Development and Validation of an RP-HPLC Method for the Estimation of Tivozanib in Bulk and Pharmaceutical Dosage Forms

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Abstract:** In the sequential treatment landscape for advanced renal cell carcinoma (RCC), tivozanib (marketed as Fotivda) is a highly selective oral vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor (TKI) that has become an essential therapeutic drug. Its strong and specific suppression of VEGFR-1, VEGFR-2, and VEGFR-3 phosphorylation is the main component of its unique therapeutic profile. Tivozanib efficiently inhibits angiogenesis, the process of creating new blood vessels necessary for tumor growth and metastasis, by blocking these important receptors. At higher concentrations, it also shows inhibitory activity against other kinases like c-kit and platelet-derived growth factor receptor beta (PDGFR-β). This focused mechanism and its very long half-life of about 111 hours could permit a more consistent blockage of the VEGFR pathway compared to earlier-generation multi-targeted TKIs. The randomized, open-label, Phase III TIVO-3 research provides the crucial clinical data in favor of Tivozanib administration. In this study, tivozanib monotherapy (1.34 mg daily for 21 days on/7 days off) was directly compared to sorafenib in a difficult-to-treat patient group with advanced RCC that had relapsed or refractory and had failed two or three systemic regimens, including previous VEGFR inhibitors. By showing a statistically significant and clinically relevant improvement in progression-free survival (PFS), the TIVO-3 trial achieved its main goal. The median PFS for patients treated with tivozanib was 5.6 months, while the median PFS for patients treated with sorafenib was 3.9 months (Hazard Ratio [HR] 0.73; p=0.016). Additionally, Tivozanib produced a better objective response rate (ORR) of 18%, which was more than twice as high as the 8% that sorafenib produced. The strong increase in PFS and ORR demonstrated the treatment's therapeutic effect in this highly pre-treated context, even if overall survival (OS) did not significantly differ between the two groups. Tivozanib has a usually acceptable safety and tolerability profile, which makes it a good choice for long-term use. In clinical studies, weariness, diarrhea, decreased appetite, and—most importantly—dose-related hypertension are the most often reported side effects.

***Keywords:** Tivozanib, Fotivda, Tyrosine Kinase Inhibitor, VEGFR, Angiogenesis, TIVO-3, Progression-Free Survival, and Renal Cell Carcinoma.*

I.INTRODUCTION

Tivozanib is used as a white to light brown powder known as hydrochloride monohydrate. It is essentially insoluble in water and poorly soluble in ethanol, methanol, and aqueous acids. It is neither optically active nor hygroscopic. Tivozanib, sold under the brand name Fotivda, is a drug used for the treatment of relapsed or refractory advanced renal cell carcinoma (RCC). It is an oral inhibitor of VEGF receptor tyrosine kinase. Fatigue, hypertension, diarrhea, decreased appetite, nausea, dysphonia, hypothyroidism, coughing, and stomatitis are among the most frequent adverse effects. Because it is teratogenic, it should not be taken while pregnant [1]. A diverse collection of malignancies, renal cell carcinoma (RCC) has unique histological characteristics, molecular changes, prognoses, and therapeutic responses [2]. The Food and Drug Administration (FDA) prohibited the anti-angiogenic tyrosine kinase inhibitor tivozanib (Fotivda) from entering the US market. On the other hand, the European Medicines Agency (EMA) approved it for the treatment of adult renal cell carcinoma. The following study aims to (i) critically assess the evidence supporting the approval of tivozanib and (ii) analyze the diffusion of this evidence in the literature through a citation analysis, in light of the regulatory authorities' contradictory decisions [3,4]. Renal cell carcinoma (RCC) is the most common type of cancer of the urinary system. RCC can arise as a second primary cancer (second RCC) or as the original cancer (first primary RCC, first RCC). The incidence of the second RCC ranges from 10.9 to 28.9%, making it one of the most common SPMs. Leukemia, non-Hodgkin's lymphoma, and kidney cancer were revealed to share the five most common components of SPCs. Following prostate cancer, the three most common SPCs (lung, bladder, and colorectal cancers) and the majority of other primary malignancies had the same rankings and proportions in both groups. Consistent SPC trends are observable [5]. VHL mutation-HIF upregulation-VEGF transcription pathway is the main one connected to the development of renal cell cancer. Vascular endothelial growth factor receptors (VEGFR receptors) are a key target for tyrosine kinase inhibitors, which suppress the growth of tumors. Its primary function is to inhibit the phosphorylation of vascular endothelial growth factor receptors (VEGFR)-1, VEGFR-2, and VEGFR-3. Additionally, it suppresses other kinases such as platelet-derived growth factor beta (PDGFR) and c-kit [6]. About 80–90% of kidney cancer cases in the US are renal cell carcinoma (RCC), which is responsible for thousands of fatalities each year. The most prevalent subtype of RCC, accounting for 70–80% of diagnoses, is clear cell RCC. Since the 2005–2006 approval of kinase inhibitors such as sorafenib and sunitinib, the standard of therapy for advanced RCC has undergone significant change. Since then, a number of other immune checkpoint inhibitors (such nivolumab and pembrolizumab combos) and kinase inhibitors (including pazopanib, axitinib, and cabozantinib) have been authorized for use as first- and second-line therapies [7].

