INTRODUCTION

Sorafenib (SOR) is 4-[(4-chloro-3-(trifluoromethyl)phenyl)carbamoyl]amino)phenoxy]-N-methylpyridine-2-carboxamide (Fig.1.a). Its molecular formula is C_{21}H_{20}F_{3}ClN_{2}O_{3}. SOR is an anti-cancer drug that is a protein kinase inhibitor. A thorough literature survey reveals that few analytical methods are reported for the determination of SOR in bulk & pharmaceutical preparations and in biological fluids, which include, UV spectrophotometric method\cite{1-2}, RP-HPLC\cite{3-8}, HPTLC\cite{9}. High-Performance liquid chromatography with tandem mass spectrometry\cite{10,11}. However, most of the available methods have limitations such as arduous sample preparations, increased solvent consumption, long run times for biological samples, low sensitivity, uneconomical, and also have reduced symmetry. The present study focused on minimizing these limitations and developing a simple, accurate, precise, and reliable RP-HPLC method for the estimation of SOR in the pharmaceutical dosage form.

ABSTRACT

A sensitive, rapid, precise, accurate high-performance liquid chromatographic method was developed for the estimation of Sorafenib (SOR) in the tablet dosage form. Chromatographic separation of SOR was carried out utilizing thermo-scientific model C18 column (4.6 mm i.d. X 250 mm; 5 µm particle size) (based on 99.99 % ultra-high purity silica) using mobile phase that consisting of acetonitrile: methanol (40:60 v/v) at a flow rate of 1.0 mL/min. The absorption maximum (λmax) of SOR in the mobile phase was found to be 265.5 nm. It had a retention time of 3.223 min. The calibration curve was in linear function of the drug in the concentration range of 2-10 µg/mL (r² = 0.999) for the optimized method. The regression equation for SOR was found to be Y = 68228 x + 8071. The Detection Limit (DL) & Quantitation Limit (QL) results of SOR were found to be 0.526 µg/mL and 1.594 µg/mL respectively. The developed method was validated in pursuance of ICH Q2 (R1) guidelines. The method was linear, precise, accurate with recoveries in the range of 98 - 102 %, and minimum values of % RSD indicate the accuracy of the method. The detailed quantitative results of the study show that this method is precise, accurate, and cost-effective. Thus, the developed RP-HPLC method can be successfully feasible for the routine quality control analysis of SOR in a pharmaceutical dosage form.

Keywords: Sorafenib, RP-HPLC, Pharmaceutical formulation, Validation.

MATERIALS AND METHODS

Chemicals and Reagents

SOR sample was obtained as a gift sample from Hetero Labs Ltd., Hyderabad, India. Soranib 200 mg, marketed tablet formulation manufactured by Cipla Ltd., Purchased from a local pharmacy store. HPLC graded Methanol, Acetonitrile, Water and AR grade HCl, Sodium Hydroxide, Hydrogen Peroxide were purchased from Merck specialties Pvt. Ltd., Mumbai, India. Other excipients were prepared in our laboratory.

Instrumentation and optimization of chromatographic conditions

For UV detection of the samples, ELICO SL-210 UV spectrophotometer with 1 cm matched quartz cells were used for all spectral and absorbance measurements, and solutions were prepared in methanol. For HPLC, the chromatographic system consists of Agilent technologies - 1260 series with G1311C Quat pump VL, Thermo scientific C_{18} column, 1260 series with G11511D DAD VL detector was used. The data was acquired and processed by utilizing EZ chrome elite software. Chromatographic separation was performed on Thermo-scientific model C_{18} column (4.6 mm i.d. X 250 mm; 5 µm particle size) (based on 99.99 % ultra-
high purity silica). Using mobile phase that consisting of acetonitrile: methanol (40:60 v/v) at a flow rate of 1.0 mL/min, the runtime was set for 12 min and the eluted drug was detected at 265.5 nm with PDA detector. The sample injection volume was 20 µL. The column was maintained at a constant temperature of about 25°C.

