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Article

A Novel Spectrophotometric Approach for the Determination of Vitamin C Using Thiosemicarbazone and Its Application to Pharmaceutical Preparations

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Abstract: A new spectrophotometric method has been developed for the determination of Vitamin C in aqueous solutions. The method is based on the oxidative coupling reaction of Vitamin C with a Thiosemicarbazone reagent in an acidic medium and in the presence of potassium periodate. This reaction yields a stable, water-soluble, dark orange-colored complex exhibiting maximum absorbance at a wavelength of 490 nm. The method obeys Beer's law within a concentration range of (3 to 120 μg/mL), with a molar absorptivity of (4174.24 $L \cdot mol^{-1} \cdot cm^{-1}$) and a Sandell's sensitivity index of (0.0421 $\mu g/cm^2$); the limit of detection (LOD) is (0.0753 μg/mL), and the limit of quantitation (LOQ) is (0.251 μg/mL). The method has been successfully applied to the determination of Vitamin C in pharmaceutical tablets.

Keywords: Vitamin C, Thiosemicarbazone reagent, Potassium periodate, Spectrophotometric, oxidative coupling reaction.

1. Introduction

Ascorbic acid (also called Vitamin C) is a naturally occurring organic compound with antioxidant properties. It is a white solid but impure samples can appear yellowish. Ascorbic acid is one form ("vitamer") of vitamin C. The name is derived from *a-* (meaning "no") and scorbutus (scurvy), the disease caused by a deficiency of vitamin C. Being derived from glucose, many animals are able to produce it, but humans require it as a nutritional supplement [1] , The chemical formula is (R)-5-((S)-1,2- dihydroxyethyl)-3,4-dihydroxyfuran-2(5H)- one, molar mass 176.12 g mol-1, Density 1.65 g/cm3, melting point 190-192 °C, with solubility in water about 33 g/100 mL.It is a water-soluble vitamin which can be found in many biological systems and foodstuffs (fresh vegetables and fruits, namely, citrus). Ascorbic acid plays an important role in collagen biosynthesis, iron absorption, and immune response activation and is involved in wound healing and osteogenesis. It also acts as a powerful antioxidant which fights against free-radical induced diseases [2 -6] , Vitamin C, scientifically referred to as L-ascorbic acid, is a water-soluble vitamin. It has a potent capability to reduce substances and performs a significant role as a co-enzyme in internal hydroxylation processes. Vitamin C is present in two distinct forms: the oxidized form, referred to as dehydroascorbic acid, and the reduced form, known as ascorbic acid. This is a commonly used food

ingredient that serves several functional purposes. Most of these functions rely on its oxidation-reduction capabilities. This substance's functional functions include serving as an antioxidant, a nutritious food additive, a reducing agent, a stabilizer, a color stabilizer, and a modifier [7-9], Vegetables and fruits are good sources of vitamin C, one of the most essential vitamins for human nutrition [10], Ascorbic acid, a vital water-soluble vitamin for humans, plays a crucial role in constructing the body's framework. This nutrient actively participates in the development of blood vessel walls, dental structures, connective tissues, and skeletal system. It further aids the body to absorb amino acids and iron [11], Due to the significant and extensive A.A use, several analytical methods have been suggested for its quantification in various matrices and concentrations. The methods used in determination of A.A include voltammetry [12 -13], fluorometry [14], potentiometry [15 -16], spectrophotometry [17 - 18] , flow injection (FI) analysis [19 - 20], and chromatography [21 - 22], The molecular structure of Vitamin C is presented in Figure 1 :

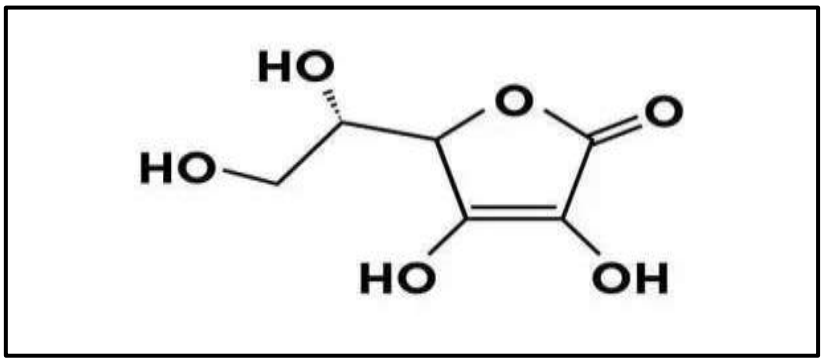


Figure 1. Molecular structure of Vitamin C.

