IHJPAS. 36 (3) 2023

Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq

Review:-Determination of vitamin E Concentration in Different Samples

Maha A. Mohammed
Department of Chemistry, College of Education for Pure Sciences Ibn-Al-Haitham, University of Baghdad, Baghdad, Iraq.

Elham N. Mezaal*
Department of Chemistry, College of Education for Pure Sciences Ibn-Al-Haitham, University of Baghdad, Baghdad, Iraq.

Kawther Ahmed Sadiq
Department of Chemistry, College of Education for Pure Sciences Ibn-Al-Haitham, University of Baghdad, Baghdad, Iraq.

***Corresponding Author:** elham.n.m@ihcoedu.uobaghdad.edu.iq

Article history: Received 26 November 2022, Accepted 18 Januarey 2023, Published in July 2023.

doi.org/10.30526/36.3.3113

Abctract

Vitamins are a type of essential and important nutrient in the human body. It also plays an essential role in the health and protection of the human body. They share physiological functions with many chemicals, and their deficiency or increase endangers human health. Therefore, it is required to evolve and use modern methods to estimate the concentration of vitamins, even if their concentration is very low, and these include the vitamin E group tocopherols, tocotrienols, isomers, esters, and derivatives. They disagree not in their ability as anti-cancer agents but rather in their physiological as well as chemical relations, unlike vitamin A and vitamin D. The richest source of vitamin E is vegetable oil. Vitamin E, classified as a vitamin, dissolves in fat. It is pointed out in different types of foods involving vegetable oils, meat, eggs, cereals, and poultry, in addition to fruits. Some of the vital signs and symptoms of a vitamin E deficiency include neurological defects such as dysfunction of the brain, nerves, spinal cord, and muscles; muscle pain and weakness; muscle deterioration, including cardiomyopathy or weak heart muscle; low birth weight; difficulty moving the eyes up and down; poor vision at night; loss or lack of sense of vibration; and a feeling of numbness or tingling.

Keywords: High Pressure Liquid Chromatography, Vitamin E, Vitamin C, Gas Chromatography.

1. Introduction

Vitamin E, which is known as 2, 5, 7,8-tetra methyl-2-[(2R, 8R) 4,8,12-trimethyl tricycle] 3, 4-dihydro- 2H- chromene-6- ol, inclusive eight particles presented via ring from chromanol with side chain from a phytol show matching jobs with four tocopherols α, β, γ, besides δ with four tocotrienols α, β, γ, also δ. Tocopherol has saturated side chain α, β, γ also δ prefixes designate the site of groups for methyl on the ring of chromanol [1]. Vitamin E is a fat soluble vitamin and is important in human metabolism. At first assimilated by the blood stream, vitamin E is able to prohibit oxidative reactions in some biomolecules as well as unsaturated fatty acids. Vitamin E is important to protect the health of the human body from the occurrence of oxidative reactions because it leads to the formation of free radicals, which cause mutation in the DNA. It also reverses vitamins A and D. The oils of vegetables are considered the richest sources of vitamin E [2]. Several methods have been developed for the separation and estimation of vitamin E using paper, columns, thin layers, gas liquids, and high-pressure liquid chromatography (HPLC). Gas-liquid chromatography (GC-LC) effective method for determining vitamin E than other vitamins that dissolve in fat.

2.The methods used in determination of vitamin E

Several methods were used for the determination of tocotrienols and tocopherols by utilizing natural stage and adverse stage HPLC [3, 4]. Natural-stage high pressure liquid chromatography dissolves four tocotrienols with four tocopherols. Reverse-stage HPLC does not dissolve β also γ tocotrienols with tocopherols [5].

