

## A Simple and Rapid HPLC Method for the Simultaneous Determination of Vitamin-A and Vitamin-E in Tablet Dosage Forms

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### ABSTRACT

A simple and precise high performance liquid chromatographic method has been developed and validated for the simultaneous determination of vitamin A (vit.A) and vitamin E (vit.E) in bulk and pharmaceutical dosage forms. Chromatography was carried out at ambient temperature on a 4.6mm×150mm, 5µm Altech prevail-C18 column with mobile phase acetonitrile and methanol (75:25) at a flow rate of 1.0 ml/min. The UV detection was carried out at 220 nm. The retention times were 3.41 min. and 9.07 min. for vit.A and vit.E respectively. Vit.A and vit.E were separated with good resolution (3.54) and minimal tailing (1.25 and 1.28 respectively), without interference of excipients. The method was validated according to ICH guidelines and the acceptance criteria for accuracy, precision, linearity, specificity and system suitability were met in all cases. The method was linear in the range of 1–200µg/ml for vit.A and 1-500 µg/ml for vit.E.

**Key words:** Vitamin A, Vitamin E, Validation, HPLC

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### INTRODUCTION

Vitamin A (vit.A), 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexan-1-yl)2,4,6,8)-natetraenol, is an essential human nutrient. Vit.A has a beta-ionone ring to which an isoprenoid chain is attached, essential for vitamin activity. Retinol, the animal form of vit.A is a yellow fat soluble vitamin with importance in vision, regulation of gene expression, immunity and growth and development. Vitamin A is the collective name for a number of substances of structure related to all-trans-retinol and having similar biological activity (Fig. 1). In tablet dosage forms, the dietary ingredient vitamin A is generally used in the form of retinyl esters mostly as all-trans-retinyl acetate and all-trans-retinyl palmitate containing minor amounts of 13-cis and 9-cis isomers, incorporated in solid carriers or excipients. The activity of vitamin A is expressed in retinol equivalents (RE) or in International Units (IU). Initially, the activity of vitamin A in International Units (IU) was based on bioassays and international standard materials that are no longer available,[1,2] therefore The United States Pharmacopeia (USP) discontinued the use of IU for potency of vitamin A.

Vitamin E (vit.E), 2,5,7,8-tetramethyl-2-[(2R,8R) 4,8,12-trimethyl tridecyl] 3,4-dihydro-2H-chromene-6-ol, encompasses eight molecules composed by a chromanol ring and a phytol side chain displaying identical functions: four tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ) and four tocotrienols ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ). Tocopherols have saturated side chain.  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  prefixes indicate position of methyl groups on chromanol ring [3].  $\alpha$ -tocopherol is the most abundant in nature (Fig. 2) [4]. One  $\alpha$ -tocopherol molecule can trap two peroxy radicals responsible of lipid oxidation initiation [5]. Hence, this molecule protects membrane lipids against oxidation [6]. Vitamin E has also a positive effect on fertility [7]. Vitamin E quinone possesses anti-clotting activity through the inhibition of vitamin K dependant carboxylase which regulates blood clotting [8].  $\alpha$ -Tocopherol can use SR-B1 to enter in enterocyte [9]. Vitamin E RDI is 15 mg/day and deficiency might occur in case of fat malabsorption or for premature infants. It is usually characterized by neurological problems due to poor nerve conduction, which are reversible by supplementation.

Literature reveals that colorimetric method [10], HPLC methods in pharmaceutical formulations,[12-15] in animal products [16-18] and biological fluids [19-28] are been reported. However, the present

reported method describes simple and rapid LC method for simultaneous determination of vit.A and vit.E. The aim of the present study was to develop a simple, specific, accurate and precise isocratic HPLC method for the simultaneous determination of vit.A and vit.E in bulk and pharmaceutical dosage forms.

## MATERIALS AND METHODS

### Chemicals and Reagents:

Vit.A and vit.E were obtained from Sigma Aldrich, Mumbai (India). Methanol and acetonitrile (HPLC grade) were obtained from Fisher scientific India Pvt. Ltd. Mumbai (India). All the chemicals and reagents were of analytical or reagent grade.

### HPLC instrumentation and conditions:

Integrated HPLC system Waters separation module 2695 consisted of auto injector and Waters 2696 PDA detector with mobile phase acetonitrile and methanol (75:25) and 4.6mm×150mm, 5µm Altech prevail-C18 column was used as stationary phase. The flow rate was 1.0 ml/min and the detector was set at 220 nm. All analyses were made at ambient temperature and the volume of solution injected was 10µl. Chromatograms were recorded and integrated on PC installed with Empower software.

