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RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION OF TIVOZANIB BY RP-HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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Abstract

The estimation of Tivozanib by RP-HPLC methodology was established using a simple, concise, and accurate method. Use of stationary Discovery C18 150mm x 4.6 mm, 5.mm, mobile phase 0.1% chromatographic conditions Orthophosphoric acid was mixed with acetonitrile in a 50:50 ratio, with a flow rate of 1.0 ml/min, a detection wavelength of 320 nm, a column temperature of 30 Oc, and mobile phase as the diluent. A retention time of 2.702 minutes was discovered. The standard was injected six times to study system suitability parameters, and the findings fell far short of the threshold for acceptance. An analysis of linearity between levels of 25% and 150% revealed an R2 value of 0.999. Precision for the Method was found to be 0.8, 0.6, and 0.9 for medium level of accuracy. 0.05 g/ml and 0.16 g/ml, respectively, are the LOD and LOQ. The assay of a commercial formulation was conducted using the procedure and 100.10% was found. Studies on the degradation of tivozanib were conducted, and under every circumstance, the purity threshold was higher than the purity angle and within the acceptable range. Even though the complete approach has not been tested yet, it can be used in order to do further analysis of Tivozanib routinely.

Introduction:-

The most typical type of urinary system cancer is renal cell carcinoma (RCC)[1]. RCC can developasafirst1^{o}cancer (firstprimaryRCC,1st RCC) or as a second primary cancer (second RCC). One of the most prevalent SPMs is the 2nd RCC, with an incidence of 10.9–28.9% [2,3]. The five most predominant components of SPCs' were found to be alike for leukemia, non-Hodgkin's lymphoma, and kidney cancer. The three most prevalent SPCs (lung, bladder, and colorectal cancers) as well as most of other primary cancers had the same rankings and proportions in both populations after prostate cancer. There markable consistent SPC patterns[4].The kinase inhibitor tivozanib (Fotivda) was approved by the Food and Drug Administration for adult patients with advanced renal cell carcinoma (RCC) that had relapsed or refractory after two or more prior systemic therapies[5].It is an VEGF receptor tyrosine kinase [6]inhibitor The primary pathway linked to the development of renal cell carcinoma is VHL mutation-HIF up regulation-VEGF transcription. Tyrosine kinase inhibitors, which stop tumor growth, have an important target in the

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form of vascular endothelial growth factor receptors (VEGFR receptors) [7,9]. The main effect is to prevent vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3 from becoming phosphorylated. It also inhibits other kinases like c-kit and platelet derived growth factor beta (PDGFR)[7,11]. Serum soluble VEGFR2(sVEGFR2)levels decreased overtime and became more pronounced with tivozanib exposure in clinical studies, suggesting that's VEGFR2 could be used as a pharmacodynamic indicator of VEGFR inhibition[8].Demethylation, hydroxylation, N-oxidation, and glucuronides are the metabolites[10], To create a new RP-HPLC method for determining Tivozanib's stability and to create a validated method in accordance with ICH guidelines.

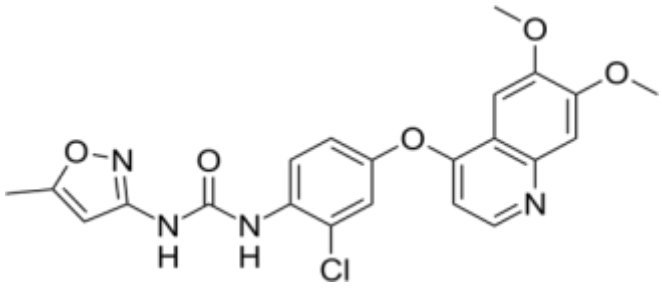

Fig 1:- Structure of Tivozanib.

