A NEW ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF SORAFENIB IN PURE FORM AND PHARMACEUTICAL DOSAGE FORM BY USING RP-HPLC

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ABSTRACT

A rapid and highly sensitive reversed phase high performance liquid chromatographic method has been developed for quantitative estimation of Sorafenib in pharmaceutical preparations. The method has been validated according to ICH guidelines with respect to accuracy, precision, specificity and linearity. The method was developed by using an isocratic condition of mobile phase comprising Acetonitrile and Methanol was taken in the ratio of 20:80% v/v for 10 minutes at a flow rate of 1.0 mL/min over Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm column at ambient temperature. The Method Precision was found to be within the limits. Intra-day and inter-day precision studies of the new method were less than the maximum allowable limit (RSD% of 2.0 according to ICH). The method showed linear response with correlation coefficient (r2) value of 0.9995. Therefore, it was found to be accurate, reproducible, sensitive and less time consuming and can be successfully applied for the assay of Sorafenib in bulk and marketed formulations.

Key Words: Sorafenib, RP-HPLC, Method Development, Validation, Robustness.

INTRODUCTION

Sorafenib (rINN), marketed as Nexavar by Bayer, is a drug approved for the treatment of advanced renal cell carcinoma (primary kidney cancer). It has also received "Fast Track" designation by the FDA for the treatment of advanced hepatocellular carcinoma (primary liver cancer), and has since performed well in Phase III trials. Sorafenib[1] is a small molecular inhibitor of Raf kinase, PDGF (platelet-derived growth factor), VEGF receptor 2 & 3 kinases and c Kit the receptor for Stem cell factor. A growing number of drugs target most of these pathways. The originality of Sorafenib[2] lays in its simultaneous targeting of the Raf/Mek/Erk pathway. No large changes in QTc interval were observed. After one 28-day treatment cycle, the largest mean QTc interval change of 8.5ms (upper bound of two-sided 90% confidence interval, 13.3ms) was observed at 6 hours post-dose on day 1 of cycle 2. Sorafenib interacts with multiple intracellular (CRAF, BRAF and mutant BRAF) and cell surface kinases (KIT, FLT-3, VEGFR-2, VEGFR-3, and PDGFR-ß). Several of these kinases are thought to be involved in angiogenesis, thus Sorafenib reduces blood flow to the tumor. Sorafenib[3] is unique in targeting the Raf/Mek/Erk pathway. By inhibiting these kinases, genetic transcription involving cell proliferation and angiogenesis is inhibited. The IUPAC Name of Sorafenib is 4-[4-[[4-chloro-3-(trifluoro methyl) phenyl] carbamoyl amino] phenoxy]-N-methyl pyridine-2-carboxamide. The Chemical Structure of Sorafenib is as following

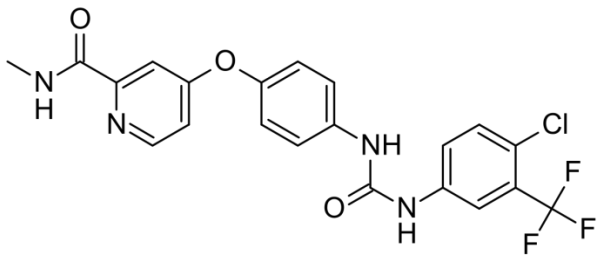


Fig-1: Chemical Structure of Sorafenib

Although several methods[31-34] have been reported previously for determination of Sorafenib in the pharmaceutical formulations, some of the methods is expensive and has some limitations in analytical uses. To overcome the limitations, the objective of the present work was to develop a simpler, accurate and rapid liquid chromatographic analytical method utilizing widely used and common column for the assay of Sorafenib in bulk and pharmaceutical formulations and to validate the method in accordance with the guidelines of FDA, USP and ICH with respect to accuracy, reproducibility, linearity and specificity.

