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COLORIMETRIC DETERMINATION OF ANTIOXIDANT VITAMINS E AND C

Ahmed Mahdi Saeed[1], Abbas Shebeeb Al-kadumi[2] and Noor Jassem Mohammed Ali[2]

[1]College of education for pure science, Diyala University, Iraq. [2]College of pharmacy, Al - Nahrain University, Iraq. E-mail: dr.ahmedalanbakey@yahoo.com

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ABSTRACT:
Determination of vitamin E (Tocopherol) and vitamin C (Ascorbic acid) by reduction ferric to ferrous ion, which was determined through reaction with potassium dichromate in acidic media using visible spectrophotometry was investigated in this research. The method was based on the use of sodium nitro prusside as a newly reagent source for ferric ion. The colour solution gave a maximum absorption at 564.4 nm, which was applied to the determination method. Conditions optimization was conducted through study different parameter such as volume of reagent, time, acid volume, temperature. The obtained results revealed that the value of R^2 was (0.99991) for both vitamins, detection limit (0.10 and 0.07), the quantitative limit (0. 33 and 0.21), the linear ranges (0.5 – 30 and 0.25 – 50) µg / mL, R.S.D (2.88 and 1.62) for vitamin E and C respectively. Results showed that the newly developed methods could be applied to determine vitamin E and C in their pure state and in pharmaceuticals with high accuracy and low cost without need any complicated treatments. Method accuracy was validated by recovery percentage mean (100.02 and 99.92 %) for vitamin C and E respectively.

Keywords: Tocopherol, Ascorbic acid, Sodium nitroprusside, Colorimetric, Antioxidant.

INTRODUCTION:

Chemically vitamin E is related to a group of compounds called Tocopherols which are consist of alpha (α)-, beta (β)-, gamma (γ)-, and delta (δ)-. The α-, β- and γ-Tocopherol has vitamin E activity, but α-Tocopherol is the most potent. The other two types Tocopherols differ only in the number and position of the CH_3 groups on the aromatic ring, β- is a 1,4- di -CH_3, and γ- a 1,2-di-CH_3 derivative. Vitamin E is stable heating agent, but it is destroyed by oxidizing agents and ultraviolet light. Oxidative rancidity of fats rapidly destroys the potency of the vitamin. The Tocopherols are excellent antioxidants and prevent the oxidation of several substances in the body, including unsaturated fatty acids. As an antioxidant, vitamin E may protect mitochondrial systems in the cell from irreversible oxidation by lipid peroxides. It may also protect tissues of lung from damage by oxidants present in strong contaminated atmospheres (Mary, 2006). Ascorbic acid is an enediol of a hexose sugar acid. The reduced of enediol form is readily oxidized to form dehydroascorbic acid. Both forms are biologically active; however, treatment with a weak alkali opens the oxide ring and produces an inactive molecule. Ascorbic acid may function in oxidation or reduction processes in the body since it is powerful reducing agents. The adrenal cortex contains appreciable amounts of ascorbic acid, which may function in the synthesis of steroid hormones in the adrenal gland. Ascorbic acid is also thought to be involved in hydrogenation reactions and in electron transport in the microsomal region of the cell (Harpers, et al, 2003). Different methods are reported for determining vita-mins such as, HPLC

(Sadilek, *et al.,* 2017) and (Zheng, *et al.,* 2016), Electrophoresis (Ting *et al.,* 2007, Paul 2003), UV-VIS spectrophotometry (Saeed *et al.,* 2017, Mohammed and Hazim, 2016), Fluorescence (Ana, 2011, Arif and Muhammad 2013), Colorimetry (Jameel 2012, Ageliki *et al.,* 2000), Photochemiluminescence (Jasmin *et al.,* 2015, Longfel and Chunxiu 2011), Potentiometry (Denglea *et al.,* 2012, Ahmad *et al.,* 2016), Voltammetry (Swaroopa and Gupta 2015, Wanida, 2014), Coulometry (Okenwa, 2014), Titerometry (Offor, 2015) and AAS (Saeed, *et al.,* 2017). The aim of this work is to develop and use simple, accurate and ease spectrophotometric method to determine the vitamin content in different pharmaceutical from available in the pharmaceutical Iraqi market, to get information about the products, which may comply or not comply with standard method requirements or official methods.

