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ORIGINAL PAPER

Colorimetric quantification of α-tocopherol (vitamin E) in pure form and different comestible samples by using newly synthesized tetrazolium salts

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Abstract
A quick, susceptible, and viable spectrophotometric procedure for the assay of α-tocopherol by newly synthesized tetrazolium reagents 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT) is reported. The reduction behavior of α-tocopherol is used to quantify it in pure form and different edibles. Colorless tetrazolium salt-reduced in the pink-colored formazan by α-tocopherol gives absorption maxima at 526 nm. The reduction process occurred in the basic medium, so the pH of the reaction medium was maintained in the range 8–9. Beer's law was obeyed in the concentration range of 0.2–15.0 µg/mL with PTMPT and 0.1–0.4 µg/mL with PTNPT. The molar absorption coefficient is 4.21×10^3 L $mol^{-1}cm^{-1}$ and 6.4×10^4 L $mol^{-1}cm^{-1}$ with PTMPT and PTNPT, respectively. Limit of detection is 0.14 µg/mL and 0.03 µg/mL, and limit of quantification is 0.42 µg/mL and 0.08 µg/mL for α-tocopherol with PTMPT and PTNPT, respectively. As the proposed method is easy and user-friendly, distinct edibles are quantified by using the proposed method, and the results show good agreement with the other existing methods. The samples were saponified first to convert α-tocopheryl acetate in its active form, i.e., α-tocopherol. Saponification had done at temperature 70 °C for 2 h on heating mental. The values of the correlation coefficient (R^2) are 0.955 and 0.998 with the relative standard deviation of 0.8% and 4.6% with PTMPT and PTNPT, respectively. The proposed method is effectively used for the assay of α-tocopherol in different pharmaceuticals, fruits, cereals, nuts, and milk samples.

Keywords α-tocopherol · PTMPT · PTNPT · Spectrophotometer · Quantification

Introduction

Naturally, vitamin E crops up in eight particular structures that fuse four tocopherols (alpha, beta, gamma, and delta) and four tocotrienols (alpha, beta, gamma, and delta). Out of all, α-tocopherol is the most powerful, frequent, and the only form of vitamin E that meets human requirements [1]. Although non-α-tocopherols have similar antioxidant nature, they are poorly recognized by the hepatic α-tocopherol transfer protein (α-TTP) [2]. Vitamin E has antioxidant behavior [3] which helps in the deactivation and inhibition of free radicals, as a result of which vitamin E plays a major role in enhancing immune response [4]. The enhanced immune system helps in preventing diseases inactive tissues of the body from the agents [5]. Bioavailability of nutrients changes with age and the kind of dietary admissions. Mostly, the eating habits regulate physiological conditions and the number of vitamins within the body. The human body does not integrate vitamins aside from vitamin D. Therefore intake of vitamin E is required and obtained through dietary routine [6]. Vitamin E has a lipophilic character, so it can protect foods by closely binds with lipids in edible products [7]. Vitamin E in supplements is usually present as α-tocopheryl acetate, a form of α-tocopherol that protects its ability to function as an antioxidant [8]. The essential sources from where one can get vitamin E are oats, natural products, and quality milk products [9]. Alongside elevated levels of basic unsaturated fats, vegetable oils [10] additionally contain regular micro-vitamin; for example, consumable oils contain noteworthy wholesome constituents [11, 12]. The insufficient amount of α-tocopherol in the body leads to the development of a disease called ataxia [13]. Quantification of

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vitamin E is troublesome in complex samples, for example, eatable material because the vitamin is available in low fixations [14]. Thusly, for the productive measurement of vitamin E one needs to perform seclusion before its assurance, and this confinement is finished by saponification methodology [15]. In the literature, an assortment of investigative systems has been utilized for the examination of vitamin E in various examples, for example, biological fluids in dosage forms, aquatic organisms, and feeds. These incorporates high-performance liquid chromatography (HPLC methods) [16–18], gas chromatography [19, 20], Fourier transform infrared spectroscopy (FTIR) [21, 22], electroanalytical techniques [23], spectrofluorimetry [24]. The evaluation of α-tocopherol in various food oils has been done by utilizing a few investigative systems, for example, FT-NIR [25]. All the above systems are very costly, tedious, hard to deal with, and not effectively accessible. The spectrophotometric technique [26–39] has been utilized as an option for the quantification of vitamin E because of its minimal effort, less time utilization, and more effectiveness. In the proposed methodology, we utilize the spectrophotometric strategy rather than some other investigative procedure because of its precision and cost-viability. In the current strategy, the reducing property of α-tocopherol has been utilized to evaluate it in distinct edibles. From literature examinations [29, 30], it has been discovered that tetrazolium salt can be easily reduced by various reducing agents. Exploring this perception, another tetrazolium salt, for example, 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl) tetrazolium bromide (PTNPT) has been synthesized. This synthesized tetrazolium has utilized as a potential spectrophotometric investigative reagent for the measurement of α-tocopherol in different pharmaceuticals and edible. The current technique includes a prompt decrease of reagents 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT) into hued formazan (λmax 526 nm) in the presence of NaOH (pH 8–9), which forms the reason for the measurement of α-tocopherol. The existing spectrophotometric techniques have genuine disadvantages; for example, some require heat and other require time for the finish of the response. In any case, the current technique is profoundly easy, exact, and precise when contrasted with these current strategies, because neither warming nor sitting tight time is required for the fulfillment of the response. We contrast our strategy and other existing writing strategies, which show great concurrence with the consequences of the proposed technique.

Materials and methods

Reagents and solutions

α-Tocopherol was snapped up from Sigma-Aldrich (assay ≥ 95.5%). Anisaldehyde (98%), 2-aminothiazol (97%), 4-nitrobenzaldehyde (98%), phenylhydrazine (97%), and *N*-bromosuccinimide (99%) were purchased from Sigma-Aldrich, while dry methanol (99.5%), DMSO, ethyl acetate (99%) were of analytical grade and purchased from CDH chemicals. Potassium hydroxide, sodium hydroxide, and glycerol (98%) were purchased from CDH chemicals. Petroleum ether used was of analytical grade and purchased from CDH chemicals. All investigated samples such as pharmaceuticals, milk samples, processed milk samples (Yakult), fruit samples, oats, nuts, syrup that were used for the quantification of α-tocopherol were purchased from a well-branded manufacturer from a local market in Kurukshetra, North West Province, India.

The stock solution of α-tocopherol (1000 µg) was prepared by dissolving 0.1 g of it in 100 mL of absolute ethanol. The working solution (50 µg/mL) for the quantification of α-tocopherol was prepared by dilution of stock solution with absolute ethanol. 0.01% solution of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 0.015% solution of 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT) were prepared by dissolving 0.01 g and 0.015 g, respectively, in 100 mL double distilled water. The solution of 60% KOH was prepared by dissolving 60 g of KOH pellets in 100 mL of double distilled water. Sodium hydroxide solution (1.0 M, 0.01 M) was prepared by dissolving the measured amount in double-distilled water.

Equipments

The measurement of absorbance at selected wavelengths was done by Systronics UV–visible Spectrophotometer 117, India, equipped with a quartz cell of 1 cm light path. FTIR spectrum of reagents 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT) was recorded with Beckman IR-20 Spectrophotometer, (Philadelphia). ESI–MS spectra of reagents were recorded with Xevo G2-S Q-T (Waters, USA). A Hanna-H198100 pH meter (UK) was used to measure the pH of solutions. All the required amount of chemicals was weighed on Afcoset 300 balance (Japan), within certain limits of 10^{-3}. Voltammogram was recorded using the IviumStat Electrochemical analyzer (Netherlands).

