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Research Article

Box-Behnken Design Employed Stability Demonstrating RP-HPLC Method Development of Sorafenib Tosylate for Rapid and Sensitive Quantification

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ABSTRACT

Sorafenib tosylate (SFN) is a tyrosine kinase inhibitor used clinically to treat liver, kidney, and thyroid carcinoma. This research aims to accurately quantify SFN using a quality-by-design (QbD) approach with reverse-phase high-performance liquid chromatography (RP-HPLC). Chromatographic settings were optimized using a Box-Behnken design, measuring responses such as retention time, tailing factor, theoretical plate, and peak area. At the same time, independent variables were flow rate, mobile phase composition, and wavelength. A C18 column (4.6 × 250 mm, 5 µm) served as the stationary phase, with a mobile phase of methanol and 0.1% *o*-phosphoric acid in a 41:59 v/v ratio. SFN detection occurred at 267 nm in isocratic mode at a flow rate of 1.1 mL min^{-1}. Method validation followed International Council for Harmonization (ICH) guidelines, yielding a coefficient of determination (R^2) of 0.9998, indicating linearity in the 5 to 25 µg mL^{-1} range. Results showed detection (LoD) and quantification (LoQ) limits of 0.04 and 0.12 µg mL^{-1}, respectively. Additionally, the method demonstrated precision, accuracy, and robustness consistent with ICH criteria. Overall, this simple, accurate, rapid, and robust RP-HPLC method is suitable for routine SFN analysis in various formulations.

Introduction

A tyrosine kinase inhibitor (TKI), sorafenib tosylate (SFN) [IUPAC: 4-[4-[3-[4-Chloro-3-(trifluoromethyl) phenyl] ureido] phenoxy]-*N*-methylpicolinamide-4-Methylbenzenesulfonate (Fig. 1.), has proven to be a highly effective orally bioavailable anti-neoplastic drug, specifically for treating hepatocellular, renal, and thyroid cancer.[1,2] Tyrosine kinase inhibitors target tyrosine kinases, crucial components in transmitting cellular growth signals.[3] At present, Europe has authorized nine tyrosine kinase inhibitors for diverse medical indications. These include lapatinib, nilotinib, pazopanib, dasatinib, erlotinib, gefitinib, imatinib, sunitinib, and sorafenib.

Even though TKIs have demonstrated effectiveness, instances of treatment ineffectiveness, adverse drug reactions, and less-than-ideal responses have been documented in their treatment.[4] The limitations of these inhibitors are probably due to a mix of factors associated with both the tumor and the host, which lead to differences in drug distribution or the emergence of resistance to these treatments.[5,6] With poor water solubility (10–20 µM) and high lipophilicity (log P of 3.8), it is permissible to classify it as a BCS category-II medication.[2,7]

For many years, liquid chromatography has transformed from being a preferred method to an affordable and nearly indispensable instrumental technique for drug analysis. Reverse-phase high-performance liquid chromatography (RP-HPLC) has recently played a crucial role in advancing analytical research due to its wide range of applications in foods, polymers, plastics, environmental monitoring,

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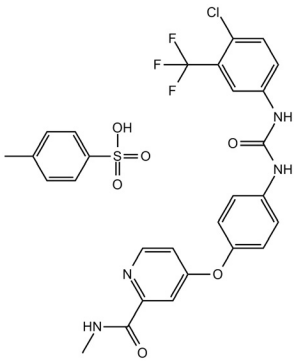

Fig. 1: Molecular structure of sorafenib tosylate

medicines, and clinical practice.[8] The unique advantage of this method lies in its rapidity, improved sensitivity, and ability to concurrently detect and operate automatically. This technique, specifically tandem mass spectroscopy (HPLC-MS), has significantly transformed the analytical quantification of drug(s) in biological samples, as well as in drug stability assessments and impurity profiling, surpassing traditional analysis methods.[9]

Numerous efforts have been documented in the existing literature to analyze SFN through liquid chromatography, specifically employing RP-HPLC,[10] high-performance thin layer chromatography (HPTLC),[11] and LC-tandem mass spectroscopy (LC-MS/MS).[12] Buffers are frequently included in the mobile phase composition of these methods. Due to this, it has been noted that column blockages often result from using these procedures with buffer salts. In contrast, the usage of only non-polar solvents in high concentrations is not cost-effective.

The design using Box-Behnken (BBD) is a valuable strategy for optimizing chromatographic conditions in analytical methods. This optimization involves a three-factor analysis organized in blocks, leading to a reduced overall number of experiments compared to alternative approaches. This technique is effective in fine-tuning chromatographic parameters. Employing the quality-by-design approach in experiments enhances result reliability, presenting a distinctive method. When applied to the HPLC analytical technique for method development and validation, QbD-optimized chromatographic conditions contribute to significant cost and time savings, which is particularly beneficial for routine analysis. In contrast to the time-consuming approach of one-factor-at-a-time (OFAT) or trial-and-error in HPLC method development, where experiments are conducted sequentially by altering one chromatographic parameter continuously until satisfactory peak resolution is achieved, the QbD approach helps manage and reduce the number of experiments, especially when multiple parameters are considered.[13-15] Numerous research literature is available to date that focuses on QbD-employed method development for SFN in biological samples and complex formulations. Hence, in this research, we established a modest analytical technique for SFN using the QbD approach by utilizing a unique mobile phase (MP) composition, which has not yet been reported. Hence, the established test method can be used to quantify the drug content in various non-biological samples and other dosage forms, and as a result, this methodology has a lower failure rate.

