Spectrophotometric Determination of Ascorbic acid in Aqueous Solutions and in Pharmaceuticals formulations

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Abstract

A new sensitive colorimetric method for determination of ascorbic acid tablet in aqueous solution. The method is based on the formation of colored azo dye by diazotization of 2, 4-dichloroaniline followed by azo-coupling reaction between the resulting product and ascorbic acid. The optimum conditions that gave maximum absorption of azo dye at535nm, with best concentration of reactant and order of addition were studied to get the highest sensitivity (ε=2315) $L.mol^{-1}.cm^{-1}$ with a wide range of calibration curves (2-70) µg/ml good repeatability (RSD% 0.68 and 1.49), the recovery (99.56 - 100.30)%, Erel% (-0.20 and 0.44) were gave , Pharmaceutically prepared tablets were measured with the new technique using standard addition method given a very good recovery % (101.25 and 98.77) with a small Erel% (-1.25 and 1.23).

Keywords: Ascorbic acid drugs, 2, 4- dichloroanilineazo coupling reaction, pharmaceutical preparation.

Introduction

The chemical formula ofAscorbic acid is (R)-5-((S)-1,2-dihydroxyethyl)[3],4-dihydroxyfuran-2(5H)-one, molar mass176.12 g mol^{-1}, Density 1.65 g/cm^3, its structural formula showing as in Fig.(1) [1,2,3].

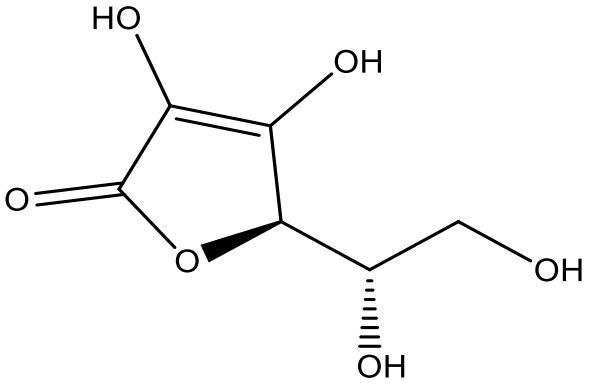

Fig.(1) The structure formula of Ascorbic acid.

Many analytical methods were used for ascorbic acid determination, including chromatography [4], spectrophotometry, high-performance liquid chromatography (HPLC) [5,6] and fluorimetry. Oxidative coupling organic reactions are used for spectrophotometric determination of several drugs such as phenylephrine hydrochloride [7,8], folic acid, and salbutamol [9], and amoxicillin catecholamine drugs [10]. Thus a successful attempt has been proposed in the article to quantify aspirin andascorbic acid simultaneously by spectrophotometer [11,12,13]. The UV spectrophotometric analysis is often preferred in quality control testing and ordinary laboratories due to its broad availability and suitability [14, 15, 16]. The objective of this study was to develop and validate a simple and specific UV spectrophotometric method for the simultaneous determination of aspirin and ascorbic acid in tablets. This method exhibited precise, accurate and cost effective assay for these drugs in mixture [17,18]. Ascorbicand uric acids were determined by coupling an amperometrictechnique with flow analysis. Voltammetric and amperometric measurements were performed in a flow cell, using gold microelectrodes on which Pd was electrochemically deposited [19, 20].

Experimental

Instruments and Equipment:

1. Double-beam UV-Visible spectro-photometer model (UV-1650 PC) SHIMADZU (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version1.10), using 1.00 cm quartz cell was used for measuring the absorption single, Ultra sonic devise (ultrasonicator) for dissolving samples, (SONOREX), (W.Germany), Ultra-pure water manufacturing device, (TORAYPURE), model uv-08 (Japan).

2. The IR spectra were measured as (KBr disc) were recorded with Shimadzu-FTIR 8300 spectrophotometer.

Chemicals and Reagents

Ascorbic acid standard material was obtained from state company of Drug industries and medical Appliances (German Vitamin C, T&D Pharma Gmbh, Germany and cetavit Vitamin C, Alshahba labs. Aleppo Syrian); all commercial drugs tablets contain 100 mg and 500mg respectively of ascorbic acid, were obtained from local pharmacies.

A standard solution of 100µg /ml ascorbic acid was freshly prepared by dissolving 0.01gm of ascorbic acid in 10ml absolute ethanol and then diluted with distilled water in 100ml; A 2, 4-dichloroaniline was obtained from (Merck), a standard solution of 100µg /ml was prepared by dissolving 0.01g of 2, 4-dichloroaniline in10ml absolute ethanol and then diluted with distilled water to 100ml. sodium nitrite (99.80% purity) form (BDH) and standard solution was prepared0.01M. Sodium hydroxide of (99% purity) from (RDL), solution of 1M was prepared by dissolving 4 gm in 100 ml distilled water.