Method development and optimization of chromatographic conditions

For HPLC development, various mobile phases containing HPLC grade water, acetonitrile, methanol in different ratios with or without buffers, and various flow rates were performed. A good symmetrical peak was found when the mobile phase comprising a mixture of acetonitrile: methanol (40:60 v/v).

Selection of detection wavelength

In the present study, the drug solutions of 10 µg/ml of SOR were prepared and scanned over a range of 200 - 400 nm. It was observed that the drug showed maximum absorbance at 265.5 nm which was chosen as the detection wavelength for the determination of SOR. The overlay spectrum (2-10 µg/mL) is shown in Figure 1.b.

Method validation

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its pre-determined specifications and quality characteristics. The method was validated as per ICH guidelines.

System suitability

The system suitability parameters like the theoretical plates, retention time, tailing factor, were studied and found satisfactory. The results are shown in Table 1.b.

Specificity

Blank, standard, system suitability, placebo, placebo spiked with the analyte, test preparations, individual impurities, and spiked test preparations were analyzed as per the method to examine the interference of blank and

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**Table 1.a: Optimized chromatographic conditions for the proposed HPLC method**

| Parameter                        | Chromatographic conditions                      |
|----------------------------------|-------------------------------------------------|
| Column                           | Thermo scientific model C18 Column               |
|                                  | (4.6 mm i.d., X 250 mm; 5 µm particle size)      |
|                                  | (based on 99.999 % ultra-high purity silica)     |
| Mobile phase                     | Acetonitrile: methanol (40: 60 v/v)              |
| Flow rate                        | 1 mL/min                                        |
| Run time                         | 12 minutes                                      |
| Detector                         | 1260 Diode Array Detector.                      |
| Detection wavelength             | UV at 265.5 nm                                   |
| The volume of the injection loop | 20 µL                                            |
| Temperature                      | Room temperature (25 °C)                         |

**Table 1.b: System suitability parameters**

| System suitability parameters | Limits          | SOR  |
|-------------------------------|-----------------|------|
| Tailing factor (T)            | ≤ 2.0           | 1.122|
| Number of theoretical plates  | NLT 2000        | 8768 |
| Theoretical plates per meter (N)* | -              | 1,66,592|
| Retention time*               | -               | 3.223 minutes |
| SD for peak area and RT       | 0.0228          |
| % RSD                          | NMT 2.0         | 0.5628|

*Average of five determinations, SD = Standard deviation, RSD = relative standard deviation.
placebo with SOR peaks. The peak purity angles are mentioned in Table 1.c, and the specificity study of SOR is mentioned in Table 2. The results of the sample and placebo chromatograms are represented in Figure 1.b & Figure 2 respectively.

**Table 1.c: Peak Purity**

| Sample                          | Analyte | Purity angle | Purity threshold |
|---------------------------------|---------|--------------|-----------------|
| Standard preparation            | SOR     | 0.138        | 0.222           |
| Placebo + analyte preparation  | SOR     | 0.138        | 0.227           |
| Test preparation                | SOR     | 0.132        | 0.229           |
| Spiked test preparation         | SOR     | 0.136        | 0.222           |

There should be no significant NMT 0.2 % of target concentration from blank, placebo, and known impurities with the analyte. The Peak purity of the analyte peak should meet the requirement. The purity angle shall be less than the purity threshold. There is no interference observed to analyte peaks, and the peak purity value complies, thus proving the specificity of the method. The results of the specificity study are mentioned in Table 2.

**Table 2: Results of specificity study for SOR**

| Name of the solution                  | Retention time (R) minutes |
|---------------------------------------|----------------------------|
| Mobile phase (blank)                   | No interference at RT of analyte peak |
| Placebo                               | No interference at RT of analyte peak |
| SOR 10 µg/mL (sample)                 | 3.223 minutes              |

**Method precision & Intermediate precision**

For method precision, six test preparations were analyzed as per the methodology representing a single batch, and the assay was determined for the same. The % RSD for assay of six test preparations should not be more than 2.0. The results are well within acceptance criteria, and the % RSD observed for assay values indicates the precision of the method. The resultant values for the drug are given below in Table 2.b.

