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

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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 preparation 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 standard 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

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 therapeutic 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) related 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...
reported that in the literature that Vitamin E has protective
effect against cardiovascular, neurological and inflammato-
ry diseases (4,8,9), and an incidence reducing effect against
some diseases such as fibroplasia, bronchopulmonary dyspla-
sia and hemolytic anemia (10,11). In literature, there are a lot
of studies about in vitro and in vivo antitumor potential, anti-
oxidant 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, de-
scribes eight lipophilic, naturally occuring compounds contain-
ing four tocopherols and four tocotrienols (α, β, γ and δ). The
well-known function of vitamin E is antioxidant activity. Vita-
min 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-to-
copherol is given in Figure 1 (3,17).

For the determination of α-tocopherol, several methods have
been reported in different samples such as foods (18), cos-
metics (19), biological fluids (20–22), natural compounds (23)
and pharmaceutical preparations (24) in the literature. Chro-
matographic methods such as gas chromatography, liquid
chromatography and spectroscoptic methods such as mass
spectrometry, UV-Vis spectrophotometry, flourescence spec-
photometry 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 specificity for determination of phar-
aceutical preparations (24).

In this work, it was aimed to develop a validated, sensitive and
simple UV spectrophotometric method for the quantitative de-
termination 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 Inter-
national 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 be-
tween 250 and 400 nm.

α-Tocopherol (CAS number 10191-41-0) and methanol (CAS
number 67-56-1) was purchased from Sigma Aldrich (Ger-
many). 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 concentra-
tion 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 pharmaceutical 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 guide-
lines (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 re-
spectively. Intra- and interday precisions were tested at three
concentration levels (25, 50, 75 µg/mL) of α-tocopherol. Ac-
curacy of the method was examined by recovery studies per-
formed at three concentrations. Recovery and RSD were calcu-
lated for commercial capsule form.

**RESULTS**

**Spectrophotometric method**

Methanol was used as blank solution in the study. α-Tocopher-
ol’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 cali-
bration 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 cali-
bration curves along with the standart deviation of slope and
intercept on the ordinate (n=6).
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               | $y=0.0058x+0.2198$                |
| Standard deviation of slope       | 0.002                             |
| Standard deviation of intercept   | 0.090                             |
| Correlation coefficient (r)       | 0.999                             |
| LOD (µg/mL)                       | 2.228                             |
| LOQ (µg/mL)                       | 6.752                             |

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

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

**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   |

**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 different 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 reproducible 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.
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 quantitative determination of α-tocopherol in different samples such as natural plants (23), pharmaceutical preparations (24) and human plasma (20,21). Spectrophotometric and chromatographic (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 observed 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 precision 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 precise and accurate for the quantitative 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 commercial 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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**Ethics Committee Approval**: Ethics committee approval is not required because of no material or experimental animal that would require permission.

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Özgül Artuç
Determination of α-Tocopherol

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