There are few RP-HPLC methods have been reported in the literature for the determination of Tivozanib in bulk and pharmaceutical dosage form by RP-HPLC[12,13,14].An effort has been made to create an RP-HPLC method for the quantitative determination of tivozanib in bulk and pharmaceutical dosage form that is straight forward, specific, quick, precise, and affordable. The International Conference on Harmonization's (ICHQ2 (R1) guidelines have been used to validate this method.[15]

Materials And Methods:-

Chemicals and reagents

Tivozanib pure drugs (API) brought from Merck India limited (GOA), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the chemicals and solvents mentioned above are from Rankem.

Instrumentation:

The instrument which was used in the study was HPLC (Waters 2695 with PDA detector 2996) which was integrated and monitored using Empower 2 software. electronic balance, sonicator, hot air oven, digital pH meter and UV-Visible chamber.

Buffer Preparation: -

0.01N Potassium dihydrogen ortho phosphate-Accurately 1.36gm of Potassium dihydrogen Ortho phosphate weight in a 1000ml of Volumetric flask was added about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

Preparation of Standard stock solution:

Accurately weighed 6.7mg of Tivozanib transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10minutes.Flasks were made up with diluents and labeled as Standard stock solution (134µg/ml of Tivozanib).

Preparation of Standard working solution:

1ml of Tivozanib from each stock solution was pipetted out and taken in to a 10ml volumetric flask and made up with diluent. (13.4µg/ml of Tivozanib).

Preparation of Sample stock solution:

10 capsules of reference standards were weighed and the average weight to each Capsule was calculated, then the weight equivalent to1capsule was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25min, further the volume was made up with diluent and filtered by HPLC filters (134 µg/ml of Tivozanib).

Preparation of working sample solution: 1ml of sample stock solution which was transferred to10ml volumetric flask and made up with diluent. (13.4µg/ml of Tivozanib)

Table 1:- Chromatographic conditions.

Flowrate	1.0 ml/min
Column	Discovery C18 (250mm x4.5 mm, 5µm)
Wavelength	320nm
Column temperature	30°C
Injection volume	10.0µL
Run time	5mins
Diluent	Acetonitrile: 0.1% OPA (50:50)

Observation:-

Tivozanib eluted at 2.702 min respectively with good resolution (Fig 2). Plate count and tailing factor was very reasonable, so this method was optimized and validated.

Degradation:

According to ICH recommendations and standard industrial practice, forced deterioration is typically carried out in conjunction with a control sample under various stress condition, including acid, alkali, peroxide, heat, and UV. Although there are no established standards for industrial degradation, it is recommended that 5to30 percent of degradation be reached under any of the applied stress conditions. The goal of the degradation to be accomplished by stress testing is to replicate the stability circumstances of the control room temperature[16]. To conduct the forced degradation experiment, standard stock solutions of Tivozanib was exposed to various stress conditions, including 1 mL of 20% H_2O_2 (for oxidative degradation), 1 mL of 2N HCL (for acidic degradation) and 1mLof2NNAOH (for acidic degradation) (for basic degradation). The produced solutions were refluxed for 30 minutes 60^{o}C. To examine the descent, the standard solutions were also subjected to UV radiation and temperature conditions. The resulting solutions were diluted to yield13.4µg/ml of Tivozanib for degradation studies. To examine sample stability, 10µl samples were fed into the system and chromatograms were obtained.

Method Validation:-

The method was validated in accordance with ICH recommendations Q2R1. System appropriateness, specificity, linearity, accuracy, precision, LOD& LOQ, and hardiness are amidst the validation parameters.

Results And Discussion:-

System suitability parameters:

The system suitability parameters were determined by preparing standard solutions of Tivozanib (13.4ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined the %RSD for the area of six standard injections results should not be more than 2%.

Specificity:

Interference has been checked here in the method which has been improved. At their tension times of these drugs using this method, we shouldn't observe interfering peaks in the placebo or blank groups. Thus, it was claimed that this method was specific.

Linearity:

Inject 6 standard solutions containing Tivozanib at concentrations ranging from 3.35ppm to 20.10 ppm to show the linearity of the assay method. Produce a graph that shows peak area versus concentration. The calculated slopewas55112x+756.1, and the correlation coefficient was 0.999. The results were shown in table 2 and fig 6.

