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A NEW METHOD FOR THE ESTIMATION OF TIVOZANIB IN PHARMACEUTICAL FORMULATION BY USING RP-HPLC METHOD

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Abstract
The present paper describes a simple, accurate and precise reversed phase High Performance Liquid Chromatography (HPLC) method for rapid and simultaneous quantification of Tivozanib. The chromatographic separation was achieved on Symmetry C_{18} (150x4.6mm, 3.5µm) column. Mobile phase contained a mixture of Hexane sulphonic acid and Acetonitrile in the ratio of 40:60 v/v, flow rate 1.0ml/min and UV detection at 226nm. The proposed method shows a good linearity in the concentration range of 20-120µg/ml of Tivozanib under optimized conditions. Precision and recovery study results are in between 98-102%. In the entire robustness conditions %RSD is below 2.0%. Degradation has minimum effect in stress condition and solutions are stable up to 24hrs. This method is validated for different parameters like Precision, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), ruggedness, robustness and forced degradation study were determined according to the ICH Q2B guidelines. All the parameters of validation were found to be within the acceptance range of ICH guidelines.
Key words: RP-HPLC, Validation, Tivozanib.

INTRODUCTION

Tivozanib is used in form of the hydrochloride monohydrate, which is a white to light brown powder [1]. It is practically insoluble in water and has low solubility in aqueous acids, ethanol and methanol. It is not hygroscopic and not optically active [2, 3]. Tivozanib, sold under the brand name Fotivda, is a medication used for the treatment of relapsed or refractory advanced renal cell carcinoma (RCC) [4, 5]. It is an oral VEGF receptor tyrosine kinase inhibitor [6, 7]. The most common side effects include fatigue [8, 9], hypertension [10, 11], diarrhea, decreased appetite, nausea, dysphonia [12], hypothyroidism [13], cough, and stomatitis [14]. It should not be taken during pregnancy as it is teratogenic [15], embryotoxic and fetotoxic in rats. Administration of a single dose of tivozanib with rifampicin, a strong inducer of the enzyme CYP3A4 [16, 17], cuts the biological half-life and total exposure (AUC) of tivozanib in half, but has no relevant influence on highest concentrations in the blood. Combination with ketoconazole, a strong CYP3A4 inhibitor, has no relevant effects. A quinoline [18] urea derivative, tivozanib suppresses angiogenesis [19] by being selectively inhibitory against vascular endothelial growth factor (VEGF) [20, 21]. It is designed to inhibit all three VEGF receptors.

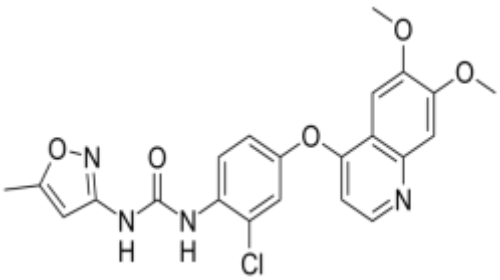

Fig.1: Tivozanib

MATERIALS AND METHODS

Chemicals

Acetonitrile, Ortho Phosphoric acid (OPA) and water (HPLC grade), were purchased from Merck (India) Ltd. Worli, Mumbai, India. All APIs of Tivozanib as reference standards were procured from Torrent Pharma, Ahmadabad.

Equipment

Waters alliance -2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions

Chromatographic separation was carried out in isocratic mode at room temperature using Symmetry C_{18} (150x4.6mm, 3.5µ) column. The mixture of Hexane Sulphonic acid of pH=5 adjusted with Acetonitrile 40:60 v/v at a flow rate of 1.0ml/min was used as a mobile phase. The injection volume was 10µl and eluent was monitored at 226nm using PDA detector. The run time was 4min.

Selection of wavelength

The absorption spectra of solution of Tivozanib are scanned over the range of 75-450nm by using PDA detector and the spectra was recorded. The spectrum was shown in fig.3.