Materials And Methods

Chemicals

Khandelwal Laboratories Pvt. Ltd. (Mumbai, India) provided SFN as a complimentary sample. Sorafenib tosylate (containing 200 mg per tablet) was purchased from Aprazer Healthcare Pvt. Ltd. (New Delhi, India). *O*-phosphoric acid and analytical-grade methanol were acquired from Merck Specialties Pvt. Ltd. (Mumbai, India). Using Milli-Q (Millipore GmBH, Germany) filtration, Milli-Q HPLC grade water was filtered and utilized. The analytical method optimization process exclusively utilized HPLC-grade analytical solvents, chemicals, and reagents.

Instrumentation

A high-performance RP-HPLC system, an Agilent Tech gradient system (Model 1100, Canada) equipped with a quaternary pump (G130A quaternary gradient) and a PDA detection system (G13148 DAD), was employed with an autosampler for sample handling. The operation of this system was managed through CHEMSTATION software (version 10.1) to control instrument functions. The temperature of the column was kept at room temperature. To ensure precision, all standards were meticulously weighed using a standardized ME204/AD4 weighing balance (Mettler Toledo, Switzerland).

Selection of Wavelength

SFN at a selected concentration, 20 µg mL^{-1} was prepared by dissolving it in methanol to make a primary standard solution and aimed to generate an ultraviolet-visible spectrum. After that, a quartz cell cuvette containing an aliquot of that concentration was scanned using UV-2600, Shimadzu, Japan (a double-beam spectrophotometer) to identify the maximum absorption wavelength (λ_{max}) within the 200 to 800 nm range, using methanol as a blank. The final step involved obtaining the UV-absorption spectrum of the drug to ascertain the isosbestic wavelength and determine the maximum absorption of the drug.

Chromatographic Conditions

Sorafenib was satisfactorily separated on chromatogram using an Agilent reverse phase column c-18 (dimensions 4.6 × 250 mm and 5-micron particle size) previously saturated with the blended mobile phase. Operating in an isocratic mode, the MP is composed of a blend of 0.1% *o*-phosphoric acid and methanol of HPLC grade, with a ratio of 59:41 v/v, respectively. Prior to analysis, the MP was filtered through a 0.45 µm membrane filter using a

vacuumed filtration assembly, and then it was sonicated for 20 minutes. The flow rate (FR) was kept stagnant at 1.1 mL min^{-1}, the column temperature was controlled at 25 ± 0.5°C, and a 20 µL volume was utilized for injection.

Samples and Standard Solution Preparation

The standard stock solution of SFN at a concentration of 500 µg per mL was made using the mobile phase and then covered by aluminum foil for storage at room temperature until analysis. Subsequently, secondary stock solutions having 5, 10, 15, 20, and 25 µg mL^{-1} were generated by appropriate dilutions using the primary stock solutions with the mobile phase.

Risk Assessment Studies

Risk assessment is utilized to evaluate how various factors affect the quality characteristics of the designated analytical technique. Essential method parameters in the current HPLC analysis were employed as critical analytical attributes (CAA) to depict formal connections within the quality profile of the target method before initiating the risk assessment. This method assists in classifying potential sources of problems, flaws, variations, discrepancies, or failures to pinpoint their underlying causes. The risk assessment study is pivotal in extracting vital information regarding the possibility and risk linked to specific factors throughout the analysis process.[16] Initially, the likelihood of subsequent failure was determined, and potential interactions with CAAs were examined by risk assessment. Seven distinct CAA (i.e., %organic modifier in the MP, detection wavelength, FR, injecting volume, type of elution, column type, and temperature) were taken into account and later classified into high, medium, and low-risk categories to facilitate screening and prioritization. Following this evaluation, a subset of factors was chosen, and ultimately, three of them (i.e., %organic modifier in the MP, FR, and λ_{max}) were selected for systematic optimization using the software Design Expert® (Stat-Ease Inc., Minneapolis, USA) version 13.0.0.5.

