EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 7
82-87
Year: 2017

SPECTROPHOTOMETRIC DETERMINATION OF VITAMIN E VIA FORMATION OF GOLD COMPLEX

Ahmed Mahdi Saeed[1*], Abbas Shebeeb Al-Kadumi[2] and Noor Jassem Mohammed Ali[3]

[1]College of Education for Pure Science - Diyala University.
[2]College of Pharmacy – Al - Nahrain University.
[3]College of Science - Diyala University.

***Author for Correspondence: Dr. Ahmed Mahdi Saeed**
College of Education for Pure Science - Diyala University.

Article Received on 02/05/2017 **Article Revised on 24/05/2017** **Article Accepted on 14/06/2017**

ABSTRACT
Two different methods were used for the determination of vitamin E (Tocopherol) via complex formation with gold ions in solutions using UV - VIS spectrophotometry and flame atomic absorption spectrophotometry (AAS). The absorption maxima of vitamin E – gold complex was found to be at (535 nm) and this wavelengths was selected for the analysis of vitamin E as standard and formulated samples. The optimum conditions were investigated through the study of different parameter such as pH, temperature, concentration, time and complex formula. Stability constants for the complex was (5.91×10^5). The purposed method obeyed Beer-Lambert's Law in the concentration range of (2 - 40 µg/mL), (1 – 22 µg/mL) with R^2 values of (0.9991, 0.9992) for UV-VIS and atomic spectroscopic methods respectively. The relative standard deviation (1.95, 1.59), detection limits (0.18, 0.044) and recoveries (96.86 and 101.66%) for the first and second method respectively. The methods were applied for the estimation of the active gradient of the vitamin E in different samples of formulated dosage. The accuracy of method was validated by mean percentage recovery which was found to be in the acceptable range.

KEYWORDS: Tocopherol, Spectrophotometric, Determination, Pharmaceutical.

INTRODUCTION
Vitamin E consists of two families of compounds, the tocopherols and tocotrienols, characterized by a 6-chromanol ring and an isoprenoid side chain. The members of each family are designated alpha (α)-, beta (β)-, gamma (γ)-, or delta (δ)- according to the position of methyl groups attached to the chroman nucleus. Therefore, 8 stereo isomers of the large vitamin E family are possible but only the RRR-form occurs naturally. Tocopherols and tocotrienols are differentiated by their phenyl "tails" as these are saturated in the tocopherols but unsaturated in the tocotrienols . α-Tocopherol is the most active.

Vitamins E consist of eight naturally occurring Tocopherols, of which α - Tocopherol is the most active. Chemical name 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol.[1] The primary function of vitamin E is as an antioxidant in prevention of the nonenzymic oxidation of cell components, for example, polyunsaturated fatty acids, by molecular oxygen and free radicals. Vitamin E is the generic descriptor for two families of compounds, the Tocopherols and the Tocotrienols. The different vitamins (compounds having similar vitamin activity) have different biologic potencies; the most active is D-α-Tocopherol, and it is usual to express vitamin E intake in milligrams of D–α-Tocopherol equivalents. Synthetic DL–α-Tocopherol does not have the same biologic potency as the naturally occurring compound.[2] Many analytical methods for determining vitamin E reported such as, High Performance Liquid Chromatography HPLC[3 – 11], UV-VIS spectrophotometry[12, 13], fluorescence[14, 15], colorimetric[16], photochemiluminescence.[17] Aim of this work is to use the ease and accurate spectrophotometric method for the determine the vitamin content in tablet samples from different pharmaceutical 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
Ultraviolet-Visible spectrophotometer UV-VIS:(Jasco V-650 spectrophotometer), England. Fourier transforms spectrophotometer FTIR: (Perkin Elmer Spectrum 65 FT-IR spectrophotometer), Germany. Flame atomic absorption spectrophotometer (FAAS) :Shimadzu (AA-

670) Japan. Electronic balance: KERN ACJ / ACS, Germany.

Materials and reagents

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

Preparation of Tocopherol standard solutions (100 µg/mL)

A stock drug solution (1000 µg/mL) was prepared by dissolving 0.100 g weight of Tocopherol standard powders in 100 mL acetone. Working standard drug solutions were prepared by diluting 10 mL of stock solution to 100 mL with acetone 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 (DDW).

