Spectrophotometric Determination of Vitamin C Using Indirect Oxidation with a New Organic Dye

Asmaa Ahmed Mohammed Alrashidy [1], Omar Adnan Hashem [1], Kalid Abdul-Aziz ALBadrany [1*]

Abstract

Background: In this study, the concentration of vitamin C was determined in various sources such as vitamins tablets, fruit juices, but particularly in natural fresh fruits e.g orange, lemon, mandarin, green lemon and lime as well as industrial juices from different companies. Methods: A simple, sensitive and accurate spectrophotometric method was developed for the determination of vitamin C in pharmaceutical preparations. The method is based on the oxidation of vitamin C by excess potassium iodate (KIO3) in acid medium and unreacted iodate reacts with an organic dye (A1) to give a yellow color and the resulting iodine is extracted with toluene and measured spectrophotometrically at 430 nm. Results: A study was performed on various important parameters of the new dye such as the volume of the dye, type of oxidizing reagents, effect of volume, effect of sulfuric acid, effect of reaction time on the bleaching and oxidation process. Calibration curves were plotted after a diluted dye solution was analyzed within an appropriate Beer's law concentration interval (3-90 µg/ml) for each dye species. Molar absorptivities were obtained with values of 12500.521 L.mol^-1.cm^-1, mean recovery rate was 98.05 %. Conclusion: The developed procedure is successful in the analysis of Ascorbic acid content in pharmaceutical formulations and natural samples. The results obtained were found to be in good agreement with certified values and standard addition procedure. The simplicity and effectiveness of this procedure to estimate pure vitamin C content with accuracy make it a useful tool in various applications.

Keywords: Beer's law, new organic dye, Vitamin C, Spectrophotometric method, Ascorbic acid properties, Analytical techniques, Calibration curve

Significance | The research focuses on the indirect determination of vitamin C in various pharmaceutical formulations, as well as in natural fruit samples and commercially available juices. For the first time, a novel organic dye was utilized in the process, and it was found that the method was indeed effective for the quantification of vitamin C in these products.

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1.Introduction

Vitamin C, as known as L-ascorbic acid, has an essential importance among other vitamins in different scientific disciplines due to its remarkable properties. With its water solubility, antioxidant, reducing power, vitamin C plays multiple crucial roles in various field of chemistry, biochemistry, pharmacology, medicine etc. (Sania et al., 2012). Ascorbic acid can be reversibly oxidised to form L-dehydroascorbic acid (DHA), which also has significant biological activities. While the conversion of dehydroascorbic acid into ascetic acid in human body to be more physiologically important (Mohammed Idaan Hassan AL Majidi & Hazim Y-ALQubury, 2016).

Clarke (2011) states that protein metabolism is altered due to vitamin C deficiency therefore it affects many physiological systems. Research shows that higher intakes of vitamin C are linked with lower levels of cardiovascular disease factors such as CHD, and elevated blood pressure (WHO, 2014). Vitamin C is found in a variety of different foods, however, the prime source is any fruit or vegetables that are orange in colour. 100% fruit juices should be

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treated as additional sources to make up the five portions of fruit and vegetables required daily (FSA, 2015; WHO, 2014).

Vitamin C, which is commonly known as ascorbic acid or ascorbate, is a type of carbohydrates that is soluble in water. Knowing the facts that the molecular formula is C6H8O6, the molecular weight is 176.13 g/mol, and the boiling point is around 190°C (RATH, 1993). Vitamin C usual needs to consume from outside of the human body as unable to produce it (Fenech et al., 2019). As a result, lots of quantitative techniques were development on evaluation of vitamin C in selected matrixes. These techniques for an example, can be analyses by titration method (Nerdy Nerdy, 2018; Intisar El Sharaa & Samira Ben Mussa, 2019), an iodimetry titration (Nishanta Shrestha et al., 2015) and developing of new electrochemical sensors (Fatemeh, et al., 2016; Vahid, et al., 2016; Baghizadeh., et al., 2015; Gheibi., et al., 2015) or using high-performance liquid chromatography (HPLC) (MAZUREK & JAMROZ., 2015; Klimczak & Gliszczyńska-Świgło., 2015).

The study demonstrated a novel simple spectrophotometric method for the determination of Vitamin C using Potassium iodate as oxidant. In this method, iodide reacts with iodate to form iodine. This iodine oxidizes ascorbic acid sodium salt. In acidic media the reaction proceeds, the resulting iodine bleaches the dye. The actual reaction takes place with the molecular iodine and ascorbic acid at acidic pH by giving dehydroascorbic acid. The reaction rate follows the simple second order law at constant pH. The following work will describes the determination of vitamin C in pure powder form and pharmaceutical formulations, Neutral sample and Industrial sample.

