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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR THE DEVELOPMENT AND VALIDATION OF TIVOZANIB IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
Tivozanib determination in pharmaceutical dosage form using RP-HPLC technique Run through Kromosil C18 (4.6mm x 150mm, 5µm) the chromatogram. Methanol taken in the 60:40 ratio was pumped through column at a flow rate of 1.0ml/min in mobile phase including 0.1% Perchloric acid. Temperature maintained at thirty-degree Celsius. Selected a wavelength optimised at 230.0 nm. Tivozanib's retention time came up as 2.696min. %RSD of the Tivozanib turned out to be 0.6%. PercentageMethod accuracy of Tivozanib shown to be 0.6% RSD.For Tivozanib, recovery was 100.67%. Regression equation of Tivozanib is y = 52314x + 17011. LOD, LOQ values derived from this equation were 0.08, 0.24. Retention durations were lowered and run time was lowered; so, the new approach was straightforward and cost-effective that one could apply in routine Quality control tests in different industries.
Key Words: Tivozanib, Method development, Validation, RP-HPLC.

INTRODUCTION[1-10]
Renal cell carcinoma (RCC) is the predominant form of kidney cancer in adults and is dominantly caused by genetic changes that promote angiogenesis, the process of generating new blood vessels, therefore facilitating tumour development. By inhibiting vascular endothelial growth factor receptors (VEGFR), the repression of angiogenesis has emerged as a crucial approach in the treatment of advanced renal cell carcinoma (RCC).
Tivozanib is a pharmaceutical compound acting as an oral tyrosine kinase inhibitor (TKI) that specifically acts on vascular endothelial growth factor receptors (VEGFR) genes 1, 2, and 3. These receptors play a crucial role in angiogenesis, the mechanism by which new blood vessels mature to provide nutrition to tumours, therefore facilitating their proliferation and spread to distant sites. Through the inhibition of VEGFR signalling, tivozanib efficiently reduces tumour development by severing the blood flow to the tumour. The main use of this therapy is in the management of advanced renal cell carcinoma (RCC), a malignancy that significantly relies on angiogenesis induced by VEGF.
In 2021, the U.S. Food and Drug Administration (FDA) granted approval to Tivozanib for the treatment of patients with relapsed or resistant renal cell carcinoma (RCC). The approval primarily relied on the findings of the TIVO-3 clinical study, which demonstrated that tivozanib greatly enhanced progression-free survival (PFS) in patients who had undergone two or more previous chemotherapeutic treatments, as compared to sorafenib.
Tivozanib stands out among current VEGFR inhibitors due to its exceptional selectivity for VEGFR, leading to a reduced occurrence of off-target adverse effects, and its comparatively extended half-life, enabling once-daily administration. Clinical studies have shown advantageous effectiveness, especially in individuals with recurrent or resistant illness. Furthermore, the medication has demonstrated promise in mitigating certain adverse effects

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generally linked to VEGFR inhibitors, such as hypertension and diarrhoea, which are frequently observed with less specific drugs like sunitinib and pazopanib.

With a lengthy half-life of roughly five days, the pharmacokinetic profile of tivozanib ensures persistent inhibition of VEGFR and enables the medication to maintain therapeutic levels with a constant once-daily dosage. This feature enhances its potential as a durable therapy for renal cell carcinoma (RCC). Moreover, current studies are assessing the effectiveness of tivozanib when used with other cancer treatments, such as immune checkpoint inhibitors, to investigate its wider applicability in the field of oncology.

Nevertheless, similar to other anti-angiogenic agents, tivozanib is not devoid of potential hazards. Frequently seen negative consequences encompass hypertension, tiredness, and hand-foot syndrome. Strategic patient selection and diligent monitoring are crucial to mitigate hazards and optimise therapy results. Notwithstanding these difficulties, tivozanib continues to be a significant choice in the management of advanced renal cell carcinoma (RCC), especially for patients who have not responded to previous treatments.

