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Novel method for determination of Vitamins E and C by Cloud-point extraction

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***Abstract*---**A new, simple, fast and accurate spectrophotometric method has been found for the determination of vitamin E and C in their complexes by cloud point method. The Beer-Lambert Law was complied with for a range of concentrations ranging from 5-50 µg.ml-1 for vitamin E and 5-40 µg.ml-1 for vitamin C, and the value of the correlation coefficient R2: 9987 and 9997, and the value of the molar absorption coefficient Ɛ: 24421.25 and 10760.93, Sandel's sensitivity was 0.0176 µg.cm-2 and 0.0164 µg.cm-2 for vitamins E and C, respectively, while the detection limit was 0.0008 and 0.0009, and the quantitative limit was 0.0026 and 0.0030 for vitamins E and C, respectively. The method has been successfully applied for the determination of vitamin E and C in their pure forms and pharmaceutical preparations.

***Keywords*---** cloud point extraction method, vitamin E, vitamin C.

Introduction

The cloud point extraction method-CPEM is one of the extraction methods described firstly by Watanabe and Tanaka in 1978, and developed by Saitoh, *et al*[1,2]. It is an eco-friendly assay for the separation and pre-concentration of abundant analytes that presents many benefits over the classic liquid-liquid extraction[3]. The method is inexpensive, rapid, selective, and accurate precise. It is classified as a green extraction method as it consumes no or minimum

quantities of poisonous organic solvents[4] . The traditional CPEM depends on the feature of nonionic surfactant to form micelles in aqueous media at heating above a fixed temperature named cloud temperature or cloud point[5], or by salting out phenomenon[6]. This procedure depends on the separation into an aqueous phase as the first phase and a surfactant-rich one as the second phase is then conceivable by centrifugation[7]. Many researcher used this method for determination of many vitamins, in which Ortega,*et al* developed CPE and micellar chromatographic-MC methods for the determination of vitamins A and E [8]. while Heydari, *et al*[9], developed a new, simple, and effective ion-pair cloud-point extraction connected with a gradient high-performance liquid chromatography-HPLC method for the evaluation of water-soluble vitamins which include vitamin B1(thiamine), vitamin B3(niacinamide), vitamin B6, (pyridoxine), and vitamin B2,(riboflavin) in urine and plasma. So the present study aimed to develop a new method for the determination of Vitamins E and C by Cloud-point extraction.

Procedure

The extraction process is carried out to form a cloud point for vitamin C and E by taking a 10ml solution containing a certain concentration of the dye with a certain concentration of each vitamin and adding the buffer solution, then adding the 10% of surface material (Triton X-114), heating in a water bath until the cloud point. It is separated by a centrifuge, and then placed in an ice bath for 5 min. The water layer is separated and the cloud point layer is dissolved with 5ml of ethanol, and the absorption is measured at the specified wavelength.

- The maximum wavelength:
  The maximum wavelength was determined for performing spectroscopic studies for a series of experiments by choosing the above method by mixing 1ml of 10 ppm of vitamins solution(E and C) with 1ml of 10 ppm Bromophenol blue dye solution with 0.5 ml of buffer solution and then add 1 ml of 10% Triton X-114 into a volumetric flask and fill the volume to 10ml with the appropriate solvent (Acetone for vitamin E and water for vitamin C). The spectrum was measured for the colored product, as it gave the maximum absorbance at 594 and 434 nm for vitamins E and C, respectively. As shown in Fig. 1 and 2.
- Study the optimum conditions for extracting the cloud point:
  - Effect of pH: The pH has an important effect on the interconnection between the vitamins under study and the dye to form the complex. In order to choose the optimum pH for the cloud point extraction process, a number of buffer solutions were prepared with a pH value ranging between 3-9 in a series of volumetric bottles of 10 ml and at a certain concentration for vitamins and dye, adding 0.5ml of the buffer solution to each flask with an appropriate volume of 10% Triton X-114 10%, incubated in a water bath at the appropriate temperature and time, and then centrifuged to separated the aqueous layer from the organic layer (cloud point) in a number of cycles and an appropriate time and then incubated in the ice bath for 5min and then the aqueous layer is decanted out, the organic layer remains, and it is dissolved with an appropriate solvent in a volume of 5 ml, and the absorption is measured

at the maximum wavelength that was previously determined, as shown in Fig 3.