Procedure:

2 ml of ascorbic acid standard solution 100 µg/mL and 0.75 ml of 1M sodium hydroxide solutions were added to 5 ml of 2, 4-dichloroaniline and 0.5 ml of 0.01M sodium nitrate and 0.5 ml of 1M HCl were mixed and completed with distilled water to the mark in 10ml volumetric flask and shacked for 5minutes, cooling ice bath for 5 minute, after 10minutes the yellow color is completely developed and the absorbance measurement was carried out at a wavelength at 535nm, against a blank solution prepared with same method but without ascorbic acid.

The procedure of ascorbic acid in pharmaceutical preparations:

Tablet: ten tablets were weighted and take 200mg of ascorbic acid after powdered (equivalent one tablet) to dissolving with 10 ml absolute ethanol and then diluted in 50 ml volumetric flasks and diluted up to the mark to obtained 200 µg/mL.

Oral solution:4 ml was taken from container containing 400 µg/mL of ascorbic acid was transferred in to 200 ml volumetric flasks and diluted up to the mark with distilled water. Working standard prepared by dilution and the recommended procedure was used for ascorbic acid for its determination.

Result and Discussion

The colored product after coupling of ascorbic acid with 2, 4-dichloroaniline in the presence of sodiumhydroxidehave maximum absorption at 535 nm while the blank at these wavelength shows zero absorbance as shown in (Fig.(2)).

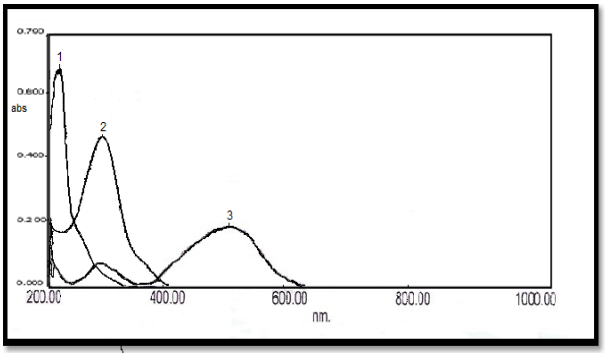

Fig. (2) (1) Absorption spectrum of the 2,4-dichloroaniline.(2) Absorption spectrum of ascorbic acid (3) Absorption spectrum of the azo dye formed, ascorbic acid (40µg/ml) and 2, 4-dichloroaniline.

The influence of various reaction variables on the color development was tested to establish the most favorable conditions.

The optimum conditions:

The effect of order of addition:

In order to have high sensitivity and a linear range for the calibration curve, That the best of the order of addition high absorbance at the wavelength 535nm with sequence volumes were taken 5ml of 100µg ml^{-1} 2, 4-dichloroaniline, 0.5 ml of 1M HCl, 0.5ml of 0.01 M sodium nitrite 2ml of 100µg ml^{-1} of ascorbic acid and 0.75ml of sodium hydroxide.

The effect of time and stability:

The color intensity reached maximum after formation of azo dye of ascorbic acid. The color obtained was stable for at least 1 day and this stability, period was sufficient to allow several measurements to be performed sequentially.

The linear calibration graph for ascorbic acid is obtained in Fig. (3).

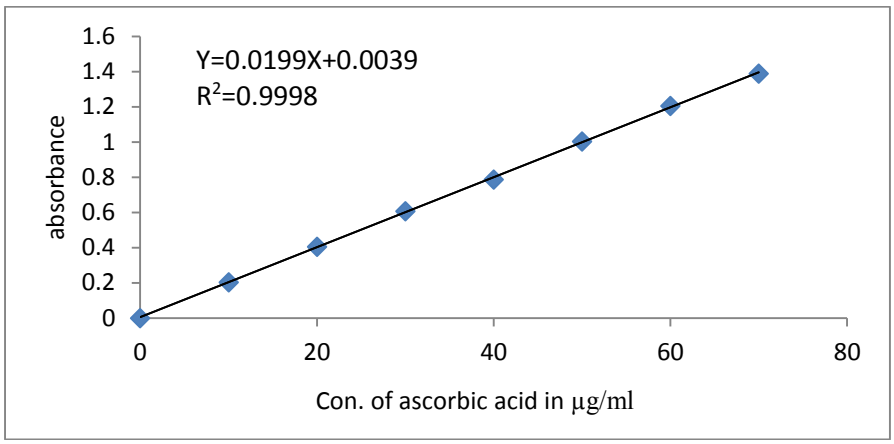

Fig.(3) Calibration curve of ascorbic acid with UV spectrophotometry.

The absorption of different concentration rang of 2-70 µg/ml with correlation coefficient obeyed beer's low that the conditional absorptivity of the product formed was found (2513) $Lmol^{-1}cm^{-1}$. The results was reported in Table (1).

