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QBD APPROACH FOR METHOD DEVELOPMENT AND VALIDATION OF TIVOZANIB BULK DRUG AND IT'S FORMULATION BY USING RP-HPLC

[1] Miss. Shivani Ravindra Wagh, [2]Prof. R. J. Jadhav
[1]Student, [2]Assistant Professor
Department of Pharmaceutical Chemistry
K.Y.D.S.C.T.S College of Pharmacy, Sakegaon, Bhusawal, Dist- Jalgaon, Maharashtra, India

Abstract:
Attempts were made to develop RP-HPLC method for estimation of Tivozanib from tablet. For the RP - HPLC Agilent (S.K) method Gradient System UV Detector and C18 column with 250mm x4.6 mm i.d and 5µm particle size methanol: Methanol+0.05% OPA (80+20% v/v) was used as the mobile phase for the method. The detection wavelength was 220 nm and flow rate was 0.8 ml/min. In the developed method, the retention time of Tivozanib were found to be 4.280 min.The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Tivozanib in bulk drug as well as in formulations.
Keywords: Tivozanib, RP-HPLC, Chromatogram.

Corresponding author:

Prof. R. J. Jadhav,
Assistant Professor
Department of Pharmaceutical Chemistry
K.Y.D.S.C.T.S College of Pharmacy, Sakegaon, Bhusawal, Dist- Jalgaon, Maharashtra, India

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1. INTRODUCTION:

Quality is the heart of pharmaceutical industry. Quality is one of the fundamental criteria in addition to safety and efficacy for any entity to be qualified and approved as a drug. For ensuring consistency of performance of pharmaceutical products and systems, the recent emphasis has been on building the quality rather than merely testing it. This philosophy forms the basis of Quality by Design (QbD).

The twenty-first century began with the pharmaceutical industry using manufacturing technologies that have been employed since the 1940s and did not make significant changes in manufacturing process unless significant compliance or costs saving advantages could justify the high costs and long cycle time needed to gain approval. This often resulted in inefficient, overly expensive processes that were ultimately not in the best long-term interests of patients. As a result, the FDA (Food and Drug Administration) and other agencies around the world have embraced a new paradigm for regulation. The desired state was to shift manufacturing from being empirical to being more science, engineering, and risk based. Juran is often credited with introducing the concepts behind Quality by Design (QbD).1.

ANALYTICAL CHEMISTRY

Analytical Chemistry is a measurement of science consisting of a set of powerful ideas and methods that are useful in all fields of science and medicine. It seeks ever improved means of measuring the chemical composition of natural and artificial materials. This branch of chemistry, which is both theoretical, and a practical science, is practiced in a large number of laboratories in many diverse ways while analytical method, is a specific application of a technique to solve an analytical problem. Methods of analysis are routinely developed, improved, validated, collaboratively studied and applied. The discipline of analytical chemistry consists of qualitative and quantitative analysis.

Qualitative analysis – Information regarding the presence or absence of one or more components of the sample.

Quantitative analysis– Information regarding the amount of components of the sample, however the required information is finally obtained by measuring some physical property that is characteristically related to the compound of interest. (Nash et al).

2. DRUG PROFILES

Tivozanib:

Molecular formula: $C_{22}H_{19}ClN_4O_5$

Molecular weight: 454.863 g/mol

Structure:

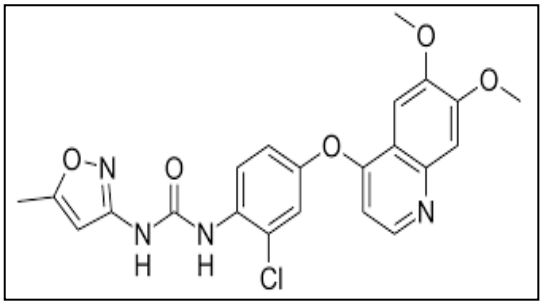

Fig. No.1: Chemical Structure of Tivozanib

Description:

Tivozanib is a white to off-white powder.

IUPAC Name:

1-[2-chloro-4-(6, 7-dimethoxyquinolin-4-yl) oxyphenyl]-3-(5-methyl-1,2-oxazol-3-yl)urea

Mechanism of action:

The VHL mutation-HIF up regulation-VEGF transcription is the main pathway implicated in the growth of renal cell carcinoma. Vascular endothelial growth factor receptors (VEGFR receptors) are important targets for tyrosine kinase inhibitors, which halt the growth of tumours. Tivozanib is a tyrosine kinase inhibitor that exerts its actions by inhibiting the phosphorylation of vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2 and VEGFR-3 and inhibits other kinases such as c-kit and platelet derived growth factor beta (PDGFR β). The above actions inhibit tumour growth and progression, treating renal cell carcinoma.

3. MATERIALS AND METHODS:

Selection and Procurement of Drug

In method development and validation of preservatives following chemicals and reagents were used**.**

Table 1: List of Reagents and Chemicals used

Sr. No.	Name of chemicals
1	Tivozanib
2.	Acetonitrile (HPLC grade)
3.	Methanol (HPLC grade)
4.	1 % OPA (HPLC grade)
5.	water (HPLC grade)

Selection of Analytical Technique

HPLC was selected as analytical technique for estimation of Tivozanib.

- **Instruments:**

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, (DAD) &Gradient Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 250mm; 5µm),a 20µl and UV730D Absorbance detector and running chemstation 10.1 software.

❖ **Stock preparations**:

➢ Stock I : Standard Sample Preparation

Std. 10 mg Tivozanib in 10 ml Methanol = 1000 µgm/ml

Take 40.22 mgs in 10 ml Methanol i.e= 1000 µgm/ml

(a) Chromatographic conditions:

The following chromatographic conditions were established by trial and error and were kept constant throughout the experimentation.

