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Research Article

DEVELOPMENT AND VALIDATION OF STABILITY INDICATING UPLC METHOD FOR QUANTIFICATION OF ANTI OXIDANT IN COMPLEX DOSAGE FORMS

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ABSTRACT

A simple, hi-sensitive, accurate, precise and specific UPLC method was developed for the identification and quantification of antioxidant from complex dosage forms. The chromatographic separation employs the isocratic elution by using C_{18} column by using the mobile phase containing mixture of Methanol, Acetonitrile, Tetrahydrofuran and Buffer, with the flow rate of 0.4 mL/min. The analyte was detected and quantified at the wavelength of 291 nm by using photo diode array detector. The method was validated as per ICH guidelines and found to be specific, precise, linear and accurate. The peak purity of analyte with the aid of photo diode array detector was satisfactory. Hence this method was concluded that stability indicating method.

Keywords: Antioxidant, Complex Molecules, Stability Indicating UPLC method.

INTRODUCTION

Alphatocopherol [1] is chemically designed as ((2R)-2,5,7,8-Tetramethyl-2-[(4R,8R)-(4,8,12-trimethyltridecyl)]-6-chromanol) baring the molecular weight of 430.7. Tocopherols are considered to be powerful antioxidants [2] and widely used in the manufacturing of complex molecules like microspheres and liposomes.

The literature survey reveals that, alphatocopherol was reported in Ph.Eur [1]. There are several methods were reported in different journals for estimating alphatocopherol with different methods [3,4]. But is no quantification method was available by liquid chromatography.

Regulatory agencies recommend the use of stability indicating methods [5] for the quantification of stability samples [6]. This requires stress studies in order to generate the potential related impurities under stressed conditions, method development and validation [7]. With the evident of the International Conference on Harmonization (ICH) guidelines [8], requirements for the establishment of stability indicating methods have become more clearly mandated. Environmental conditions including light, heat and the susceptibility of the drug product towards hydrolysis or oxidation can play an important role in the formation of potential impurities. Stress testing can help identifying degradation products and provide important information about intrinsic stability of the drug product.

Therefore, here we developed new stability indicating UPLC method to quantify the alphatocopherol present in the formulation. Chemical structure of alphatocopherol was shown in Figure-1.

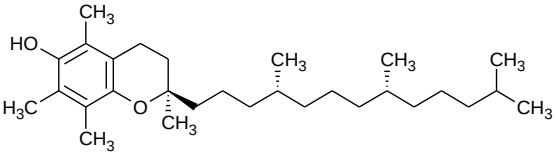

Fig. 1: Structure of Alphatocopherol

MATERIALS AND METHODS

Reagents and materials

Alphatocopherol active pharmaceutical ingredient kindly supplied by Dr.Reddy`s laboratories, Hyderabad. acetonitrile, methanol, tetrahydrofuran, and ammonium acetate were obtained from Merck Limited, Mumbai. High purity de-ionized water was obtained from Millipore, Milli-Q purification system.

Instrumentation

Acquity UPLC System equipped with auto sampler and binary gradient pump with in-built degasser used. It was connected with photo diode array detector and operated with Empower software.

Chromatographic Conditions

ACQUITY UPLC BEH C18, 1.7µm, 2.1 x 100mm UPLC column was used as stationary phase maintained at 25°C. The mobile phase involved a variable composition, mixture of methanol, acetonitrile, tetrahydrofuran and ammonium acetate buffer in the ratio of 68:23:3:6 *v/v/v/v* respectively. The mobile phase was pumped at the flow of 0.4 mL/min. The optimum wavelength selected was 291nm which represents the wavelength of maximum response analyte in order to permit the optimum determination.

Standard Solution:

Solution containing alphatocopherol (4 µg/mL) of working standard.

Sample Solution

Sample solution containing the alphatocopherol (4 µg/mL).

OPTIMIZATION OF METHOD

Selection of Stationary Phase

It is clearly confirming that the alphatocopherol is highly non polar in nature. Hence reverse phase chromatography was selected. Initially both C_8 and C_{18} stationary phases were tested with the particle size of 5µm and lower. But comparatively adequate separation, good peak shape and shorter runtime was attained with C_{18} stationary phase with 1.7 µm particle size. But the stationary phase is not only the parameter which can give better chromatography. Mobile phase, pH and organic modifies also plays very important role which leads to the best separation, peak shape and with good system suitability.

Selection of Mobile Phase

Initially development was started with potassium phosphate buffer, sodium phosphate buffer. Some of closely eluting peaks which are

coming from complex matrix of formulation were not separating completely and peaks were little bold. Then good separation was observed with ammonium acetate buffer. When mixture of buffer, methanol and acetonitrile was used as mobile phase, poor peak shape was observed and elution of peaks were delayed. But when tried with the combination of methanol, acetonitrile, tetrahydrofuran and buffer yields very sharp peak shape with lesser runtime.

