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DETERMINATION OF α–TOCOPHEROL (VITAMIN E) IN OILS BY SPECTROPHOTOMETRIC METHOD

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Abstract. The Emmerie Engel's method is used for the determination of α tocopherol (Vitamin E) in cottonseed oil, coconut oil, palm oil and wheat germ oil. The method has been found to be quite accurate, reproducible and less time consuming as compared to other analytical methods. Standard graph has been drawn. The results have been discussed

There are two major classes of methods available to the analyst for the estimation of α-tocopherol namely biological and chemical. Biological mehtods[1] have the advantage of supplying an accurate estimate of the biopotency of the sample without elaborate separation of the individual tocopherols. On the other hand they suffer the disadvantage of requiring longer periods of time for completion and are susceptible to more variables than do the chemical methods. There are a large number of chemical methods[2] available. A relatively rapid procedure has been described for the determination[3] of alpha tocopherol in pharmaceutical products, animal feeds, plant tissues, animal tissues and in blood plasma by Engel[4] spectrophotometrically. This method is basically the reduction of ferric to ferrous by the tocopherols with the formation of a red complex of the Fe^{++} with α–α′ dipyridyl. It suffers the disadvantage of being interferred with by any oxidizing or reducing material present. Despite this it is still the most widely used method for the determination of tocopherols. In the present studies the Emmrie Engel's method[4] has been used for the determination of α-tocopherol in oils.

Experimental

Equipment

(i) Beckman D.B. spectrophotometer. (ii) Amber coloured conical bottles of 50 ml capacity fitted with ground glass stoppers preferably, these bottles should be painted black on the outside.

Reagents

(i) *Absolute Ethyl Alcohol.* Purified by the Lund Bjerrum method[5] with magnesium metal and iodine. The alcohol is distilled off directly into the vessel to be stored. The purity of the alcohol exceeds 99.95%, provided adequate precautions are taken to protect the distillate from atmospheric moisture.

(ii) *Petroleum Ether*[6] *(40-60)°*. Shake one litre with 100 ml of concentrated H_2SO_4 for 15 min on a mechanical shaker. Allow the phases to separate and decant the petroleum ether into a distilling flask containing potassium hydroxide pellets and zinc granules. Redistil (40-60°) discarding first and last 50 ml.

(iii) *Ferric Chloride Solution.* 0.2% in absolute ethyl

(iv) *α–α′ –Dipyridyl Solution.* 0.5% in absolute ethyl alcohol.

Standard Stock Solution of α-Tocopherol (S).

Accurately weighed 101 mg of α-tocopherol-acetate (100 mg of α-tocopherol) dilute this with 50 ml absolute ethyl alcohol. This solution contains 2.0 mg of α-tocopherol per ml.

Procedure[7]

Blank. Into a reaction bottle palce 3 ml petroleum ether, 2ml absolute ethyl alcohol; 1 ml α–α′–dipyridyl solution and 1 ml ferric chloride solution. Swirl and allow to stand exact 10 minutes from the time the ferric chloride solution was added. Decant into a quartz cell (1 cm). Set the transmission to 100% in the spectrophotometer.

Standard. Add to a reaction bottle 3 ml petroleum ether, 1.5 ml α–α′–dipyridyl solution and 1 ml ferric chloride solution. Swirl and allow to stand exactly 10 min from the time the ferric chloride solution was added and decant into spectrophotometer cell, and read the absorbance at 580 mμ. Similarly take the absorbance of the standard solution by taking 0.25 ml, 0.5 ml, 0.75 ml, 1.0 ml, 1.25 ml and 1.5 ml at a time. Now plot the concentration in mg against absorbances at 580 mμ. This is the standard graph. Typical data for various concentrations of α-tocopherol and their absorbances at 580 mμ is given in Table 1. The data has been plotted and is given in Fig. 1 which shows a Linear Relationship.

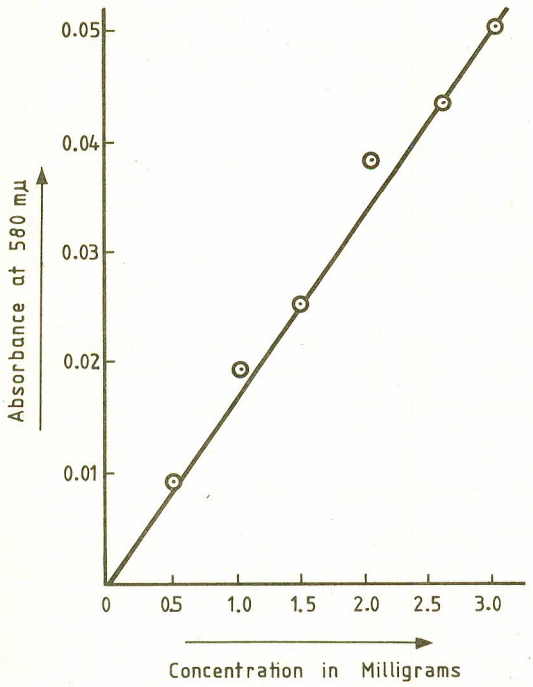

Fig. 1. Plot of absorbance at various concentrations of α-tocopherol.

