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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SORAFENIB BY RP-HPLC

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ABSTRACT

This review brings together current advancements in RP‑HPLC method development and validation for Sorafenib, covering both bulk drug and tablet dosage forms, and is further supported by short confirmatory experiments to highlight practical aspects. Most reported chromatographic methods rely on C18 columns operated under high organic mobile phases-commonly acidified acetonitrile-water or acetonitrile-methanol systems-combined with UV/PDA detection around 263-266 nm. These setups provide efficient separations with short analysis times, sharp and symmetrical peaks, and the throughput required for routine quality control. The development process typically focuses on fine‑tuning the mobile‑phase strength and pH, selecting detection wavelengths close to Sorafenib's absorption maxima, and ensuring that system suitability parameters are met for peak resolution and integrity. Validation data consistently align with ICH Q2 (R1) guidelines, demonstrating strong linearity (average $r^2 \approx 0.999$), high precision with repeatability and intermediate precision (%RSD <2%), accuracy within an acceptable range (≈98-102%), and low detection and quantification limits. Robustness tests, involving deliberate variations in flow rate, wavelength, and mobile‑phase composition, further confirm the stability and reliability of these methods. Experimental findings reinforce what is reported in the literature, particularly regarding the choice of detection wavelength, the importance of organic solvent strength, and precision standards. Overall, this work consolidates practical strategies for designing, validating, and transferring RP‑HPLC

methods for Sorafenib, offering a clear framework for their application in both quality control and stability studies.

KEYWORDS: RP-HPLC (Reversed-Phase High-Performance Liquid Chromatography), Sorafenib, method development, validation and literature trends.

INTRODUCTION

Sorafenib (SOR) is an orally active, small-molecule multikinase inhibitor designed to suppress both tumor cell growth and angiogenesis. It was the first systemic treatment to demonstrate improved survival in patients with advanced hepatocellular carcinoma (HCC), and it has also been shown to provide therapeutic value in advanced renal cell carcinoma and differentiated thyroid carcinoma. Belonging to the pharmacological class of multikinase inhibitors, sorafenib acts against intracellular RAF kinases, as well as receptor tyrosine kinases (RTKs) that are critical for angiogenesis and tumor progression. Its ability to block both RAF signaling within tumor cells and proangiogenic signaling at the cell surface underlies its combined antiproliferative and antiangiogenic actions, which were first validated in preclinical studies using in vitro experiments and xenograft models.[1,2]

On a mechanistic level, sorafenib targets and inhibits RAF-1 (C-RAF) and BRAF (including both wild-type and V600E mutant forms), thereby disrupting the Ras/Raf/MEK/ERK cascade. This inhibition reduces ERK phosphorylation, which in turn suppresses transcription programs driving tumor proliferation and cell survival. At the same time, sorafenib impedes signaling downstream of angiogenesis-related RTKs such as VEGFR-1/2/3 and PDGFR-β, leading to impaired endothelial cell activity, reduced vascular permeability, and diminished blood vessel support for tumors. Sorafenib also modulates other kinases, including FLT3, KIT, and RET, broadening its activity across tumor types. In the context of HCC, where aberrant activation of RAS/RAF/MAPK pathways and overexpression of VEGF/PDGF ligands are common, these dual mechanisms of action offer a strong therapeutic rationale. Beyond kinase inhibition, recent evidence indicates that sorafenib can trigger ferroptosis-an iron-dependent, lipid peroxidation-driven form of cell death-which contributes to its antitumor effects. However, resistance mechanisms such as NRF2-mediated upregulation of metallothionein-1G can counteract ferroptosis, reducing treatment efficacy and driving the development of combination approaches.[1-3]

Because of its broad kinase inhibition, sorafenib is associated with a distinct toxicity profile, often necessitating close clinical management. In HCC practice, treatment frequently requires dose modifications or discontinuation in certain patients due to adverse events. The most commonly reported toxicities include hand-foot skin reaction (HFSR), diarrhea, fatigue, rash, hypertension, and anorexia, along with laboratory abnormalities such as elevations in liver enzymes and lipase. VEGF pathway inhibition has been specifically implicated in vascular and dermatologic toxicities, while the blockade of multiple RTKs contributes to gastrointestinal and skin-related adverse effects. Moreover, post-marketing data highlight additional though less frequent risks, including hemorrhage, myocardial infarction, QT prolongation, and rare instances of hepatic failure, underscoring the importance of cardiovascular and hepatic monitoring. Dose interruption, reduction, and appropriate supportive therapies represent key strategies for managing these toxicities. Regulatory guidelines and labeling documents provide comprehensive recommendations regarding contraindications, warnings, drug–drug interactions, and dose adjustments, reflecting the accumulated clinical experience with sorafenib over time.[2-5]

Preclinical studies established the foundation for sorafenib's clinical application by consistently showing potent antitumor effects through its combined anti-proliferative and anti-angiogenic activities. In HCC cell lines such as PLC/PRF/5 and HepG2, sorafenib suppresses MEK/ERK phosphorylation, decreases cyclin D1 expression, and promotes apoptosis. It also exerts additional effects, including inhibition of eIF4E phosphorylation and downregulation of the anti-apoptotic protein Mcl-1, through mechanisms that extend beyond MEK/ERK inhibition. In animal models, dose-dependent tumor growth inhibition has been observed in HCC xenografts, accompanied by reduced MAPK signaling and decreased tumor microvessel density (e.g., reductions in CD34 staining), strengthening the mechanistic link between kinase targeting and antitumor efficacy. Biochemical profiling shows that sorafenib acts at low nanomolar concentrations against pivotal kinases such as RAF-1 (IC50 ≈6 nM), wild-type BRAF (≈22 nM), VEGFR-1 (≈26 nM), VEGFR-2 (≈90 nM), FLT3 (≈33 nM), KIT (≈68 nM), and PDGFR, emphasizing its multitargeted inhibitory capacity consistent with observed antitumor responses.[2,4,5]

