Research Article

Development and Validation of UV- Spectrophotometric Method for Determination of Sorafenib in Pharmaceutical Dosage Form and its Degradation Behaviour Under Various Stress Conditions

Panchumarthy Ravisankar*, P. Srinivasa Babu, SK. Munaza Taslim, K. Kamakshi, R. Lakshmi Manasa
Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, Guntur, A.P, India.
***Corresponding author's E-mail:** banuman35@gmail.com

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ABSTRACT

The objective of this study is a cost effective, precise, accurate, simple stability indicating UV-Spectrophotometric method was developed for the estimation of Sorafenib in tablet dosage form. Instruments used ELICO Double beam SL 210 Ultra violet-Visible spectrophotometer consisting two matched quartz cells with one cm light path was utilized for measuring of absorbance of Sorafenib. Sorafenib shows highest λmax at 265.5 nm. Beer's law was found over a concentration range of 2-10 µg/ml with superior correlation coefficient (r2 = 0.9998). The Limit of Detection (LOD) and Limit of Quantitation (LOQ) were found to be 0.3741 µg/ml and 1.1337 µg/ml respectively. The results of the Sorafenib recovery analysis were found to be 99.9580 ± 0.02095 to 99.9787 ± 0.0106. Percentage assay of Sorafenib tablets (Sorafenat) got more than 99.88 %. Sorafenib was subjected to alkali, acidic, oxidation, thermal, UV light degradation. Sorafenib is more unstable in acidic, oxidation, thermal and stable in alkaline and ultra violet (UV) light irradiation. The Proposed spectrophotometric method was validated as per the ICH Q1A (R2) guidelines. While estimating the Sorafenib in tablet formulation there was no interference of additives & excipients. Hence this method can safely be employed for the routine quality control analysis of Sorafenib in bulk and tablet formulations.

Keywords: Forced degradation, Sorafenib, Method development, UV spectroscopy.

INTRODUCTION

The chemical name for Sorafenib is 4-[4-({[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl} amino) phenoxy]-N-methyl pyridine-2-carboxamide. Sorafenib is used in the treatment of renal cell carcinoma & colon cancer.

From the extensive literature survey reveals that not many UV analytical methods published to describe the quantification of Sorafenib by LC-MS/MS[1], LC-MS[2-6], HPTLC[7-8], UPLC[9], HPLC-MS[10], RP-HPLC[11], UV-Spectrophotometric method[12]. Infact the published UV method utilizes only methanol as solvent. But there is no stability indicating UV method with acetonitrile and methanol as solvent hitherto. These stability indicating methods would be helpful in establishing the stability data of these drugs in bulk and tablet dosage forms. Generally this UV technique is less expensive and with inherent simplicity. Quick development in the pharmaceutical industries, producing more number of new drugs and formulations in different parts of world has been increasing. For getting effective and safe drug formulation to consumers direly needed. So innovative novel analytical methods compulsory for controlling their quality and amount of drug in pharmaceutical dosage forms particularly it plays an vital role in the case of powerful drugs. So the author inclined to select a novel, fast stability indicating UV spectrophotometric analytical method to quantify Sorafenib in bulk and tablet dosage forms. Figure 1 shows the structure of Sorafenib.

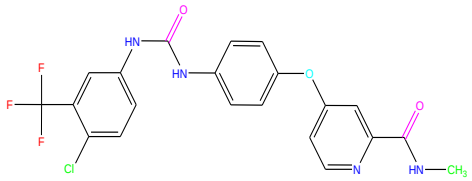

Figure 1: Chemical structure of Sorafenib.

MATERIALS AND METHODS

Instruments utilised ELICO Double beam SL 210 UV - ultra violet-Visible spectrophotometer containing 2 matched quartz cells with one cm light path was utilized for measuring of absorbance of Sorafenib. Essae vibra AJ 0.001 g balance was used for weighing. Ultra sonicator bath Model no - 91250, PCI Ltd., Mumbai were used in this study.