**Table 2.b: Compiled data of Method Precision & Intermediate Precision**

| Injection No. | Method Precision | Intermediate Precision |
|---------------|------------------|------------------------|
| 1             | 98.3             | 99.3                   |
| 2             | 99.6             | 99.6                   |
| 3             | 98.4             | 98.2                   |
| 4             | 99.4             | 98.5                   |
| 5             | 99.1             | 99.4                   |
| 6             | 98.7             | 99.6                   |
| Mean          | 98.1667          | 99.1000                |
| % RSD         | 0.540332         | 0.605449               |
| Cumulative RSD | 1.14578        |                        |

**Precision at different levels**

Precision at different levels of the analytical method was determined in the concentration range of 50 %, 100 %, 150 %, their values are mentioned in Table 2.c.

**Table 2.c: Precision at 50 %, 100 %, 150 % (Precision at different levels)**

| S. No. | 50%       | 100%      | 150%      |
|--------|-----------|-----------|-----------|
| 1      | 145328    | 288941    | 427689    |
| 2      | 145742    | 287465    | 428864    |
| 3      | 146230    | 288129    | 428513    |
| 4      | 145896    | 288752    | 428567    |
| 5      | 145246    | 289148    | 427614    |
| 6      | 145847    | 288465    | 428954    |
| Mean   | 14571.8   | 288483.33 | 428366.83 |
| % RSD  | 0.25417   | 0.2129125 | 0.13530080 |
**Linearity**

The linearity of SOR was determined in the concentration range of 2 to 10 µg/mL. The linearity data, calibration curve results are shown in Table 3 and Figure 2.a. The linear regression data and ANOVA studies are shown in Figure 2.b.

**Table 3:** Linearity data of Sorafenib by HPLC

| S. No. | Concentration (µg/mL) | Area  |
|--------|-----------------------|-------|
| 1.     | 0                     | 0     |
| 2.     | 2                     | 145011|
| 3.     | 4                     | 288592|
| 4.     | 6                     | 428834|
| 5.     | 8                     | 546704|
| 6.     | 10                    | 686130|
| 7.     | Intercept             | 8071.047619|
| 8.     | Slope                 | 68228.15714|
| 9.     | CC                    | 0.999505|
| 10.    | Squared CC            | 0.999010|

**Figure 2.a:** Calibration graph of Sorafenib by RP-HPLC

\[ y = 68228x + 8071 \quad R^2 = 0.999 \]

**Table 3.a:** Results of Accuracy study

| % Recovery Level | Amount Added (mg) | Amount Recovered (mg) | % Recovery |
|------------------|-------------------|-----------------------|------------|
| 1                | 12.14             | 12.09                 | 99.5       |
| 50 %             | 12.21             | 12.11                 | 99.1       |
| 3                | 12.09             | 11.96                 | 99.6       |
| 1                | 24.53             | 24.11                 | 98.2       |
| 100 %            | 24.65             | 24.49                 | 99.3       |
| 3                | 24.72             | 24.61                 | 99.5       |
| 150 %            | 36.91             | 36.78                 | 99.6       |
| 3                | 36.89             | 36.72                 | 99.5       |

**Accuracy (Recovery studies)**

A known amount of drug was spiked with placebo at three different levels in triplicate preparations. The samples were then analyzed as per the proposed standard method. The accuracy studies are mentioned in Table 3.a. The precision at accuracy results is mentioned in Table 3.b.