2.1- Utilizing chromatography techniques

Derivatives of vitamin E (tocopherols) can be extensively analyzed using high-performance liquid chromatography (HPLC) method that utilizes natural and reverse-phase columns by using various detectors such as UV-Vis, fluorescence, electrochemical, and mass spectrometric. The preference of the analytical method depends on the nature of the forficate and the sample matrix, as Afaf and Jelena mentioned [6]. Korchazhkina and his colleagues [7] elucidated that the removal of vitamin E obtained from human milk using hexane with and without preform has been compared. Milk extractions were analyzed using HPLC technology with a C18 column and a UV detector. Using all methods, a considerable link was obtained ($P < 0.01$) between levels of -tocopherol and γ-tocopherol in milk from humans, in spite of saponification, which resulted in the highest recovery of the internal standard, δ-tocopherol 99.6 ± 4.0%, also important for ameliorating the assurance of the data. The detection limit for methods including saponification, extraction, and HPLC analysis is 0.65 μg / ml of -tocopherol in milk. A suggested saponification stride was an unpretentious modification for mode on account of it being completed in the toke igniter as the remove, engross, and 30 min also did not include the utilization of stable gas. The development of a modest and rapid method for estimating the amount of vitamins in complex matrices is important for monitoring the quality of foods eaten by humans, including milk of children. In this research, after submitting the method for determining vitamin E from the milk of children, its tenor was a comparison with label claims. In this study, the scattered liquid-liquid microextraction DLLME procedure, together with acetonitrile and chloroform as scattered and stripped-off solvents, respectively, was utilized to insulate and clean up vitamin E in the milk of child models in the absence of saponification. A reversed phase in HPLC consisting from column C18 is a fixed-phase constant-phase combination of acetonitrile: methanol: water (91:8:1%). As such, moving phases utilize the standard additive style. It was used to estimate vitamin E using a UV detector at the wavelength of 296 nm. After validating the method under optimal conditions,

the presented style gave a linear range with a determination coefficient R^2= 0.99 and acceptable precision and accuracy. The results showed that the developed method was suitable for monitoring and knowing the quality of the milk of children. Benefits for DLLME compared with the saponification operation and liquid-liquid extraction are reduced consumption of organic solvents, and the proposed method is simple and quick to estimate vitamin E in children's milk. The developed method was applied for the analysis of vitamin E within the milk of children in the market, which showed significant differences between the named content and the obtained content of the samples [8]. In 2013 [9]**,** a sensitive and rapid separation method using high-performance liquid chromatography on a homogeneous column evolved for the quantification of vitamin E (acetate) in nutritional supplements from commercial samples. 20 µ l the volume of sample, the filtered solution is injected directly into the device of HPLC.

The separation of vitamin E (acetate) from the rest of the components was carried out in a single column 100 × 4.6 mm with mobile moving methanol and water at a rate of 98:2 V / V respectively, flow rate of 2 ml / min, temperature 30^{o}C, the detector measured at 290 nm. Under optimal conditions, the calibration curve has been measured with perfect linearity, with the correlation coefficient of vitamin E (acetate) equaling r= 0.9992 for n= 6 among summit areas with concentrations of vitamin E (acetate). The accuracy of the method is calculated by calculating the recovery (Rec%), which equals 96.4–103.6% in nutritional supplements from commercial samples, and the relative standard deviation RSD% is found to be 1.1-3.6 %. The developed method proved to increase the productivity of the model through the preparation operation, with a low time for the analysis process of 3.5 min. In 2011 [10], their study consisted of improving a method to verify, quantify, and estimate the complete isomeric structure of vitamin E α-, β-, γ- also δ-tocopherols with tocotrienols in six types of cooked and raw vegetables, three herbs, the eggs row and cooked, vegetable oils canola, olive with soybean, flaxseed with sorghum [flour also seeds], and soy flour via HPLC with a fluorescence detector.

Various conditions were selected for extraction and analysis. The best method for analysis consists of extraction directly from the solvents hexane and ethyl acetate at a rate of 85 or 15 V/V. A method analysis using a plain phase column together with moving molecules consisting of hexane, isopropanol, and acetic acid at rates of 98.9, 0.6, and 0.5 together with isocratic elution and detection from fluorescence A good segregation of each vitamin E isomer was received, together with adequate quantification in all nutrient analyses. Linearity for each isomer ranges from 2.5-137.5 ng / ml, R^2 bigger than 0.995, and recovery ranges from 91.3-99.4%. Limit of detection is 21.0- 48.0 ng / ml for tocopherols and 56.0 - 67.0 ng / ml for tocotrienols, while the limit of quantification is 105.0 – 240.0 ng / ml for tocopherols and 280.0-335.0 ng / ml for tocotrienols. This method was considered fast, simple, and reliable. Vitamin E isomers were preserved when compared to validated methods involving saponification. Another analytical method was performed by Mehmet et al. (2012) [11], utilizing RP-HPLC with a UV-detector for the estimation of vitamin E isomers in grape seeds.