Table .2: chromatographic conditions (HPLC) details used during method Development

1.	HPLC	Agilent (S.K)Gradient System
2.	Software	Chemstation
3.	Column	(Agilent) C18 column (4.6mm x 250mm)
4.	Particle size packing	5 µm
5.	Stationary phase	C-18 (Agilent)
6.	Mobile Phase	MEOH : Water (0.05% OPA)
7.	Detection Wavelength	220 nm
8.	Flow rate	0.8 ml/min
9.	Temperature	25° C (Ambient)
10.	Sample size	20 µl
11.	pH	3.
12.	Run Time	15 min
13.	Filter paper	0.45 µm

4. EXPERIMENTAL WORK:

METHOD DEVELOPMENT OF HPLC:

❖ **List of Trials :**

Table .3: Selection of mobile Phase

Sr.no	Mobile Phase
1.	90% MEOH +10% 1% Acetic Acid Water, Flow 0.7,220 nm. ml/min abs at 249 nm (column 100mm X 4.6, 2.5 µm)
2.	80% MEOH +20% 1% Acetic Acid Water, Flow 0.7,220 nm ml/min abs at 249 nm (column 100mm X 4.6, 2.5 µm)
3	70% MEOH +30 %(0.05% OPA) Flow 1,220 nm. ml/min abs at 249 nm (column 100mm X 4.6, 2.5 µm)
4	50% MEOH +50% (0.05% OPA) Water, Flow 1, 220 nm .ml/min abs at 249 nm (column 100mm X 4.6, 2.5 µm)

Chromatogram of Trial-1:

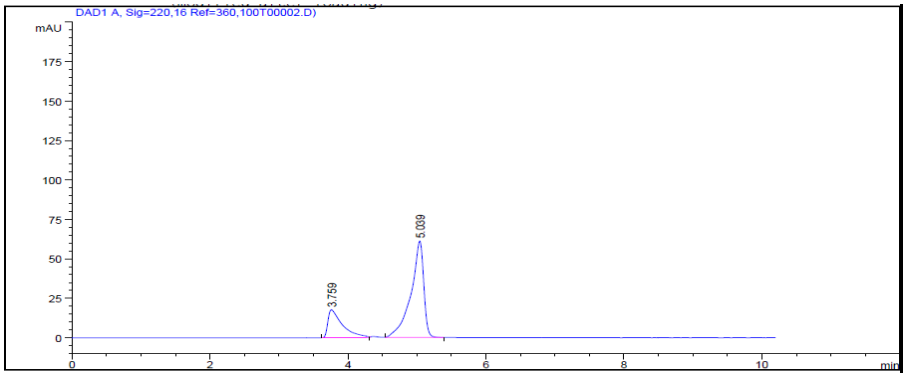

Fig.No.2 Chromatogram of Trial 1

Table 4: Chromatogram of Trial 1

RT[min]	Area[mV*s]	TP	TF	Resolution
3.759	261.38818	1957	0.28	-
5.039	795.11902	4683	2.19	4.03

Observation: Splitting is observed and TF is greater than 2. Chromatogram not acceptable.
Conclusion: Method rejected

Chromatogram of Trial-2

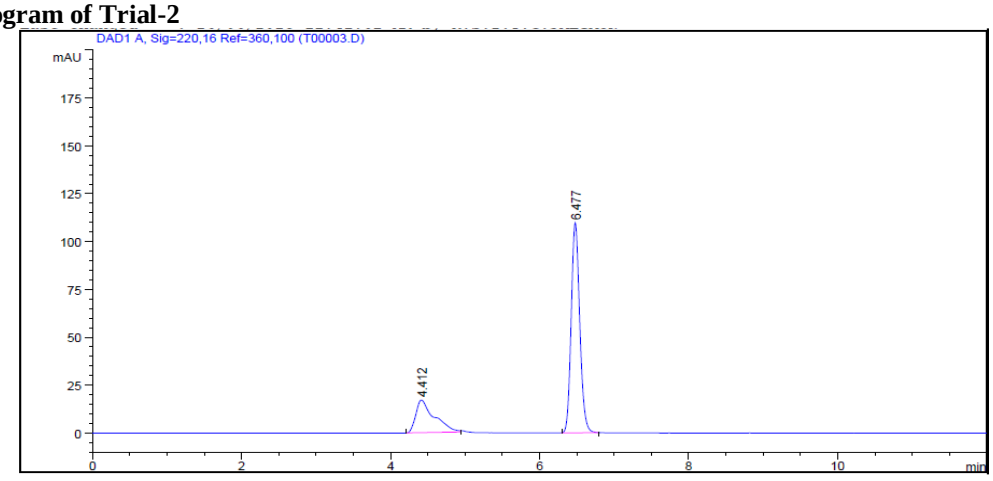

Fig.No.3 Chromatogram of Trial 2

Table 5: Chromatogram of Trial 2

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.412	293.82129	2184	0.40	-
2	6.477	874.65790	16137	0.83	7.09

Observation: Splitting is observed. Chromatogram not acceptable.
Conclusion: Method rejected

Chromatogram of Trial-3

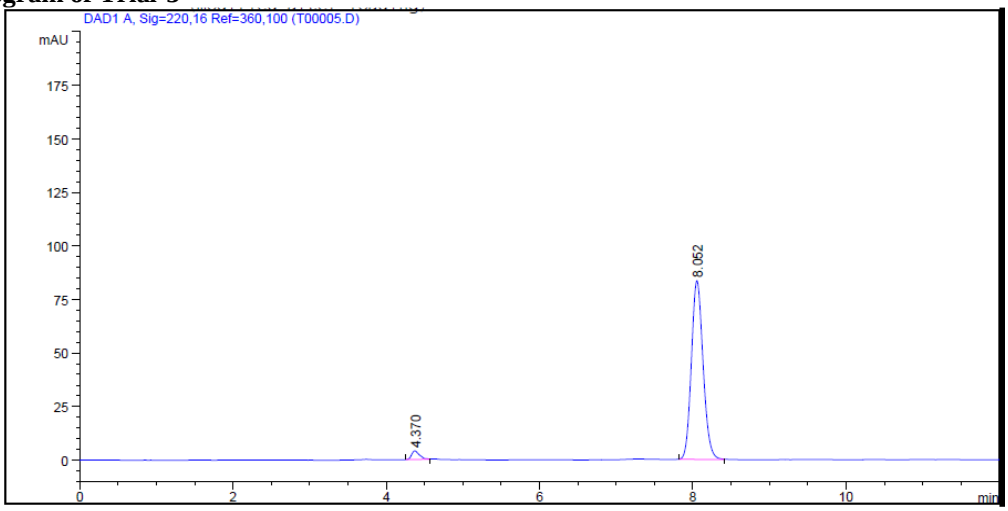

Fig.No.4 Chromatogram of Trial 3

Table 6: Chromatogram of Trial 3

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.370	28.90981	9072	0.61	-
2	8.052	887.38696	13744	0.85	16.04