Fig. 1: Structure of tivozanib

Monograph:

- Generic name: Tivozanib hydrochloride
- Brand name: Fotivda
- Drug class: Anti-angiogenic agent; Tyrosine kinase inhibitor (TKI)
- Synonyms / codes: AV-951, KRN-951
- Chemical class: Small-molecule tyrosine kinase inhibitor (VEGFR inhibitor). (Primary sources: FDA label; DrugBank) [8,9,10].
- Chemical formula: $C_{22}H_{19}ClN_4O_5$
- Molecular weight: 438.86 g/mol
- IUPAC name: 1-[(6-chloro-1H-indol-2-yl) carbonyl]-4-[7-methoxy-2-(3-methoxyphenoxy) quinazolin-4-yl] piperazine [11,12].
- Mechanism of action:

  VEGF secretion by tumor cells

  ↓

  Binding to VEGFR-1, VEGFR-2, VEGFR-3

  ↓

  Activation of tyrosine kinase signaling

  ↓

  Angiogenesis → Tumor growth

  ↓

  Tivozanib inhibits VEGFR phosphorylation

  ↓

  Blocks angiogenic signaling

  ↓

  Reduced tumor vascularization and growth [13].

Uses:
Advanced renal cell carcinoma (RCC) is the main condition for which tivozanib, an oral vascular endothelial growth factor receptor (VEGFR) tyrosine kinase inhibitor (TKI), is utilized. By specifically suppressing VEGFR-1, VEGFR-2, and VEGFR-3, it prevents angiogenesis and the formation of tumors.

Approved Uses:
Adult patients with advanced renal cell carcinoma (RCC) should start taking tipozanib as their first line of treatment. With the exception of other VEGFR-targeted medications, it is also authorized for patients who have already had one systemic treatment for RCC.

Investigational or Off-Label Uses:
Combination Therapy: To improve the therapeutic response in metastatic RCC, tivozanib is being tested in conjunction with immune checkpoint inhibitors such nivolumab.
Other Solid Tumors: Research is investigating tivozanib's possible use in the treatment of colorectal, ovarian, and hepatocellular carcinomas, where VEGF-driven angiogenesis is important [14,15].

II.MATERIALS AND METHODS

Equipment:
- Sonicator:

A sonicator is an ultrasonic apparatus that creates ultrasonic cavitation in liquids by transforming electrical energy into mechanical vibrations. An ultrasonic generator, piezoelectric transducer, probe (horn), sample vessel, and cooling system make up its essential components [15]. To agitate liquids for processes including cell disruption, mixing, emulsification, or particle size reduction, a sonicator uses ultrasonic waves. Through the use of a transducer and probe, electrical energy is transformed into high-frequency mechanical vibrations. Strong local energy is produced via cavitation, which is the development and violent collapse of microbubbles caused by these vibrations. This energy disperses particles, breaks up cells, and degasses or homogenizes liquids. Amplitude, duration, and pulse mode are important characteristics to regulate intensity and avoid overheating [16,17,18,19].