In this study the Thiosemicarbazone reagent was used in spectrophotometric determination of Vitamin C, the goal of this research was to develop a swift sensitive, simple and efficient process for quantitative estimation of Vitamin C in its pure and pharmaceutical formulations.

2. Materials and Methods

Instrumentation

Spectrophotometric analyses were conducted using a T90 UV–Visible spectrophotometer (PG Instruments Ltd., UK) equipped with 1 cm quartz cells. An analytical balance (Sartorius 210S, Kern) was used for accurate weighing of chemicals, while a water bath was utilized to maintain the required reaction temperature.

Reagents and Materials

All chemicals and solvents employed throughout this study were of analytical reagent grade and obtained from Aldrich and Fluka without any additional purification. A stock solution of Vitamin C (1000 μg mL^{-1}) was prepared by dissolving (0.100 g) of the pure drug in distilled water. The solution was then transferred to a (100 mL) volumetric flask and diluted to volume with distilled water. Working standard solutions were obtained by appropriate dilution of this stock solution (1.0×10^{-3} M) Thiosemicarbazone reagent was prepared by dissolving (0.0158 g) of the reagent in (5 mL) of dimethylsulfoxide, followed by dilution to the mark with distilled water in a (50 mL) volumetric flask and (1.0×10^{-2} M) Potassium periodate was prepared by dissolving (0.2300 g) of the Oxidizing agent in followed by dilution to the mark with distilled water in a (100 mL) volumetric flask. A working Vitamin C solution (300 μg mL^{-1}) was prepared by diluting the previously

prepared (1000 μg mL^{-1}) stock solution with distilled water in a (100 mL) volumetric flask. In addition, (0.1M) aqueous solutions of hydrochloric acid (HCl), nitric acid (HNO_3), sulfuric acid (H_2SO_4), and acetic acid (CH_3COOH) were freshly prepared and used during the experimental investigations.

Sample preparation

For the analysis of the pharmaceutical formulation, ten Vitamin C tablets, each labeled to contain (500 mg of Vitamin C), were accurately weighed, giving a total weight of (8.1579 g). The tablets were finely powdered, and an amount of the powder equivalent to (0.0490 g) was transferred to a (100 mL) volumetric flask. The powder was first dissolved in diluted with distilled water and filtered to remove any insoluble excipients. Finally, the filtrate was made up to the mark with distilled water to obtain a (300 μg mL^{-1}) Vitamin C solution. Additional working solutions of lower concentrations were prepared by appropriate serial dilution of this stock solution using distilled water.

3. Results and Discussion

The proposed spectrophotometric method is based on the reaction between Vitamin C and Potassium periodate in an acidic medium, resulting in the formation of a yellow-colored from the oxidation of Vitamin C then coupling with Thiosemicarbazone reagent in the formation of a Orange-colored. The resulting product exhibits a maximum absorption peak at (490 nm), which was used for quantitative analysis. For the assay, aliquots ranging from (0.1 to 4.5 mL) of a (300 μg mL^{-1}) Vitamin C solution were transferred into separate (10 mL) volumetric flasks. Subsequently, (1.0 mL) of (1.0×10^{-2} M) Potassium periodate solution, (0.5 mL) of (0.1 M) hydrochloric acid were added and (1 mL) of (1.0×10^{-3} M) with Thiosemicarbazone reagent. The reaction mixture was then diluted to the calibration mark with distilled water and mixed thoroughly to allow complete color development. The absorbance of the resulting Orange solution was measured at (490 nm) against a reagent blank. To obtain the optimum analytical performance, the experimental variables affecting Oxidative coupling formation—including reagent volume, acid concentration, reaction medium, and other relevant parameters—were systematically investigated and optimized.