2 – MATERIALS AND METHODS

2.1-Instruments: UV-VIS spectrophotometer (Jasco V-650 spectrophotometer), England. Electronic balance: KERN ACJ / ACS, Germany.

2.2- Chemicals: The used chemical materials were of analytical grade, Tocopherol and Ascorbic acid standard materials were provided by state company for drug industries and medical appliance SDI Samarra-Iraq.

2.3 - Preparation of tocopherol and ascorbic acid standard solutions (100 µg/mL): A stock drugs solutions (1000 µg/mL) were prepared by dissolving 0.100 mL tocopherol standard liquid

and 0.100 mg ascorbic acid in 100 mL acetone. Working standard drug solutions (100 µg/mL) were prepared by diluting 10 mL of stock solution to 100 mL with acetone in a 100 mL volumetric flask.

2.4 - Procedure for vitamin assay in pharmaceuticals tablets: Twenty formulated sample tablets were accurately weighed and emptied or crushed to a powder. Amount equivalent to 0.1 g was weighed, dissolved in acetone, transferred to a 100 mL volume- tric flask and completed to the mark with the same solvent. Known volumes containing the appropriate amount of vitamin E and vitamin C corresponding to range of calibration curves were further transferred in a 10 mL flask, analyzed at the same λ_{max} that applied for standard measurements. The straight-line equation was applied to calculate vitamin E and vitamin C concentration.

3 - RESULTS AND DISCUSSION

3.1-Maximum absorbance wavelength determination: UV-VIS spectra of solutions were carried out, maximum absorbance was found at λ_{max} (310 nm) for Tocopherol, (265.6 nm) for Ascorbic acid as shown in Fig 1, (255.8,365 nm) for potassium dichromate and (266,352.6 nm) for sodium nitroprusside as shown in Fig 2 and (564.4 nm) for deep green solution after mixing of vitamins with reagent as shown in Fig 3.

