

## DETERMINATION OF VITAMIN C CONTENT IN DIETARY SUPPLEMENTS

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**Abstract:** This research focused on determination of vitamin C content in pharmaceutical preparations using the titrimetric method of analysis. The content of vitamin C (ascorbic acid) was measured in twelve preparations. Pharmaceutical preparations that were used for the analysis were supplied in retail, on the territory of Republic of Srpska. For the determination of a substance content, the volumetric method, iodometry, was used. The method is based on determining the volume of a reagent with a known concentration needed for complete reaction with the test substance. In the iodometry, iodine is reduced by ascorbic acid and when it all is reacted, iodine that is in excess forms a blue-black complex with starch indicator, which marks the ending point of the titration.

Based on the results obtained during the experimental work, it is evident that the percentage content of vitamin C in the compositions is in the interval from 95.24 to 99.64 %. Deviations from the prescribed values are not larger than 5 %. This analysis concluded that the highest percentage of vitamin C include samples of DM (99.64 %), Biofar's (99.64 %) and Pliva sample tablets with sustained release (99.50 %). The lowest percentage was determined in a sample Eunova-Multi-Vitalstoffe (95.24 %).

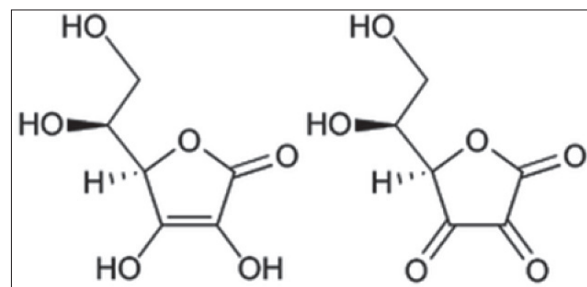
**Keywords:** Vitamin C, Supplements, Volumetric analysis method.

### Introduction

Ascorbic acid (C<sub>6</sub>H<sub>8</sub>O<sub>6</sub>) contains from 99.0% to 100.5% (R) -5 - [(S) -1,2-dihydroxyethyl] -3,4-dihydroxy-5H-furan-2-one. White or almost white, crystalline powder that changes color upon exposure to air. Almost no smell, and the taste is sour. The powder is easily soluble in water, moderate in ethanol, and practically insoluble in ether. It melts at about 100 ° C, with decomposition. It is kept in a tightly closed container that is not made of metal and protected from light. Identification details of ascorbic acid can be found in the fifth Yugoslav Pharmacopoeia (Ph. Jug. V, 2000).

Ascorbic acid is one of the components of vitamin C. Its name is derived from *a-* (meaning - not) and *scorbutus* (scurvy), a disease caused by a deficiency of vitamin C. It is a derivative of glucose that many plant and animal products, and people need to bring in their diet. Ascorbic acid is oxidized in air oxygen, particularly in the presence of heavy metal ions (for example: copper, iron and the like). Activity of Vitamin C is generally destroyed by heat treatment of food, especially where there are traces of metals, such as

copper, but it is resistant to freezing. It is degraded by cooking. Vitamin C is probably one of the most volatile among water soluble vitamins (Lešková, Kubíkoviá, Kováčiková, Košická, Porubská, Holčíková, 2006). In nature, there are only L-form of ascorbic acid and dehydroascorbic shown in Figure 1.



L-ascorbic acid L-dehydroascorbic acid

Figure 1. Structure of ascorbic acid and dehydroascorbic acid (Grujić, i sar., 2014)

Vitamin C plays a major role in the body. Vitamin C is a unique because it performs a number of physiological functions in the human body and the only one among the vitamins that play a role in almost all of the body functions (Gropper, Smith, Grodd, 2005). Vitamin C acts as an electron donor for the eight different enzymes (Levine, Rumsey, Wang, Park, Daruwala, 2000). An appropriate amount of an antioxidant in food, such as vitamin C, reduces the risk of cancer. It is important to know that ascorbic acid increases the body's resistance to viral and bacterial infections, including allergies, and that is effective in the treatment of respiratory diseases and other diseases (Audera, Patulny, Sander, Douglas, 2001). Ascorbic acid is well known for its antioxidant activity. When the human body generates more free radicals than antioxidants, the condition is called oxidative stress (McGregor, Biesalski, 2006). Oxidative stress has an effect on cardiovascular disease, hypertension, chronic inflammatory disease, diabetes (Goodyear-Bruch, Pierce, 2002; Mayne, 2003) and other diseases. Vitamin C in the daily diet can help smokers in preventing cancer. Therefore, the smoker needs for Vitamin C are high (McEvoy et al., 2012). Vitamin C can contribute to reducing the risk of cardiovascular disease, preventing the occurrence of stroke and contribute to the slight decrease in blood pressure (Fotherby, Williams, Forster, Craner, Ferns, 2000).