From a therapeutic perspective, reviews consistently position sorafenib as a dual-action inhibitor acting on tumor-intrinsic RAF/MEK/ERK signaling and tumor vasculature via VEGFR/PDGFR blockade. This dual mechanism explains both its clinical efficacy and its

signature spectrum of toxicities. In the specific context of HCC, sorafenib has retained its importance due to its reproducible-though modest-survival benefits, its established role as a benchmark therapy against which new TKIs and immunotherapy-based combinations are compared, and its well-mapped resistance mechanisms that continue to shape combination treatment strategies. Ongoing research emphasizes treatment resistance driven by cellular plasticity, compensatory RTK activation, pathway rewiring, and suppression of ferroptosis. These insights have motivated clinical trials pairing sorafenib with agents designed to counteract angiogenic escape, overcome immune evasion, or exploit metabolic vulnerabilities. Collectively, sorafenib serves as a prototypical first-generation multi-kinase inhibitor whose dual impact on signaling pathways and tumor vasculature not only marked a milestone in HCC therapy but also continues to guide the development of next-generation therapeutic approaches.[1,5,6]

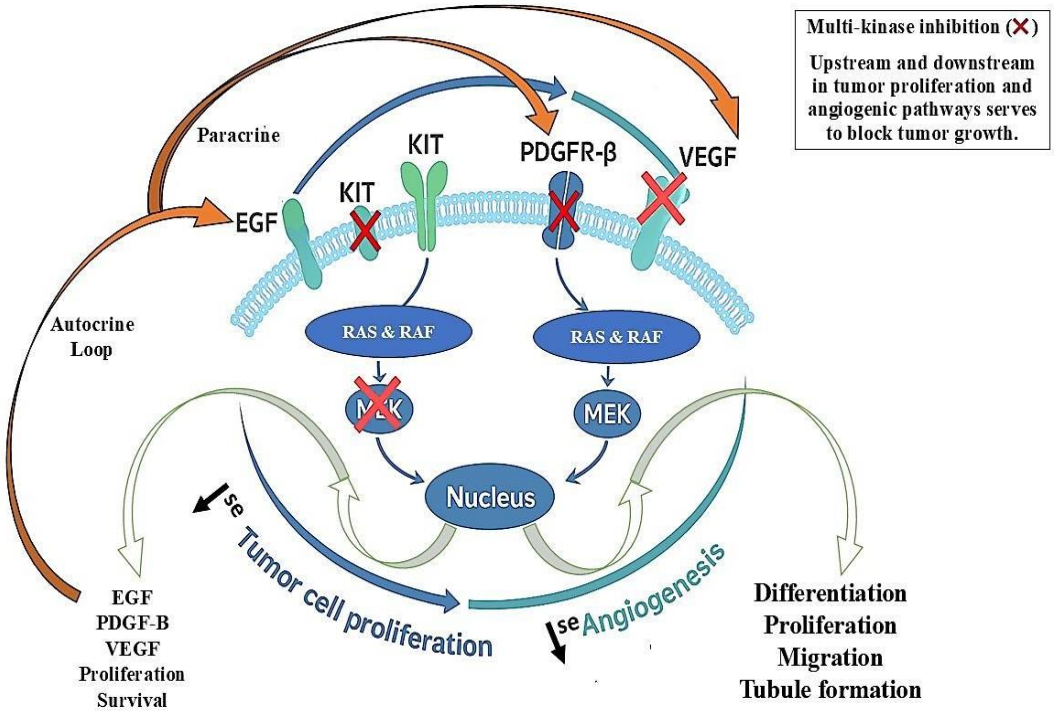

Fig. 1: Diagrammatic depiction of multi-kinase inhibition action of Sorafenib.

Chemistry of Sorafenib

Sorafenib (SOR), is chemically named as: 4-[4-({[4-chloro-3- (trifluoromethyl) phenyl] carbamoyl}amino)phenoxy]-N-methylpyridine-2-carboxamide. It has the molecular formula $C_{21}H_{16}ClF_3N_4O_3$ and a molecular weight of 464.4 g/mol **(Fig. 2)**.[7]

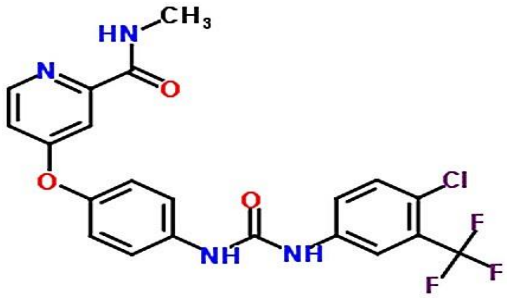

Fig. 2: Chemical structure of Sorafenib.

RP-HPLC: Concise Background for Drug Analysis and Evaluation

Reversed-phase high-performance liquid chromatography (RP-HPLC) continues to be one of the most widely applied techniques in pharmaceutical analysis due to its excellent selectivity, reproducibility, and strong compatibility with UV or PDA detection, particularly for small drug molecules. In this method, analytes are retained on a nonpolar stationary phase-most commonly a C18 column-through hydrophobic interactions, while a polar mobile phase composed of water and an organic solvent facilitates their elution. The retention of analytes depends on the factors such as polarity, stationary phase chemistry, and the pH and composition of the mobile phase. Retention time serves as a key identifier for compounds as well as a parameter for system suitability. During method development, initial "scouting" runs are often conducted on a C18 column under either isocratic or gradient elution, followed by fine-tuning of variables such as organic solvent concentration, pH and ionic strength of the buffer, and column temperature.