Precision:

Repeatability:

Various no. of samples has been taken from a sample stock solution, and 6 working sample solutions of the identical concentrations (13.4µg/ml Tivozanib) were constructed. Each injection was given from each working sample solution, and the results are shown in table3. The average area, standard deviation, and %RSD for the

medication were computed and found to be 0.4% for Tivozanib. The system precision was Passed for these procedures in the precision limit was less than "2 %." Table 3 shows the information results.

Intermediate Precision:
Multiple samples were taken from a sample stock solution, and six working sample solutions of the same concentrations (13.4µg/ml of Tivozanib) was prepared. Each injection from each working sample solution was given on the following day of the sample preparation, and the obtained areas are listed in table 4. The average area, standard deviation, and % RSD for the medication was computed and found to be 0.4% for Tivozanib. Because the precision limit was less than "2%" the intermediate precision was used for this procedure. Table 4 shows the information results.

Accuracy:
The conventional addition procedure was used to create three levels of accuracy samples. Triplicate injections were administered at each degree of accuracy, and the mean % recovery for Tivozanib was found to be 99.53%. Table 5 shows the outcomes. Because satisfactory recover values were achieved, the accuracy for this approach was passed.

Robustness:
Robustness conditions like Flow minus(0.8ml/min), Flow plus(1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was continued, and samples were injected in duplicate manner. Parameters such as System suitability have been observed to be not affected much and all tribes the parameters were passed. %RSD was within the limit. Table 6 shows the data.

Assay:
Tivozanib had a label claim of Tivozanib1.34mg per unit formulation. The mentioned formulation was used for the assay. The average %assay achieved for Tivozanib was 99.86%.

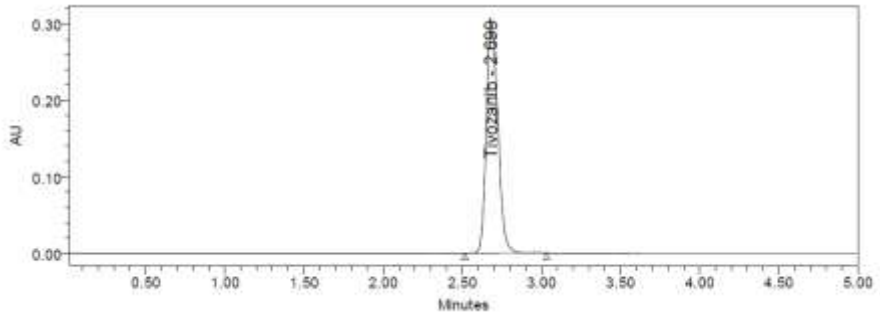

Fig 2:- Assay Chromatogram of reference standard.

Degradation Studies:
Degradation studies were performed with the stock standard solution and the degraded samples were analyzed using the proposed method. Assay % of Tivozanib in the injected samples was calculated and all the samples passed the limits of degradation. The results were shown in table 7.

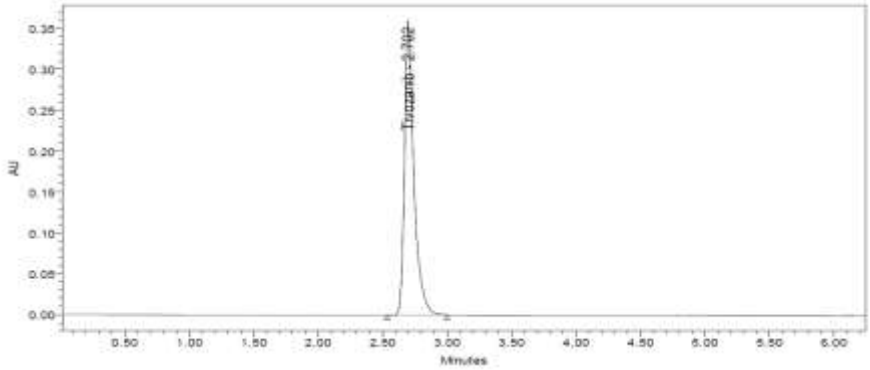

Fig 3:- Optimized Chromatogram.