Experimental

Synthesis of tetrazolium salts

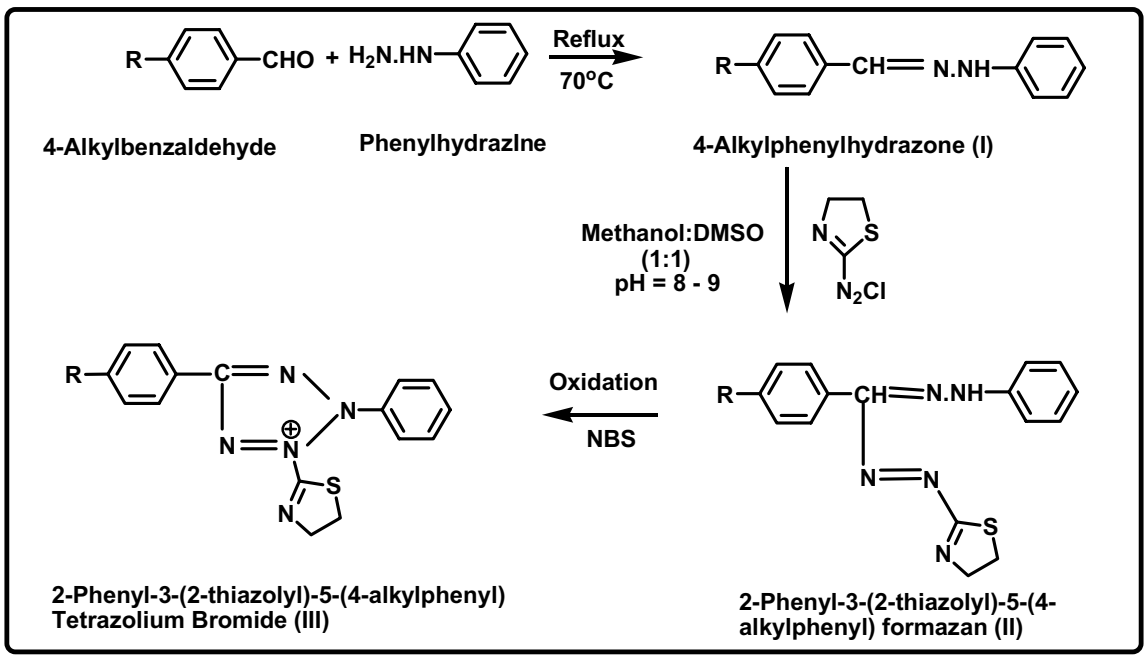
Synthesis of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl) tetrazolium bromide and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide comprises three different steps [29]. Firstly, 4-alkylphenylhydrazone was synthesized by the reaction of 4-alkylbenzaldehyde and phenylhydrazine. The second step involved the diazotization of 4-alkylphenylhydrazone with 2-aminothiazol. The final step is the conversion of 2-phenyl-3-(2-thiazol)-5-(4-alkylphenyl) formazan (II) into 2-phenyl-3-(2-thiazolyl)-5-(4-alkylphenyl)tetrazolium bromide. The outlines for the synthesis of 2-phenyl-3-(2-thiazolyl)-5-(4-alkylphenyl)tetrazolium bromide are illustrated in Scheme 1.

Step 1: synthesis of 4-alkylphenylhydrazone (I)

2.0 g of 4-alkylbenzaldehyde (0.01 mol) was dissolved in 30 mL dry methanol taken in a 100-mL round-bottom flask. Then, 1.6 mL of phenylhydrazine (0.01 mol) was added dropwise with the addition of 1–2 drops of glacial acetic acid in the above reaction mixture in a round-bottom flask. The mixture contents were refluxed for 2 h at 70 °C. The contents of the flask were cooled, and the yellow-colored precipitates of 4-alkylphenylhydrazone were filtered. The precipitates with a small quantity of methanol (2–3 mL) were washed, and the product of the reaction was dried at room temperature for 4 h [yield 95%, melting point 180–182 °C for R = OCH_3 and yield 96%, melting point 184–185 °C for R = NO_2].

Step 2: synthesis of 2-phenyl-3-(2-thiazolyl)-5-(4-alkylphenyl) formazan (II)

The preparation of 2-Phenyl-3-(2-thiazolyl)-5-(4-alkylphenyl)formazan (II) was done using the diazotization procedure. For this, 1.5 g of (I) and three times of anhydrous sodium acetate in (1:1) methanol/DMSO solution were added. The reaction mixture was stirred, and the temperature of contents was maintained at 0°C (**solution I**). Diazonium salt solution was prepared by dropwise addition of 0.69 g of sodium nitrite in water into 1.0 g of 2-aminothiazolyl in dilute HCl at temperature < 5°C (**solution II**). Then, prepared diazonium salt solution (**solution II**) was added into the above solution (**solution I**) with constant stirring at 0°C. The pH of the solution was maintained slightly basic (pH 8.0–9.0) because formazan formation takes place in a basic medium. The contents of the reaction mixture were stirred at room temperature overnight. Blue or black precipitates of formazan that is insoluble in water were separated out. The precipitates were filtered, they were washed with distilled water, and they were recrystallized with absolute ethanol [yield 53.2%, melting point 141-142°C for R = OCH_3 and yield 52.7%, melting point 145-146°C for R = NO_2].



Scheme 1 Synthesis of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT) (R = OCH_3 for PTMPT and R = NO_2 for PTNPT)

Step 3: synthesis of 2-phenyl-3-(2-thiazolyl)-5-(4-alkylphenyl) tetrazolium bromide by the oxidation of formazan (III)

Synthesis of 2-phenyl-3-(2-thiazolyl)-5-(4-alkylphenyl) tetrazolium bromide was done by the oxidation of formazan synthesized in step 2. For this, the weigh amount (0.29 g) of formazan was dissolved in 30 mL of ethyl acetate and *N*-bromosuccinimide was added in it for its oxidation. As the color of formazan disappears, the formation of tetrazolium salt is confirmed. The ethyl acetate evaporated, the dry precipitates were dissolved in methanol, and this solution was filtered using carbon black. 1/3 of the original solution evaporated, and then ethyl acetate (15–20 mL) was added in it. The resulting solution was allowed to stand overnight. Faint yellow or colorless precipitates of tetrazolium salt seperates out [yield 65%, melting point 193–195 °C for $R = OCH_3$ and yield 58.2%, melting point 189–190 °C for $R = NO_2$].

The FTIR spectrum of the reagents PTMPT and PTNPT has been recorded in the range between 4000 and 400 cm^{-1} at room temperature with Beckman IR-20 Spectrophotometer. The spectrum has been recorded at room temperature using potassium bromide pellets. The IR absorption band appears at: for PTMPT IR data (cm^{-1}): 1697(C = N), 1180(CH_3O-Ph), 1605(C = C), 3400–3000(N–H stretching), 2970(C–H stretching) (Fig. 1) and for PTNPT IR (cm^{-1}): 1697(C = N), 1528(N–O stretching), 1180(H_3C-O-Ph), 3400–3000(N–H stretching) (Fig. 2).

The ESI–MS^+ spectra of the reagents PTMPT and PTNPT recorded in D_2O with Xevo G2-S Q-T (Waters, USA) give a base peak at 336.4178 and 351.0871 which is under [M − 2] peak. This indicates the molecular weight of the synthesized reagent is 338.4178 and 353.0871 for PTMPT and PTNPT, respectively. Mass spectroscopy is found to be in good agreement with the proposed structure of tetrazolium salt PTMPT and PTNPT; MS–ESI–TOF (m/z) found in 338.4172 and 353.0871, respectively, shown in Figs. 3 and 4, respectively.

