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Determination of Vitamin C via Formation of Gold Complex Using Different Spectrophotometric Methods

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ABSTRACT

Determination of vitamin C (Ascorbic acid) via complex formation with gold using UV-VIS spectrophotometry and atomic absorption spectrophotometry (AAS) was investigated in this research. The absorption maxima of vitamin C – gold complex was found to be at (599 nm) and this wavelength was selected for the analysis of vitamin C as standard and in formulated samples. The optimum conditions were investigated through the study of different parameter such as concentration, time, pH, temperature, and complex formula. Complex stability constants was found (2.6192 x10^6). The methods are linear in the range of (2-45 µg/mL) and (1–22 µg/mL) with R^2 values of (0.9993, 0.9992) for UV-VIS and AAS methods respectively. The relative standard deviation (0.48, 0.82), detection limits (0.24, 0.09) and recoveries (100.34 and 100.32 %) for the UV-VIS and FAAS respectively. The methods were applied for the estimation of the active gradient of the vitamin C in different samples of formulated dosage. Accuracy of methods was validated by mean percentage recovery which was found to be in the acceptable range.

Keywords: Ascorbic acid, FAAS, Determination, Pharmaceutical.

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INTRODUCTION

Vitamin C is L-ascorbic acid (chemically: 2-oxogulonolactone). The two hydroxyl groups have acidic properties. By releasing a proton, ascorbic acid therefore turns into its anion, ascorbate. Vitamin C is particularly abundant in fresh fruit and vegetables. Many soft drinks and foodstuffs also have synthetic ascorbic acid added to them as an antioxidant and flavor enhancer. In the body, ascorbic acid serves as a reducing agent in variations reactions. Even higher doses of the vitamin have a protective effect against infections [1]. The active form of vitamin C is ascorbic acid. The main function of ascorbate is as a reducing agent in several different reactions. Vitamin C has a well-documented role as a coenzyme in hydroxylation reactions. Vitamin C is, therefore, required for the maintenance of normal connective tissue, as well as for wound healing. Vitamin C also facilitates the absorption of dietary iron from the intestine [2]. Vitamin **C** is a vitamin for human beings and other primates, the guinea pig, bats, passerine birds, and most fishes and invertebrates; other animals synthesize it as an intermediate in the uronic acid pathway of glucose metabolism. Both ascorbic acid and dehydroascorbic acid have vitamin activity[3].

Vitamin C was determined by many reported analytical methods such as Chromatography [4- 7], Electrophoresis [8, 9], Flow injection [10] , Coulometry [11], Voltammetry [12, 13], Potentiometry [14, 15], Spectrophotometry [16 - 20] and Colorimetry [21]. Aim of this work is to use the precise and accurate spectrophotometric method for the determine the ascorbic acid content in different pharmaceutical from for different companies available in Iraqi pharmaceutical market, to give information about these products, which may or may not comply with the requirements of the standard method or other official methods.

EXPERIMENTAL

Instruments

Spectrophotometer UV-VIS (Jasco V-650 spectrophotometer), England. Fourier transforms spectrophotometer FTIR (Perkin Elmer Spectrum 65 FT-IR spectrophotometer), Germany. Flame atomic absorption spectrophotometer (AAS) Shimadzu (AA-670), Japan. Electronic balance (KERN ACJ / ACS), Germany.

Materials and reagents

All chemicals used were of analytical reagent grade and Ascorbic acid standard material was provided from state company for drug industries and medical appliance (SDI) Samarra Iraq.

Preparation of ascorbic acid standard solutions (100 µg/mL)

A stock drug solution (1000 µg/mL) was prepared by dissolving 0.100 g ascorbic acid standard in 100 mL distill water. Working standard drug solution (100 µg/mL) was prepared by diluting 10 mL of stock solution to 100 mL with distill water in a 100 mL volumetric flask.

Metal ions standard solutions (100 µg/mL)

Standard 100 µg/mL Au (III) solutions were prepared by diluting 10 mL of 1000 µg/mL Au (III) stock solution provided for atomic absorption spectrometric analysis ($HAuCl_4$ solution in 3% HCl) to 100 mL with double distill water.