Fig. 2: Diagram of sonicator

- UV-Visible spectroscophotometer:

UV-Visible spectroscophotometer analyzes a sample's electronic transitions by measuring how much ultraviolet or visible light it absorbs. A monochromator selects a certain wavelength of light from a UV and visible bulb, which is then sent through the sample in a cuvette. A photodetector detects the transmitted light after molecules in the sample absorb light at specific wavelengths. Both qualitative and quantitative analysis are made possible by the relationship between absorbance and concentration as established by the Beer-Lambert law. A spectrum of absorbance versus wavelength is the end result, which provides details about the concentration, conjugation, and molecule structure [20,21,22,23,24].

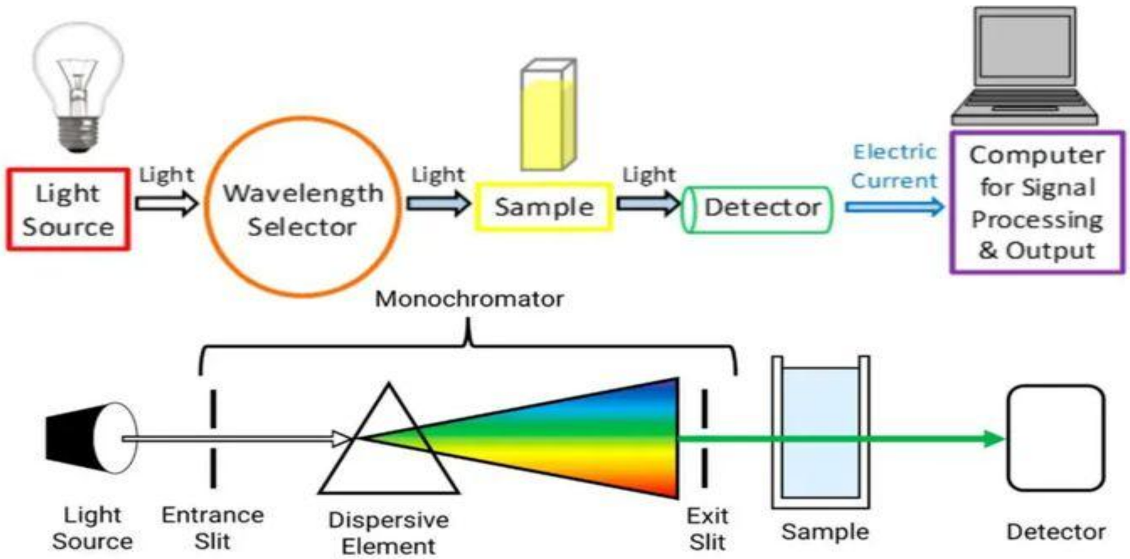

Fig.3: Diagram of UV-Vis spectroscophotometer

- High-Performance Liquid Chromatography (HPLC):

Components in a mixture can be identified, separated, and quantified using High-Performance Liquid Chromatography (HPLC). After the sample is injected into a stationary phase-containing column, it is carried through by a liquid mobile phase that is under high pressure. Separation results from the many ways that components interact with the stationary phase. A chromatogram is created by measuring the eluted chemicals using a detector. Identification is aided by retention time, and quantification is made possible by peak area or height. Pharmaceutical, environmental, food, and biological analyses all make extensive use of HPLC [25,26,27,28,29].

