Development and Validation of novel Chromatographic method for estimation of Sorafenib in Pharmaceutical dosage form

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ABSTRACT
Objective: A sensitive, rapid, accurate and precise novel RP-HPLC method was developed for the estimation of Sorafenib in pharmaceutical dosage form.
Methods: Chromatographic separation of Sorafenib was carried out utilizing PHENOMENOX C_{18} (25 cm × 0.46 cm, 5 μm particle size) using mobile phase that consisting of Acetonitrile : Water (pH 3.5 adjust with o-phosphoric acid) in portion of 80:20 at a flow rate of 1.0 mL min^{-1}.
Results: The maximum absorption (λmax) of Sorafenib in Mobile phase was found to be 265 nm. It had a retention time of 5.630 min. The linear calibration curve of the drug in the concentration range of 5-25 μg mL^{-1} (r^2= 0.999) for the optimized method. The regression equation for Sorafenib was found to be Y= 42,972.03x – 5,009.35. The Detection limit (DL) and Quantitation limit (QL) results of Sorafenib were found to be 0.0133 μg mL^{-1} and 0.0404 μg mL^{-1} respectively. The developed method was validated in pursuance of ICH Q2 (R1) guidelines.
Conclusion: The developed method was accurate, validate and precise with recoveries in the range of 98 – 102 %. Minimum values of % RSD indicate the accuracy of the method. The detailed quantitative results show that this method is novel, sensitive as well as cost effective.
KEY- WORDS: Sorafenib, RP- HPLC, Validation, Quantitative.

I. INTRODUCTION
Sorafenib belongs to Anticancer Drug that is a protein kinase inhibitor. Chemical name of Sorafenib is 4-[4-[[4-chloro-3-(trifluoromethyl) phenyl] carbamoylamino] phenoxy]-N-methyl-pyridine-2-carboxamide. The chemical formula and Mol. Wt. are $C_{21}H_{16}ClF_3N_4O_3$ and 464.83 g mol^{-1} respectively. It is official in IP/ USP/ EU. It contains freely solubility in Methanol and Acetonitrile. It is used in treatment of Primary Kidney and Liver Cancer. [1-5]
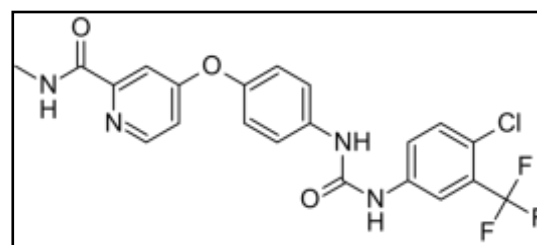

Fig. 1: Structure of Sorafenib

Detail Review of Literature survey reveals that reported conventional methods in bulk and pharmaceutical formulation which include, UV Spectroscopic method[6], RP- HPLC[7-10], and HPTLC[11-12] were found to be more time consuming and expensive.

II. MATERIAL AND METHODS
Instrumentation
The chromatographic method was developed and validated on Shimadzu LC- 10 AT (Shimadzu Corporation, Kyoto, Japan). This HPLC system consists of PDA detector and 20μL fixed loop injector. Analytical chromatographic data were collected and processed using Lab solution software. Selected Mobile phase was degassed using Frontline electronics- FS 5 ultra sonicator.

Chemicals and Reagents
Reference standard sample of Sorafenib was procured from Shashi Pharma, Chhatral. Marketed formulation of Sorafenib Tablets 200 mg, marketed by Cipla Ltd., Purchased from Local Pharmacy store. Water, Methanol and Acetonitrile, was HPLC grade purchased from Finar Ltd. Potassium di-hydrogen orthophosphate and o-phosphoric acid was Analytical grade purchased from Ranbaxy chemicals.

Chromatographic conditions

The Chromatographic separation was carried out using a Mobile phase consisting of a mixture of Acetonitrile: Water, pH 3.5 adjusted with o- phosphoric acid in ratio of 80:20 at a flow rate of 1.0 mL min^{-1}. The eluted drug was detected at 265 nm with PDA detector. The sample injection volume was 20 µL. The HPLC system was maintained at temperature 25 ± 2 °C.

Method Development of RP- HPLC Method
Selection of Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. An ideal wavelength is the one that gives good response for the drug that are to be detected. In the present study, drug solution of Sorafenib (5 µg mL^{-1}) was prepared in Methanol. This drug solution was than scanned in UV region of 200-400 nm and maximum Absorbance was recorded.

Selection of Mobile Phase

After considering the varying combinations of various mobile phases, Acetonitrile: Water, pH 3.5 adjusted with o- phosphoric acid was finalized as it was showing no tailing and good peak shape.

