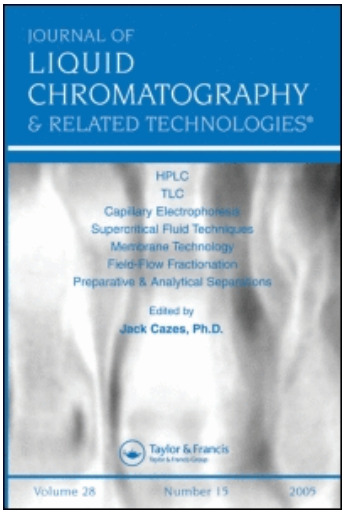

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High Performance Liquid Chromatography of Fat-Soluble Vitamins. II. Determination of Vitamin E in Pharmaceutical Preparations and Blood

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF FAT-SOLUBLE VITAMINS. II. DETERMINATION OF VITAMIN E IN PHARMACEUTICAL PREPARATIONS AND BLOOD

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ABSTRACT

∝ - tocopherol acetate (Vitamin E-acetate) is extracted, separated and determined from pharmaceutical preparations and from biological materials in nanogram range in less than 25 minutes. The extraction of Vit. E-acetate is performed in a fully automated, electronically controlled extraction apparatus. A reversed phase high-performance liquid chromatography (HPLC) using a column of lichrosorb RP18 and methanol as eleuent has been developed for the separation and quantitative determination of Vit. E-acetate in formulations. The described extraction and determination methods are suitable for the analysis of Vit. E-acetat alone and in the presence of other water - and

Fat-soluble Vitamins in pharmaceutical preparations and are reproducible with coefficient of variation of ~3%. Vitamin A-acetate can be used as internal standard.

INTRODUCTION

The efficiency of HPLC for separation and determination of numerous pharmaceutical compounds and especial Water- and Fat-soluble Vitamins is well established.[1-11] Vitamin E is classified as a fat-soluble Vitamin and is one of the important therapeutic interest compounds.[12] The use of the published methods for determination of vitamin E in pharmaceutical preparations especial in multivitamins formulations and in biological fluids, however, is often complicated by the large excess of pharmaceutical exipients and by the very low concentration particularly in biological materials. Therefore, in this paper simple, specific and sensitive methods are developed which allow the extraction, separation and determination of vitamin E-acetate in pharmaceutical preparations and in biological fluids in the nanogram range within 15-25 minutes.

Experimental :

Electronically Controlled Extraction Apparatus*

(*Produced by W. Krannich K. G., 3400 Gottingen, Ellihauser Weg 17, West Germany).

The apparatus is composed of the glass set for simultaneous Extraction of 3 samples as described in a previous report[2]. The qualification of this apparatus has been proven in the extraction of many active substance such as steroids and vitamins from pharmaceutical preparations[13,14, 1, 2].

High-performance Liquid Chromatography (HPLC) :

Reversed phase HPLC was carried out using a Knauer compact apparatus with a variable wave-length spectrophotometer detector and a syringe-loaded loop injections valve with an internal volume of 50 μl. A stainless steel column 100 x 4,6 mm i.d. packed with vertex lichrosorb RP18, 7 μm, and methanol as eluent were used. The spectrophotometer detector was used to measure the absorbance at 288 nm. The sensitivity of the detector was 0,04 AUFS. The flow rate was 2 ml.min^{-1} and the pressure 70-80 bar. The chromatograms were recorded on a knauer recorder with a 100 mv span set at a chart speed of 10 mm.min^{-1}. Vitamin A-acetat can be used as internal standard.

Materials and Reagents :

Methanol used for extraction and chromatography was obtained from E. Merck (Darmstadt, GFR). Standard substances of vitamins E-acetate and A-acetate were kindly supplied by Pfizer Pharmaceutical Co. (Cairo, Egypt). The following Pharmaceutical preparations were purchased locally.

1) Vitamin E capsules containing 5 mg vit-E-acetate per capsule.

2) Vitamin E dragees containing 100 mg vit. E-acetate per dragee.