Procedure for the vitamin E assay in pharmaceuticals tablets

Twenty tablets from formulated sample were accurately weighed and crushed to a powder. Amount equivalent to 0.1 g was weighed, dissolved in acetone, transferred to a 100 mL volumetric flask and completed to the mark with the same solvent. Known volume containing the appropriate amount of vitamin E corresponding to the range of the calibration curve was further transferred in 25 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 E concentration and it's weight. For AAS assay the measurements were carried out for purple precipitate isolated by centrifuge and dissolved in 5 mL acetone, the relationship between absorbance and concentration were plotted. The linear equation was used for calculate vitamin E concentration.

RESULTS AND DISCUSSION

Determination wavelength of maximum absorbance

The UV-VIS spectra of solutions were carried out, the maximum absorbance was found at λ_{max} (310 nm, 244 and 314 nm, 535 nm) for Tocopherol, Au^{+3} aqueous solution and Tocopherol – Au complex respectively as shown in Fig 1.

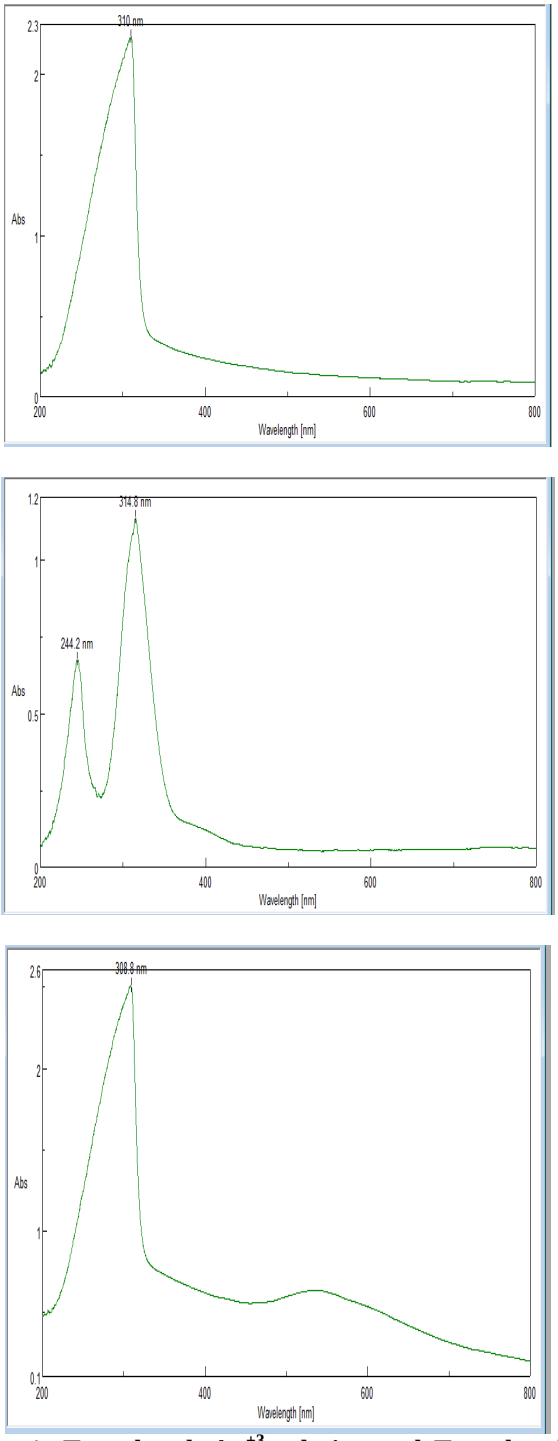

Fig: 1: Tocopherol, Au^{+3} solution and Tocopherol - Au complex spectrum.

Optimization conditions

Effect of pH was investigated after adjusting the value at the range of (2 - 12) by adding HCl or NaOH solution. Absorbance were recorded at λ_{max} of 535 nm versus blank solution. Results obtained revealed that the best pH value are (7 - 8) as shown in Fig 2. That may be due to the deprotonation of hydroxyl group was occur.