According to Pharmacopoeia Yugoslavia (Ph. Jug. V, 2000), the average single dose of vitamin C is 0.05 g (prophylactic) and 0.5 g (for therapy). The recommended dosage for men is 90 mg per day and for women 75 mg per day. During pregnancy, it takes about 85 mg per day while breastfeeding 120 mg per day. Tobacco destroys vitamin C in the body, because of which smokers should consume up to 200 mg of vitamin C per day.

However, there are several categories of the population which cannot 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, Blanch, Curiel, Pérez Díez, 2011; Zofková, Nemcikova, Matucha, 2013; Fanian, Mac-Mary, Jeudy, Lihoreau, Messikh, Ortonne 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).

In the chemical analysis different methods of qualitative and quantitative analytical chemistry are used (Harris, 1987). 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, Marjanović, Popov-Raljić, 2007).

For the determination of ascorbic acid, a wide range of techniques and methods is available: spectrophotometric method, IR spectroscopy (Araya, Mahajn, Jain, 1998), volumetric methods (AOAC, 2005), colorimetry, fluorometry, chromatography (Bushway, King, Perkins, Krishnan, 1988; Eitenmiller, Ye, Landen, 2008), and electrochemical techniques (Ogulnesi, Okie, Azeez, Obakachi, Osunsanmi, Nkenchor, 2010), each with its own advantages and disadvantages.

## Materials and Methods

Samples used in the process of volumetric determining are:

- Vitamin C 500 mg tablets, STRONG NATURE (Manufacturer: Elephant CO., Serbia; Series: C53/S 04 06; Shelf life: 06. 2015.)
- Vitamin C 500 mg tablets, ALKALOID (Manufacturer: Alkaloid, Serbia; Series: 60422 0213; Shelf life: 02. 2015.)
- Vitamin C 500 mg tablets, GALENIKA (Manufacturer: GALENIKA a.d., Serbia; Series: 00963; Shelf life: 03. 2016.)
- Protect Vitamin C 500 mg tablets, ESENSA (Manufacturer: Esensa d.o.o., Serbia; Series: 3521112; Shelf life: 11. 2014.)
- Plivit C 500 mg extended release tablets, PLIVA (Manufacturer: Teva Operation Poland sp z.o.o., Poland; Series: 16002913; Shelf life: 01. 2016.)
- Plivit C 500 mg tablets, PLIVA (Manufacturer: Croatia; Series: 6164102; Shelf life: 10. 2014.)
- Vitamin C Depo-time release capsule, 300 mg, Das gesunde PLUS, dm-drogeriemarkt d.o.o. (Manufacturer: Germany; Series: 1222805; Shelf life: 10. 2015.)
- Vitamin C 180 mg effervescent tablets with orange flavor, Protection, SENSILAB (Manufacturer: Serbia; Series: 06230, L270313; Shelf life: 03. 2015.)
- Aspirin Plus C, Acetylsalicylic acid 400 mg + 240 mg Ascorbic acid, effervescent tablets, BAYER (Manufacturer: Austria, Series: (B) BTADS60/2, 81473080; Shelf life: 09. 2016.)
- MultiVita, MultivitaPLUS orange, effervescent tablets, 60 mg of Vitamin C, MULTIVITA (Manufacturer: Serbia; Series: B305824; Shelf life: 02. 2016.)
- Calcium Complex 600, Laboratori BIOFAR, effervescent tablets, 60 mg of Vitamin C (Manufacturer: France; Series: L2269/7; Shelf life: 09. 2015.)
- EUNOVA-MULTI-VITALSTOFFE, Langzeit 50+, 152 mg of Vitamin C (Manufacturer: Hemofarm, Serbia; Series: 990320; Shelf life: 02. 2015.)

Supplements that were used to determine the content of vitamin C (ascorbic acid) were purchased at retail, in the territory of Republic of Srpska and were taken at the pharmacy “Moja apoteka” in dm-drogeriemarkt.

In this paper we used a volumetric analysis method based on redox reactions, or oxidation-reduction method, called iodometric (Jelikić-Stankov, 2000).

