Determination of Vitamins D3, E and C in a mixture by HPLC

Ayman Sadiq Al-Samarrai[1], Shatha Younes Al-Samarrai[2], Rafah Razooq Hameed Al-Samarrai[3]
[1,2]Department of chemistry, college of Science, Tikrit university, Selah El-dean, Iraq
[3]Department of applied chemistry, College of applied science, University of Samarra, Selah El-dean, Iraq
Email: ayman.sa.j@uosamarra.edu.iq

Abstract
A new analysis method has been found for the determination of vitamin E, D3, and C in one mixture using HPLC technology and separation in a short time and high accuracy using a mobile phase that is easy to prepare and apply, does not contain a buffer solution and does not contain expensive materials. using column C18 (dimensions: 150mm*4.6mm, 5µm), The mobile phase consists of methanol only with a flow rate of 1.5 ml/min with a UV detector at a wavelength of 280 nm, and the retention time of vitamins C, D3 and E was 0.909 min , 3.685 min and 4.972 min respectively, The method had an accuracy between 95.59475% - 102.604% with a linear range between 20-180 µg.ml-1 , 80-400 µg.ml-1 and 5–20 ng.ml-1, The correlation coefficient R2 9994, 0.9990 and 0.9996 , A limit of detection was 0.001101 ng/ml, 0.13532 ng/ml and 0.123338 ng/ml , A limit of quantification 0.003669 ng/ml , 0.451066 ng/ml and 0.411125 ng/ml for Vitamin C, D3, and E, respectively .
Keywords: HPLC; Vitamin D3, Vitamin E and Vitamins C

Introduction

Chromatography is one of the most important separation techniques used to analyze and quantify a substance or mixture of substances, which is classified into several techniques (1,2), One of the most widespread and important types is high-performance liquid chromatography -HPLC, which follows liquid chromatography-LC techniques, which are widely used to analyze organic, inorganic, and biological compounds. In this technique, the first phase (mobile phase) is pumped through a column that holds with the packing of small porous particles with a second phase (stationary phase) under high pressure to speed up the process of separating the components [3,4]. HPLC technique is based on the principle of distributing the components of the model between two phases, one of which is the stationary phase and the other mobile phase, which consists either of one solvent or a mixture of more than one solvent [5], and it is a common method for the analysis of many biological compounds such as amino acids, monosaccharides, fatty acid, vitamin ...etc [6-8].

Vitamins are classes of highly complex compounds, found in foodstuffs in trace amounts, organic in nature, essential for normal metabolic pathways, and loss cause disorders while re-equipment of these nutrients can heal the deficiency symptoms [9,10]. Vitamins are classified according to their solubility to soluble in water (such as Vit. B1.B2, B3, B5, B6, B9,B12 and VitC) and Vitamins soluble in lipids(such as Vit. A, E, D and K) [9]. Vitamins are essential for cell growth and development, vision, promotion of proper cell communication, and antioxidant activity [11]. Throughout the years, different methods for vitamins determination were developed, using different techniques, such as colorimetric methods or liquid chromatography. So, the present study aimed to improve the HPLC Analysis method of Vitamin D3, E, and C in a mixture [12] which the structures are shown in Figures1,2,3(13).