Observation: High RT. Chromatogram not acceptable.
Conclusion: Method rejected

❖ **List of QbD Trials :**

Table. 7: Transaction factor levels for FFD response

Levels	Methanol	Flow rate
-1	80	0.8
0	81	0.9
+1	82	1.

Preparation of standard stock solution:-
Tivozanib standard stock solution: (Stock I)
An accurately weighed quantity, 10 mg of Tivozanib (TVZ) was dissolved in methanol in a 10 ml Volumetric flask and volume made up to 10 ml to produce a solution of 1000 ug/ml. and 15 min sonicate to dissolve it and remove the unwanted gas, further an aliquots portion of Tivozanib stock solution were mixed in volumetric flask in 10 ml and volume was adjusted up to mark with mobile phase from the resulting solution 0.1ml was transferred to 10 ml volumetric flask And the volume was made up to the mark with MEOH: Water (0.05% OPA), prepared in 80 ml MEOH and 20 ml Water (0.05% OPA))solvent.

HPLC used for chromatographic condition applies on the Preparation of standard solution:-

Preparation of std. Tivozanib solution: (Stock I)
An accurately weighed quantity, 10 mg of Tivozanib (TVZ) was dissolved in methanol in a 10 ml volumetric flask and volume made up to 10.0 ml to produce a solution of 1000 ug/ml. From the freshly prepared standard stock solution (1000 ug/ml), 0.05-0.25 ml stock solution was pipetted out in 10 ml of volumetric flask and volume was made up to 10 ml with mobile phase to get final concentration of 5-25 ug/ml.

Selection of mobile phase:
Each mobile phase was vacuum degassed and filtered through 0.45µ membrane filter. The mobile phase was allowed to equilibrate until 0.05% OPA by baseline was obtained. The standard solution containing mixture of Tivozanib was run with different individual solvents as well as combinations of solvents were tried to get a good separation and stable peak. From the various mobile phases tried, mobile phase containing MEOH and Water (0.05% OPA) was selected since it gave sharp, well resolved peaks with symmetry within the limits and significant reproducible retention time for Tivozanib. Chromatograms of Tivozanib

Calibration Experiment

- **HPLC Method :**

a) Preparation of Calibration curve standard:

The above standard stock solution (5-25 µg/ml) of Tivozanib was diluted with mobile phase to yield five calibration curve (cc) standards with concentrations of 5,10,15,20 and 25 µg/ml of Tivozanib

- **UV Spectrophotometric method:**

a) Selection of detection Wavelength:
Standard solutions were scanned in the range of 200-400 nm , against 10 ml MEOH and volume make with water solvent system as reference Tivozanib were showed absorbance maxima (lambda max) at 220 nm respectively.

b) Calibration standard drug and regression equation data:
From the standard stock solution of Tivozanib, different concentration were prepared respectively in the range of 5 - 25 µg/ml for of Tivozanib measured at 220 nm. The calibration curves were plotted and Regression equation data presented

c) Calibration runs and regression analysis:
These calibration standard solutions were analyzed in three replicates using the under mentioned chromatographic conditions.

- Analytical column: Agilent C18 Column (250 mm x 4.6mm, 5µm particle size).
- Injection volume : 20µl.
- Flow rate : 0.8 ml/min.
- Mobile phase : MEOH: Water (0.05% OPA) (80:20 % V/V).
- Detection : 220 nm.

At the end of the calibration runs, the chromatograms of CC standards were processed to give the peak areas for of Tivozanib. Least square linear regression analysis was used to define the functional relationship between the two variables-peak area of the drug (Y-axis) and concentration of the corresponding

Validation of method for analysis of Tivozanib
The developed method was validated as per ICH guidelines.

Linearity:
Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range,

Determination:
The linearity of the analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range. Area is plotted graphically as a function of analyte concentration.

Acceptance Criteria:

- The plot should be linear passing through the origin. Correlation Coefficient should not be less than 0.999. The Result are shown in;
- **Preparation of standard stock solution for linearity**:

Weight 10 mg of Tivozanib were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. Sonicated for 10 min with occasional swirling. 0.1 ml of this solution diluted up to 10 ml volumetric flask with diluents was added to make up the volume.

Preparation of linearity solution:
A series of standard preparations of working standard of were prepared**.**

Table 8: Table of linearity for RP -HPLC Method

Concentration	
Sr.No	**Tivozanib**
1	5
2	10
3	15
4	20
5	25

Accuracy (recovery):
The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte. The accuracy of an analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay,
Acceptance Criteria:
Mean recovery should be in the range of 98-102%.
The Relative Standard Deviation should not be more than 2.0%.

- **Preparation of standard stock solution:**

10 mg of Tivozanib working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume 0.1 ml of this solution diluted up to 10 ml with diluent

- Application of proposed method for analysis of Capsule formulation:
- Accuracy

Table. 9: Table of Accuracy for HPLC Method

Sample	Amount added
Accuracy 80%	8
Accuracy 100%	10
Accuracy 120%	12

Repeatability:
Precision of the system was determined with the sample of RP-HPLC for. Six replicates of sample solution containing 25 µg/ml of Tivozanib were injected and peak areas were measured and %RSD was calculated.is was repeated for five times
Precision:
Precision of an analytical method is the degree of agreement among Individual test results when the procedure is applied repeatedly to multiple Samplings of a homogenous sample. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation.