ANALYTICAL BACKGROUND[11]

Tivozanib inhibits growth factor receptors, treating renal cell carcinoma. It is chemically known as 1-{2-chloro-4-[(6,7-dimethoxyquinolin-4-yl)oxy]phenyl}-3-(5-methyl-1,2-oxazol-3-yl)urea

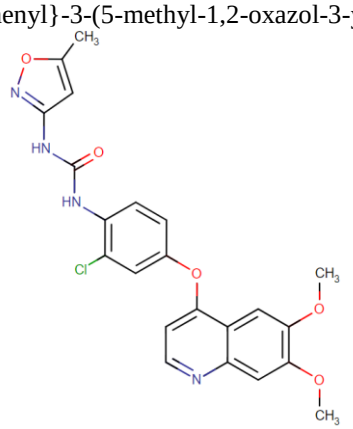

Figure 1 structure of Tivozanib

High Performance Liquid Chromatography (HPLC) plays a crucial role in the validation of Tivozanib, In the review of literature, more economical methods were observed [14-16], hence a simple, cost-effective stability-indicating simultaneous estimation of Tivozanib by RP-HPLC in pharmaceutical dosage form must be developed and validated as per the guidelines of ICH (Q2 specification).

MATERIALS:

Tivozanib pure drug (API), Tivozanib formulation (Fotivda), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

INSTRUMENTATION

The development and method validation were conducted using a WATERS HPLC, specifically the model 2695 SYSTEM, equipped with a Photo diode array detector. The system also included an automated sample injector and the Empower 2 software.

Table 1: Chromatographic Conditions:

Mobile phase	Acetonitrile: OPA (60:40 v/v)
Flow rate	1 ml/min
Column	Discovery C18 (4.6 x 250mm, 5µm)
wave length	230 nm
Column temperature	26°C
Injection volume	10µL
Run time	10.0 min
Buffer	OPA

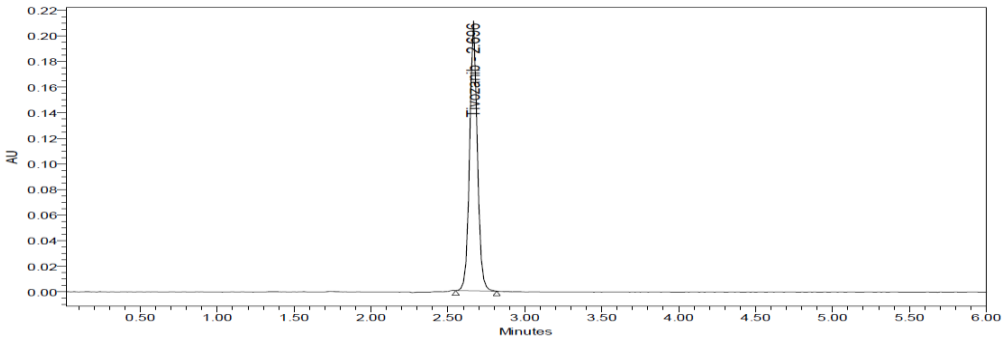

Figure 2: Optimized Chromatogram

Methods:

Preparation of Standard stock solutions: Accurately weighed 13.4mg of Tivozanib transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (134µg/ml of Tivozanib)

Preparation of Standard working solutions (100% solution): 1ml of Tivozanib from stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (13.4µg/ml of Tivozanib)

Preparation of Sample stock solutions: 5 Capsules were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters.(134 µg/ml of Tivozanib)

Preparation of Sample working solutions (100% solution): 1ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (13.4µg/ml of Tivozanib)

Validation:

System suitability parameters:

The system suitability parameters were determined by preparing standard solution of Tivozanib (13.4 ppm) and the solution 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 (Selectivity): Checking of the interference in the optimized method. We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So, this method was said to be specific. Representative chromatogram is shown in Figure 3 and experimental data is given in Table 2