EXPERIMENTAL

INSTRUMENTS USED

Table-1: List of Instrument used

S. No.	Instruments/Equipments/Apparatus
1.	HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
2.	ELICO SL-159 UV – Vis spectrophotometer
3.	Electronic Balance (SHIMADZU ATY224)
4.	Ultra Sonicator (Wensar wuc-2L)
5.	Thermal Oven
6.	Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
7.	P^H Analyzer (ELICO)
8.	Vacuum filtration kit (BOROSIL)

CHEMICALS / REAGENTS USED

Table-2: List of Chemicals used

S.No.	Name	Specifications		Manufacturer/Supplier
		Purity	Grade	
1.	Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
2.	HPLC Grade Water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
3.	Methanol	99.9%	HPLC	Loba Chem; Mumbai.
4.	Hydrochloric Acid	99.9	A.R.	Sd fine-Chem ltd; Mumbai
5.	Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
6.	Sodium Hydroxide	99.9	A.R.	Sd fine-Chem ltd; Mumbai
7.	Ethanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai
8.	Octanol	99.9	A.R.	Sd fine-Chem ltd; Mumbai

Method Development and its Validation for Sorafenib by RP-HPLC

Selection of Wavelength

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Sorafenib, so that the same wave number can be utilized in HPLC UV detector for estimating the Sorafenib. The scanned UV spectrum is attached in the following page,

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Sorafenib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase[4].

Optimization of Chromatographic Conditions:

The chromatographic conditions[5] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table-3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile = 100	1.0ml/min	284nm	Very Low response	Method rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Methanol : Water = 50 : 50	1.0ml/min	284nm	Low response	Method rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile: Water = 80 : 20	1.0ml/min	284nm	Tailing peaks	Method rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Phosphate Buffer : Acetonitrile = 75:25 (pH-4.8)	1.0ml/min	284nm	Resolution was not good	Method rejected
Symmetry ODS (C_{18})	Phosphate	1.0ml/min	284nm	Tailing peak	Method

RP Column, 250 mm x 4.6 mm, 5µm	Buffer : Methanol = 40:60 (pH-4.0)				rejected
Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm	Acetonitrile : Methanol = 20:80	1.0ml/min	284nm	Nice peak	Method accepted

Preparation of Mobile Phase:

200ml of HPLC Grade Acetonitrile and 800ml of HPLC Grade Methanol were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration[6].

Method Validation

Selectivity/Specificity

Selectivity[7] of an analytical method is its ability to measure accurately an analyte in the presence of interferences that may be expected to be present in the sample matrix.

Precision

Precision of a method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple samplings.

Precision[8] is measured by injecting a series of standards or analyzing series of samples from multiple samplings from a homogeneous lot. From the measured standard deviation (SD) and Mean values, precision as relative standard deviation[9] (% RSD) is calculated.

Accuracy

The accuracy of an analytical method is the degree of agreement of test results generated by the method to the true value.

Accuracy[10] is measured by spiking the sample matrix of interest with a known concentration of analyte standard and analyzing the sample using the "method being validated." The procedure and calculation for Accuracy (as% recovery[11]) will be varied from matrix to matrix and it will be given in respective study plan or amendment to the study plan.

Linearity

The linearity[12] of an analytical method is its capability to elicit check consequences which might be at once, or with the aid of well described mathematical adjustments, proportional to the concentration of analytes in within a given range.

Linearity is determined by injecting a series of standards of stock solution/diluted stock solution using the solvent/mobile phase, at a minimum of five different concentrations in the range of 50–150% of the expected working range. The linearity graph[13] will be plotted manually/using Microsoft Excel or software of the computer (Concentration vs. Peak Area Response) and which will be attached to respective study files.

Range

The range[14] of an analytical method is the interval between the upper and lower levels that have been demonstrated to be determined with precision, accuracy and linearity using the set method. This range will be the concentration range in which the Linearity test is done.

Limit of detection and Limit of quantitation

The term LOD[15] is defined as the lowest concentration at which the instrument is able to detect but not quantify and the noise to signal ratio for LOD should be 1:3. The term LOQ is defined as the lowest concentration at which the instrument is able to detect and quantify. The noise to signal ratio for LOQ[16] should be 1:10.

