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DETERMINATION OF CALCIUM CONTENT IN DIETARY SUPPLEMENTS

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Summary

Calcium is a macro element that is very important for the human body: its content and circulation in the body is large, it serves as the electrolyte, it has a building role and participates in the process of metabolism. The European Union, the World Health Organization (WHO) and the Ministry of Food and Drug (Food and Drug Administration, FDA) gave the RDA (Recommended Dietary Allowances,) for this macro element. The absorption and bioavailability of the calcium may vary depending on a number of factors, and because all of the foregoing it is consumed by means of different supplements.

The aim of this study was to determine the content of calcium in the various diet products using the volumetric analytical method of analysis. Supplements that were analyzed are divided into two groups. The first group consists supplements in which the calcium is present in the form of different chemical compounds, and the second group consists of supplements of a number of different manufacturers in which the calcium is in the form of calcium carbonate.

Calcium content, obtained by applying the method above, which ranged from 95.11% to 99.80% compared to the theoretical value. Results were analyzed using the t-test, while not producing a statistically significant difference.

Keywords: calcium, supplements, volumetric analysis method

Introduction

In the chemical analysis different methods of qualitative and quantitative analytical chemistry are used (Harris, 1987). According to the type of physical size, which in the final analysis is the measurement methods of quantitative chemical analysis and it is divided into two main groups: classical methods of analysis and instrumental methods of analysis. Classical methods include gravimetric and volumetric methods of analysis, while the instrumental methods of analysis based on the measurement of physical quantities that are directly related to the amount of determined substances, such as: conductometry, potentiometry, photoelectric photometry, spectrophotometry, etc. (Vindakijević and Sladojević, 2005). Group of methods determining the volume of solution of known concentration of the substance, which came in response to the tested ingredient in foods, called volumetric methods (volumetric titration). The best known are the neutralization reaction, the redox and complexometric titration, which can be used for determining the content of certain vitamins and minerals in food (Grujić et al. 2007).

Calcium (Ca) is a metal that is of all of the mineral matter is the most present in a human body (Hass, 1992; Grujić and Miletić, 2006). Of the total body mass 1.5-2.0% is calcium. Most calcium is incorporated into bone (98%), about 1% is incorporated in the teeth, where it is in the form of $(PO_4)_2 \cdot Ca(OH)_2$ $Ca_5(PO_4)_3(OH)$, but is usually written $Ca_{10}(PO_4)_6(OH)_2$. Bones contain 150 mg Ca /g of dry matter (Bronner, 1994; Otten et al., 2006). The remaining 1% of the total calcium is in the tissues and body fluids, or in soft tissues is 35 mg Ca /g of dry matter. A man weighing 70 kg in the body is about 1.54 kg of calcium.

Calcium plays several important roles in the human organism. The main role of calcium can be represented by the following: Calcium is a component of bones and teeth, calcium regulates the contraction and relaxation of muscles, calcium regulates the functioning of the nerve tissue, the calcium is responsible for the clotting of blood and takes part in the regulation of blood pressure

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and the signal transmission. Magnesium, together with calcium has a role in the functioning of the blood, the work of the nerves and muscles, in particular the regulation of muscle contraction and heart rate and conduction of nervous impulse. Calcium is excreted in the urine and feces (Grujić and Miletić, 2006).

The total amount of calcium consumed is not absorbed. Only 20-50% of the entered calcium is absorbed. The amount of calcium absorbed depends on the type of food, food composition and physiological state of the individual. In order to be able to absorb calcium from the food the presence of vitamin D, of phosphorus, and magnesium is necessary. Together with calcium in the bones and teeth phosphorus is embedded. Vitamin D is required to make the calcium (and phosphorus) absorbed in the digestive tract. Together with some hormones, vitamin D regulates calcium levels in the blood. The balance of calcium in the blood is essential for life, especially for the heart condition. The normal state of the blood calcium is about 10 mg /100 cm3 of blood. The calcium in the blood is in the ion form of Ca^{2+} (5.5 mg), attached to a protein (about 4.0 mg) or linked to the phosphate and citrate (approximately 0.5 mg) (Grujić and Miletić, 2006).