Preparation of Mobile Phase

The Mobile phase was prepared by mixing Acetonitrile and water in the proportion of 80: 20 v/v, pH 3.5 of Mobile phase was adjusted with o- phosphoric acid. The prepared Mobile phase was filtered through a 0.45 µm nylon membrane filter. The Mobile phase was degassed by sonication.

Preparation of Stock and Working standard solution

Accurately weight 1 mg Sorafenib (RS) into 10 mL volumetric flask. Dissolved and diluted it upto 10 mL with acetonitrile to get a stock solution containing 0.1 mg mL^{-1} (100 µg mL^{-1}) of Sorafenib. From above solution aliquot 0.5 mL, 1 mL, 1.5 mL, 2 mL and 2.5 mL into 10 mL volumetric flask and volume was made upto acetonitrile to get a working standard solution containing 5, 10, 15, 20 and 25 µg mL^{-1}.

Validation of developed Chromatographic method

The method was validated as per ICH guidelines. [13-14]

Specificity

Specificity of method establish by the peak purity study. Peak purity values were obtained to be more than 0.998. Peak Profiling values indicating that there are non- interference of any other peak of degradation product or impurities.

Linearity and Range

Linearity was assessed by analysis of standard solution in a range of 5-25 µg mL^{-1} Sorafenib. Standard Calibration curve was plotted and Correlation coefficient (r^2) was found.

System Precision (Repeatability)

For RP- HPLC analysis, six replicate injections of sample were injected over a short period of time. Repeatability is also termed as intra- assay precision. Repeatability study carried out using Sorafenib solution containing 15 µg mL^{-1} concentrations. Then average peak area and % RSD were calculated.

Intermediate Precision

Intermediate Precision of analytical method demonstrates by Intraday and Interday Precision.

Intraday Precision

In Intraday Precision, Standard solution containing Sorafenib (10, 15, 20 µg mL^{-1}) was injected three in same day (0 hr, 3 hr and 6 hr) and then average peak area and % RSD were calculated.

Interday Precision

In Interday Precision, Standard solution containing Sorafenib (10, 15, 20 µg mL^{-1}) was injected in three different day (Day- 1, 2 and 3) and then average peak area and % RSD were calculated.

Accuracy (Recovery study)

To measure accuracy of analytical method, recovery studies were carried out using standard addition method with different level 80 %, 100 % and 120 %. The results of recovery studies indicated that the method is accurate for the estimation of Sorafenib.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of Detection is a lowest concentration in a sample that can be detected but not necessarily quantified under the optimized experimental conditions. The Limit of Quantitation

is lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy.

Robustness

The robustness of method was determined under variable conditions. The robustness of developed analytical method was established by illustrating its reality against consider changes in the optimized chromatographic conditions.

Assay

Twenty Sorafenib tablets were accurately weighted and average weight was calculated. The tablets were grinded and mixed well. Aliquot quantity of the powder equivalent to the average weight of the tablets 200 mg of Sorafenib were transferred to 100 mL volumetric flask. Sorafenib was extracted by addition of 80 mL Acetonitrile. Above solution was sonicated for 20 minutes and fill upto mark with same solvent. From above solution, dilute 0.1 mL to 10 mL to have a concentration of 20 µg mL^{-1}. Then the solution was analyzed using the proposed Chromatographic method.

III. RESULT & DISCUSSION
Method development

In this research work, firstly, maximum absorbance peak was recorded at 265 nm. (Figure) Therefore, the detection wavelength of the detector was set at this wavelength for further analysis. The method development trials were initiated by C_{18} column having 25 cm X 0.46 cm, 5 µm diameter.
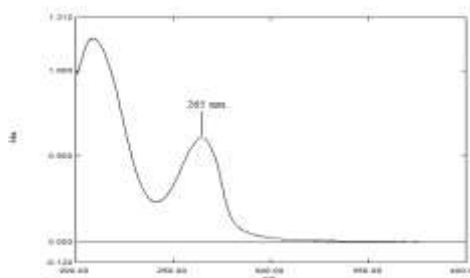

Fig. 2: UV Spectra of Sorafenib (5 µg mL^{-1})

Optimization of Chromatographic conditions

Optimization of Chromatographic conditions for estimation of Sorafenib by proposed gradient RP- HPLC method is shown in Table I.