2. Materials and methods

2.1 Chemicals

Chemicals and solvents used in the study were purchased from Aldrich and Fluka, USA. Melting points were determined using a Stuart melting point apparatus. Infrared spectra were obtained using KBr discs and a Shimadzo FTIR-8100 spectrophotometer. ThirteenC-NMR spectra were acquired using an MHZ spectrometer with DMSO-d6 as a solvent, while 1H-NMR spectra were obtained using the same apparatus. The purity of compounds was assessed using TLC on alumina sheets percolated in silica gel (type 60 F254 Merck, Darmstadt, Germany).

Chemicals and solvents were used without further purification. Ascorbic acid (Vitamin C) was provided by the State Company for Drug Industries and Medical Appliance (SDI), Samarra, Iraq. Distilled water was employed in the preparation of all solutions.

2.2 Instruments

All spectrophotometric measurements were conducted using a T90 UV-Visible Spectrophotometer manufactured by PG Instrumental Ltd. (UK). A 10mm quartz cell was utilized for these measurements. For precise weight measurements, a Sartorius Balance 210S kern was employed.

2.3 Solutions

To prepare a 10-2M solution of the new organic dye A1 (C10H9N5O3, M.wt 247.07 g/mol), 0.2470 g of the substance was accurately weighed and dissolved in 5 mL of ethanol. The solution was then quantitatively transferred to a 10 mL volumetric flask and diluted to the mark with ethanol. This process ensured the precise preparation of the desired concentration of the organic dye solution.

For the preparation of the stock solution of vitamin C, a concentration of 300 µg/mL (M) was targeted. The appropriate amount of vitamin C was dissolved in 10 mL of distilled water and then transferred to a 100 mL volumetric flask. The volume was adjusted to the mark with distilled water, and the solution was stored in the dark for a minimum of ten days to ensure stability. Working solutions of various concentrations were subsequently prepared by serial dilutions with distilled water, providing a range of concentrations for calibration and analysis.

To prepare a 1.0x10-2M solution of potassium iodate (KIO3), 0.2140 g of pure KIO3 was accurately weighed and dissolved in distilled water. The solution was quantitatively transferred to a 100 mL volumetric flask and diluted to the mark with distilled water. This process ensured the precise preparation of the desired concentration of the KIO3 solution.

Additionally, solutions of 1.0 M hydrochloric acid, nitric acid, and sulfuric acid were prepared by diluting the respective acids with distilled water in 100 mL volumetric flasks. This step ensured the availability of standardized acid solutions for use in the experimental procedures, allowing for accurate control of the reaction conditions and pH adjustments as needed.

2.4 Sample Preparation

Ten Forvita C tablets, each containing 500 mg of vitamin C, were weighed and finely granulated (6,500 g). The granules were then dissolved in a small volume of distilled water and brought up to 100 mL to obtain a 5000 µg/mL solution. Working solutions were prepared by serial dilutions with distilled water.

Samples of natural fruits (orange, lemon, and mandarin) and industrial juices from various companies were collected for quantifying the vitamin C concentration in each.

2.5 Synthesis of 3-methylpyrazol-5-one

To produce 3-methylpyrazol-5-one, hydrazine hydrate (1 mole) was added dropwise to a conical flask containing 0.5 mole of ethylene acetoacetate in ethanol (40 mL) while stirring. The reaction mixture was maintained at 60°C and stirred for an additional eighty minutes. Crystallization was completed by lowering the mixture into an ice bath, and the resulting solid was rinsed with ice-cold ethanol.

2.6 Synthesis of 3-methyl-4-((4-nitrophenyl)diazenyl) pyrazol-5-one

The synthesis of 3-methyl-4-((4-nitrophenyl)diazenyl) pyrazol-5-one involved two steps. First, 4-nitroaniline (0.5 mole) was dissolved in distilled water with HCl (3 mL). In a separate step, 3-methylpyrazol-5-one was dissolved in 10% NaOH solution. The dizonium salt from the first step was then added dropwise to the solution from the second step. Crystallization was achieved by chilling the mixture in an ice bath, and the resulting solid was rinsed with cold ethanol.

4. Results

Benzene diazonium salt was prepared from its corresponding amine base following the scheme (1). Scheme (2) outlines the synthesis of 3-methyl-4-((4-nitro phenyl) diazenyl)pyrazol-5-one. Pyrazoline condenses to give compounds (A), which used to this study.

