Int J Pharm Bio Sci 2012 July; 3(3): (P) 322 - 327

Research Article **Pharmaceutics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

A VALIDATED RP – HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF VITAMIN A AND VITAMIN E IN MULTIVITAMIN TABLET DOSAGE FORM

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ABSTRACT

A simple, selective, rapid, precise and accurate reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of vitamin A and Vitamin E in multivitamin tablet dosage form. The method was carried out on waters 2695 separation module & waters 2996 PDA detectors with Xterra C 18 column (150 X 4.6 mm) using Acetonitrile : methanol and water pumped at a flow rate of 1ml/min In the ratio of 85 : 15 : 5 as mobile phase at 30^{o}C. The detection carried out at 280 nm. The intra and inter day precision was found to be less than 2% showing high precision of the assay method. The % recovery of the method was greater than 98% and RSD did not exceed 2% indicating high degree of accuracy of the proposed HPLC method. The %RSD for the robustness testing was also less than 2%. The proposed HPLC method can be used for the estimation of Vitamin A and Vitamin E in combined multivitamin tablet dosage forms.

KEYWORDS : Vitamin A, Vitamin E, RP – HPLC,

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INTRODUCTION

Vitamins are an extremely diverse range of organic compounds present in minute amounts in natural foodstuffs. They are vital in the enzyme reactions that are necessary for carbohydrate, fat, and protein metabolism. Vitamins are relatively unstable, affected by factors such as heat, light, air, other food components, and food processing conditions [1, 2]. If this intake is insufficient or if special dietary requirements exist, multivitamin preparation should be taken in order to prevent vitamin deficiency. Numerous such preparations, often formulated as film coated dragee or effervescent, are available in the market. Requirement on sufficient input of vitamins and hence, keeping health of individual, results in a need for accurate quantitative measurements of vitamins in food. Likewise the content of vitamins in pharmaceutical preparations needs to be checked in order to ensure correct intake and the accuracy of the label statements. Loss of vitamins in pharmaceutical preparations can be related to the specific formulation, technology of manufacturing, and storage. Because of their critical role in nutrition and their relative instability, qualitative and quantitative analyses are important issues as well as a challenging task for food manufacturers. HPLC is preferred for vitamin separation because of its high selectivity [3]. Recent studies show various applications in determining vitamins in different sample sources [4-6].

Nowadays, there is a growing need for more rapid and specific methods for vitamin analysis. Individual vitamins can be chromatographed isocratic ally, as well as certain combinations of two or more vitamins; the simultaneous chromatography of more complicated mixtures may require a gradient elution. Determination can be carried out by normal-phase, ion exchange or ion-pairing chromatography, RP chromatography being the most common method. In contemporary time HPLC, connected with different detection techniques, is the leading analytical method for the quantification of vitamins as well as for most of other analytes, thanks to the possibility of rapid separation and quantification. A use of some newly developed RP stationary phases allows separation of polar vitamins without the necessity of ion-pair reagent addition. For example, a use of a stationary phase for basic compounds involving a ligand with amide groups (RP-C18) provides good separation for simultaneous determination of the B-group vitamins [7, 8] Sample preparation and pre-run sample stabilization are the most important steps to ensure that subsequent HPLC analysis is effective. Sample preparation has to be carefully optimized especially for vitamins subject to degradation due to light, oxidizing reagents, pH, heat, and others. Especially for vitamins executing function of antioxidants, which makes them unstable by their nature, it is necessary to use suitable methods to acquire accurate results. Considering sample matrix and evaluated vitamins, appropriate type of extraction (direct extraction, LLE, SPE, SFE, etc.) should be chosen[9]. Fat-soluble vitamins are very hydrophobic and must be dissolved in organic solvents. It is important to optimize such parameters as solvent used, sample/solvent ratio, particle size of sample (pulverization of sample), time of extraction, and temperature for sample preparation and if necessary to assure protection from light and O2.The object of the study was to develop a simple, precise, rapid and accurate reverse phase HPLC method for the determination of Vitamin A and Vitamin E in combined Pharmaceutical dosage forms.

MATERIALS AND METHODS

Reagents and chemicals

Vitamin A & Vitamin E (Arvind Remedies, Thiruvallur) Acetonitrile & methanol- HPLC grade, Water- Double distilled was used in this study.

Instruments used

A HPLC (waters alliance 2695 separation module with waters 2996 Photo diode array detector) equipped with waters empower software for data processing and RP- C 18 column (150 X 4.6 mm, 5 µm particle size) was used.