Table 1: Optimized Chromatographic conditions

Parameters	Chromatographic conditions
Column	Phenomenex C_{18} (25 cm X 0.46 cm, 5 µm)
Mobile phase	Acetonitrile: Water (80: 20 V/V) adjust pH 3.5 With o-Phosphoric acid
Flow rate	1.0 mL min^{-1}
Detection Wavelength	265 nm
Injection volume	20 µL
Run time	15 minutes
Column Temperature	Room Temperature (25 ± 2 ^{o}C)

Method validation of Chromatographic method
System suitability parameters

The system suitability parameters like Theoretical Plates per column (N), Tailing factor (T) and Retention time were studied and found satisfactory. Obtained results are shown in Table 2.

Table 2: System suitability parameters

Parameters	Observed value	Acceptance Criteria
Theoretical plates per column (N)	9492.11	> 2000
Tailing factor (T)	1.13	< 2
Retention time (min)	5.630	---

Linearity and Range

Linearity was assessed by analysis of standard solution in a range of 5-30 µg mL^{-1} Sorafenib. Calibration curve for Sorafenib was found to be y= 42,972.03x – 5,009.35. The correlation coefficient (r^2) was found to be 0.999. The linearity data and calibration curve results are shown in Table 3 and Fig. 3. Linearity Chromatogram of 5-25 µg mL^{-1} Sorafenib is shown in Fig. 4.

Table 3: Linearity data for Sorafenib (5– 25 μg mL^{-1})

Concentration (μg mL^{-1})	Peak Area (Mean ± S.D.)	% R.S.D.
5	209182.2 ± 116.87	0.055
10	423576 ± 181.20	0.042
15	642251.8 ± 200.27	0.031
20	855668.8 ± 250.32	0.029
25	1067329.7 ± 232.09	0.021

Sorafenib

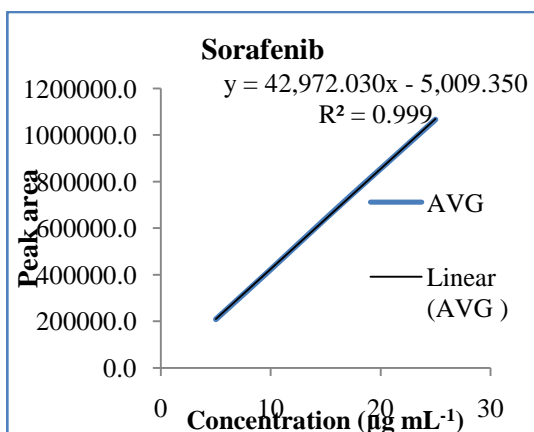$y = 42{,}972.030x - 5{,}009.350$
$R^2 = 0.999$

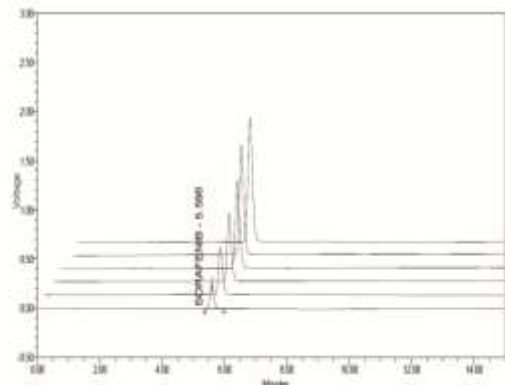**Fig. 3: Standard Calibration curve for Sorafenib**

Fig. 4: Linearity Chromatogram of 5-25 μg mL^{-1} Sorafenib

System Precision (Repeatability)
Repeatability study carried out using Sorafenib solution containing 15 μg mL^{-1} concentrations. Then % RSD was found to be 0.034.

Table 4: Repeatability data for Sorafenib

Sorafenib Conc. (μg mL^{-1})	Repeatability		
	Area	Mean (n= 6) ± SD	% RSD
15	642032	642276.3 ± 220.98	0.034
	642205		
	642026		
	642478		
	642528		
	642389		

Intermediate Precision
Intermediate Precision demonstrated by Intraday Precision and Interday Precision.

Intraday Precision
In Intraday Precision, % RSD was found to be 0.025- 0.027.

Interday Precision
In Interday Precision, % RSD was found to be 0.028-0. 048.

Table 5: Intraday and Interday data for Sorafenib

Sorafenib Conc. (μg mL^{-1})	Intraday	Interday
	Peak area (Mean, n= 6) ± SD, %RSD	Peak area (Mean, n= 6) ± SD, %RSD
10	423583.3 ± 106.82, 0.025	423402 ± 203.66, 0.048
15	642094.6 ± 173.80, 0.027	642211.6 ± 184.36, 0.028
20	855787 ± 227.29, 0.026	855696.3 ± 255.67, 0.029

Accuracy
The amount of Sorafenib was calculated and % recovery found satisfactory.

