

**DEVELOPMENT AND VALIDATION OF STABILITY INDICATING HPLC METHOD  
FOR ESTIMATION OF SORAFENIB TOSYLATE**

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Article Received on 18/03/2018

Article Revised on 09/04/2018

Article Accepted on 30/04/2018

**ABSTRACT**

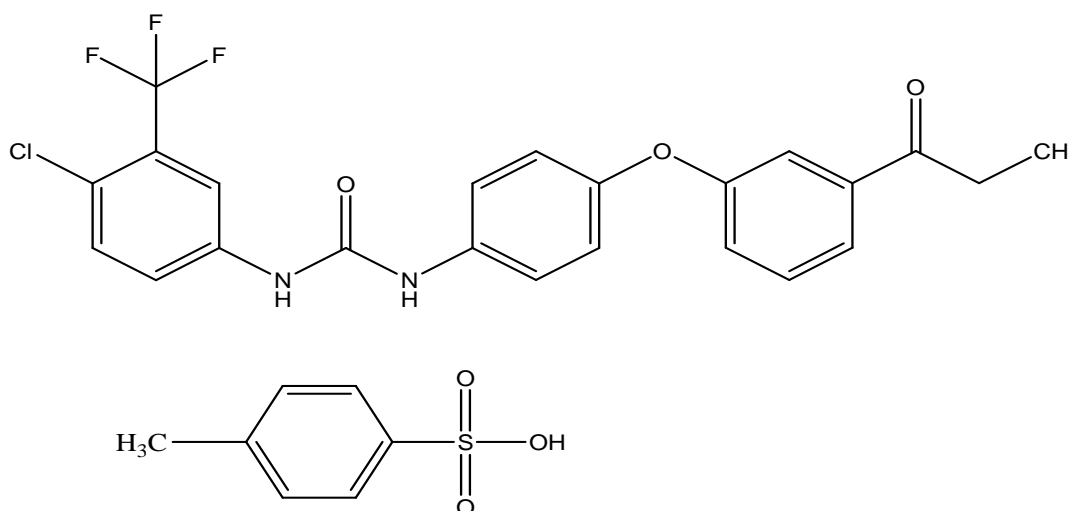
Simple, rapid validated stability indicating HPLC method for estimation of Sorafenib tosylate was successfully developed. The separation was achieved by using a mobile phase of Methanol: Water (pH 3 adjusted with Glacial Acetic Acid) in the ratio of 80:20 v/v using HiQ SiL C18 column (150 × 4.6 mm i.d. 5 μm) at 1 ml/min as flow rate. The detection was carried out at 265 nm using PDA detector. The retention time observed was 5.5±0.2 min. This drug was subjected to stress conditions as per ICH Q1A (R2). Linearity was found to be in the concentration range of 10-50 μg/ml with  $r^2 = 0.9909$ . The suitability of this HPLC method for quantitative estimation of Sorafenib was proved by validation in accordance with requirements of ICH guidelines Q2A (R1).

**KEYWORDS:** Sorafenib Tosylate, HPLC, Stress degradation, Validation, ICH guidelines.

**INTRODUCTION**

Chemically Sorafenib tosylate is 4-[4-[[4-chloro-3-(trifluoromethyl) phenyl] ureido] phenoxy] - N - 2 - methyl pyridine - 2 - carboximide 4 - methylbenzenesulfonate. Drug was approved for the treatment in primary kidney cancer and advanced primary liver cancer.<sup>[1, 2, 3]</sup> Sorafenib is white crystalline powder with  $C_{28}H_{24}ClF_3N_4O_6S$  as molecular formula. Sorafenib tosylate is soluble in Methanol.<sup>[4, 5]</sup> The ICH guideline for stability testing of new drug substances and products requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance. Forced degradation is a process that

involves degradation of drug products and drug substances at conditions more severe than accelerated conditions to determine the stability of the molecule.<sup>[6]</sup> Literature survey reveals that there are papers on pharmacokinetic study<sup>[7]</sup>, Bioanalytical HPLC<sup>[8]</sup>, HPLC-SIM<sup>[9, 10]</sup> and HPTLC-SIM of Sorafenib tosylate.<sup>[11]</sup> There is no similarity in results reported in literature survey according to the ICH guidelines, therefore the aim of the present work is to develop an accurate, specific, and reproducible stability indicating HPLC-PDA method for determination of Sorafenib tosylate and to develop a validated stability-indicating assay method.



**Fig. 1: Structure of Sorafenib tosylate.**

## MATERIALS AND METHODS

### Reagents and Chemicals

The working standard Sorafenib tosylate was provided by NATCO pharma (Hyderabad, India). The reagents used for present study are as follows Methanol HPLC Grade (MeOH), Distilled water, Hydrochloric acid (HCl), Sodium hydroxide (NaOH), 6% w/v Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were of analytical grade from LOBA CHEMIE PVT. LTD., Mumbai, India.