### Standard and Sample Preparation:

Vit.A and vit.E standard stock solution was prepared by transferring accurately about 2.5mg of vit.A and 25mg of vit.E reference standards to a 100ml volumetric flask. To this 70ml of mobile phase was added and sonicated for 15 minutes to solubilize vit.A and vit.E. The solution was diluted to volume with the mobile phase to give final concentration of 25ppm and 250ppm for vit.A and vit.E respectively.

The sample solution was prepared by taking tablets (label claim 2.5mg and 25mg each of vit.A and vit.E respectively). The twenty tablets were powdered and powder equivalent to average weight of twenty tablets was taken in 100ml volumetric flask and to this 70ml of mobile phase was added and sonicated for 15 minutes to solubilize it. The solution was diluted to volume with the mobile phase and filtered through 0.45µm membrane filter and further analyzed by using above mention HPLC conditions.

The amount of vit.A and vit.E per tablet was calculated from the peak areas of vit.A and vit.E in the chromatograms of the test solution and standard solution, respectively.

### Procedure for calibration curve:

The contents of the mobile phase were filtered before use through 0.45µm filter paper, and pumped from the respective solvent reservoirs to the column at a specified flow rate Prior to injection of the drug solutions, the column was equilibrated for at least 30 min with the mobile phase flowing through the systems Then, 20µl of each of standard and sample solutions were injected into the HPLC system for six times to get the chromatograms. The retention time and average peak areas of each drug were recorded. Calibration curve was plotted by taking concentration on X-axis and peak areas on Y-axis.

## RESULT AND DISCUSSION

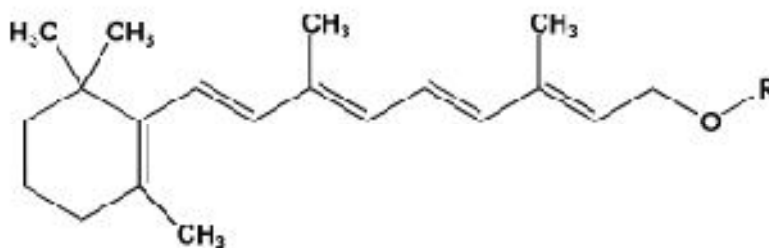
### Optimization of the Method

To develop a suitable and robust HPLC method for the determination of vit.A and vit.E, different mobile phases, methanol: water, acetonitrile: water, acetonitrile: methanol were used in different composition of the mobile phases (30:70, 40:60, 50:50, 60:40, 80:20) at different flow rates (1.0, 1.2, 1.5, 1.8 ml/min). The mobile phase acetonitrile: methanol in the ratio of 75:25 at a flow rate of 1.0 ml/min gave sharp peaks with minimum tailing and good resolution. Vit.A and vit.E were eluted at retention time around 3.42 and 9.07 respectively with symmetric peak shape. Altech prevail-C18 4.6mm×150mm, 5µm, column at a detection wavelength of 220nm was used for chromatographic detection.

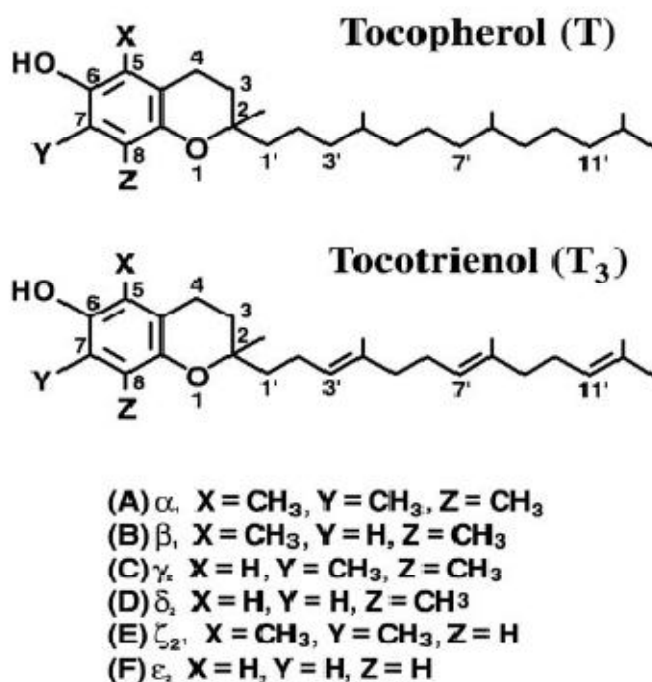
**Table No. 1 - System suitability and validation parameters**

Parameters	Vit.A	Vit.E
Tailing factor	1.25	1.28
Resolution (R)	3.5	--
Theoretical plates	4652	5643
Precision (n = 6) % R.S.D	0.14	0.04
Accuracy Mean recovery (%)	98.98	100.02