Optimization

The examination of how independent variables influence dependent responses, as discussed earlier, was conducted using the QbD. Finding failure modes and developing a reliable approach with workable design space for every stage of product life cycle management are the main goals of BBD in the current research. This methodology enabled the determination of suitable experimental runs and interaction effects among the variables impacting the dependent outcomes. After evaluating all seven factors, the experimental setup involved three factors, each with low, medium, and high levels: (A) organic modifier (methanol conc.), (B) flow rate, and (C) wavelength. The effects of these factors, both individually and in combination, were assessed on retention time (Rt) (i.e., the drug's ability to get separated from the stationary phase), peak area (Pa) (i.e., an indicator of the drug's concentration), theoretical plates (Tp) (i.e., the pointer for method performance and mobile phase suitability), and tailing factor (Tf) (i.e., shows the degree of peak symmetry) as the dependent variables.[17] Seventeen distinct experimental compositions were employed to optimize the factor levels (Table 1). At the last, outcomes under various conditions were analyzed and conclusions were drawn from the results.

Assay Validation

The analytical method developed to identify and quantify sorafenib tosylate was validated following the International Council for Harmonization (ICH) technical standards for pharmaceuticals of human use.[7] As part of the validation process, a method's specificity, linearity and range, accuracy, precision, sensitivity, and robustness were all assessed. However, the system suitability assessment was conducted under United States Pharmacopeia (USP) guidelines.[18]

Specificity

The developed method's specificity was assessed by determining SFN in tablets. The presence of interference peaks at the analytes Rt was evaluated in the chromatogram. Moreover, the developed method's specificity was assessed by subjecting SFN to force degradation, aiming to examine potential interference from degradation products at the Rt of SFN.

Linearity and range

The concentration of SFN was measured exactly and dissolved in 20 mL of the MP to produce the primary stock solution, which had a 0.5 mgmL^{-1} concentration. Then, different working concentrations were made, having 5, 10, 15, 20, and 25 µgmL^{-1} by diluting the solutions as mentioned previously. For the assessment of linearity and range, these working solutions with varying concentrations of SFN were subjected to two injections each. A concentration versus Pa response plot was generated after the Pa for each concentration was noted. Plotting Pa against each concentration and the response factor against the concentration for each calibration standard allowed the range of the developed method for SFN to be determined.

Accuracy

The percentage recovery of SFN in quality control (QC) samples at the low (LQC), middle (MQC), and high-quality control (HQC) levels was used to measure accuracy. For each concentration, duplicate samples containing known SFN concentrations of 4, 5, and 6 µgmL^{-1} for LQC, MQC, and HQC, correspondingly, were made. The %recovery and %RSD were then calculated.

Precision

Intra-day (repeatability) and inter-day (intermediate) precision are used to assess an analytical method's precision, where %RSD was used for its evaluation. QC samples at three distinct concentrations were examined in duplicate to look into intra- and inter-day precision. The same day was used to prepare and evaluate the LQC for SFN at 5 µg mL^{-1}, the MQC at 15 µg mL^{-1}, and the HQC at 25 µgmL^{-1} for repeatability. Three separate quality control samples were produced and evaluated on three successive days while keeping the same analytical, and experimental settings to achieve inter-day precision.

Sensitivity

By using a visual determination approach, the LoD evaluates the analytical method's sensitivity. The formulas 3.3 x SD/Slope and 10 x SD/Slope were used to determine the LoD and LoQ, respectively.

Robustness

The robustness was assessed by intentionally fluctuating chromatographic parameters, including variations in the FR, wavelength, and MP composition. The MP composition was adjusted within a range of ± 1%, the FR within ± 0.04 mL per minute, and the λ_{max} by 2 nm. The impact of these modifications on Rt and Pa was analyzed to determine the robustness of the method.

System suitability

Suitability testing was done following USP guidelines.[18] to make sure the chromatographic system was suitable for the intended analysis. Three injections of a standard solution containing 15 µg mL^{-1} of SFN were used in the test to ascertain the parameters, which included Tp, Pa, Rt, and height-equivalent theoretical plates.

Stress studies

To assess the degradation characteristics, SFN underwent exposure to various stress conditions. Acid hydrolysis was induced by subjecting SFN (20 µg) to 5 mL of 0.1N HCl, basic hydrolysis to 5 mL of 0.1N NaOH, and neutral hydrolysis to 5 mL of water, all at room temperature for durations of 2 and 5 hours. Oxidative studies were conducted using 3% H_2O_2 at room temperature for 2 and 5 hours. Before injecting samples in HPLC, they were neutralized. Further dilutions with the MP were prepared, and the samples were subsequently examined using HPLC.

Results And Discussion

Risk Assessment Studies and Optimization by Using BBD

BBD is used in this HPLC method to optimize and finalize the chromatographic conditions. In contrast, analysis of variance (ANOVA) was employed to examine the consequence of the procedure's critical elements. Among the factors affecting the dependent variables, the independent factors were chosen for screening. For every factor, there were three levels used. The independent factors that had numerical values were the detection wavelength (266, 267, and 268 nm), FR (1, 1.1, and 1.2 $mLmin^{-1}$), and methanol (40, 41, and 42%). The *f*-value and *p-value* of the drug's Rt, and Pa were used to evaluate the results of the built-in ANOVA model. In parallel, the response surface model graphs for the overlay plot, contour, residuals vs. run, and predicted vs. actual.