RESULTS AND DISCUSSION

Development of a Method

Selection of Wavelength

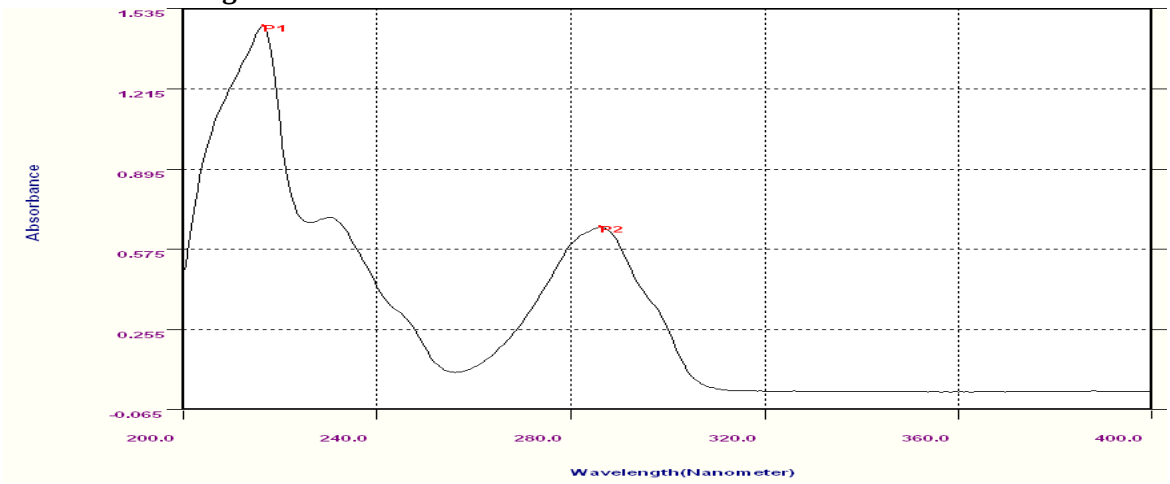


Fig-2: UV spectrum for Sorafenib

Observation: While scanning the Sorafenib solution we observed the maxima at 284nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions

The Optimum Chromatographic conditions[17] obtained from experiments can be summarized as below:

Table-4: Summary of Optimized Chromatographic Conditions

Mobile phase	Acetonitrile : Methanol = 20:80
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm
Column Temperature	Ambient
Detection Wavelength	284 nm
Flow rate	1.0 ml/ min.
Run time	10 min.
Temperature of Auto sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10µl
Type of Elution	Isocratic
Retention time	4.783 minutes

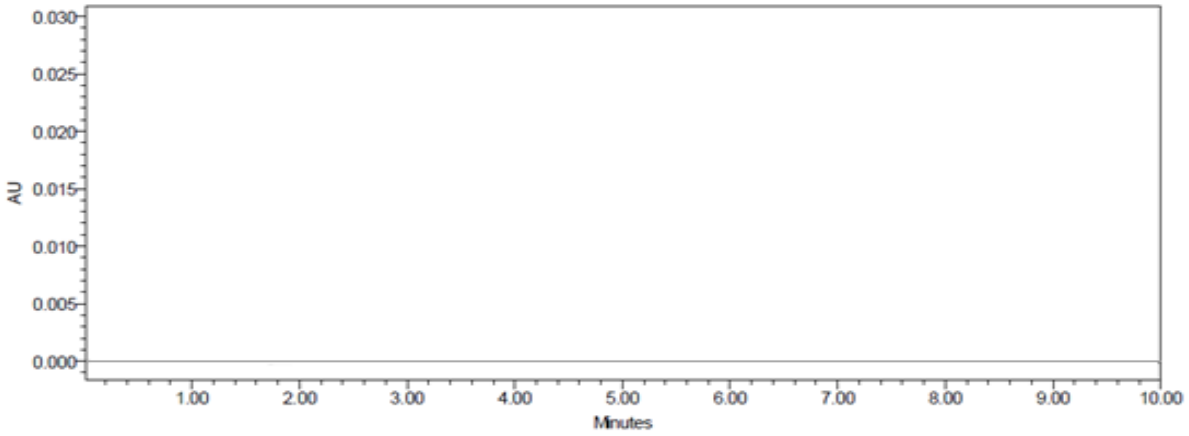
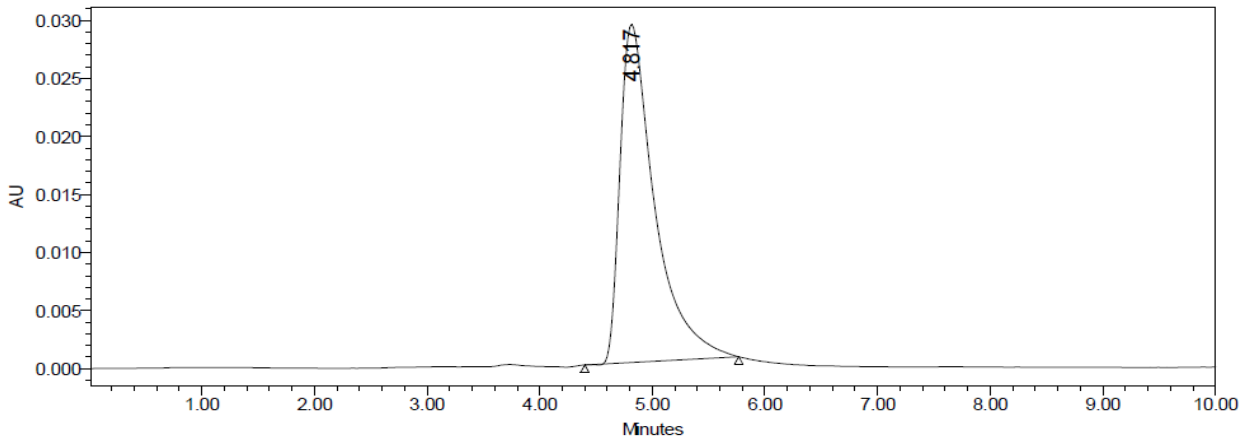