Optimization of Reaction conditions

Effect of Thiosemicarbazone Reagent Volume: -

The optimum volume of Thiosemicarbazone reagent was established by investigating volumes ranging from (0.1 to 5.0 mL). Maximum absorbance at (490 nm) was obtained when (1.5 mL) of the reagent was used, indicating complete formation of the colored Oxidative coupling under the selected conditions. Consequently, volume was adopted for the remainder of the study. The experimental results are presented in Figure 2:

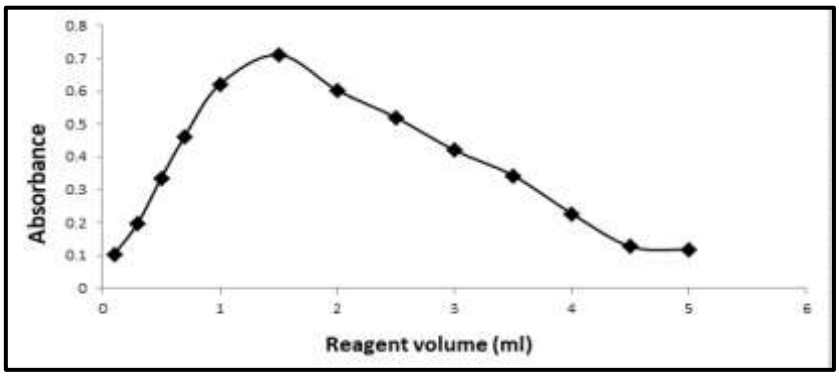


Figure 2. The effect of reagent amount on absorbance.

1. Effect of Acid Type

The effect of various acids on the Oxidative coupling reaction was evaluated using HCl , HNO_3 , H_2SO_4 , and CH_3COOH**.** Among the acids tested, hydrochloric acid provided the greatest color stability and the highest absorbance of the reaction product. Therefore, it was chosen for further analytical studies. corresponding results are presented in Table 1:

Table 1. Effect of Acid Type.

Selection of the Acid Type	Abs.	λmax.
Hydrochloric acid	0.711	490
Nitric acid	0.653	490
Acetic acid	0.491	490
Sulfuric acid	0.613	490

2. Effect of Hydrochloric Acid Volume

The effect of (0.1 M) hydrochloric acid volume on the spectrophotometric response was evaluated over the range of (0.1–5.0 mL)**.** As shown in Figure 3, the absorbance of the Orange Oxidative coupling reaction with increasing acid volume and reached its maximum at (1 mL). Consequently, (1.0 mL) of hydrochloric acid was chosen as the optimum volume for all further experiments.

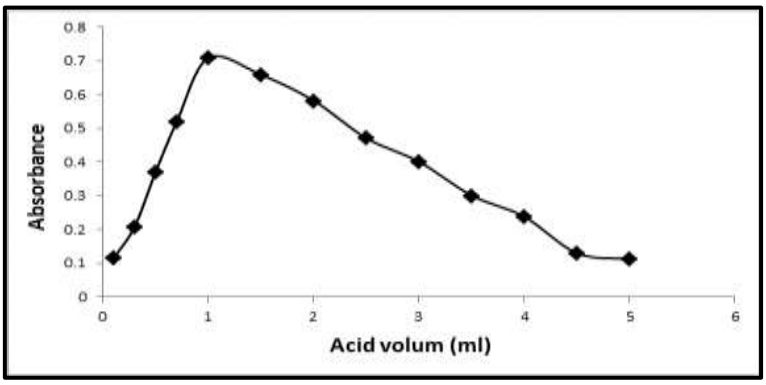


Figure 3. Effect of Hydrochloric Acid Volume.

3. Effect of Oxidizing agent volume

The effect of (1.0 × 10^{-2} M) Potassium periodate volume on the spectrophotometric response was evaluated over the range of (0.1–5.0 mL). As shown in Figure 4, the absorbance of the increasing Oxidized agent volume and reached its maximum at (1.5 mL). Consequently, (1.5 mL) of Potassium periodate was chosen as the optimum volume for all further experiments.