Retention Time (Rt)

When analyzing the SFN retention time using an ANOVA (linear model), the values of *f* and *p* were found to be 436.39 and < 0.0001, respectively. The model is significant for A and B. The model terms in this case were (A) the methanol % and (B) the mobile phase flow rate. The model terms are significant if the value of *p* is less than 0.05. The adjusted R^2 and predicted R^2 values in fit statistics are 0.9879 and 0.9813, respectively. It is acceptable to have a discrepancy of less than 0.2 between these numbers. Since 64.999 is more than 4, the appropriate precision number, the model may navigate the design space. In terms of coded factors, the final equation is expressed as Rt = A + B. The model terms had respective values of (– 0.0929) and (– 0.3320), and the mean Rt was +3.86 minutes. By comparing the factor coefficients, the coded equation may ascertain the relative benefits of the elements. The retention time color point value is used to display the predicted vs. actual and residuals *vs*. run in Figs 2 and 3 (Rt) for reference. While there linear correlation graphs are shown in Fig. 4.

Peak Area (Pa)

The ANOVA findings for the linear model of the Pa values of 17 trials are shown in Table 2. The model demonstrated a significant *f-value* of 31.28 and an observed *p-value* of less than 0.0001. Both A and C model terms were significant, as indicated by their *p-values* being less than 0.0500, confirming the overall significance of the model. The lack of fit *f-value* of 12.48 suggests significant noise, which is undesirable. The statistics of model fit revealed an adjusted R^2 of 0.8502 and a predicted R^2 of 0.7516, with a difference of less than 0.2. This implies the model is reliable for exploring the design space, as evidenced by an appropriate precision value of 19.602. The response value and relative influence of each factor are determined by the coefficient values in the coded equation, with factors A, B, and C having coefficients of +213.85, +47.60, and -178.08, respectively. Figs 2 and 3 (Pa) illustrate the predicted versus actual values and the residuals versus run, highlighting the peak region through color point values. also Fig. 5 represents graphs of their linear correlations. The outcomes of risk assessment studies based on CAA are depicted in Tables 1 and 2, show that keeping the MP, FR, and λ_{max} parameters as 41%, 1.1 mL/min, and 267 nm, respectively, have an insignificant impact on the dependent

Table 1: CAA parameters

Runs	*MP (Methanol-% v/v)*	*FR (mL min^{-1})*	*λ_{max} (nm)*
1	0	0	0
2	+1	+1	0
3	0	-1	+1
4	0	0	0
5	-1	0	+1
6	0	+1	+1
7	+1	0	+1
8	0	-1	-1
9	-1	0	-1
10	0	0	0
11	0	0	0
12	-1	+1	0
13	+1	-1	0
14	0	0	0
15	-1	-1	0
16	0	+1	-1
17	+1	0	-1

Level of factors to be studied	*Low (-1)*	*Medium (0)*	*High (+1)*
Methanol (% volume)	40	41	42
FR (mL min^{-1})	1	1.1	1.2
λ_{max} (nm)	266	267	268

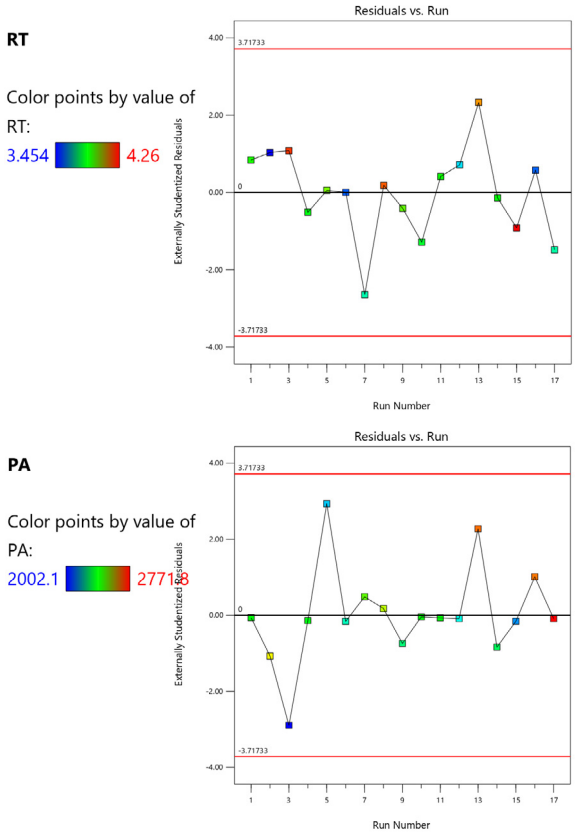

Fig. 2: Response surface technique residuals in BBD trials for SFN compared to run of peak area (Pa) and retention time (Rt)

Table 2: Independent factors based on BBD and their impact on dependent variables (Responses)