Sample. Place a sample equivalent to approximately 1 mg of α-tocopherol in a 250 ml boiling flask equipped with a reflux condenser. Add 100 ml ethyl alcohol, attach the condenser and allow to boil vigrously for 10 min. Add 1 g KOH pellets through the condenser, followed by 100 ml of distilled water through the condenser. Remove flask and add 25 ml petroleum ether cool and transfer to a separatory funnel. Allow the phases to separate and withdraw the aqueous phase into another separatory funnel. Repeat the extraction with 2 x 25 ml of purified petroleum ether. Wash the combined extract with 2 x 50 ml of 1% KOH in 50% ethyl alcohol. Then wash the combined extracts with 2 x 100 ml of distilled water. Pass the extract through anhydrous sodium sulphate in a small funnel into a 50 ml volumetric flask. Rinse the separatory funnels, filter, sodium sulphate with sufficient peroleum ether to make volume. After saponification the unsaponifianle part is subjected to column chromatography on $MgHPO_4$. This method[8] will separate tocopherol mixture into three fractions : *alpha, beta plus gama,* and *delta* tocopherols. Carotene and vitamin A in small quantities will not interfere. Chromatorgaphy is carried on in a chromatographic tube allowing a column width of 12 mm and a height of 20 cm. An aliquot of the petroleum ether extract from the appropriate previous saponification step is added in the column. This system is closed and apply nitrogen pressure. Allow the solid to pack. Open the system and repeat until a column height of about 20 cm is obtained. Top the colums with 1 cm of anhydrous sodium sulphate. Apply pressure until the meniscus is just above the top of the column. Add an aliquot of the petroleum ether extract from the appropriate previous saponification step. Apply pressure until the meniscus is just above the top

TABLE 1. ABSORBANCE OF α–TOCOPHEROL AT VARIOUS CONCENTRATIONS, VOLUME OF PETROLEUM ETHER, α–α–DIPYRIDYL AND FERRIC CHLORIDE BEING 3 ml, 1 ml AND 1 ml RESPECTIVELY FOR EACH RUN.

No.	Volume of standard in ml	Concentration of standard in mg	Volume of absolute ethyl alcohol in ml	Absorbance at 580 mμ
1.	0.25	0.5	1.75	.009
2.	0.50	1.0	1.50	.020
3.	0.75	1.5	1.25	.025
4.	1.0	2.0	1.0	.039
5.	1.25	2.5	0.75	.043
6.	1.50	3.0	0.50	.050

TABLE 2. COMPOSITION OF α–TOCOPHEROL IN VARIOUS OILS.

No.	Kind of oils	Volume of unsaponifiable portion of oil in petroleum ether in ml	Absorbance of one ml of sample solution at 580 mμ	Concentration of α-tocopherol in mg/100g	Lit. value of α-tocopherol in mg/100g	References
1.	Cotton seed	50	.015	50	69	9
2.	Coconut	50	.010	40	43	10
3.	Palm	50	.015	50	54	9
4.	Wheat germ	50	.018	60	67	9

of the solid. Add 75 ml of petroleum ether, apply pressure and pass the solvent through the column. This aliquot will contain the carotene and related compounds which is discarded. Now add 100 ml of 2% diethyl ether in petroleum ether and pass through the column. This fraction will contain the α-tocopherol. Boil the aliquot down to 50 ml under nitrogen and proceed with the spectrophotometric determination.

Discussion

In author's experience there is not any singly method that is suitable for the determination of α-tocopherol in all types of samples. The choice of specific method will be largely determined by the nature of the sample, the equipment available and the manner in which the results are to be expressed. The Emmerie Engel's method for the oils was selected due to the easy availability of the equipment and chemicals. Another advantage of this method is that it takes very short time to complete the reaction and also for the determination. There are two major disadvantages of this method that is it is slightly sensitive to light and heat. It controlled properly one can get reliable and reporducible results in a reasonable time.

References

1. H. Gottlieb, F.W. Quacken bush and H. Steen bock, J. Nutr., **25**, 433 (1943).
2. Meyer Freed, *Determination of alpha-Tocopherol Method of Vitamin Assay* (Inter Science Publishers, New York, 1966),p. 365.
3. G. Lambersten and O.R. Brackkan, Analyst., **84**, 706 (1959).
4. A. Emmerie, C. Engel, Rec. Trav. Chim., **57** 1351 (1938).
5. E. Vogel, *Purification of Organic Solvents Practical Organic Chemistry* (Longman Publishers, New York, 1956),p. 167.
6. Reference 2, p. 367.
7. Reference 2, p. 390.
8. F. Bro-Rasmussen and W. Hjarde, Acta. Chem. Scand., **11**, 44 (1957).
9. E.W. Eckey, *Vegetable Fats and Oils* (Reinhold Publishing Corporation 1954).
10. W. Lange, J.Am. Oil Chem.Soc., **27**, 414 (1950).