This method depends on the separation and extraction of the solid from the liquid (solid-liquid) in a column of ODS, and the sample is monitored analytically using a UV detector at a wavelength of 295 nm. The HPLC separation of tocopherols is checked at 12 min, together with the

development of n-hexane and isopropyl alcohol 99.99: 0.01 V / V. RSD for n=10 at concentration of 500 ng/ml equals 2.57% -3.30 % and relative error equals 0.84% - 6.54 %. Limit of detection: 25 ng / ml for α-tocopherol, 43 ng / ml of γ- and 83 ng / ml for δ-tocopherols. In 2016 [12], it was shown that the presented chromatography method is appropriate for the estimation of tocopherols in grape seeds and can also be utilized for the estimation of tocopherols in different samples. N-hexane is extracted without saponification, which saves a large amount of solvent, time, and stage of analysis, and prevents a lack of tocopherols. Except for the δ - tocopherols, which cannot be detected, direct extraction of hexane and chromatographic separation showed high efficiency in achieving extraction at high repeatability for all samples. More commonly found in grape seeds is α - catechol range from 2.31-36.42 mg / g. The difference in the amount of tocopherols is large; this information can be utilized as an easy and simple way to distinguish between grape varieties. An amount of tocopherols has been calculated using the standard addition method. HPLC method was used for the microscale estimation of alpha and gamma-tocopherols in leaves, flowers, and fresh beans from Maringa oleifera is mentioned. Optimized conditions for RF- HPLC together using a UV detector are as follows: column, 25 cm x 0.46 cm, heat of column equal 25^{o} C , moving phase 20, 80 V / V mix from methanol and acetonitrile, 1 ml/min tidal rate RSD% is 5.6% for - tocopherols and 4.9% for γ- gamma-tocopherols [13]. A fast and simple RP-HPLC method was developed for simultaneous determination of each trans, retinol, and α-tocopherols in human serum, utilizing retinol acetate as the internal standard at a concentration of 0.5 µg / ml. The extraction of the liquid phase is applied at 250 µl from serum together n- hexane and dichloromethane mix 70:30 (V/V) at the second step, utilizing ethanol and methanol mix 95:5 (V/V) for precipitation of protein and butyrate hydroxyl toluene as stabilizer for preparation of the sample. For each analysis, columns C18 (150 mm x 4.6 mm, 5 µm), C18 (150 mm x 3 mm, 3 µm) and C18 (30 mm x 4.6 mm, 10 µm) manufactured by Perkin Elmer were used. Analysis and measurement were carried out at 292 nm wavelength using methanol and water 99: 1 (V/V). The mode of isocratic as moving phase utilized a 1.5 ml/min flow rate and also 1ml/min for each of the columns of 5 µm and 3 µm. Perfect separation for each analysis was achieved in a time of 3-6 min on 3 and 5 µm columns by injecting 20 µl of sample inside HPLC using an automatic sampling device while maintaining the temperature of the column oven at 25^{o}C [14]. In 2019, a new method and simple HPLC have been developed for simultaneous assessment of ubidecarenone and vitamin E acetate in the form of capsules. The method provides accurate separation between drugs and a short retention time. The results indicated that the intended technique is exact, durable, and sensitive.

The sample recovery process was good and agree with the label claim, and the results indicated that there was no involvement of formulation excipients in the determination. Therefore, the results of the proposed study confirmed that the developed method is a suitable technique for simultaneous estimation of ubidecarenone and vitamin E acetate in the form of a compound dose [15]. The RP-HPLC is a selective bioanalytical method for determining vitamin E in a dried blood spot (DBS) model, extraction of vitamin E from the DBS model utilizes liquid-liquid extraction with 100% ethanol as the solvent to reconstitute the residue. An internal standard of α- tocopherols acetate was used. The sample was analyzed directly in HPLC with a C18 (250 × 4.6 mm × 5 µm) column phenomenex. Moving phase was consist of methanol with water 99:1 (V / V) and influx rate 1.4 ml / min. at 292 nm wavelength [16].