**Table 3.b:** Results of Precision at Accuracy study

| S. No. | Concentration % of spiked level | % recovery | % RSD  |
|--------|---------------------------------|------------|--------|
| 1      | 50 %                            | 99.4       | 0.2661 |
| 2      | 100 %                           | 99.0       | 0.7070 |
| 3      | 150 %                           | 99.6       | 0.5798 |

**Robustness**

The robustness of the method was determined for the system suitability and assay value under variable conditions. The robustness of the analytical method was established by demonstrating its reliability against deliberate changes in the chromatographic conditions. The robustness of the method of SOR is mentioned in Table 3.c.
Table 3.c: Results of Robustness study

| Parameters                          | Optimized | Used          | Retention time (min) | Plate count $ | Peak asymmetry # | Remarks |
|-------------------------------------|-----------|---------------|----------------------|---------------|-----------------|---------|
| Flow rate (± 0.2 mL/min)            | 1.0 mL/min| 0.8 mL/min    | 3.298                | 8820          | 1.11            | *Robust |
|                                     |           | 1.0 mL/min    | 3.223                | 8768          | 1.12            | *Robust |
|                                     |           | 1.2 mL/min    | 3.19                 | 8720          | 1.11            | *Robust |
| Detection wavelength (± 5 nm)       | 265 nm    | 260 nm        | 3.223                | 8768          | 1.20            | Robust  |
|                                     |           | 265 nm        | 3.223                | 8768          | 1.13            | Robust  |
|                                     |           | 270 nm        | 3.223                | 8764          | 1.13            | Robust  |
| Mobile phase composition Methanol: | 60:40 v/v | 55:45 v/v     | 3.327                | 8779          | 1.10            | *Robust |
| Acetonitrile (± 5 %)                |           | 60:40 v/v     | 3.223                | 8768          | 1.12            | *Robust |
|                                     |           | 65:35 v/v     | 3.209                | 8789          | 1.17            | *Robust |

Acceptance criteria (Limits): $^\text{a}$Peak Asymmetry < 1.5, $^\text{b}$Plate count > 2000, * Significant change in Retention time

LOD and LOQ

Limit of Detection is the lowest concentration in a sample that can be detected but not necessarily quantified under the stated experimental conditions. The limit of quantitation is the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy. The LOD and LOQ are 0.526 μg/mL and 1.594 μg/mL respectively.

RESULTS AND DISCUSSION

The above-mentioned SOR is relatively polar, an RP-HPLC method was used. The column for the separation was a C$_{18}$ column that has an internal diameter of 4.6mm, length of 250 mm, and 5µm particle size. Multiple numbers of trials were performed using various buffer solutions with various compositions of methanol, ethanol, acetonitrile, and HPLC grade water and variable flow rates. Eventually, optimum separation was obtained with a mixture of acetonitrile and methanol (40:60 v/v). The mobile phase flow rate was adjusted at 1mL/min, and the detection wavelength was set at 265.5 nm. Thus, a proper chromatographic peak was obtained with excellent symmetry and the least peak tailing. The chromatograms of various concentrations were shown in Figure 3 – Figure 3.d.
System suitability was conducted as per the methodology system suitability solution, and six replicates of standard preparation were injected into HPLC. The tailing factor was found to be 1.122. The number of theoretical plates was 8768, the number of theoretical plates per meter was 1,66,592. The retention time was found out to be 3.223 minutes, and the % RSD was calculated to 0.5628. The results were well within the acceptance criteria, and the study concludes the suitability of the analytical system for analysis.

For specificity studies, blank, standard, system suitability, placebo, test preparations, individual impurities, placebo spiked with the analyte, and spiked test preparations were analyzed to examine the interference of blank and placebo with SOR peaks. The purity angle for standard preparation is 0.139, for placebo plus analyte preparation is 0.138, for test preparation is 0.132 and spiked test preparation is 0.137. No interference was observed in analyte peaks, and peak purity values comply, thus proving the specificity of the method.

The precision of the method was examined by using System precision, Method, and Intermediate precisions. Various levels of concentration were taken in six replicate samples. For Method and Intermediate precisions, the % RSD was found to be 98.166 and 0.60544. The % RSD of the System precision was found to be 0.02967. The precision at different levels was mentioned in Table 2.C. The results are well within the acceptance criteria, and the % RSD observed for the replicate injections indicates the precision of the HPLC used, assay values indicate the precision of the method.