Sample preparation

Pharmaceutical formulations

Most of the pharmaceutical samples contain α-tocopherol in its stable form, i.e., α-tocopherol acetate. To quantify α-tocopherol in different pharmaceutical formulations, saponification has been done to get α-tocopherol in free form. To saponify, five capsules (each capsule contains 400 mg of α-tocopherol) had been taken, which had been mixed thoroughly to get homogeneity. Into a 100-mL round-bottom flask, 0.1 g of an oily mass of α-tocopherol and 2 mL of KOH solution (60%), 10 ml glycerol, and 25 ml absolute ethanol had been taken. The reaction mixture has been refluxed for 2 h at 70 °C [15]. Then, the reaction mixture was cooled to room temperature followed by its extraction in separating funnel by addition of 30 mL petroleum ether in the reaction mixture for 10 min. The aqueous layer was removed, and the ether layer evaporated to dryness and then the dried mass was dissolved in 100 mL of absolute ethanol to get a stock solution of concentration 1000 µg/mL, which was further diluted with absolute ethanol to get the working solution of 50 µg/mL. The solution so prepared has been used for the quantitative analysis of α-tocopherol.

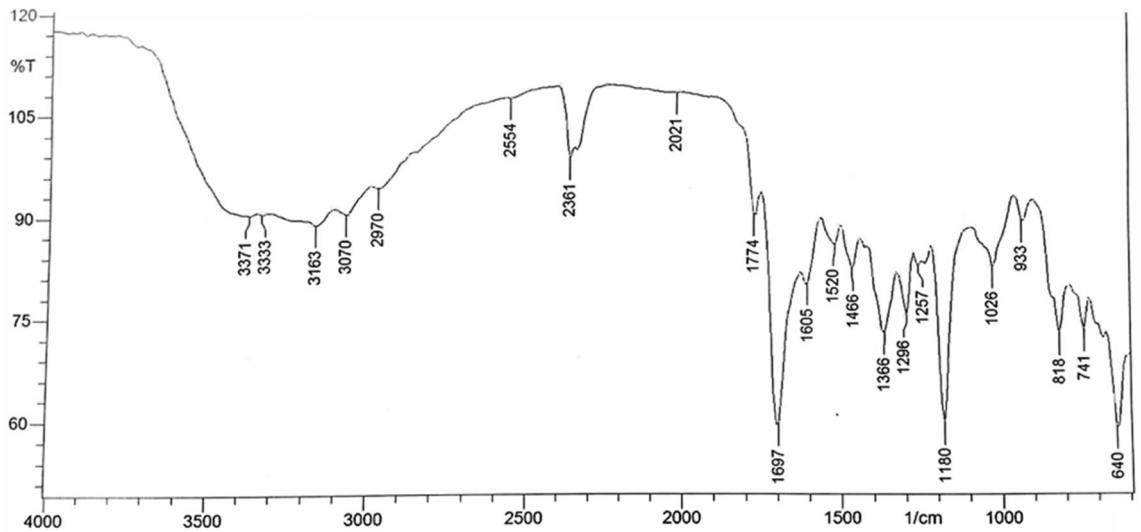


Fig. 1 Infrared spectra of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT)

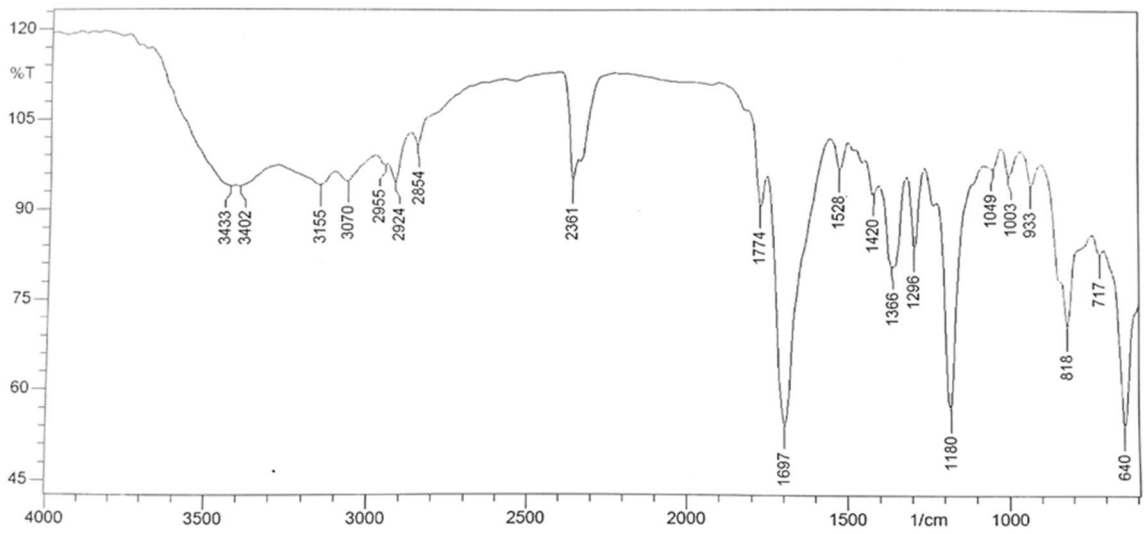


Fig. 2 Infrared spectra of 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT)

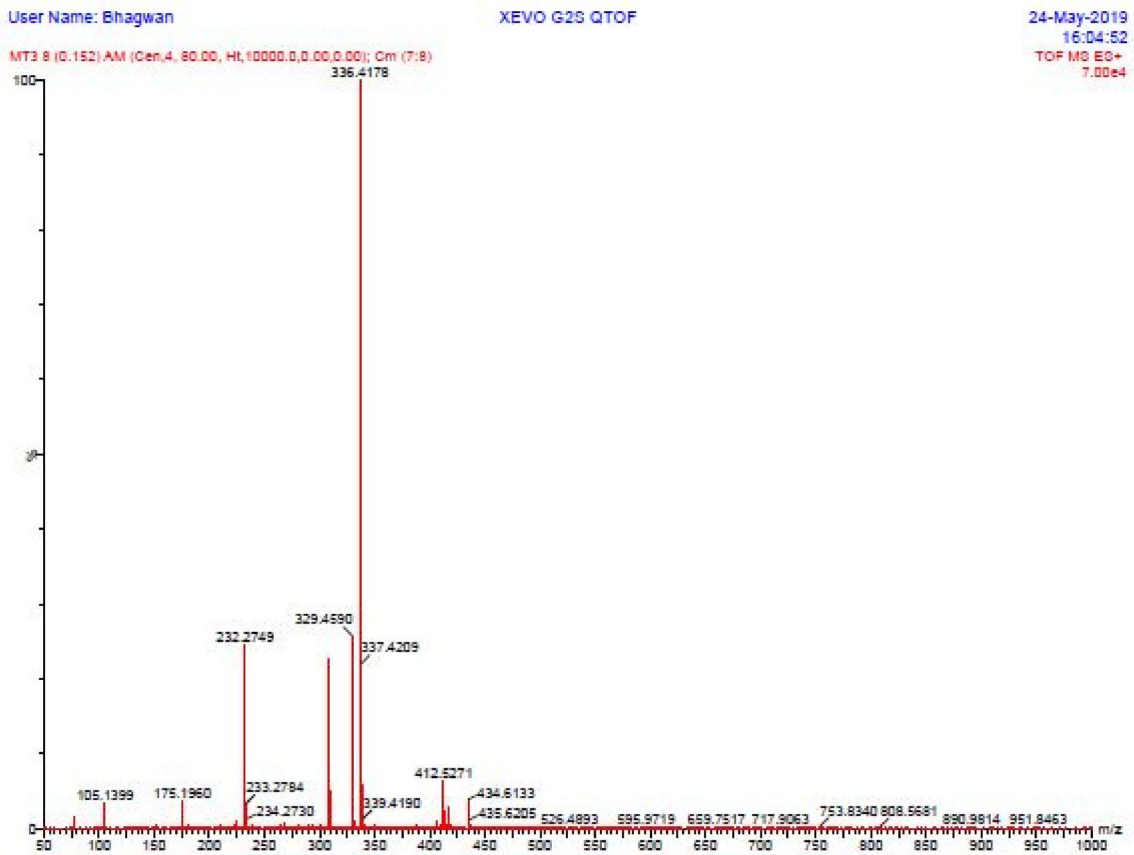


Fig. 3 Mass spectrum of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT); MS–ESI-TOF (m/z) for $C_{17}H_{16}N_5OS$ calcd: 338.4178