Table: 2 System suitability parameters for Tivozanib

S no	Tivozanib		
Inj	RT(min)	USP Plate Count	Tailing
1	2.662	12943	1.05
2	2.665	12940	1.04
3	2.667	13175	1.04
4	2.673	13507	1.12
5	2.673	13464	1.09
6	2.678	13088	1.05

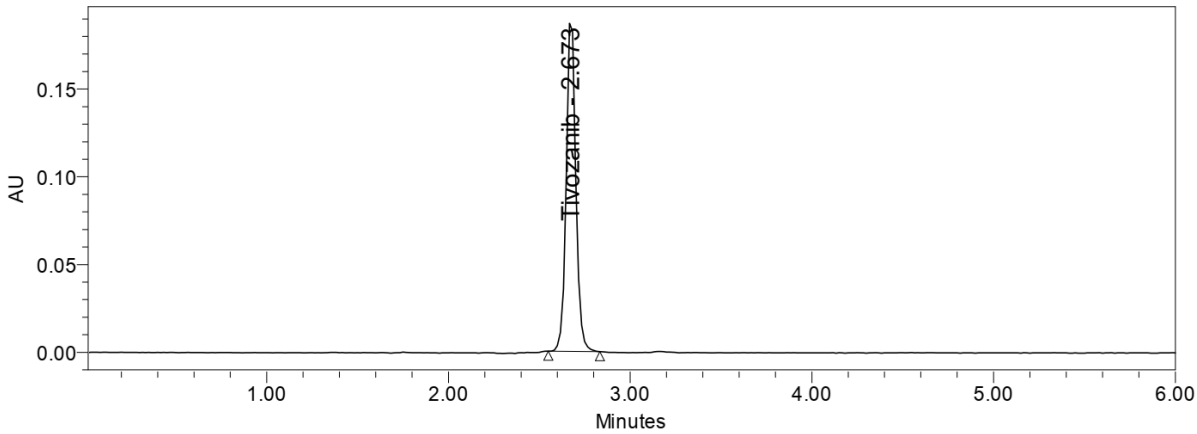

Figure 3: System Suitability Chromatogram of Tivozanib

Table 3: Specificity Data

Peak name	Rt	Area	USP plate count	Tailing
Tivozanib	2.697	700471	13036	1.03

Specificity:

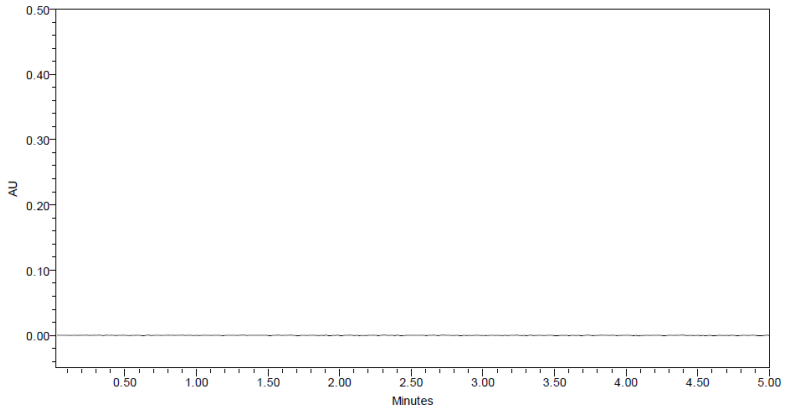

Figure 4 Chromatogram of blank

The forced degradation conditions are mentioned in Table 4 and the results are mentioned in Table 5

Table 4: Forced degradation conditions for Tivozanib

Stress condition	Solvent	Temp(^{0}C)	Exposed time
Acid	2N HCL	60^{0}c	30 mins
Base	2N NAOH	60^{0}c	30 mins
Oxidation	20% H_2O_2	60^{0}c	30 mins
Thermal	Diluent	105^{0}c	6 hours
Photolytic	Diluent	-	-
Hydrolytic	Water	60^{0}c	

From the results, degradation peaks were observed when the samples were exposed to acid. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.