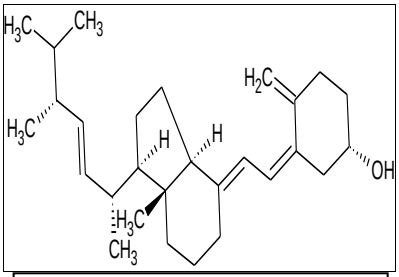


Figure 1: Structure of vit. D3

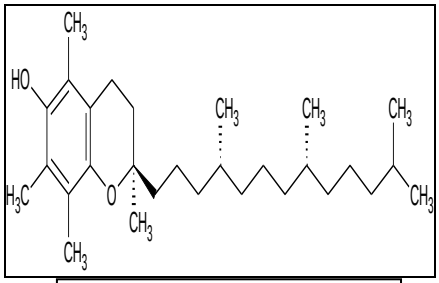


Figure 2: Structure of vit E

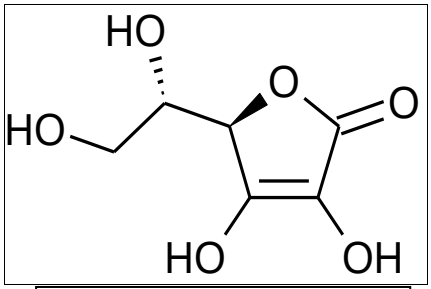


Figure 3: Structure of vit. C

Material and Methods

-Instruments: HPLC and UV/Vis Spectrophotometer 1800 from Shimadzu were used in the study.

-Chemical materials: Methanol, Ethanol, and other materials with high purity were used in the study which are shown in Table 1.

doi.org/10.31838/hiv22.02.634 Received: 01.04.22, Revised:13.06.22, Accepted: 06.08.22

Table 1 : Chemical materials used			
Chemicals compounds	Chemical formola	Purity	Supplying company
Ethanol	CH_3CH_2OH	99.9%	Biosolve
Methanol	CH_3OH	99.9%	Biosolve
Vitamin C	$C_6H_8O_6$	Pure	
Vitamin D3	$C_{27}H_{44}O$	$2.5*10^{-8}$% D_3	
Vitamin E	$C_{29}H_{50}O_2$	44.1% E	
Aerosil	SiO_2	100.1%	Evonik
Avicel PH 102	$C_{14}H_{26}O_{11}$	Pure	JRS
Magnesium stearate	$Mg(L_1H_{35}O_2)_2$	Pure	Valaji
Maize starch	$C_{27}H_{48}O_{20}$	Pure	Sunor
Sod. Starch Glycolate (type A)	$C_2H_3NaO_3$	4.1%(Na)	Hangzhu
Talc	$Mg_3Si_4O_{10}(OH)$	pure	Valaji

-Solvent selection: The solubility of the vitamins was studied; Ethanol was chosen as a solvent for vitamins D3 and E. They were completely dissolved in ethanol, and distilled water as a solvent for vitamin C, as it was completely dissolved in it, after using several solvents.

-Standard solutions: 1000 µg.ml^{-1} standard solutions of the vitamins C, E, and 25 ng.ml^{-1} of vitamin D_3 were prepared from the pure standard substance as a stock solution, which was used to prepare different concentrations (ranging between 2.5-25 ng.ml^{-1} for D3 and 100-1000 µg.ml^{-1} for E and 20-200 ppm for C), then the samples were injected into the HPLC column by 50 µl syringe.

-Preparation of pharmaceutical solutions: Pharmaceutical solutions of vitamin D3 and E were prepared using oil capsules containing 2000 IU and 400 IU for each of them respectively. while the concentration of vitamin C in tablets was 500ppm, In which ten tablets were weighed and the average weight of one tablet for each vitamin was taken and placed in an appropriate volumetric flask and dissolved with the appropriate solvent to obtain a stock solution with a final concentration of 50 ng.ml^{-1} of vitamin D3 and 1000 µg.ml^{-1} of vitamin E and 1000 µg.ml^{-1} of vitamin C.

-Preparation of the mobile phase: Methanol for HPLC, gradient grade, ≥99.9% only was used in the study, which gave good separation of the mixture of vitamins under investigation after using several solvents as a mobile phase.

-Preparation of solutions of pharmaceutical additives

solutions for common pharmaceutical additives were also prepared according to their percentage in the tablets under investigation, the pharmaceutical additives were dissolved in ethanol, incubated for 10min in ultrasonic, and then the solution was filtrated by micro-filter.

Results and discussion

Choosing the optimal conditions for the proposed method

1- Choose the wavelength of the absorption spectrum of the vitamins mixture:

The absorbance of vitamin D3, E, and C were measured against their blank. The three vitamins under investigation(D3,E,C) showed maximum absorbance at different wavelengths 270nm , 286nm , and 290nm respectively. The study also includes measuring the absorbance of vitamins in the mixture (against blank), at wavelengths 254-300 nm, the results indicate that the best separation was at 280 nm as shown in Figure 4.