Materials

Sorafenib unadulterated drug was procured as gift sample by Hetero Drugs Ltd., Hyd, Telangana, India. The Sorafenib tablets consisting 200 mg of Sorafenib procured from local market. ACN and MEOH were acquired from E. Merck specialties private Ltd., Mumbai, India.

Selection of solvents

Numerous trails were performed to find out the perfect solvent system for dissolving the drug. The solvents such as ACN, MEOH, double distilled water and dimethyl sulfoxide [DMSO] were tried depending on the solubility of the Sorafenib. Sorafenib is soluble in organic solvents such as ACN, DMSO, and methanol and insoluble in distilled water. Based on the solubility methanol and

acetonitrile were preferred all the way through the experiment.

Selection of detection wavelength

To estimate the maximum λ_{max}, Sorafenib 10 µg/ml of working standard solution was prepared and scanned in UV wavelength range of 200 - 400 nm utilizing as a blank. It was observed that the drug showed maximum absorbance at 265.5 nm which was chosen as the detection wavelength for the determination of Sorafenib.

Preparation of stock and working standard solution

Sorafenib 10 µg/ml standard stock solution was prepared by transferring accurately weighed 10 mg of standard Sorafenib to 10 ml volumetric flask and dissolved in MEOH and ACN. The volume was made upto the mark with MEOH and ACN. From this solution 1 ml was exactly transferred into a 10 ml volumetric flask and volume was made upto the mark with MEOH and ACN. Again from the above solution 1 ml was precisely transferred into a 10 ml volumetric flask and volume was made upto the mark with MEOH and ACN. Working standard solutions of Sorafenib was prepared by suitable dilution of the stock solution (10 µg/ml) with the MEOH and ACN.

Preparation of Calibration curve

A calibration curve was plotted over a concentration range of 2-10 µg/ml for Sorafenib. Exactly measured standard solution of Sorafenib (2, 4, 6, 8, and 10 ml) was shifted to a series of 10 ml volumetric flasks and the volume was filled upto 10 ml with MEOH and ACN. Calibration curve was done by plotting Sorafenib concentration on X-axis and their respective absorbances on Y-axis. Calibration data is shown in table 1. The optical characteristics are shown in table 2. Figure 2 shows the overlain spectrum of Sorafenib. Figure 3 shows the calibration curve of Sorafenib.