The efficiency of absorption of calcium from most food is about the same. Absorption of calcium may be less if the used foods are rich in oxalic acid (spinach, sweet potatoes and beans) and foods rich in phytic acid (unleavened bread, raw beans, seeds, nuts, soy isolate, and others) (Otten et al., 2006). Some organic acids (oxalic acid, phytic and others) with Ca^{2+} ions form the salt hardly soluble and thus hinder its absorption (Kerstetter et al., 2005). Efficiency of calcium absorption depends on the age of the individual and the greater the younger the person. Certain differences in the absorption of macro elements were observed due to differences in gender of the person, which is probably a consequence of the state of hormones. Absorption calcium in men, irrespective of age, is greater than for women of the same age (Hope et al., 1992; Bygrave and Benedetti, 1993).

Absorption of calcium is in inverse proportion to the amount entered calcium; if the input decreases the extent of absorption efficiency increases. However, the increase in absorption at extremely low calcium intake was not able to fully cover losses due to insufficient intake through food (Otten et al., 2006).

Foods in which the ratio of Ca:P is in the range of from 2:1 to 1:2 allow optimum absorption of Ca. If this relationship is different will be disrupted calcium intake (Allen, 1982; Anderson, 1991).

The main sources of calcium in the human diet are milk, cheese, meat, fish, vegetables and cereals, and its deficiency can lead to various disorders and diseases (osteoporosis, rickets, neurological disorders) (Szefer and Nriagu , 2007).

Chronic calcium deficiency can occur due to insufficient intake or poor absorption in the gut. Then there is its resorption from bone. In this way the health of the bone is endangered. Chronic calcium deficiency is regarded as one of the causes of the reduction of bone mass, osteoporosis and frequent bone fractures. In the United States annually more than 1.5 million bone fractures associated with osteoporosis happen (Otten et al., 2006).

Since absorption of calcium is dependent on many factors, the question of how much of the macro elements should be determined as a reference input. In setting the RDA for calcium Food and Nutrition Board of the National Academy of Sciences USA is estimated that 12-50% of the initial amount of calcium is available for use. RDA is set on a four-fold higher dose (800 mg Ca / day) so that this element was sufficient for the needs of the organism.

However, there are several categories of the population which can not provide optimal amounts of all necessary nutrients through the food. In addition, there are numerous situations in which people have the need for increased intake of certain nutrients. In the category of the population that has a need for a greater intake of vitamins and minerals are athletes, the elderly, pregnant women, menopausal women, people who do heavy physical work or those who are under great mental effort, and patients, convalescents, etc. (Gómez et al., 2011; Zofková et al., 2013; Fanian et al., 2013).

In these situations, the use of supplements can help, not only to avoid disease of deficient diet, but also to supplement the daily diet, and their

use is to improve the health of consumers (Anon, 2014).

The chemical forms of calcium whose use is permitted in the manufacture of nutritional supplements are: calcium-acetate, calcium L-ascorbate, calcium-bisglicinat, calcium-carbonate, calcium-chloride, calcium-citrate-malate, calcium citrate, calcium-gluconate, calcium-glycerophosphate, calcium-lactate, calcium-pyruvate, calcium salt of phosphoric acid, calcium-succinate, calcium L-lisinat, calcium-maltat, calcium-oxide, calcium L-pidolate, calcium L-treonat, calcium-hydroxide, calcium-sulfate (Anon, 2014).

Materials and Methods

Samples used in the process of volumetric determining are:

- "Calcium - gluconate ampoules, 1000mg/10ml",
- "Calcium-citrate, supplement",
- "Eunova-Multi-Vitalstoffe Langzeit 50%",
- "Calcium Complex 600",
- "Calcium with lemon flavor",
- "Effervescent tablets of calcium with orange flavor",
- "Calcimed Hermes 500 mg, effervescent tablets",
- "Calcium plus, 20 effervescent tablets with calcium and vitamins" and
- "Calcium, Vitamin D3".