3) Heparinized blood : 6 samples of 20 ml heparinized blood with 0,1 mg vitamin E-acetate per sample.

Preparations of Standards and Samples :

Standards substances (pure substances) : 75 mg of each of vitamin E-acetate and vitamin A-acetate as internal standard were weighed to 0.01 mg and dissolved in 100 ml methanol. 5 ml of this solution was pipetted accurately into 100 ml calibrated flask and diluted to volume with methanol. Volume of 5 ml of this solution was pipetted into 100 ml calibrated flask and diluted to volume with methanol. Consequently the concentration of this end solution is 7,5 $\mu g.ml^{-1}$.

Procedure for Pharmaceutical Preparations :

The contents of one capsule or one pulverised dragee was transferred accurately into the extraction apparatus and extracted twice with a total volume of 100 ml methanol.

A solution of vitamin A-acetate was added as internal standard and the solution was further diluted so that the final concentration of vit. E-acetate and vit. A-acetate about 6-10 $\mu g.ml^{-1}$.

Procedure for Heparinized blood :

The blood samples were centrifuged for 5 min at 1260g. The plasma decanted from the coagulum and the coagulum again centrifuged for 5 min at 1260g after mixing with 5 ml 0,9% aqueous sodium chloride solution. After decanting, the supernatant was transfered accurately into the extraction apparatus and was treated with 100 ml acetone-methanol (1:1). The deproteinized plasma was filtered and after transfer in a new inter-

changeable filter funnel in the extraction apparatus the organic solvent mixture was drawn off in vacuum at room temperature. After addition of 0,15 mg vitamin A-acetate as internal standard the two vitamins were extracted from the remaining deproteinized plasma 3 times with a total volume of 100 ml chloroform - methanol (1:1).

Calculation :

The percentage recovery of vitamin E-acetate in pharmaceutical preparations can be calculated by using either the calibration method or the external standard method as described in the first part of this series[1] or by the internal standard method according to the following equation :

$$\text{Recovery of vit. E-acetate}(\%) = \frac{Ps/PI \ \ (\text{Sample})}{Ps/PI \ \ (\text{Standard})} X \ 100$$

Where Ps is the peak area for the vitamin E-acetate and PI is the peak area for internal standard (Vit. A-acetate).

Results :-

Figures (1) and (2) show the HPLC separation pattern of Vitamin E-acetate. Figure (3) shows the calibration lines of Vitamin E-acetate and Vitamin A-acetate. Table (1) gives the reproducibility of HPLC determination of vitamin E-acetate. The standard deviation and the coefficient of variation based on six determination of Vit. E-acetate from standard solution is summarized in this table. Table (2) presents the results of HPLC determination of vitamin E-acetate in pharmaceutical preparations and in heparinized blood. Table (3) gives the retention times and the capacity factor K' of the vitamins E-acetate and A-acetate.