The IR spectra of the compounds contain the 1525-1573 cm-1 bond for CC units are and from 1585 to1595cm-1 for the (C=N) group, 1660-1668 cm-1 obtained for the C=O stretching and 3058-3060cm-1 for the N-H, 3380 cm-1 for the Ar-H stretch.

There are two singlets in this molecule. There are also multiple peaks around 7 which means that there are a lot of hydrogen environments around 7. All the way to 10 reflects carboxylic acid environment and 13 reflects nitrogen and proton environment.

The recorded of C13-NMR spectra of compound I reveals the appearance of two signals corresponding to (C3)C=O, (C1)C=N and (C2)CH-N at δC = 173.99 ppm(b), δC = 164.88 ppm(e) and δC= 105.48 ppm(f) and the methyl group's carbon (CH3) (C10) at δC = 16.78 ppm while the carbon atoms of the phenyl ring give the signals at δC = 121.73–146.05 ppm. (Figure 1)

Improving the Impact of New Dye (A1) Volumes

Accurate determination of the optimal volume of the new organic dye (A1) is crucial for obtaining reliable results. Among various volumes tested, 2.0 mL emerged as the most effective, yielding maximum absorbance. Deviating from this volume, either by using less or more dye, resulted in diminished absorbance, potentially leading to inaccurate results (refer to Figure 3, 4, 5).

Selecting Oxidizing Reagents

The choice of oxidant reagent holds paramount importance. After meticulous selection, KIO3 was identified as the optimum oxidant, underscoring the significance of careful reagent selection for successful reaction outcomes. (Table 1, 2).

Determining KIO3 Volume

Experimentation was conducted to optimize the volume of KIO3 for subsequent stages. Findings revealed a titration-dependent reaction, with the most significant bleaching effect observed at 2 mL of 0.02 M KIO3 (see Figure 4). Accurate titrations are indispensable for determining the precise volume necessary for complete oxidative bleaching of TCP.

Influence of Acid

Vitamin C exhibits high susceptibility to oxidation. Exposure of drink mix samples to air highlights the critical factors affecting vitamin C stability, namely, the type of acid and pH value. Notably, HCl demonstrated effectiveness as an acid catalyst, emphasizing the importance of selecting optimal conditions for color stability and accurate readings.

Effect of Time on Bleaching and Oxidation

Results suggest that the oxidation process is kinetically controlled, necessitating examination of time's impact on oxidation. Extending the reaction period to 20 minutes achieved complete oxidation of the dye, emphasizing the significance of adequate reaction time for accurate determination. Stable dye behavior beyond 20 minutes further validates the reliability and reproducibility of the method. (Table 1, 2)

Order of Addition

Adhering strictly to the prescribed order of oxidizing reagent addition is imperative to maintain the reliability of the reaction. Following this order enhances assay consistency and ensures the attainment of expected results. (Table 5, 6, 7, 8, 9).

Construction of Calibration Curves

Developing calibration curves is pivotal for accurately determining vitamin C concentration. Preparing standard solutions and measuring absorbance facilitate the creation of precise calibration curves essential for achieving accuracy in quantification. (Figure 6).

Presentation of Absorption Spectrum

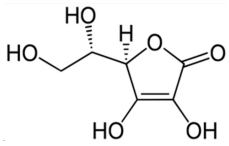
The final absorption spectrum visually represents response dynamics and the progression of the oxidation reaction, offering valuable insights into reaction products.

Precision and Accuracy

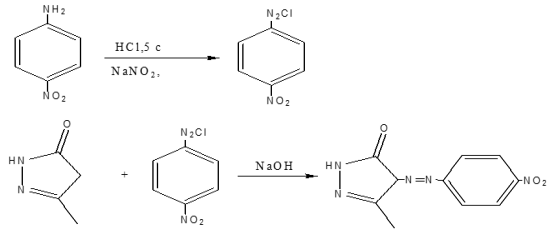
The method's reliability hinges on its accuracy and precision. With observed recovery of the vitamin C assay at 98.05% close to the acceptable range (100%), the method's accuracy is affirmed. Moreover, the method exhibits sensitivity and reliability as evidenced by its detection limit performance. (Table 3, 4).

5. Discussion

Although the methodology for determining ascorbic acid (vitamin C) has been extensively documented in literature, its effectiveness heavily relies on the optimization of various parameters. The SMRP (Spectrophotometric Method with Rapid Estimation) technique demonstrates promise within a pH range of 3 - 4.7 for quantifying vitamin C across diverse pharmaceutical formulations, including effervescent tablets, chewable tablets, hard skin vitamin C tablets, and those from at least three distinct sources. Additionally, it finds utility in assessing vitamin C content in various fruit juices, encompassing both natural and synthetic varieties. (Table 5, 6, 7, 8).