2. 2 . Gas Chromatography

A quick, simple, and accurate method has been developed for determining of vitamin E in cereal and biscuit products. The detection limit and quantification were calculated. Utilizing the source material FAPAS T10112QC, precision and accuracy for the method were estimated, and the specified values were consistent with approved values. Vitamins in cereals and biscuits can be estimated using this method presented in the application by using gas chromatography with flame ionization detection (GC-FID). The results proved that most cereals are rich in vitamin E. Bakery products that contain vanilla and nuts give an increased quantity of vitamin E, while bakery products that contain fruits give a lower content of vitamin E [17]. An efficient and rapid gas chromatography-mass spectrometry (GC-MS) method has been developed to identify isomers α - , β -, γ - and δ as well as tocotrienols, in addition to tocopherol acetate nutritional supplements and functional foods. Vitamin E isomers were extracted directly from the sample without saponification by mixing together solvents for methanol and hexane 7:3 (V/V). A good separation was obtained by utilizing column VF-5MS (30 mm, 0.25 mm, 0.25 mm) for 13 min. Mass spectrometry was run in full scan mode and SIM mode, using specific detection of the effect of ionized electrons on distinct ion pairs during retention time. Linearity of method ranged from 0.1 mg/ml to 40 mg/ml, and the correlation coefficients was higher than 0.997, and the detection limit was found to be 0.09- 0.46 ng / ml and limit quantification: 0.29–1.52 ng / ml. Relative standard deviation RSD for 1 mg/ml of the intraday and interday of method was 4.9%–8% and 2.1%–4.9%, respectively. The recovery rate of the method was 83.2%-107% together with RSD 1.1%-8.4% [18].

2. 3. Thin layer chromatography

Vitamin E is soluble in fat; it is found naturally in some foods and is beneficial to nutritional supplements and nutrients because of its biological job as an antioxidant. Several methods are available to analyze vitamin E. It was used for the quantitative determination of vitamin E by private HPLC and GC, which identified the different isomers of vitamin E. A loss average for the same vitamin through food processing and transformation also presents complications in the development of highly sensitive methods for its separation. So, HPLC, despite its effectiveness, is relatively costly and ponderous. The objective of this study was to measure the advantage of high-performance thin layer chromatography (HPTLC) in vitamin E [19]. The methods obtainable utilizing thin layer chromatography (TLC) are sensitive enough to determine the simultaneous isomers for vitamins. Using HPTLC, various isomers of vitamin E were determined. This technique is considered an alternative to other technologies such as GC and HPLC. The history of vitamins is considered the most important chapter in the history of biochemistry for its profound impact on health and the understanding of the catalytic processes that occur in the metabolism of living organisms. It is known that there is a direct relationship between food and disease. Several methods have been developed to determine the content of fat soluble vitamins in pharmaceutical preparations. It is considered one of the recommended methods, although it is less accurate than other methods, but it finds its rightful place among the methods of analysis when the expensive equipment for other methods is not available [20].

Developing a new method for determining the presence of sunflower oil in olive oil is done by preparing a mixture of olive oil and sunflower oil in the following proportions: 5%, 10%, 15%, and 20% sunflower oil in olive oil. This mixture was analyzed using RP-HPLC paired with a

fluorescence detector. The chromatographic system consists of C18 and a moving phase of methanol and acetonitrile 50:50 (V/V) that does not require saponification sample preparation or the addition of antioxidants. Using a fluorescence detector, the excitation wavelength was set at 290 nm and the emission wavelength at 330 nm. In oil of olive adulterated with oil of sunflower, a concentration of α- tocopherol increases linearly. The method is selective, simple, sensitive (RSD% = 2.65%), and it can detect oil of sunflower in oil of olive [21].