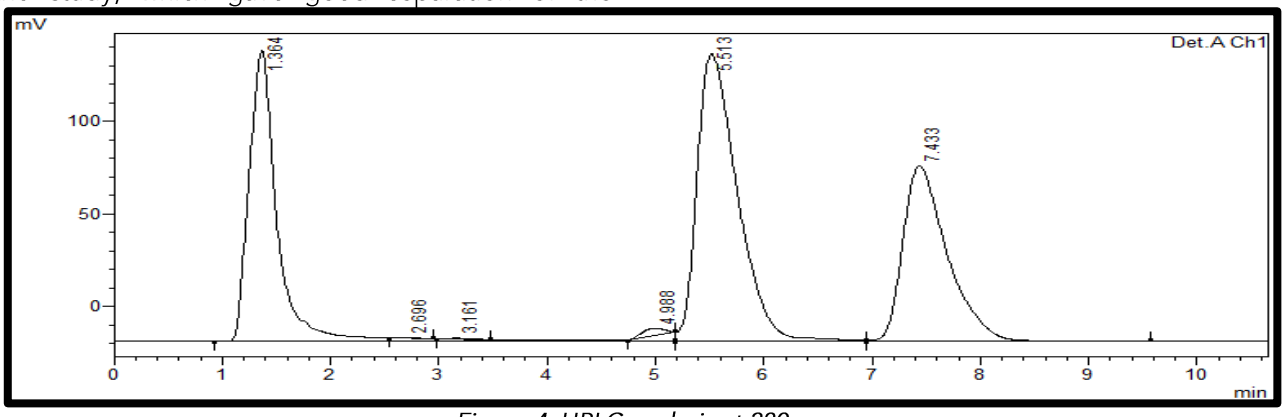


Figure 4: HPLC analysis at 280 nm

2-Column selection

The study includes the use of different types of columns (L1, L3, L7, L11) to obtain the best separation, different concentrations of vitamins mixtures were injected (30 ng.ml^{-1}, 80 µg.ml^{-1}, and 20 µg.ml-1), with a flow rate of 1.0 ml/min and the injection volume 50 µl. According to the separation

efficiency, peak area value, and retention time at 280nm, the results indicate that the best separation was carried out by using L1 column (150mmx4.6mm; 5 µm) , Figure 5.

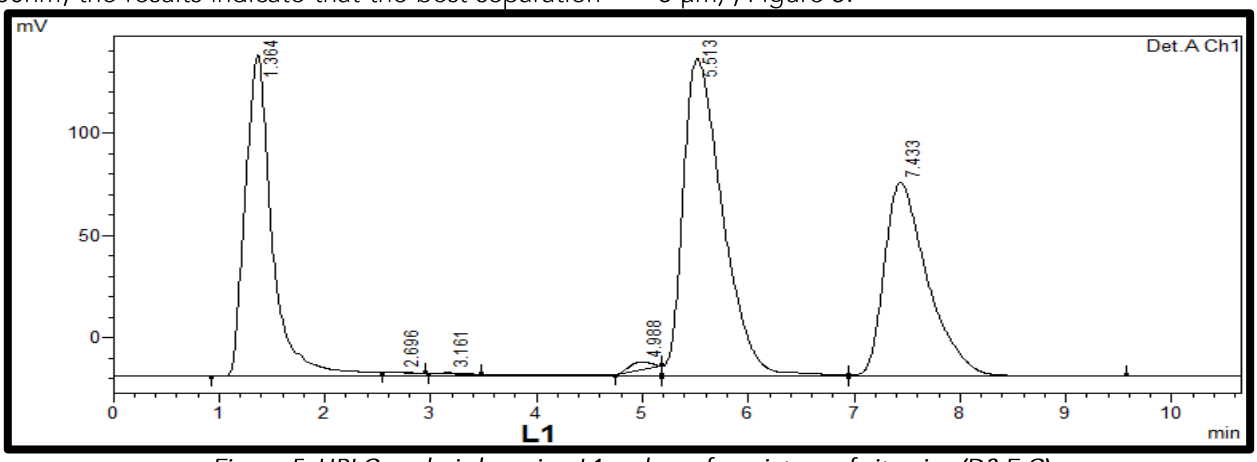


Figure 5: HPLC analysis by using L1 column for mixture of vitamins (D3,E,C)