Procedure for the vitamin C assay in pharmaceuticals tablets

Ten tablets from formulated sample were accurately weighed and crushed to a powder. Amount equivalent to 0.1 g was weighed, dissolved in distill water, transferred to a 100 mL volumetric flask and completed to the mark with distill water. Known volume containing the appropriate amount of vitamin C corresponding to the range of the calibration curve was further transferred in 10 mL flask and analyzed at the same λ_{max} applied for standard measurements against blank solution. The equation of straight line was applied to calculate vitamin C concentration and it's weight. For AAS assay the measurements were carried out for

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black precipitate isolated by centrifuge and dissolved in 5 mL acetone, the relationship between absorbance and concentration was plotted. The linear equation was used for calculate vitamin C concentration.

RESULTS AND DISCUSIONS

Determination wavelength of maximum absorbance

UV-VIS spectra of solutions were carried out, the maximum absorbance was found at λ_{max} (265 nm, 314 and 344 nm, 599 nm) for ascorbic acid, Au^{+3} aqueous solution and ascorbic acid – Au complex respectively as shown in Fig 1.

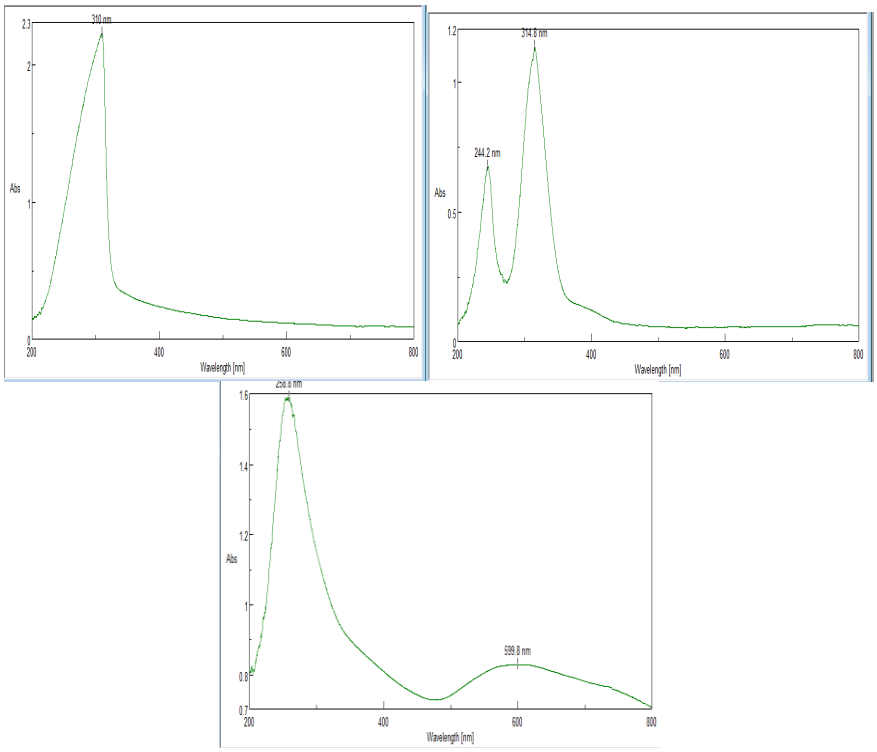

Fig: 1: Ascorbic acid, Au^{+3} solutions and ascorbic acid-Au complex spectrum.

Optimization conditions

pH effect was investigated after adjusting the value at the range of (2-12) by adding HCl or NaOH solution. Absorbance was recorded at λ_{max} of 599 nm versus blank solution. Results obtained revealed that the best pH value was 10 as shown in Fig 2.

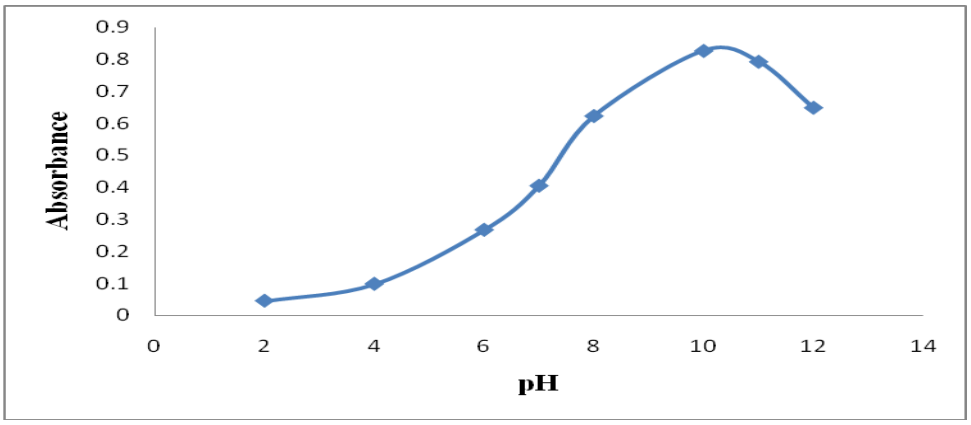

Fig: 2: pH value effect.