- Effect of Bromophenol blue volume: The volume of the dye affects the process of cloud point formation. To choose the optimum volume of the dye to form the complex, a series of volumetric flask (10 ml capacity) were prepared at a specific concentration of each vitamin as well as the dye and the optimum pH and an appropriate volume of 10% Triton X-114. Incubated in a water bath at an appropriate temperature and time. The aqueous layer was separated from the organic layer (cloud point) by centrifugation with several cycles and an appropriate time and then incubated in an ice bath for 5 minutes then the aqueous layer was decanted out and the organic layer remains and dissolved with a 5ml of suitable solvent and its absorbance was measured at the optimum wavelength that has been determined previously.
- Effect of Temperature: The formation of the cloud point depends mainly on the temperature, in which the interaction between the complex formed and the surface active substance Triton X-114 is hydrophobic or electrostatic or both, and the separation of phases is due to the competition between the influence of internal energy and entropy, so the separation process is due to an increase in the accumulation the micelles with increasing temperature and the separation of phases is at a certain degree due to the internal stress of the micelle and at the optimum temperature they are in a state of more attraction. A series of the volumetric flask (capacity of 10ml) were prepared at a certain concentration for each vitamin, the optimum dye volume, the optimum pH, and a specific volume of Triton X-114, and the volume was completed in the volumetric bottles at the mark and then incubated in a water bath with different temperatures ranging from 20-90 ^{o}C and the appropriate time, and the aqueous layer is separated from the point cloud layer by centrifugation at each degree, then the cloud layer is dissolved with 5ml of an appropriate solvent, then the absorbance is measured at the specified wavelength as shown in Fig. 4.
- Effect of heating time: The effect of time on the extraction process is important to the process of cloud point formation, after determining the optimum pH, optimum dye volume, and optimum temperature, a series of volumetric bottles (with 10 ml capacity) were prepared at a certain concentration for each vitamin, optimum dye volume, optimum pH and a certain volume of active surfactant. At a concentration of Triton X-114 and complete the volume in a volumetric flask, incubated in a water bath at the appropriate temperatures for a different time between 5-20 min. The absorbance is determined at a specific wavelength.
- Effect of Triton X100 volume: The effect of the surface active substance Triton X-114 was studied for the cloud point extraction process and its effect on the extraction process. Different volume of Triton X100 (0.5-3 ml) were taken and incubated in a water bath at the optimum temperature and time, then separated and dissolved in 5ml of appropriate solvent, and then the absorbance was measured at the optimum wavelength, as shown in Table (1).
- The effect of centrifugation time: The centrifugation process is one of the most important factors for the process of separation and extraction from

the aqueous solution after the formation of the cloud point. A series of volumetric flasks (10 ml in capacity) were prepared and at a certain concentration for each vitamin, as well as the optimum dye volume, the optimum pH function, the optimum temperature ،and time, and then the solutions are placed in the centrifuge at different time(10-40)min. Incubation after centrifugation in an ice bath for 5 min, and the aqueous layer was separated from the cloud point layer by pouring it, and the layer was dissolved with a 5ml suitable solvent, and its absorbance were measured at the optimum wavelength.

- The effect order of additions: The effect of the sequence of additions on the intensity of absorbance of solutions was studied, as all possible successive additions of the reactants were tested with different sequences of each vitamin, dye, buffer solution and surfactant, which were studied in the previous paragraphs, and the highest absorption was obtained as shown in Table (2).
- Study Stoichiometric ratio: The Stoichiometric ratio between each vitamin and the dye was studied to find out the structural formula of the complex formation through the Job method (continuous changes method). Vitamin and dye and by changing the volumes oppositely in the range of 0.1-0.9 ml so that the final volume of each vitamin and reagent is equal to 1ml and at the optimum pH and the optimum surface material volume (Triton X-114), and the volume is completed in the volumetric flask with the appropriate solvent and incubated in a water bath At the optimum temperature and time until the formation of the cloud point and then separating it by centrifugation with the number of cycles and the optimum time, then incubated it in an ice bath for 5 minutes and decant the aqueous layer and remaining the cloud point layer and dissolved with 5ml of the appropriate solvent, then the absorbance was measured for it at the optimum wavelength and the absorbance value is drawn against the volumetric ratio of each vitamin and dye and the correlation ratio is found from the curve to determine the expected shape as shown in Fig 5.
- Calibration curve: After confirmation of the optimum conditions, a calibration curve was prepared for the spectral determination of each vitamin with the dye by preparing a series of solutions) 10ml in capacity) in the volumetric flask containing graduated volumes of each vitamin, the optimum concentration and volume of the dye, buffer solution, and the optimum volume of the surfactant Triton X-114 . And complete the volume to the mark (acetone for vitamin E and water for vitamin C), and it is placed in the water bath at the optimum temperature and time to form the cloud point layer, and then separated in the centrifuge at the optimum number of cycles and time, then placed in an ice bath for 5 minutes and separated The aqueous layer is separated from the organic layer (the cloud point) and it is dissolved in 5ml ethanol. The absorbance was measured for each concentration and the concentrations are calculated according to the volume of ethanol(5ml) used, and the relationship between the absorbance against concentration is drawn to get a straight line and the slope value of the calibration curve as in Fig 6.
- Accuracy and Precision: Accuracy and Precision were calculated for all concentrations of the calibration curve, which are 18, 5, 8, 12 µg/ml(five