Scheme 1. Vitamin C structure



Scheme 2. synthesis of 3-methyl-4-((4-nitrophenyl)diazenyl) pyrazol-5-one

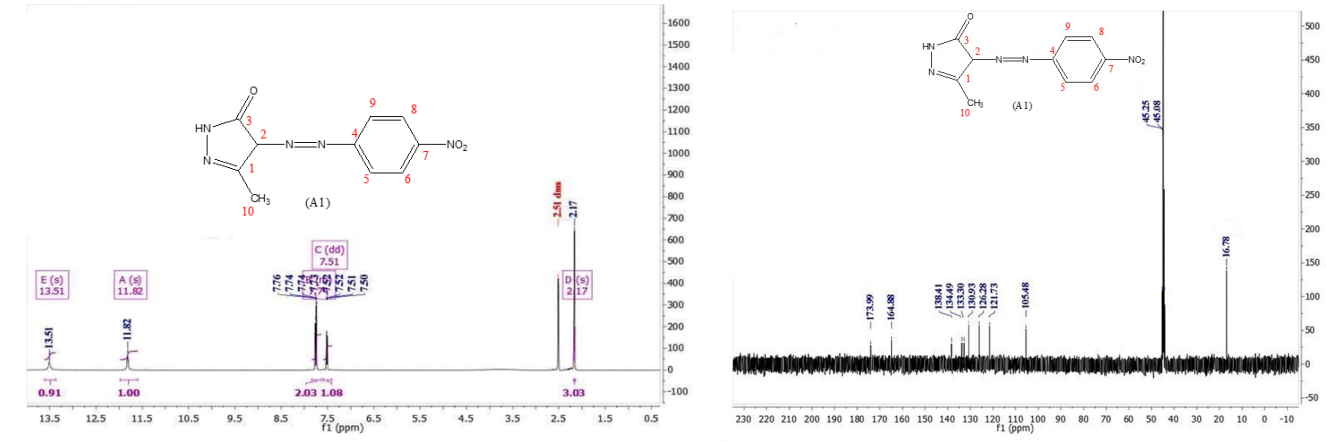


Figure 1. H-NMR spectrum for compound (A1)