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Temperature effect was carried out at the range of (15 – 45˚C). The optimum temperature recorded at 30˚C as shown in Fig 3.

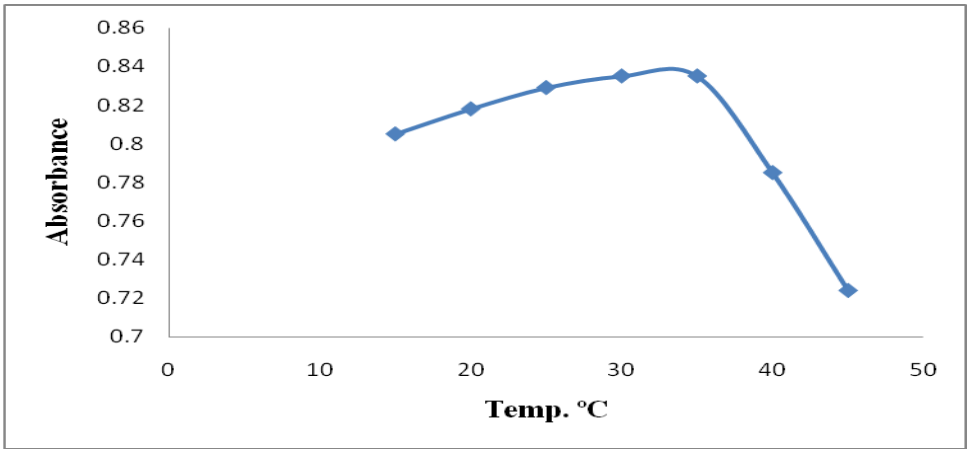

Fig: 3: Temperature effect.

The best time for complexation reaction was found to be at 15 minute as clear in Fig 4.

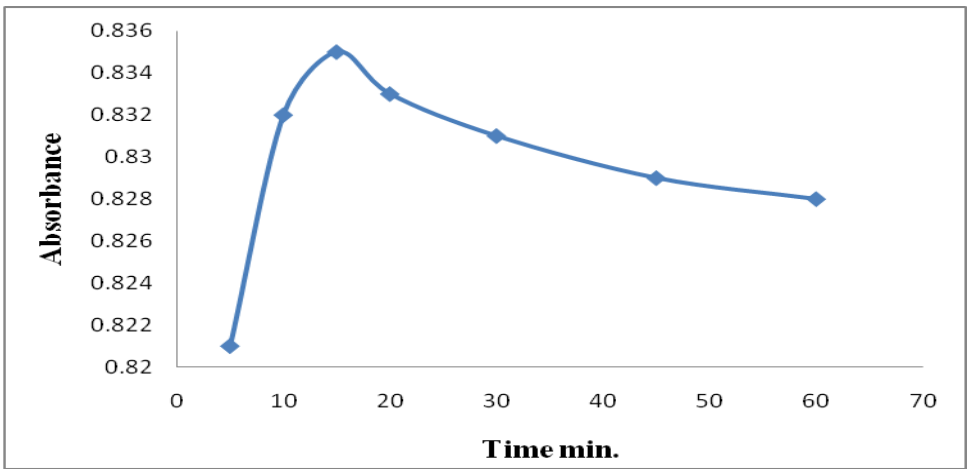

Fig: 4: Time of reaction effect.

Figure 5 shown the effect of gold ion concentration, which indicate that the optimum concentration is 30 μg/mL.