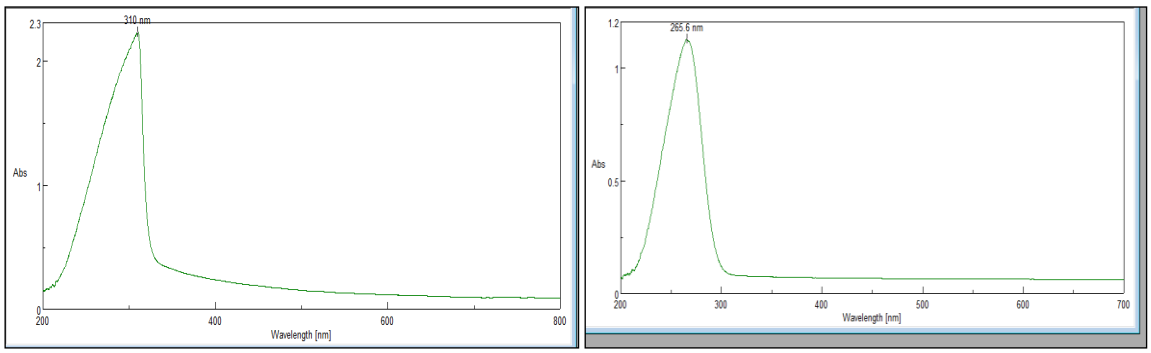

Fig. 1: Tocopherol and Ascorbic acid spectra

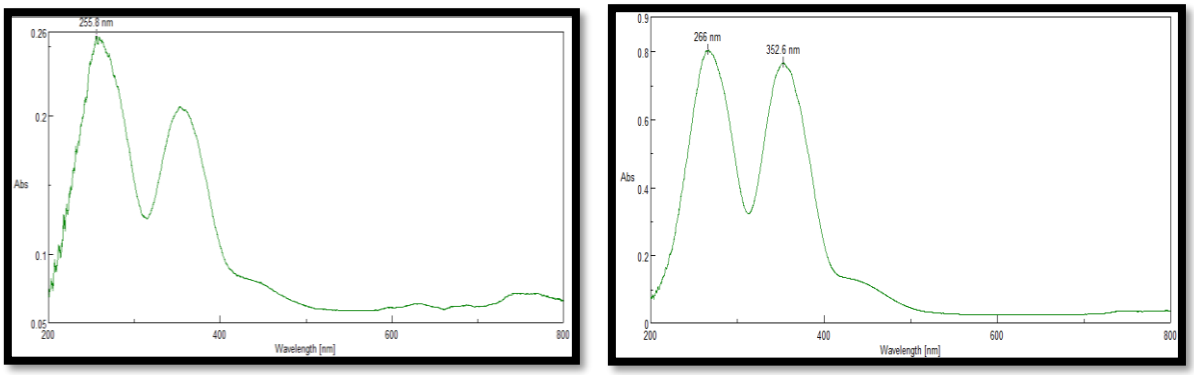

Fig. 2: Potassium dichromate and Sodium nitro prusside solution spectra.

Fig. 3: Resulting spectrum for mixture and its picture

3.2 – Type of solvent: Effect of solvent was investigated using water, methanol and ethanol. Results obtained revealed that the best solvent was methanol as shown in Fig 4.

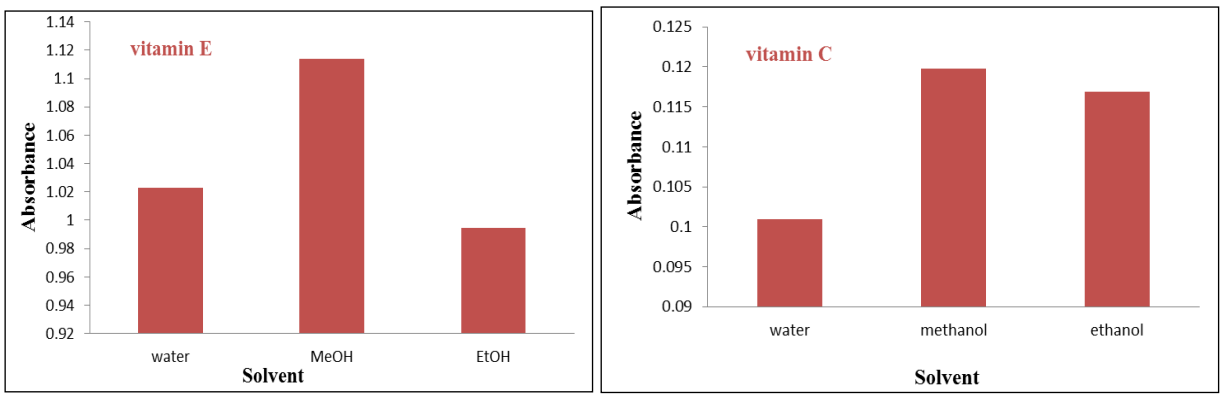

Fig. 4: Solvent effect.

3.3- Sequence of additions: Table 1 bellow, illustrates the sequence addition effect on absorbance of mixed solution, the absorbance data showed the optimum sequence of addition of vitamin solutions to the ferric solution reagent SNP first, followed by adding potassium dichromate solution and final addition of sulphuric acid solution.

Table 1: The sequence addition effect on absorbance of mixed solution

Vitamin E			Vitamin C		
NO.	**Order Of Addition**	**Absorbance**	**NO.**	**Order Of Addition**	**Absorbance**
1	**V+SNP+ D+A**	**1.3409**	**1**	**V+SNP+ D+A**	**0.1767**
2	**D+V+SNP+A**	**0.3672**	**2**	**D+V+SNP+A**	**0.1302**
3	**SNP+D+V+A**	**0.3027**	**3**	**SNP+D+V+A**	**0.1605**
4	**SNP+V+D+A**	**1.3598**	**4**	**SNP+V+D+A**	**0.1808**

3.4 - Optimum time: Figure 5 showed that the optimum time was 25 and 15 min. for vitamin E and vitamin C respectively.