## Results and discussion

Experimental determination of the content of ascorbic acid in the pharmaceutical preparations according to the volumetric method of analysis is shown in Table 1.

**Table 1.** Results of determining the content of ascorbic acid in the samples analyzed

Sample (mass of vitamin C per tablet or capsule)	The average mass (of the tablet or capsule) (mg)	The mass of which contains 100 mg of ascorbic acid (mg)	The average volume of spent iodine solution (ml)	The mass of ascorbic acid in the sample (mg)	Recovery value (%)
<b>S. NATURE</b> (500 mg/tablet)	0.7805	0.1561	11.10	97.7355	97.73
<b>ALKALOID</b> (500 mg/tablet)	0.5523	0.1105	11.18	98.4692	98.47
<b>GALENIKA</b> (500 mg/tablet)	0.6828	0.1366	11.20	98.6160	98.62
<b>ESENSA</b> (500 mg/tablet)	0.7993	0.1599	11.21	98.7260	98.73
<b>PLIVA e.r.<sup>1</sup></b> (500 mg/tablet)	0.7394	0.1479	11.30	99.4965	99.50
<b>PLIVA</b> (500 mg/tablet)	0.6023	0.1205	10.90	95.9745	96.00
<b>dm</b> (300 mg/cap.)	0.4568	0.1523	11.32	99.6430	99.64
<b>SENSILAB</b> (180 mg/tablet)	3.9008	2.1671	11.00	96.8550	96.85
<b>BAYER</b> (240 mg/tablet)	3.1837	1.3266	11.20	98.6160	98.62
<b>MULTIVITA</b> (60 mg/tablet)	3.6822	6.1370	11.00	96.8550	96.85
<b>BIOFAR</b> (60mg/tablet)	5.4852	9.1421	11.32	99.6433	99.64
<b>EUNOVA</b> (150 mg/capsule)	0.5287	0.3478	10.82	95.2402	95.24

The results show that deviations from the values listed in the analyzed supplements are not large and do not exceed 4.76% (with sample EUNOV-MULTI-VITALSTOFFE). This may be the reason for low loss in the transmission of the sample, weigh or dissolution of samples, given the high sensitivity of analytical balances. The sample EUNOVA is spent and at least ml of a solution of iodine (10.82 ml). The second sample with the lowest percentage of vitamin C is PLIVA (96%), and the volume in the titration is 10.90 ml, unlike PLIVA sustained release tablet, manufactured in Poland, which is among the highest percentage in this experimental analysis and amounts 99.50%.

Samples dm-drogeriemarkt (Depo-time-release capsules, 300 mg) and a Lab-BIOFAR (effervescent tablets, 60 mg) contain the highest percentage of the ascorbic acid, which is 99.64%.

The results showed that consumption of resources for the titration should be about 11.36 ml, for the content of ascorbic acid in the sample to be 100% and was 100 mg.

Figures 1 and 2 present the contents of ascorbic acid in pharmaceutical preparations containing an active substance only, ascorbic acid (Figure 1) and in compositions in which ascorbic acid is in combination with other active substances (Figure 2). In the graphs we can see that other active substances do not influence the course and results of the analysis and do not interact with vitamin C in the volumetric determination.

Samples containing ascorbic acid as an active ingredient are: Vitamin C 180 mg effervescent tablets with orange flavor, Protection, SENSILAB; Vitamin C Depo-time release capsule, 300 mg, Das gesunde PLUS, dm-drogeriemarkt; Plivit C 500 mg tablets, PLIVA; Plivit C 500 mg extended release tablets, PLIVA; Protect Vitamin C 500 mg tablets, ESENSA; Vitamin C 500 mg tablets, GALENIKA; Vitamin C 500 mg tablets, ALKALOID and Vitamin C 500 mg tablets, STRONG NATURE.