Simple, sensitive, and rapid method development of estimation of vitamin E and C into pure and pharmaceutical formulations via the spectrophotometric method This method depends on the formation of complex charge transfer by reaction among Fe^{+3} $[FeNH_4(SO_4)_2.12H_2O]$ and vitamins in the presence of K_3 Fe $(CN)_6$ to configure complexes colored blue greenish, giving the greatest absorption at 743nm. The optimum conditions have been studied in terms of PH, volume, temperature, and reaction time. A linear dynamic range is 0.05-28 μg / ml, 0.5-28 μg /a limit of detection (LOD) of 0.01 μg/ml, 0.09 μg/ml and a LOQ 0.033 μg/ml, 0.297 g/ml, respectively, of vitamin C and vitamin E. The correlation coefficient (R^2) was found to be 0.9993, percentage linearity R^2%= 99.93, and RSD % was less than 0.3% for n=3. This method has been successfully applied for the estimation of vitamin C and E in pharmaceutical preparations. A new method managed to be admitted as an alternative analytical method of estimation for vitamin C and E in their pure forms and dosages [22].

Although there are many ways to quantify the antioxidant vitamins A, C, and E, there are no analytical methods to be practiced on a large scale where there are errors or limitations or an expensive device is required. Therefore, they developed modifications to the spectrophotometric methods for the estimation of all of these vitamins that you could not originally apply to lab exercises in different aspects. This method depends on reactions between vitamin C and a reagent of phosphotungstate, a reaction for vitamin E with batophenanthroline and FeCl3, $FeCl_3$ a vitamin A reaction with H_3PO_4. The tests showed the complete validity of the analytical information obtained while preserving the benefits of a native method. As a result, it was successfully implemented in the clinical analysis routine. Detailed adjustments to the estimation of vitamins in foodstuffs can also be used, for example: in milk, juices, and extracts of solid foods [23].

A fast, simple, and sensitive method of determining tocopherol in its pure form and dosage by utilizing UV-Vis and atomic absorption spectroscopy by reaction with gold ions in solution. The vitamin E complex with gold is absorbed at a wavelength of 535 nm, so it was chosen for the analysis of vitamin E. The optimum conditions were studied in terms of PH, concentration, temperature, and complex formation time. The method obeys Beer's law for the range of concentrations 2- 40 μg / ml also 1-22 μg / ml of UV-Vis and atomic absorption spectroscopy, respectively, R^2=0.9991 for UV-Vis and R^2=0.9992 for atomic absorption. RSD% equals 1.95, 1.59, LOD equals 0.18, 0.044 μg / ml and Rec% equals 96.86%, 101.66% for UV-Vis and atomic absorption spectroscopy, respectively. The method was applied to estimate the effective dosage of vitamins in various samples using compound doses. The accuracy of the method was verified by calculating the average recovery percentage, which was within the acceptable range [24].

The UV-Vis technology is capable of detecting and characterizing small changes in the concentration of mixed E vitamins as a score for its irradiation [25], together with a comparison of the results with the FTIR technique. The results of vitamin E vaccination resulting from radiation showed a linear relationship with radiation dose, with concentrations ranging from 3–

29% and doses ranging from 25–150 KGY. Copper (II)-neocuproine spectrometry can be used, as it has been shown that it allows the determination of various reducing agents for the estimation of vitamin E. This method obeys Beer's law for the range of concentrations of 2.4×10^{-6} - 9.0×10^{-6} M from tocopherols and RSD% =2.1%. The results obtained were compared with other methods, such as HPLC, by calculating a t-test, which showed that the developed method is not fundamentally different from the other methods. The resulting molar absorbance of copper (II)-neocuproine at wavelength 450 nm versus blank indicated the presence of vitamin E oxidation, which could be slightly enhanced by solvent delay, and the entire copper (II)-neocuproine is considered a strong oxidizer [26].

In this research, the physical properties of vitamin A and vitamin E were determined utilizing spectroscopic analysis of various tuber extracts from Lavezares, in northern Samar, Philippines. As a score of physical characterization for various dioscorea types in tuber extracts, it was found to have a boiling temperature 97.6° C, white color, a pH equal 4.9, density of 0.81 g/ml, solubility in ethanol, and miscibility with water. While tuber extracts have a boiling point 98.6° C, they are brown, smell like tea, have a density of 0.86 g / ml also Ph= 5.6, are solubilized in ethanol, and are miscible with water. UV-Vis is utilized to estimate vitamin A and E content for dioscorea types, and the extracts were compared using water and ether. The analysis shows the type contains good natural vitamins [27].

