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Quantitative Determination of α-Tocopherol in Pharmaceutical Soft Capsule by Spectrophotometry

Farmasötik Yumuşak Kapsülde α-Tokoferolün Spektrofotometri ile Kantitatif Tayini

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ABSTRACT

Objective: Vitamin E is an essential micronutrient for maintaining a healthy status and preventing disease. The purpose of this study was to develop and validate a simple, sensitive and easily applicable spectrophotometric method for determination of α-tocopherol in pharmaceutical preparations.

Material and Method: The quantitative determination of the α-tocopherol in pharmaceutical prepation was carried out using the maximum absorbance value measured at 290 nm. Calibration graphs were constructed by plotting the absorbance against the corresponding concentration of standart α-tocopherol samples at five different concentrations (10-100 µg/mL).

Results: The amount of α-tocopherol in the pharmaceutical soft capsule was calculated as 101.572% (203.145 IU/capsule) (Evicap soft capsule labelled content: 200 IU/capsule).

Conclusion: It suggested that the developed spectrophotometric method in this study is accurate, sensitive, precise, reproducible and easily applied to soft capsules and the other pharmaceutical preparations.

Keywords: Spectrophotometry, pharmaceutical soft capsule, α-tocopherol

ÖZ

Amaç: E vitamini sağlıklı durumun korunması ve hastalıkların önlenmesi için gerekli bir mikro besin maddesidir. Bu çalışmanın amacı, farmasötik preparatlarda α-tokoferolün belirlenmesi için basit, duyarlı ve kolayca uygulanabilir bir spektrofotometrik yöntem geliştirmek ve doğrulamaktır.

Gereç ve Yöntem: Farmasötik preparatlarda α-tokoferolün kantitatif tayini 290 nm'de ölçülen maksimum absorbans değeri kullanılarak gerçekleştirildi. Kalibrasyon grafikleri, beş farklı konsantrasyonda (10-100 µg/mL) standart α-tokoferol örneklerinin absorbanslarına karşılık gelen konsantrasyonların çizilmesiyle oluşturuldu.

Bulgular: Farmasötik yumuşak kapsüldeki α-tokoferol miktarı %101,572 (203,145 IU/kapsül) olarak hesaplandı (Evicap yumuşak kapsül etiket içeriği: 200 IU/kapsül).

Sonuç: Bu çalışmada geliştirilen spektrofotometrik yöntemin doğru, duyarlı, hassas, tekrarlanabilir olduğu ve yumuşak kapsül ve diğer farmasötik preparatlara kolayca uygulanabileceği ileri sürülmektedir.

Anahtar Kelimeler: Spektrofotometri, farmasötik yumuşak kapsül, α-tokoferol

INTRODUCTION

Vitamin E discovered by Evans and Bishop (1922) is an essential micronutrient soluble in fat and must be provided to the human body on regular basis to prevent deficiency and maintain a healthy status (1,2).

Vitamin E is very important for health promotion, disease prevention and terapeutic applications due to its chemical and biological antioxidant activity (3).

Vitamin E is a classical antioxidant due to properties free-radical scavenger (4) and has been used in treating reactive oxygen species (ROS) releated diseases (5). Vitamin E derivatives have been shown to be potent inducers of apoptosis in cancer cells because of their antioxidant activity. In addition, Vitamin E derivatives have been shown induce protective effects and prevent apoptosis in some experimental model systems (1,3). In addition to protective effects against some types of cancer (6,7), it has been

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reported that in the literature that Vitamin E has protective effect against cardiovascular, neurological and inflammatory diseases (4,8,9), and an incidence reducing effect against some diseases such as fibroplasia, bronchopulmonary dysplasia and hemolytic anemia (10,11). In literature, there are a lot of studies about *in vitro* and *in vivo* antitumor potential, antioxidant activity, antiradical activity and cytotoxicity of Vitamin E (12-16).

Vitamin supplements attract a lot of attention due to these effects. Numerous vitamin preparations often formulated are available on the market and can be taken easily.

Vitamin E is the name for molecules with α-tocopherol, describes eight lipophilic, naturally occuring compounds containing four tocopherols and four tocotrienols (α, β, γ and δ). The well-known function of vitamin E is antioxidant activitiy. Vitamin E requirements in humans are limited only to α-tocopherol because only α-tocopherol has been shown to reverse human vitamin E deficiency symptoms. Chemical structure of α-tocopherol is given in Figure 1 (3,17).

