Quantitative Determination of α-Tocopherol and α-Tocopheryl Acetate in Pharmaceutical and Supplementary Soft Capsules by High Performance Liquid Chromatography

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Abstract

Aim: This study aims to determine the amounts of α-tocopherol (α-toc) and α-tocopheryl acetate (α-tocAc) in soft pharmaceutical capsules and supplements using a chromatographic method. Additionally, method validation parameters for both analytes were evaluated.

Method: Analyses were performed using a chromatographic method. As part of the method validation, recovery (% recovery), repeatability (% RSD), linear regression coefficient (R^2), limit of detection (LOD), and limit of quantification (LOQ) values were determined.

Results: The recovery values were found to be above 95%. Repeatability (%RSD) was calculated as <3%. The linearity (R^2) values were obtained as 0.999. For α-toc: LOD: 0.131 µg/mL and 2.228 µg/mL, LOQ: 0.396 µg/mL and 6.752 µg/mL. For α-tocAc: LOD: 0.260 µg/mL, LOQ: 0.787 µg/mL.

Conclusion: The chromatographic method used in this study enabled the determination of α-toc and α-tocAc with high accuracy and repeatability. This method can be considered a reliable alternative for the analysis of these compounds in pharmaceutical products.

Keywords: α-tocopherol, α-tocopheryl acetate, HPLC, soft capsule.

Farmasötik ve Takviye Edici Yumuşak Kapsüllerdeki α-Tokoferol ve α-Tokoferil Asetat'ın Yüksek Performanslı Sıvı Kromatografisi ile Kantitatif Tayini

Öz

Amaç: Bu çalışmada, yumuşak farmasötik kapsüller ve takviyelerde bulunan α-tokoferol (α-tok) ve α-tokoferil asetat (α-tokAs) miktarlarının kromatografik yöntem kullanılarak belirlenmesi amaçlanmıştır. Ayrıca, her iki analit için yöntem doğrulama (validasyon) parametreleri değerlendirilmiştir.

Yöntem: Analizler kromatografik bir yöntemle gerçekleştirilmiştir. Yöntem validasyonu kapsamında geri kazanım, tekrarlanabilirlik, doğrusal regresyon katsayısı, LOD ve LOQ değerleri belirlenmiştir.

Bulgular: Geri kazanım değerleri %95'in üzerinde bulunmuştur. Tekrarlanabilirlik (%RSD) <%3 olarak hesaplanmıştır. Doğrusallık (R^2) değerleri 0.999 olarak elde edilmiştir. α-tok için: LOD: 0.131 µg/mL ve 2.228 µg/mL, LOQ: 0.396 µg/mL ve 6.752 µg/mL α-tokAs için: LOD: 0.260 µg/mL, LOQ: 0.787 µg/mL.

Sonuç: Çalışmada kullanılan kromatografik yöntem, α-tok ve α-tokAs miktarlarının yüksek doğruluk ve tekrarlanabilirlik ile belirlenmesini sağlamıştır. Yöntem, farmasötik ürünlerdeki bu bileşiklerin analizi için güvenilir bir alternatif olarak değerlendirilebilir.

Anahtar Sözcükler: α-tokoferol, α-tokoferil asetat, HPLC, yumuşak kapsül.

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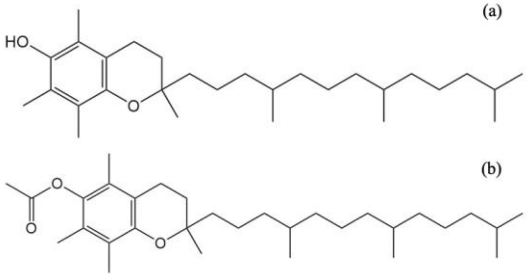
Introduction

Vitamins are organic nutrients that are required in low amounts for biochemical reactions in the body, and their absence in the diet can lead to inadequate growth and development. These organic nutrients differ in their chemical composition, physiological effects and nutritional importance[1]. Vitamin E is a fat-soluble vitamin consisting of 4 tocopherols (α, β, γ and δ tocopherols) and 4 tocotrienols (α, β, γ and δ tocotrienols)[2]. The IUPAC name of α-toc was ((2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12trimethyltridecyl]-3,4-dihydro-2H-1 benzopyran-6-ol[3]. The IUPAC name of α-tocAc was (2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-4,8,12-trimethyltridecyl]-3,4-dihydro-2H-1- benzopyran-6-yl acetate. The chemical structures of α-tocopherol (α-toc) and α-tocopheryl acetate (α-tocAc) are given in Figure 1.