times for each concentration) in order to express the accuracy and precision of the method used in terms of the ratio of relative error (REC%), the ratio of standard deviation RSD% and the ratio Retrievable Rec% respectively.

- Applications: Two concentrations of each vitamin were prepared by taking 1 and 2 ml of the drug solution with a concentration of 100 µg/ml in a volumetric flask (10 ml capacity), then these solutions were treated with the same procedure for preparing the titration curve, and the absorbance was measured and compensated in the calibration curve.

Results and Discussion

Spectroscopic study of the complex formation for each vitamin with the reagent

To certain results for the interaction between the vitamins under study and the dye, the absorbance spectrum was drawn with a cloud point to determine the wavelength of the optimum absorbance against the blank solution. A new absorbance peak was shown at 594 nm for the vitamin E complex (Fig 1) and 434 nm for the vitamin C complex (Fig 2), in which Bromophenol Blue dye in ethanol gives the optimum absorbance at 424 nm, and 592 nm.

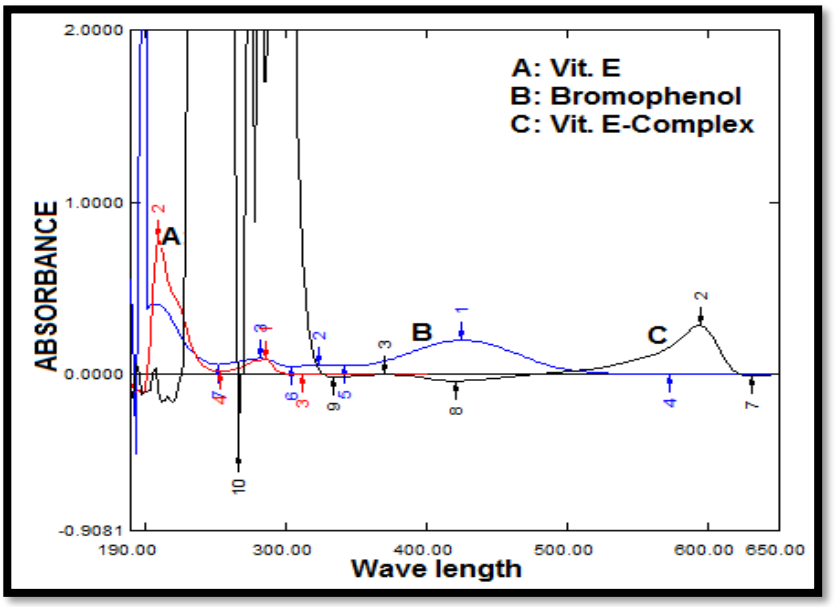


Fig. 1. The complex spectra (C), Vitamin E(A) and dye(B)