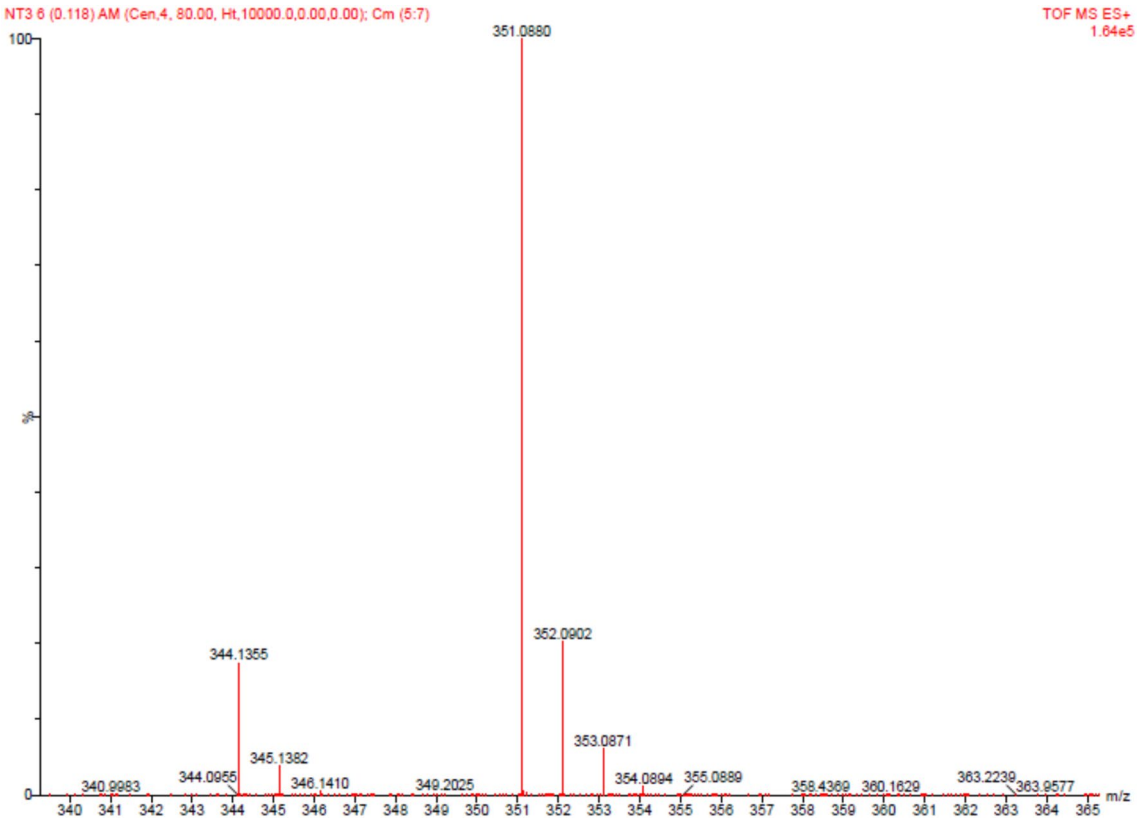


Fig. 4 Mass spectrum of 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT); MS–ESI-TOF (m/z) for $C_{16}H_{13}N_6O_2S^+$ calcd: 353.0815

Milk samples

For the quantification of α-tocopherol weighed milk sample equivalent to 0.1 g (cow milk, buffalo milk, Yakult processed milk, protein powder, infant milk powder) was transferred to a 100-mL round-bottom flask, for saponification, as described for the pharmaceutical formulations. The resulting dried mass after the saponification procedure has been then dissolved in 100 mL absolute ethanol. This solution has been further diluted with 100 mL absolute ethanol to get the working solution.

Nuts sample

Nuts that contain a remarkable amount of α-tocopherol. 0.1 g of dried, well-crushed nut samples (almond, cashew nuts, and pistachio) have been transferred to a 100-mL round-bottom flask for saponification following the above procedure, and after that, the dried mass has been dissolved in 100 mL of absolute ethanol taken in measuring flask. The resulting solution has been further diluted with 100 mL absolute ethanol to get a solution, which has been used for further analysis.

Miscellaneous sample

Each of the other investigated samples, which have not been quoted above, i.e., Proteinx, Quaker Oats, and cloves, also contains a significant amount of α-tocopherol. To quantify α-tocopherol, 0.1 g (solid sample), 2 mL (Mac total Syrup) of each sample has been taken in the round-bottom flask and saponification has been done using the above procedure. The resulting viscous mass has been then dissolved in 100 mL of the absolute ethanol, which has been diluted with absolute ethanol up to 100 mL to get the working solution and analyzed by the prescribed procedure.

Experimental procedure

Quantification of α-tocopherol using 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl) tetrazolium bromide (PTMPT)

Aliquots of 50 µg of α-tocopherol were added to a 10-mL volumetric flask. Then, 2.0 mL of reagent (PTMPT, 0.01%) solution was added to it followed by the addition of 1.0 mL of 1 M sodium hydroxide solution. The pink-colored

formazan formed because of the reduction of colorless tetrazolium reagent by α-tocopherol. The pink-colored formazan gives absorption maxima at 526 nm.

Quantification of α-tocopherol using 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl) tetrazolium bromide (PTNPT)

To construct the calibration graph using tetrazolium salt, the analytical procedure was as follows: To 10 mL of volumetric flask aliquots of 50 µg of α-tocopherol were added followed by the addition of 2 mL of tetrazolium salt solution (0.015%) and 0.02 mL of 0.01 M sodium hydroxide solution. The volume of the volumetric flask was raised to 10 mL using a DMSO solvent containing NH_4OH (1 drop of ammonia in 100 mL of DMSO). The complex gives absorption maxima at 526 nm.

Quantification of α-tocopherol in different pharmaceuticals, milk samples, nuts, cereals, and fruits samples

In different edible samples, quantification of α-tocopherol has been done by following the same procedure as described above, for the pure α-tocopherol form. The absorbance intensity observed for these samples is equivalent to the amount of α-tocopherol present in them, calculated by using the calibration graph. A comparison of our results with the amount of α-tocopherol was made, and the labeled and the observed values from the newly proposed method show good agreement with other existing methods.