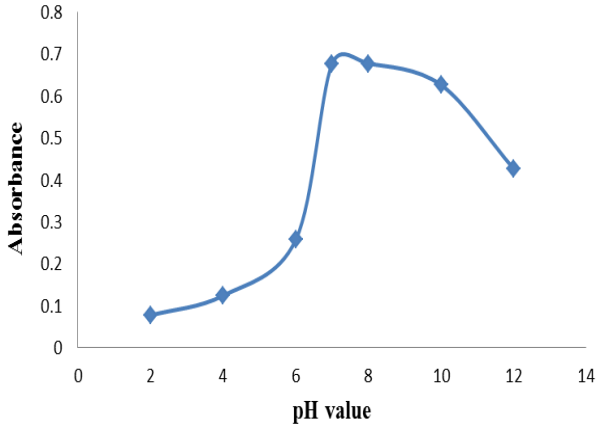

Fig: 2: pH value effect.

Temperature effect was carried out at the range of (20 - 35 ºC). The optimum temperature recorded at 20 ºC as shown in Fig 3.

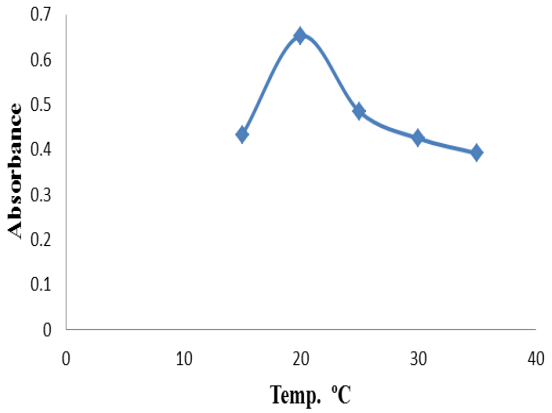

Fig: 3: Temperature effect.

The best time for complexation reaction was found to be at 30 minute as clear in Fig 4.

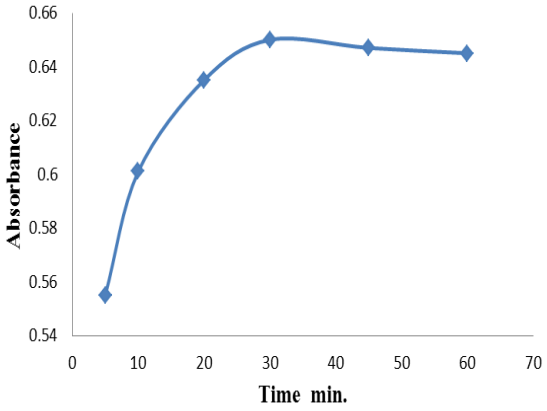

Fig: 4: Time of reaction effect.

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

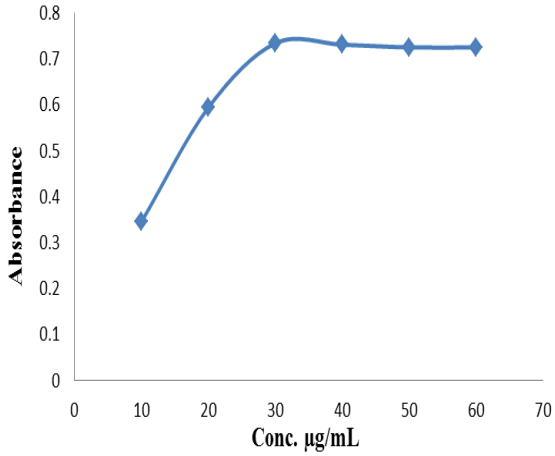

Fig: 5: Concentration of gold effect.

Estimation of molecular formula for complex

To determine the ratio of gold to Tocopherol in the formed complex, 2.94 x 10^{-3} M solution of both Tocopherol and gold ion 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 5.91 x 10^{5} 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 first point.