Table 5: Degradation profile results

Degradation Condition	% Drug Un Degraded	% Drug Degraded
Acid	96.09	3.91
Base	96.13	3.87
Oxidation	95.48	4.52
Thermal	98.16	1.84
Photolytic	98.48	1.52
Hydrolytic	99.60	0.40

Limit of detection (LOD) The detection limit is considered as very low level of concentration of an analyte in a sample that can be detected, but not necessarily quantitated.

Limit of quantitation (LOQ): The limit of quantitation is considered as the lowest concentration of an analyte in a sample that can be determined with acceptable precision and accuracy of the method.

The LOD values obtained for Tivozanib are listed in Table 6.

Table 6: Summary of limit of detection

Sample	Conc (µg/ml)
LOD	0.08
LOQ	0.24

Linearity: The linearity of the method was demonstrated for Tivozanib by analyzing the solutions ranging from 25% to 150% of the specification limit (Table 7). The correlation coefficient for Tivozanib was 0.999. This indicates good linearity (Figures 8).

Linearity:

Calibration data is given in table 7 and regression data in table 8 and calibration curve in figure 5

Table 7: Calibration data of Tivozanib

Tivozanib	
Conc (µg/mL)	**Peak area**
0	0
3.35	194577
6.7	369239
10.05	537632
13.4	709817
16.75	906590
20.1	1064489

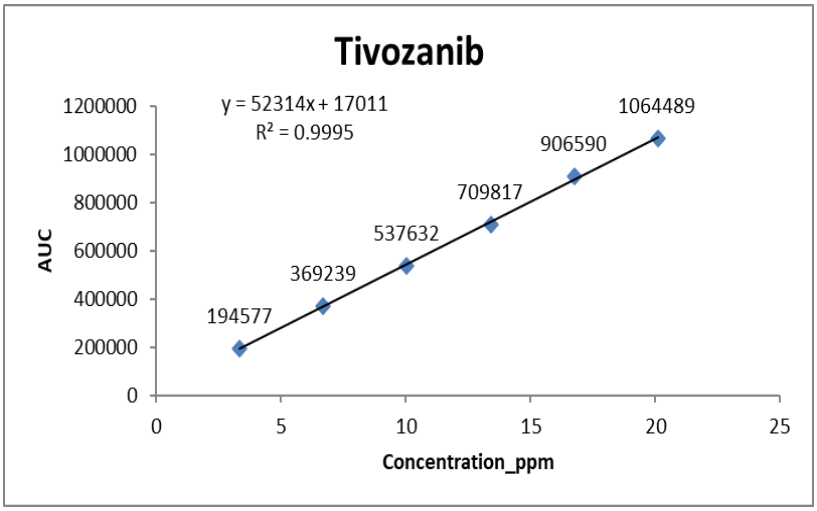

Figure 5: Calibration curve of Tivozanib

Table 8: regression data

Parameter	**Tivozanib**
Conc range (µg/mL)	3.35-20.1µg/ml
Regression Equation	y = 52314x + 17011
Co-relation	0.999

Accuracy: The accuracy of the method was determined by using solutions containing spiked samples of Tivozanib at 50%, 100% and 150% of the working strength. All the solutions were prepared in triplicate and analysed. The percentage recovery results obtained for each impurity was listed in Table 9.

Table 9 Accuracy table of Tivozanib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% recovery
50%	6.7	6.81	101.71
	6.7	6.80	101.55
	6.7	6.68	99.63
100%	13.4	13.45	100.39
	13.4	13.33	99.50
	13.4	13.59	101.43
150%	20.1	20.31	101.04
	20.1	20.04	99.71
	20.1	20.32	101.10
Mean % recovery			**100.67**

System Precision: The system precision was performed by analyzing six replicate injections of standard solution at 100% of the specified limit with respect to the working strength of Tivozanib. Results of peak area are summarized in Table 10

Table 10 System precision table of Tivozanib

S. No	Area of Tivozanib
1.	703130
2.	696828
3.	695132
4.	701834
5.	692716
6.	698110
Mean	**697958**
S.D	**3963.3**
%RSD	**0.6**