Analyzed samples were obtained in the free market in Bosnia and Herzegovina and Serbia.

"Calcium-gluconate ampoules, 1000mg/10ml" is the supplement in which calcium is bound in the form of calcium-gluconate for injection ($C_{12}H_{22}CaO_{14}\cdot H_2O$). Calcium-gluconate for injection contains from 99.0% to 101.0% of calcium-D-gluconate, monohydrate. White, crystalline, or granular powder, little soluble in water, and easily soluble in hot water (Pharmacopoes Jugoslavica, 2000).

Data from the packaging - Manufacturer: Monico Spa; Country of origin: Italy; Importer for BiH: Pharmacy Medicus, Prijedor; Shelf life: 04.2015., Series: 12DA109.

"Calcium citrate, supplement" is a composition in which the calcium is bonded in the form of calcium citrate ($C_{12}H_{10}Ca_3O_{14}$), a colorless or white crystalline powder of organic tricarboxylic acids.

Data from the packaging - Manufacturer: Natural Wealth Nutrition Corp., Bohemia, NY; Country of origin: United States; Importer for BiH: M and D Company, Čitluk; Shelf life: 10.2016., Series PB 751001B.

"Eunova-Multi-Vitalstoffe Langzeit 50%" is a supplement in which the calcium is bonded in the form of a chemical compound of calcium-hydrogenphosphate ($CaHPO_4$), and calcium-D-pantothenate ($C_{18}H_{32}CaN_2O_{10}$). Anhydrous calcium-hydrogenphosphate contains from 98.0% to 101.0% $CaHPO_4$, calculated on the dry substance. It occurs as a white, crystalline powder or as colorless crystals, almost insoluble in water and alcohol. It dissolves in dilute hydrochloric acid and dilute nitric acid (Pharmacopoes Jugoslavica, 2000). Calcium-D-pantothenate is a white, almost odorless, slightly hygroscopic powder. It is easily soluble in water, soluble in glycerol, slightly soluble in ethanol and practically insoluble in ether and chloroform (The Chemical Company, 2005).

Data from the packaging - Manufacturer: Hemofarm; Country of origin: Serbia; Shelf life: 02.2015., Series: 990320.

"Calcium Complex 600" is a supplement in which calcium occurs as calcium-citrate ($C_{12}H_{10}Ca_3O_{14}$) and calcium-glycerophosphate ($C_3H_7CaO_6P$).

Data from the packaging - Manufacturer: Biofar, Nahterre; Country of origin: EU, France; Importer for BiH: Pharma Swiss, Serbia; Shelf life: 09.2015., Series: L2259/7.

"Calcium with lemon flavor", "Effervescent tablets of calcium with orange flavor", "Calcimed Hermes 500 mg, effervescent tablets", "Calcium plus, 20 effervescent tablets with calcium and vitamins" and "Calcium, Vitamin D3" are supplements in which the calcium is bonded in the form of calcium-carbonate ($CaCO_3$). Calcium-carbonate contains from 98.5% to 100.5% of $CaCO_3$, calculated relative to the dry substance. It is white powder, virtually insoluble in water (Pharmacopoes Jugoslavica, 2000).

Data from the packaging:

"Calcium with lemon flavor" - Manufacturer: Sunlife GmbH, Horelhof, Germany; Country of