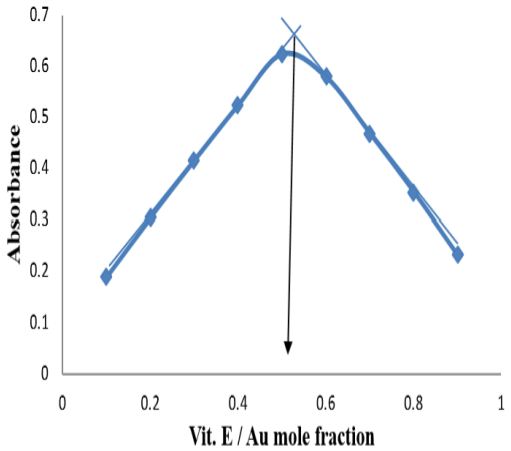

Fig: 6: Job method plot.

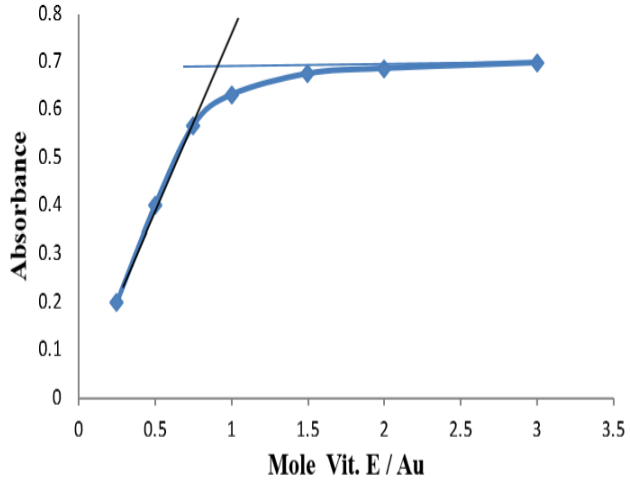

Fig: 7: Mole ratio method plot.

The FTIR spectra showed that the gold ion is coordinated to the hydroxyl group and ether oxygen of Tocopherol, leading to the suggested structures shown in Fig 8.

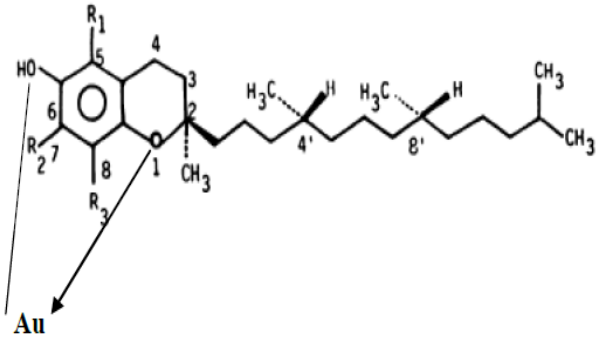

Fig: 8: Suggested structure for the Au - Tocopherol complex.

Preparation of calibration curves

A series of standard solutions of Tocopherol (1 - 50 µg/mL) were prepared after experimental conditions have been adjusted. The absorbance of complex in each case was recorded at the recommended λ_{max} and plotted against the concentration of Tocopherol. 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 an R^2 of (0.9991, 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.

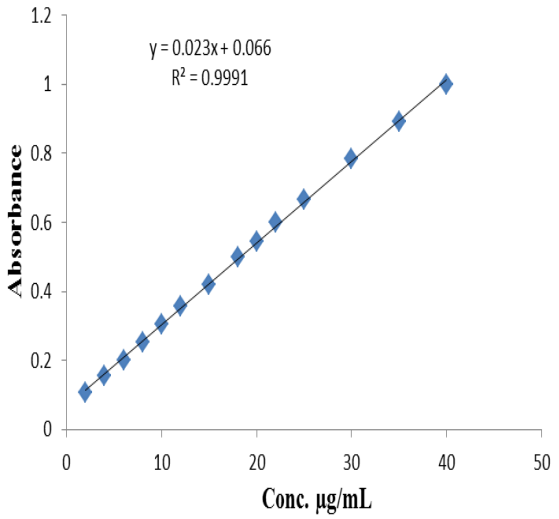

Fig: 9: UV calibration curve of Vitamin E.