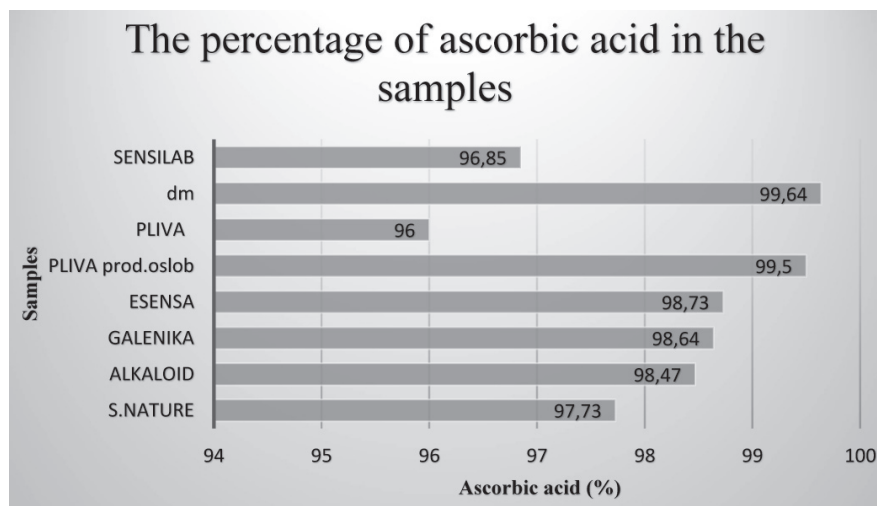


Figure 2. The percentage of ascorbic acid in the samples containing only ascorbic acid as an active ingredient

The samples used for volumetric analysis of ascorbic acid in combination with other active substances are:

- EUNOVA-MULTI-VITALSTOFFE, Langzeit 50+, 152 mg of Vitamin C, calcium-hydrogenphosphate, calcium-D-pantothenate, vitamin B<sub>5</sub> and calcium (120 mg per capsule);
- Calcium Complex 600, Laboratori BIOFAR, effervescent tablets, 60 mg of Vitamin C, calcium (600 mg), phosphorus (300 mg), silicon (2000 µg) and vitamin D<sub>3</sub> (5 µg);
- MultiVita, MultivitaPLUS orange, effervescent tablets, MULTIVITA, 60 mg of Vitamin C, niacin (20 mg), pantothenic acid (10 mg), vitamin E (5.4 mg), vitamin B<sub>6</sub> (2 mg), vitamin B<sub>1</sub> (1.5 mg), vitamin B<sub>2</sub> (1.5mg), folic acid (400 µg) and vitamin B<sub>12</sub> (6 µg);
- Aspirin Plus C, Acetylsalicylic acid 400 mg + 240 mg Ascorbic acid, effervescent tablets, BAYER.

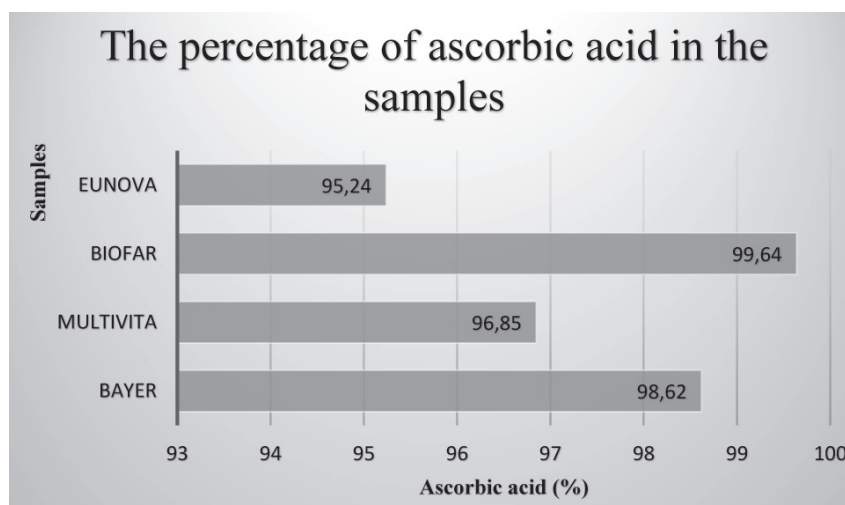


Figure 3. The percentage of ascorbic acid in the samples which in addition to ascorbic acid also contain other active ingredients

## Conclusions

Based on volumetric analysis of the samples we conclude that the percentage content of ascorbic acid is between 95.24 to 99.64%.

It has been found that the highest percentage of vitamin C is included in the samples of dm-drugriemarkt (99.64%), Biofar-a (99.64%) and the sample of Pliva sustained release tablet (99.50%). The

lowest percentage was determined in a sample EUNOV-MULTI-VITALSTOFFE (95.24%), which is quite difficult to dissolve in an ultrasonic bath.

By comparing the results of the analysis, we conclude that the other active ingredients in supplements do not affect the course and results of the analysis, do not interact with vitamin C in the volumetric determination. Content of ascorbic acid in the tested pharmaceutical compositions is in accordance with the values indicated on the package. From these results we can conclude that the method of analysis can be applied in the process of determining the content of vitamin C in pharmaceutical preparations.

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