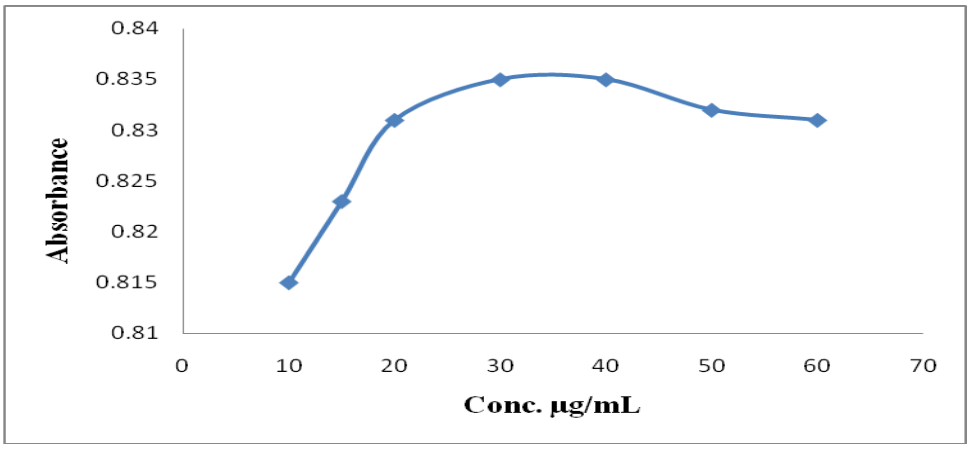

Fig: 5: Concentration of gold ion effect.

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Estimation of molecular formula for complex

To determine the ratio of gold to ascorbic acid in the formed complex, 2.94 x 10^{-3} M solution of both ascorbic acid and gold ions were used. Two methods were applied (Mole ratio and Job methods). The results obtained Fig (6 and 7), revealed that the ratios of complexation for both methods were (1:1). The stability constant value for complex equal to 2.6192x10^{6} was calculated depending on mole ratio curves according to the next equation:

$$k = \frac{(A_1 - A_3)(A_2 - A_3)}{(A_2 - A_1)^2 C},$$

Where k is the formation constant, C is the molar concentration, A_1 is the absorbance which represents two tangents intercept, A_2 is the absorbance which represents the highest absorbance, A_3 is the absorbance of the first point.

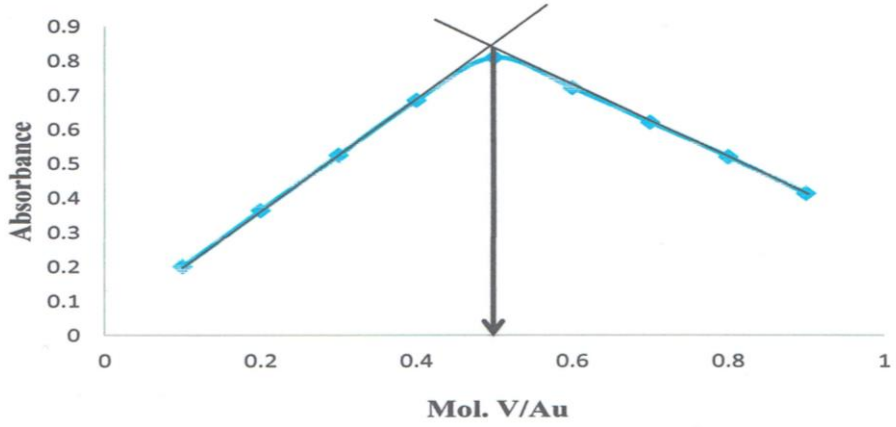

Fig: 6: Job method.

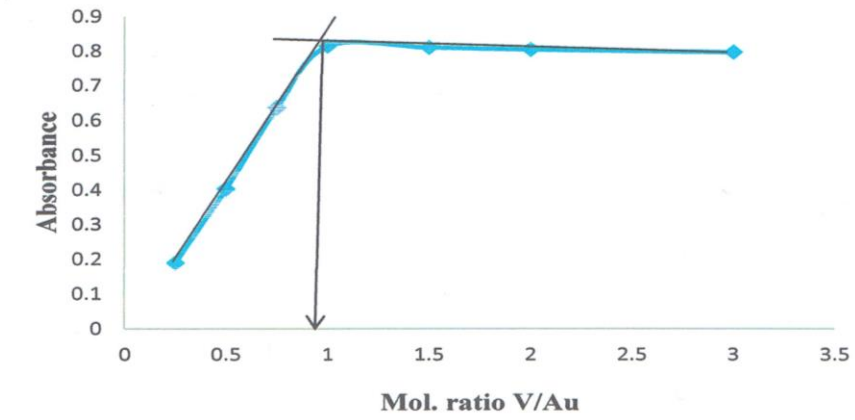

Fig: 7: Mole ratio method.

