Study of Vitamin C amount in pharmaceutical industries and its matching with manufacturer's specifications in the prospectus for different companies.

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Abstract

Vitamin C (Vit C) participates in many biological processes and is an important antioxidant for the human body. It is existent in some species of vegetables and fruits and the most taken nutritional supplement in world, especially during COVID 19. This study aims to estimate amount of Vit C in different pharmaceutical forms and compare it with that aforementioned label on the container for different pharmaceutical companies with different Brand names. These pharmaceutical forms of Vit C were bought from pharmacies in Alhoban-Taiz,Yemen, and were divided to three categories according to the content of Vit C (240, 500, and 1000 mg). After that, they were analyzed using iodometric titration method based on an oxidation-reduction reaction between vitamin C and potassium iodate solution, pure Vit C was used as standard. According to the obtained results, it was found that the calculated contents of Vit C in the pharmaceutical forms were different than those the manufacturer's specification in the prospectus, the decreases were ranged between 9.12 –23.92% less than those demanded amounts.

Key words: Vitamin C, Pharmaceutical industries, Manufacturer, prospectus, specification.

1. Introduction

Vitamin C (ascorbic acid) is one of the essential water-soluble nutrients, which cannot be synthesized by humans due to loss of a key enzyme in the biosynthetic pathway [1, 2], so it must be taken from outside [3]. It can be found either in fresh fruits (e.g: orange, tangerine, lemon, kiwi,…) and vegetables naturally or in medical forms such as normal tablets, effervescent tablets and liquid vials. It is the most widely taken supplement [4,5]. For nearly fifty years, it has been stated that Vit C has a very important role, especially in the function of immune system cells [6]. It is one of the most ubiquitous vitamins ever discovered. Besides plays a paramount role as an antioxidant and free radical scavenger, it has been suggested to be an effective antiviral agent [7].

Vit C has a number of activities that could conceivably contribute to its immune-modulating effects. It is a highly effective antioxidant, due to its ability to readily donate electrons, thus protecting important biomolecules (proteins, lipids, carbohydrates, and nucleic acids) from damage by oxidants generated during normal cell metabolism and through exposure to toxins and pollutants (e.g., cigarette smoke) [8]. Vit C is also a cofactor for a family of biosynthetic and gene regulatory monooxygenase and dioxygenase enzymes [9,10]. The vitamin has long been known as a cofactor for the lysyl oxidase and prolyl hydroxylases required for stabilization of the tertiary structure of collagen, and is a cofactor for the two hydroxylases involved in carnitine biosynthesis, a molecule required for transport of fatty acids into mitochondria for generation of metabolic energy [10]. In addition, ascorbic acid has been widely used in the pharmaceutical, chemical, cosmetic and food industry as antioxidant. Though daily requirements of Vit C are changeable according to the age, sex and conditions, it is around 75 to 90 mg per day for healthy adults and no more than 200mg per day is recommended [11]. The recommended daily intake of vitamin C is 110 mg/day for men and 95 mg/ day for women [12]. However, in a person exposed to infectious agents and under physical stress, this amount may be insufficient. In addition, it recommends 400 mg per day for individuals 50+ years of age to strengthen the immune system [13]. A diet that supplies 100–200 mg/day of vitamin C provides adequate to saturating plasma concentrations in healthy individuals and should cover general requirements for the reduction of chronic disease risk [14-16]. Due to the low storage capacity of the body for the water-soluble vitamin, a regular and adequate intake is required to prevent hypovitaminosis C.

There is no treatment for COVID-19 yet. Vit C has become a potentially helpful Candidate for COVID-19 treatment due to its antioxidant, anti-inflammatory and immunomodulatory properties. To date, oral Vit C has been shown to reduce both the duration and frequency of respiratory infections. In addition, intravenous Vit C has been shown to reduce hospital stay, stay in mechanical ventilation and intensive care, and reduce mortality. The benefits of using Vit C in COVID-19 have been shown due to the low cost of Vit C, the high safety profile, and the increased need for Vit C during inflammation [13]. The need for Vit C has increased during viral infections such as COVID-19. Studies have shown that low vitamin C is associated with high

oxidative stress. Since Vit C is reduced in cases of pneumonia and sepsis, studies have been conducted showing the effectiveness of Vit C in these groups [17].

Due to importance and role of Vit C for the human, especially during COVID 19, so, this study carried out to determine quality of Vit C producing from different pharmaceutical industries.