### Instrumentation

The method development and validation of RP-HPLC method was performed on JASCO HPLC system comprising of model PU 2080 Plus pump, Rheodyne sample injection port with 20µl loop, using HiQ SiL C<sub>18</sub> Column with MD 2010 PDA detector. The chromatogram was recorded with Borwin- PDA software (version 1.5). Shimdazu Model AY-120 Balance was used for weighing. Other instruments used were UV-Visible Double beam spectrophotometer make Jasco Model V-730, Elga Lab water purification system(PURELAB UHQ-II), Hot Air Oven (Kumar Laboratory Oven), Photo stability chamber (Make Newtronic. Model IC DAC version 1.2).

### Preparation of Stock Solution

Standard stock solution of Sorafenib tosylate was prepared by dissolving 10 mg of drug in 10 ml of methanol to get concentration of 1000 µg/ml. It was suitably diluted with methanol to get concentration of solution 100 µg/ml. working standard of solution was prepared containing Linearity range 10-50 µg/ml using MeOH as solvent.

### Selection of detection wavelength

From standard stock solution, appropriate dilution was made using Methanol and scanned over range of 200-400 nm.

### Optimized chromatographic conditions

The various mobile phases tried consisted of Phosphate Buffer pH 6: ACN, Ammonium acetate Buffer pH 7: Methanol in various proportions. The peak shape was not proper so the buffer was replaced by water having pH3. The optimized mobile phase consisted of water (previously adjust the pH 3 with Glacial Acetic Acid): Methanol (20:80 v/v). It was then filtered through 0.45µm membrane filter using vacuum filtration assembly. Then sonicated using ultrasonic water bath for 10 min.

### Stress Degradation Studies of Bulk Drug

The forced degradation studies were carried out on bulk drug substance in order to prove the stability-indicating property and selectivity of the developed method. The API was subjected to hydrolysis under different pH, oxidative, thermal and photolytic conditions. Optimization of conditions was done by changing strength of reagent and duration of exposure to achieve degradation in 10 to 30 % range.

### Acid Treatment

2 ml working standard solution of Sorafenib tosylate (100 µg/ml) was mixed with 2 ml of 1N hydrochloric acid (HCl) and 6 ml of methanol to get final concentration of 20 µg/ml. the solution was kept at room temperature overnight.

### Alkali treatment

2 ml working standard solution of Sorafenib tosylate (100 µg/ml) was mixed with 2 ml of 1 N sodium hydroxide (NaOH) and 6 ml of methanol to get final concentration of 20 µg/ml. The solution was kept at room temperature overnight.

### Oxidative degradation

2 ml working standard solution of Sorafenib tosylate (100 µg/ml) was mixed with 2 ml of 6% w/v Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and 6 ml of methanol to get final concentration of 20 µg/ml. The solution was kept at room temperature overnight.

### Thermal degradation

Thermal degradation was performed by keeping drug in oven at 60°C for period of 8 hours. A sample was withdrawn, weighed and dissolved in methanol to get solution of 20 µg/ml.

### Photolytic degradation

Sorafenib tosylate was exposed to UV light (200 watt hours/square meter) and cool white fluorescent light (1.2 million lux hours). Sample was weighed, dissolved in methanol and diluted to concentration of 20 µg/ml.

## RESULTS AND DISCUSSION

### Selection of analytical wavelength

It was observed that Sorafenib tosylate showed considerable absorbance at 265 nm hence this wavelength was chosen for detection.