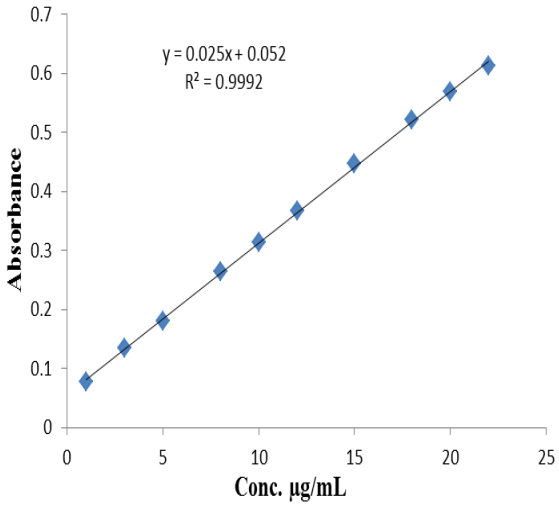

Fig: 10: AAS calibration curve of Vitamin E.

Table 1: Calibration curves statistical calculation.

Statistical factors	Value	
	UV- VIS method	AAS method
Linear equation	y = 0.023 [X] + 0.066	y = 0.025 [X] + 0.052
Slope (m)	0.023	0.025
Intercept	0.066	0.052
Correlation coefficient "R^2"	0.9991	0.9992
Percentage linearity (R^2%)	99.91	99.92
Correlation coefficient (r)	0.9995	0.9996
Intercept standard error	0.26	0.19
Intercept standard deviation	0.33	0.25
"R.S.D."	1.95	1.59
"LOD" µg/mL	0.18	0.044

"LOQ" µg/mL	0.594	0.145
Linearity range µg/mL	2.0 - 40	1 - 22
Molar Absorptivity L. mol.$^{-1}$. Cm^{-1}	1285	1102
Calculated (t) values $t_{cal.} = \frac{/r/\sqrt{n-2}}{\sqrt{1-r^2}}$	115.33 >>> 2.14	101.07 >>> 2.23

Accuracy and precision of proposed method

Vitamin E was determined at three different selected concentrations (10, 20, 30 and 5, 10, 15 µg /mL) for UV and AAS methods respectively. The obtained results are tabulated in (Table 2), which indicated that the proposed methods for the determination of vitamin E 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 E µg/mL		% Recovery		% Error	R.S.D n = 4
	Taken	Found				
UV– VIS.	10	10.29	102.90	Mean = 101.29 S.D. = 1.95	2.90	2.01
	20	20.36	101.80		1.80	1.28
	30	29.75	99.17		0.83	0.69
AAS	Vitamin E µg/mL		% Recovery		% Error	R.S.D n = 4
	Taken	Found				
	5	5.08	101.60	Mean = 99.93 S.D. = 1.59	1.60	1.51
	10	9.98	99.80		0.20	1.85
	15	14.76	98.40		1.60	1.58

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 methods

Method	% Recovery			
	New Method	R.S.D n = 4	Official Method [18]	R.S.D
UV – VIS.	101.29	1.95	99.31	2.24
AAS	99.93	1.59		

Quantitative assessment of vitamin E in tablets

Pharmaceutical formulations of vitamin E has been analyzed as described under recommended procedure, a good accuracy and precision were obtained as shown in Table 4. Obtained results were confirmed the reality and the applicability of the proposed method for the determination vitamin E of in pharmaceutical formulations. The results indicate that the recovery percentages for applying methods are with an acceptable range of (101.29 and 99.93) for standard vitamin E sample and the quantity of vitamin E in tablets was accepted within the normal percentage according to official method. Recovery percentages for vitamin E in formulate tablets were found to range from 96.86 – 101.66 % for the two methods, which confirmed the validity of the method for analysis the drugs in pharmaceutical formulations.

Table 4: Estimated quantity of vitamin E tablets sample.

Methods	Vitamin E Company	Label Claim mg/ tab.	Mean amount found mg/tab.	% Mean amount found	R.S.D n = 4
UV - VIS	PHILVITAIE	400	387.44	96.86	1.33
AAS			406.64	101.66	1.58

CONCLUSIONS

The proposed study involved simple, fast, precise and accurate methods for the determination of Tocopherol in pure and dosage form by ultraviolet-visible and atomic absorption spectroscopy via complexion with gold ion in solutions .The 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.