- Result of Intraday and Inter day Precision studies on RP-HPLC for Tivozanib

Intra-day precision:
Sample solutions containing 10 mg of Tivozanib three different concentration (5µg/ml, 15µg/ml, and 25µg/ml) concentration of Tivozanib, Tivozanib were analyzed three times on the same day and %R.S.D was calculated.
Inter-day precision:
Sample solutions containing 10 mg of Tivozanib three different concentration (5µg/ml, 15µg/ml, and 25µg/ml) concentrations Tivozanib. Tivozanib were analyzed three times on the next day and %R.S.D was calculated.

- Acceptance criteria:

The Relative Standard Deviation should not be more than 2% for test

Robustness:
Preparation of standard stock solution:
15 µg/ml of Tivozanib working standards were weighed and transferred to 10 mL volumetric flask & diluent was added to make up the volume. 0.15 ml of this solution diluted up to 10 ml with diluent. The mobile phase composition was changed in (±1 ml/ min^{-1}) proportion

Detection Limit
Where,
σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve and S.D. was used should be calculated from the y- intercepts of regression line in calibration curve.

Quantitation Limit

Based on the S.D. of the response and the slope of calibration curve, the quantitation limit (QL) was calculated as

Where,

σ = the S.D. of the y-intercepts of regression lines. S = the slope of the calibration curve.

$$QL = \frac{10\sigma}{}$$

Analysis of marketed formulation

To determine the content of Tivozanib in marketed Capsules (label claim 10 mg of Tivozanib), 20 Capsules powder weighed in 107.8 Gms and average weight of powder was calculated in 0.5.39 gms Capsules powder equivalent to weigh in 40.22 mg the drug was extracted from the Capsules powder with 10 mL MEOH.

5. RESULT AND DISCUSSION:

Preliminary studies on Tivozanib

Melting point

The procured reference standard of Tivozanib was found to melt in the range of 220-233^{0}C respectively.

Solubility

The drug was found to be

- Freely soluble in Methanol,Acetonitrile,DMSO,water
- Insoluble in ether

UV Spectroscopy

UV absorption of 10 µg/mL solution of Tivozanib in Methanol was generated and absorbance was taken in the range of 200-400 nm. λmax of Tivozanib in Methanol was found to be 220 nm.

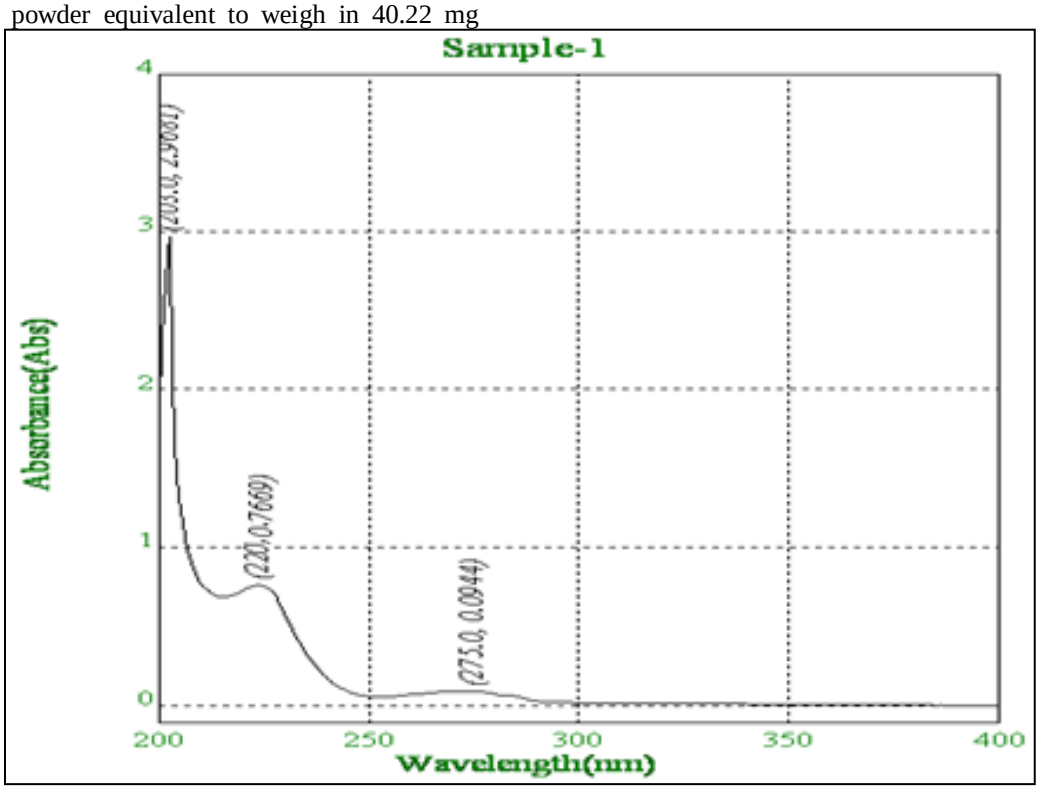

Fig No.5: UV Spectrum of Tivozanib

Studies on the chromatographic behaviour of Tivozanib

Table 10: Chromatographic behavior of Tivozanib mobile phase of various compositions.

Fig. No.	Column used	Mobile phase, Flow Rate and Wavelength	Observation	Conclusion
1	C_{18}(Agilent (250 ×4.6mm, 5.0µ)	90% MEOH +10% 1% Acetic Acid Water, Flow 0.7,220 nm.	Sharpe peaks were not obtained (splitting)	Hence rejected
2.	C18(agilent) (250 ×4.6mm, 5.0µ)	80% MEOH +20% 1% Acetic Acid Water, Flow 0.7,220 nm .	Sharpe peaks were not obtained	Hence rejected
3	C18(agilent)(250 ×4.6mm, 5.0µ)	70% MEOH +30 %(0.05% OPA) Flow 1,220 nm.	Sharpe peaks were not obtained	Hence rejected
4	C_{18}(agilent)(250 ×4.6mm, 5µ)	50% MEOH +50% (0.05% OPA) Water, Flow 1, 220 nm .	Sharpe peaks were not obtained	Hence rejected
5	C_{18}(agilent)(250 ×4.6mm, 5µ)	40% MEOH +60% (0.05% OPA) Water, Flow 1, 220 nm	Sharpe peaks were not obtained	Hence rejected
6	C_{18}(agilent)(250 ×4.6mm, 5µ)	80% MEOH +20% (0.05% OPA) Flow 0.7, 220 nm.	Sharpe peaks were obtained	Hence selected

Thus, from the above, it has been observed that, using mobile phase of Methanol + Water (0.05% OPA) (80+20% v/v) 220 nm, 0.8 ml, gave adequate retention time at 4.280 min. with good peak shape (Theoretical plates of 6373 of Tivozanib).