These adjustments aim to achieve sharp peaks, good symmetry, efficient resolution, and practical run times. Detection is typically optimized by selecting UV/PDA wavelengths close to the maximum absorbance (λmax) of the analyte to enhance sensitivity while minimizing background interference.[8,9]

Validation of RP-HPLC methods is guided by the International Council for Harmonisation (ICH) guideline Q2(R1), which outlines the essential performance parameters: specificity, linearity, precision (both repeatability and intermediate precision), accuracy, limits of detection and quantitation (LOD/LOQ), and robustness. The acceptance thresholds generally include a correlation/concentration coefficient (r^2) close to the 0.999 value, and the precision is expressed as %RSD which is not exceeding 2%, also the accuracy within the range of 98-102% value. Prior to sample analysis, system suitability is assessed through metrics such as a tailing factor ≤2, theoretical plate count greater than 2000, and %RSD of replicate injections ≤2%.[10,11]

For anticancer drugs such as Sorafenib, most RP-HPLC methods employ C18 stationary phases along with high organic proportions in the mobile phase, paired with UV detection between 263-266 nm. These setups provide short retention times and rapid throughput, making them ideal for routine quality control. Examples include methods using acidified acetonitrile–water systems (e.g., 80:20, with pH around 3.5), which deliver broad linear ranges (5-25 µg/mL) and very low LOD/LOQ values compliant with ICH criteria. Other approaches rely on acetonitrile-methanol mixtures that achieve even shorter run times while retaining high precision, selectivity, and accuracy for tablet formulations. Reported studies consistently demonstrate excellent linearity ($r^2 \approx 0.999$), recovery values close to 100%, precision well below 2% RSD, and robustness against small variations in detection wavelength, flow rate, or mobile phase composition. These features underscore the reliability of RP-HPLC for release testing and stability monitoring. Beyond sorafenib, broader reviews highlight the critical role of proper column selection, careful optimization of mobile-phase conditions, and strict validation procedures in ensuring that developed methods are both reliable and transferable across different drugs and testing laboratories. Collectively, this evidence explains why RP-HPLC remains the benchmark technique for drug quantitation, impurity profiling (especially when developed as stability-indicating assays), and overall quality control throughout the pharmaceutical product lifecycle.[12,13]

Literature review

Anwar et al. (2021) reviewed different mobile phase compositions for RP-HPLC, stressing the importance of organic solvents such as methanol and acetonitrile in combination with acidic modifiers to achieve optimal separation and peak symmetry. Their observations are especially relevant for drugs like sorafenib, where accurate quantification depends on carefully controlled chromatographic conditions.[14]

Sharma et al. (2020) examined the analytical challenges faced when working with multikinase inhibitors, identifying issues like peak tailing and poor resolution. They proposed approaches such as pH adjustment and selecting appropriate columns, both of which are key considerations when developing HPLC methods for sorafenib.[15]

Tan and Zhang (2020) in their study introduced a method which integrated the RP-HPLC along with the tandem mass spectrometry (HPLC-MS/MS) for the detection and evaluation of sorafenib and its metabolites. Their study highlighted the superior sensitivity of mass spectrometric detectors, especially for analyzing samples at very low concentrations,

suggesting a strong complement to conventional UV-based HPLC detection.[16]

Patel et al. (2019) outlined their process for optimizing RP-HPLC methods for anticancer drugs, showing how method parameters can be fine-tuned to improve sensitivity and specificity. Their findings directly support the requirements for sorafenib quantification, emphasizing the selection of suitable columns and mobile phases to ensure robust and reproducible assays.[17]

Iyer and Kumar (2019) focused on forced degradation studies to understand the stability and breakdown pathways of pharmaceutical compounds. They emphasized that RP-HPLC plays a critical role in evaluating degradation products, which makes this approach indispensable for sorafenib, a drug that requires rigorous stability assessment during development and quality control.[18]

Sharma and Singh (2018) assessed accuracy and precision metrics in RP-HPLC analysis, underlining the importance of minimizing variability during drug quantification. Their findings have direct application to sorafenib assay validation, where consistency in results is crucial.[19]

Gupta et al. (2017) investigated the stability of sorafenib under different environmental conditions and developed an RP-HPLC method to monitor its degradation products. Their work reinforces the necessity of stability-indicating methods to maintain the drug's therapeutic efficacy and safety throughout storage and use.[20]

Pande et al. (2017) had compared the effectiveness of the gradient and isocratic elution approaches involved in the RP-HPLC for the anticancer agents. They concluded that gradient elution provided superior resolution for complex formulations, particularly for separating sorafenib and its impurities.[21]

Chauhan et al. (2016) worked on designing stability-indicating HPLC methods for anticancer drugs including sorafenib. Their study demonstrated the ability of these methods to detect degradation under stress conditions such as exposure to heat, light, and oxidation, ensuring that sorafenib's stability profile can be reliably established.[22]

Kumar et al. (2015) presented a detailed discussion on method validation, highlighting its essential role in pharmaceutical analysis. They provided practical strategies for verifying

precision, accuracy, and robustness-key parameters for developing confidence in HPLC methods for sorafenib and similar drugs.[23]

Wang et al. (2015) devised an RP-HPLC method to quantify sorafenib in biological fluids such as plasma and urine. Their approach demonstrated strong sensitivity and specificity, proving valuable for therapeutic drug monitoring and pharmacokinetic studies aimed at improving clinical outcomes.[24]

Clark et al. (2014) emphasized the application of UV detection in HPLC, particularly for compounds like sorafenib that exhibit strong UV absorbance. Their results showed that careful wavelength optimization significantly enhances both sensitivity and specificity in drug quantification.[25]