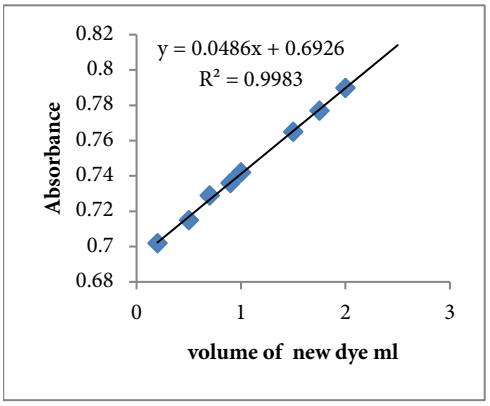


Figure 3. The effect of new organic dye amount on absorbance

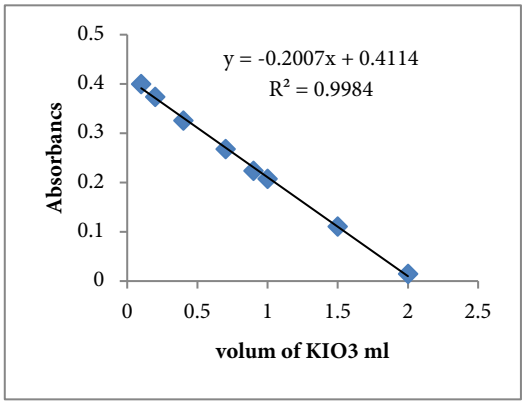


Figure 4. The effect of KIO3 volume on new organic dye absorbance

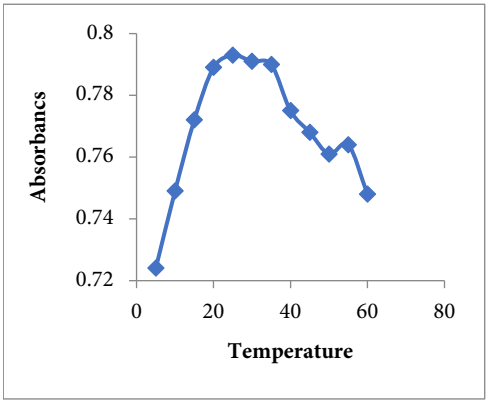


Figure 5. The effect of temperature on new dye absorbance

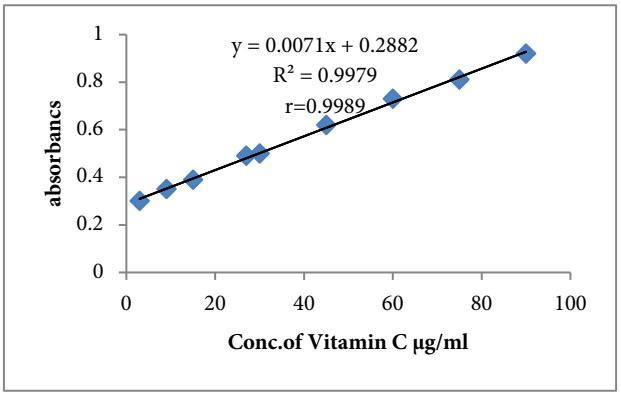


Figure 6. Calibration curve for determination of Vitamin C

Table 1. The effect of oxidant reagents

Oxidant reagents	Absorbance
KIO_3	0.792
KI	0.683
NaI	0.692
$FeSO_4.7H_2O$	0.589

Table 2. The effect of acid on oxidation

Type of acid	Absorbance	λmax
H_2SO_4	0.722	431
HNO_3	0.705	430
HCl	0.791	430
CH_3COOH	0.678	430

Table 3. Results of accuracy and precision. *n=6

Conc of Vitamin C µg /ml	Conc of Vitamin C Observed*	Recovery,*%	Er%
9	8.7	96.72	/ 3.28"
30	29.83	99.44	/ 0.56"
75	73.94	97.99	/ 2.009

Table 4. Results of Detection limits (*A. ALRashidy, K. ALBadrany& G. ALgaragoly, 2020) ** A Al_Taiee, A. Al-rashidy & M. Shareef 2019)

Concentration µg /ml	P	S	LOD* µg/ml	LOQ** µg/ml
3	0.0071	0.001	0.464	1.408""

Table 5. Determination of vit C in pharmaceutical. * n=3

Conc of vit C µg/mL	Conc of vit C Observed µg/mL*	Erel%
10	9.6	-3.09
45	45.18	0.406
80	78.14	-2.323

Table 6. Determination of Vit C in juices of fruits. * n=3

sample	Absorbance*	Conc. of vit C Observed µg/ml
orange	0.825	75.605
lemon	0.785	69.971
mandarin	0.704	58.563

Table 7. Determination of Vit C in industrial juices of fruits. * n=3

Industrial juices (Iraqi &turkey products)	Absorbance*	Conc. of Vit C Observed µg/ml
Randiy Orang	0.802	72
Fresh Lemon	0.759	66.30
Fanta Mandarin	0.694	57.15

Table 8. Standard additions result

Type of Drug	Conc. of Vit.C present µg/ml	Conc. of Vit.C measured µg/ml	Recovery %	Erel%
Tablets (Forvita C ,Ascorbic acid)	78	81	96.15	3.84

The proposed SMRP method boasts simplicity, expedited execution, reduced hazards compared to conventional wet chemical methods, versatility, and potential for automation, thereby enhancing analysis throughput. Successful construction of calibration curves across a spectrum of matrices underscores the method's efficacy in diverse analytical scenarios, further solidifying its status as a preferred assay for vitamin C.

Comparison studies with alternative methods validate the proposed method's reliability and suitability. It exhibits robust performance alongside established techniques, positioning it as a viable option for routine quality control analysis in pharmaceutical laboratories.

6. Conclusion

The spectrophotometric technique presented at 430 nm emerges as a dependable and efficient means for quantifying microgram amounts of vitamin C. By leveraging an oxidation reaction with excess KIO3 and measuring residual vitamin C using a novel organic dye (A1), the method achieves exceptional precision and agreement. Notably, its simplicity, high sensitivity, and compatibility with aqueous solutions confer significant advantages from an analytical perspective.

Moreover, the method's lack of pre-treatment requirements, specialized working conditions, organic solvents, or temperature controls renders it practical, straightforward, and cost-effective. Its versatility extends to the determination of various vitamin C forms in pure form, pharmaceutical formulations, natural fruit sorbets, and industrial juices, enhancing its utility across different laboratory and industrial settings.

In summary, the proposed method represents a valuable and reliable tool for quantifying vitamin C across diverse applications, offering significant benefits to laboratories and industries engaged in such analyses.

Author contribution

O.A. performed the practical procedures, whereas A.A. secured the data and helping in manuscript writing and also performed editorial stat analysis and designs for the methodology. K.A.A. conceived the main idea of the manuscript and designed the study. All authors read and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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