Table 2:- System suitability parameters.

S.No.	Tivozanib		
Inj	RT (mi)	USP Plate Count	Tailing
1	2.692	10608	1.24
2	2.699	10461	1.27
3	2.700	10489	1.24
4	2.702	10473	1.25
5	2.703	10645	1.24
6	2.705	10516	1.26

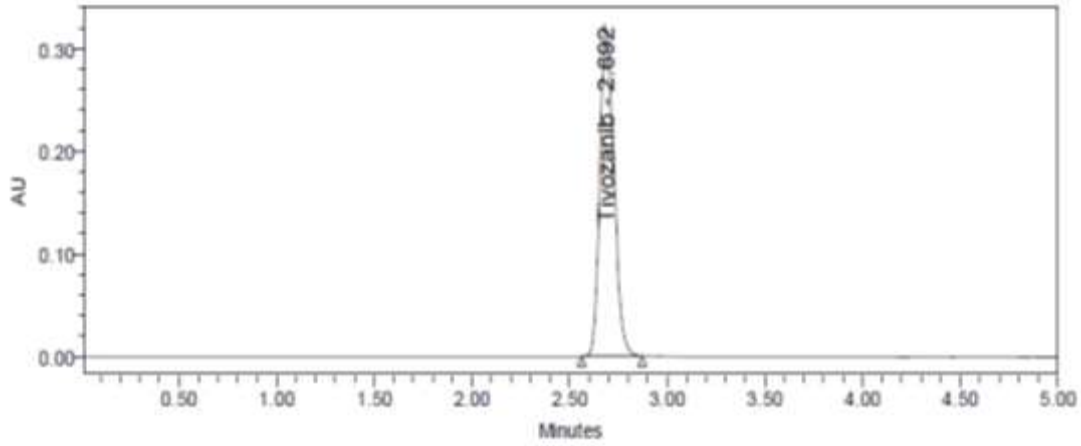

Fig 4:- Standard solution chromatogram.

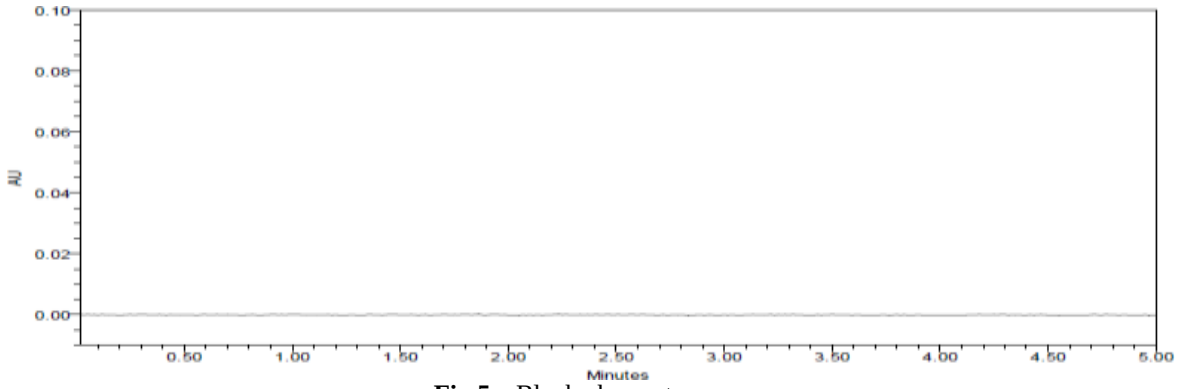

Fig 5:- Blank chromatogram.