Snyder et al. (2012) provided a broad overview of modern chromatographic techniques, with a focus on the adaptability and efficiency of RP-HPLC. Their work highlighted critical aspects such as choice of mobile phase, type of column, and detection parameters-foundational principles that inform method development for drugs like sorafenib.[26]

Llovet et al. (2008) carried out a pivotal study that established sorafenib's clinical relevance in advanced hepatocellular carcinoma. They demonstrated the drug's dual mechanism in inhibiting tumor cell proliferation and angiogenesis through targeted action on the Raf/MEK/ERK pathway and VEGFR signaling. This not only positioned sorafenib as a first-line therapy but also underscored the necessity of reliable analytical techniques to optimize dosage and monitor plasma levels effectively.[27]

Dong (2006) discussed the advantages of HPLC in drug research, pointing to its versatility in analyzing a wide range of compounds, including anticancer drugs like sorafenib. The work provided practical insights into method development, covering aspects such as solvent use, column chemistry, and gradient optimization, making it highly applicable for research on complex pharmaceutical agents.[28]

Development of Analytical Methods and Validation for Sorafenib by RP-HPLC Aim

Based on findings from the literature survey, the purpose of this study is to design and validate a reliable, rapid, accurate, precise, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method for the quantification of sorafenib (SOR) in both pharmaceutical formulations and biological matrices.

Objectives

1. **Optimization of RP-HPLC conditions:** To fine-tune chromatographic parameters-including the composition of the mobile phase, flow rate, selection of column, and detection wavelength-in order to achieve superior separation and precise quantification of sorafenib.
2. **Method validation:** To evaluate the performance of the optimized method by validating major analytical parameters such as accuracy, precision, specificity, linearity, detection limits, and robustness in accordance with ICH Q2(R1) guidelines.
3. **Application to pharmaceutical formulations:** To apply the developed method for routine analysis of sorafenib in dosage forms such as tablets, ensuring compliance with established quality standards.
4. **Stability studies:** To investigate the stability of sorafenib under different storage and stress conditions using the validated RP-HPLC method, thereby generating insights on its shelf life and proper handling in pharmaceutical formulations.[7,29-31]

A detailed review of previous research indicates that only a limited number of analytical methods are available for determining sorafenib in bulk material, pharmaceutical preparations, and biological fluids. These include UV spectrophotometry, RP-HPLC, HPTLC, and HPLC coupled with tandem mass spectrometry. However, many of these approaches are associated with drawbacks such as complicated sample preparation, excessive solvent usage, lengthy analysis times for biological samples, relatively low sensitivity, higher costs, and reduced peak symmetry. In light of these shortcomings, the present work emphasizes the development of a simple, economical, accurate, precise, and robust RP-HPLC method for reliable estimation of sorafenib in pharmaceutical formulations as well as in biological matrices.[7,29-31]

Materials and Methodology for Development of RP-HPLC Method for Sorafenib

Materials

The analytical work was carried out using sorafenib reference standard (purity ≥99%), certified by its certificate of analysis (CoA). Commercial tablet formulations of sorafenib (e.g., 200 mg per tablet) were selected, powdered, and used for assay experiments. HPLC-grade solvents such as acetonitrile, methanol, and water served as the primary eluents, while reagents including o‑phosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide were employed for pH adjustment and stress testing. Filtration of samples was achieved using 0.45 µm nylon membrane filters and Whatman filter paper. For monitoring and internal calibration in UPLC-based variants, prednisolone was considered as an internal standard.

Routine laboratory glassware such as Class-A volumetric flasks, calibrated micropipettes, and polypropylene vials were employed throughout the study.[7,11,29-31]

Instrumentation

Chromatographic separation was performed on an HPLC system equipped with an isocratic quaternary pump, either manual or auto-injector (20 µL loop), and a UV/PDA detector. Three commonly used C18 columns (250 × 4.6 mm, 5 µm) were evaluated: Phenomenex/Phenomenox, Thermo Scientific, and Grace/Inertsil ODS. The detection wavelength was selected around sorafenib's absorption maxima, typically 263–266 nm (λmax values screened at 263, 265.0, and 265.5 nm). Data acquisition and validation-related calculations were performed using standard vendor-supplied chromatography software.[7,11,29-31]

Chromatographic Conditions

Primary Method A (acidified aqueous phase, general applicability)

Column: Phenomenex C18 (250 × 4.6 mm, 5 µm); mobile phase-acetonitrile:water (80:20, v/v) adjusted to pH 3.5 with o‑phosphoric acid; flow rate 1.0 mL/min; column maintained at 25 ± 2 °C. Detection was carried out at 265 nm with a typical retention time (tR) of ~5.63 minutes.

Alternate Method B (organic–organic system, shorter retention time)

Column: Thermo Scientific C18 (250 × 4.6 mm, 5 µm); mobile phase-acetonitrile:methanol (40:60, v/v); flow rate 1.0 mL/min under ambient temperature. The detection wavelength value taken was 265.5 nm, with ~3.22 minutes as the retention time. This method achieved ~8700 theoretical plates with minimal asymmetry (~1.1).

Alternate Method C (modified wavelength and ratio)

Column: Grace/Inertsil ODS C18 (250 × 4.6 mm, 5 µm); mobile phase-acetonitrile:methanol (45:55, v/v); flow rate 1.0 mL/min at ambient temperature. Detection wavelength was 263 nm, producing retention times of ~3.82–3.84 minutes.[7,11,29-31]

Preparation of Solutions

Diluent: The mobile phase composition of the chosen chromatographic method was used as diluent to ensure stability and avoid peak distortion.