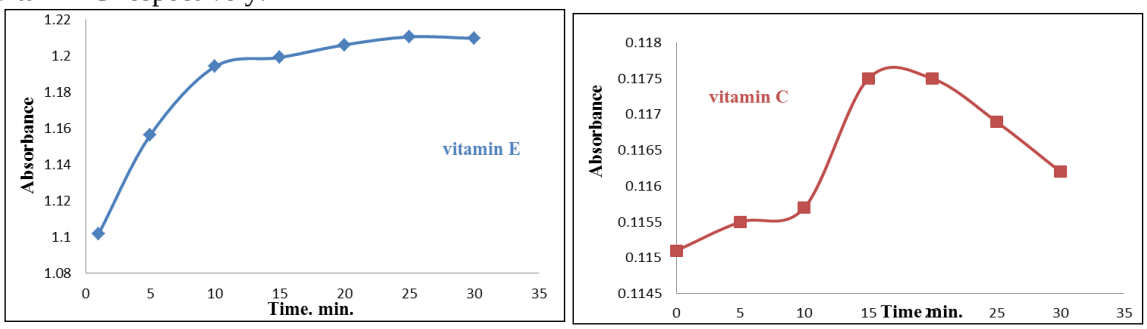

Fig. 5: Optimum time

3.5-Volume of sodium nitro prusside: The obtained optimum volumes of sodium nitro prusside were 2 and 1 mL for vitamin E and vitamin C respectively as shown in Fig.6.

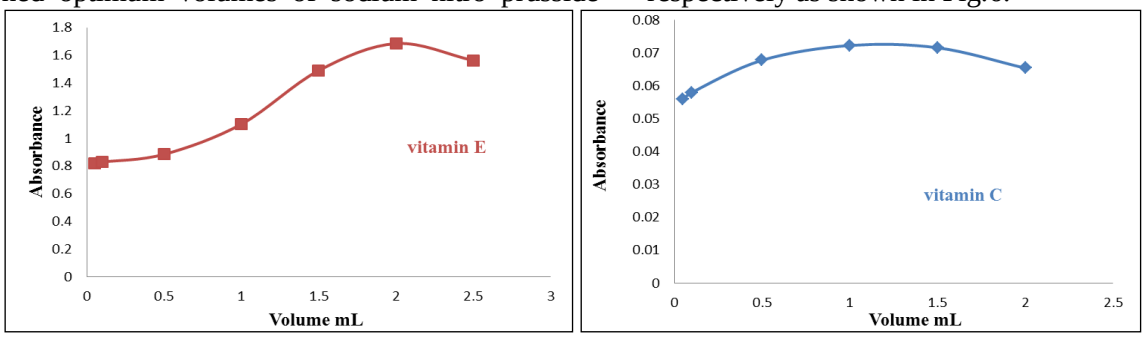

Figure 6: Volume of nitro prusside

3. 6 - Potassium dichromate volume: In this study the obtained results revealed that 1 and 0.8 mL were the optimum volumes of dichromate for vitamin C and vitamin E respectively.

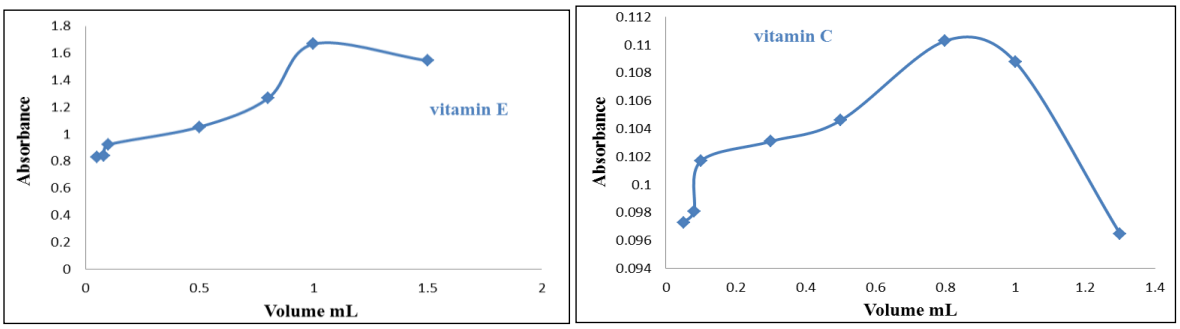

Fig. 7: Volume of potassium dichromate

3.7 - Acid volume: Figure 8 showed that the best sulphuric acid volume was 1 mL for the two vitamins.