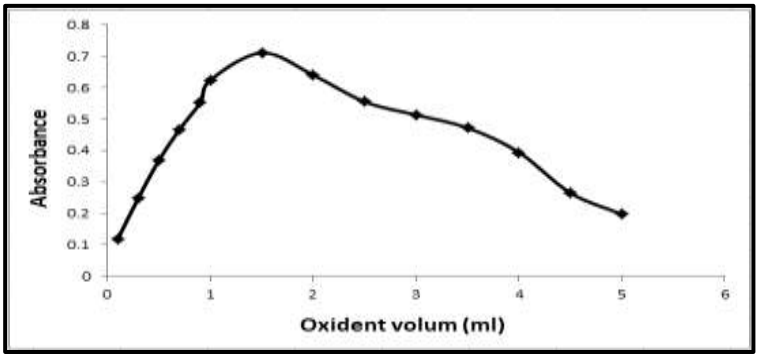


Figure 4. Effect of Potassium periodate Volume.

Selection of the Optimum Temperature

The influence of reaction temperature on the oxidation coupling was evaluated under different temperature conditions. As illustrated in Figure 5, the highest and most stable absorbance was obtained at room temperature (25–30 °C). Therefore, all subsequent experiments were carried out at room temperature to ensure optimum analytical performance.

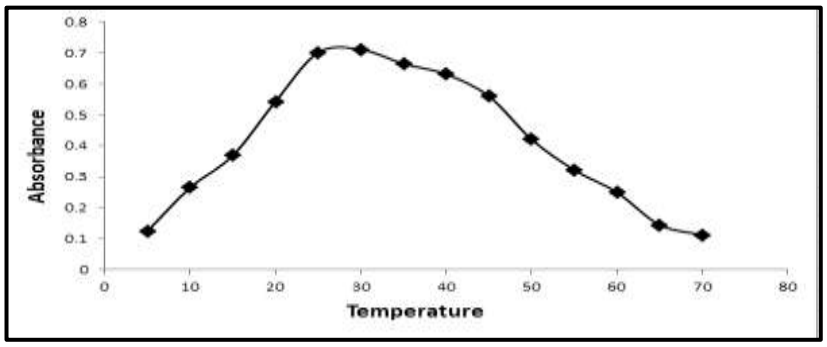


Figure 5. The effect of Temperature.

Stability of the Reaction Product

The stability of the resulting product was investigated over various time intervals. Specifically, (1 ml) of Vitamin C, (1.5 ml) of Potassium periodate, (1 ml) of hydrochloric acid and (1.5 ml) of Thiosemicarbazone reagent were the solution was transferred to a 10 mL volumetric flask and diluted to the calibration mark with distilled water (as shown in Table 2). The product was found to remain stable for approximately 60 minutes (one hour), a duration considered sufficient for performing the necessary calculations.

Table 2. Stability of the Reaction Product.

time / minutes	Direct	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min.	60 min.
Abs.	0.703	0.705	0.706	0.708	0.709	0.711	0.712	0.712	0.710	710.0	0.708	0.707

Effect of the Order of materials Addition

The effect of varying the order in which the reaction components were added was systematically investigated. As shown in Table 3, the highest absorbance was achieved when the materials were added according to sequence (2). Accordingly, this order was selected for all subsequent analytical measurements.

Table 3. The effect Order of Materials addition.

No.	Order of addition	Absorbance
1	P + T + H + C	0.684
2	C + P + H + T	0.711
3	H + T + P + C	0.692
4	T + C + P + H	0.678
5	C + H + T + P	0.656

(C) Vitamin C, (P) Potassium periodate, (H) Hydrochloric acid, (T) Thiosemicarbazone.

Establishment of the Calibration Curve: -

A calibration curve was established by preparing a series of (10 mL) volumetric flasks containing Vitamin C at concentrations ranging from (3 to 120 μg mL^{-1}). To each flask, (1.5 mL) of (1.0×10^{-2} M) Potassium periodate solution (1.0 mL) of (0.1 M) hydrochloric acid and (1.5 mL) of (1.0×10^{-3}M) Thiosemicarbazone reagent were added. The reaction mixtures were allowed to stand for (20 min) to ensure complete color development. Subsequently, the solutions were diluted to the calibration mark with distilled water, mixed thoroughly, and their absorbance was measured at (490 nm) against a reagent blank. The calibration curve was constructed by plotting absorbance versus Vitamin C concentration. Under the optimized experimental conditions, the method exhibited excellent linearity over the concentration range of (3–120 μg mL^{-1}), with a correlation coefficient (R^2) of (0.9983). The calculated molar absorptivity and Sandell's sensitivity were (4174.24 L mol^{-1} cm^{-1}) and (0.0421 μg cm^{-2}), respectively. The resulting calibration curve is presented in Figure 6:

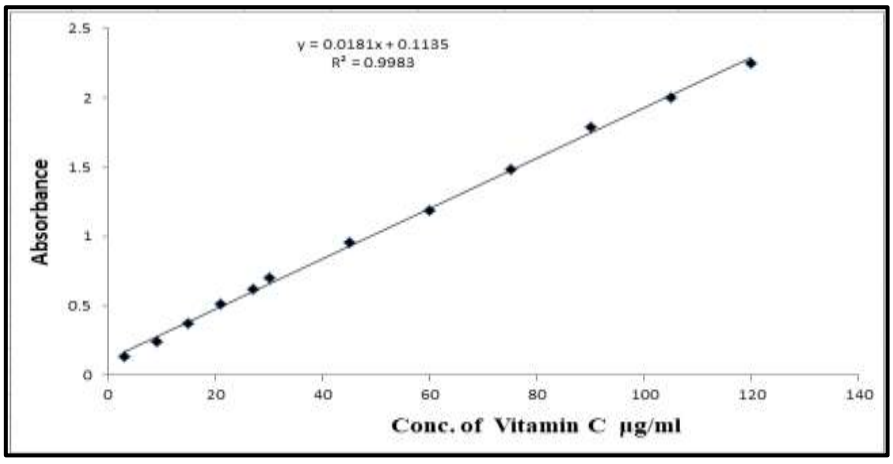


Figure 6. Standard calibration curve of Vitamin C.

Absorption Spectrum of the Final Reaction Product

Figure 7: displays the absorption spectrum of the colored Oxidized coupling formed from the reaction between Vitamin C and Thiosemicarbazone.

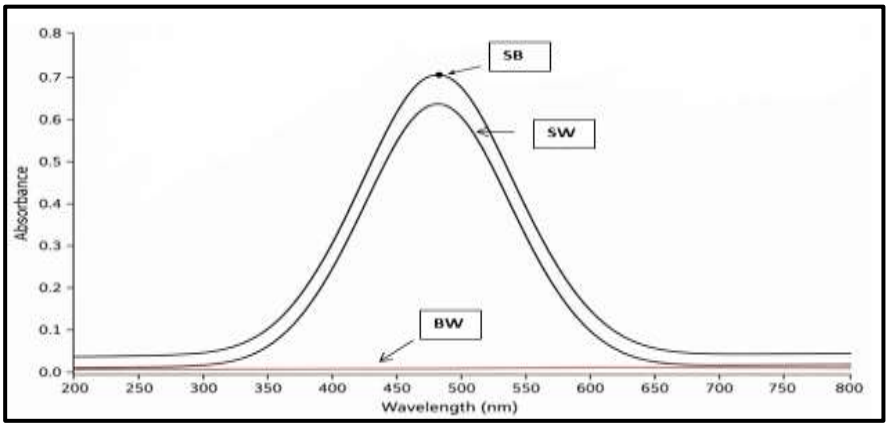


Figure 7. Absorption spectrum of the final reaction product.
SB = sample solution vs blank solution
SW = sample solution vs distilled water
BW= blank solution vs distilled water

Accuracy and precision: -

The reliability of the proposed analytical method was evaluated in terms of accuracy and precision by performing six replicate measurements at (490 nm) for three Vitamin C concentrations selected from within the Beer's law range. The method exhibited satisfactory analytical performance, yielding a mean recovery of (101.67%), which reflects its excellent accuracy and precision. The detailed results are listed in Tables 4 and 5**.** Table 4 includes the theoretical and measured concentrations, together with the percentage recovery and relative error (Er%)**,** whereas Table 5 presents the calculated values of the limit of detection (LOD) and limit of quantification (LOQ).