Estimation of vitamins E and C via stenography for the ion of ferric to the ion of ferrous during reaction with potassium dichromate in an acid medium by using Vis-spectrophotometry This method depends on the utilization of a modern reagent, sodium nitroprusside, as a source for ferric ions. A colored solution gave the maximum absorption at 564.4 nm, which was applied in the estimation method. Conditions are improved by studying various variables such as time, volume of acid, volume of reagent, and temperature. The score obtained displays the value of $R^2 = 0.99991$ for each vitamin: LOD=0.1, 0.07 µg/ml; LOQ = 0.33, 0.21 µg/ml, linear range 0.5 - 30 µg/ml and 0.25 - 50 µg/ml, RSD %= 2.88 %, 1.62 %; recovery % = 99.92%, 100.02%, for E vitamin and C vitamin, respectively. A score proves that it is possible to apply the newly developed methods for estimation of E and C vitamins in their pure state and into pharmaceutical preparations together to increase accuracy, reduce cost, and eliminate the need for complex treatments [28].

In this study, the covering period for antioxidant C vitamins and α-tocopherol in plasma and blood for the Bengali population was a limitation, as was the comparison with the source periods in literature. Healthy volunteers aged 18–68 years underwent extensive clinical procedures and were included in the study. UV-Vis was used to estimate the C vitamin, also α-tocopherol. The number of volunteers was 71 healthy Bengalis, 31 males and 40 females, and it was found that the average vitamin C in the plasma was 0.65 mg/dl. The average of α-tocopherol was 6.35 mg/L in this study, which is higher than the natural threshed amount of it, but less than other population groups. This study managed to set a nonspecific coverage period between the sexes of antioxidant vitamins, and the periods were less than the specified period in another population group [29].

A rapid method using ultra performance liquid chromatography (UPLC) from human plasma has been upgraded for the estimation of vitamin E isomers. A method consisted of extracting the hydrophilic liquid portion of the plasma sample of injection in a system UPLC. The UPLC system is armed with a detector fluorescent with an excitement wavelength equal to 296 nm and an emission wavelength equal to 330 nm, and column C18 contains the separate particles. The mobile phase consists of H_2O 1% and methanol 99% grade HPLC, together with a flow rate of 0.4 ml /

min. The summit was reached after 3.5 min, and a 5 µl sample was required for injection. The results showed that the UPLC system is capable of separating, detecting, and identifying six summits, viz α-, γ-, δ- and -tocopherol, as well as tocotrienol. The linear range was 1–10 µg/ml from the total of vitamin E and $r^2 > 0.9996$ of all isomers [30].

Vitamin E is a fundamental micronutrient for maintaining a healthy body, and it is also forbidden for illness. The aim of this study is to validate, develop, and test the reliability of a sensitive and easy-to-apply spectrophotometric method for the determination of α- tocopherol in commercially manufactured soft capsules. This method can be easily applied linearly to commercial pharmaceutical samples containing α- tocopherol. Results showed that the suggested method may be an alternative to the routine method of estimation [31].

A simple method of UV-spectrophotometric estimation of tocopheryl acetate in bulk with micro-emulsion maintenance has been developed. Tocopheryl acetate is freely soluble in ethanol and gives the greatest absorption at 286 nm. The suggested method obeyed Beer-Lambert's Law at range concentrations 1 - 12 µg/ml with LOD=0.093 µg/ml and LOQ=0.236 µg/ml. Reproducible results are obtained with a coefficient of variation of less than 2% [32].

3.Conclusion

In this review, several available methods are discussed for the separation and determination of vitamin E, such as separation by column chromatography (GC, LC, and HPLC) and separation by paper or thin-layer chromatography. Chromatography was successfully used to estimate vitamin E in different samples compared to other vitamins, which are also fat-soluble.

Acknowledgement

After completing the review, the author thanks and appreciates their colleagues for their efforts in completing its preparation, and they also extend their thanks and appreciation to everyone who contributed to this work, even in one word. Finally, thanks and appreciation to all the researchers whose achievements were presented in this review.