Results and discussion

Various spectrophotometric techniques have been utilized for the measurement of α-tocopherol in pharmaceuticals and various edibles. In the vast majority of these current strategies, metal–ligand complexation has been utilized for the measurement, where α-tocopherol reduces metal particles present in higher oxidation states to lower oxidation states, which at that point formed a colored complex with some particular reagents. Fe(III) reduced to Fe(II) with the assistance of α-tocopherol [32], and the reduced metal particle structure forms complex with the specific reagent, which has absorption maxima at 562 nm. Cu (II)-neocuproine framework was used for the quantification of α-tocopherol [36], and the absorption maxima for Cu (II)-neocuproine was found at 450 nm. The reagents bathocuproine and 2, 4, 6-Tris-(2-pyridyl)- s-triazine builds complex with Cu (I) and Fe (II) ions separately [33]. The quantitative investigation has been done in the presence of buffer (pH 4), and the maximum absorbance for the buildings framed has been found at 479 nm and 595 nm separately for both the complexes. Extraction was finished utilizing n-hexane for α-tocopherol evaluation in palatable oils and ghee tests. Likewise, ferrozine-Fe(II) framework has been utilized for the evaluation of vitamin A and α-tocopherol on a similar example. It was presumed that the greater part of these techniques, include metals in the investigation. By exploring the literature, we have developed two new strategies [26, 34] for the quantitative investigation of α-tocopherol in different pharmaceutical and consumable samples. In this way, we infer that these spectrophotometric techniques are roundabout one. Moreover, these systems require either sitting tight time or warming for the finish of the investigation, just as the contribution of metal particles makes these methods dull and profoundly helpless for impedance in the examination. Execution of an immediate technique for the test of α-tocopherol was finished utilizing triphenyltetrazolium salt as a systematic reagent [31]. This method requires warming for 10 min on a water shower at 90 ± 2 °C, for the reduction of tetrazolium salt by α-tocopherol. Therefore, an easy system, which is without holding up time just as warming, is required for the quantitative examination of α-tocopherol.

In the present investigation, we have evaluated α-tocopherol by utilizing its reducing property. The synthesis of new tetrazolium salts was finished utilizing literature strategies [29, 30]. In writing, extraordinary tetrazolium salts have been utilized for the measurement of α-tocopherol. In every one of those strategies, either warming or waiting time is required for the reduction of a dismal tetrazolium salt into relating hued formazan by α-tocopherol [31]. The two recently incorporated tetrazolium salts get reduced immediately by the α-tocopherol (Fig. 5) within the sight of a modest quantity of base (pH 8–9) neither warming nor hanging tight time is required for the proposed technique. The measure of α-tocopherol was determined by estimating the absorbance of the shaded formazan utilizing a UV–visible spectrophotometer. We contrast our technique and the current writing strategy, which shows great concurrence with the after-effects of the present method.

Optimization of different reaction variables

The reduction of tetrazolium salts, i.e., PTMPT and PTNPT, requires an appropriate amount of base. For PTMPT as the amount of base increases, the absorption values increase up to 1.0 mL of 1 M NaOH solution; after that, further addition of base will decrease the absorption value. In the case of PTNPT, as the amount of 0.01 M base increases, the absorption increases up to 0.02 mL and further addition of base shows a decrease in absorption value. So, 0.02 mL of 0.01 M was considered sufficient for the quantification of α-tocopherol using PTNPT. The effect of reagent concentration has also been studied, and it has been found that

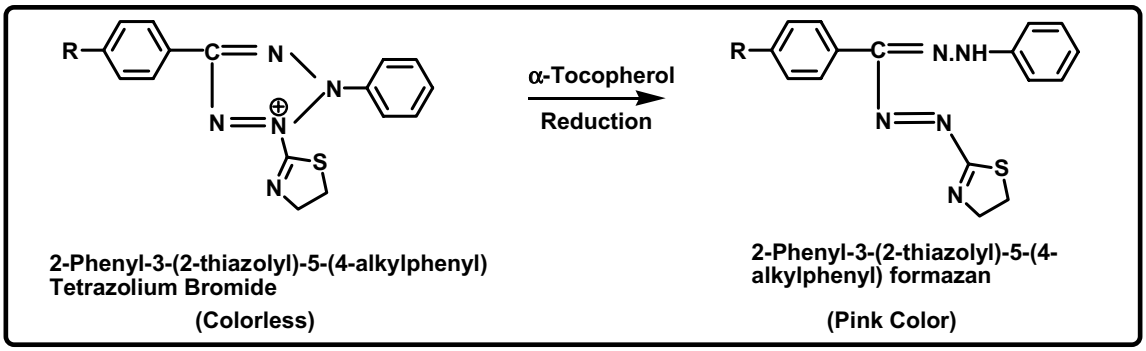


Fig. 5 Reduction of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl) tetrazolium bromide (PTNPT) (R = OCH_3 for PTMPT and R = NO_2 for PTNPT)

as the amount of tetrazolium salt solution increases, there was an increase in the absorption value. By optimizing all the factors, 2 mL of both the reagents PTMPT and PTNPT was found to be adequate for maximum color development. To ensure the maximum reproducibility, the reagent must be added before the addition of NaOH. Otherwise, low color intensity was observed due to the incomplete oxidation–reduction reaction. It has been noted that α-tocopherol has been easily oxidized by air due to its photochemical activity. To overcome this difficulty, the experiments were conducted in the dim light/in the darkened room/or covering the flask with aluminum foil that prevents the photochemical oxidation of α-tocopherol.

Analytical points of merit

The optimization of different reaction variables has been done to get the maximum absorption value. The calibration graph was constructed between the absorbance of the reduced PTMPT/PTNPT and the concentration of α-tocopherol, which shows a linear relationship in between the concentration range 0.2 µg/mL to 15 µg/mL for PTMPT and 0.1 µg/mL to 0.4 µg/mL for PTNPT.

The different statistical parameters had been optimized, and satisfactory results are listed in Table 1.

Calibration graph for the quantification of α-tocopherol at 526 nm

The UV–visible absorption spectra for α-tocopherol with both the reagents PTMPT and PTNPT were obtained by scanning of pink-colored formazan from 400 to 700 nm using a UV–visible spectrophotometer. The maximum absorption of formazan for both the reagents was obtained at 526 nm (Fig. 6). The calibration graph for α-tocopherol using both the reagents was constructed by the addition of aliquots of 0.01% PTMPT and 0.015% PTNPT in 10-mL volumetric flask containing aliquots of 50 µg/5 µg of α-tocopherol for PTMPT and PTNPT, respectively. The addition of sodium hydroxide to the above contents gives pink-colored formazan with absorption maxima at 526 nm. The calibration graph for α-tocopherol with

Table 1 Statistical parameters for the quantification of α-tocopherol

Sr. no.	Parameter	Value		Parameter	Value	
		PTMPT	PTNPT		PTMPT	PTNPT
1.	$\lambda_{max.}$ (nm)	526	526	Correlation coefficient (r)	0.955	0.998
2.	Beer's limit (µg/mL)	0.2–15.0	0.1–0.4			
3.	Molar absorptivity (L $mol^{-1}cm^{-1}$)	4.21×10^3	6.4×10^4	Relative standard deviation (%)	0.87	4.6
4.	Sandell's sensitivity (µg/cm^2)	0.11×10^3	0.006×10^{-3}	LOD	0.14	0.03
5.	Standard deviation	0.030	0.016	LOQ	0.42	0.08
6.	Regression equation	$Y = 0.013x + 0.202$	$Y = 0.167x + 0.008$			
7.	Slope (m)	0.013	0.167			
8.	Intercept (c)	0.202	0.008			

$Y = mx + c$ where x is in µg/mL

LOD = $3S_a/b$ (S_a = standard deviation of response, b = slope of calibration curve)

LOQ = $10S_a/b$ (S_a = standard deviation of response, b = slope of calibration curve)