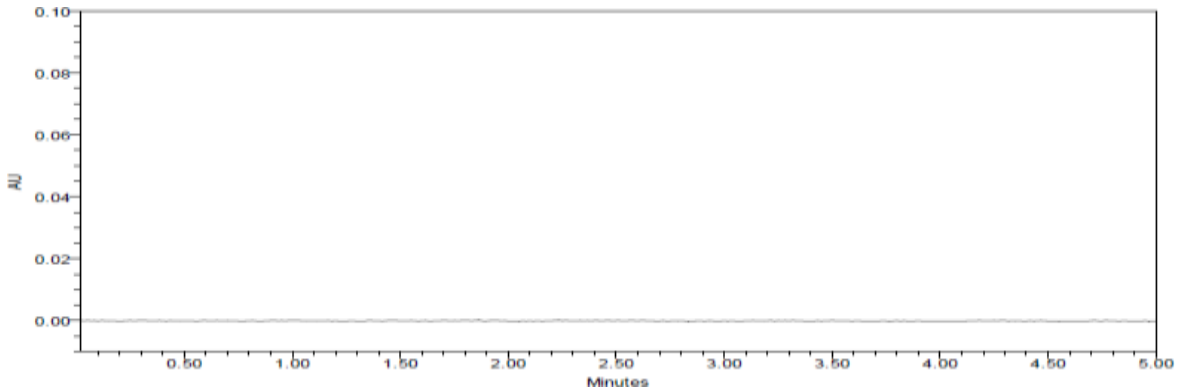

Fig 6:- Placebo chromatogram.

Table 3:- Linearity table for Tivozanib.

Tivozanib	
Conc (µg/mL)	**Peak area**
0	0
3.35	532790
6.7	1120830
10.05	1691958
13.4	2152598
16.75	2738880
20.10	3234514

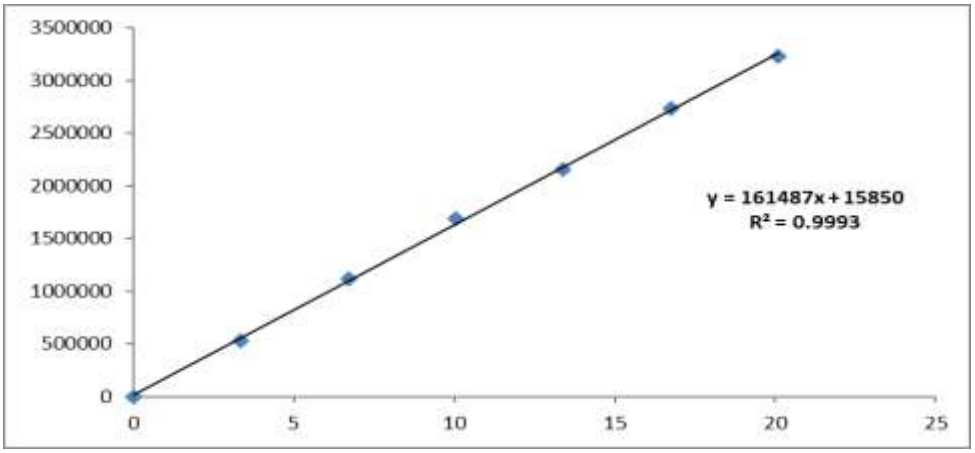

Fig 7:- Calibration curve of Tivozanib.

Table 4:- Repeatability for Tivozanib.

S.no.	Tivozanib
1	2115763
2	2132356
3	2132356
4	2165198
5	2135087
6	2128485
Mean	2134874
S.D	16360.4
%RSD	0.8

Table 5:- Intermediate Precision for Tivozanib.

S.no	Tivozanib
1	2138465
2	2124516
3	2140488
4	2129774
5	2160175
6	2154557
AVG	2141329
STDEV	13822.1
%RSD	0.6

Table 6:- Accuracy for Tivozanib.

%Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	%Recovery	Mean %Recovery
50%	6.7	6.67	99.58	100.05%
	6.7	6.60	98.49	
	6.7	6.78	101.14	
100%	13.4	13.41	100.10	
	13.4	13.44	100.31	
	13.4	13.28	99.13	
150%	20.1	20.02	99.60	
	20.1	20.19	100.45	
	20.1	20.43	101.63	

Table 7:- Robustness Data.