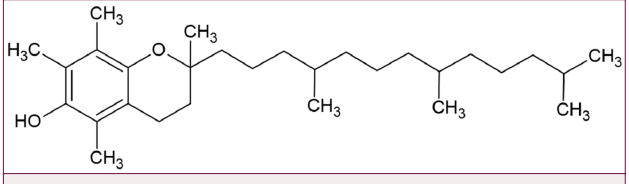

Figure 1. Chemical structure of α-tocopherol.

For the determination of α-tocopherol, several methods have been reported in different samples such as foods (18), cosmetics (19), biological fluids (20–22), natural compounds (23) and pharmaceutical preparations (24) in the literature. Chromatographic methods such as gas chromatography, liquid chromatography and spectroscophic methods such as mass spectrometry, UV-Vis spectrophotometry, flourescence spectrophotometry have been widely used for the quantification of tocopherols (25). Although mostly chromatographic methods such as high performance liquid chromatography (HPLC) are used, spectrophotometric methods are also highly preferred due to their simplicity and specifity for determination of pharmaceutical preparations (24).

In this work, it was aimed to develop a validated, sensitive and simple UV spectrophotometric method for the quantitative determination of α-tocopherol and also to apply the developed method to the commercial pharmaceutical preparations. For this purpose, the proposed method was validated according to International Council for Harmonisation (ICH) guideline (26) in terms of precision, linearity and accuracy.

MATERIAL AND METHOD

Instrumentation and chemicals

Ultraviolet visible spectrophotometer (Shimadzu UV Visible Spectrophotometer UVmini-1240) with local control software was used for determination of α-tocopherol. UV spectra of the solutions were recorded in 1 cm quartz cells at the range between 250 and 400 nm.

α-Tocopherol (CAS number 10191-41-0) and methanol (CAS number 67-56-1) was purchased from Sigma Aldrich (Germany). Evicap soft capsule (labelled content: 200 IU α-tocopherol/capsule) was purchased from pharmacy (Istanbul, Turkey).

Preperation of stock and quality control solutions

The α-tocopherol stock solution was prepared at a concentration of 5 mg/mL in methanol. For preparation of the quality control samples the stock α-tocopherol solution was diluted with methanol at the concentrations of 10, 25, 50, 75 and 100 µg/mL. Methanol was used blank solution. All solutions were stored 4 °C for 2 weeks.

Assay of pharmaceutical soft capsule

Three pharmacetical soft capsules (200 IU α-tocopherol in a capsule) were diluted to 30 mL with methanol and sonicated for 30 seconds. The mixture was filtered and than completed to 50 mL with methanol. The amount of α-tocopherol in capsule was calculated using regression equation.

Method validation

The developed method was validated according to ICH guidelines (26). Calibration curves were constructed by plotting the absorbance against to the corresponding concentration of quality control samples. Limit of quantification (LOQ) and limit of detection (LOD) were determined as 10 σ/s and 3.3 σ/s respectively. Intra- and interday precisions were tested at three concentration levels (25, 50, 75 µg/mL) of α-tocopherol. Accuracy of the method was examined by recovery studies performed at three concentrations. Recovery and RSD were calculated for commercial capsule form.

RESULTS

Spectrophotometric method

Methanol was used as blank solution in the study. α-Tocopherol's UV spectrum of in methanol showed maximum absorbance at 290 nm. The maximum absorbance of α-tocopherol was broader at low concentration. So, concentration of minimum quality control solution was 10 µg/mL in the study.

Assay validation

Linearity, LOD and LOQ

For spectrophotometric determination, linearity ranges were 10-100 µg/mL in methanol with a correlation coefficient (r) of 0.999 for α-tocopherol. The regression equation was found to be y=0.0058x+0.2198 for α-tocopherol. Standard graphic calibration curve and correlation coefficient (r) value were given in Figure 2. The statistical parametres of calibration curves were given in Table 1. The regression equation was calculated calibration curves along with the standart deviation of slope and intercept on the ordinate (n=6).