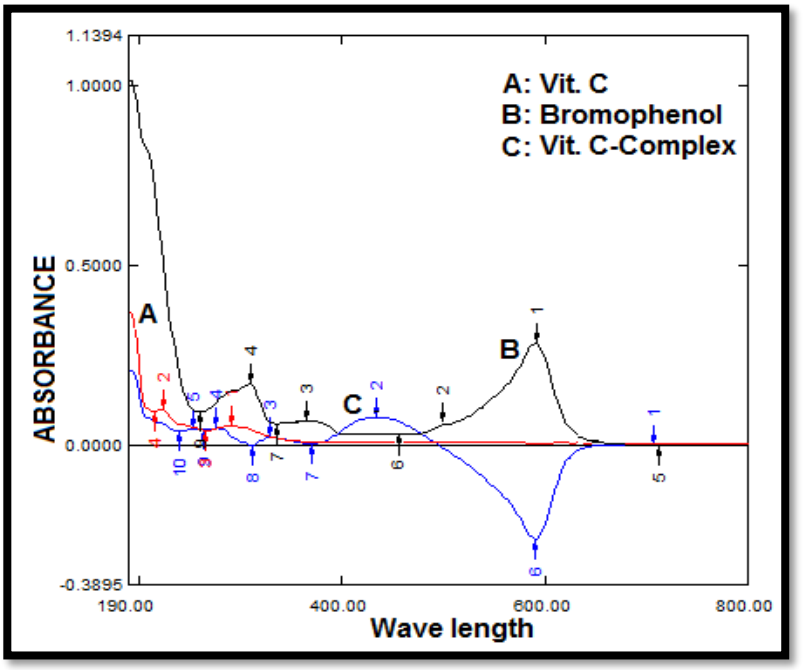


Fig. 2. The complex spectra (C), Vitamin C(A) and dye(B)

Study of optimal conditions for vitamin E and C extraction

The effect of pH: The effect of pH on the complex formation at pH ranging 3-9 was studied and the extraction process was completed for the cloud point and the absorbance was measured for it. It was found that the best pH is 6 and 5.5 for vitamin E and C, respectively, which gave the highest absorbance value as in Fig 3.

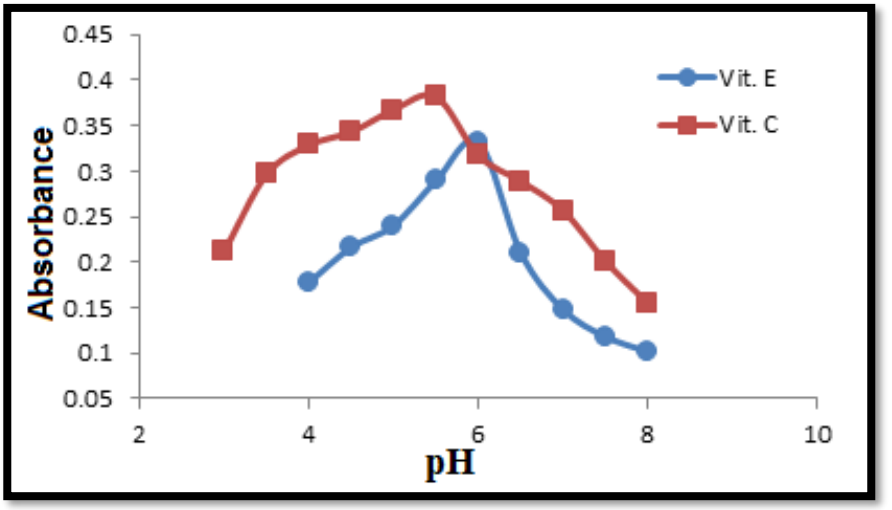


Fig.3. The optimum pH for vitamins E and C

The effect of temperature: Temperature is one of the important factors in the extraction process to form the point of the cloud. This study showed that the temperature has a direct effect on the formation of the complex between the vitamin and the dye. The temperature range used in the current study was 20-90°C, as the cloud began to form at a temperature of 40°C and was completed at 60°C,and it disintegrated at 90 ° C for vitamin E, whilefor vitamin C, the cloud began to appear at 35°C and completed at 50°C, and then began to disintegrate at 70°C, as shown in Fig4.

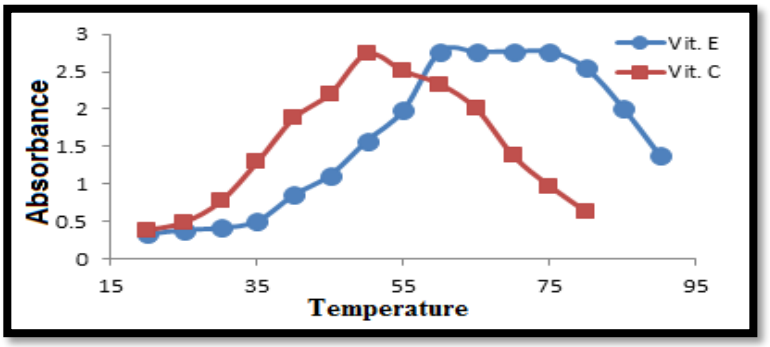


Fig.3. The effect of temperature to the vitamins E and C complexes

The effect of heating time: The heating time at the optimum temperature has an important effect on the formation of cloud points with high extraction efficiency. In which the effect of time ranging (0-20)min was studied and the absorbance was measured after extraction process for cloud point, the results indicate that the best time for extraction efficiency with the optimum absorbance were 13 and 15 min for vitamin E and C, respectively.