Preparation of mixed standard solution

Solution A

Weigh 50 mg of Vitamin A acetate powder transfer into a 50 ml standard flask. Add 2 ml of 50% KOH solution. Shake for 2 minutes and make up the volume with methanol and filter

Solution B

Weigh 62.5 mg equivalent to Vitamin E acetate powder. Transfer into a 50 ml volumetric flask. Add methanol and sonicate for 2mins then make up the volume with methanol and filter.

5 ml of solution A and 5 ml of solution B was pipetted out into a 25 ml volumetric flask and made up to the volume with methanol.

Preparation of sample solution

Weigh 20 tablets and crush. Weigh accurately the powder equivalent to 50 mg of Vitamin A acetate in a 50 ml volumetric flask. Add 2ml of 50% KOH solution and shake for 5 minutes. Make up the volume with methanol. Filter and dilute 5 ml to 25 ml with methanol.

Chromatographic conditions

The optimum composition of mobile phase consisting of Acetonitrile, methanol and water in the Ratio of 85: 10: 5. Filter through 0.45 µ filter and degassed by using sonicator for 10 minutes. The floe rate of the mobile phase was set to 1 ml/min and UV detection was carried out at 280 nm. All determinations were performed at constant column temperature (30o C). The retention time for Vitamin A and Vitamin E under the optimized chromatographic condition was found to be 2.928 and 9.267 minutes respectively.

RESULTS AND DISCUSSION

Assay of formulations *(16,17)*

Twenty tablets were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 50 mg of Vitamin A acetate, was transferred to a 100ml volumetric flask. Add 2ml of 50%KOH solution and shake for 5 mins. Make up the volume with methanol. This solution was filtered through a 0.22 µm membrane filter. A suitable aliquot of this solution was transferred to 25ml volumetric flask, volume made up with methanol and analysed under the optimized chromatographic conditions. The results are given in table no 1

Validation of HPLC method *(10-15)*

The proposed HPLC method was validated as per ICH guidelines. Linearity of data was evaluated by serially diluting the stock solutions of Vitamin A and Vitamin E in the range of 160 µg/ml to 240 µg/ml. Triplicate dilutions of each concentration were injected in to the HPLC in triplicate. The linearity of calibration graphs and adherence of the system to beer`s law was validated by high value of correlation coefficient.

The accuracy of the method was determined by performing recovery studies at 80, 100 and 120 % of the test concentrations. The percentage of recovery was calculated. The precision of the method was demonstrated by intraday and interday studies. To evaluate the robustness of the developed RP-HPLC method, small deliberate variations in the optimized method parameters like the effect of flow rate, mobile phase ratio, and wave length were studied. The results of validation and system suitability studies are given in table no : 2. Figure 1 shows the reversed-phase analysis of vitamin A acetate, and vitamin E acetate performed on a C18 (150*4.6mm) 5µm (xettra) column using methanol. In this analysis, a mixture of vitamin A acetate and vitamin E acetate was separated by reversed-phase HPLC using the xettra C18 column (Figure 2). All chromatograms exhibit excellent resolution