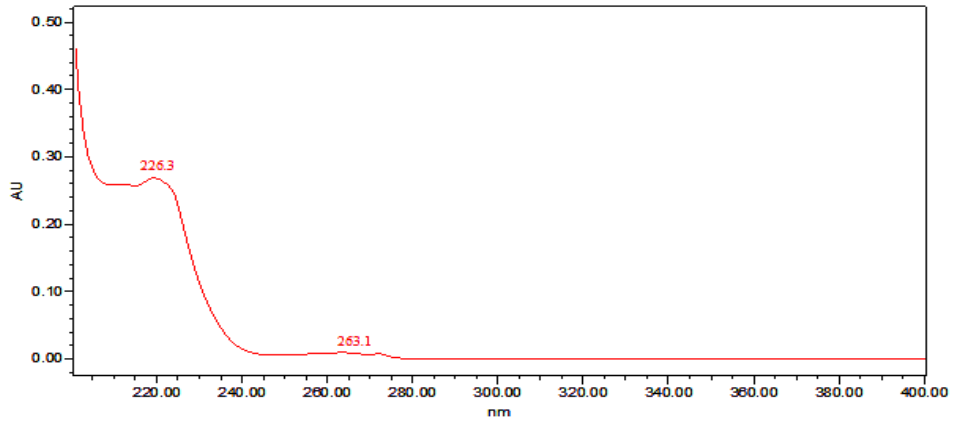

Fig. 2: PDA Spectrum of Tivozanib

Preparation of Standard solution:

Accurately weigh and transfer 8 mg of Tivozanib working standard into a 10 ml clean dry volumetric flask add diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)
Further pipette 1 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent. (80ppm of Tivozanib)

Preparation of Sample solution

Accurately weighed and transfer equivalent to 10.27mg of Tivozanib sample into a 10mL clean dry volumetric flask add diluent and sonicate it up to 30 mins to dissolve, and centrifuge for 30min. to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45 micron Injection filter. (Stock solution). Further pipette 1 ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent. (80ppm of Tivozanib)

Validation Procedure

The analytical method was validated as per ICH Q2 (R_1) guidelines for the parameters like system Precision, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) and forced degradation.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components, which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with Tivozanib.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision

Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves. The LOD and LOQ for Tivozanib and dapsone were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ for calculated as 3.3s/n and 10s/n respectively as per ICH guidelines where s/n indicates signal-to-noise ratio.

Stress Degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q_1A (R_2). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (±0.2), organic content in the mobile phase (±10%). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

RESULTS AND DISCUSSION

Method Validation

In this method system suitability, linearity, precision, accuracy, LOD, LOQ, robustness, forced degradation are validated for the selected drugs Tivozanib drugs. The proposed method having standard solution and sample solution chromatograms are shown.