Fig-3: Chromatogram for Blank Solution



Fig–4: Chromatogram of Sorafenib in Optimized Condition

Analytical Method Validation

The developed method was validated according to ICH guidelines[27] Q2 (R1) for parameters such as linearity, repeatability, precision, accuracy and, limit of detection and limit of quantification.

1. Linearity:

To evaluate the linearity, serial dilution of analyte were prepared from the stock solution was diluted with mobile phase to get a series of concentration ranging from 60-140µg/ml. The prepared solutions were sonicated. From these solutions, 10µl injections of each concentration were injected into the HPLC system[18] and chromatographed under the optimized conditions. Calibration curve was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

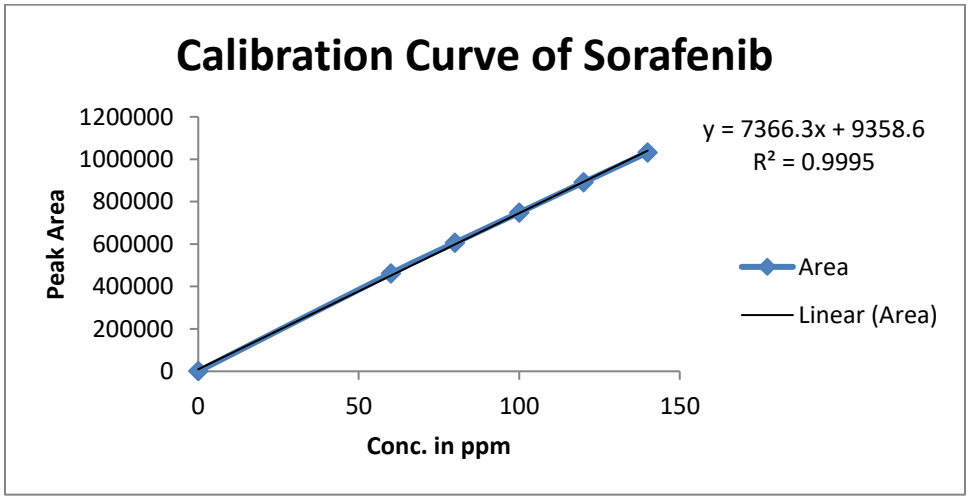


Fig-5: Calibration Curve of Sorafenib

Table-5: Linearity Data for Sorafenib

Conc. (µg/ml)	Area
0	0
60	461404
80	606157
100	748506
120	891041
140	1032196

2. Accuracy: The accuracy of the method was determined by recovery studies[19] and the percentage recovery was calculated. The recoveries of Sorafenib were found to be in the range of 99.91 %. The proposed Liquid Chromatographic method was applied to the determination of Sorafenib. The results for Sorafenib comparable with the corresponding labeled amounts.

Table-6: Shown Accuracy Observation of Sorafenib

Accuracy	Amount Added	Amount Recovered	Peak Area	% Recovery	Mean Recovery
80%	80	80.798	604517	100.997	99.6%
	80	80.673	603598	100.841	
	80	80.756	604213	100.945	
100%	100	99.933	745471	99.933	
	100	100.083	746574	100.083	
	100	100.365	748652	100.365	
120%	120	120.290	895415	100.241	
	120	120.201	894762	100.167	
	120	120.442	896541	100.368	

3. Precision:

Repeatability: The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug.

Sorafenib (API). The percent relative standard deviation[20] was calculated for Sorafenib are presented in the table-7.