References

1. Kareti, S. R.; Nargesh, K. K.; Nikhil, V. B.; Bhanoji, R.M. E; Srinivasa, K .; Ajay K. P. A. Simple and rapid HPLC method for the simultaneous determination of vitamin-A and vitamin-E in tablet dosage forms, *Research Journal of Chemical and Environmental Sciences*, **2013**, *1, 2-6.*
2. Brock, G.; Joe M. Quantifying vitamin E in vegetable oils with reversed-phase high-performance liquid chromatography, *Concordia College Journal of Analytical Chemistry*, **2011**, *2, 44-50.*
3. Abidi, S.L. Chromatographic analysis of tocol-derived lipid antioxidants, *Journal of Chromatography A*, **2000**, *881,197–216.*
4. Ruperez, F. J.; Martin, D.; Herrera, E.; Barbas C. Chromatographic analysis of alpha-tocopherol and related compounds in various matrices, *Journal of Chromatography A*, **2001**, *935, 45-59.*
5. Gimeno, E.; Castellote, A. I.; Lamela-Raventos, R. M.; de Latorre, M. C.; Lopez-Sabater, M. C. Rapid determination of vitamin E in vegetable oils by reversed-phase high-performance liquid chromatography, *Journal of Chromatography A*, **2000**, 881, *251–254.*

6. Afaf, K.; Jelena J. HPLC Determination of Vitamin E in Fortified Food. *Wiley Online Library*, **2011**, *211-221*.

7. Korchazhkina, O.; Jones, E.; Czauderna, M.; Spencer, S. A.; Kowalczyk, J. HPLC with UV detection for measurement of vitamin E in human milk. *Acta Chromatographical*, **2005**, *16, 48-57*.

8. Fatemeh, S.; Ali, S.; Hadi, V.; Mahboob, N. A. Fast and simple method for determination of vitamin E in infant formula by dispersive liquid-liquid microextraction combined with HPLC-UV. *Food Analytical Methods*, **2019**, *12, 23–31*.

9. Ivana, B.; Lenka, K.; Dalibor, Š.; Lucie, H.; Petr, S. A fast HPLC method for determination of vitamin E acetate in dietary supplements using amonolithic column, *Food Analytical Methods*, **2013**, *6, 380–385*.

10. Helena, M. P.S.; Michele, G.; Daniela da, S. O.; Ceres, M. D. L.; Bárbara de, L. R.; Sebastião, C. C. B. Method for simultaneous analysis of eight vitamin E isomers in various foods by high-performance liquid chromatography and fluorescence detection, *Journal of Chromatography A,* **2011**, *1218 (47), 8496-8502*.

11. Mehmet, E. Ş.; Ali, Ç.; Kenan D. Determination of vitamin E isomers of grape seeds by high-performance liquid chromatography-UV detection, *Journal of Chromatographic Science*, **2012**, *50 (2), 97-101*.

12. Barbora, C.; Lenka, K. K.; Dagmar, S.; Bohuslav, M.; Petr S. Recent advances in the determination of tocopherols in biological fluids: from sample pretreatment and liquid chromatography to clinical studies, *Analytical and Bioanalytical Chemistry*, **2016**, *408 (10), 2407-2424*.

13. Sánchez-Machado, D. I.; López-Cervantes, J.; Ríos, V. N. J. High-performance liquid chromatography method to measure alpha- and gamma-tocopherol in leaves, flowers, and fresh beans from Moringa oleifera, *Journal of Chromatography A*, **2006**, *1105 (1-2),111-114*.

14. Khan, A.; Khan, M. I.; Iqbal, Z.; Shah, Y.; Ahmad, L.; Watson D. An optimized and validated RP-HPLC/UV detection method for simultaneous determination of all-trans-retinol (vitamin A) and alpha-tocopherol (vitamin E) in human serum: comparison of different particulate reversed-phase HPLC columns, *Journal of chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, **2010**, *878 (25), 2339-2347*.

15. Caroline, G. A.; Prabha, T.; Jagadeeswaran, M.; Srinivasan; Sivakumar, T. Analytical method development for simultaneous determination of ubidecarenone and vitamin E acetate in capsule dosage form by HPLC, *International Journal of Pharmacy and Pharmaceutical Sciences*, **2019**, *11, 79-84*.

16. Archana, M. N.; Vichika, R. P.; Falguni, B. T. Chromatographic method for estimation of vitamin E from dried blood spot sample, *Future Journal of Pharmaceutical Sciences*, **2020**, *6(8), 2-9*.