Table 1: Calibration data of Sorafenib

S. No	Concentration (µg/ml)	Absorbance (nm)
1	2	0.2462
2	4	0.4774
3	6	0.6995
4	8	0.9423
5	10	1.1866

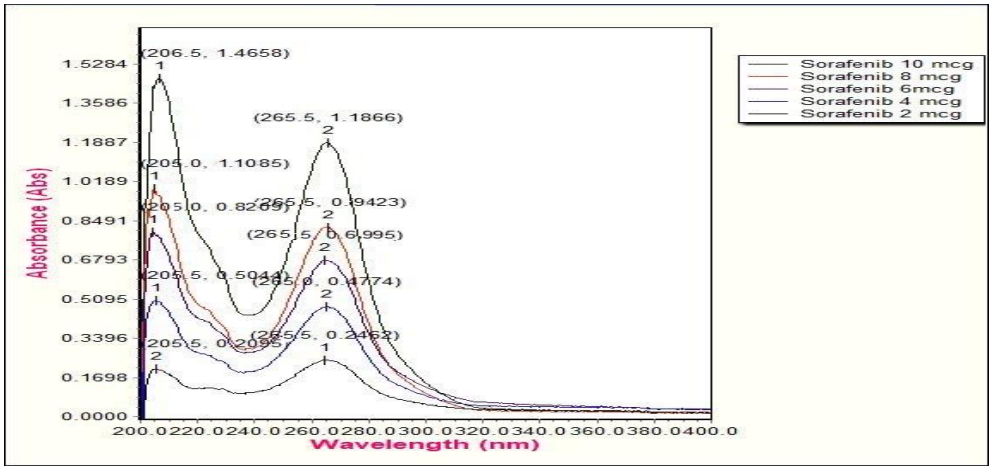

Figure 2: Overlay Spectrum of Sorafenib

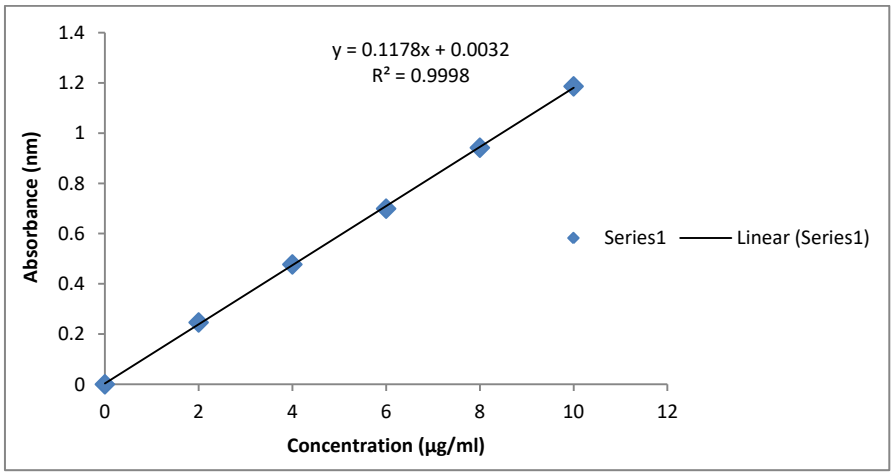

Figure 3: Calibration curve of Sorafenib

Forced Degradation Studies

To evaluate the stability indicating property of the proposed UV method stress studies were done under ICH[9-11] recommended conditions. Forced degradation[12] of Sorafenib was carried out by exposing the bulk sample to Thermal, UV irradiation alkaline, acidic, oxidative conditions.

Figure 4a: Irradiation with UV light

Figure 4b: Thermal degradation

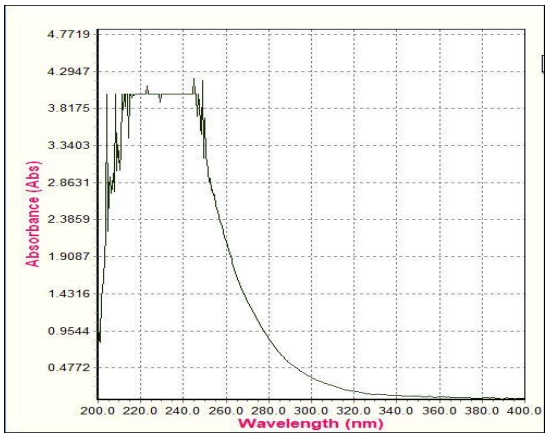

Figure 4c: Oxidative degradation

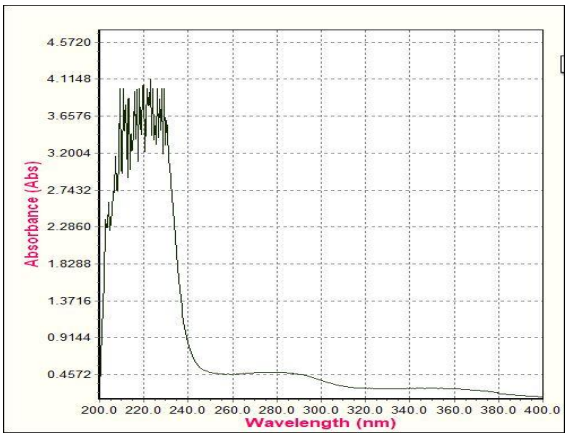

Figure 4d: Alkaline degradation

Figure 4e: Acidic degradation

Irradiation with UV light

A sample powder of Sorafenib (10 mg) was exposed to UV light for 48 hours. The material was dissolved in 10 ml methanol and acetonitrile. The solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was suitably diluted and absorbance was recorded and represented in figure 4a.