	Independent variables			*Responses*			
	A	*B*	*C*	*R1*	*R2*	*R3*	*R4*
S. No.	*Methanol (%)*	*Flow rate (mL min^{-1})*	*Wavelength (nm)*	*Retention time*	*Peak area*	*Theoretical plates*	*Tailing factor*
1	41	1.1	267	3.978	2380.98	6968	0.84
2	42	1.2	267	3.454	2174.60	6912	0.85
3	41	1	268	4.216	2702.05	7152	0.83
4	41	1.1	267	3.942	2374.69	7011	0.84
5	40	1.1	268	3.954	2454.12	6906	0.85
6	41	1.2	268	3.528	2244.34	6894	0.85
7	42	1.1	268	3.718	2456.39	7266	0.84
8	41	1	266	4.188	2529.52	7389	0.83
9	40	1.1	266	3.935	2298.86	7006	0.85
10	41	1.1	267	3.923	2382.81	7176	0.85
11	41	1.1	267	3.967	2380.62	7135	0.85
12	40	1.2	267	3.633	2213.70	6762	0.86
13	42	1	267	4.140	2188.18	7778	0.80
14	41	1.1	267	3.952	2318.56	7148	0.85
15	40	1	267	4.260	2113.53	7836	0.81
16	41	1.2	266	3.533	2681.42	7634	0.82
17	42	1.1	266	3.727	2171.86	7003	0.80

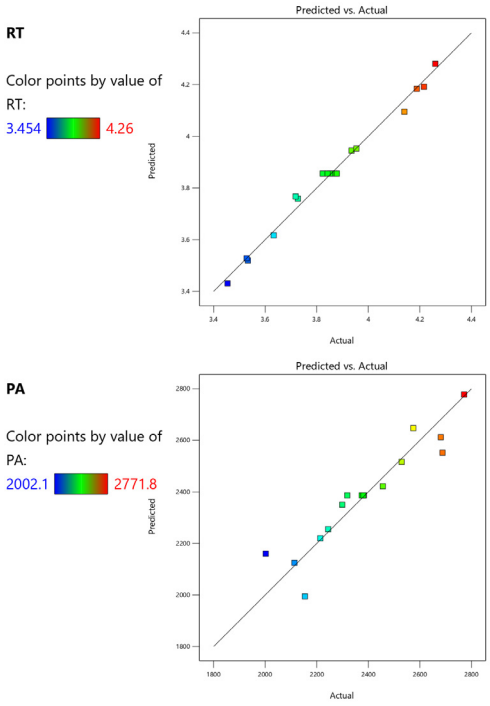

Fig. 3: BBD experiments for SFN, response surface approach predicted *vs*. actual retention time (Rt) and peak area (Pa)

variable (where Rt and Tf remain the same). While making changes in the MP and FR, it especially affects the Pa, Tp, and Tf. Hence, for method development and validation the optimized conditions are utilized.

Method Development

All prepared samples were covered using aluminum foil to prevent external exposure and contamination throughout the experiment. Following thorough optimization, the RP-HPLC method development for SFN quantification began. The MP ratio of 59:41 v/v was employed, consisting of 0.1% *o*-phosphoric acid as an aqueous MP and methanol as an organic MP. The previously specified chromatographic conditions were applied. Absorbance measurements for SFN were obtained at λ_{max} of 266 nm, as illustrated in Fig. 6., with a determined retention time of 3.9 minutes. System suitability parameters, including Rt, Pa, Tp, Tf, and height equivalent theoretical plate, were all observed to fall within tolerable limits.

Method Validation

Specificity

The chromatogram of sorafenib tosylate showed no interference from any additional peak at the 3.9-minute Rt (Fig. 7). However, during forced degradation studies of SFN under oxidation conditions, interference from degradants was observed that had no major impact on the retention time of SFN. Consequently, it was found that the RP-HPLC validated and developed technique was specific to sorafenib tosylate.