The FTIR spectra showed that the gold ion is coordinated to the two hydroxyl group of ascorbic acid, leading to the suggested structures shown in Fig 8.

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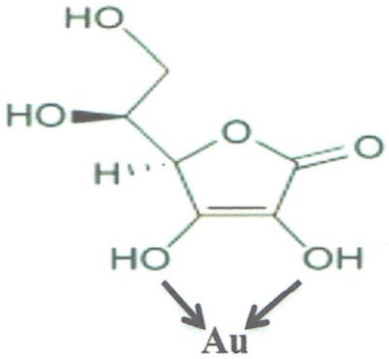

Fig: 8: Suggested structure for Ascorbic acid-Au

Preparation of calibration curves

A series of standard solutions of ascorbic acid (2- 50 µg/mL) and (1-25 µg/mL) were prepared for UV and AAS methods respectively. After the experimental conditions have been adjusted, the absorbance of complex in each case was recorded at the recommended λ_{max} (599 nm). The calibration curves in Fig (9 and 10) were obtained by plotting absorbance versus known concentrations. The results in (Table 1) showed that the values of t_{cal} are larger than t_{tab} values. The methods were linear with R^2 of (0.9993, 0.9992) for the UV–VIS and AAS methods respectively, indicating that there is a strong correlation between the variation of concentration and response. Linearity was determined by the regression analysis.

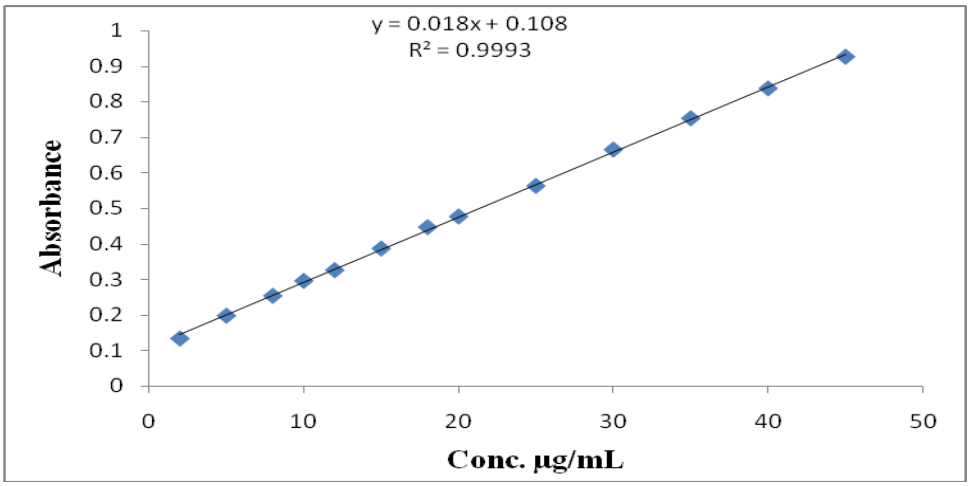

Fig: 9: UV-VIS calibration curve

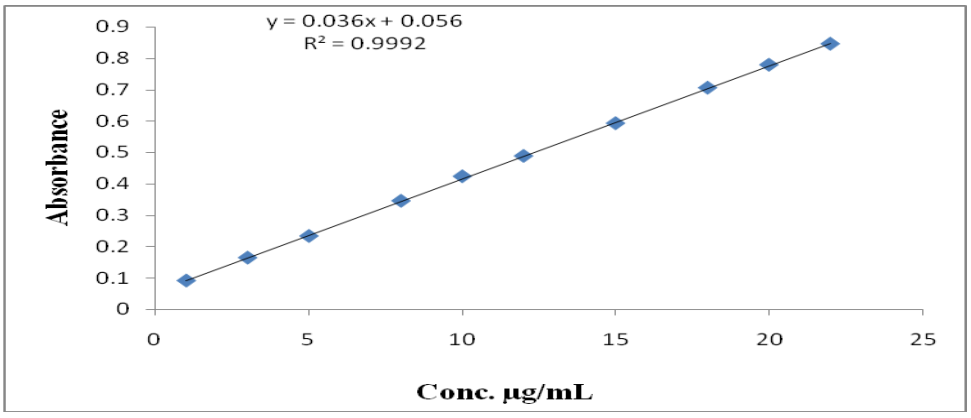

Fig: 10: AAS calibration curve

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Table 1: Statistical calculation for calibration curves.