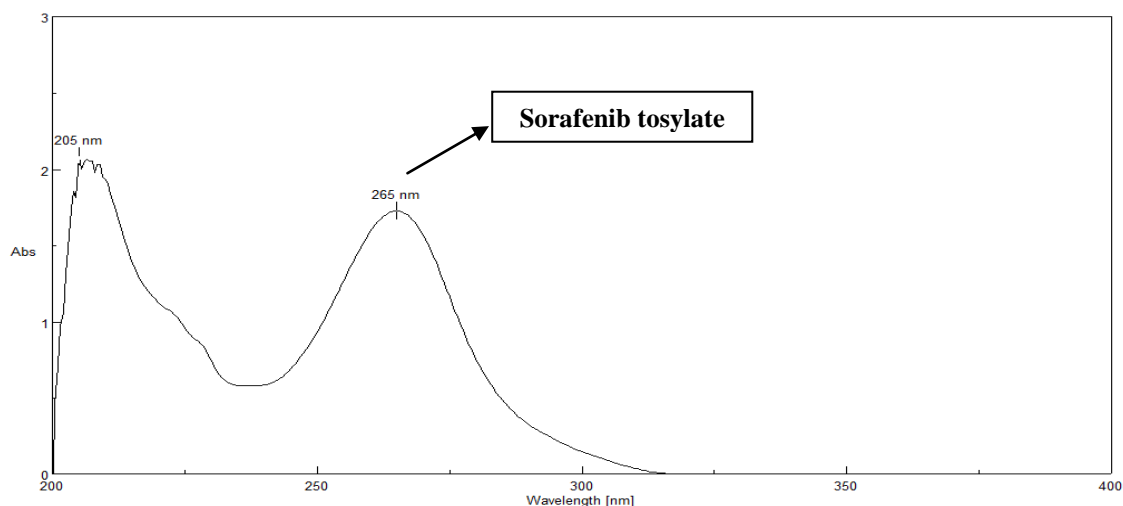


Fig. 2: UV spectrum of Sorafenib tosylate (20ppm)

Table no. 1: Chromatogram and system suitability parameter of drug.

Name	RT(Min)	Conc. (µg/ml)	Area	Theoretical Plates	Asymmetry	Resolution
Sorafenib tosylate	5.7±0.2	20	1696459.10	5092.16	1.72	2.06

Table no. 2: Optimization of chromatographic condition.

Sr. No.	Parameter	Conditions used for Analysis
1	Column	HIQ SIL C18 column (150 x 4.6 mm i.d, 5 µm)
2	Mobile phase	Methanol: Water (pH 3 adjusted with glacial acetic acid) 80:20 v/v
3	Flow rate	1 ml/min
4	Detection Wavelength	265 nm
5	Sample Volume	20µl
6	Column temperature	Room temperature

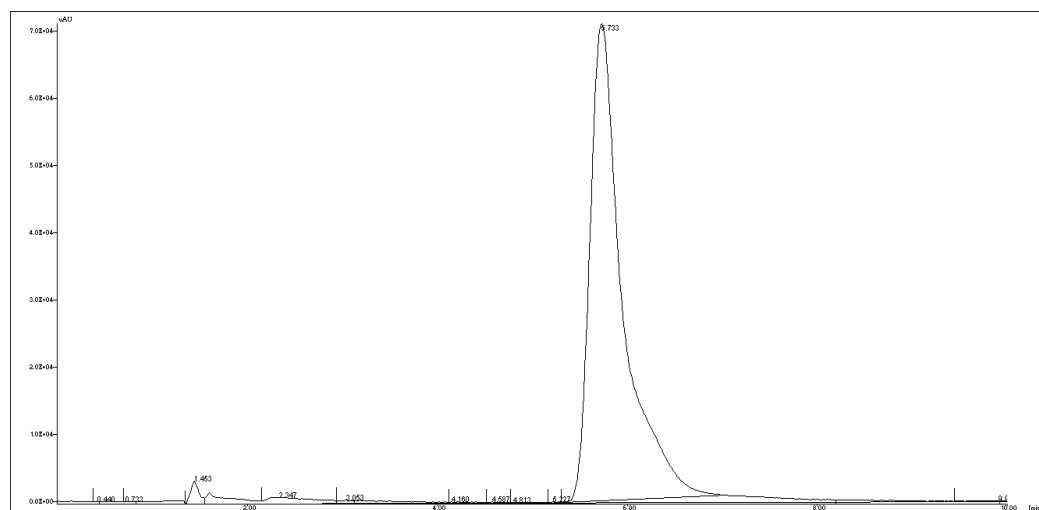


Fig. 3: Chromatogram of standard Sorafenib tosylate (20ppm).

#### Result of forced degradation studies

Degradation were seen in Acidic, Alkaline and oxidation condition. No degradation seen in Thermal and photolytic conditions for pure Sorafenib tosylate in HPLC. Also there is no separate degradation peak in any condition. It was confirmed by multiwavelength analysis peak purity comparison.

These degradation studies indicated that Sorafenib tosylate was susceptible to hydrolysis under acidic, basic pH and also to oxidative conditions. Relatively stable in other stress conditions.