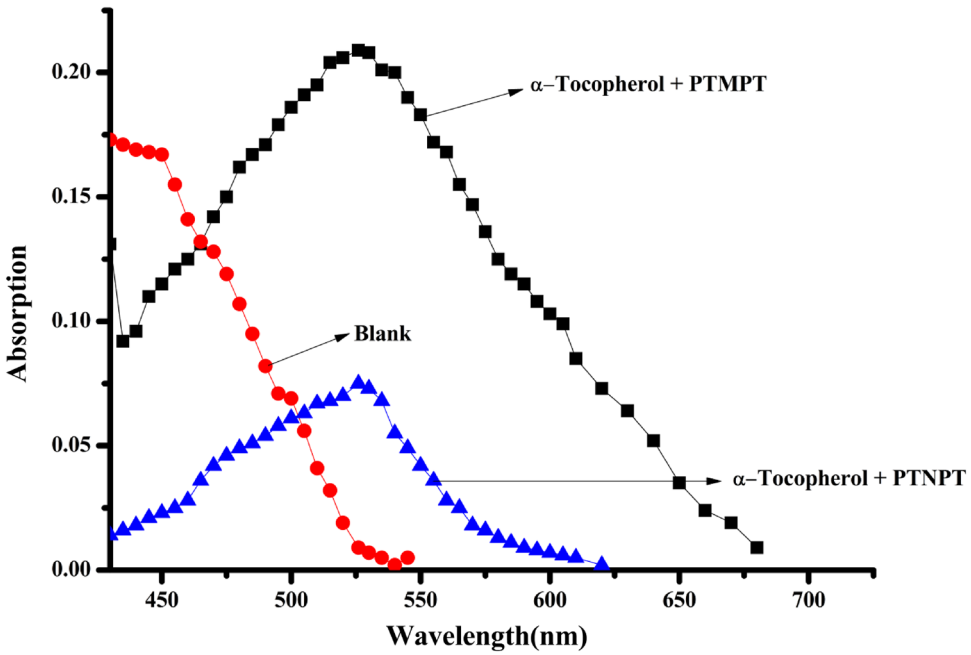


Fig. 6 Absorption spectra of reduced 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide (PTMPT) (0.01%, 2 mL) and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl)tetrazolium bromide (PTNPT) (0.015%, 2 mL) against reagent blank; α-tocopherol (50 µg/mL,3 mL), NaOH (1.0 M, 1.0 mL) for PTMPT and α-tocopherol (5 µg/mL, 0.8 mL), NaOH (0.01 M, 0.02 mL), total volume of solution = 10 mL

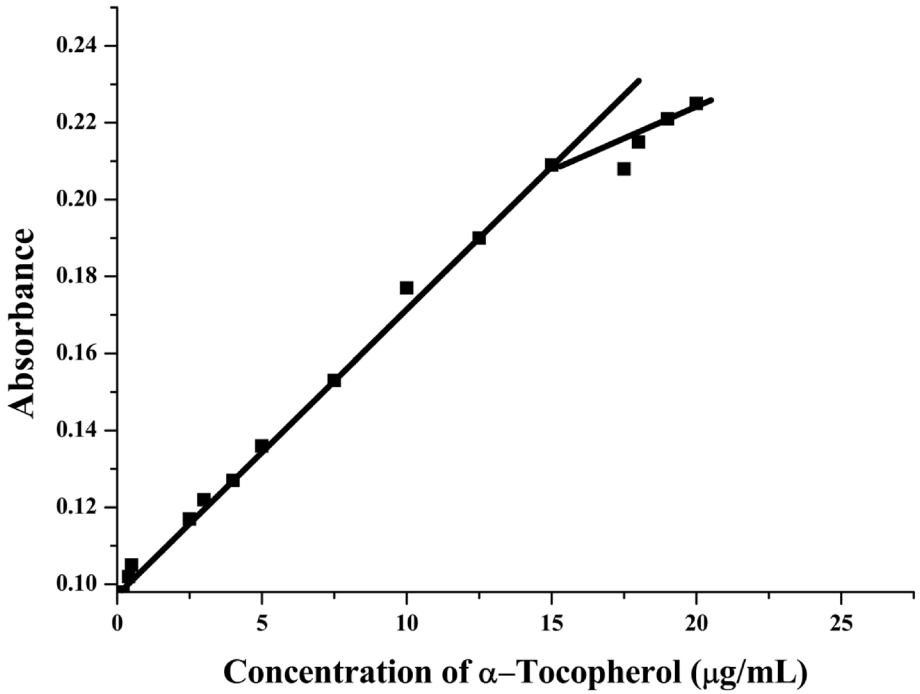


Fig. 7 Calibration graph: absorbance versus concentration of α-tocopherol, PTMPT (0.01%, 2 mL), NaOH (1.0 M, 1.0 mL), total volume of solution = 10 mL

2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl)tetrazolium bromide and 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl) tetrazolium bromide is shown in Figs. 7 and 8, respectively.

Cyclic voltammetric characteristics

Cyclic voltammetric studies had been done using the IviumStat Electrochemical analyzer to measure the reduction of PTMPT/PTNPT by α-tocopherol. The peaks observed in the cyclic voltammogram of the reagent in the presence and absence of α-tocopherol give an idea of electron change during the reduction process. The voltammogram of α-tocopherol + PTMPT/PTNPT + NaOH was recorded in ethanol at room temperature using KCl as supporting electrolyte, having a scan rate of 0.1 V/s, within the potential range - 0.1 V to + 1.0 V. The graph between I/mA and E/V for

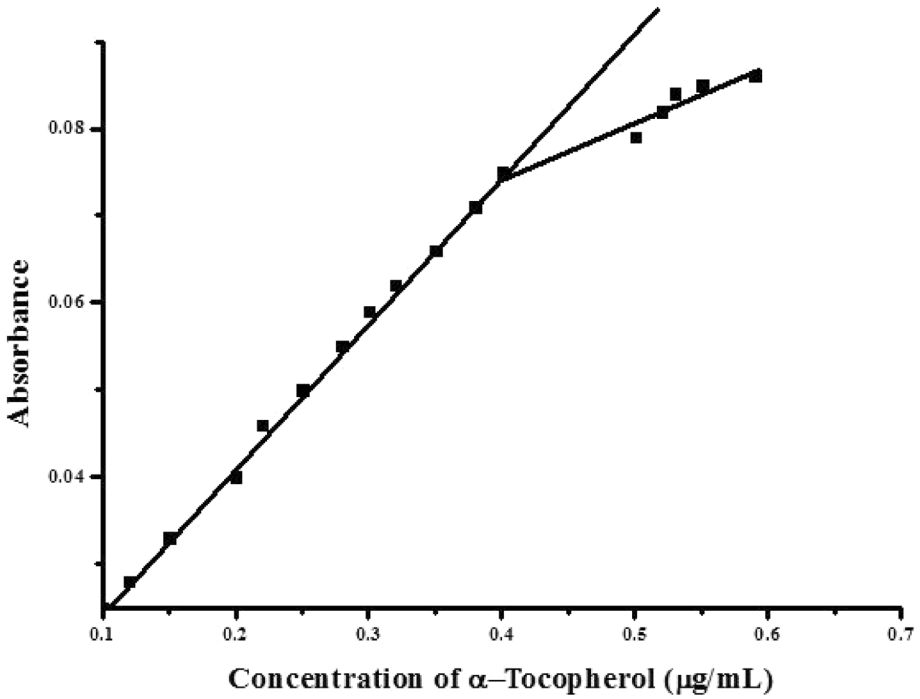


Fig. 8 Calibration graph: absorbance versus concentration of α-tocopherol, PTNPT (0.015%, 2 mL), NaOH (0.01 M, 0.02 mL), total volume of solution = 10 mL