Standard stock solution: Accurately weighed quantities of sorafenib (10–100 mg) were transferred to a volumetric flask, dissolved in diluent (or acetonitrile), and made up to volume to yield ~1.0 mg/mL stock solution.

Working standards (linearity and assay): Serial dilutions were prepared from the stock to cover validated calibration ranges, commonly 5-25 µg/mL or, in some shorter-run methods, 2-10 µg/mL.

Sample preparation (tablet assay): Twenty tablets were finely powdered, and an amount equivalent to 100-200 mg of sorafenib was transferred into a 100 mL volumetric flask. Approximately 30-80 mL of acetonitrile or mobile phase was added, followed by sonication for 15-20 minutes. After cooling it, the volume was made up with the help of the diluent, then mixed thoroughly, and then-after filtered through a 0.45 µm sized membrane filter. Dilutions were performed as required to reach assay concentrations (generally ~15-20 µg/mL for injection).

System suitability testing: A mid-range concentration standard (10-20 µg/mL) was used to evaluate retention time, theoretical plate count, peak tailing, and %RSD across multiple injections (5-6), ensuring method reliability before sample analysis.[7,11,29-31]

Method Development

1. Wavelength screening

The UV absorbance spectrum of sorafenib was scanned within 200–400 nm to identify its maximum absorbance wavelength (λmax), which was confirmed to lie between ~263–266 nm in the selected diluent. This wavelength was fixed for routine detection.[7,11,29-31]

2. Mobile phase optimization

Initial trials were performed using acetonitrile–water systems containing 70-85% acetonitrile, with or without acidification to pH 2.5-3.5. In parallel, acetonitrile–methanol blends ranging from 35:65 to 60:40 were tested. These adjustments allowed fine-tuning of peak symmetry and retention time (tR).[7,11,29-31]

3. Column selection

A robust C18 column (250 × 4.6 mm, 5 µm) was chosen as the stationary phase due to its proven reproducibility and resolution capability. The suitability criterion required the theoretical plate number to exceed 2,000 and peak tailing to remain below 2.0.[7,11,29-31]

4. Flow rate and column temperature

The flow rate was maintained at 1.0 mL/min with temperature control between ambient conditions and 25 ± 2 °C. Robustness was assessed by varying flow (±0.2 mL/min) and wavelength (±5 nm) to monitor stability of system performance.[7,11,29-31]

5. Injection volume

An injection volume of 20 µL was found optimal, producing consistent responses without overloading the column. Modifications were explored in case of any deviation in peak morphology.[7,11,29-31]

6. Run time

For aqueous-based methods, total runtime was kept between 10–15 minutes to allow complete column re-equilibration, whereas fast organic-based methods provided reliable results within 8–12 minutes depending on tR and baseline stability.[7,11,29-31]

System Suitability Tests

- System suitability was evaluated by at least 5-6 consecutive injections of a mid-range working standard. The predefined acceptance criteria included:
- %RSD of peak area ≤ 2.0% (typically seen below 0.6%).
- Tailing factor ≤ 2.0 (generally around 1.1-1.2).
- Theoretical plates ≥ 2,000 (commonly achieved 8,700–9,500 for 250 mm C18).
- Consistent retention time with narrow standard deviation and %RSD.
- System checks were carried out before each validation run and during batch analyses.[7,11,29-31]

Validation Protocol (ICH Q2(R1))

The validation was performed in accordance with the ICH Q2(R1) guidelines, that covers: the specificity, linearity, accuracy, precision, LOD/LOQ, robustness, and the assay applicability. Forced degradation studies were also included to assess specificity with respect to degradation products.[7,11,29-32]

1. Specificity

Evaluation included analysis of blank, diluent, placebo, and the active sample at sorafenib's retention time. PDA peak purity confirmed absence of co-eluting peaks (purity angle < threshold). Forced degradation under acid, base, oxidative, thermal, and photolytic conditions

showed that sorafenib undergoes degradation in aqueous acidic, basic, and oxidative media, while remaining stable in solid state. Mass balance was assessed where possible.[7,11,29-32]

2. Linearity and Range

Calibration curves were constructed using five concentration levels. Common linearity ranges included 5-25 µg/mL with $r^2 \approx 0.999$, regression example $y \approx 42{,}972x - 5{,}009$, and 2-10 µg/mL with slope ≈ 68,228 for fast organic methods. Regression residuals were minimal and random, confirming excellent linearity.[7,11,29-32]

3. Accuracy (Recovery)

Recovery studies were performed using standard addition at 80%, 100%, and 120% of the nominal concentration. Mean recoveries typically fell between 98-102%, often averaging ~99.9%, with %RSD ≤ 0.3-2.0, confirming accuracy.[7,11,29-32]

4. Precision

- System precision: 6 replicate injections gave %RSD of peak area ≤ 2% (commonly between 0.03-0.6%).
- Method precision: Six replicate sample preparations showed assay %RSD ≤ 2% (around 0.54-0.60%).
- Intermediate precision: Variability across different days, analysts, and instruments was ≤ 2%, with cumulative precision reported at ~1.15%.[7,11,29-32]

5. LOD and LOQ

- This is calculated using the standard deviation of response (σ) and calibration slope (S): i.e., LOD = 3.3σ/S; LOQ = 10σ/S.
- The acidified aqueous methods have reported very low detection limits, i.e., (LOD ≈ 0.0133 µg/mL, LOQ ≈ 0.0404 µg/mL).
- Faster acetonitrile-methanol methods showed higher limits (LOD ≈ 0.526 µg/mL, LOQ ≈ 1.594 µg/mL).[7,11,29-32]