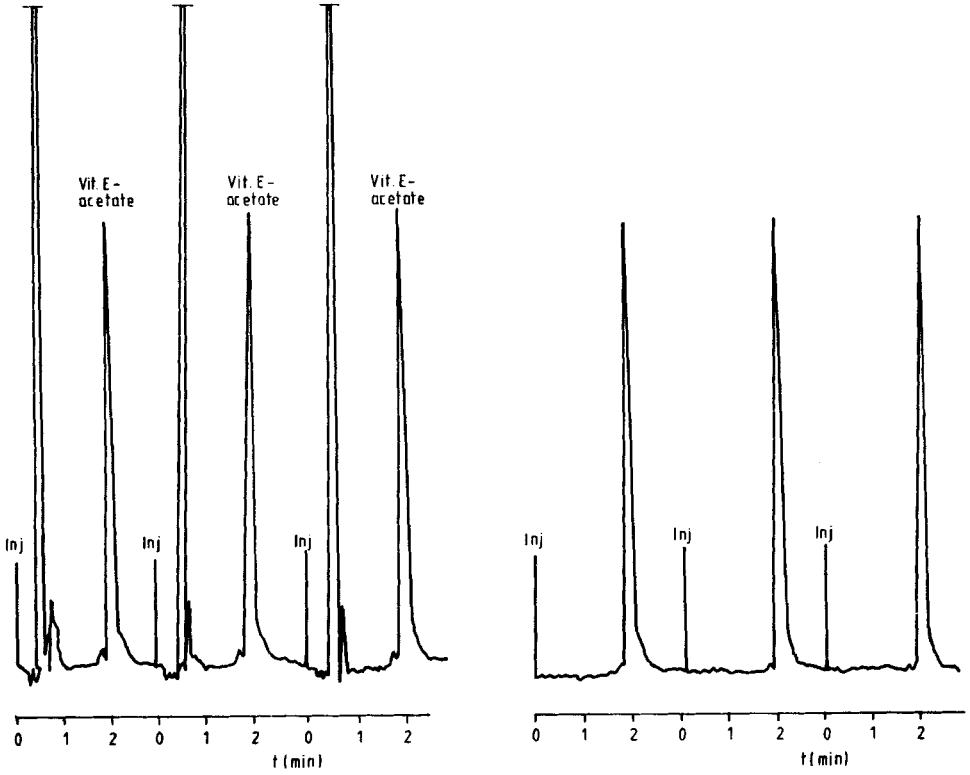

Fig. (1) : Separation of Vitamine E-acetate from Prep.1 Left side and from stander solution right side.
Amount injected = 37,5 ng.
Conditions : Lichrosorb RP18, 7 um, 100 x 4,6 mm i.d., Eluent : Methanol; Flow rate = 2 ml. min^{-1};pressure= 75 bar; detector sensitivity = 0,04 AUFS; detector wave length= 288 nm.

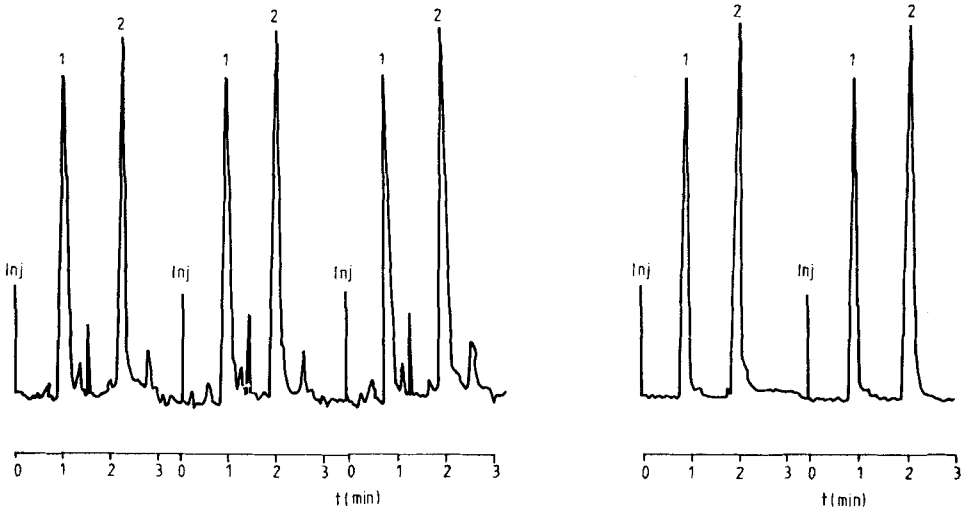

Fig. (2) : Separation of vitamin E-acetate from heparinized blood Left Side and from standard right side 1 vit. A-acetate (internal standard) 2 = Vit. E-acetate. Amount injected = 37,5 ng vit. A-acetate; 25 ng vit. E-acetate Conditions : as in Fig. (1) detector wavelength for vit. A-acetate = 320 nm.

TABLE (1): Repreoducibility of HPLC determination of Vitamin E-acetate carried out with pure substance (The results given are the means of 6 determinations).