The effect of TritonX-114 volume: The effect of the surfactant active substance (Triton-X114) volume was studied within the range 3-0.5ml. After performing the cloud point extraction process under the specified conditions, the absorbance results for each volume were shown in Table(1). The optimum absorbance was at the volume of 1 ml for vitamin E and 0.5 ml for vitamin C.

Table1
The effect of Triton-X114 volume

Vol. of TX-114	Abs. of Vit. E	Abs. of Vit. C
0.5	0.2925	0.3857
1	0.3345	0.3655
1.5	0.3169	0.3569
2	0.3074	0.3501
2.5	0.2898	0.3488
3	0.2787	0.3412

The effect of centrifugation time: The effect of the centrifugation time to separate the active phase was investigated by centrifugation at 4000 rpm at

different time period ranging from 10 to 30 min. In which the time 30min is the optimum for vitamins E and C, because it gave the best separation process for the cloud layer.

The sequence of additions: The effect of the sequence of additions on the absorbance was studied and it was found that the sequence of additions had an important effect on the absorption of the products formed, and the following table shows the sequence of additions of vitamins E and C, as in Table 2.

Table 2
Show the sequence of additions

Order of Addition	Abs.	Order of Addition	Abs.
E+BROMO+BUFFER	0.3312	C+BROMO+BUFFER	0.3799
E+BUFFER+BROMO	0.3159	C+BUFFER+BROMO	0.3722
BROMO+E+BUFFER	0.2548	BROMO+C+BUFFER	0.3535
BROMO+BUFFER+E	0.2166	BROMO+BUFFER+C	0.3363
BUFFER+E+BROMO	0.2231	BUFFER+C+BROMO	0.3687
BUFFER+BROMO+E	0.2199	BUFFER+BROMO+C	0.3265

Conjugation ratio between vitamins E and C with dye according to Jobe method (continuous changes method): Job's method of continuous changes was depend upon the purpose of finding the structural formula of the extracted complex by mixing equal concentrations and different volumes of each vitamin and dye to final volume equals to 1 ml. The extraction method was followed previously and the absorbance was measured at optimum wavelength and the curve between absorbance and volume ratio of each vitamin to the ratio of the sum of the volume of the vitamin and the dye. The results indicate that the conjugation ratio is 1:1 for each vitamin, as shown in Fig. 5.

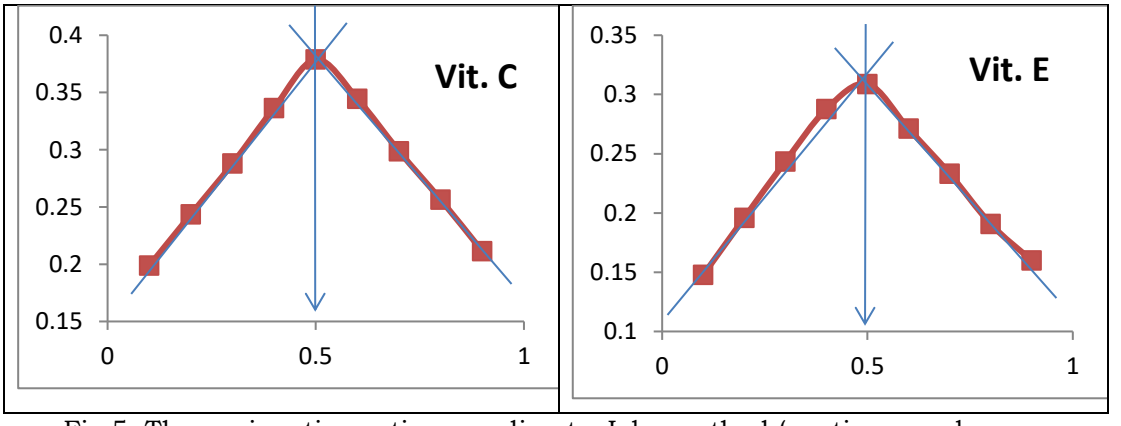
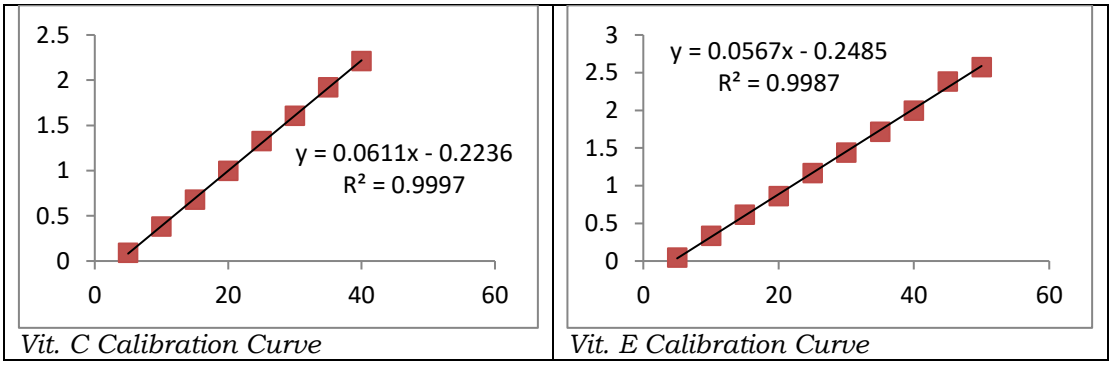


