scientific and medical community and for regulatory bodies such as FAO and WHO. Their deficiency is the origin of diseases and alterations in growth; only a few milligrams of them are required per day and when they pass a certain threshold of concentration they become toxic.[12]

A growing interest has been directed towards heavy metals, due to their high toxicity and persistence in the environment as they are naturally present in soils, although most of the time these concentrations do not pose a risk to human health. However, some of them are bioaccumulative and in recent years human activities have increased the number of emissions into the environment.[3]

When analyzing the elemental concentration in the raw material, it can be assumed that the results obtained in this research are in correspondence with the results reported by Rodríguez-Heredia, et al.[12] These researchers analyze that soybean processing industries are characterized by dischargingwastewater into the environment, generally of organic origin, associated with the nature of the technological process itself. In their study on the evaluation of the quality of wastewater from the Soybean Processing Company, they reported average values obtained for the heavy metals analyzed in the wastewater, which do not constitute a threat to the ecosystem of the Bay of Santiago de Cuba, being within the norms.[13]

Gonzalez, et al.[14] conducted a study to know the effect on the biological and physicochemical characteristics of soils and the probable accumulation of heavy metals in fertigation plants as a cause of heavy metal contamination of foodstuffs, they analyzed soybean crops, and showed the presence of several metals, some heavy metals such as chromium, copper, mercury, molybdenum, nickel and zinc.

Cuban Standard NC 493:2012(8) takes into account the updated and recommended criteria of the international market. It establishes the principles and procedures applied and recommended by the Codex Alimentarius in relation to metal contaminants present in food, as well as the maximum permitted levels, acceptable daily intake (ADI) and provisional tolerable weekly intake (PTWI).(9) Concern regarding lead present in nutritional supplements is especially focused on long-term manifestations in the hemo-lymphopoietic system and the nervous system, where clinical manifestations are imperceptible. In addition, auditory, cardiovascular, nephrological and hematological effects have been demonstrated in children exposed to lead.

Dietary reference values (DRVs) are estimates of daily amounts of nutrients that meet the needs of healthy people. They are only used to issue recommendations on nutrient intake and as a basis for information when establishing healthy dietary guidelines, but should be used for nutritional supplementation.[9] In the authors' opinion, the results of this study could serve as a reference for future research to quantify the potential damage to health that would result from daily supplementation and to establish DRVs.

In the present investigation, the estimated concentrations of metals were compared with the limits established in that standard,[8] being regulated contaminants of less toxicological significance Iron (Fe) (ADI 0.8), Zinc (Zn) (ADI 1.0) and Copper (Cu) (ADI 0.5), which exceeded the permissible limit for Cu. The values referred for Lead (Pb) (PTWI 0.025) and Cadmium (Cd) (PTWI 0.007), considered of risk by the bioaccumulative effect, and which are toxic at any concentration,[12] showed the first one in risk limits, which deserves investigations to evaluate the impact on health that an administration in therapeutic regimen would have.

Despite the scientific advances obtained, human exposure to heavy metals and other toxic substances cannot be totally avoided, which constitutes a public health concern at global, regional and local levels.[3] However, an exhaustive characterization of the raw material and the proposal of methods to minimize the presence of heavy metals in it are needed.

The presence of heavy metals in the soybean lecithin samples tested could be explained by deficiencies during grain collection, storage and industrial processing. The use of metal containers with an undocumented content of heavy metals could be one of the causes of the transfer of such contaminants to the grains and their derivatives. Other estimated causes are the deterioration of industrial processing equipment, as a generator of cross-contamination, the use of pesticides that have not been withdrawn from the production chain because other products have become more expensive, and the presence of heavy metals in crop soils.[14]

Non-essential and toxic metals produce reversible or irreversible alterations in the organism, which can even be lethal.[12] Of these non-essential heavy metals or those with no known biological function, the presence of certain quantities in living beings leads to organic dysfunctions.[11]

The systematic and critical review in the databases of the effects of soy lecithin in the last ten years suggests further research in the field of pharmacology and toxicology of this product, as the results concerning its properties and uses are contradictory and inconclusive.[2] It is therefore important to carry out properly designed and approved clinical trials to demonstrate its therapeutic actions and help to further disseminate the advantages of its application.
The results so far analyzed may raise questions about the safety of soy lecithin, in terms of the content of metals with bioaccumulative effects, which should be corroborated by further research. On the other hand, the dosage of a product is based on the amounts frequently used in available clinical trials or in historical practice; however, in natural products it is not always clear what the optimal doses are, so efficacy and safety must be balanced.

Therefore, the analysis of all factors related to the presence of metals in soybean oil refining by-products should be taken into account, from the possible transfer during the technological process, the contamination of soils with chemicals, which then influence the metabolism of plants, to the cultivation and storage of the grain by suppliers, in order to make it suitable for human consumption in nutritional and/or epidemiological terms.

CONCLUSIONS

The values of metals determined by ICP OES are within the range reported as permissible according to the Cuban standard (NC 493:2015) except for lead (Pb) and copper (Cu). These will allow evaluating the impact on health that would have the administration of the nutritional supplement in therapeutic regimen and the analysis of factors related to the presence of metals in soybean oil refining by-products for future research.

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