There are many analytical methods from them; high performance liquid chromography, spectrofluorimetric, Spectrophotometric and an Electro-Activated Pencil Graphite Electrode [18-21] .In the study present the amount of vit C was evaluated in given samples using iodometric titration method. In this method the reaction between iodine and starch suspension, will indicate the endpoint by producing the blue-black product.

This study aims to estimate amount of Vit C in different pharmaceutical forms of Vit C and compare them with that aforementioned label on the container for different pharmaceutical industries with different Brand names. Therefore, there is a need to find an accurate, reliable, rapid, and easy-to implement method for measuring the amount of ascorbic acid in a sample.

2. Materials and methods

2.1. *Chemical materials and apparatus*

In this study was used the following materials and apparatus: Volumetric flask**,** Beaker, Burette, Graduated cylinder, Erlenmeyer flask, Balance, Heater, Oven, Distilled water, Potassium iodate (KIO_3) (UNICHEM, company), Potassium iodide (KI) (Merck, Germany), Starch powder, Vit C standard, Vit C samples (tablets, effervescent tablets and liquid vials) with concentrations 240,500 and 1000 mg for different pharmaceutical companies as shown in the tables 1,2,and 3.

2.2. Preparation solutions

2.2.1. Preparation of potassium iodate solution

For preparation of 0.004 M potassium iodate solution, 1g of KIO_3 powder was dried in oven at 100 °C for 24 h, then 0.434 g was dissolved in 500 ml of distilled water a 1000 ml Volumetric flask, and was titrated by standard solution of sodium thiosulphate (0.1N), after that was used for titration of pure Vit C and required Vit C samples.

2.2.2. Preparation of starch solution

Addition of 0.25 g of starch powder in 50 ml warm distilled water, As the starch is insoluble in cold water and needs to be boiled to stay in solution.

2.2.3. Preparation of potassium iodide solution

For preparation of 0.6 M potassium iodide solution, 10g of KI powder was dissolved in 100 ml of distilled water in a 100 ml Volumetric flask.

2.2.4. Preparation of hydrochloric acid solution

For preparation of 1 M hydrochloric acid solution, 11.8 M of hydrochloric acid solution was diluted using 85 ml and dilute it by distilled water until 1000 ml in a 1000 ml volumetric flask.

2.2.5. Preparation of Vitamin C samples solutions

Samples of Vit C were prepared from different pharmaceutical forms (tablets, effervescent tablets and ampule) for different companies, dissolving them separately in 50 ml of distilled water in a 100 ml volumetric flask.

2.3. Experimental procedures

Vit C content was determined by using iodometric titration method according to Mohammed, Ch. et al [4]. Required samples and standard of Vit C were dissolved in 50 ml of distilled water with agitation for complete solubility. after that 5 ml of both standard and sample was taken from each solution in a 50 ml Volumetric flask, then 5 ml of potassium iodide, 5 ml of hydrochloric acid, 1 ml of starch solution were added, and titrant containing potassium iodate solution was run against analyte containing either sample or standard and then titration was started. The endpoint was noted when analyte appears blue color. Each assay was performed in triplicate.

The solubility of iodine is increased with iodide and tri-iodide is occurred:

$$I_{2\,(aq)} + I^- \longleftrightarrow I_3^- \qquad (1)$$

After that I_3^- oxidizes vitamin C to dehydroascorbic acid:

$$C_6H_8O_6 + I_3^- \rightarrow C_6H_6O_6 + 3I^- + 2H^+ \qquad (2)$$

When Vit C is present, I_3^- is converted to iodide and no color change is observed. The endpoint is production of a blue-black color which occurs as a result of the reaction of iodine with starch suspension.

2.4. Calculation

In the beginning of the experiment 5 ml of sample was taken from 100 ml of prepared solution containing 1000 mg of Ascorbic acid. As 43 ml of potassium iodate is required for the color change, the dilution was done 10 times to that of the solution. Example: for sample of Vit C 1000 mg (effervescent tablets).

Average of potassium iodate volume for three times = 43 ml

Moles Number of iodate which reacted to form iodine was calculated according to the equation:

$$n = c \times v$$

Where n is moles number, c is concentration, v is volume of iodates solution

$$n = 0.004 \times \left(\frac{43}{1000}\right) = 0.000172\ mol/L$$

Calculation of iodate ions, iodide ions and formed iodine was performed according to reaction equation (1), Vit C amount which reacted with iodates was calculated according to reaction equation (2).