17. Ioannis, N. P.; Ioannis, K. K.; Lila, P. k.; Charalampos, P. Determination of Vitamin E in Cereal Products and Biscuits by GC-FID, *Foods*, **2018**, *7(3), 1-6*.

18. Dan Lu; Yi Yang; Xin Wu; Li Zeng; Yong Xin Li; Chengjun Sun. Simultaneous determination of eight vitamin E isomers and a-tocopherol acetate in functional foods and nutritional supplements by gas chromatography – mass spectrometry, *Analytical Methods*, **2015**, *7, 3353-3362*.

19. Hari, R. K.; Janaka, R. Quantitative determination of vitamin E isomers using gas chromatography, high performance liquid chromatography and high-performance thin layer chromatography, *Indian Journal of Nutrition and Dietetics*, **2017**, *54, 1-2*.

20. Ana, M. H.; Mihaela, F. M.; Cristiana, R.; Mihaela, I.; Vasile, M. TLC Applications on separation and quantification of fat-soluble vitamins, *Romanian Biotechnological Letters*, **2009**, *14 (5), 4615- 4619.*

21. Baker, S. M.; Gad male, D. K.; Toche, R. B.; Gaikwad, V. B. Rapid determination of alpha-tocopherol in olive oil adulterated with sunflower oil by reversed phase high-performance liquid chromatography, *Journal of Food Science and Technology*, **2015**, *52 (5), 3093–3098*.

22. Ahmed, M. S.; Mohammed, J. H.; Noor, J. M. A. Sensitive spectrophotometric method for determination of vitamins (C and E), *International Journal of Pharmaceutical Sciences and Research*, **2018**, *9 (8), 3373-3377*.

23. Maciej, R.; Krzysztof, G. Modifications of spectrophotometric methods for antioxidative vitamins determination convenient in analytic practice, *Acta Scientia rum Polonium, Techno logia Alimentaria*, **2007**, *6 (3), 17-28*.

24. Ahmed, M. S; Abbas, S.; Noor, J. M. A Spectrophotometric determination of vitamin E via formation of gold, *European Journal of Biomedical and Pharmaceutical sciences*, **2017**, *4, 82-87.*

25. Rowell, S.; Oral, E.; Mouratoglou, O. k. Detection of vitamin E in irradiated uhmwpe by UV-visible spectroscopy Poster No. 1186, *ORS Annual Meeting*, **2011**, *1186.*

26. Esma, T. R.; Apak; Esma, G.; Kevser, S. Spectrophotometric determination of vitamin E (alpha- -tocopherol) using copper (II)-neocuproine reagent, *Talanta*, **1997**, *44 (2), 249-255*.

27. Jonalyn, E. E.; Karina, M. R.; Merle, N. T.; Rolando, A. D. Spectrophotometric analysis of vitamin A and vitamin E in different species of dioscorea tuber, *International Journal of Trend in Scientific Research and Development*, **2019**, *3, 837-839.*

28. Ahmed, M. S.; Abbas, S.; Noor, J. M. A. Colorimetric determination of antioxidant vitamins E and C, *Pakistan Journal of Biotechnology*, **2018**, *15 (2), 451-457.*

29. Sanghamitra, C.; Indranil, C. Determination of coverage interval of antioxidant vitamins (vitamin C & vitamin E) in plasma and serum of Bengali population, *Biochemistry Research International*, **2013**, *1, 1-4.*

30. Shahidee, Z. A.; Mohammad, J. I.; Mohd, H. R.; Nuraliza, A. S. Determination of vitamin E isomers in plasma using ultra performance liquid chromatography, *The Malaysian Journal of Analytical Sciences*, **2012**, *16 (1), 71- 78.*

31. Farmasötik, Y. Quantitative determination of α-tocopherol in pharmaceutical soft capsule by spectrophotometry, *Experimed*, **2020**, *10 (2), 72-76.*

32. Sayad, I. W.; Md Ismail, M.; Tadwee, I. Simplistic spectroscopic method for determination of α-tocopherylacetate in bulk and formulated microemulsion, *International Journal of Pharmaceutical Research & Allie.*