Table-7: Repeatability Data for Sorafenib

S. No.	INJECTION	PEAK AREA
1	Injection 1	743826
2	Injection 2	745277
3	Injection 3	742506
4	Injection 4	747576
5	Injection 5	746715
6	Injection 6	741278
7	**Average**	**744529.6667**
8	**SD**	**2440.4116**
9	**% RSD**	**0.32777**

Intermediate Precision:

The Intermediate Precision[21] consists of two methods:-

Intra Day: In Intra Day process, the 80%, 100% and 120% concentration are injected at different intervals of time in same day.

Inter Day: In Inter Day process, the 80%, 100% and 120% concentration are injected at same intervals of time in different days.

Table-8: Results of intra-assay & inter-assay

Conc. of Sorafenib (API) (µg/ml)	Observed Conc. of Sorafenib (µg/ml) by the proposed method			
	Intra-Day		Inter-Day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
80	80.38	0.56	80.45	0.56
100	100.17	0.71	100.50	0.77
120	120.89	0.89	120.91	0.85

Observations: The intra & inter day variation of the method was carried out for standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Sorafenib revealed that the proposed method is precise.

4. LOD and LOQ:

The LOD and LOQ parameter was evaluated by mistreatment the slope of line and variance obtained from accuracy studies[22].

The detection limit (LOD) and quantization limit (LOQ) may be expressed as:

$$\textbf{L.O.D. = 3.3(SD/S).}$$

$$\textbf{L.O.Q. = 10(SD/S)}$$

Where, SD = Standard deviation of the response

S = Slope of the calibration curve

The slope S may be estimated from the calibration curve[23] of the analyte.

The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.07 & 0.21µg/ml respectively.

5. System Suitability: System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. Following system suitability test parameters[24-26] were established. The data are shown in Table-9.

Table-9: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Retention Time	RT > 2	Sorafenib=4.783
2	Asymmetry	T ≤ 2	Sorafenib=1.35
3	Theoretical plate	N > 2000	Sorafenib=2865
4	Tailing Factor	T<2	Sorafenib=1.37

6. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate 1.0 ml (± 0.1ml/min), Wavelength of detection 284 (±2nm) & organic phase content in mobile phase (±5%) studied to determine the robustness[28] of the method are also in

favour of (Table-10, % RSD < 2%) the developed RP-HPLC method for the analysis of Sorafenib (API).

Table-10: Result of Method Robustness Test

Change in parameter	% RSD
Flow (1.1 ml/min)	0.45
Flow (0.9 ml/min)	0.38
More Organic	0.76
Less Organic	0.65
Wavelength of Detection (286 nm)	0.98
Wavelength of detection (282 nm)	0.93

7. Estimation of Sorafenib in Pharmaceutical Dosage Form

Twenty Tablets were taken and the I.P. method was followed to determine the average weight. Above weighed tablets were finally powdered and triturated well. A quantity of powder equivalent to 25 mg of drugs were transferred to 25 ml volumetric flask, make and solution was sonicated for 15 minutes, there after volume was made up to 25 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with mobile phase. The solution was filtered through a membrane filter (0.45 μm) and sonicated to degas[29]. The solution prepared was injected in five replicates into the HPLC system and the observations were recorded.

The Assay[30] data are shown in Table-11.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg}$$

Where:

AT = Peak Area of drug obtained with test preparation

AS = Peak Area of drug obtained with standard preparation

WS = Weight of working standard taken in mg

WT = Weight of sample taken in mg

DS = Dilution of Standard solution

DT = Dilution of sample solution

P = Percentage purity of working standard

Table-11: Recovery Data for estimation Sorafenib in Soranib Tablet

Brand Name of Sorafenib	Labelled amount of Drug (mg)	Mean (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Soranib Tablet (Cipla Pharma)	200mg	199.786 (± 0.856)	99.857 (± 0.516)

Result & Discussion: The amount of drug in Soranib Tablet was found to be 199.786 (± 0.856) mg/tab for Sorafenib & % Purity was 99.857 %.

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Sorafenib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (methanol, acetonitrile, water, 0.1N NaOH, 0.1NHCl). Detection wavelength was selected after scanning the standard solution of drug over 200 to 400nm. From the U.V spectrum of Sorafenib it is evident that most of the HPLC work can be accomplished in the wavelength range of 284 nm conveniently. Further, a flow rate of 1 ml/min & an injection volume of 10µl were found to be the best analysis. The result shows the developed method is yet another suitable method for assay which can help in the analysis of Sorafenib in different formulations.

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