



DETERMINATION OF SORAFENIB IN BULK AND TABLET FORMULATION BY A NEW VALIDATED REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A rapid, precise, economical and accurate reverse phase HPLC method was developed and validated for estimation of Sorafenib (SFN) in bulk and formulations. The chromatographic resolution was achieved using acetonitrile and 0.1M disodium phosphate buffer (55: 45 V/V) as a mobile phase, UV detection at 248 nm, Interesil ODS C18 column and flow rate 1mL/min. The extraction recovery of Sorafenib from its formulation dosage form (tablets) was found to be greater than 99.48% and the calibration curve was linear ($r^2 = 0.9998$) over Sorafenib concentration ranging from 20 to 120 $\mu\text{g}/\text{mL}$. The method had an accuracy of greater than 99%. Limit of detection and limit of quantification were found to be 0.7466 $\mu\text{g}/\text{mL}$ and 2.3300 $\mu\text{g}/\text{mL}$ respectively. The developed method was validated statistically and recovery studies were found to be satisfactory.

Keywords: Sorafenib, Renal cell carcinoma (RCC), Hepatocellular carcinoma [HCC] and colorectal cancer [CRC], RP-HPLC, CC, LOD AND LOQ.

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INTRODUCTION

Sorafenib (SFN) is a novel, oral multi-kinase inhibitor that blocks serine/threonine and receptor tyrosine kinases in both the tumor and the vasculature. Single-agent activity and acceptable tolerability were initially demonstrated with sorafenib in Phase I studies in patients with a variety of advanced, refractory solid tumors, including renal cell carcinoma (RCC), colorectal cancer (CRC), and hepatocellular carcinoma (HCC). Dose-limiting toxicities (DLTs) included diarrhea, fatigue and skin toxicity, but not bone marrow suppression. Phase II studies have also demonstrated activity of sorafenib in advanced RCC and HCC, and evidence of significantly prolonged progression-free survival over placebo in a Phase III trial in RCC was recently presented. Good tolerability with manageable side-effects was confirmed in this Phase III trial in approximately 900 patients.

A few publications are available for the determination of sorafenib. HPLC-UV method was reported for the determination sorafenib in human plasma and application to cancer patients in routine clinical practice¹. A number of publications were available in clinical studies like Phase-I, Phase-II and III²⁻⁵, but no RP-HPLC method is available to determine the drug in pharmaceutical formulations. So the author made an attempt and succeeded in developing a new method which was precise and accurate. This method was effective to produce better retentions, very sharp and symmetrical peak shapes and exhibit very good sensitivity.

EXPERIMENTAL

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC20AT and LC20AT VP series HPLC pumps, with a 20 μl injection of sample loop (manual), and SPD20 A VP UV –visible Detector. The output signal was monitored and integrated using ShimadzuClass VP version 6.12 SP1 software. Intersil ODS C18 (250 x 4.6 μm , 5 μm) column was used for separation.

Standards and chemicals

Sorafenib and its formulations were purchased from Natco Pharmacy and standard sample was gifted by Chandra labs. HPLC grade chemicals and solvents such as acetonitrile, potassium dihydrogen phosphates are purchased from Merck chemicals.

Preparation of standard drug solution

50mg of Sorafenib and an amount of its formulation equivalent to 50mg was accurately weighed and transferred in two separate 50 mL of volumetric flask containing 25mL of mobile phase sonicated for 25 min, diluted with mobile phase up to the lower meniscus mark and filtered it through 0.45µm membrane to get this stock solution (1mg per mL)

Chromatographic conditions

The mobile phase used in this study was a mixture of acetonitrile and disodium phosphate (buffer PH~4 with phosphoric acid) 55:45 V/V, then the content was solicited for 30 min for degassing purpose and then filtered through 0.45 µ (pore diameter) Whatman filter paper. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0mL/min. The eluents were monitored at UV max 248 nm. The column temperature was maintained ambient throughout the experiment.

Preparation of Calibration of Standards

Calibration standards were prepared by spiking working standard into mobile phase containing 25mL volumetric flask to yield concentrations of 20, 40, 60, 80,100 and 120 µg/mL. A 20 µL aliquot was injected into the analytical column. The resultant peak areas of the drug were measured. Calibration curve was plotted between peak area and drug concentration (Fig.3). Slope, intercept and correlation coefficient of the data were presented in Table-1.

Recovery of Sorafenib from its Formulation

The finely powdered formulation dosage and accurately weighed sample of formulation equivalent to 50 mg sorafenib was extracted with acetonitrile in a 50mL volumetric flask using ultra sonicator. This solution was diluted with mobile phase, so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates. The percent of recovery of the drug from its formulations was shown in Table 2.

Table-1: Linearity studies between concentration Sorafenib and peak area

Concentration (µg /mL)	Peak Area	Linearity Parameters
20	312.967	Slope (1): 16.566
40	664.704	
60	988.364	
80	1323.096	Intercept: 6.8392
100	1658.644	
120	1968.890	Correlation coefficient: 0.9998

Table-2: Recovery of Sorafenib in formulation tablet by developed HPLC Method

Type of Formulation	Labeled Amount (mg)	Recovered Amount	% Recovery
Tablet	100	99.48	99.48

*Each value is the average of five determinations

Table-3: Precision Studies

Concentration (µg/mL)	Peak Area	%RSD
100µg /mL	1788.225	0.209589

*Each value is the average of five determinations

RESULTS AND DISCUSSION

Specificity and selectivity of the method was assessed by preparing a drug concentration of 100 μ g/mL from pure drug stock (Fig.1) and commercial sample stock in selected mobile phase and analyzed (Fig-2).