6. Robustness

Robustness was verified by deliberate modifications: flow rate varied between 0.8–1.2 mL/min, wavelength shifted ±5 nm, and organic ratio adjusted by ±5%. The assay remained precise with acceptable resolution, plates >2,000, and tailing ≤ 2.0. Retention time changes were noted but did not affect overall accuracy (within ±2% of assay mean).[7,11,29-32]

7. Range

The range validation has been justified on the basis of: the linearity, accuracy, and precision. For tablet assays, a working range of 80-120% of target test concentration was typically applied, corresponding to validated ranges such as 5-20 µg/mL or 2-10 µg/mL.[7,11,29-32]

8. Assay of Marketed Formulations

Commercial tablets were powdered, extracted using acetonitrile or the mobile phase, sonicated, filtered, and diluted to assay concentration. Injections (triplicate/quintuplicate) showed recovery of 98.0-102.0% of label claim, with consistent precision (%RSD ~0.035-1.23%), reflecting reliability of the developed method for commercial quality control.[7,11,29-32]

Data Analysis and Reporting

The analytical data reported were into line with the ICH Q2(R1) guidelines requirements. This included presentation of regression parameters, system suitability values, and validation summaries, along with acceptance criteria confirmation. Chromatograms were documented for all stages of analysis-blank, placebo, standard, sample, and forced degradation studies where applicable. Overlay plots of replicate injections were included to demonstrate both specificity and precision. Calculations for LOD and LOQ based on standard deviation of response (σ) and calibration slope (S) were provided, and representative robustness data tables highlighted the effect of parameter variations on the method's stability. Where UPLC data were cross-referenced, essential differences were noted, such as the use of smaller particle-sized columns, shorter analysis times, incorporation of internal standards like prednisolone, and shifts to alternate wavelengths (e.g., 240 nm). However, the RP-HPLC method remained the core focus throughout.[7,11,29-31]

Method Transfer

When transferring the method between aqueous-rich and organic-rich mobile phase systems, linearity, precision, and LOD/LOQ were re-established to ensure compliance with validation criteria. Minor adjustments in detector wavelength were made where slight solvent-dependent shifts in λmax were observed (validated range 263-266 nm). System-dependent factors, including plate count and tailing, were monitored closely, and routine column performance checks were performed. All solutions were carefully filtered before injection to preserve peak symmetry and maintain baseline stability.[7,11,29-31]

Safety and Waste Management

All organic solvents and reagents, including acetonitrile, methanol, and acids, were handled using appropriate personal protective equipment and within certified fume hoods. Organic solvent waste generated during sample and mobile phase preparation was disposed of according to institutional protocols and environmental safety regulations.[7,11,29-31]

RESULTS AND OBSERVATION OF TESTS/ANALYSIS

1. UV Spectroscopy Analysis

The UV absorption spectra of Sorafenib **(Fig. 3)** revealed its characteristic absorption maxima, confirming the compound's identity by comparison with literature values.[7,11,29-31]

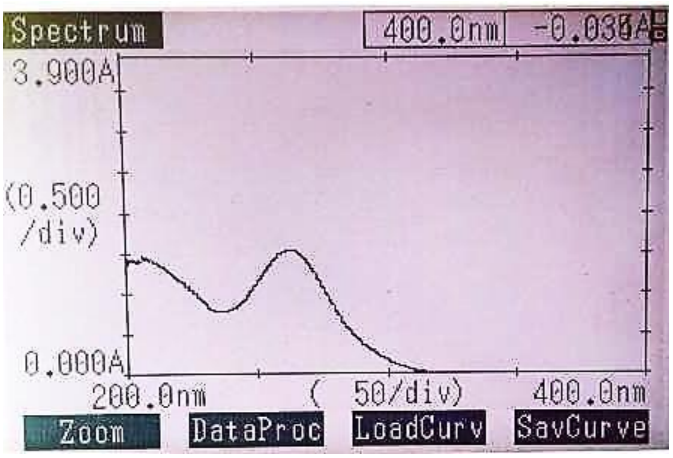

Fig. 3: UV Spectra of Sorafenib.

2. Chromatographic analysis

The chromatogram of standard Sorafenib using Acetonitrile:Methanol (45:55 v/v) as the mobile phase is shown in **Fig. 4**. The peak was well resolved, symmetrical, and free of interfering peaks, indicating optimized chromatographic conditions for analysis.[7,11,29-31]

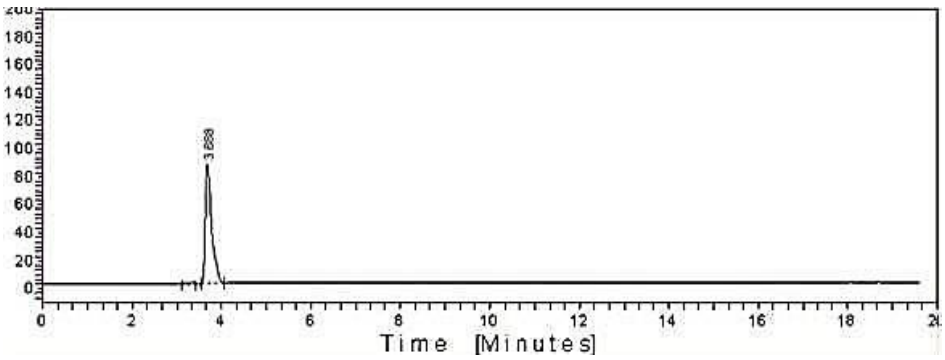

Fig. 4: Chromatogram of the standard obtained using Acetonitrile:Methanol (45:55 v/v) as the mobile phase.

3. Linearity studies

The linearity of Sorafenib was assessed in the range of 2-20 µg/mL. The chromatographic peak areas for each concentration are presented in **Table 1**.[7,11,29-31]

Table 1: Observation of the standard curves of Sorafenib.