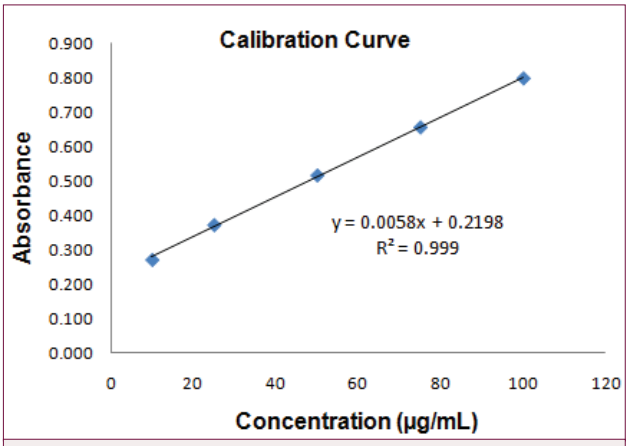

Figure 2. Standart graphic calibration curve.

LOD and LOQ values of the α-tocopherol was determined using calibration standards. LOQ and LOD of the proposed method were 2.228 and 6.752 µg/mL for α-tocopherol, respectively. The linearity parameters of the method were presented in Table 1.

Table 1. Validation parameters of the proposed method (n=6).

Parameters	α-tocopherol
λ (nm)	250-400
Maximum absorption (nm)	290
Linearity range (µg/mL)	10-100
Regression equation	y=0.0058x+0.2198
Standart deviation of slope	0.002
Standart deviation of intercept	0.090
Correlation coefficient (r)	0.999
LOD (µg/mL)	2.228
LOQ (µg/mL)	6.752

Precision and accuracy

Precision and accuracy of the proposed method was determined by analysing the quality control samples in the same day and on three diffrent days at three different concentration (25, 50, 75 µg/mL) (n=6).

Precision of the method was expressed by relative standard deviation (RSD %). Interday and intraday precision values (RSD %) were found in the range of 0.876-1.308 and 0.566-1.349, respectively.

Accuracy of the method was expressed by mean percent recovery (R%). Interday and intraday accuracy values (R%) were found in the range of 99.563-102.425 and 100.927-102.368, respectively.

Intraday and interday precision were found to be less than 1.35% and accuracy values were found to be about 100% (Table 2). These results showed that the developed method was validated and reproduciple with good precision and accuracy.

Analysis of commercial soft capsule

In this study, α-tocopherol in commercial soft capsule was analysed according to the validated method at three different concentration (25, 50, 75 µg/mL) (n=6). The mean recovery values was found between 100.084-103.920% range for the different concentrations of α-tocopherol. It was observed that the developed method was reproducible with good accuracy (Table 3) for soft capsule.

Table 3. Recovery values of the commercial soft capsule containing α-tocopherol (n=6).

Added (µg/mL)	Found±SD	R (%)	RSD (%)
25	25.178±0.003	100.084	0.692
50	51.960±0.005	103.920	0.903
75	75.063±0.005	100.713	0.742

SD: Standart deviation. RSD: Relative standart deviation. R: Recovery

Table 2. Intraday and interday precision values of the proposed method (n=6).

Interday precision				Intraday precision			
C (µg/mL)	**Found±SD**	**RSD (%)**	**R (%)**	**C (µg/mL)**	**Found±SD**	**R (%)**	**RSD (%)**
25	24.891±0.003	1.308	100.889	**25**	25.293±0.002	100.927	1.349
50	51.213±0.004	0.707	102.425	**50**	51.184±0.004	102.368	0.771
75	75.667±0.009	0.876	99.563	**75**	75.695±0.009	101.172	0.566

C: Concentration, SD: Standart deviation. RSD: Relative standart deviation. R: Recovery