Statistical factors	Value	
	UV- VIS method	AAS method
Linear equation	y = 0.018 [X] + 0.108	y = 0.036 [X] + 0.056
Slope (m)	0.018	0.036
Intercept	0.108	0.056
Correlation coefficient "R^2"	0.9993	0.9992
Percentage linearity (R^2%)	99.93	99.92
Correlation coefficient (r)	0.9996	0.9996
Intercept standard error	0.21	0.25
Intercept standard deviation	0.31	0.29
"R.S.D.%"	0.48	0.82
"LOD" µg/mL	0.24	0.09
"LOQ" µg/mL	0.78	0.28
Linearity range µg/mL	2–45	1–22
Molar Absorptivity L. mol.$^{-1}$. Cm^{-1}	2844	5280
Calculated (t) values $t_{cal.} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	125.29 >>> 2.16	99.98 >>> 2.23

Accuracy and precision of proposed method

Vitamin C was determined at three different selected concentrations (10, 20, 30 µg /mL) and (5, 10, 15µg /mL) for UV – VIS and AAS respectively. The obtained results are tabulated in (Table 2), which indicated that the determination of vitamin C using these methods are quite satisfactory in reality with respect to the procedure and parameters calculated.

Table 2: Accuracy and precision of proposed method.

Method	Vitamin C µg/mL		% Recovery		% Error	%R.S.D n = 3
UV– VIS	Taken	Found				
	10	9.88	98.80	Mean =100.34	1.20	0.91
	20	20.41	102.05	S.D. =1.97	2.05	0.26
	30	30.33	101.16		1.16	0.27
FAAS	Vitamin C µg/mL		% Recovery		% Error	%R.S.D n = 3
	Taken	Found				
	5	4.97	99.40	Mean = 100.32	0.60	1.33
	10	9.93	99.30	S.D. = 1.83	0.70	0.67
	15	15.34	102.26		2.26	0.44

T-test carried out as shown in Table 3, indicated that there was no significant difference between the developed method and the official one at 95% confidence interval as the calculated t-value is less than tabulated one.

Table 3: Comparison between the new method and official method (HPLC).

Method	New Method		Official Method [7]	
	% Recovery	R.S.D n = 3	% Recovery	R.S.D n = 3
UV – VIS	100.34	1.97	99.59 - 101.93	1.22
FAAS	100.32	1.83		

Quantitative assessment of vitamin C in tablets

Pharmaceutical formulations of vitamin C has been analyzed as described under recommended procedure, a good accuracy and precision were obtained as shown in Tables 4. Obtained results are confirmed the reality and the applicability of the proposed method for the determination of vitamin C in pharmaceutical formulations. The results indicate that the recovery percentages for applying methods are with an acceptable

range of (99.58 - 101.93%) for standard vitamin C sample and the quantity of vitamin C in tablets are accepted within the normal percentage according to official method. Recovery percentages for vitamin C in formulate tablets were found to range from (99.32 - 101.17 %), which confirmed the validity of the method for analysis the drugs in pharmaceutical formulations.

Table 4: Estimated quantity of vitamin C tablets.

Methods	Vitamin C Company	Label Claim mg/ tab.	Mean amount found mg/tab.	%Mean amount found	R.S.D n = 3
UV - VIS	**Furat Pharma tablet , Iraq**	250	252.68	101.07	0.48
AAS			248.31	99.32	0.82
UV - VIS	**Cetavit tablet , Alshaba Syria.**	500	505.85	101.17	0.19
AAS			496.95	99.33	0.46

CONCLUSIONS

A simple and rapid UV spectrophotometric and atomic absorption spectroscopy method were developed and validated for the quantitative determination of ascorbic acid in pure and bulk pharmaceutical formulations via complexation with gold. The obtained results indicate that the quantity of ascorbic acid in tablets is accepted within and close the normal percentage 99.58 %-101.93%, according to the official method. The methods were linear with an R^2 of (0.9993, 0.9992) for UV VIS and AAS respectively. Percentage errors, detection limits and linearity obtained from AAS indirect method was lower than UV-VIS, but higher relative standard deviation than UV-VIS method.

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