Selection of a mobile phase solution

To choose the mobile phase solution, required conducting several practical experiments and preparing several solutions of the mobile phase to choose the optimal one. The results showed that the best solution for the mobile phase is using methanol alone, where the best separation peaks, the best retention time, and the best peak area was obtained, and the results obtained are shown In Figure (6):

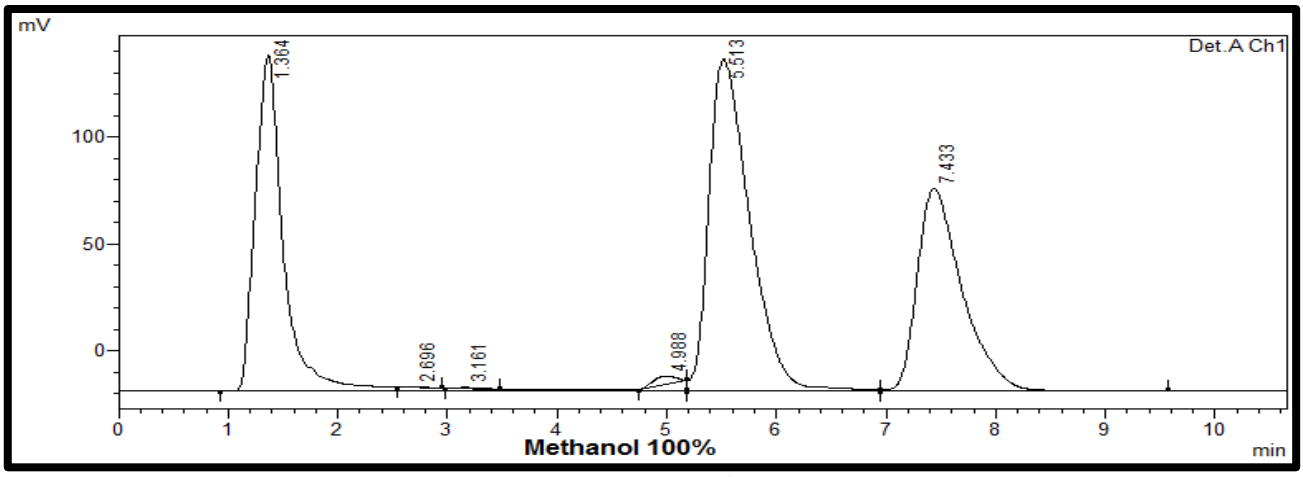


Figure 6 shows the best solution for the mobile phase

mobile phase flow rate

A test of the mobile phase flow rate was conducted by changing the flow rate which is 0.5, 0.75, 1, 1.5 ml/min for the mixture of vitamins under study. The flow rate of 1.5 ml/min showed the best peaks and the best retention time, as shown in figure (7) :