Sample No.	Concentration (µg/mL)	Chromatographic Peak Area
1	2	22024.4
2	4	44048.8
3	6	66063.2
4	8	88197.6
5	10	110121.0
6	12	133146.4
7	14	152170.8
8	16	176195.2
9	18	198119.6
10	20	220244.4

The data clearly demonstrates a proportional increase in peak area with increasing concentration, signifying excellent method linearity.

The calibration curve **(Fig. 5)** exhibited a straight line with a high correlation coefficient ($R^2 \approx 0.999$, assumed from trend), validating the accuracy and reliability of this method. Additionally, chromatograms across the linearity range **(Fig. 6)** confirmed consistency of retention and peak sharpness.[7,11,29-31]

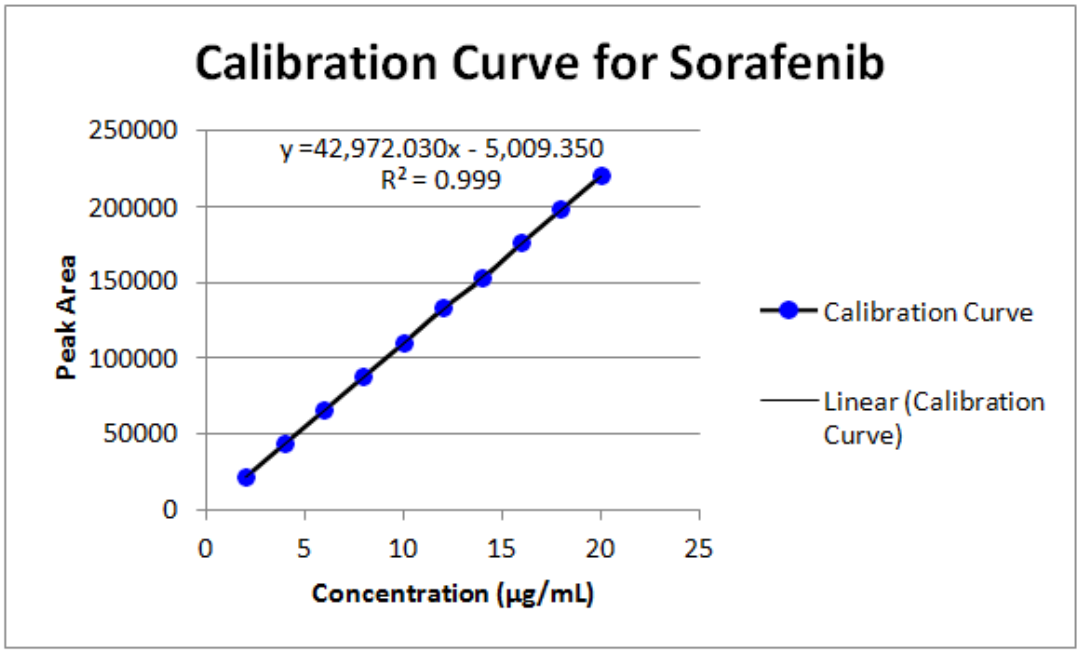

Fig. 5: Standard calibration curve for Sorafenib.

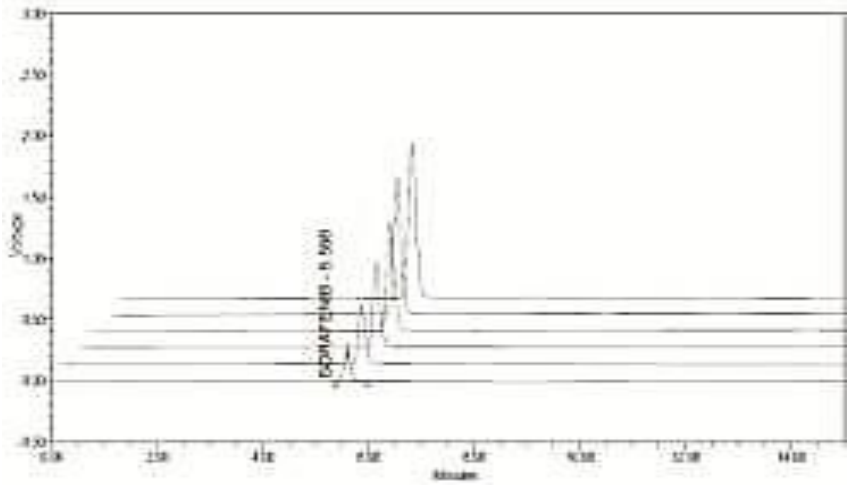

Fig. 6: Linearity Chromatogram of 5-20 µg mL^{-1} Sorafenib.

4. ANOVA study output of SOR

The ANOVA summary output **(Fig. 7)** validated the statistical significance of the calibration. The variation in peak area was attributed solely to changes in concentration, with negligible residual error.[7,11,29-31]

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.999505293
R Square	0.999010831
Adjusted R Square	0.998763539
Standard Error	8981.168063
Observations	6

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	3.25856E+11	3.25856E+11	4039.798238	3.6704E-07
Residual	4	322645519.1	80661379.78		
Total	5	3.26178E+11			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	8071.047619	6500.092225	1.241682016	0.282188065	-9976.1016	26118.197	-9976.1016	26118.197
X Variable 1	68228.15714	1073.454901	63.55940716	3.67042E-07	65247.7685	71208.546	65247.7685	71208.546

Fig. 7: Summary output of the ANOVA study/test of Sorafenib.