Table 6: Accuracy data for Sorafenib

Conc. Level	Amt. Taken (μg mL^{-1})	Amt. added (μg mL^{-1})	Amt. Recovered (μg mL^{-1})	% Amt. Found ± S.D. (μg mL^{-1})	% RSD
80 %	10	8	7.99	99.93 ± 0.14	0.14

100 %	10	10	9.98	99.89 ± 0.12	0.12
120 %	10	12	11.99	99.97 ± 0.23	0.23

LOD and LOQ:
The LOD and LOQ are found to be 0.0133 μg mL^{-1} and 0.0404 μg mL^{-1}.

Table 7: LOD and LOQ data for Sorafenib

LOD = 3.3 × (SD/ Slope)	LOQ = 10 × (SD/ Slope)
= 3.3 × (173.70 / 42967.70) = 0.0133 μg mL^{-1}	= 10 × (173.70 / 42967.70) = 0.0404 μg mL^{-1}

Robustness
The robustness of method is demonstrated in the table 8.

Table 8: Robustness data for Sorafenib

Conditions		Area	Mean	S.D.	% R.S.D.
Flow rate (mL min^{-1})	0.8	641896	642124.33	198.076	0.030
	1	642250			
	1.2	642227			
% mobile phase (ACN)	78	642057	641993.00	238.528	0.037
	80	642193			
	82	641729			
pH of phosphate buffer	3.3	642189	642385.66	286.196	0.044
	3.5	642254			
	3.7	642714			

Assay
Assay of Sorafenib Tablets were found within standard range.

Table 9: Assay of Sorafenib tablets

Area of Standard Sorafenib			855361		
Label claim (mg)	Mean Area* of Sample	Mean Result* (mg)	Average* % Assay	S.D.	% RSD
200	855083.8	199.93	99.96 %	0.035	0.035

* Average of five determination (n=5)
Summary of Method Validation parameters of Sorafenib

Table 10: Summary of validation parameters for Sorafenib

Parameters		Sorafenib
Concentration Range		5-25 μg mL^{-1}
Regression equation		y = 42735x - 21953
Regression co-efficient		0.999
LOD		0.0133 μg mL^{-1}
LOQ		0.0404 μg ml^{-1}
Repeatability (% RSD)		6421199 ± 109.35, 0.0017
Intraday precision (n=3) (Mean ± SD, % RSD)	**10 μg mL^{-1}**	423583.3 ± 106.82, 0.025
	15 μg mL^{-1}	642094.6 ± 173.80, 0.027
	20 μg mL^{-1}	855787 ± 227.29, 0.026
Interday precision (n=3) (Mean ± SD, % RSD)	**10 μg mL^{-1}**	423402 ± 203.66, 0.048
	15 μg mL^{-1}	642211.6 ± 184.36, 0.028
	20 μg mL^{-1}	855696.3 ± 255.67, 0.029
% Recovery	**80 %**	99.93%
	100 %	99.89%
	120 %	99.97%
Assay of formulation	**Marketed**	99.96 %

IV. CONCLUSION

An Accurate, Precise and reliable HPLC method for estimation of Sorafenib using Photodiode array detector has been developed and validated according to the ICH guidelines. An adequate separation and symmetric peak for Sorafenib were obtained with a mobile phase containing a mixture of Acetonitrile and Water (adjust pH 3.5 with o- phosphoric acid) in ratio of 80: 20 V/V was delivered at flow rate of 1.0 mL min^{-1} to get better repeatability and reproducibility. Wavelength selected for Sorafenib estimation is 265 nm. The retention time of Sorafenib was found

to be 5.63 min. Forced Degradation studies were conducted in aqueous acid, base and peroxide. Solid sample was observed for photo stability and high temperature. Sorafenib is stable under solid state, but degrade under acidic, basic and oxidative aqueous conditions which found to be within acceptance range. The method fulfilled the requirements to be considered a realizable method, including all validation parameters- Specificity, Linearity, Precision, accuracy, robustness, LOD and LOQ. This method is linear over concentration range of 5- 25 µg mL^{-1}. The system suitability, Precision and accuracy values are within the acceptable limits. The obtained results indicate that this method can be used for routine analysis of Sorafenib in the Pharmaceuticals.

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CONFLICT OF INTERESTS
Authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ABBREVIATIONS
UV: Ultra- violate**; RP-HPLC:** Reverse phase high performance liquid chromatography; **IP:** Indian Pharmacopoeia; **USP**: United State Pharmacopoeia; **EU:** European Pharmacopoeia; **ICH:** International Council for Harmonisation; **LOD:** Limit of Detection; **LOQ:** Limit of Quantitation; **RSD:** Relative standard deviation.

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