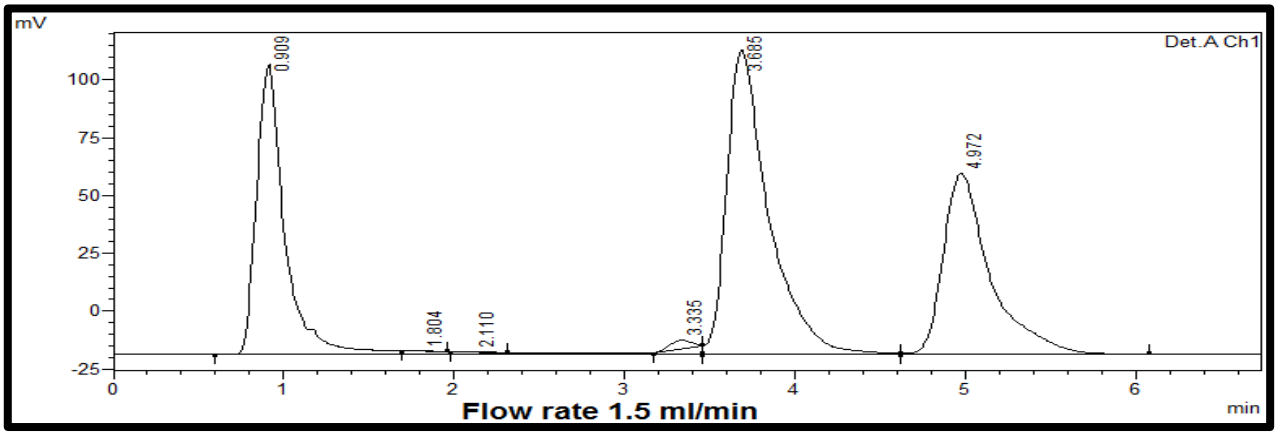


Figure 7 shows the best mobile phase flow rate

column temperature selection

The effect of the change in temperature on the proposed method was studied by changing the temperature on the column installed in the method (L1) by injecting 50 microliters of the mixture containing the vitamins understudy at temperatures

30, 35, 37, 40, 45 and 50 °C . The results showed that there is a slight decrease in the retention time, but the best separation is at the temperature of the laboratory, shown in Figure (8):

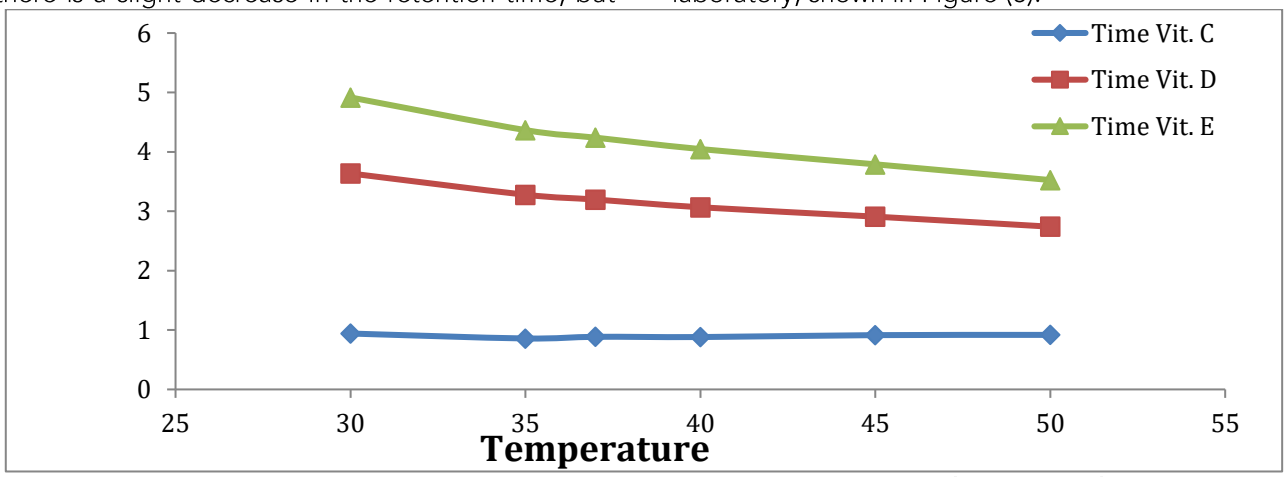
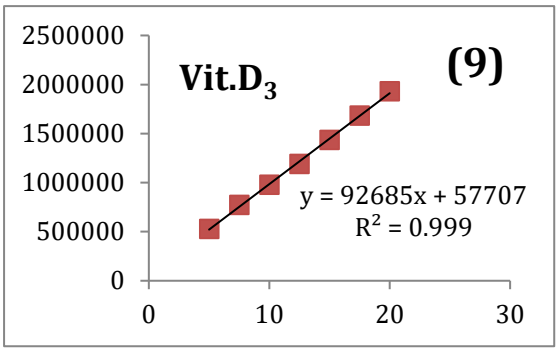
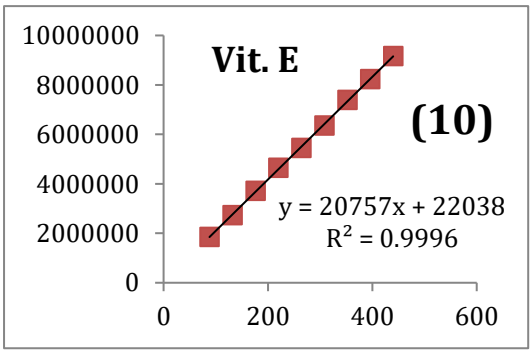