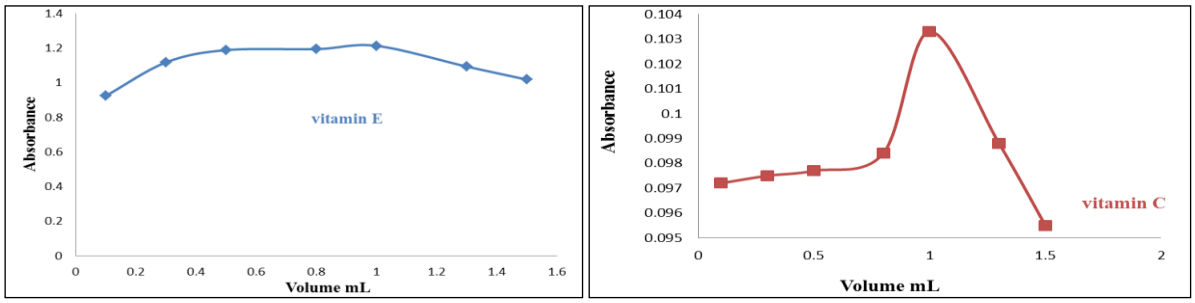

Fig. 8: Sulphuric acid volume

3. 8 - Temperature effect: Obtained results revealed that the 25 ºC was optimum temperature for reaction as shown in figure 9.

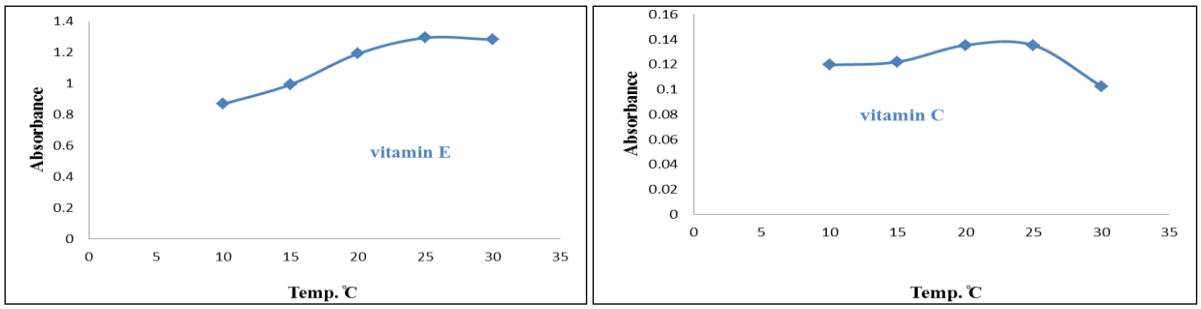

Fig. 9: Optimum temperature

3. 9–UV–VIS spectrophotometric calibration curve: A serious of standard vitamin solutions ranges 0.5-30 and 0.25-50µg/mL were prepared, add to the sodium nitro prusside solution, followed by adding potassium dichromate solution to react with the resulting ferrous ion in sulphuric acid medium, after adjusting all optimum conditions studied previously, record absorbance of deep green color solution at maximum wavelength 564.4nm and plot the calibration curve as in Fig. 10.