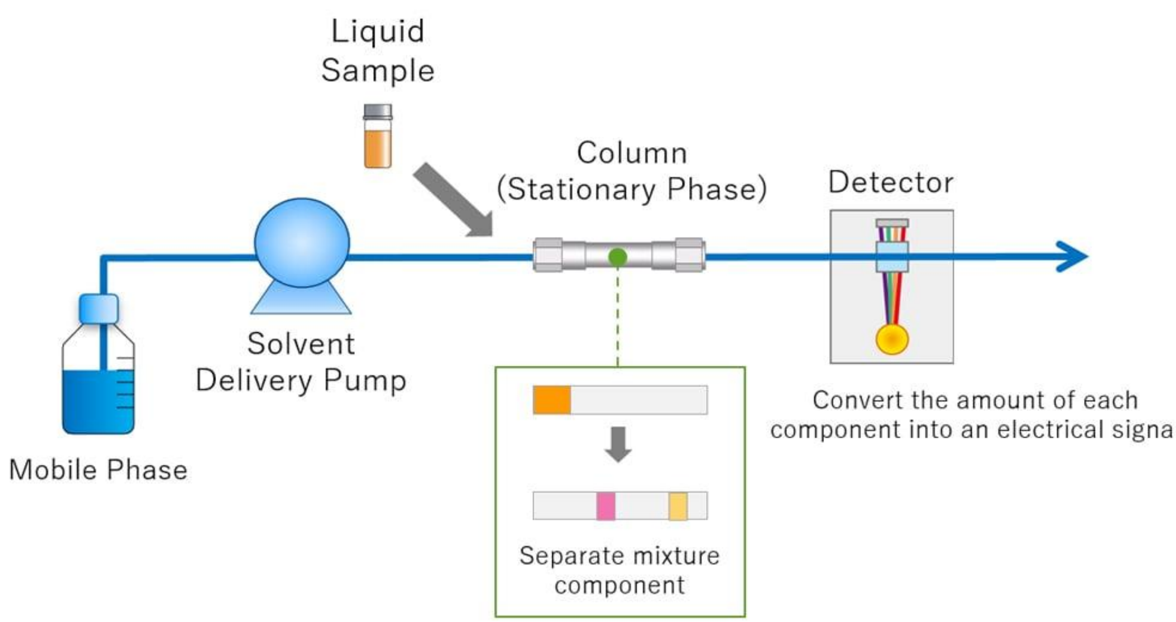

Fig.4: High-Performance Liquid Chromatography (HPLC)

Chromatographic condition:
The set of particular parameters and settings utilized to perform a chromatographic separation is referred to as chromatographic conditions. How well the analyte (compound of interest) is separated, identified, and measured during analysis depends on these factors [30,31,32,33].

Selection of wavelength:
The process of determining the wavelength of light a material (analyte) absorbs the most in order to detect and quantify it in spectroscopic or chromatographic analysis (such as UV or HPLC with UV detector) is known as wavelength selection. It entails determining the ideal wavelength at which the molecule exhibits the highest absorbance (λmax) and the least amount of interference from solvents or other substances [34,35].

Validation procedure:
The methodical process of demonstrating that an analytical approach or method is appropriate for its intended use and continuously yields accurate and dependable findings. Put more simply, it is the methodical procedure used to verify that a method produces precise, accurate, and repeatable measurements [35,36,37,38,39].

System suitability:
A series of checks made to make that the analytical system is operating correctly either before to or during sample analysis. It confirms that the system is operating at a level suitable for the planned analysis. System appropriateness, to put it simply, verifies that your GC, HPLC, or other analytical device is prepared and equipped to generate accurate findings. The following are typical system suitability parameters: The USP plate count (efficiency) gauges the effectiveness of the column. Checks peak symmetry with the USP tailing factor. %RSD of duplicate injections: a measure of accuracy Before evaluating Tivozanib, for instance, system appropriateness makes sure the chromatographic technology separates the medication precisely and consistently [40].

Specificity:
The capacity of an analytical technique to measure and unambiguously identify the target analyte even when it is accompanied by other potentially present components, such as contaminants, degradation products, excipients, or matrix components [35].