Table (1)
Analytical parameters for calibration graph for ascorbic acid.

Parameters	*Value*
Linearity range(µg/ml)	2-70
Regression equation	Y=0.0199X+0.0039
Slope	0.0199
intercept	0.0039
Correlation coefficient(r)	0.9998
Linearity(%R^2)	99.98
Molar absorptivity ($L\ mol^{-1}cm^{-1}$)	2513

Accuracy and Precision:
Determination of the pure ascorbic acid was carried out through replicate analysis of 5 times for 10µg/mland70µg/ml the results of recovery %, RSD%(Relative Standard Deviation), E_{rel} % (error relative) and C.L. (Confidence Limit at 95% confidence level and 5 degrees of freedom (t=2.57) are shown in Table (2) below:

Table (2)
Accuracy and precision results for the proposed methods*.

Amount (µg/ml) of A.A		*%RSD*	*%Rec.*	*% $E_{rel.}$*	*C.L.*
Taken	*Found*				
20	20.02±0.0032	0.68	100.3	0.3	20.02±0.00399
70	69.53±0.0087	1.49	99.56	-0.44	69.53±0.01086

mean foe 5 independent analysis.

Composition of the formula structure:
Ascorbic acid form colored product after coupling with an electrophilic of 2, 4-dichloroaniline.The composition of the formula structure of azo dye was studied by the mole ratio method [19]. A mole ratio of 1:1 was found for the formation of ascorbic acid with 2, 4-dichloroaniline Fig.(4).

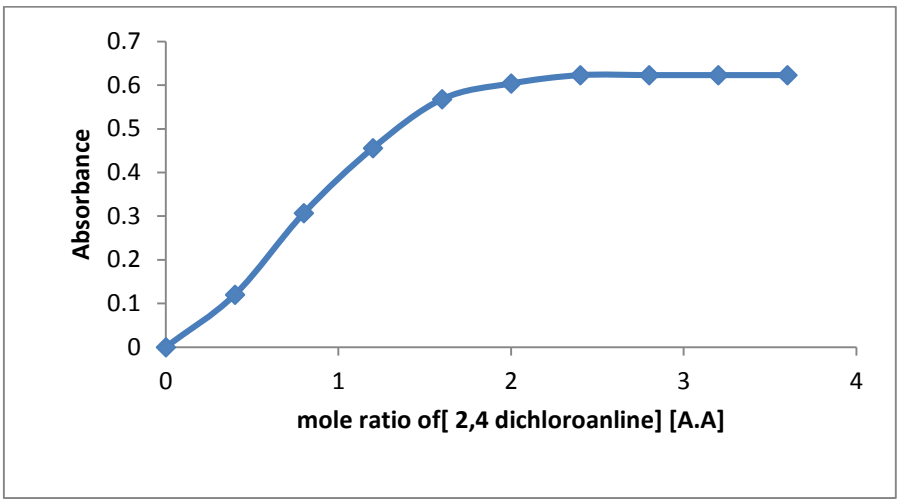

Fig. (4) Mole ratio of the azo dye of ascorbic acidwith 2, 4-dichloroaniline.

The stability constants were calculated by comparing the absorbance of solution containing a twice amount of ascorbic acid with 2, 4-dichloroaniline that the average conditional stability constant of the dye in water, under the described experimental conditions is $0.48x10^4$. The color intensity reached maximum after formation of azo dye and the color obtained was stable for at least 24 hours when used (FTIR) for the identification of the complex formed that the measurements obtained show in Fig. (5).

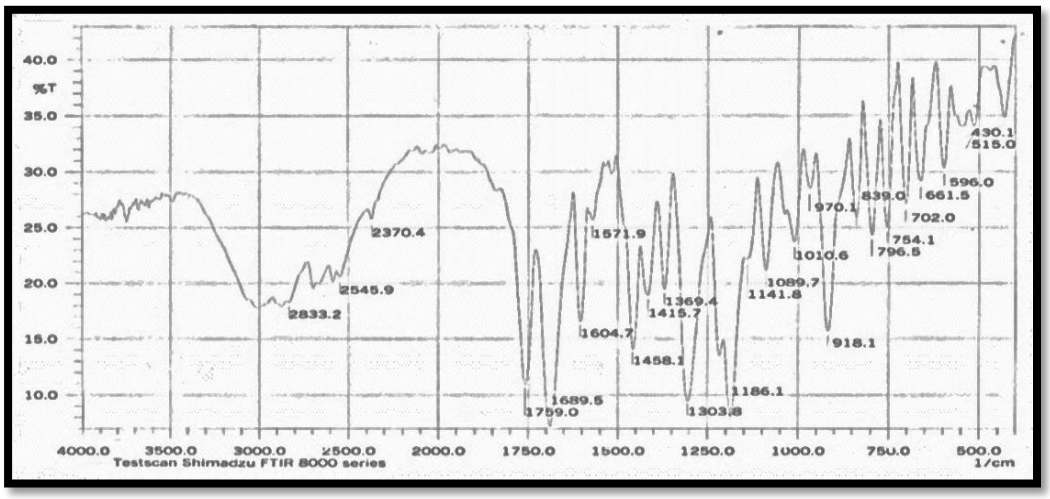

Fig. (5) The (FTIR) spectrum of the complex formed.