Table 4. Evaluation of Accuracy and Precision.

Concentration of Vitamin C µg /ml	Concentration of Vitamin C measured *	Recovery.*%	Er. %
21	**21.5**	**102.38**	**2.38**
45	**45.9**	**102**	**2**
75	**75.49**	**100.65**	**0.653**

*N=6

Table 5. Results of (L.O.D) and (L.O.Q).

Concentration. µg /ml	L.O.D µg/ml	L.O.Q µg/ml
3	0.0753	0.251

Stoichiometry of the Reaction Product

The stoichiometric composition of the colored reaction product and the combining ratio between Vitamin C and Thiosemicarbazone were determined using the continuous variation (Job's) method and the molar ratio method. In both approaches, equimolar solutions of Vitamin C and Thiosemicarbazone **(**1.0×10^{-3} M**)** were employed, For Job's method, a series of (10 mL) volumetric flasks was prepared by mixing different volumes of the Vitamin C solution **(**0.5–4.5 mL**)** with complementary volumes of the Thiosemicarbazone reagent **(**4.5–0.5 mL**)**, while maintaining a constant total volume of the reactants. Subsequently, (1.0 mL) of hydrochloric acid solution and (1.5 mL) of Potassium periodate solution was added to each flask, and the mixtures were diluted to the calibration mark with distilled water. The absorbance of the resulting solutions was measured at (490 nm), The experimental results, presented in Figure 8, revealed that Vitamin C reacts with Thiosemicarbazone in a (1:1), confirming the stoichiometric composition of the formed Oxidation coupling.

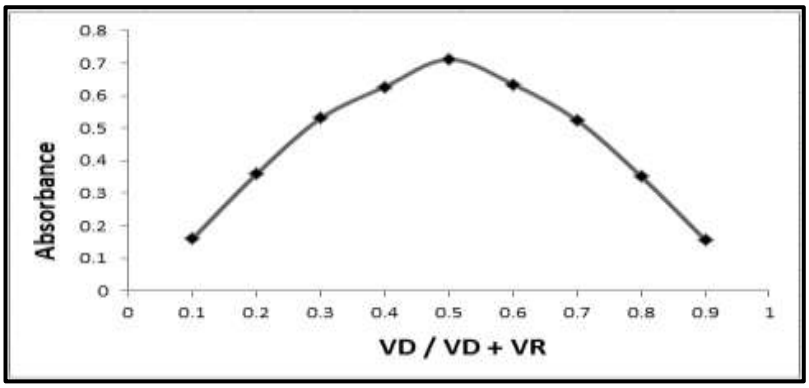


Figure 8. Continuous variations in output method (Job's method).

In the molar ratio method, a constant volume of (2.0 mL) of (1.0×10^{-3} M) Vitamin C solution was transferred into a series of (10 mL) volumetric flasks. Increasing volumes **(**0.5–4.5 mL**)** of (1.0×10^{-3} M) Thiosemicarbazone reagent solution were then added. Afterward, (1.0 mL) of hydrochloric acid and (1.5 mL) of Potassium periodate solution was introduced into each flask, and the solutions were diluted to the calibration mark with distilled water. The absorbance of the resulting solutions was recorded at (490 nm) using the corresponding reagent blank as the reference. The obtained data agreed well with those obtained by the continuous variation (Job's) method, demonstrating that Vitamin C and Thiosemicarbazone reagent react in a (1:1) stoichiometric ratio. The results are illustrated in Figure 9:

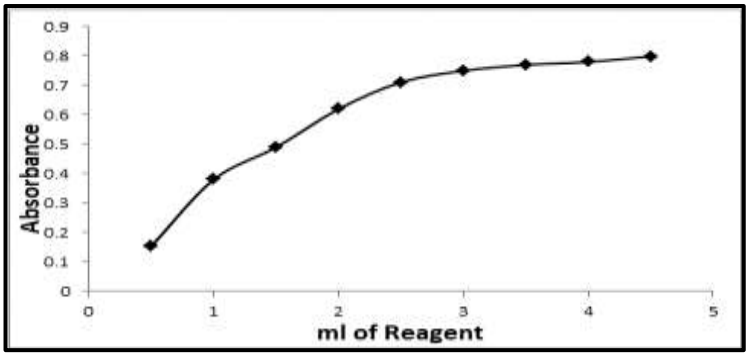


Figure 9. Molar ratio method for the product.