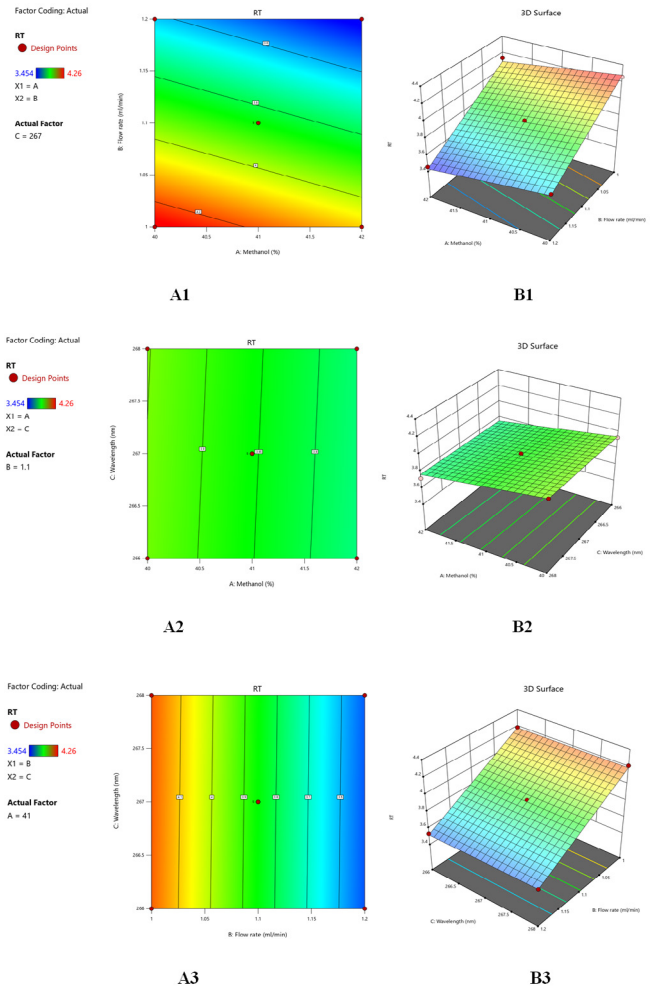

Fig. 4: Plot of a linear correlation between the related graphs and the true (A1-A3) and measure (B1-B3) values

Linearity and range

For SFN, the linearity range was identified as 5-25 μgmL^{-1}, as illustrated in Fig. 8. The calibration curve for SFN displayed a remarkable correlation coefficient (R^2) of 0.9998, signifying a robust linear association between SFN concentration and the recorded response (*y*-values). The equation of the line derived from the calibration curve (Equation. 1.), with y representing the measured response and x representing the SFN concentration, emphasized the method's calibration suitability for accurately determining low and intermediate SFN concentrations.

* y = 101.85x + 41.588..... Eq. 1.

Accuracy and precision

The trial assessed these parameters of the newly developed RP-HPLC method, with three repetitions performed for each of the three QC levels. The findings outlined in Table 3 reveal that the %RSD numbers for both precision and accuracy was less than 1%, ranging within the limits given by the FDA, demonstrating the precise and accurate nature of the method developed.

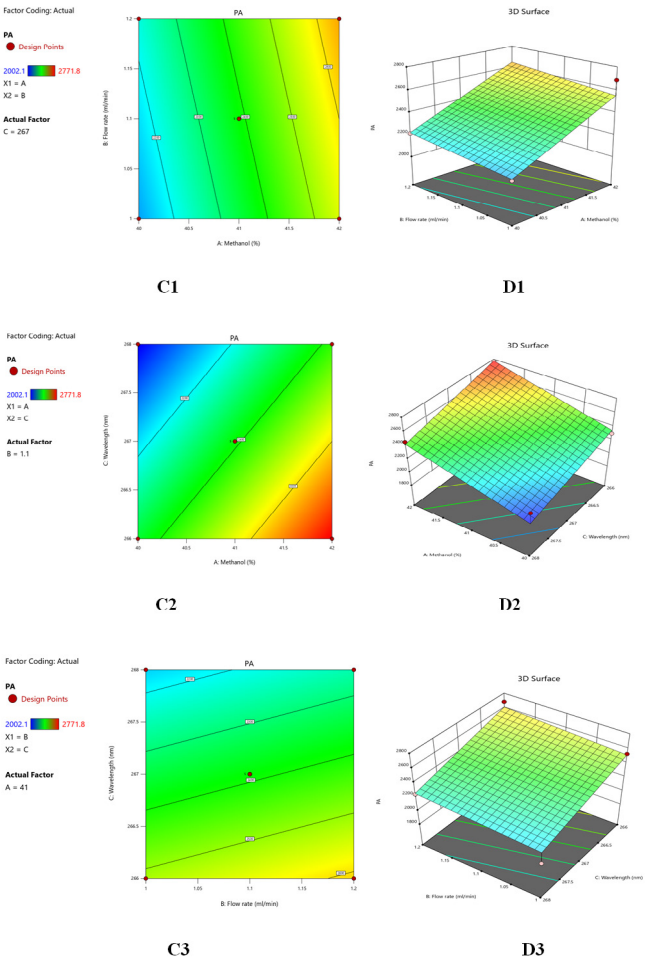

Fig. 5: Plot of a linear correlation between the related graphs and the true (C1-C3) and measure (D1-D3) values

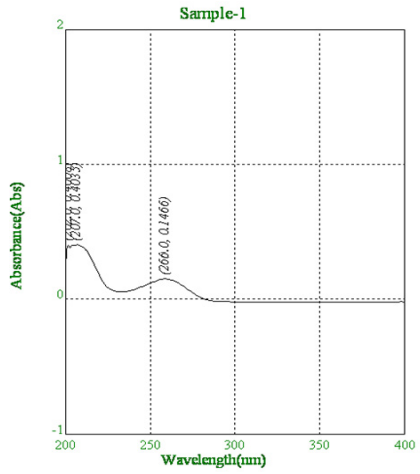

Fig. 6: Uv-visible spectrum of sorafenib tosylate

Sensitivity

The LoQ was 0.12 µg mL^{-1} and the LoD was 0.04 µg mL^{-1} achieved during the analysis, emphasizing the analytical method's excellent sensitivity and reliability. These findings affirm the method's ability to precisely identify