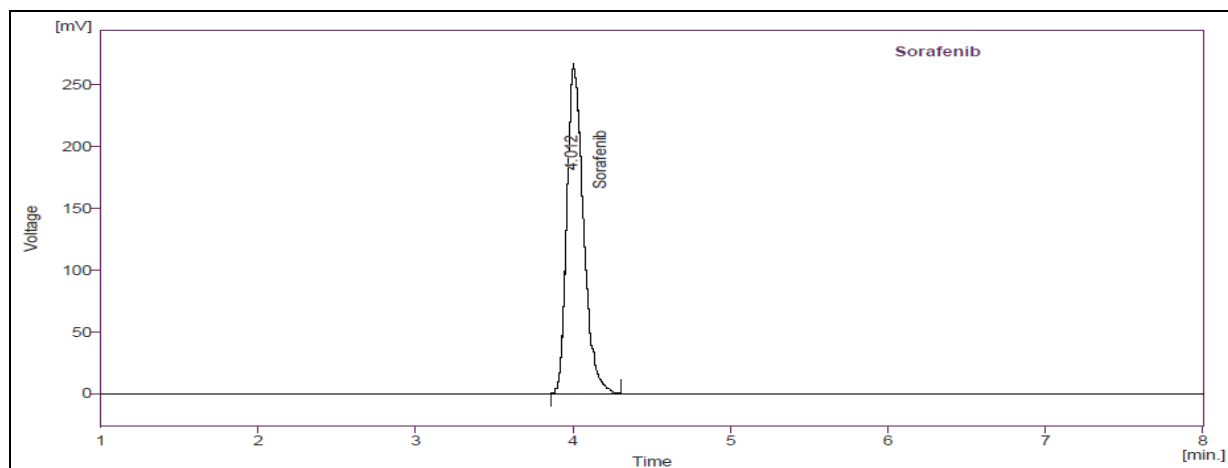


Fig.-1: A typical chromatogram of Sorafenib standard

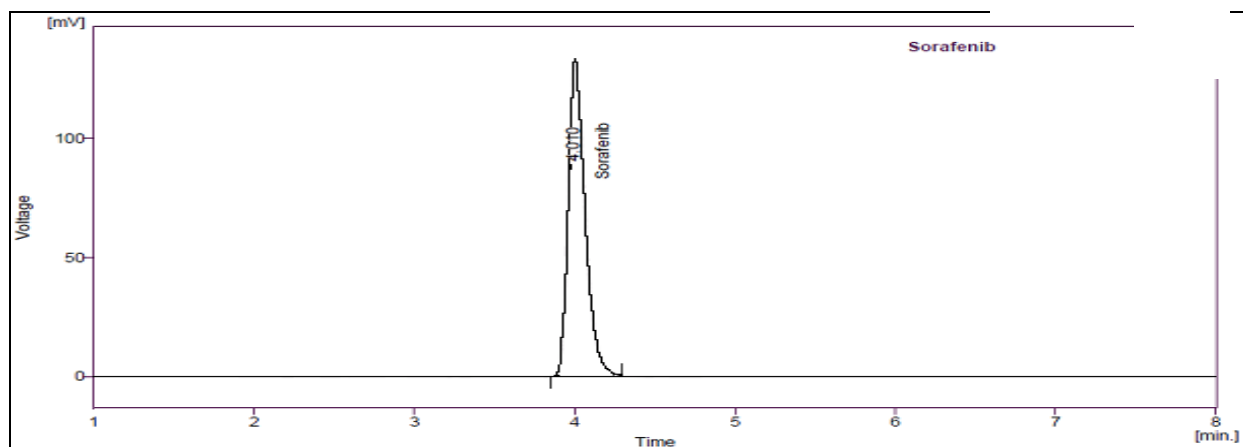


Fig.-2: A typical chromatogram of Sorafenib formulation



Fig.-3: Linearity plot between concentration of the drug and peak area

Table-4: Accuracy Studies

Mixture of pure and formulation(%)	Concentration of formulation in $\mu\text{g/mL}$	% of Recovery of pure drug	%RSD
80	80	99.125	0.025234
100	100	99.655	0.017496
120	120	100.505	0.019342

*Each value is the average of five determinations

The HPLC chromatograms recorded for the drug matrix showed almost no other peaks within a retention time 4.010 min. The developed HPLC method is selective for Sorafenib. The method is linear in the concentration range 20 to 120 $\mu\text{g/mL}$ (Fig.3). Intra day precision was studied by five replicate measurements at three different concentration levels over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation (%RSD) of the drug at different concentration levels for five injections was less than 2.0. Precision and accuracy data were shown in Table-3 and Table-4 respectively. For system suitability, five replicates of standard sample were injected and different parameters were studied (Table 5). The tailing factor for Sorafenib was always less than 2.0.

Table-5: System Suitability

S.No.	Parameters	Values
1.	Theoretical Plates (N)	6941.00
2.	LOD, $\mu\text{g/mL}$	0.74660
3.	LOQ, $\mu\text{g/mL}$	2.33000

CONCLUSIONS

Under the presently prescribed conditions, the recoveries of sorafenib were found to be 98.12 to 100.50%. This indicates that commonly used excipients in pharmaceutical formulations were not interfering in the proposed method. This method is very useful for determination of sorafenib in pharmaceutical dosage forms.

The observation of % RSD less than 2.0 for both intra- and inter-day measurements also indicates high degree of precision. In the present method, we have established linearity range of 20-120 $\mu\text{g/mL}$; this linearity range covers all the strengths of sorafenib hence this method can be applied for quantifying the low levels of sorafenib in pharmaceutical dosage forms.

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