INTRODUCTION

Tivozanib, sold under the brand name Fotivda, is a medication used for the treatment of relapsed [1, 2] or refractory advanced renal cell carcinoma (RCC) [3-5]. It is an oral VEGF receptor tyrosine kinase inhibitor [6]. The most common side effects include fatigue [7], hypertension [8, 9], diarrhea, decreased appetite [10], nausea, dysphonia [11], hypothyroidism [12, 13], cough [14], and stomatitis [15]. Tivozanib must not be combined with St. John's Wort, an inducer of the liver enzyme [16, 17] CYP3A4. It should not be taken during pregnancy as it is teratogenic [18, 19], embryotoxic and fetotoxic in rats. Administration of a single dose of tivozanib with rifampicin, a strong inducer of the enzyme CYP3A4 [20, 21], 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. The clinical significance of these findings is not known. A quinoline urea derivative, tivozanib suppresses angiogenesis [22, 23] by being selectively inhibitory against vascular endothelial growth factor (VEGF) [24, 25]. It is designed to inhibit all three VEGF receptors [26, 27]. After tivozanib is taken by mouth, highest blood serum levels are reached after 2 to 24 h. The total AUC is independent of food intake. When in the bloodstream, over 99% of the substance are bound to plasma proteins, predominantly albumin. Although the enzymes CYP3A4 and CYP1A1 [28] and several UGTs are capable of metabolising the drug, over 90% circulate in unchanged form. The metabolites are demethylation, hydroxylation and N-oxidation products and glucuronides [29]. The biological half-life is 4.5 to 5.1 d; 79% being excreted via the faeces, mostly unchanged, and 12% via the urine, completely unchanged. Tivozanib is used in form of the hydrochloride monohydrate. The aim of the study is to estimate the plasma ingredient Tivozanib by using RP-HPLC.

MATERIALS AND METHODS

Chemicals

Acetonitrile, HPLC-grade formic acid, water were purchased from Merck India Ltd, Mumbai, India. API of Tivozanib standard was procured from Glenmark, Mumbai.
Till today there are no HPLC methods were reported in the literature. Hence we developed a method for the quantification of Tivozanib. The developed HPLC method was utilized for the estimation of the drug by in vitro method. Different extractions were tried using acetonitrile, methanol, and dimethylformamide.

Validation procedure
The analytical parameters such as system suitability, precision, specificity, accuracy, linearity, robustness, LOD, LOQ, forced degradation and stability were validated according to ICH Q2 (R1) guidelines [31-34].

Preparation of buffer
1 ml of formic acid is dissolved in 1 lt of HPLC grade water and filter through 0.45 µ filter paper.

Chromatographic conditions
The HPLC analysis was performed on reverse phase HPLC system with isocratic elution mode using a mobile phase of acetonitrile and 0.1% formic acid (50:50 v/v) and X-bridge phenyl (150x4.6 mm, 3.5 µ) column with a flow rate of 1 ml/min.

Diluent
Mobile phase was used as diluent.

Preparation of the standard solution
For standard stock solution preparation, add 70 ml of diluents to 13.4 mg of Tivozanib taken in a 100 ml volumetric flask and sonicate for 10 min to fully dissolve the contents and then make up to the mark with diluent. 5 ml of solution is drawn from the above normal stock solution into a 50 ml volumetric flask and diluted up to the level.

Preparation of the sample solution
For sample solution preparation, add 70 ml of diluents to 52.8 mg of Tivozanib sample (each tablet contains 1.34 mg of Tivozanib) taken in a 10 ml volumetric flask and sonicate it for 20 min to fully dissolve the contents and then make up to the mark with diluent. 1 ml of solution is drawn from the above sample stock solution into a 10 ml volumetric flask and diluted up to the level.

RESULTS AND DISCUSSION
The main analytical challenge during development of a new method was to separate active Pharma ingredients. In order to provide a good performance the chromatographic conditions were optimized.

System suitability
In System suitability injecting standard solution and reported USP tailing and plate count values are tabulated in table 1 [35].

| System suitability parameter | Acceptance criteria | Tivozanib |
|-----------------------------|---------------------|----------|
| USP Plate Count             | NLT 2000            | 4257     |
| USP Tailing                 | NMT 2.0             | 1.21     |
| USP Resolution              | NLT 2.0             | -        |
| % RSD                       | NMT 2.0             | 1.35     |

![Fig. 2: Chromatogram of standard](image)

![Fig. 3: Chromatogram of blank](image)
Specificity
In this test method placebo, standard and sample solutions were analyzed individually to examine the interference. The below fig. shows that the active ingredient was well separated from blank and their excipients and there was no interference of placebo with the principal peak. Hence the method is specific.

Linearity
The area of the linearity peak versus different concentrations has been evaluated for Tivozanib, as 10, 25, 50, 75, 100, 125, 150 percent respectively. Linearity was performed in the range of 1.34-20.1µg/ml of Tivozanib. The correlation coefficient achieved was greater than 0.9991.