Chromatogram of Final Trial:

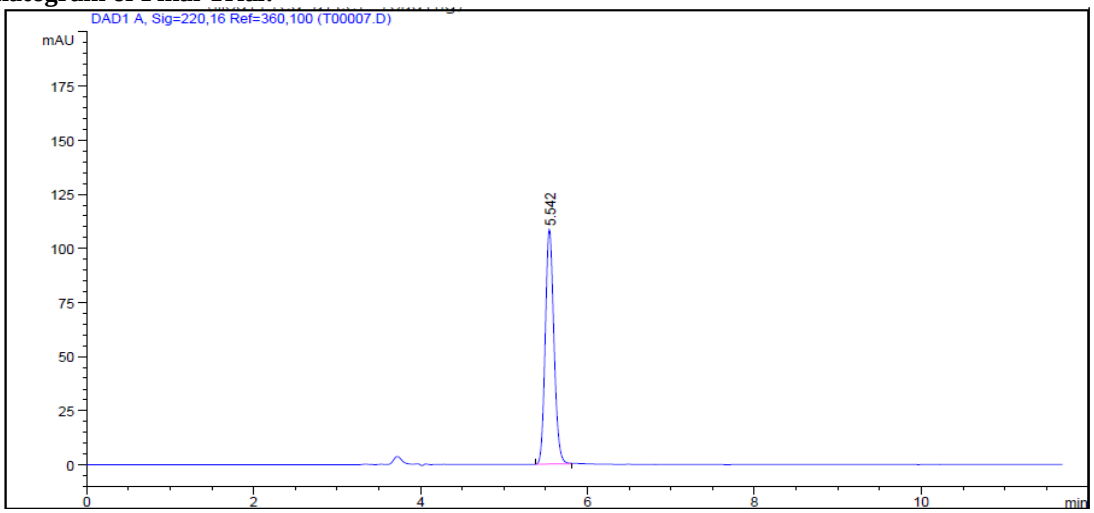

Fig.No 6: Representative Chromatogram of Tivozanib using 80 % Methanol + 20 0.05% OPA -220- 0.7ML- 20 MCG as mobile phase.

Table11: Chromatogram of Tivozanib using 80% Methanol+ 20 % 0.05% OPA

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	5.542	781.94818	14588	0.84	-

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR ANALYSIS OF TIVOZANIB:

Chromatogram of QBD Trial-1:

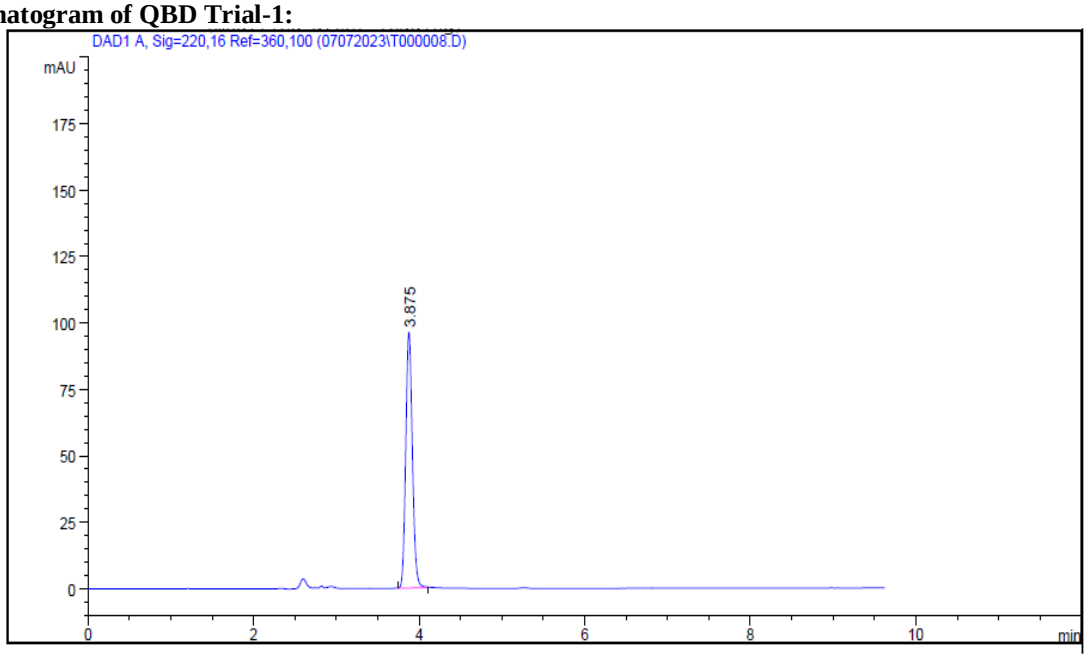

Fig.No 7: Representative Chromatogram of Tivozanib using 80 % Methanol 220nm- 0.9ML

Table 12: Chromatogram of QBD Trial

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.875	537.00903	11977	0.85	-

Chromatogram of Trial-2 :