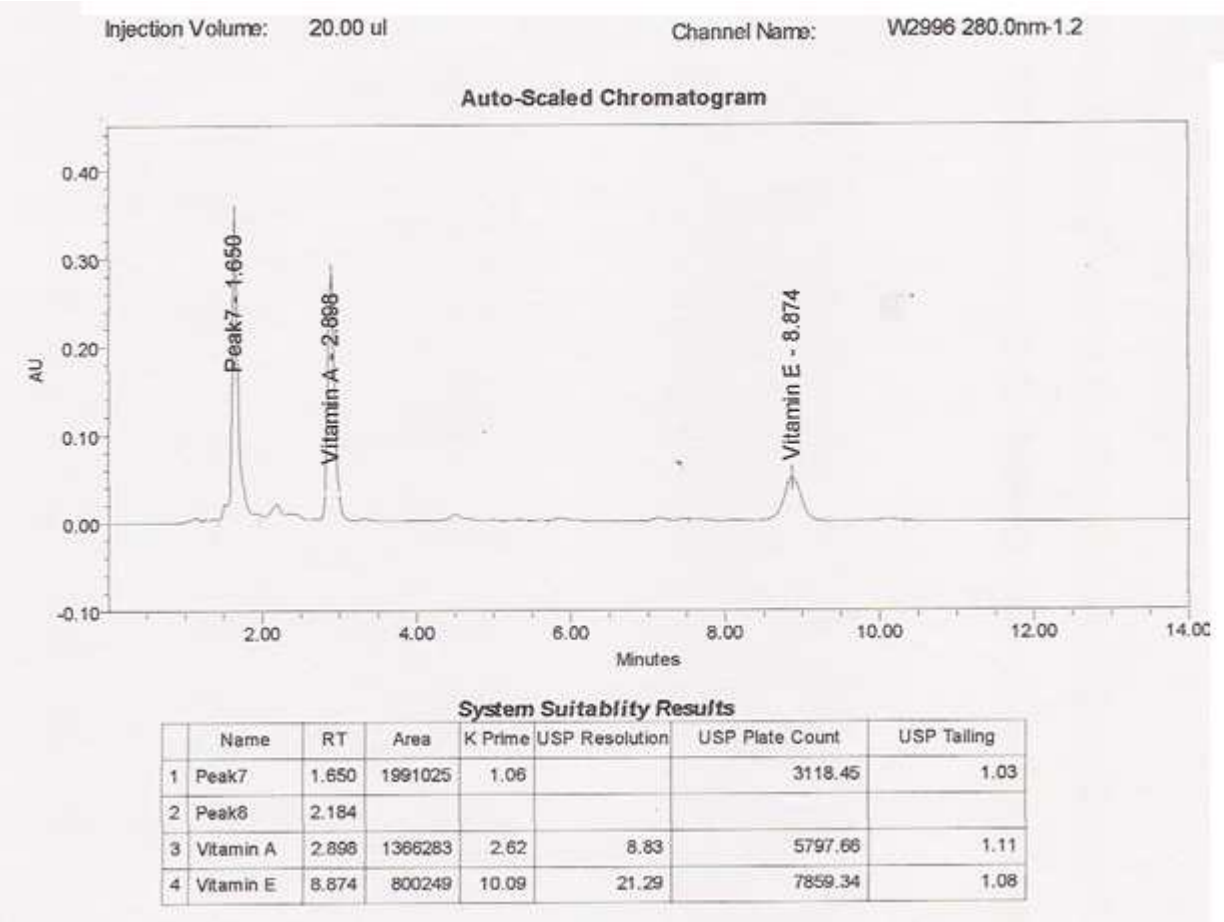
and peak shape. The fat-soluble vitamins in the real sample – Multivitamin chewable tablet – were separated on the xettra C18 column (Figure 3) and studied. In this, Vitamin E acetate and vitamin A acetate were clearly identified.

Table no 1
Assay of formulations

Components	Amount present (mg)	Amount found (%)	Standard deviation	% RSD	Mean recovery
Vitamin A	140	137.58	0.502	0.58	100.72
Vitamin E	110	109.53	0.8582	0.783	99.86

Table no : 2.
Validation of HPLC method

Parameter	Vitamin A	Vitamin E
Linearity range	160 – 240 µg/ml	160 – 240 µg/ml
Correlation coefficient (r)	0.9997	0.9991
Slope (m)	10262.27	11614.27
Intercept (c)	5985.661	5408.714
LOD (µg/ml)	1.441	4.42
LOQ (µg/ml)	4.368	13.4
Retention time	2.928	9.267
Resolution factor	6.72	20.65
Precision (%RSD)		
Interday	0.58	1.46
Intraday	0.17	1.27
Tailing factor	1.16	0.90
Mean % recovery	100.72	99.86



System Suitablity Results

	Name	RT	Area	K Prime	USP Resolution	USP Plate Count	USP Tailing
1	Peak7	1.650	1991025	1.06		3118.45	1.03
2	Peak8	2.184					
3	Vitamin A	2.898	1366283	2.62	8.83	5797.66	1.11
4	Vitamin E	8.874	800249	10.09	21.29	7859.34	1.08

Figure no 1

(a,b,c): Reversed-phase analysis of vitamin A acetate, and vitamin E acetate in multivitamin tablet formulation

CONCLUSION

The proposed method was found to be simple and linear in the concentration range of 160 – 240 µg/ml for Vitamin A and Vitamin E. The peak areas of the drug were reproducible as indicated by the low coefficient of variation. Also the %RSD for both the tablet analysis and recovery studies was less than 2% indicating high degree of precision and accuracy of the proposed method. The results of the robutness study also indicated that the method is robust and is unaffected by small variations in the chromatographic conditions. Hence, the developed RP-HPLC method is simple, accurate, precise and robust can be employed successfully for the routine estimation of Vitamin A and Vitamin E in multivitamin formulations.

ACKNOWLEDGEMENT

We thank M/s ARVIND REMEDIES LTD, Thiruvallur, Chennai, for providing all facilities to carry out this work.

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