Figure 1. Chemical structure of α-toc (a) and α-tocAc (b)



Alpha tocopherol is the main component of vitamin E and is known to have antioxidant properties. Alpha tocopheryl acetate is the acetylated ester form of alpha tocopherol. Alpha tocopherol acetate acts as an antioxidant by protecting cells from free radical damage[4,5]. Alpha tocopherol acetate has the same effect by converting from ester form to tocopherol form, but it becomes active more slowly. Since it is in ester form, it is a slightly more stable component than tocopherol. Therefore, this form is more preferred in cosmetic products[6–8].

Vitamin E serves as the primary lipophilic antioxidant in the plasma, membranes, and tissues across all mammals. Among its homologues, α-toc is the most prevalent form found in humans[9]. It plays a critical role in preventing lipid peroxidation and is recognized for mitigating stress-induced apoptosis[10,11]. Numerous studies have demonstrated that α-toc enhances antioxidant defenses within the biological system, making it beneficial for individuals experiencing anxiety and depression. Moreover, vitamin E supplementation is associated with minimal side effects[12]. As a potent fat-soluble antioxidant, α-toc effectively interrupts chain reactions in biological membranes, stabilizes cell membranes, and shields cellular components from damage caused by oxygen-free radicals and lipid peroxidation. Additionally, due to its strong antioxidant capacity, vitamin E aids in neutralizing free radicals generated by radiation exposure[13].

Vitamin E supplements are typically provided in the form of α-tocAc, a derivative of α-toc with antioxidant properties[14]. The U.S. Food and Drug Administration classifies tocopherols and α-tocAc as generally recognized as safe substances. Tocopherols, when utilized as antioxidants in food products, are readily absorbed and metabolized by the body. While they exhibit low toxicity, with LD50 values around 2000 mg/kg[15], excessive intake of vitamin E may interfere with the absorption of other fat-soluble vitamins,

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including vitamins A, D, and K. Additionally, high doses of vitamin E might exert a pro-oxidant effect under certain conditions[16].

Quantification of lipophilic vitamins in clinical or biological samples is of medical and epidemiological importance. However, there are some difficulties in the simultaneous determination of these compounds, such as their susceptibility to photooxidation, the presence of cis- and trans-isomers, and their varying polarity. Due to the complex matrix of clinical and biological samples, an additional sample pretreatment step is needed before their determination[17]. Various techniques such as voltametric[18], spectrophotometric[19] and chromatographic[20,21] methods, have been reported in the literature for the determination of α-toc in pharmaceutical preparations.

In this study, α-toc and α-tocAc in pharmaceutical preparations and supplements were quantitatively determined by high-performance liquid chromatography (HPLC). The analytical method applied was precise, accurate, linear, selective, and robust.

Material and Methods

Reagents and Chemicals

α-toc (CAS number 10191-41-0) and HPLC-grade methanol (CAS number 67-56-1) were purchased from Sigma Aldrich (Germany). α-tocAc was purchased from Doğa İlaç (Istanbul), Evicap soft capsules (200 IU α-toc per capsule) and Nature's Bounty (400 IU 268 mg^{-1}) were purchased from the pharmacy (Istanbul, Turkey), Doppel herz Omega-3 (20 mg Vitamin E per capsule) was purchased from the pharmacy (Deutschland, Germany).

Analytical Instruments and Conditions

Agilent 1200 instrument with UV/Vis detector was used for the determination of α-toc and α-tocAc in pharmaceutical preparation and supplements by HPLC, and ChemStation software was used for data collection and analysis. HPLC consists of a piston pump, degasser, column oven, UV-DAD detector and autosampler quadruple system. Glass vials of 1.5 mL were used in the autosampler. Chromatographic separation was performed using a C18 column (GL Sciences, Japan) (150 mm x 4.6 mm, 3 µm). Mobile phase: methanol (100%, v/v), flow rate: 2 mL/min, injection volume; 10 µL, column temperature: 25 ^{o}C, chromatographic detection wavelength; 285 nm. DK SONIC brand (DK-300H) ultrasonic bath, Radwag (AS 220.R2) brand analytical balance for all weighing, and Eppendorf (Research plus) brand pipettes were used for pipetting.