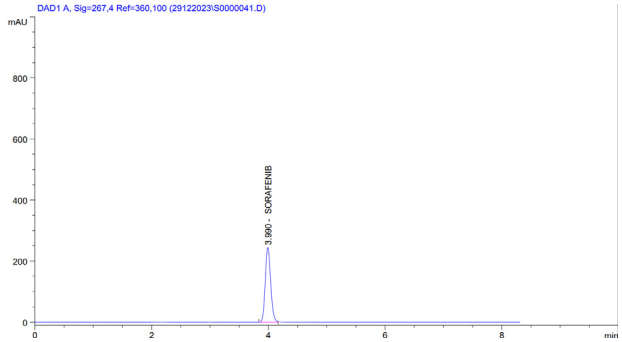

Fig. 7: RP-HPLC- chromatogram of SFN

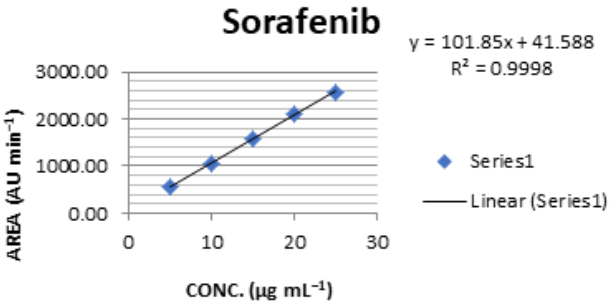

Fig. 8: Calibration curve of SFN

the substance even at low levels and to measure its concentration with commendable accuracy.

Robustness

In the developed method, intended changes were made, i.e., MP composition, FR, and wavelength. The results (Table 4) show no major impacts on the Rt of the drug. This represents the developed method is robust for SFN.

System suitability

The system suitability test elements were thoroughly observed and found to fall within tolerable limits, indicating the suitability of both the system and chromatographic conditions for this method. Analysis of the system suitability results revealed no notable variance in the Pa and Rt of SFN across three consecutive injections (Table 5). %RSD values for all parameters were below 1%, underscoring the high precision of the chromatographic instrument and ensuring accurate results.

Stress studies

An analysis was conducted to examine the %degradation of SFN under different stress conditions (Table 6). Minimal degradation was noted across all parameters examined. However, a notable increase in degradation was observed particularly in 0.1 N NaOH and oxidative stress environments after 5 hours. Some previously reported studies have demonstrated that SFN is susceptible to degradation under strongly acidic, alkaline, and oxidative hydrolysis conditions, especially when subjected to high temperatures.

Table 3: Accuracy results of SFN

Theoretical content (μg mL^{-1})	*Extra drug added to an analyte (μg)*	*Conc. detected (Mean ± SD)*	*% Recovery (Mean ± SD)*	*%RSD*
5	4	3.98 ± 0.06	99.58 ± 0.16	0.16
5	5	4.97 ± 0.05	99.92 ± 0.10	0.10
5	6	5.98 ± 0.12	99.86 ± 0.20	0.19

Method precision outcomes of SFN

	Inter-day			*Intra-day*	
Conc. of analyte (μg mL^{-1})	*Conc. detected (Mean ± SD)*	*% RSD*	*Conc. of analyte (μg mL^{-1})*	*Conc. detected (Mean ± SD)*	*%RSD*
LQC 5	5.24 ± 0.01	0.28	5	4.62 ± 0.99	0.02
MQC 15	14.05 ± 0.99	0.24	15	15.09 ± 0.06	0.04
HQC 25	25.06 ± 0.96	0.13	25	24.29 ± 0.87	0.03

Two notable separate peaks were observed in the chromatogram of oxidative studies showing the presence of 2 degraded products having retention times of 2.1 and 4.5 minutes (Fig. 9c.). Also, the findings suggested that the drug undergoes more significant hydrolysis in alkaline environments (Fig. 9b.). The results of mass spectroscopy for SFN conducted by Teenu and colleagues,[19] which show the presence of the m/z 311 fragments in the SFN mass spectrum reacted with NaOH and H_2O_2, are consistent with this data. These fragments are most likely the result of the breakage of the ether bond inside the SFN molecule. Following treatment with NaOH, the mass spectrum of SFN exhibits a characteristic m/z 499 fragment, which may be explained by the addition of a methanol and H_2O molecule from the test samples after the SFN molecule lost a chloride ion. Notably, the occurrence of the molecular ion peak is evident under all stress conditions (NaOH, HCl, and H_2O_2), underscoring the resilient nature of the SFN molecule against these stressors. Overall, degradation studies further support the higher stability of the SFN molecule, even when exposed to harsh conditions such as HCl, NaOH, and H_2O_2.

Method Optimization

A novel RP-HPLC technique was developed by employing the QbD approach to refine chromatographic conditions. The focus was on optimizing two key parameters: methanol concentration and flow rate. Through the implementation of the Box-Behnken design method, these responses were effectively fine-tuned using three independent variables. Subsequently, the method was further refined based on the chosen factors. As a result, a resolved, symmetric peak devoid of retention time interference was obtained. The method is simple, robust, affordable, precise, and accurate due to the innovative and reliable optimum conditions.