Accuracy:
The degree to which the value determined by the analytical procedure and the actual or recognized reference value agree. To put it another way, accuracy indicates how true or accurate your measurement. Accuracy in RP-HPLC analysis of Tivozanib, for instance, is assessed by comparing the amount of medication found in a sample to the actual amount added. Typically, it is stated as a percentage of recovery [35,41.42].

Precision:
How well a set of measurements taken from several samplings of the same homogenous sample under certain conditions agree with one another. To put it another way, precision indicates how reliable or consistent your findings are when the same sample is measured more than once. To assess precision in RP-HPLC analysis of Tivozanib, for instance, the same concentration of Tivozanib is injected repeatedly, and the percentage RSD (relative standard deviation) is calculated. A low percentage RSD denotes great precision [35,43,44,45,46].

Linearity and range:
The capacity of an analytical technique to produce test findings that are exactly proportionate to the analyte concentration in the sample within a specified range is known as linearity. Put more simply, it indicates that the analytical signal (such as the peak area in RP-HPLC) rises proportionately to the drug's concentration (such as that of Tivozanib) [35].
Range is the difference between the sample's higher and lower analyte concentrations for which the analytical technique has shown satisfactory linearity, accuracy, and precision. Put more simply, it's the range of concentrations that the method consistently performs well. For instance: The validated range of Tivozanib is 10 µg/mL to 100 µg/mL if measured reliably by an RP-HPLC technique [35].

LOD and LOQ:
LOD is the lowest concentration of an analyte in a sample that, given the specified experimental conditions, can be detected but not necessarily measured. To put it another way, it indicates the

lowest amount of a medication (like tivozanib) that the technique can accurately identify [35].

The lowest quantity of analyte in a sample that can be quantitatively identified under the specified experimental conditions with appropriate precision and accuracy is known as the limit of quantification, or LOQ. Put more simply, it indicates the lowest drug concentration that the technique can accurately detect. LOD and LOQ, for instance, assist guarantee that the RP-HPLC analysis of Tivozanib can identify and measure even extremely low drug quantities in a sample [35].

Stress degradation:

Exposing a pharmacological molecule or product to harsh environments in order to assess its chemical stability and find possible degradation products. To put it simply, stress degradation illustrates how a medication (like tivozanib) degrades in the presence of extremes such as heat, light, acidic or basic environments, and oxidation. It aids in the creation of an analytical technique that indicates stability. Stress degradation, for instance, aids in confirming that the RP-HPLC method is stability-indicating, which means it can identify Tivozanib even in the presence of degradation products [35,47].

Robustness:

An analytical technique's dependability under typical operating settings is demonstrated by its capacity to withstand minor, intentional changes in method parameters and conditions. For instance, to verify robustness for RP-HPLC analysis of Tivozanib, one can adjust the flow rate (e.g., ±0.1 mL/min) or column temperature (e.g., ±5 °C) slightly and see if the retention duration, peak area, and resolution stay satisfactory [35,48].

III.DISCUSSION

The analytical evaluation of Tivozanib by RP-HPLC demonstrated high accuracy, precision, and specificity, confirming the method's suitability for routine pharmaceutical analysis. The linearity study indicated a direct correlation between concentration and peak area within the validated range, ensuring reliable quantification. Low values of LOD and LOQ confirmed the method's sensitivity for detecting even minimal drug concentrations. Stress degradation studies under acidic, basic, oxidative, and thermal conditions revealed that Tivozanib is moderately stable, validating the method as stability-indicating. The robustness test proved that small, deliberate changes in flow rate, temperature, or wavelength did not significantly affect analytical performance. These findings collectively suggest that the developed RP-HPLC method is simple, rapid, and reproducible for assessing Tivozanib in bulk and formulations. Moreover, the validated parameters comply with ICH guidelines, ensuring accuracy in quality control and stability testing during pharmaceutical development and regulatory evaluation

IV.CONCLUSION

The developed RP-HPLC method for Tivozanib was found to be accurate, precise, and robust as per ICH guidelines. It effectively determined the drug in bulk and formulations, proving stability-indicating and suitable for routine quality control and analytical evaluation in pharmaceutical research and development.

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