From the previous two equations (1) and (2), it be noted that 1 mole of iodates reacted with 3 moles of Vit C

$$\text{Mol of Vit C} = 3 \times 0.000172 = 0.000516\ mol/L$$

Final, amount of Vit c was calculated converting (mole/L) to (mg/50ml)

$$\text{Mass of Vit C} = M \times n = 0.000516 \times 176.12 = 0.090877\ g = 90.877\ \text{mg}$$

$$90.877\ \text{mg of Vit C}\ \ In\ \ 5\ ml\ of\ sulotion\ ,$$

$$but\ amount\ of\ Vit\ C\ \ In\ 50\ ml\ of\ sulotion\ is\ 90.877 \times 10 = \ \ 908.77\ mg$$

3 Results and discussions

The results of this study were divided to three groups based to concentration of studied samples of Vit C

3.1. First group

This group included five pharmaceutical companies producing Vit C with concentration 1000 mg.

Table 1 summarizes the results of the analytical determinations performed on Vit C samples (1000 mg) for five companies.

From the table, it was observed that calculated amounts of Vit C were 908.8 mg (90.88 %) for modern pharma. co, 845.4 mg (84.5 %) for Sedico pharma. co, 828.5 mg (82.85 %) for Zhejiang Pharma. co, 818 mg (81.8 %) for Development Company for Chemicals, and 807.3 mg (80.73 %). The decreases in this group were ranged between 9.12 –19.27% less than those demanded amounts, as shown in the table 1 and Figure 1.

Table 1 : Amount of Vit C of different pharmaceutical forms (1000 mg) for different companies

Pharmaceutical Company	**Brand name**	**country**	**Pharmaceutical form**	**Calculated amount of Vit C (mg)**	**Calculated amount of Vit C %**	**Reduced amount (%)**
Modern pharma co	Cal-vit-C	Yemen	effervescent tablets	908.8	90.88	9.12
Sedico pharma. co.	Vitamin C - sedico	Egypt	Effervescent powder	845.4	84.5	15.5
Zhejiang Pharma	Vitamin C	China	Effervescent tablets	828.5	82.85	17.15
Development Company for Chemicals	VITACID CALCIUM	Yemen	Effervescent tablets	818	81.8	18.2
Pharco pharma. co	FAWAR C	Egypt	Effervescent granules	807.3	80.73	19.27