origin: EU, Germany; Importer for BiH: Dugi Commerce, Široki brijeg, Interpromet; Shelf life: 06.2016., Series: L3163/5.
"Effervescent tablets of calcium with orange flavor" - Manufacturer: dm-drogeriemarkt, Karlsruhe, Germany; Country of origin: EU, Germany; Importer for BiH: dm drogeriemark, Ilidža; Shelf life: 01.2016., Series: L4028.
"Calcimed Hermes 500 mg, effervescent tablets" - Manufacturer: Hermes Arzeneimiittel, Munich; Country of origin: EU, Germany; Importer for BiH: Oktal Pharma, Ilidža; Shelf life: 10.2016., Series: 3108351.
"Calcium plus, 20 effervescent tablets with calcium and vitamins" - Manufacturer: Multivita, Vršac, Serbia; Country of origin: Serbia; Importer for BiH: ATACO, Mostar; Shelf life: 01.2016., Series: B304832.
"Calcium, Vitamin D3" - Manufacturer: Bifar, Nanterre- France; Country of origin: EU, France; Importer for BiH: Blagoleks, Bijeljina; Shelf life: 10.2016., Series: L3296/5.
Determination of calcium in these diet products was performed using standard complexometric analysis methods (Rajaković et al., 2000).

Results and discussion

Complexometric calcium determination is based on the reaction:

$$Ca^{2+}(aq) + H_2Y^{2-}(aq) + 2H_2O \leftrightarrow CaY^{2-}(aq) + 2H_3O^{+}(aq),$$
$$CaY^{2-}(aq) \leftrightarrow Ca^{2+}(aq) + Y^{4-}(aq)$$

For the complexometric determination of calcium is used as an indicator murexide. The sensitivity of this reaction is high. Titration with the murexide is done in a very alkaline environment (pH = 11-13), and the change in color of the indicator from red-purple to blue-violet is very easy to see. In a insufficient alkaline environment transition color is not sharp, and in a overly alkaline results are too low (Rajaković et al., 2000). For each analyzed sample is made five solutions of the calcium salt, at least five titrations were done, and from the volume of spent Complexone III middle values were calculated (Table 1 and 2).

Table 1. Content of calcium in dietary supplements in which the calcium is bonded in the form of different chemical compound

Dietary supplement	The mass of the analyzed sample (g)	Volumes of spent reagents (ml)	Calcium content (mg)
Calcium-gluconate ampoules, 1000mg/10ml	0.0186	4.60	183.60
Calcium-citrate, supplement	1.0245	4.87	194.41
Eunova-Multi-Vitalstoffe Langzeit 50%	0.5287	3.00	119.00
Calcium Complex 600	5.4852	14.33	572.00
Calcium, Vitamin D_3	4.4917	12.30	491.00

Table 2. Content of calcium in dietary supplements in which the calcium is bonded in the form of calcium-carbonate

Dietary supplement	The mass of the analyzed sample (g)	Volumes of spent reagents (ml)	Calcium content (mg)
Calcium with lemon flavor	3.9928	12.03	481.00
Effervescent tablets of calcium with orange flavor	4.1162	9.53	380.43
Calcimed Hermes 500 mg, effervescent tablets	3.8928	12.50	499.00
Calcium plus, 20 effervescent tablets with calcium and vitamins	3.7517	6.07	243.00
Calcium, Vitamin D3	4.4917	12.30	491.00

The results of the tests indicate no significant deviation between the values that are listed in the products and the value obtained by applying the used titrimetric methods of analysis (Figure 1 and 2). Statistical calculation of the t-test ($t=1.2087<t_{(4\ i\ 0.05)}=2.78$) it was found that the obtained difference in the values of the content of calcium, bound in the form of different chemical compounds, and the theoretical values of the content of calcium is not statistically significant. The value of t-test ($t=1.0995<t_{(4\ i\ 0.05)}=2.78$) for calcium in the form of calcium-carbonate also showed a statistically not substantively difference ($P>0.95$).