Fig.5. The conjugation ratio according to Jobe method (continuous changes method) between vitamins E and C with dye

The effect of interactions: The effect of many additives on the vitamin E and C complex was studied under the optimal conditions for its formation, by adding

these interfering substances in concentrations equal to the concentration of each vitamin after adding each vitamin directly, and then following the subsequent additions according to the extraction method mentioned previously. The absorbance of the organic layer is measured after extraction and compared with the absorbance of each vitamin. The results showed that the absorbance values of the complex did not change after adding the interfering materials, and this means that the additives have no effect on the process of forming the complex.

Calibration curve: The calibration curve was prepared for each vitamin at optimal conditions that were previously studied. The Beer-Lambert Law was obeyed with range of concentrations 5-50 µg.ml-1 for vitamin E and 5-40 µg.ml-1 for vitamin C, and the value of the correlation coefficient R^2 are 9987 and 9997, and the value of the molar absorption coefficient Ɛ: 24421.25 and 10760.93, Sandel's sensitivity was 0.0176 µg.cm-2 and 0.0164 µg.cm-2 for vitamins E and C, respectively, while the detection limit was 0.0008 and 0.0009, and the quantitative limit was 0.0026 and 0.0030 for vitamins E and C, respectively. This indicates an excellent linear relationship between absorbance and concentration, as shown in Fig 6.



Vit. C Calibration Curve *Vit. E Calibration Curve*

Fig 6. The calibration curve of each vitamins

The accuracy and precision: From the results obtained, it was found that the proposed method has good accuracy and precision. The accuracy advantage was achieved through the relative return, which ranged between 97.865% - 103.774% for vitamin E, and between 98.286% - 102.716% for vitamin C. The precision of the method was achieved through the optimal value of the relative standard deviation, which ranged between 0.00615 - 0.15472 for vitamin E, and between 0.0052 - 0.0783 for vitamin C, as these results confirm the possibility of applying the proposed method analytically.

The standard addition method: Vitamins E and C were estimated in the solution of their pharmaceutical preparations by the standard additive method at optimal conditions that were previously studied. The results indicate the efficiency of the used method, as the Rec% values of the vitamin E complex were 96.32% and the vitamin C complex 96.4%, while The value of ratio standard deviation RSD% ranged between 0.0069-0.0155 and 0.0024-0.0503 for vitamin E and C complexes, respectively.

Applications

The suggested method was applied by performing five repetitions for each measurement of the complex of each vitamin, taking into account all the optimal conditions that were previously studied. Where they were conducted for two different concentrations of each drug were chosen to fall within the concentrations of the titration curves, and these concentrations are 20, 30 µg.ml-1 for each vitamin, as the ratio of recall was 101.58% and 99.12% for vitamin E, and 103.69% and 102.13% for vitamin C, either The ratio standard deviation was 0.0049 and 0.0076 for vitamin E, and 0.0050 and 0.0157 for vitamin C

Conclusion

From all the above results we can conclude that the suggested method is a simple, fast, accurate, safe, inexpensive, reproducible and low environmental impact method, so may be used to determine the concentration of vitamin E and C in its complexes.

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