Method Development

The HPLC method developed underwent validation following the guidelines set by the ICH. The proposed

Table 4: Robustness experiment results

Conditions	*Pa*	*Rt (minutes)*
MP ratio (40:60)	2880.9	4.1
MP ratio (42:58)	2833.1	3.9
FR - 1.09 mL min^{-1}	2862.6	4.0
FR - 1.13 mL min^{-1}	2761.1	3.9
λ_{max} 266 nm	2739.5	3.9
λ_{max} 268 nm	2822.5	3.9

Table 5: System suitability results for SFN (n = 3)

S. No.	*Rt (minutes)*	*Pa*
1	3.9	1582.8159
2	3.9	1583.4354
3	3.9	1560.4966
Average	3.9	1575.5826
SD	0.44	
%RSD	0.03	

Table 6: Outcomes of stress studies

Stress study parameter	*%degradation after 2 hours*	*%degradation after 5 hours*
0.1N HCl (Acid degradation)	14.85	15.08
0.1N NaOH (Basic degradation)	14.82	19.61
Neutral hydrolysis	4.3	4.5
3% H_2O_2 (Oxidative studies)	16.61	19.34

method for analyzing SFN demonstrated linearity, accuracy, precision, robustness, and sensitivity, meeting the criteria for determining SFN effectively. Validation parameter results fell within acceptable ranges, indicating the method's reliability and suitability for routine SFN analysis, whether alone or within formulations. Notably, this study marks the first utilization of a blended MP

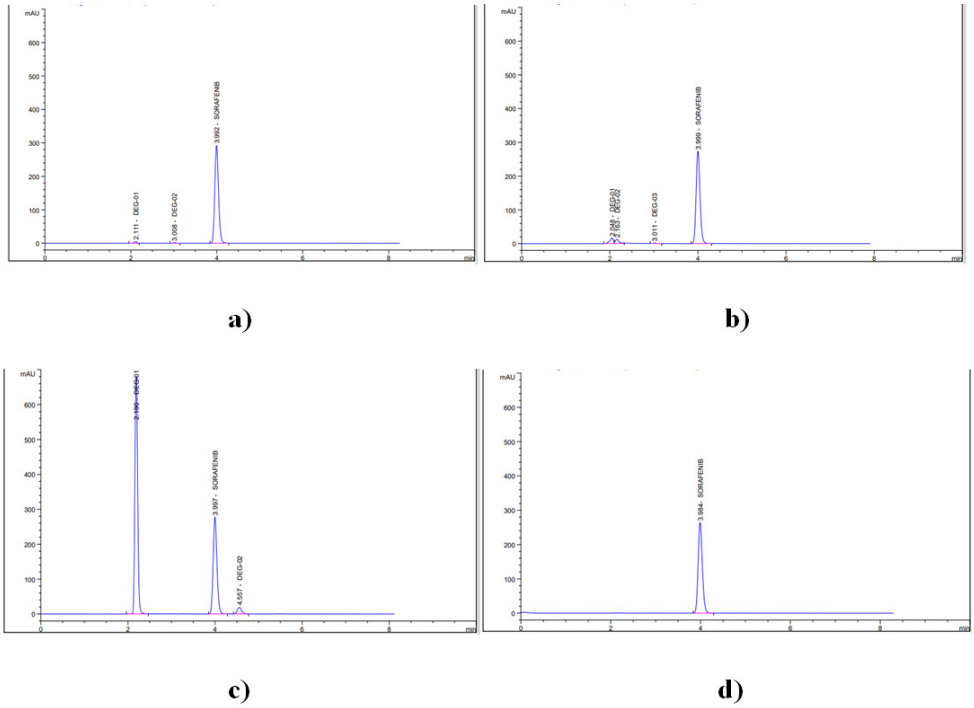

Fig. 9: Stress studies and their chromatogram: a) Acidic and b) Alkaline hydrolysis c) Oxidative degradation d) Neutral hydrolysis

consisting of methanol and 0.1% *o*-phosphoric acid, employing a QbD approach. Consequently, the method is thoroughly optimized for various chromatographic parameters through QbD principles, in addition to being straightforward, cost-effective, precise, and accurate.

Conclusion

The HPLC technique was devised following quality by-design principles, as outlined in ICH Q8 and Q8 (R2) guidelines, and subsequently confirmed. Additionally, a reverse-phase HPLC method was effectively created and enhanced using Box-Behnken design to achieve optimal chromatographic conditions, including a mixture of methanol and 0.1% *o*-phosphoric acid (41:59 v/v), a retention time of 3.9 minutes, and a rate of flow having 1.1 mL min^{-1}, resulting in favorable outcomes. This method underwent validation for the determination of SFN in tablets, demonstrating outstanding analytical performance characterized by specificity, linearity, precision, accuracy, and robustness. Thus, this method offers a dependable and sensitive means of quantifying SFN in solid dosage forms.

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