The reaction is followed by disappearance of (NH_2) absorption band at (3100-3400) cm^{-1} withappearance of (N=N) absorption band at (1450-1460) cm^{-1}.

Pharmaceutical Applications:

Two pharmaceutically preparation were tested using Standard Addition Method (SAM), the results of thelinearity studies of ascorbic acid are shown in Table (3).

Table (3)
Summary of linearity studies of A.A. in pharmaceutically preparation using SAM.

Pharmaceutical preparation	*Regression equation*	*Slope*	*Intercept*	r^2	*Correlation coefficient (r)*
CetavitVit C	y = 0.019x + 0.087	0.019	0.087	99.80	0.9989
German Vit C	y = 0.014x + 0.428	0.014	0.428	98.00	0.9899

The results Pharmaceutical preparation of are reported in Table (4). The results were reliable and accurate.

Table (4)
The results of pharmaceutical preparation accurate.

Pharmaceutical preparation	*Amount added (µg/ml)*	*Amount recovered (µg/ml)*	*Recovery %*	E_{rel}%
CetavitVit C	2	2.05	101.25	1.25
German Vit C	60	59.50	98.77%	1.23

Interference Studies

The effect of interference by common organic compound was determined by measuring the absorbance of a dye solution containing 2 ml of $6.5X10^{-4}$ M of glucose and various amount of other species such as p-phenylendiamine, O-aminophenol,4-chloronitro aniline, resocinol, paracetamol, and starch. The results showed that 4-chloro nitro aniline major of common compound do interfere the results are given in Table (5).

Table (5)
Effect various interferences of organic compounds on the determination of ascorbic acid (µg/ml).

Interference	*Without addition*	*Glucose*	*o-aminophenol*	*resocinol*	*paracetamol*	*starch*	*4-chloro nitro aniline*	*p-phenyler diamine*
Absorbance	0.464	0.103	0.167	0.179	0.168	0.146	0.199	0.122

Analytical application

The proposed method have been used of two types of drugs containing of ascorbic acid (tablet and oral) which they gave good accuracy and precision the proposed method was compared with British pharmacopeias standard method , since F-test, T-test showed that two no significant differences between the proposed method and official method the results obtained were tabulated in Table (6).

Table (6)
Determination of ascorbic acid in pharmaceutical preparation by the proposed method and comparison with British pharmacopeia method.

method	*pharmaceutical preparation**	*RSD %*	*Recovery %*	*% E*
Proposed method	Tablet* CetavitVit C	4.3	99.87	0.125
	Oral German Vit C	2.55	99.96	1.2
British pharmacopeia method[18]	Tablet*CetavitVit C	2.75	99.13	0.86
	Oral German Vit C	3.25	99.55	0.45

***mean three determinations.**

Conclusion

The great number of methods described in the literature for the analysis of ascorbic acid, the proposeddiazotization and method for the determination of ascorbic acid in pharmaceutical preparation which was reported in this paper is simple, rapid, inexpensive, and can used in pharmaceutical industries and research institutions. The procedure is easier to execute and requires less sample handling than methods currently described in the literature. Statistical comparison of the results with the proposed diazotization method showed goodagreement indicates in accuracy and precision.

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الخلاصة

طريقة جديدة لتحديد اللونية الحساسة من فيتامين C قرص في محلول مائي. هذه الطريقة اعتمادا على تكوين صبغة آزو الملونة الصفراء من اقتران التفاعل بين ٢، ٤-dichloroaniline تليها آزو والناتج مع حمض الاسكوربيك. تمت دراسة الظروف المثلى التي أعطت أقصى امتصاص الصبغةnm 535 الآزو، مع أفض لتركيز المتفاعلة وترتيب بالإضافة إلى الحصول على أعلى حساسية (٢٣١٥) مول$^{-1}$. لتر. سنتميتر$^{-1}$مع مجموعة واسعة من منحنيات المعايرة (٢-٧٠) ميكروغرام/مل التكرار جيدة %(RSD٠,٦٨-١,٤٩)، أعطى الانتعاش٪ (٩٩,٥٦-١٠٠,٣٠)، تم قياس أقراص صيدلانية أعدت مع التقنية الجديدة باستخدام بنسبة معينة و٪ الانتعاش جيد جدا (٩٨،٧٧-١٠١) مع تحديد الثقة (١,٢٥ - ١,٢٣)٪.