Preparation of Stock and Quality Control Solutions

The stock solution of α-toc and α-tocAc for HPLC determination was prepared in methanol at a concentration of 50 µg/mL. Standard solutions were prepared by diluting the stock solution with methanol at concentrations of 1, 2, 4, 6, 8 (α-tocAc), 0.5, 1, 2, 4, 6, 8 (α-toc) µg/mL. Methanol was used as a blank solution. All solutions were stored in the refrigerator at +4 ^{o}C until analysis.

Procedure for Pharmaceutical Preparation and Supplements

Pharmaceutical soft capsules and supplements were cut open with a sharp knife, and the capsule contents were transferred to a volumetric flask with methanol. The knife and capsules were carefully washed with methanol, and the remaining α-toc and α-tocAc

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were transferred to the flask[19,22,23]. The final volume was diluted to 50 mL with methanol. The resulting mixture was dissolved in an ultrasonic bath at room temperature (24-25°C) for 30 min and then diluted to 50 mL with methanol. The prepared solutions were filtered through a 45 µm filter into 1 mL HPLC vials and kept refrigerated at +4°C until analysis. The same procedure was performed for the blank solution using solvent only.

Method Validation

Chromatographic method was validated following the protocols specified in ICH guidelines Q2(R1)[24]. Validation parameters are linearity, accuracy, precision, specificity, LOD, LOQ.

Linearity

Concentration curves of α-toc and α-tocAc solutions prepared in the concentration range of 0.5-8 and 1-8 µg/mL were plotted (peak area versus concentration for chromatographic method). Evaluation of linearity was performed by regression analysis using the least squares method.

Precision

Intraday precision analysis was performed by HPLC method for four tablet samples (n=4) prepared at specific concentrations. For inter-day precision, measurements were performed on five consecutive days, and analyte concentration and relative standard deviation (%RSD) were calculated.

Accuracy

The accuracy of the method was determined by recovery studies. Accuracy study was performed by analyzing the sample at the determined concentration and comparing the values after addition at known concentrations. Recovery percentages were calculated by HPLC methods.

Limit of Detection and Limit of Quantification (LOD, LOQ)

LOD and LOQ values were calculated to evaluate the sensitivity of chromatographic method. LOD; 3.3σ/m, LOQ; 10σ/m were calculated according to the formulae. σ is the standard deviation (intercept) of the y-axis intercept of the calibration curve, and m is the slope of the calibration curve.

Marketed Formulation Analysis

Soft capsule samples containing α-toc and α-tocAc were prepared as described in the preparation of pharmaceutical preparations section and analyzed by HPLC.

Results

The linearity of the method was established over the analytical range of 0.5-8 µg/mL for α-toc and 1-8 µg/mL for α-tocAc. An excellent correlation between the analyte peak area and the standard concentration was obtained, with $R^2 \geq 0.999$ for all standard curves (Figure 2, Figure 3, Table 1).

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Figure 2. Standard graphic of calibration curve of HPLC-UV (α-toc)

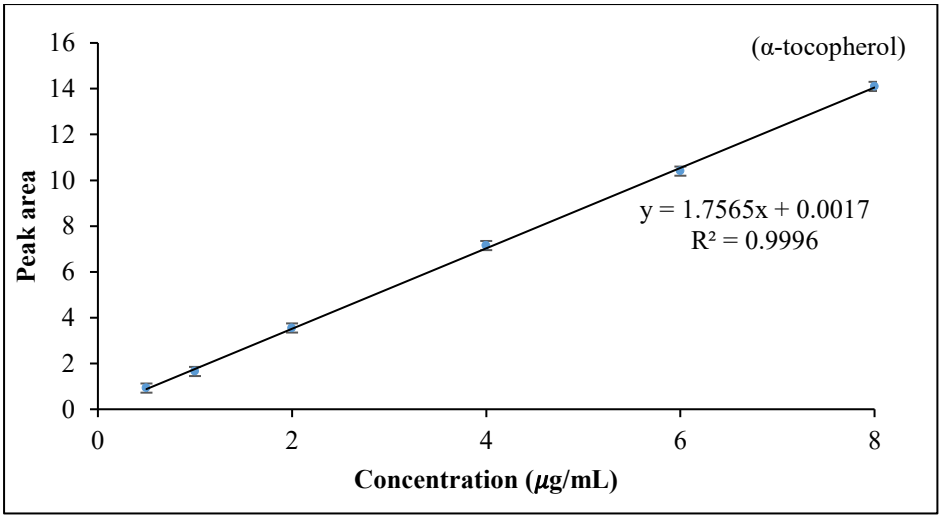

Figure 3. Standard graphic of calibration curve of HPLC-UV (α-tocAc)