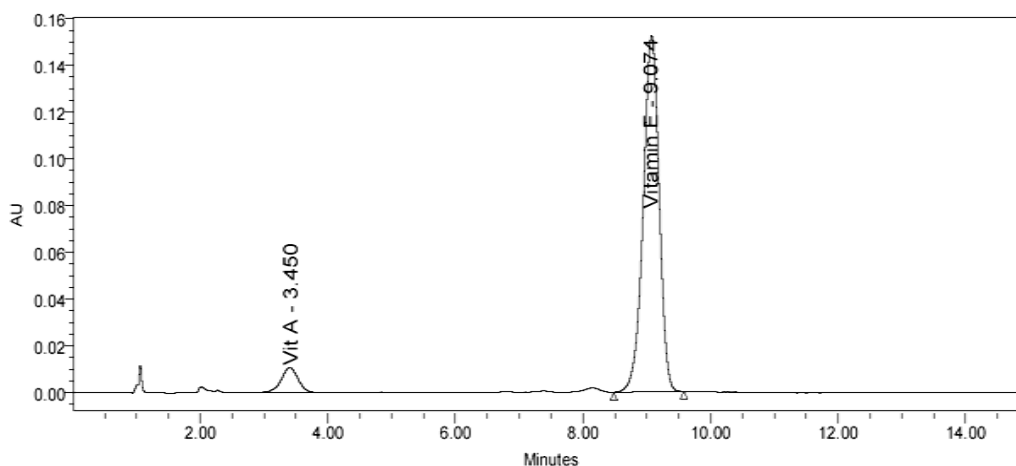
R.S.D. = relative standard deviation.



**Figure No.1:** Structure of vitamin A: R=H, all-trans-retinol; R=CO-CH<sub>3</sub>, all-trans-retinyl acetate; R=CO-C<sub>15</sub>H<sub>31</sub>, all-trans-retinyl palmitate. 3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexan-1-yl) 2,4,6,8)-natetraenal-3-dehydroretinol



**Figure No. 2:** Structure of vitamin E 2,5,7,8-tetramethyl-2-[(2R,8R) 4,8,12-trimethyl tridecyl] 3,4-dihydro-2H-chromene-6-ol



**Figure No. 3:** HPLC chromatogram for vit.A and vit.E by the proposed method

### Method validation

The method was validated according to the ICH guidelines[29]. The described method has been validated for linearity, precision, accuracy, specificity, LOD and LOQ, system suitability parameters, ruggedness and robustness

#### Linearity

Vit.A and vit.E showed linear calibration curves in the range of 1–200µg/ml and 1-500 µg/ml respectively. Regression equation for vit.A was,  $y = 11847x + 7842.2$  ( $R^2 = 0.9998$ ) and for vit.E,  $y = 138261x + 50496$  ( $R^2 = 1$ ).

#### Accuracy

This experiment was performed at three levels, in which sample stock solutions were spiked with standard drug solution containing 80, 100 and 120% of labeled amount of both vit.A and vit.E (2.5mg vit.A and 25mg vit.E) in tablets. Three replicate samples of each concentration level were prepared and the % recovery at each level ( $n = 3$ ), and mean % recovery ( $n=9$ ) were determined (Table 1). The mean recovery was 98.98 and 100.02 % for vit.A and vit.E respectively.

#### Precision

Instrumental precision was determined by six replicate determinations of standard solution and the relative standard deviations were 0.14% for vit.A and 0.04% for vit.E.

Method precision or intra-assay precision was performed by preparing six different samples involving different weightings. Each solution was injected in triplicate under the same conditions and the mean values of area under curve for each solution were taken. The R.S.D. values were 0.46% for vit.A and 0.98% for vit.E

#### Robustness

Robustness of the proposed method was estimated by changing: (i) mobile phase composition from acetonitrile: methanol (70:30) to acetonitrile: methanol (80:20); (ii) changing the flow rate from 1.0 ml to 0.8 and 1.2ml/min. System suitability parameters in Table 1 were found to be within acceptable limits.

#### System suitability parameters:

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The system suitability parameters like theoretical plates (N), resolution (R), and tailing factor (T) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of vit.A and vit.E in pharmaceutical formulations was validated or not. System suitability is usually developed after method development and validation has been completed. The obtained value of theoretical plates (N) in this method was 4652 for vit.A and 5643 for vit.E. The resolution of vit.A and vit.E was found to be 3.54 and the tailing factors were 1.25 and 1.28 for vit.A and vit.E respectively.

### CONCLUSION

The proposed HPLC method is simple, rapid, specific, accurate and precise for simultaneous determination of vit.A and vit.E in pure and its pharmaceutical dosage forms. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested non-interference of formulation excipients in the estimation. A representative chromatogram is shown in fig-1. The proposed method can thus be used for routine analysis of vit.A and vit.E in combined dosage forms and can also be used for dissolution or similar studies.

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