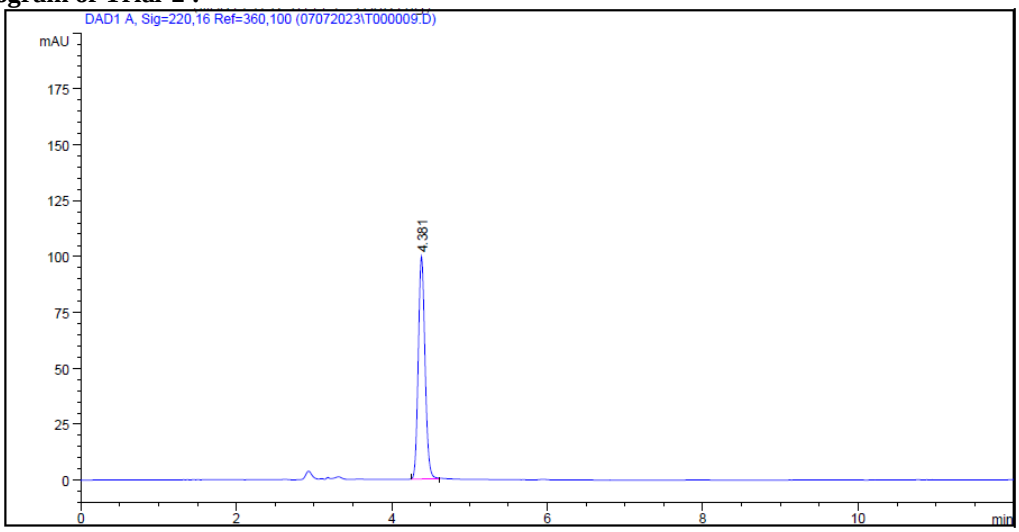

Fig.No 8: Representative Chromatogram of Tivozanib using 80 % Methanol 0.8Ml

Table 13: Chromatogram of QBD Trial-2

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.381	602.41718	12502	0.85	-

Chromatogram of Trial-3 :

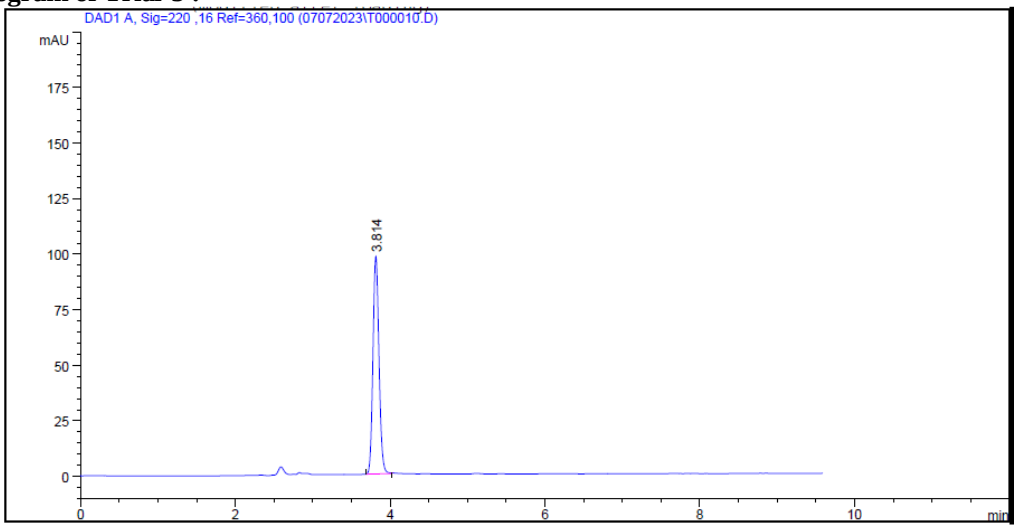

Fig.No 9: Representative Chromatogram of Tivozanib using 81 % Methanol - 0.9 ML

Table 14: Chromatogram of QBD Trial

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	3.814	531.31812	12012	0.86	-

Statistical data analysis (DOE)

The layout of actual design of DOE with the subsequent response results are shown in **table no.26** as given below,

Table 15: Layout of Actual Design of DOE

Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4
A:MeOH	**B:Flow Rate**	**RT**	**PA**	**TP**	**TF**
%	ml/min	min	AUC		
80	0.9	3.875	537.0090	11977	0.85
80	0.8	4.381	602.4171	12502	0.85
81	0.9	3.814	531.3181	12012	0.86
82	0.8	4.241	594.0190	13263	0.86
82	1	3.382	477.1806	11195	0.87
81	1	3.462	477.0061	11159	0.87
81	0.8	4.293	590.6330	12922	0.86
80	1	3.485	474.4526	10767	0.86

ANOVA for response surface Quadratic model

The analysis of variance (ANOVA) was performed to identify the significant and insignificant factors. The results of ANOVA for the retention time of DOE are as following

Table No- 16: ANOVA table for retention time of Tivozanib

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.15	5	0.2300	702.95	< 0.0001	significant
A-MeOH	0.0255	1	0.0255	77.88	0.0031	
B-Flow Rate	1.11	1	1.11	3406.63	< 0.0001	
AB	0.0003	1	0.0003	1.05	0.3817	
A^2	0.0001	1	0.0001	0.3751	0.5835	
B^2	0.0094	1	0.0094	28.82	0.0127	
Residual	0.0010	3	0.0003			
Cor Total	1.15	8				

The **Model F-value** of 702.95 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 17: Response 1: RT

Std. Dev.	0.0181	**R^2**
Mean	3.85	**Adjusted R^2**
C.V. %	0.4697	**Predicted R^2**
		Adeq Precision

The **Predicted R^2** of 0.9897 is in reasonable agreement with the **Adjusted R^2** of 0.9977; i.e. the difference is less than 0.2.

Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 67.191 indicates an adequate signal. This model can be used to navigate the design space.