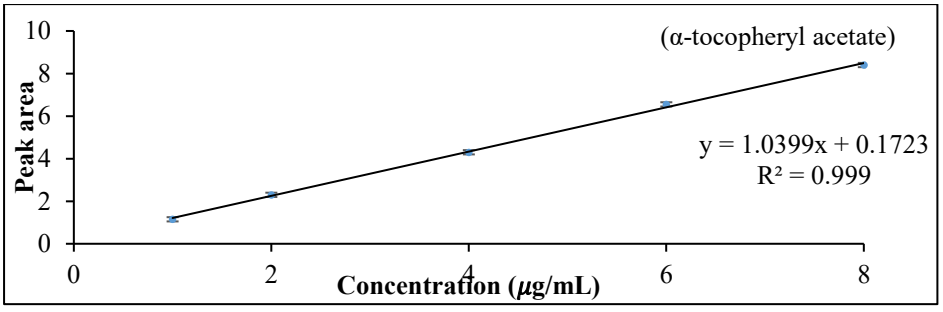

Table 1. Linearity data obtained by HPLC for α-toc and α-tocAc

Regression parameters	**α-toc**	**α-tocAc**
Number of points	6	5
Regression coefficient (R^2)	0.9996	0.999
Slope	1.7565	1.0399
Intercept	0.0017	0.1723
Concentration range (µg/mL)	0.5-8	1-8

It was observed that the quantification of α-toc and α-tocAc by chromatographic method provided strong theoretical plates (>7.0) and peak tailing factor (<1.0) when methanol was used as the mobile phase. Chromatographic conditions were isocratic mobile phase containing 100% methanol on a GL Sciences C18 column at 25°C and 2 mL/min flow rate. Under these chromatographic conditions, the retention times of α-toc and α-tocAc were 3.5 and 5.0 min, respectively (Figure 4, Figure 5).

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Figure 4. HPLC-UV chromatograms of α-toc (0.5-8 µg/mL)

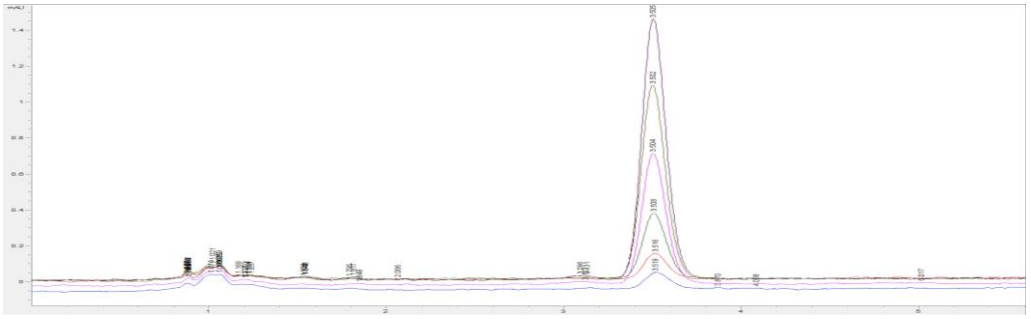
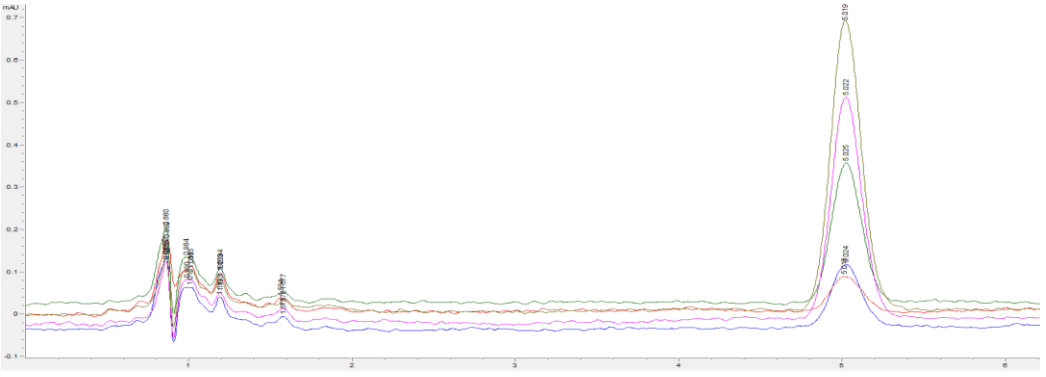