The stoichiometric studies confirmed that the reaction between Vitamin C and Thiosemicarbazone reagent proceeds in a (1:1) ratio The proposed reaction is shown schematically in Figure 10:

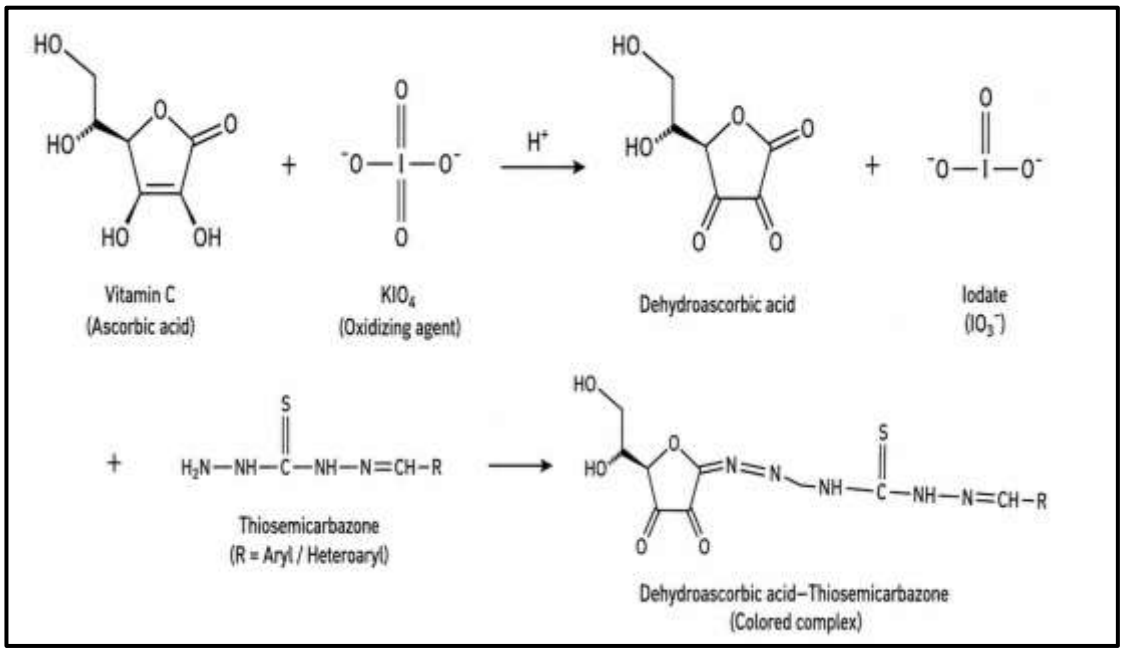


Figure 10. Final reaction equation.

Applications

The applicability of the developed method was evaluated by analyzing Vitamin C in commercial tablet formulations. Ten tablets (total weight 8.1579 g) were finely powdered, and (0.0490 g) of the powder was dissolved in distilled water. The solution was diluted to (100 mL) with distilled water, filtered, and the clear filtrate was used to prepare three different concentrations for analysis. The obtained results are presented in Table 6:

Table 6. Application of the Proposed Method to Vitamin C Tablets.

Concentration. of Vitamin C μg/mL	**Concentration. of Vitamin C measured μg/mL***	Er.%
15	14.44	- 3.7
60	58.37	- 2.71
90	91.51	1.67

* N=3

Table 6: Application of the Developed Method to the Determination of Vitamin C in Pharmaceutical Formulation.

4. Conclusion

The proposed spectrophotometric method provides a simple, sensitive, and reliable approach for the determination of Vitamin C in aqueous solutions. The method exhibits good linearity over a wide concentration range, high molar absorptivity, and low LOD and LOQ values. The formation of a stable, water-soluble colored complex allows accurate spectrophotometric measurements at 490 nm. The successful application to pharmaceutical tablets demonstrates that the method is suitable for routine determination of Vitamin C in pharmaceutical preparations.

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