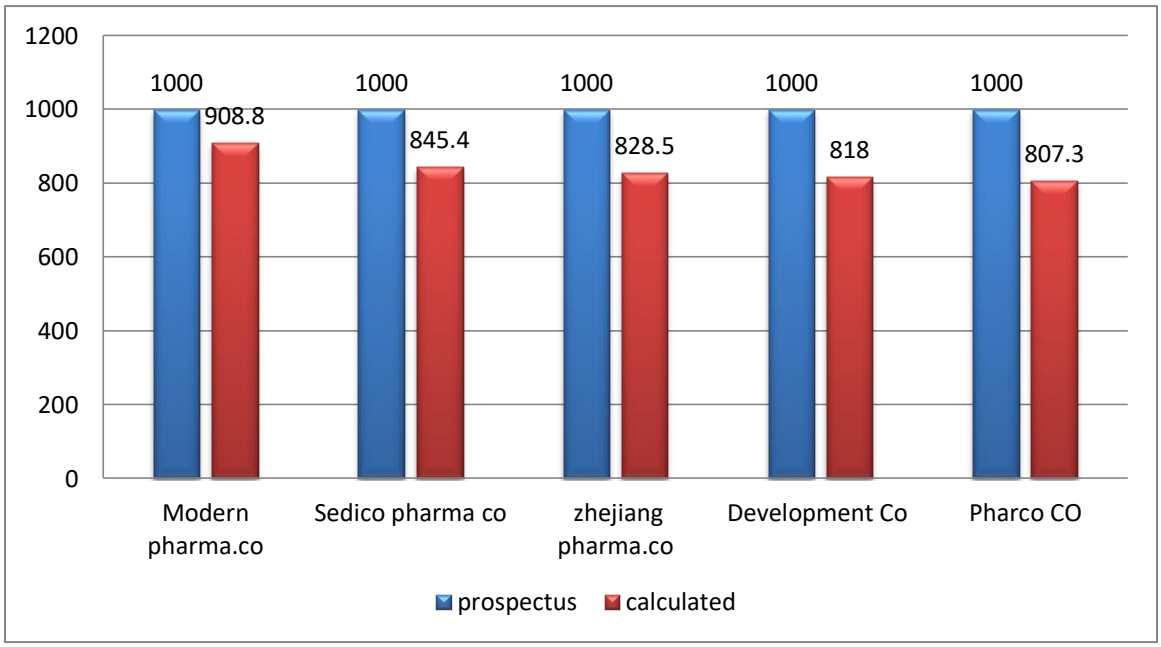

Figure 1: Amount of Vit C (mg) and deviation from the prospectus

3.2. Second group

This group included four pharmaceutical companies producing Vit C with concentration 500 mg. The results of this group were collected in table 2, which shows that calculated amounts of Vit C were found to be 443.8 mg (88.76 %) for Yedco pharma co, 420.6 mg (84.12 %) for Shiba-Pharma co, 386.76 mg (77.35 %) for Shaphaco pharma co, and 380 mg (76.08 %) for Rayman pharma co. the decreases in this group were ranged between 11.24 –22.65% less than those demanded amounts, as shown in the table 2 and Figure 2.

Table 2 : Amount of Vit C of different pharmaceutical forms (500 mg) for various companies

Pharmaceutical Company	**Brand name**	**country**	**Pharmaceutical forms**	**Calculated amount of Vit C (mg)**	**Calculated amount of Vit C (%)**	**Reduced amount (%)**
Yedco pharma.co	Vitamin C-Yedco	Yemen	effervescent powder	443.8	88.76	11.24
Shiba-Pharma.co	Vitamin C	Yemen	Effervescent tablet	420.6	84.12	15.88
Shaphaco pharma.co	Muvit C	Yemen	tablets	386.76	77.35	22.65
Rayman pharma.co	Remo-C	Yemen	Ampule	380	76.08	23.92

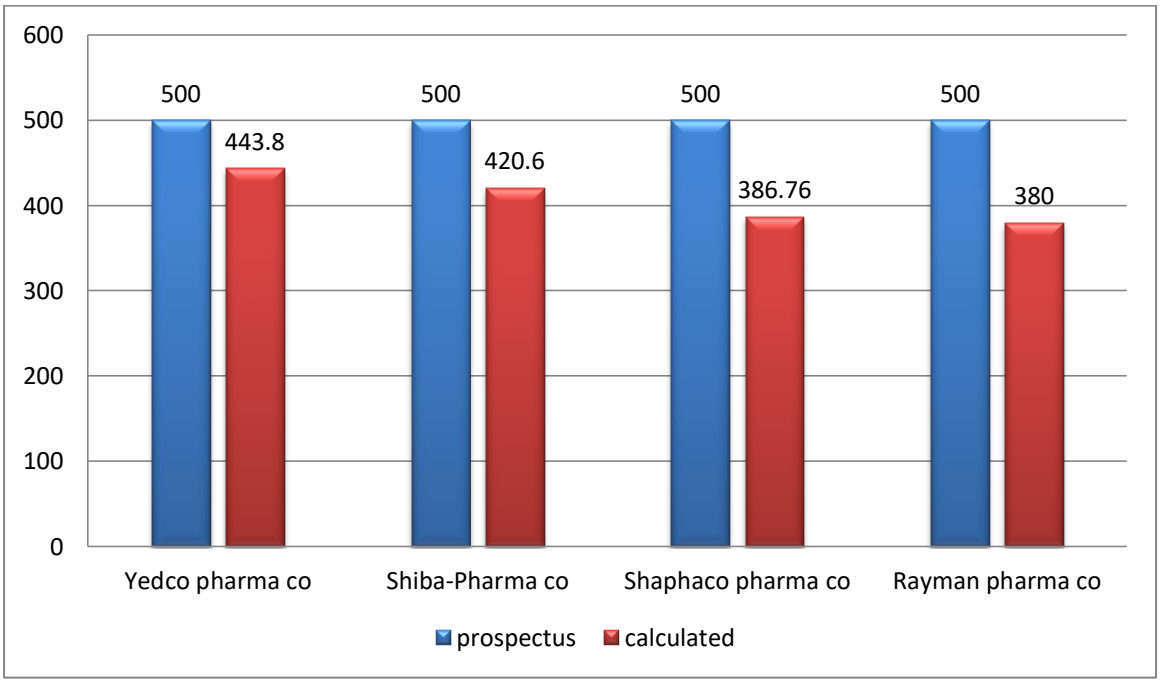

Figure 2: Amount of Vit C (mg) and deviation from the prospectus

3.3. Third group

This group included two pharmaceutical companies producing Vit C with concentration 240 mg. Table 3 presents results this group, the table illustrates that the amounts of Vit C were found to be 213.5 mg (88.94 %) for Bayern-pharma co. while 211.34 mg (88.05 %) for Sanotact-pharma co. the decreases in this group were ranged between 11.06 –11.95% less than those demanded amounts.