Influence of Column Compartment Temperature

During the stress study of sample solution, one of the degradant impurity was formed and eluting very adjacent to the alphatocopherol peak. Extended study reveals that the impurity is very sensitive to the column temperature. At lower temperature that impurity got separated and passed peak purity. Hence column oven temperature was finalized as 25°C.

After extensive and repeated experimental studies, the method finalized with ACQUITY UPLC® BEH C18, 1.7µm, 2.1 x 100mm column using the mixture of methanol, acetonitrile, tetrahydrofuran and ammonium acetate buffer in the ratio of 68:23:3:6 *v/v/v/v* respectively as mobile phase. The mobile phase pumped through column at the flow rate of 0.4 mL/min. The column compartment maintained at the temperature of 25°C. Good detector response for detecting alphatocopherol found at 291 nm. The typical chromatogram standard and test (Fig. 3 and Fig. 4) represents the peak shape and separation of all peaks from each other.

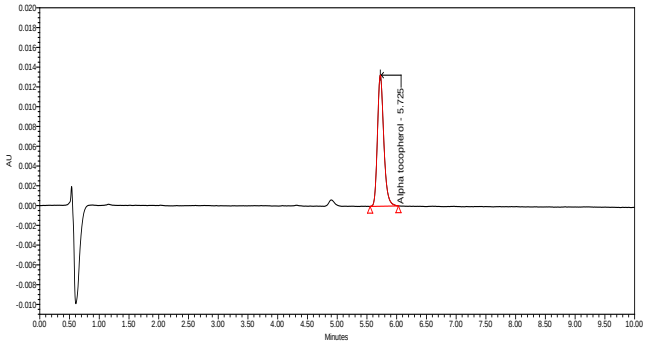

Fig. 3: Specimen Chromatogram of Standard

VALIDATION

The developed method was validated according to ICH guidelines [9], with respect to specificity, accuracy, precision (repeatability and intermediate precision), linearity, range and robustness. System suitability features also assessed.

System Suitability

The system suitability test performed according to USP 30 [10] and BP 2007 [11] indications. The observed RSD values for the replicate injections of standard peak of alphatocopherol meets acceptance criteria. USP plate count and USP tailing factor are also determined. The results obtained are all within acceptable limits.

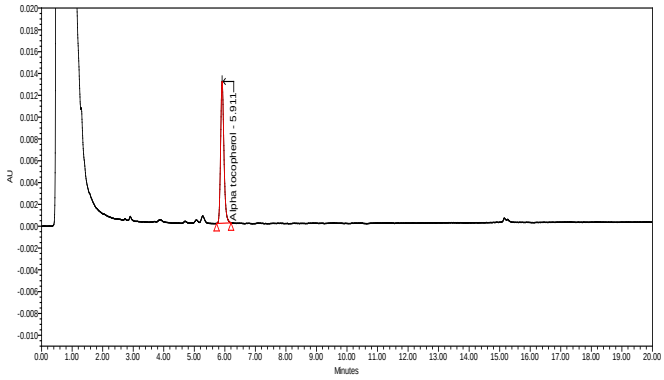

Fig. 4: Specimen Chromatogram of Test

Specificity

Specificity is the ability of the analytical method to unequivocally assess the analyte response in the presence of its other peaks of impurities. The peak purity indices for the analytes in stressed solutions were determined with PDA detector under optimized chromatographic conditions found to be better ("purity angle is lesser than purity threshold" and "No Flag" with empower software) indicating that no additional peaks were co-eluting with the analyte peak and evidencing the ability of the method to assess unequivocally the analyte of interest in the presence of potential interference.

The analyte peak meets the specification, visibly confirmed standard and test in Fig. 3 and Fig. 4.

Stress Study of Sample

Stress studies were performed on samples to provide the stability-indicating property and specificity of the proposed method. Degradation study was attempted at the stress conditions of heat (60°C), acid (0.1 N Hydrochloric acid), base (0.1 N Sodium hydroxide), water hydrolysis by reflecting with water and oxidation (30% Hydrogen peroxide) to evaluate the ability of the proposed method to separate all the degradant impurities formed during the stress study of sample from the interest peak. All degradant impurities formed were completely separated from alphatocopherol peak with acceptable peak purity. The details of the stress study in Table-1, indicates that the method is stability indicating.