Final Equation in Terms of Coded Factors

RT =+3.81-0.0652A-0.4310 B+0.0092AB-0.0078A^2-0.0687B^2

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

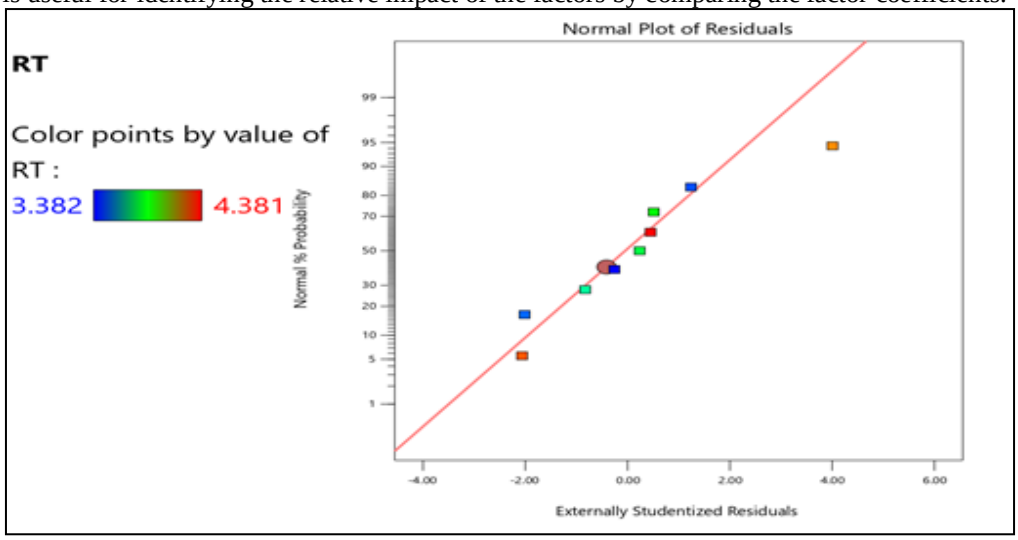

Fig. No 10: color point by value of retention time Residuals of Normal plot

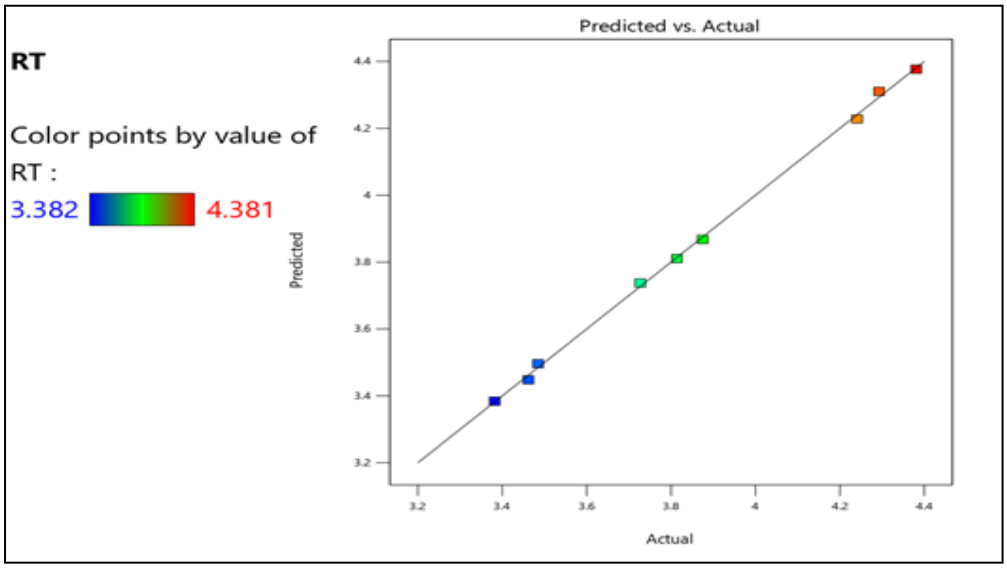

Fig.No- 11: color point by value of retention time Predicted vs Actual

Table 18: Response 2: PA

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	21485.55	2	10742.77	329.16	< 0.0001	Significant
A-MeOH	73.56	1	73.56	2.25	0.1840	
B-Flow Rate	21411.99	1	21411.99	656.07	< 0.0001	
Residual	195.82	6	32.64			
Cor Total	21681.37	8				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

Calibration experiment
RP-HPLC Method:
The data obtained in the calibration experiments when subjected to linear regression analysis showed a linear relationship between peak areas and concentrations in the range 5-25 µg/mL for Tivozanib

Table 19: Linearity data for Tivozanib

Method	Conc. µg/ml	Peak area(µV.sec)		Average peak area (µV.sec)	S.D. of Peak Area	% RSD of Peak Area
		1	2			
RP-HPLC Method	5	306.23	305.14	305.685	0.77	0.25
	10	717.2	719.77	718.485	1.82	0.25
	15	1142.18	1138.1	1140.14	2.88	0.25
	20	1607.44	1605.4	1606.42	1.44	0.09
	25	2032.3	2041.15	2036.725	6.26	0.31
	Equation		y = 87x-143.5			
	R^2		0.9999			