The linearity of SOR was determined in the concentration range of 2 µg/mL to 10 µg/mL of the test concentration. The squared correlation coefficient value was found to be 0.99901, which is well within the limit.

To determine the accuracy of the SOR, the drug was spiked with a placebo at three different levels in triplicate preparations. The results of accuracy are mentioned in Table 3.a, and the results of precision at accuracy are given in Table 3.b. The mean % recovery at each level was found out to be within limits i.e., 98.0 % to 102.0 %.
The robustness of the HPLC was determined for the suitability and assay value under multiple variable conditions like flow rate change, wavelength change, and change in mobile phase composition. The results are mentioned in Table 3.c. The LOD and LOQ of SOR were found out to be 0.526 μg/mL and 1.594 μg/mL, respectively.

**Application of the developed method for marketed formulation (Assay)**

For the assay of pharmaceutical formulation, 20 tablets of Soranib marketed formulation (SOR 200 mg) were weighed, the average weight was calculated, and a quantity of tablet powder equivalent to 100 mg of SOR was accurately weighed and transferred into a 100 mL volumetric flask containing 30 mL of the mobile phase. The solution was ultra-sonicated for about 15 minutes, filtered through a Whatman filter paper (0.45 μm) nylon filter, and the filtrate was made up to volume with the mobile phase. The concentration was 1 mg/mL. Transfer 1 ml of the filtered sample solution to 10 mL volumetric flask and made up to volume with mobile phase to get 100 μg/mL. This is used as a working solution for the preparation of the assay. Then 0.2 ml of this solution is transferred into a 10 ml volumetric flask and made up to volume to obtain 2 μg/mL which is used for the assay. The assay results are presented in Table 4. a. The representative sample chromatogram of SOR is shown in Figure 4.a.

**Table 4.a: Assay results of marketed formulation**

| S. No. | Formulation   | Labeled claim | Amount found | Mean % recovery ± SD | % RSD |
|--------|---------------|---------------|--------------|----------------------|-------|
| 1      | Soranib tablets | 200 mg/tablet | 199.9 mg/tablet | 99.95 ± 12           | 1.23  |

*Average of six determinations, SD denotes standard deviation, RSD denotes % relative standard deviation.

**Figure 4.a: SOR sample chromatogram**

**SUMMARY:**

To summarize the methods employed and the results obtained in the study of SOR, it is mentioned below in Table 4.b.

**Table 4.b: Summary of the RP-HPLC of Sorafenib**

| S. No. | Validation Parameter | Acceptance Criteria | Results               |
|--------|----------------------|---------------------|-----------------------|
| 1      | System Suitability   | 1. The ICH regulation between degradants peak and SOR peak in the system suitability should not be less than 2.0.  
2. The ICH tailing factor for SOR peak in standard preparation should not be less than 2.0.  
3. The ICH theoretical plate count for the SOR peak in standard preparation should not be less than 3000.  
4. The % Relative standard deviation in six replicate injections of standard preparation should not be more than 2.0 %. | Complies |
| 2      | Specificity          | 1. There should not be any significant interference (NMT 0.2 % of the target concentration) from blank, placebo, and impurities with the analyte.  
2. Peak purity of the analyte peak should pass (Purity angle should be less than Purity threshold). | No interference observed, and peak purity complies |
| 3      | Precision            | % RSD for six replicate injections should not be more than 2.0. | SOR: % RSD - 0.02967 |

1. System precision
CONCLUSION

The current study demonstrated a validated RP-HPLC method for the estimation of SOR available as the tablet dosage form. The method was completely validated and showed satisfactory results. The method was free from the interference of the other active ingredients and additives used in the formulation. The RP-HPLC method for the determination of SOR has various advantages like less solvent consumption, low retention time, good peak symmetry accurate, precise and robust. The results of the study indicate that the developed method was found to be accurate, precise, linear, sensitive, simple, economical, and reproducible, which has a short run time, which makes the method rapid. Hence it can be concluded that this method may be employed for the routine quality control analysis of SOR in active pharmaceutical preparations.

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