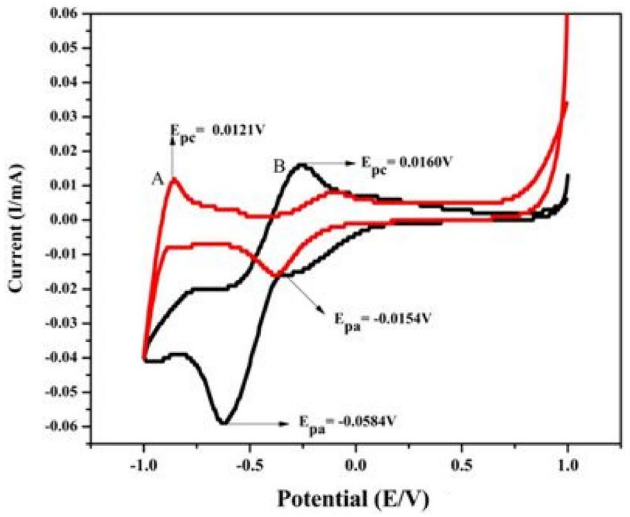


Fig. 9 Cyclic voltammogram of 2-phenyl-3-(2-thiazolyl)-5-(4-methoxyphenyl) tetrazolium bromide (PTMPT) in the absence[A] and presence[B] of α-tocopherol (15 μg/mL), using KCL as supporting electrolyte

reagent in the presence of α-tocopherol and reagent alone is represented by curves **A** and **B**, respectively. From the graph, it is evident that the reduction potential for α-tocopherol with the reagent and the reagent alone is of having different values. The potential of curve (**A, B**) observed in the absence of α-tocopherol had Epa = -0.0154 V and Epc = 0.0121 V and in the presence of α-tocopherol had Epa = -0.0584 V and Epc = 0.0160 V for PTMPT (Fig. 9) and the potentials of curve (**A, B**) observed in absence of α-tocopherol had Epa = -0.023 V and Epc = 0.011 V and in the presence of α-tocopherol had Epa = -0.036 V and Epc = 0.027 V for PTNPT (Fig. 10).

Interferences

Some metals also have a reducing character that can interfere in the quantification of α-tocopherol. To eliminate these interferences, the extraction of samples in petroleum ether must be done after saponification. Water-soluble impurities and vitamins such as vitamin C were removed by this method because it is the vitamin E, which is soluble in petroleum ether, while other vitamins not. The other probable error was removed by standard addition method, and the experimental error for the vitamin E samples was minimized by making a triplicate set of observations because all the samples were prepared similarly.

Statistical tools for validation of method

The validation of the proposed method has been carried out by ICH recommendations 18.

Linearity and range

The present method describes the quantification of vitamin E using newly synthesized tetrazolium salts. The variables involved in the quantification of α-tocopherol was validated and optimized to evaluate the linearity of the

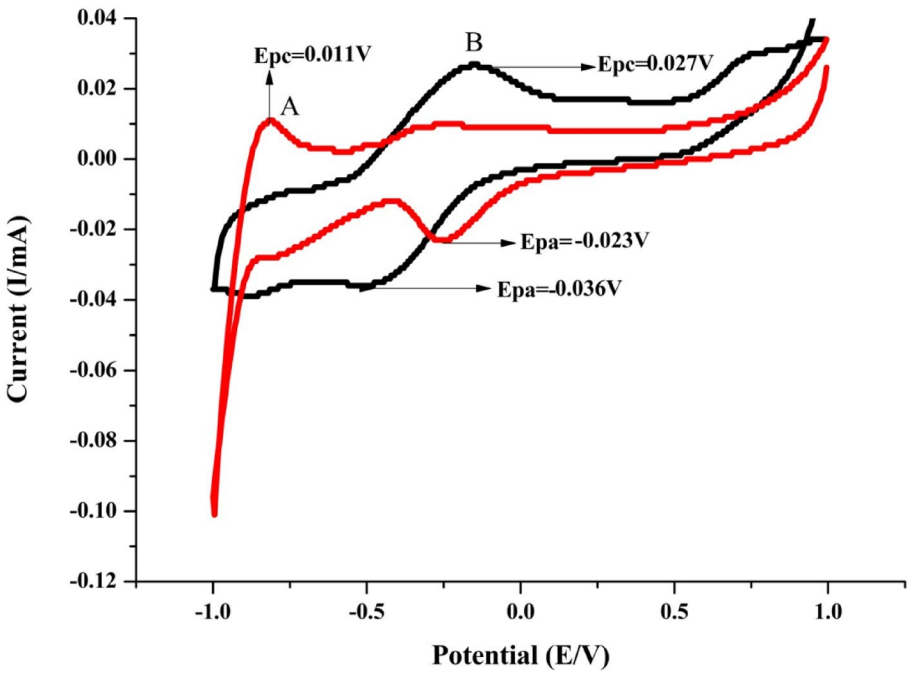


Fig. 10 Cyclic voltammogram of 2-phenyl-3-(2-thiazolyl)-5-(4-nitrophenyl) tetrazolium bromide (PTNPT) in the absence[A] and presence[B] of α-tocopherol (0.4 µg/mL), using KCL as supporting electrolyte

proposed method by analyzing the different concentration of vitamin E. Beer's law was obeyed in the range of concentration 0.2–15.0 µg/mL for PTMPT and 0.1–0.4 µg/mL for PTNPT. Indication of good linearity was observed when the value of the correlation coefficient was found near unity ($R^2 = 0.955$ for PTMPT and $R^2 = 0.998$). The other parameters like the intercept, slope, molar absorptivity, Sandell's sensitivity, and correlation coefficient were calculated and are presented in Table 1.

Precision and accuracy

The measurement of precision was done using standard addition method, and for each concentration, absorbance measurement was repeated thrice. The proposed analytical method was found to have high precision and have % RSD less than 2. Percentage recoveries of the vitamin E formulations were attributed to the accuracy of the proposed method (Tables 2 and 3). For each solution, the regression equation was used to calculate the concentration values ($n = 3$). The high accuracy of the proposed method was characterized

Table 2 Quantitative analysis of α-tocopherol in pharmaceutical formulations using PTMPT

Sr. no.	Pharmaceutical sample		Amount of vitamin present (C_P) (µg)	Amount of vitamin added (C_A) (µg)	Amount found (C_F) (µg)	Recovery %	Relative standard deviation (RSD) %
	Brand	Manufacturer					
1.	Enew	Merck Ltd. (CM Care)	20	4.0	23.8	100.8	± 0.6
			20	10.0	29.6	101.3	± 2.8
			20	12.0	31.7	100.9	± 0.8
2.	Evitek-400	Vintek Pharmaceuticals	20	4.0	24.5	97.9	± 1.2
			20	10.0	31.6	94.9	± 0.4
			20	12.0	33.3	96.1	± 0.4
3.	Native Forte	Franco Indian Pharmaceutical Ltd.	20	4.0	23.6	101.6	± 1.1
			20	10.0	29.9	100.3	± 0.8
			20	12.0	32.7	97.8	± 1.4
4.	EVOC-400	Indizen Pharmaceutical	20	4.0	25.1	95.6	± 0.8
			20	10.0	31.3	95.8	± 0.6
			20	12.0	32.8	97.5	± 0.5

% Recovery = $C_P + C_A/C_F$ for three replicate measurements ($n = 3$)

Table 3 Quantitative analysis of α-tocopherol in pharmaceutical formulations using PTNPT

Sr. no.	Pharmaceutical sample		Amount of vitamin present (C_P) (μg)	Amount of vitamin added (C_A) (μg)	Amount found (C_F) (μg)	Recovery %	Relative standard deviation (RSD) %
	Brand	Manufacturer					
1.	Enew	Merck Ltd. (CM Care)	20	0.2	19.8	102.0	± 0.5
			20	0.3	20.1	100.9	± 0.9
			20	0.4	20.0	102.0	± 0.8
2.	Evitek-400	Vintek Pharmaceuticals	20	0.2	20.5	98.5	± 0.3
			20	0.3	19.6	103.5	± 0.7
			20	0.4	20.3	100.5	± 0.8
3.	Native Forte	Franco Indian Pharmaceutical Ltd.	20	0.2	20.6	98.1	± 0.8
			20	0.3	20.1	100.9	± 1.0
			20	0.4	19.7	103.5	± 0.8
4.	EVOC-400	Indizen Pharmaceutical	20	0.2	20.1	98.5	± 1.2
			20	0.3	19.3	105.2	± 0.8
			20	0.4	19.8	103.1	± 0.8

% Recovery = $C_P + C_A/C_F$ for three replicate measurements ($n = 3$)

by a low coefficient of variation ($R^2 = 0.985$) and % RSD (< 2). The concentrations of different samples containing α-tocopherol had been analyzed using the proposed method three times intra- and inter-day. Good results had been found, and an acceptable amount has been observed as shown in Tables 2 and 3.