Figure 5. HPLC-UV chromatograms of α-tocAc (1-8 µg/mL)



Analytical Validation

The regression coefficient (R^2) in α-toc and α-tocAc determination by the HPLC method was high (>0.999) and no significant difference in linearity was observed in the analyzed range. As a result of the analysis, %RSD values are below three (<3) (Table 2). The %RSD values obtained show that the sensitivity of the method is good.

Table 2. Validation parameters for HPLC methods

Validation parameters	**α-toc**	**α-tocAc**
Number of points	6	5
Accuracy, (mean recovery, %, n=5)	94.7	106.2
LOD (µg/mL)	0.131	0.260
LOQ (µg/mL)	0.396	0.787
Concentration range (µg/mL)	0.5-8	1-8
Intra-day precision (RSD %, n=4)	1.66	1.76
Inter-day precision (RSD %, n=20)	2.02	2.39

Method accuracy was examined using the standard addition method. Recovery values were found to be close to 100% (Table 3).

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Table 3. Recovery of α-toc and α-tocAc from pharmaceuticals (n=5)

Added	**Found**		**Recovery (%)**	
	HPLC*	**HPLC**	**HPLC***	**HPLC**
-	4.026±0.221	4.121±0.164	-	-
0.5 µg/mL	4.594±0.124	4.570±0.057	113.6	89.8
1.0 µg/mL	5.074±0.124	5.151±0.073	104.8	103
1.5 µg /mL	5.531±0.092	5.492±0.074	100.3	91.4

* α-tocAc

In the specificity analysis performed by HPLC, the peak purity of the α-toc chromatograms of the samples was observed to be over 99%. According to the results, other peaks were separated from the main peak.

The LOD values for the HPLC method were 0.131 µg/mL and 0.260 µg/mL for α-toc and α-tocAc, respectively, and the LOQ values were 0.396 and 0.787 µg/mL for α-toc and α-tocAc, respectively.

Soft Capsules Samples Analysis

HPLC method was applied to the pharmaceutical preparations Evicap soft capsules and Nature's Bounty dietary supplement soft capsules (Table 4).

Table 4. Determination of α-toc acetate and α-tocAc in real samples (n=5)

		α-toc		**α-tocAc**	
Evicap	3 µg/mL	3.112±0.341	103.7%	0.885±0.043	-
	4 µg/mL	4.029±0.157	100.7%	1.187±0.049	-
	5 µg/mL	4.883±0.142	97.6%	1.519±0.035	-
Nature's Bounty	3 µg/mL	-	-	2.995±0.061	99.8%
	4 µg/mL	-	-	4.055±0.077	101.2%
	5 µg/mL	-	-	5.160±103.2	103.2%
Doppel herz	20mg/capsule Total Vitamin E			442.6□2.75	110.5%

HPLC determination of α-toc and α-tocAc in pharmaceutical preparation and supplements were found to be a more sensitive and selective method. This method can be applied for α-toc and α-tocAc determination. The Figure 6 and Figure 7 shows the overlaid spectra of real sample and standard α-toc and α-tocAc.

α-toc and α-tocAc are compounds found in many products such as pharmaceuticals, supplements, and cosmetics. Therefore, it is very important to develop and validate simple, rapid, nontoxic, and inexpensive procedures for the determination of these compounds.