REFERENCES

1. Maryadele JO. (2006), The Merck Index, 14th ed, white house station, NJ, USA, Merck and co. Inc, 1114.
2. Harpers and et al (2003), Illustrated Biochemistry, twenty-sixth edition, 486.
3. Bin Z., Su-Yin T., Jia L., Mui H., Lionel K. and Shabbir M. (2004), Simultaneous determination of vitamin C, E, and β-carotene in human plasma by high performance liquid chromatography with

photodiode - array detection, J Pharm Pharm Sci, 30(2): 200-204.
4. Korchazhkina, E. Jones, M. Czauderna, S. Spencer and Kowalczyk, J. (2006), HPLC with UV detection for measurement of vitamin E in human milk, ACTA Chromatographia, 16: 48-57.
5. Mehmet E. and Kenan D. (2012), Determination of Vitamin E Isomers of Grape Seeds By High-Performance liquid Chromatography–UV Detection, J Chromatogr Sci, 50(2): 97-101.
6. Ivana B., Lenka K. and Dalibor Š. (2013), A Fast HPLC Method for Determination of Vitamin E Acetate in Dietary Supplements Using Monolithic Column, Food Analytical Methods, 6(2): 380–385.
7. Petr S., Dalibor S., Martin O., Radek S. and Petr S. (2017), Rapid and Simple Determination of Vitamin A and Vitamin E in Human Plasma by Column-Switching High-Performance Liquid Chromatography, 13(6): 311-315.
8. Francisco J., María J. and Ana F. (2011), Determination of vitamins E (α-, γ- and δ-tocopherol) and D (cholecalciferol and ergocalciferol) by liquid chromatography in milk, fruit juice and vegetable beverage, European Food Research and technology, 232(5): 829–836.
9. Prabakaran1, R., Chenthilnathan A. and Vikraman, S. (2014), Validation of simultaneous determination of vitamin A acetate and vitamin E acetate in multivitamin tablets by RP-HPLC, International Journal of Pharmaceutical, Biological and Chemical Sciences, 3(2): 27-34.
10. Zheng Y., Huang B. and Ren Y. (2016), Simultaneous determination of eight vitamin E isomers and vitamin A in foods by normal-phase high performance liquid chromatography, Chinese Journal of Chromatography, 34(7): 692-696.
11. Shahidee Z., Mohammad J., Mohd H. and Nuraliza A. (2012), determination of vitamin E isomers in plasma using ultra Performance Liquid Chromatography, The Malaysian Journal of Analytical Sciences, 16(1): 71 – 78.
12. Sanghamitra C. and Indranil C. (2013), Determination of Coverage Interval of Antioxidant Vitamins (Vitamin C & Vitamin E) in Plasma and Serum of Bengali Population ,Biochemistry Research International, 2013; 4-11.
13. Pilar P. (1999), Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E, 269(2): 337-341.
14. Ana M., Mihaela F., Mihaela I, Alexandru S. (2011), Quantitative analysis by fluorescence of vitamin E in pharmaceutical formulations, St. Cerc. St. CICBIA, 12(1): 25-32.
15. Saima, A. Malik, M. and Muhammad A. (2013), Spectrophotometric method for the determination of Vitamin A and E using Ferrozine-Fe (II) complex, Asian Journal of Research in Chemistry, 6(4): 334-340.
16. Jameel G., Shaheenkousar H., Swastika D., Salim A. and Kusal K. (2012), A modified simple method for determination of serum α-tocopherol (vitamin E), J Basic Clin Physiol Pharmacol, 19(1): 45-48.
17. Jasmin K., Victoria H., Josephine K., Margrit K. and Volker B. (2015), Antioxidant activities of Tocopherols - tocotrienols and lipophilic antioxidant capacity of wheat, vegetable oils, milk and milk cream by using photochemiluminescence, Food Chemistry, 175: 593–600.
18. Martínez S., Herrero V. and Negro S. (2004), Determination of Vitamin E in Biodegradable Microspheres by Reversed-Phase High-Performance Liquid Chromatography, Journal of Chromatographic Science, 42: 43-48.