	Vitamin E-acetate
Amount injected (ng)	75
Arithmetic mean (peak area cm^2) $\bar{X}$	0,68
Standard deviation of single value (cm^2) SD	0,021
Coefficient of variation (%) VK	3,1

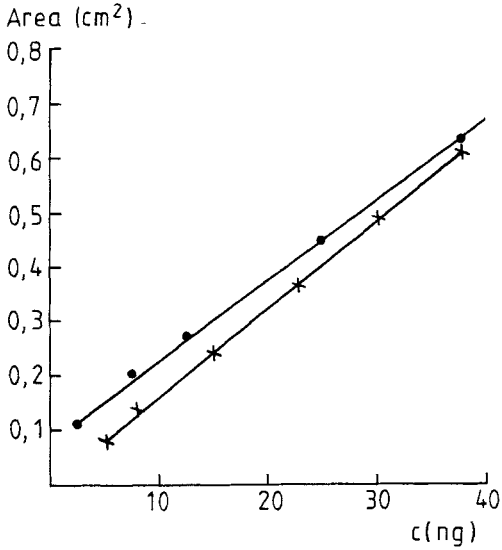

Fig. (3) : HPLC calibration line of vitamine E-acetate x-x-x and vitamine A-acetate -.-.-. Amount injected 5-37,5 ng Vit. E-acetate and 2,5 - 37.5 ng Vit. A-acetate. detector sensitivity = 0,04 AUFS.

TABLE (2) : Quantitive HPLC of Vitamin E-acetate in the Pharmaceutical Preparations. The results given are the means of 6 determinations.

Preparation	Vit.E-acetate Presents in Preparation	Amount injected (ng)	Amount Vit. E-acetate found		
			$\bar{X}$ (ng)	SD (ng)	VK (%)
1.	30mg/caps.	30	30,85	1,01	3,3
2.	100mg/Drag.	50	51,10	1,35	2,7
Heparinized blood	0,1mg/20ml.	25	24,15	0,71	2,9

TABLE (3) : Retention times of Vitamins E-acetate and A-acetate Separated on Lichrosorb RP18, 7 Λm, 100 X 4,6 mm i.d., Eluent = Methanol, Flow rate = 2 ml.min^{-1} and Pressure 75 Bar.

Vitamins	Retention time (min)	Capacity factor K'
E-acetate	2.0 - 2.1	12.7
A-acetate	0.9 - 1.0	6.3

$K' = \frac{t_R - t_o}{t_o}$; t_R = retention time of the vitamins

t_o = retention time of the eluent (unretained peak).

DISCUSSION

Table (1) indicates that the described HPLC is well reproducible and can be used for the determination of vitamin E-acetate with a coefficient of variation of $\sim$ 3%. The peak areas of 37,5 ng vitamin E-acetate separated by HPLC at a detector sensitivity of 0.04 AUFS as is shown in Fig. (1) indicates that by running at an 4 x higher sensitivity (e.g. at 0.01 AUFS) amounts of 1-2 ng of vitamin E-acetate could be determined. The determination of 5 ng at sensitivity of 0.04 AUFS is still possible as is present in Fig. (3).

Table (2) presents the advantages of the combination of the extraction apparatus and the HPLC method in respect to the analysis of vitamin E-acetate in pharmaceutical preparations and in biological materials.

The extraction apparatus allows the simulataneous extraction of the vitamins E-acetate and A-acetate (internal standard) from three pharmaceutical samples in 5 min and from three biological samples in about 15 min. The described HPLC method is rapid, accurate, sensitive and enables the determination of 1-2 ng of vitamin E-acetate in less than 5 min. The described methods are furthermore suitable for the simultaneous extraction and determination of vitamins E-acetate and A-acetate in multi-vitamins preparations and in biological materials and can be used in the pharmacokinetic and bioavailability studies of Vitamin E.

Fig. (3) shows that a linearity between the concentration and peak areas is available in the range of 5-37,5 ng. In comparison with the existing quantitative HPLC determination of vitamin E-acetate in pharmaceutical preparations and biological material the described extraction and separation methods have advantages with respect to extraction time, separation time, sensitivity and simplicity[3,4].

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