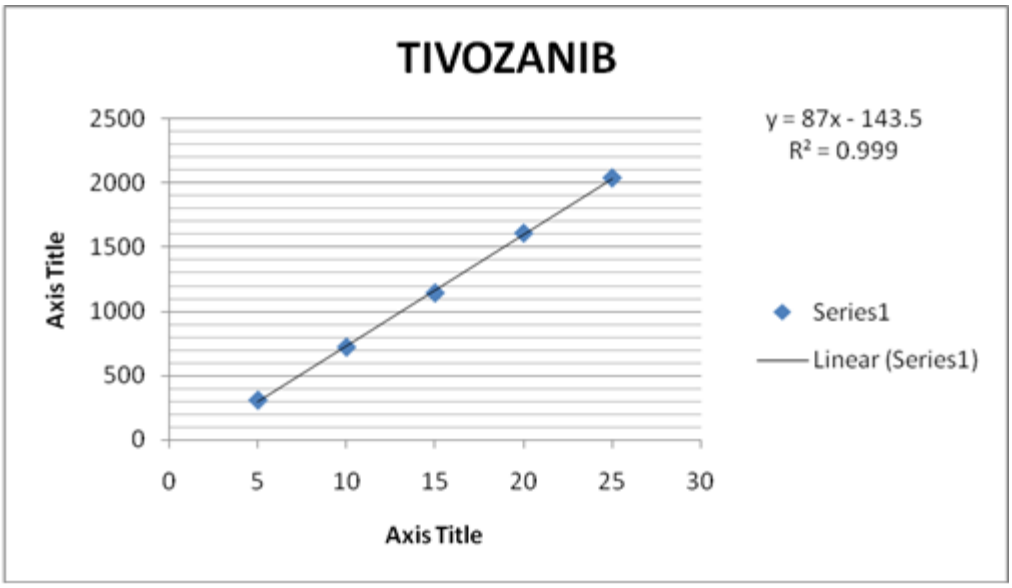

Fig.No.12: Calibration curve of Tivozanib

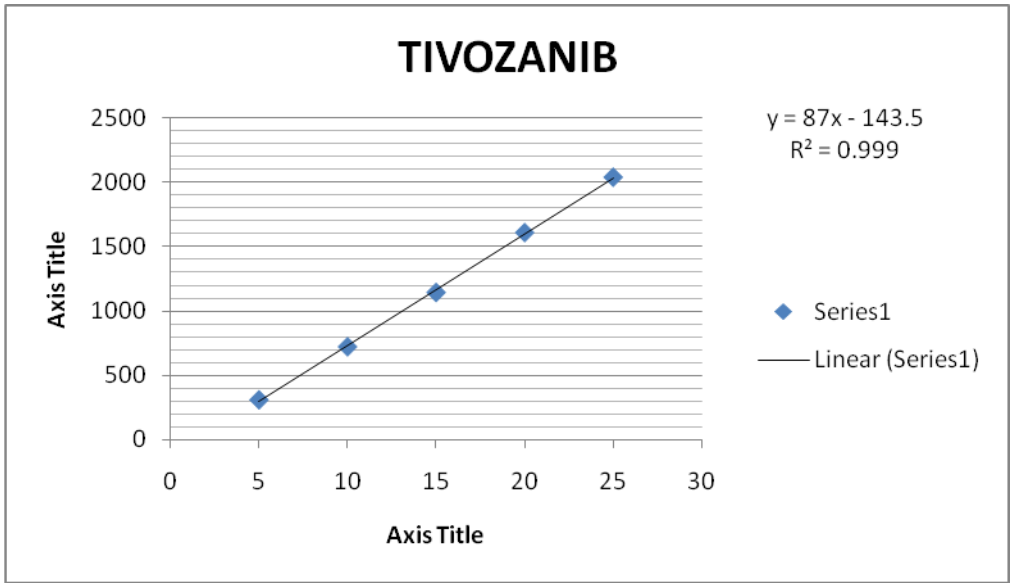

Fig.No.13. Calibration curve for HPLC method

Accuracy:-

Recovery studies were performed to validate the accuracy of developed method. To pre analyzed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed Statistical validation of recovery studies.

Accuracy 80%

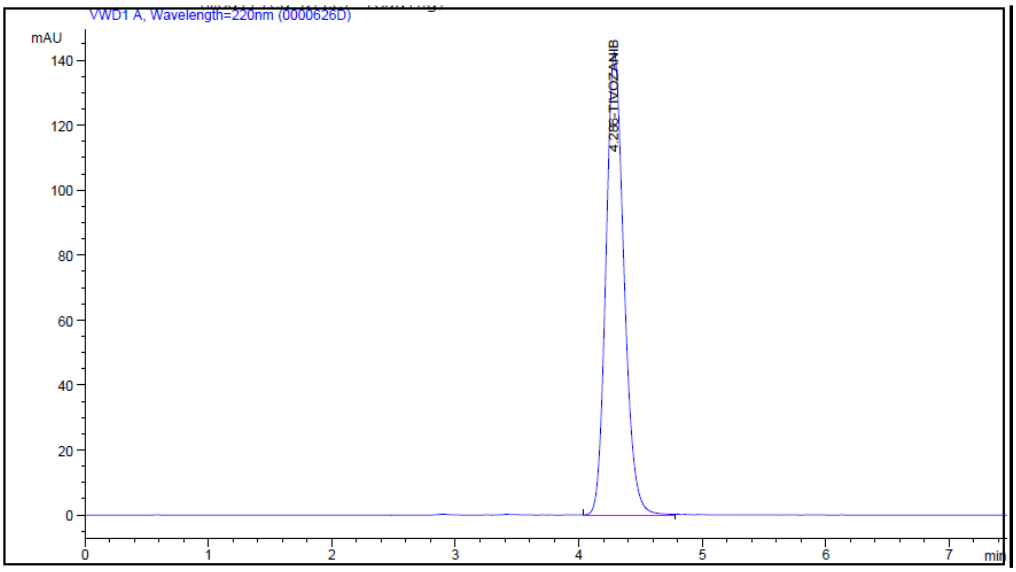

Fig. No 14: Chromatogram of Accuracy 80% -01

Table 20: Chromatogram of Accuracy 80% -01

No.	RT[min]	Area[mV*s]	TP	TF	Resolution
1	4.286	1432.96470	4308	0.80	-

6. SUMMARY AND CONCLUSION:

The present work deals with the Development and validation of RP-HPLC method using QbD approach for determination of Tivozanib by pure and Tablet dosage form.

Attempts were made to develop RP-HPLC method for estimation of Tivozanib from tablet. For the RP - HPLC Agilent (S.K) method Gradient System UV Detector and C18 column with 250mm x4.6 mm i.d and 5µm particle size methanol: Methanol+0.05% OPA (80+20% v/v) was used as the mobile phase for the method. The detection wavelength was 220 nm and flow rate was 0.8 ml/min. In the developed method, the retention time of Tivozanib were found to be 4.280 min.

The developed method was validated according to the ICH guidelines. The linearity, precision, range, robustness was within the limits as specified by the ICH guidelines. Hence the method was found to be simple, accurate, precise, economic and reproducible. So, it is worthwhile that, the proposed methods can be successfully utilized for the routine quality control analysis Tivozanib in bulk drug as well as in formulations..

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8. CONFLICTS OF INTEREST

Authors have no conflicts of interest to declare.

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