Limit of detection and quantification

Low estimations of LOD and LOQ given in Table 1 demonstrated high affectability of the developed methods. These methodologies are dependent on the SD of the reaction and the slope of the calibration curve for each utilizing theories conditions: LOD = 3.3 × SD/slope and LOQ = 10 × SD/slope.

Analytical applications

The quantification of vitamin E in different edibles has been done using the proposed method and the results are found to be in close agreement as per the labelled amount of edibles. The closeness of the results are listed in Table 4. The analyzed amount of α-tocopherol in edible material such as milk samples, nuts, and miscellaneous samples (syrup, oats, and cloves) along with the absorbance value is shown in Figs. 11 and 12.

Table 4 Vitamin E content in various comestible samples

Sr. no.	Sample	Manufacturer	Proposed method	Labeled amount on product	
			PTMPT (μg/g)	PTNPT (μg/g)	
1.	Pistachio	–	0.4	0.29	0.28
2.	Almonds	–	0.21	0.26	–
3.	Cashew nuts	–	0.32	0.28	–
4.	Fermented milk drink	Yakult	0.23	0.15	0.2
5.	Infant milk powder	Nestle India ltd.	0.38	0.36	0.33
6.	Cow milk	–	0.32	0.30	–
7.	Buffalo Milk	–	0.21	0.38	–
8.	Proteinx	Nutricia International Private Limited	0.23	0.37	0.25
9.	Mactotal syrup	Tirupati lifesciences	0.21	0.29	0.33
10.	Quaker oats	Symega food ingredients ltd.	0.15	0.36	0.46(from nutrition data)
11.	Cloves	–	0.30	0.32	0.35

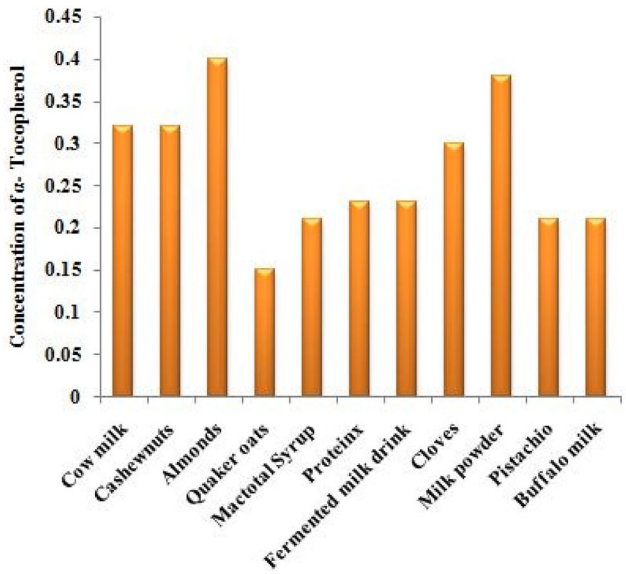


Fig. 11 Quantitation of α-tocopherol in different comestible edibles using PTMPT

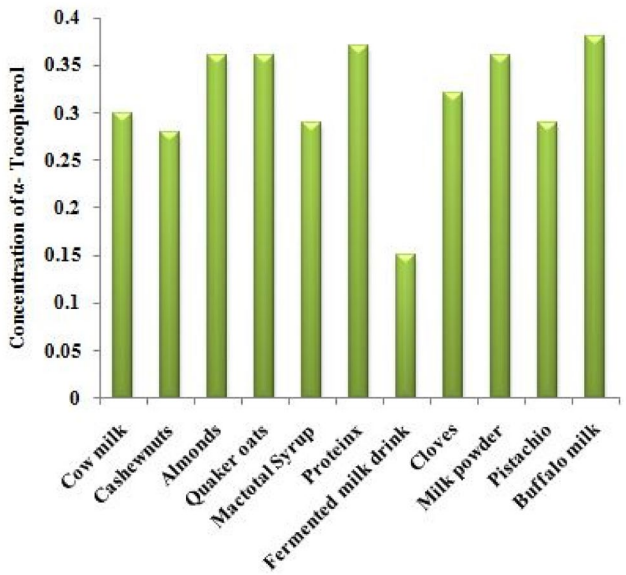


Fig. 12 Quantitation of α-tocopherol in different comestible edibles using PTNPT

Comparison of the proposed method with other spectrophotometric methods

The result of the proposed method has been compared with the other existing UV–visible spectrophotometric methods in Table 5. It was observed that the proposed method is simple, reliable, and quicker than other many spectrophotometric methods. The main advantage of the proposed method is its sensitivity and compatibility. The present procedure is not complicated and less time-consuming than other existing spectrophotometric methods.

Conclusion

Here, a simple, highly sensitive, direct, easy to handle, and facile procedure was proposed for the quantification of α-tocopherol (vitamin E) in pure form, various pharmaceutical formulations, and comestible food samples. The quantitative analysis is done by spectrophotometric method, which is cost-effective and easy to handle and less time-consuming in comparison with the literature techniques such as HPLC, UPLC and gas chromatography, which are quite expensive and not available easily. The proposed procedure is magnificent for the evaluation of α-tocopherol (vitamin E) in different eatables and is quite competent for the agile analysis of vitamin E.

Table 5 Comparison of proposed method with other reported spectrophotometric methods for the quantitation of α-tocopherol

Sr. no.	Reagent	Mode	λ_{max} (nm)	Time for completion of reaction	Remarks	References
1.	Cu(I)-bathocuproine	Indirect	479	5 min	Proper analytical parameters were not fixed	Devi et al. [33]
2.	Fe(II)-2,2′-bipyridine	Indirect	520	4 min	Less sensitive method	Emmerie and Engel [35]
3.	Cu(II)-neocuproine	Indirect	450	30 min	Less sensitive and high waiting time required	Tutem et al. [36]
4.	Fe(II)-2,4,6-Tripyridyl-s-triazine	Indirect	595	60 s	No proper analytical parameters	Devi et al. [33]
5.	Fe(II)-ferrozine	Indirect	562	2 min	Waiting time is low but heating required	Jadoon et al. [32]
6.	Phosphomolybdenum complex	Indirect	695	90 min	Heating requirement and high waiting time	Prieto et al. [37]
7.	Fe(III)-ferricyanide	Indirect	735	30 s	Extraction required and complicated steps	Jadoon and Waseem [39]
8.	Fe(II)-1-nitroso-2-naphthol	Indirect	708	60 s	Metal involved so not highly sensitive	Kumar and Kamboj [34]
9.	Fe(II)-Dmg-Py	Indirect	512	10 s	Not as sensitive as direct method	Kumar and Kamboj [26]
10.	Fe(II)-salicylic acid	Indirect	525	60 min	Highly time-consuming and less sensitive	Mir et al. [38]
11.	Tetrazolium blue	direct	526	10 min	Time-consuming and heating required	Amin Alaa [31]
12.	**PTMPT/PTNPT**	**Direct**	**526**	**Instantaneous**	**Highly sensitive, no heating required**	**Proposed method**

Bold text represents the results obtained for our reagents in the present study

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Not applicable.

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