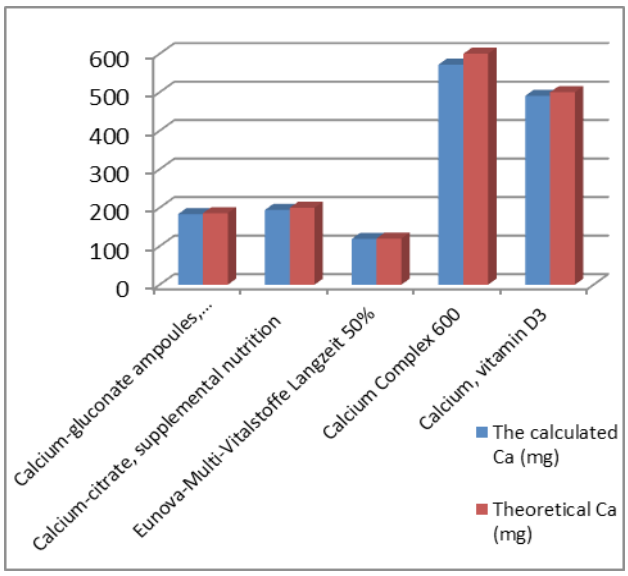

Figure 1. Calculated and the theoretically amount of calcium in supplements in which the calcium is bonded in the form of different chemical compounds

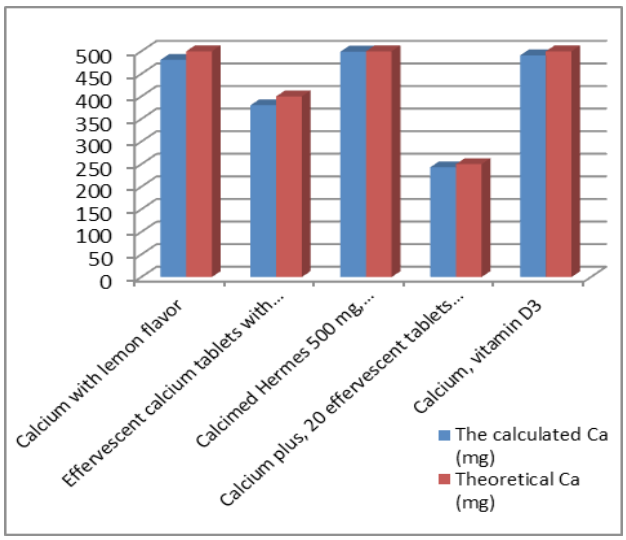

Figure 2. Calculate and the theoretical amount of calcium in supplements in which the calcium is bound in the form of calcium-carbonate

In the samples in which the content has been demonstrated that calcium is bonded in the form of different chemical compounds in the supplements, the difference ranging from 0.83% to 4.70%. With different samples of dietary supplements in which calcium is bound in the form of calcium- carbonate, the difference ranged from 0.20% to 4.80%. Recovery values of all tested dietary supplements are shown in Table 3. They were obtained as the ratio of the calculated mass of the tested calcium and theoretical mass of calcium analyzed sample multiplied by 100%. From the results it can be seen that the content of calcium, obtained by applying the methods above ranged from 95.11% to 99.80% compared to the theoretical value.

Table 3. Recovery values for all analyzed dietary supplements

Dietary supplements	**Recovery value (%)**
Calcium-gluconate ampoules, 1000mg/10ml	98.70
Calcium-citrate, supplement	97.20
Eunova-Multi-Vitalstoffe Langzeit 50%	99.17
Calcium Complex 600	95.30
Calcium, Vitamin D3	98.20
Calcium with lemon flavor	96.20
Effervescent tablets of calcium with orange flavor	95.11
Calcimed Hermes 500 mg, effervescent tablets	99.80
Calcium plus, 20 effervescent tablets with calcium and vitamins	97.20

Conclusion

Volumetry as a method of quantitative chemical analysis is in the classical methods analysis, which is still widely used in the determination of mineral matter.

In this paper we compared the results of the calcium content in the two groups of dietary supplements, while there was no statistically significant difference between the calculated and theoretical values. On that basis, it can be concluded that the volumetric analysis method suitable for the determination of calcium content in the tested pharmaceutical substances.

Deviations of the experimental data obtained in relation to the theoretical value, which is related to the amount of calcium bound in the form of different chemical compounds in the dietary supplements, amounted 0.83% to 4.70%.
The deviation obtained during the determination of calcium in the form of calcium- carbonate is of similar values, and ranges from 0.20% to 4.80%.

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