Method Precision: The precision of the method was determined by analyzing a sample of Tivozanib). Data obtained is summarized in Table 11

Table 11 Repeatability table of Tivozanib

S. No	**Area of Tivozanib**
1.	704212
2.	696479
3.	703686
4.	694720
5.	696175
6.	695630
Mean	**698484**
S.D	**4278.7**
%RSD	**0.6**

Intermediate precision: It is differently from the repeatability, the precision obtained within a single laboratory over a longer period (generally at least several months) and considers more changes than repeatability. Data obtained is summarized in Table 12.

Table 12 Intermediate precision table of Tivozanib

S. No	**Area of Tivozanib**
1.	694158
2.	689827
3.	695051
4.	686595
5.	684749
6.	697803
Mean	**691364**
S.D	**5132.7**
%RSD	**0.7**

Robustness: The chromatographic conditions were deliberately changed to evaluate the robustness of the existing method. To determine the robustness of method, system suitability solution is prepared as per methodology and injected into HPLC at different altered conditions to check the method's ability like flow rate (± 10%), column oven temperature (± 5°C) and Mobile phase (± 10%) from actual method conditions. No significant change is observed by changing flow, temperature, Mobile phase, and system suitability also complied as per methodology. The robustness results are summarized in Table 13.

Table 13 Robustness data for Tivozanib

Condition	**%RSD of Tivozanib**
Flow rate (-) 0.9ml/min	0.3
Flow rate (+) 1.1ml/min	0.3
Mobile phase (-) 65B:34A	0.3
Mobile phase (+) 55B:45A	0.3
Temperature (-) 27°C	1.1
Temperature (+) 33°C	0.5

Assay data: -

Fotivda Tablet bearing the label claims Tivozanib 1.34 mg. Assay was performed with the above formulation. Average % Assay for Tivozanib obtained was 99.98% respectively. Assay data shown in table no 8.

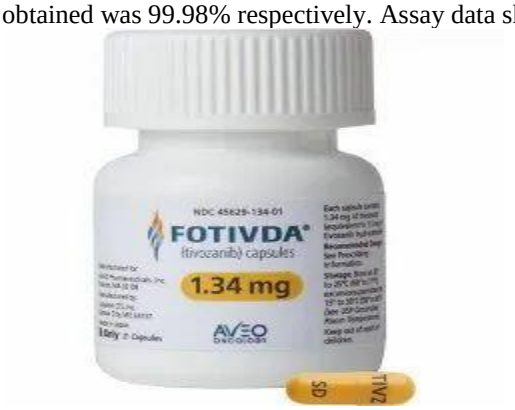

Figure 6: Tivozanib Marketed Drug

Formula to calculate assay:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{1}{10} \times \frac{10}{1} \times \frac{P}{5} \times \frac{FV}{100} \times \frac{}{L.C} \times 100$$

AT	Avergage peak area of sample in test solution
AS	Mean peak area of sample in standard solution
WS	Weight of sa,ple working standard taken in mg
P	Assay of sample working standard in % in dried basis
L.C	Label claim
FV	filled volume (1ml of a vail)

Table 14: Assay Data of Tivozanib

S.no	Standard Area	Sample area	% Assay
1	703130	704212	100.80
2	696828	696479	99.69
3	695132	703686	100.72
4	701834	694720	99.44
5	692716	696175	99.64
6	698110	695630	99.57
Avg	**697958**	**698484**	**99.98**
Stdev	**3963.3**	**4278.7**	**0.61**
%RSD	**0.6**	**0.6**	**0.61**

CONCLUSION

The results of the Tivozanib HPLC study show that this method can correctly measure the medicine's concentration and purity. This method is great for regular quality control and pharmacokinetic study because it can be used over and over again with sharp peak resolutions and uniform retention lengths. HPLC is an important tool for checking the chemical makeup of Tivozanib and making sure it works well and is safe for medical use.

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