Thermal degradation

A sample powder of Sorafenib (10 mg) was exposed to a temperature of 70°C for 48 hours in hot air oven. The drug was dissolved in 10 ml methanol and acetonitrile. The drug solution was filtered with syringe filtration disk claimed concentration of 1 mg/ml. It was diluted and absorbance was recorded. Figure 4b shows the thermal degradation study.

Oxidative degradation

Exactly 10 µg/ml of Sorafenib solution was taken into round bottom flask. The contents were then mixed with 9 ml of 30 % hydrogen peroxide solution, and the contents were allowed to proceed at room temperature (25°C) for 2 hours with intermittent shaking and absorbance was measured which is shown in figure 4c.

Alkaline and Acidic hydrolysis

Aliquot of 10 µg/ml of Sorafenib solution was transferred to a small round bottom flask. The solution was mixed with 9 ml of 0.1 N HCl or 0.1 N NaOH. The prepared solutions were subjected to reflux for 2 hours in a boiling water bath. The samples were set aside for 30 minutes for cool-down to room temperature (25°C), and absorbance was recorded as shown in figures 4d and 4e.

RESULTS AND DISCUSSION

Table 2: Summary of Optical characteristics and validation parameters

S. No	Parameters	Result
1	Detection wavelength	265.5 nm
2	Beer's Law limits (µg/ml)	2 - 10 (µg/ml)
3	Regression equation (y = mx+c)	0.117x + 0.003
4	Correlation Coefficient (r^2)	0.9998
5	Slope (m)	0.117
6	Intercept (c)	0.003
7	Precision (% RSD) System precision (n =6) Intra-day (n = 6) Inter-day (n = 6)	0.054233 0.026702 - 0.041902 0.026705 - 0.041929
8	Accuracy (% mean recovery) 80 % level 100 % level 120 % level	99.95807 99.9714 99.9787
9	Ruggedness 2 Analysts (% RSD) 2 Instruments (% RSD)	≤ 2
10	Robustness Wavelength (± 2nm) (% RSD)	≤ 2
11	LOD and LOQ	0.374139 and 1.133755

Method development and Validation

Several solvents were analysed including acetonitrile, DMSO, and methanol at 10 µg/ml concentrations. However Sorafenib was soluble and stable for minimum 72 hours at room temperature. So methanol and acetonitrile solvent was used for the estimation of detection wavelength and preparation of standard and working concentration. So as to check the proposed method to the pharmaceutical formulation, an assay of Sorafenat 200 mg tablets was used at working concentration. Assay for working concentration of sample at 265.5 nm was in limits of acceptance 98 - 102 %. According to ICH Q1A (R2) guidelines. UV spectrophotometric method developed as stated by guidelines for validation of analytical procedures. The method was validated for parameters such as linearity, precision, accuracy, specificity, robustness, ruggedness, LOD and LOQ.

Precision

In system precision 10 µg/ml concentrations of 6 replicate recordings of absorbance at 265 nm were observed on the same day and corresponding responding responses were evaluated. The mean, SD and % RSD were calculated. The repeatability data of Sorafenib is summarized. The intermediate precision or inter-day precision is evaluated by analyzing 10 µg/ml concentrations of Sorafenib fresh sample solutions were analyzed six times on 3 different days and was evaluated. Eventually the mean, SD and % Relative standard deviation were counted.

Accuracy (Recovery studies)

Recovery studies of Sorafenib were carried out by utilizing standard addition method. By preparing the known amount of standard Sorafenib drug at 3 levels (80 %, 100 % and 120 %) was added to pre-analyzed sample and again re-analyzed by duly adopting the present developing method. Infact, from the amount of Sorafenib found, % recovery was estimated.

Ruggedness

Ruggedness is determined by different analysts, instruments, laboratories. Method ruggedness may not be known when a method is first developed, but insight is obtained during subsequent use of that method. Suggested % RSD less than 2 and indicates that the method developed is rugged.