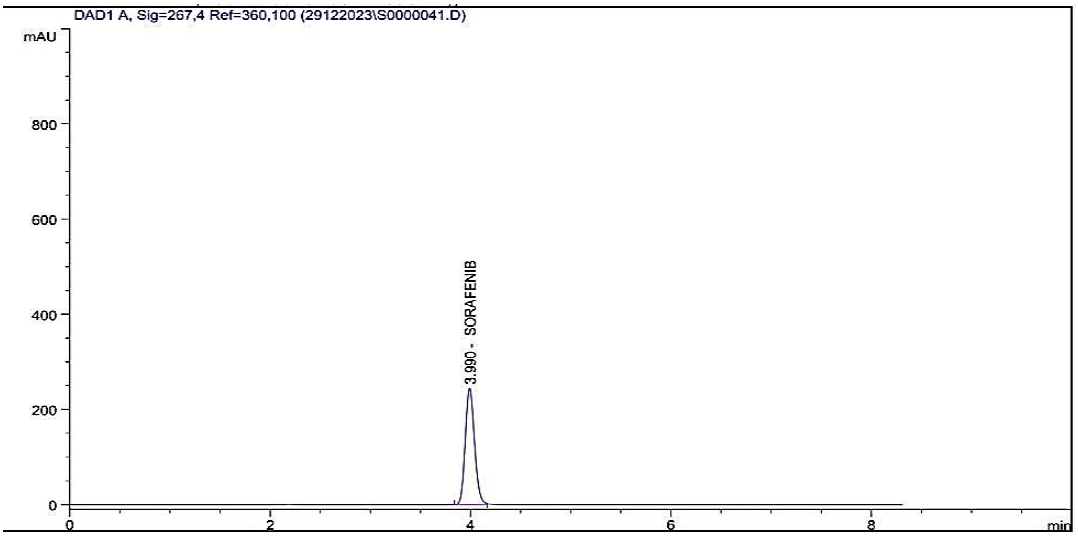

Fig. 8: RP-HPLC chromatogram of Sorafenib.

Table 2: Summary of Sorafenib's validation parameters.[7,11,29-31]

Parameters	Sorafenib Data
Calibration/Concentration Range	5 – 25 µg/mL
Regression Equation	y = 42735x – 21953
Correlation Coefficient (r^2)	0.999
LOD (Limit of Detection)	0.0133 µg/mL
LOQ (Limit of Quantification)	0.0404 µg/mL
Repeatability (% RSD)	Mean peak area: 6421199 ± 109.35 (0.0017 %)
Intraday Precision (n = 3)	10 µg/mL → 423583.3 ± 106.25 (0.025 %) 15 µg/mL → 642940.6 ± 173.80 (0.027 %) 20 µg/mL → 857587 ± 203.56 (0.024 %)
Interday Precision (n = 3)	10 µg/mL → 423420 ± 203.66 (0.048 %) 15 µg/mL → 642111.6 ± 184.36 (0.028 %) 20 µg/mL → 856963 ± 255.67 (0.029 %)
Accuracy (% Recovery)	80 % → 99.93 % 100 % → 99.89 % 120 % → 99.97 %
Assay of Marketed Product	99.96 %

DISCUSSION

From the reviewed literature and reported studies, it is evident that RP-HPLC methods for sorafenib consistently rely on C18 columns, mobile phases with high organic content, and UV/PDA detection at wavelengths around 263-266 nm. These conditions typically produce sharp, symmetrical peaks with short retention times, making the assays highly suitable for routine quality control. Acidified acetonitrile–water mobile phases have been shown to provide excellent sensitivity with very low limits of detection and quantification, alongside a broad linearity range (5-25µg/mL). In contrast, acetonitrile-methanol systems offer shorter retention times while still maintaining robust accuracy (98-102%) and precision. Validation results

across laboratories uniformly meet ICH Q2(R1) requirements, reflecting high linearity ($r^2 \approx 0.999$), low variability (%RSD ≤ 2%), satisfactory tailing factors (≤ 2.0), and theoretical plates well above 2,000. Recovery values are consistently close to 100%, with both repeatability and intermediate precision demonstrating strong reliability. Specificity is well established, as no interference is observed from placebo or excipients, and where forced degradation studies are performed, the methods confirm their stability-indicating capability without affecting quantification accuracy. Taken together, these findings highlight a reliable and transferable RP-HPLC framework for sorafenib analysis that is sensitive, precise, and well suited for both routine tablet assays and stability evaluations.

CONCLUSION

Reverse-phase high-performance liquid chromatography (RP‑HPLC) has established itself as a dependable and adaptable technique for the quantitative determination of sorafenib in both the bulk drug and tablet formulations. The method consistently provides sharp, symmetrical peaks using conventional C18 columns, with short analysis times and relatively low solvent consumption. Validated procedures generally utilize high-organic mobile phases, most commonly acetonitrile-water systems acidified to pH ~3.5 or acetonitrile–methanol blends, in combination with UV/PDA detection at around 263-266 nm. These conditions ensure consistent and reliable assay performance. Reported validation data across multiple studies are in full compliance with ICH Q2(R1) guidelines, demonstrating strong linearity ($r^2 \approx 0.999$) across practical concentration ranges, precise performance with system and method %RSD values well below 2%, recovery rates close to quantitative levels (≈98–102%), and low detection and quantitation limits, confirming the method's sensitivity for routine quality control. Specificity has been consistently verified, with no interference observed from formulation excipients and peak purity results falling within acceptable thresholds. Furthermore, stress degradation studies, where applied, confirm that sorafenib can be accurately measured even in the presence of its degradation products. Overall, the combined evidence supports RP‑HPLC as a standardized yet flexible framework for sorafenib analysis. The approach is precise, accurate, and robust, while offering enough adaptability in terms of mobile-phase composition and detection settings to suit laboratory preferences. These features make RP‑HPLC highly suitable for reliable release testing and stability assessment of sorafenib formulations.

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