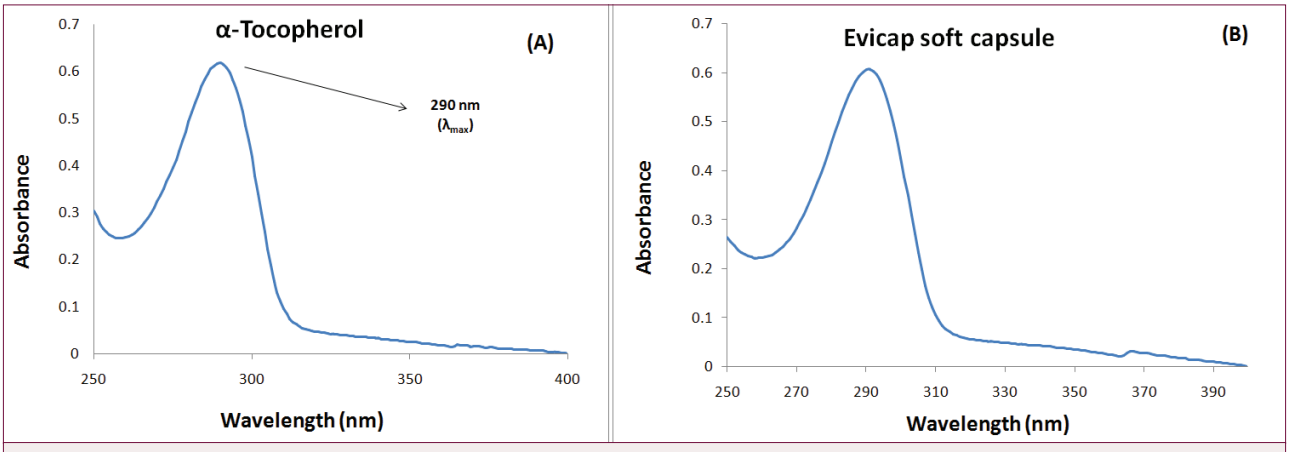

Figure 3. UV spectra of α-tocopherol (A) and commercial soft capsule (B).

UV spectrum of α-tocopherol at the concentration 100 µg/mL in methanol was given in Figure 3 (A). UV spectrum of commercial soft capsule containing α-tocopherol at the concentration 75 µg/mL was given in Figure 3 (B).

The calculated content of α-tocopherol in capsule was about 100.084-103.920% (203.145 IU/capsule) of the labelled content (200 IU/capsule).

DISCUSSION

In literature, there are several different methods (18,20,27,28) reported for the quantitaive determination of α-tocopherol in different samples such as natural plants (23), pharmaceutical preparations (24) and human plasma (20,21). Spectrophotometric and chromatografic (liquid, gas, high performance liquid chromatography etc.) methods are frequently used to determination of α-tocopherol (18,20,23,24). These methods, especially chromatographic methods require different experimental processes such as extraction and removal of excipients. Also, chemical reagent used in chromatographic methods are more expensive than others. Therefore, spectrophotometric methods that do not require these experimental procedures are cheaper and simpler than chromatographic methods.

According to the literature researches, it was ovserved that α-tocopherol determination studies were mostly done in biological fluids (27), natural plants (23), foods (18) and cosmetics (19). Although several researches for the determination of α-tocopherol in biological fluids, cosmetics etc have been reported, determination in pharmaceutical preparations is scarce.

In this study for the developed spectrometric method, the regression equation and correlation coefficient (r) were found to be y=0.0058x+0.2198 and 0.999 for α-tocopherol, respectively. LOD and LOQ values for the method were found to be 2.228 and 6.752 µg/mL, respectively. Also presicion and accuracy values of the developed spectrophotometric method were found to be less than 1.35% and about 100%, respectively. These results showed that the developed method validated for quantitative determination.

And also the calculated content of α-tocopherol in Evicap soft capsule was about 100.713-103.920% of the labelled content. These obtained results showed that a spectrophotometric method was developed and validated for the determination of α-tocopherol in commercial soft capsule formulation.

The results obtained showed that the developed and validated method is cheaper and simpler than the other methods in the literature such as chromatographic, voltammetric and spectroscopic methods (20,24,25). And also these results showed that the developed method presice and accurate for the quantitaive determination.

CONCLUSION

In the present work a simple, precise, reproducible and accurate spectrophotometric method has been developed and validated for routine determination of α-tocopherol in commercial soft capsule formulation. The presented method can be applied directly and easily to the commericial pharmaceutical formulations of α-tocopherol. The obtained results showed that the proposed method might be an alternative determination method for routine analysis.

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Peer-review: Externally peer-reviewed.

Author Contributions: Concept - G.Ö.A.; Supervision - G.Ö.A.; Materials G.Ö.A.; Data Collection and/or Processing - G.Ö.A.; Analysis and/or Interpretation - G.Ö.A.; Literature Search - G.Ö.A.; Writing - G.Ö.A.; Critical Reviews - G.Ö.A.

Conflict of Interest: The author has no conflict of interest to declare.

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Hakem Değerlendirmesi: Dış bağımsız.

Yazar Katkıları: Fikir - G.Ö.A.; Denetleme - G.Ö.A.; Gereçler - G.Ö.A.; Veri Toplanması ve/veya İşlemesi - G.Ö.A.; Analiz ve/veya Yorum - G.Ö.A.; Literatür Taraması - G.Ö.A.; Yazan - G.Ö.A.; Eleştirel İnceleme - G.Ö.A.

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