Robustness

The major part of robustness is to develop methods that allow for predictable variations in the separation parameters. For the estimation of method robustness, parameters such as variation in detector wavelength are varied within the accurate range and the quantitative influence of the variables is determined. The analysis showed % RSD less than 2 which indicates that the method established is robust.

LOD and LOQ

Limit of Detection and Limit of Quantification were calculated using following formula LOD = 3.3 * (SD) / S and LOQ = 10 * (SD) / S, where SD = standard deviation of absorbance and S = slope of the calibration.

Procedure for assay of pharmaceutical formulation

20 Sorafenib (Sorafenat) marketed tablets were accurately weighed, finely powdered and average weight of each tablet was determined and the tablet fine powder equivalent to 10 mg of Sorafenib was taken into 100 ml graduated flask and dissolved in methanol and ACN (acetonitrile) to get 100 µg/ml concentration. The solution was then sonicated for 20 min and filtered and further dilutions were done with ACN to get eventual concentration (10 µg/ml) within the linearity range and measured λ_{max} at 265.5 nm. Finally the drug content in each tablet and also bulk drug was found by utilizing the standard graph.

Table 3: Result of Assay of Pharmaceutical Formulation (Sorafenat) and bulk drug

Pharmaceutical formulation and bulk drug	Labelled claim(mg)	% Content of drug ± SD*
Sorafenat	200	99.98 ± 0.42

*Each value is mean ± deviation of five determinations, UV =ultra violet.

SUMMARY

The UV spectrum of Sorafenib was scanned in the region between 200-400 nm. The overlay spectra of Sorafenib at different concentrations (Figure 2) were appreciably absorbed maximum at 265.5 which was selected as the detection wavelength. The response of the Sorafenib was found to be linear in the ranges from 2-10 µg/ml with a good correlation coefficient of r^2 = 0.9998 and the Figure 3 shows the Sorafenib linearity calibration curve and the table 1 shows the calibration data. Optical characteristics and validation parameters of the proposed analytical method are represented in table 2. It was found that the % RSD values of intra-day and inter-day precision was 0.0378 and 0.0267 respectively pertaining to Sorafenib and the values of % RSD [< 2.0] pellucidly showed that the method was fairly precise. According to norms in vogue accuracy studies were conducted by recovery study using standard addition method at 3 different concentration levels (80, 100 and 120 %). The recovery study results were found to be within the limits and % RSD less than 2. Ruggedness was performed by changing two different analysts and two different instruments and % RSD was calculated. The % RSD clearly shows less than 2 which indicate that the method was rugged. Robustness was performed by changing two different wavelengths. Even though by changing the minor modifications the % RSD got < 2 which shows that the method was robust. In the planned method the LOD and LOQ were found to be 0.374139 µg/ml and 1.133755 µg/ml respectively which show that this method was very sensitive as they were within the permitted levels. The developed method was eventually utilized for quantification of tablet dosage form and bulk form. The mean % assay value of tablet formulation was found to be 99.98 ± 0.42. The developed method has good linearity, accuracy and precision results indicates that the high quality of the method. Forced degradation studies were done. Extensive degradation studies found in thermal and UV, little degradation found in acidic, alkaline and some degraded takes place in oxidation.

CONCLUSION

There were no UV methods have been reported (with methanol and acetonitrile as solvent) for the determination of Sorafenib in bulk as well as pharmaceutical tablet. None of the usual excipients employed in the formulation of Sorafenib dosage forms interfered in the analysis of Sorafenib by the developed method. Validation parameters are found within the limits. As a matter of fact, in this study the degradation behaviour of Sorafenib was studied by subjecting the Sorafenib to different stress conditions as per ICH guidelines. It was observed that all the statistical analysis results of % RSD values particularly precision, accuracy are observed below two which speaks that the method is precise and accurate. The results of pharmaceutical formulation asserts that the proposed method of Sorafenib suitably practicable for their determination without interfering the additives and excipients. Therefore this method was simple, precise, accurate and cost effective and in actual fact possible for routine sample analysis of Sorafenib in bulk and pharmaceutical tablets.

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