Figure (8) shows the relationship between the retention time and the temperature of the column for each vitamin

Calibration curves

Calibration curves were prepared for the mixture of vitamins understudy by drawing area versus concentration, The linearity of vitamin D3 was 5-25 ng.ml^{-1} with a constant concentration of vitamins E and C 120 µg.ml^{-1} and 20 µg.ml^{-1} respectively . While the linearity of vitamin E was 80-400 µg.ml^{-1} with a constant concentration of vitamin D3 and C: 15 ng.ml^{-1} and 20 µg.ml^{-1} respectively. And the linearity of vitamin C was 20-180 µg.ml^{-1} with a constant concentration also of vitamins D3 and E 15 ng.ml^{-1} and 120 µg.ml^{-1} respectively. which were prepared from stock solutions which have been prepared in advance using ethanol for vitamins D3 and E, and water for vitamin C and by applying the optimal conditions to the proposed method using column L1, flow rate 1.5 ml/min, wavelength 280 nm, and laboratory temperature, and as shown in Figure (9) :





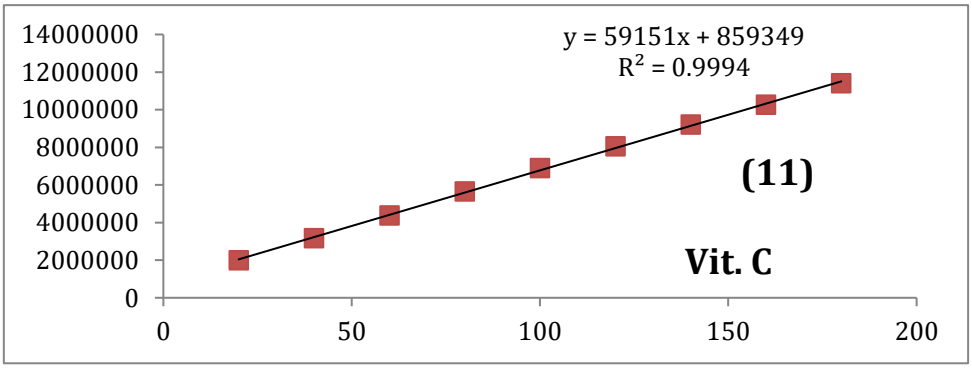


Figure (9,10,11) Calibration curve of vitamin D3 5-20 ng.ml-1 with a constant concentration of vitamins E and C 120 µg.ml-1 and 20 µg.ml-1 respectively. Calibration curve of vitamin E 80-400 µg.ml-1 with a constant concentration of vitamin D3 and C 15 ng.ml-1 and 20 µg.ml-1 respectively. And the Calibration curve of vitamin C was 20-180 µg.ml-1 with a constant concentration also of vitamins D3 and E 15 ng.ml-1 and 120 µg.ml-1 respectively.

precision and accuracy

Each concentration of the above-mentioned calibration curves was re-injected five times, and the calibration curves were prepared by drawing the relationship between the concentrations and their peak area, as the recovery values ranged between 95.59475% - 102.604% , while the value of the RSD% was confined between 0.001682 - 0.052079 .

selectivity

The proposed method can isolate and remove the interferences that may affect the separation of the vitamins under study. After stabilizing the optimal conditions the proposed method was injected into the mobile phase to ensure that there is no separation or sensitivity to the mobile phase by the chromatographic system, which may affect the separation of the vitamins understudy or It overlaps with its peaks, and a chromatogram was obtained that did not contain any separation. The effect of additives on calculating the peak area of the drug was also studied by preparing a solution consisting of pharmaceutical additives in common proportions, which are equivalent to one tablet, and there was no separation or sensitivity by the system to these additives, and no peak appeared for any of the additives. These additives do not affect the separation of these vitamins and no peak appeared during their retention time, which confirms that the method is highly selective and valid for drug estimation.