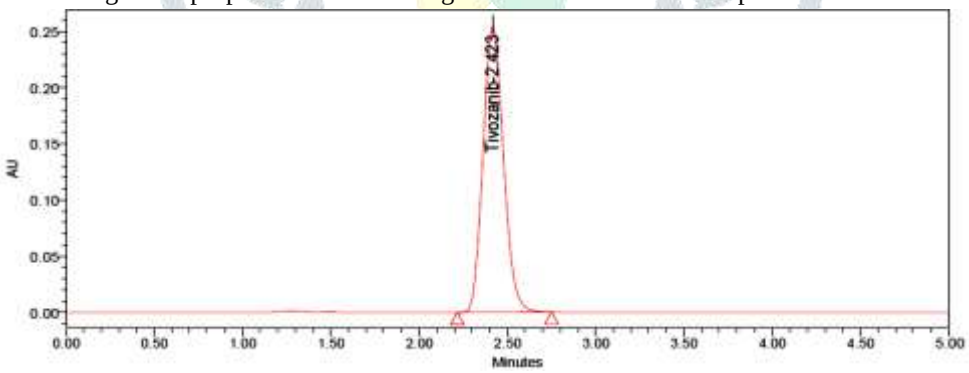

Fig. 3: Chromatogram of standard

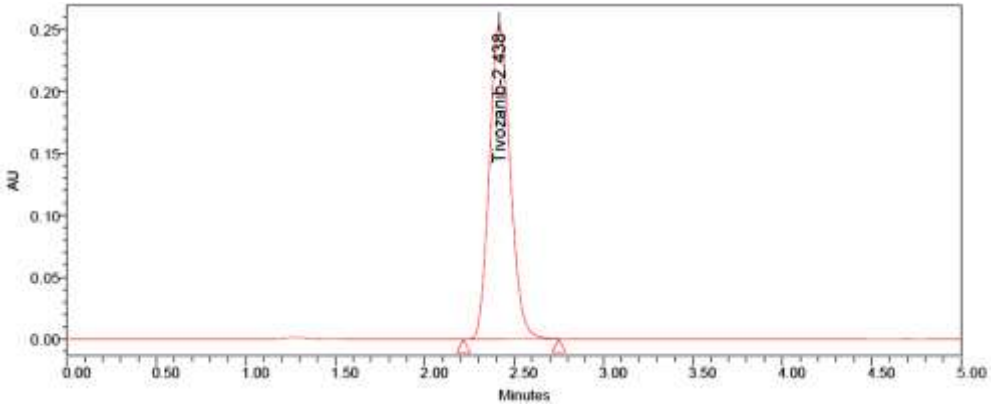

Fig. 4: Chromatogram of sample

System suitability

The HPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of the standard solution containing 226µg/ml of Tivozanib were assessed to check the system suitability. The number of theoretical plate count for Tivozanib were 1452 respectively. Tailing factor for Tivozanib were 1.05 respectively. All the parameters were found to be within limit.

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 300µg/ml of Tivozanib. Sequential dilutions were performed to the given solutions at 25, 50, 75, 100, 125 and 150% of the target concentrations. These were injected and the peak areas are used to plot calibration curves against the concentration. The correlation coefficient values of these analytes were 0.9998. The results were shown in table 1.

Table 1: Linearity study results

Analyte	Linearity range	Equation of calibration curve	Correlation coefficient
Tivozanib	20-120µg/ml	y = 46424.84x +16090.61	0.9998

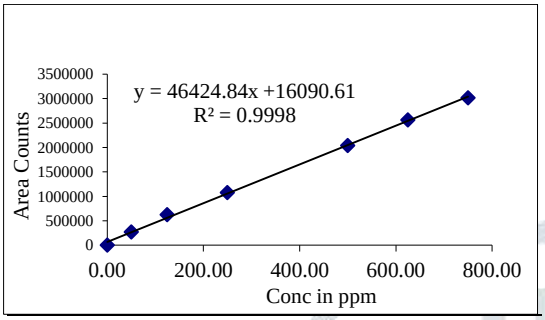

Fig. 5: Linearity plot for Tivozanib

LOD and LOQ

LOD and LOQ minimum concentration level at which the analyte can be reliably detected, quantified by using the standard formulas. LOD values for Tivozanib were 0.01µg/ml. LOQ values for Tivozanib were 0.1µg/ml.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate samples solution was injected and the peak areas obtained used to calculate mean and percentage RSD values. The present method was found to be precise as %RSD of the less than 2.0%. The results are given in table 2.

Table 2: Method Precision results

Analyte	Amount present	% RSD
Tivozanib	10	0.79

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 150,300 and 450µg/ml of Tivozanib were prepared. As per the test method the test solution was injected to three preparations each spike level and the assay was performed. The percentage recovery values were found to be in the range of 98-102%. The results are given in table 3.

Table 3: Accuracy (recovery) study results

% of target concentration	Tivozanib (% Recovery)
50	98.3
100	100.4
150	99.2
Mean (% Recovery)	99.3

Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Robustness

Robustness of the method was found to be %RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate (±20%), Organic content in mobile phase (±10%). The results are given in table 4.

Table 4: Robustness results

Drug Name	Flow Plus (1.2ml/min)	Flow Minus (0.8ml/min)	Organic Plus (66:34)	Organic Minus (54:46)
	%RSD			
Tivozanib	0.78	0.34	0.61	0.59

Forced Degradation

Forced degradation conditions such as acidic, basic, oxidation, thermal and water stress were attempted as per ICH Q1A (R2). The effect of assay on their results are shown below table 5.

Table 6: Forced Degradation results

Degradation	Tivozanib (% Degradation)
Acid	12.6
Alkali	13.6
Peroxide	15.4
Reduction	16.5
Thermal	12.2

CONCLUSION

This method described the quantification of Tivozanib in bulk and pharmaceutical formulation as per ICH guidelines. The developed method was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of sample preparation and the less expensive reagents were used. In addition Tivozanib is eluted within 6mins. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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