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Figure 6. Overlay HPLC-UV chromatogram of sample and α-toc standard

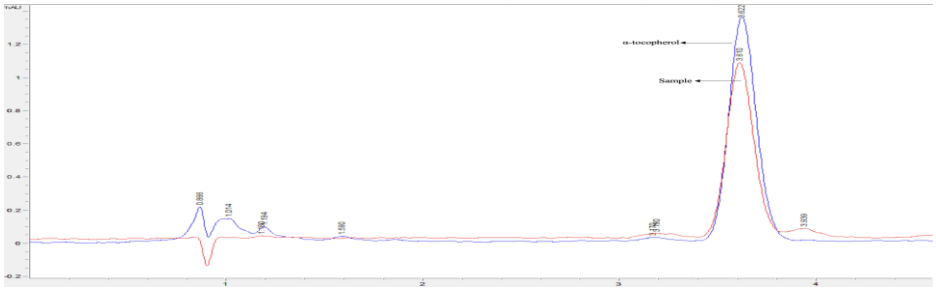
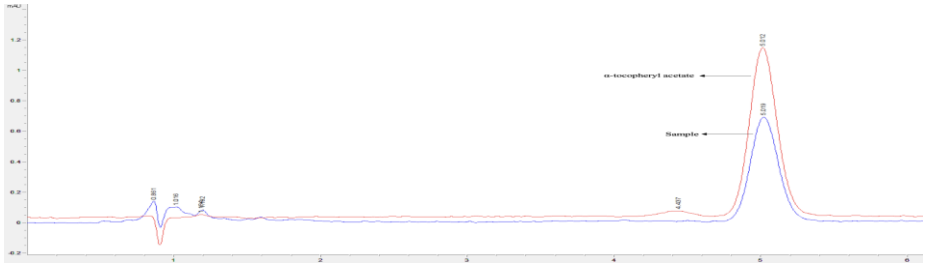


Figure 7. Overlay HPLC-UV chromatogram of sample and α-tocAc standard



Discussion

In this study, α-toc and α-tocAc in pharmaceutical preparations and supplement soft capsules were determined by chromatographic method. In the literature, there are studies in which tocopherol and its derivatives are determined from various samples by various methods[13]. The % recovery, RSD, and LOD values obtained in this study, along with some studies from the literature, are presented in Table 5.

When this study is compared with studies in the literature that applied chromatographic and spectrophotometric methods, the % recovery values were found to be consistent with the literature. When LOD values were compared, similar values were obtained with other chromatographic methods, while lower values were found compared to spectrophotometric methods. The intra-day and inter-day repeatability values obtained in this study were also consistent with the literature for both analytes, demonstrating that reproducible results were achieved.

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Table 5. Comparison of the presented study on the determination of α-toc and α-tocAc in pharmaceutical and soft capsules using HPLC with the literatüre

Study	**Analyte**	**Matrix**	**Method**	**Recovery (%)**	**RSD (%)**	**LOD**
Goudarzi[25]	Total Vitamin E	Oil	HPLC	90.2-99.76	1.11-5.12	2.3 ng/mL
Pasias[26]	α-toc	Cereal, biscuit	GC-FID	99.5	<10	0.17 mg/L
Kadioglu[27]	α-toc	Cow milk Soft capsule Film tablet	GC-FID	98.7-100.7	<20	0.30□g/mL
Demirkaya-Miloglu[28]	α-toc	Pharmaceutical capsule Human plasma	Spectrofluorimetric method	100.5-108.3	3.79	0.09□g/mL
Ashour[29]	Total Vitamin E	Pharmaceutical preparations	Spectrophotometric method	101.3	0.71-1.82	0.49□g/mL
Méjean[30]	Tocopherols tocotrienols	Soybean oil	SFC-MS	87.3-100.3	<15	21.5□g/L
Jeelani[31]	α-tocAc	Multivitamin tablet	UPLC	100.4-101	<3	-
Luhua[23]	α-toc	Capsule	HPLC	95.82	2.3	0.94 □g/mL
Moreno[20]	α-tocAc	Multivitamin capsule	HPLC	100	1.64	3.09 mg/L
Wang[32]	α-tocAc	Vitamin capsule	HPLC	103	6	-
This study	α-toc α-tocAc	Pharmaceutical Supplementary soft capsule	HPLC	89.8-113.6	1.66-2.39	0.131, 0.260 □g/mL

Conclusion

The method presented in this study is applicable for the determination of αtoc and αtocAc in supplementary and pharmaceutical soft capsules using HPLC. The obtained recovery values range from 89.8% to 113.6%. As a fat-soluble vitamin, vitamin E in the forms of αtoc and αtocAc has been determined with a low detection limit, high repeatability, good sensitivity, and a short analysis time. The simplicity and speed of the proposed procedure make it an attractive option for the determination and quality control of multivitamins in the dietary supplement and pharmaceutical preparation industries.

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Graphical Abstract

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