Table 2: Linearity of tivozanib

| S. No. | Conc. µg/ml | Tivozanib area count |
|--------|-------------|---------------------|
| 1      | 1.34        | 371255              |
| 2      | 3.35        | 826268              |
| 3      | 6.70        | 1703314             |
| 4      | 10.05       | 2350807             |
| 5      | 13.40       | 3059642             |
| 6      | 16.75       | 4068593             |
| Corr. coef |            | 0.99910            |
| Slope  |             | 236568.29           |
| Intercep |             | 20236.13            |

Fig. 4: Calibration plot of tivozanib

Accuracy
In this method, Accuracy was conducted in triplicate by analyzing active pharma ingredient sample solution at three kinds of concentration levels of 50, 100 and 150% of each at a specified limit. The percentage recovery was measured and found to be within the limit. The accuracy and reliability of the developed method was established. The percentage recovery values were found to be in the range of 99.65-100.93% for Tivozanib. The results are given in table 3.

Table 3: Results of accuracy

| S. No. | % Level | Tivozanib % recovery |
|--------|---------|----------------------|
| 1      | 50      | 99.65                |
| 2      | 100     | 100.12               |
| 3      | 150     | 100.93               |
| mean   |         | 100.23               |
| SD     |         | 0.648                |

mean±SD (n=3)

Table 4: Intraday precision results of tivozanib

| Tivozanib | Conc.(µg/ml) | Area counts | % assay as is |
|-----------|--------------|-------------|---------------|
| 1         | 1.34         | 2948264     | 99.2          |
| 2         |              | 2949491     | 99.3          |
| 3         |              | 2937437     | 98.9          |
| 4         |              | 2944641     | 99.1          |
| 5         |              | 2931517     | 98.7          |
| 6         |              | 2946124     | 99.2          |
| % RSD     | 0.238        |             |               |
| mean      | 99.07        |             |               |
| SD        | 0.22509      |             |               |

mean±SD (n=6)
In method precision study prepare six different standard solutions in the concentration of Tivozanib (13.4 µg/ml) are injected into HPLC system. Tivozanib %assay found to be in the range of 99.74-100.63.

Intraday precision
Six replicates of a sample solution containing Tivozanib (13.4 µg/ml) were analysed on the same day. Peak areas were calculated, which were used to calculate mean, SD and %RSD values. These results are given below table 4.

Intermediate precision
Six replicates of the sample solutions were studied by various researchers, and on separate days different instruments were tested.

Inter-day precision
Six replicates of a sample solution containing Tivozanib (13.4 µg/ml) were analysed on a different day. Peak areas were calculated which were used to calculate mean, SD and %RSD values. The present method was found to be precise as the RSD values were less than 2% and also the percentage assay values were close to be 100%. The results are given in table 5 [36].

LOD and LOQ
The LOD concentration for Tivozanib was 0.017 µg/ml and s/n values is 6. The LOQ concentration for Tivozanib was 0.055 µg/ml and the s/n value was 27. The method is validated as per the ICH guidelines [37]. Results of LOD and LOQ were shown in table 6.
Table 5: Inter-day outcomes of tivozanib

| S. No. | Conc.(µg/ml) | Area counts | % assay as is |
|--------|--------------|-------------|---------------|
| 1      | 13.4         | 2938262     | 98.9          |
| 2      | 2936513      | 98.8        |
| 3      | 2947542      | 99.2        |
| 4      | 2954684      | 99.5        |
| 5      | 2931509      | 98.7        |
| 6      | 2976118      | 100.2       |

%RSD 0.554
Mean 99.22
SD 0.56362

Table 6: LOD and LOQ for tivozanib

| Tivozanib | LOD | LOQ |
|-----------|-----|-----|
| Concentration | s/n | Concentration | s/n |
| 0.017 µg/ml | 6   | 0.055 µg/ml | 27 |

Robustness

The conditions of the experiment were designed to test the robustness of established system intentionally altered, such as flow rate, mobile phase in organic percentage in all these varied conditions. Robustness results for Tivozanib found to be within the limit and results are tabulated in table 7 [38].

Table 7: Robustness data of tivozanib

| Parameter name | % RSD of tivozanib |
|----------------|--------------------|
| Flow minus (0.8 ml/min) | 0.17 |
| Flow plus (1.2 ml/min) | 0.06 |
| Organic minus (-10%) | 1.20 |
| Organic plus (+10%) | 0.49 |

Stability

The sample solution was kept at room temperature and at 2-8 °C up to 24 h. Then these solutions were pumped into the device and calculate the % of deviation from initial to 24 h [39]. There was no significant deviation observed and confirmed that the solutions were stable up to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for Tivozanib drug. The results are given below table 8.

Table 8: Stability results of tivozanib

| Stability | Tivozanib | % of deviation |
|-----------|-----------|----------------|
| Initial   | 98.9      | 0.00           |
| 6 h       | 98.5      | -0.40          |
| 12 h      | 98.5      | -0.40          |
| 18 h      | 95.5      | -3.44          |
| 24 h      | 92.2      | -6.77          |

Degradation studies

The Tivozanib sample was subjected into various forced degradation conditions to effect partial degradation of the drug. Studies of forced degradation have carried out to find out that the method is suitable for products of degradation [40]. In addition, the studies provide details about the conditions during which the drug is unstable, in order that the measures are often taken during formulation to avoid potential instabilities [41].
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