Table 3 : Amount of Vit C of different pharmaceutical forms (240 mg) for various companies

Pharmaceutical Company	Brand name	country	Pharmaceutical forms	Calculated amount of Vit C (mg)	Calculated amount of Vit C %	Reduced amount (%)
Byern-pharma. co	Aspirin	Germany	effervescent tablets	213.5	88.94	11.06

Sanotact-pharma. co	Sanotact vitamin C	Germany	Effervescent tablets	211.34	88.05	11.95

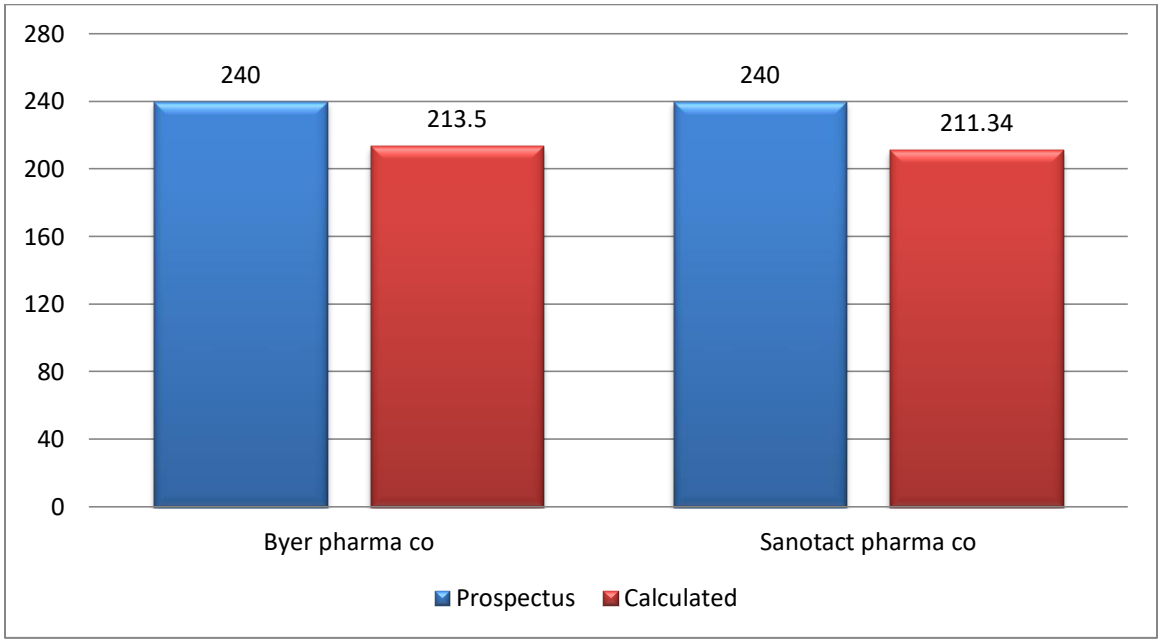

Figure 3: Amount of Vit C (mg) and deviation from the prospectus

Through the results of this study, it was noted that the calculated amounts of Vit C were close to that on packaging label for many companies, while was observed large difference between calculated amounts of Vit C and that on packaging label for another companies. The decrease was found to be from 9 % to 23.92 %.

Mohammed, Ch. et al [4] studied tablets consist 250 mg of Vit C for four different pharmaceutical companies, and reported that the reduction of Vit C were 0.8%, 6.84% , 8.46%, and 12%. While Miao, Y. et al [22] studied different forms of Vit C consist 100 mg for different pharmaceutical companies, and found that the decreases were 9.67 %, 13.72 %, 14.15 %, and 14.89 %.

4. Conclusion

Vitamin C content in different pharmaceutical forms for different pharmaceutical industries studied using iodometric titration method. The results showed that the calculated amounts of Vit C were close to those on packaging label for many companies, while it was observed that the

decrease was significant for another companies. The reduction was found to be from 9 % to 23.92 %. Modern pharmaceutical company has the nearest amount to that amount mentioned on the packaging label, so its product Cal-vit-C be considered prominent supplement for Vit C.

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