S.no	Condition	%RSD of Tivozanib
1	Flow Minus(0.8ml/min)	0.3
2	Flow Plus(1.0ml/min)	0.5
3	Mobile phase Minus (65B:35A)	0.4
4	Mobile phase Plus (55B:45A)	0.4
5	Temperature minus (25^0C)	0.6
6	Temperature plus (35^0C)	0.7

Table 8:- Degradation data.

S.no	Condition	%Undegraded	%Degraded
1	Acid	93.36	6.64
2	Alkali	95.68	4.32
3	Oxidation	95.10	4.90
4	Thermal	97.12	2.88
5	UV	98.46	1.54
6.	Water	99.22	0.78

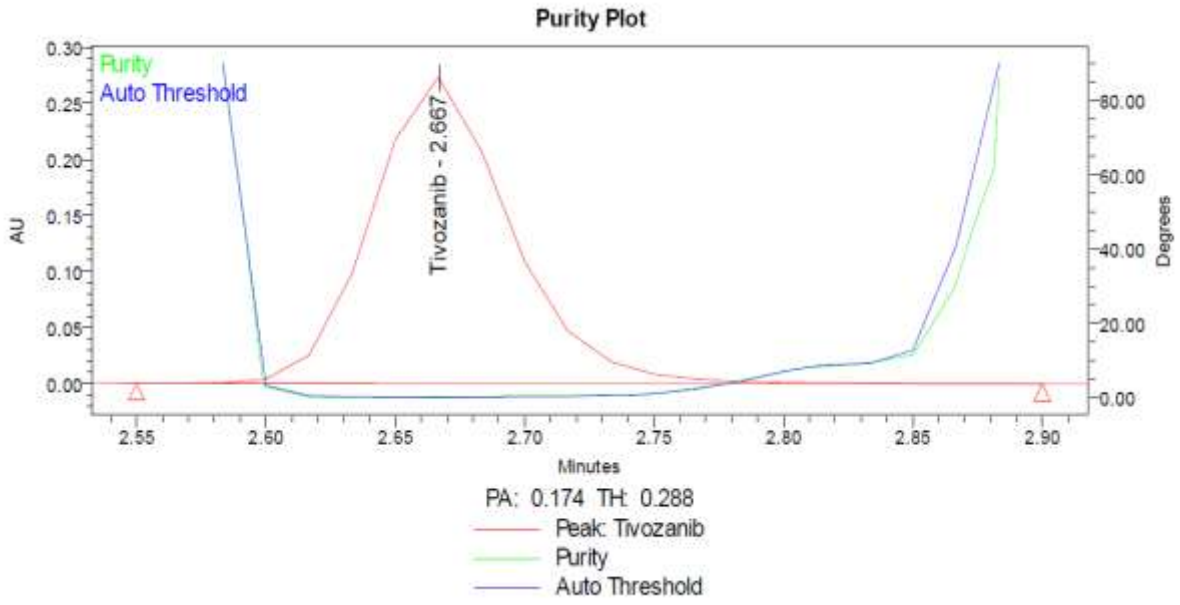

Fig 8:- Purity plots.

Conclusion:-

The estimation of Tivozanib by RP-HPLC methodology was established using a simple, concise, and accurate approach. The standard was injected six times to study system suitability characteristics, and the findings fell well short of the threshold for acceptance. An analysis of linearity between levels of 25% and 150% revealed an R^2 value of 0.999. The results showed that the method precision was 0.6, and the intermediate precision was 0.8. 0.05 g/ml and 0.16 g/ml, respectively, are the LOD and LOQ. The test of a commercial formulation was conducted using the procedure, and 100.10% was found. Degradation studies of tivozanib have been performed under every circumstance, threshold of the purity was higher compared to the purity angle and is within the suitable limit. The whole length approach has not been tested; but, if it is, it can be utilized for routine analysis of Tivozanib.

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