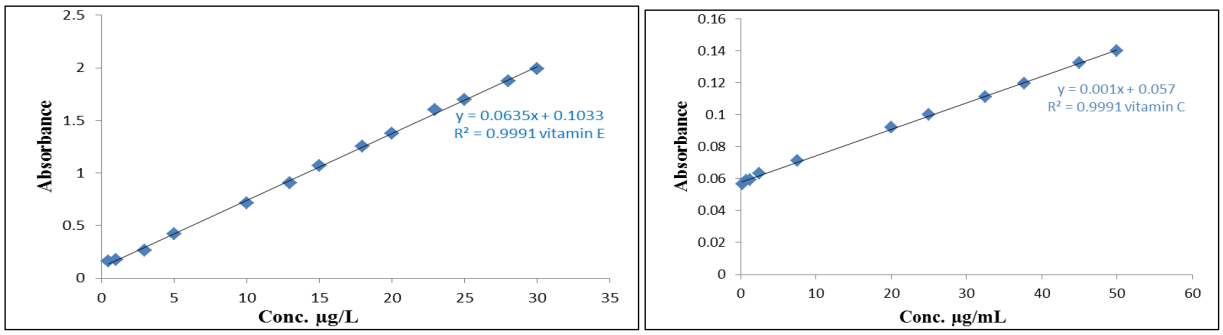

Fig. 10: UV–VIS spectrophotometric calibration curve.

Table 2 showed Statistical factors such as Linear equation, and Standard error of the intercept, standard deviation of intercept, Relative standard deviation, Linearity range, calculated (t) values so larger than tabulated values that point to strong relationship between absorbance and concentration, limit of detection and limit of quantitation.

Table 2: Statistical data for determination of vitamins.

Statistical factors	**Value**	
	Vitamin C	**Vitamin E**
Linear equation	Y=0.001[X] +0.057	Y=0.0635[X] +0.1033
Slope (m)	0.001	0.0635
Correlation coefficient "R^2"	0.9991	0.9991
Percentage linearity (R^2 %)	99.91	99.91
Correlation coefficient (r)	0.9995	0.9995
Standard error of intercept	0.001	0.011
Standard deviation of intercept	0.001	0.037
Relative standard deviation "R.S.D."	1.62	3.63
"LOD" µg/mL	0.07	0.10
"LOQ" µg/mL	0.21	0.33
Linearity range µg/mL	0.25 – 50	0.5 – 30
Calculated (t) values $t_{cal.} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	105.36 >>> 2.179	110.49 >>> 2.160

Recovery of standard materials: Recovery value was estimated after selected three solutions have different concentration in the range of standard curves of vitamins (10, 20, 30 µg/mL) for vitamin C and (5, 10 ,15 µg/mL) for vitamin E as illustrated in Table 3.

Table 3: Recovery of standard materials.

Vitamin C µg/mL		% Recovery		**% Error**	**R.S.D n = 3**
Taken	**Found**				
10	10.19	101.90	Mean = 100.02	1.90	1.01
20	20.06	100.30	S.D. =1.91	0.30	1.18
30	29.66	98.87		1.13	1.89
Vitamin E µg/mL		% Recovery		**% Error**	**R.S.D n = 3**
Taken	**Found**				
5	5.01	100.2	Mean = 99.92	0.20	1.52
10	9.79	97.90	S.D. = 2.34	2.10	1.95
15	15.16	101.67		1.67	1.68

4 - Application on commercial drug samples: The method was applied on different commercial pharmaceutical preparations from different manufactured countries as shown in Table 4.

Table 4: Application on commercial preparations.

Vitamin C Company	Label Claim mg/ tab.	Mean amount found mg/tab.	% Mean amount found	R.S.D n = 3
Iraq	250	244.6	97.84	1.11
Syria	500	510.3	102.06	2.13
Germany	75	74.06	98.75	1.07
Vitamin E **Company**	**Label Claim mg/ tab.**	**Mean amount found mg/tab.**	**% Mean amount found**	**R.S.D n = 3**
PHILVITAIE	400	404.7	101.18	1.33
MVC	100	102.8	102.8	1.44

5 - Conclusion

The newly proposed study involved simple, fast, precise and accurate methods for the determination of Tocopherol and Ascorbic acid in pure and dosage form by molecular absorption spectroscopy via oxidation reduction reaction in solutions. Accuracy of method was validated by recovery mean percentage which was found to be in the acceptable range.

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