method application

Solutions were prepared with three concentrations of a manufactured drug containing the vitamins under study (because of the unavailability of a pharmaceutical preparation containing the three vitamins alone) that mediate the concentration ranges of the calibration curves of the vitamins under study, which are 10, 12.5 and 15ng.ml^{-1} of vitamin D3 in the presence of a constant concentration of Vitamins E and C: 120 and 20 µg.ml^{-1} respectively. 160, 200 and 240 µg.ml^{-1} of vitamin E in the presence of a constant concentration of vitamins D3 and C: 15 ng.ml^{-1} and 20 µg.ml^{-1} respectively. and also 80, 100, and 120 µg.ml^{-1} of vitamin C with a constant concentration of vitamins D3 and E of 15 µg.ml^{-1} and 120 µg.ml^{-1} Respectively. The straight-line equation was applied to it, where the results showed a high accuracy of the proposed method with a Rec% that ranged between 96.20481% - 101.9039% .

Conclusions

The proposed method is simple, fast, accuracy, reproducible, reliable, inexpensive, safe, with low environmental impact and it can be used for the determination of vitamin C, D3, and E in one mixture.

References

1- Skoog, Douglas A., F. James Holler, and Stanley R. Crouch. "Principles of instrumental analysis". Cengage learning, 2017.

2- Jared L. Anderson, Alain Berthod, Veronica Pino Estevez, and Apryll M. Stalcup. Analytical Separation Science, First Edition. 2015.

3- Pederson-Bjergaard S, Gammelgaard B, Halvorsen TG. "Introduction to pharmaceutical chemistry". John Wiley & Sons; Apr29. 2019. p:185, 190.

4- Mohammed Alzuhairi . Introduction to High Performance Liquid Chromatography (HPLC) . February 2013 .

5- Al-Ghabsha, Abdul Rahim Saeed Samir, Thabet Saeed. "An Introduction to Separation Techniques in Chemistry". 1985, 117.

6- Qian, X., Nie, L., Dai, Z., & Ma, S. (2021). Determination of Free Amino Acids in Banlangen Granule and its Fractions by Solid Phase Extraction Combined with Ion-pair Hig-h Perfor mance Liquid Chromatography using a Corona-charged Aerosol Detector (SPE-HPLC-CAD). Current Pharmaceutical Analysis, 17(7), 838-846.

7- Zhao, X., He, Y., Chen, J., Zhang, J., Chen, L., Wang, B., ... & Yuan, Y. (2021). Identification and direct determination of fatty acids profile in oleic acid by HPLC-CAD and MS-IT-TOF. Journal of Pharmaceutical and Biomedical Analysis, 204, 114238.

8- Sławińska, A., Jabłońska-Ryś, E., & Stachniuk, A. (2021). High-performance liquid chromatography determination of free sugars and mannitol in mushrooms using corona charged aerosol detection. Food Analytical Methods, 14(2), 209-216.

9- Maqbool, M. A., Aslam, M., Akbar, W., & Iqbal, Z. (2017). Biological importance of vitamins for human health: A review. J. Agric. Basic Sci, 2(3), 50-58.

10- Ahmed Fathi Sayed Ahmed. Biochemistry. First Edition, Dar Al-Fajr for Publishing and Distribution: 159.

11- Zehiroglu, C., & Ozturk Sarikaya, S. B. (2019). The importance of antioxidants and place in today's scientific and technological studies. Journal of food science and technology, 56(11), 4757-4774.

12- Tyśkiewicz, K., Dębczak, A., Gieysztor, R., Szymczak, T., & Rój, E. (2018). Determination of fat- and water-soluble vitamins by supercritical fluid chromatography: a review. Journal of Separation Science, 41(1), 336-350.

13- Nelson, D. L., & Cox, M. M. (2017). Lehninger principles of biochemistry (7th ed.). W.H. Freeman.