Table 1: Stress Study of Sample

Degradation	Condition	%Assay	% Degraded	Peak Purity
Control Sample	-	100.1	-	Pass
Acid Deg	0.1 N HCl / 1mL / RT / 1hr	90.1	10.0	Pass
Base Deg	0.1 N NaOH / 1mL / RT / 1hr	95.2	4.9	Pass
Peroxide Deg	30%H2O2 / 1mL / RT / 1hr	82.4	17.7	Pass
Hydrolysis	Water / 4mL / 50°C / 1hr	90.4	9.7	Pass
Thermal Deg	60°C / 6hr	89.8	10.3	Pass
Humidity Deg	90%RH/25°C / 120hrs	87.2	12.9	Pass

Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration of analyte present in the sample preparation. The nominal concentration of solution of alphatocopherol standard is about 4 µg/mL. Response function was determined by preparing standard solution of alphatocopherol at different concentration levels ranging from 10% to 400% of target concentration.

Linearity was established by plotting graph to concentration versus corresponding response of analyte and determined the correlation coefficient. The correlation coefficients (r) exceed 0.999, the acceptable threshold suggested for linearity procedures to determine the content in Formulation [8]. The regression statistics are shown in Fig. 2

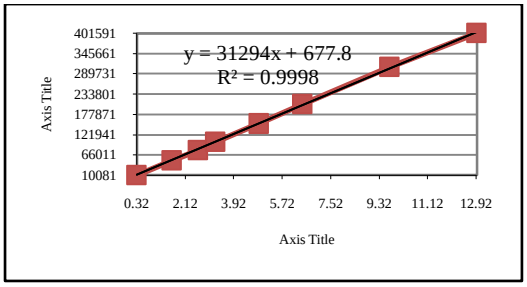

Fig. 2: Linearity Curve of Alphatocopherol

Accuracy

Accuracy was evaluated from the placebo sample prepared by standard addition method. The experiment was carried out by adding known amount of alphatocopherol corresponding to three concentrations at 50%, 100% and 150% of the specification level in placebo solution. The samples were prepared in triplicate at each level. The quantification of added analyte (%weight/weight) was carried out by using an external standard prepared at the analytical concentration

The experimental results revealed that approximately 98–102% recovery was obtained. Therefore, based on the recovery data the estimation of alphatocopherol has been demonstrated to be accurate for intended purpose and is adequate for routine analysis. The details were mentioned in Table 2.

Table 2: Accuracy of Alphatocopherol

S. No	Sample ID	% Recovery
1	50 % Sample-1	99.2
2	50 % Sample-2	95.9
3	50 % Sample-3	92.3
4	100 % Sample-1	96.6
5	100 % Sample-2	100.0
6	100 % Sample-3	100.7
7	100 % Sample-4	100.3
8	100 % Sample-5	100.8
9	100 % Sample-6	98.0
10	200 % Sample-1	96.8
11	200 % Sample-2	96.1
12	200 % Sample-3	96.6

Precision and Ruggedness

The precision of an analytical procedure expresses the degree of scatter between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The repeatability (intra-day precision) refers to the use of analytical procedure within a laboratory over a short period of time using the same operator with the same equipment. Intermediate precision (inter-day precision) involves estimation of variations in analysis when a method is used within a laboratory on different days, by different analysts. In all instances the % RSD values were less than 2%.

Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in chromatographic conditions, i.e. change in flow rate by ±0.05 mL/min, change in pH of the buffer by ±0.2 units, change in column oven temperature by ±5°C and change in organic composition of mobile phase by ±2% absolute. System suitability was verified with standard solution and the system suitability parameters were monitored. The method was demonstrated to be robust over an acceptable working range of its HPLC operational conditions mobile phase. The details were mentioned in Table 3.

Table 3: Robustness of Alphatocopherol

Parameter	Variation	Value	%RSD	Tailing Factor	USP Plate Count
Flow	Positive	0.45 mL/min	0.3	1.3	14990
	Negative	0.35 mL/min	0.1	1.3	13562
Mobile Phase pH	Positive	-	0.6	1.3	14008
	Negative	-	0.3	1.2	13869
Organic variation	Positive	0.2%	0.4	1.2	13501
	Negative	0.2%	0.5	1.2	14012
Column Temperature	Positive	25°C	0.8	1.2	13272
	Negative	35°C	1.2	1.2	14253

CONCLUSION

Suitable UPLC method was developed and validated for the quantification alphatocopherol in drug product at very low concentrations. Validation experiments provided proof that the UPLC analytical method is linear in the proposed working range as well as accurate, precise (repeatability and intermediate precision levels) and specific, being able to separate the main drug from its degradation products. The proposed method is also found to be robust with respect to flow rate, pH of buffer and wavelength. The method is very sensitive to temperature and organic composition of mobile phase. Due to these characteristics, the method has stability indicating properties being fit for its intended purpose, it may find application for the routine analysis of the alphatocopherol from its dosage forms.

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