**Table 3: Results of the stress degradation studies for standard Sorafenib tosylate.**

Sr. no.	Parameters	Condition	% Recovery	Peak purity	
				Purity front	Purity tail
1	Initial	No treatment kept overnight	-	997.971	999.342
2	Acid hydrolysis	1 N HCl overnight at RT	64.24	995.205	993.367
3	Alkali hydrolysis	1N NaOH overnight at RT	78.33	904.504	931.716
4	Oxidative stress Degradation	6% H <sub>2</sub> O <sub>2</sub> overnight at RT	71.91	993.826	992.779
5	Photolytic Degradation	UV light (200 watt hours/square meter)	115.97	991.796	994.094
		cool white fluro light (1.2 million lux hours)	105.91	997.507	997.891
6	Dry heat degradation	Hot air oven at 60°C at 8hrs	100.37	997.217	997.749

**VALIDATION OF THE METHOD<sup>[12]</sup>**

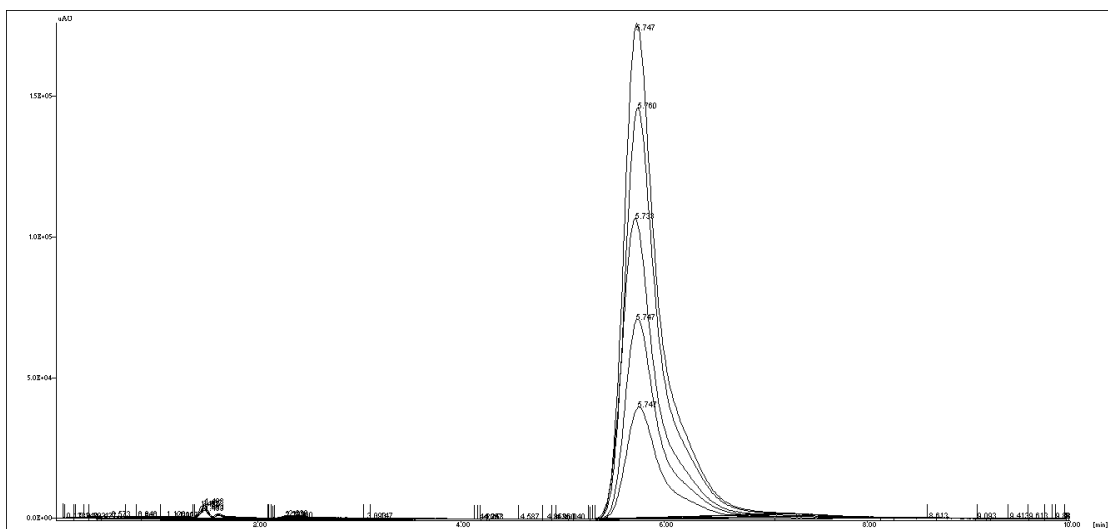
The method was validated for various parameters in accordance with ICH guideline.

**Specificity**

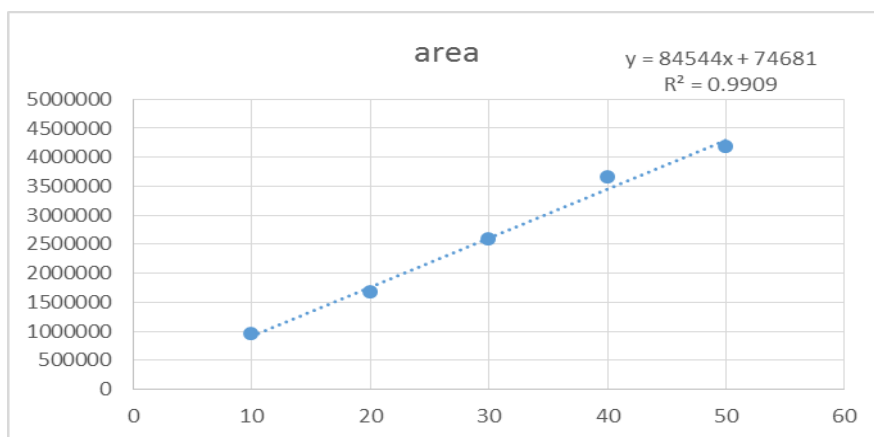
The specificity of the method was ascertained by peak purity profiling studies. The peak purity values were found to be more than 990, indicating the non-interference of any other peak of degradation product or impurity.

**Linearity**

Calibration curve was obtained in the range 10-50 µg/ml, peak area were recorded. Standard calibration graph was plotted of peak area Vs concentration injected. The equation of calibration curve found to be  $Y = 84544X + 74681$  having coefficient of correlation ( $r^2$ ) = 0.9909 shown in Figure 10.



**Fig. 9: Chromatogram of Calibration curve of Sorafenib tosylate (10-50 µg/ml).**



**Fig. No. 10: Calibration curve for Sorafenib tosylate at 265 nm.**

**Range**

The linearity range was found to be 10-50 µg/ml of Sorafenib tosylate.

**Assay**

Assay was performed on blend of bulk drug plus excipients. Assay was found to be 100.51% for Sorafenib tosylate.

**Accuracy**

To check accuracy of the method, recovery studies were carried out by adding standard drug to blank blend at three different levels 80, 100 and 120 %. The results of the recovery studies indicated that the method is accurate for estimation of drug in the blend. The results obtained are shown in table 3.

**Table 4: Recovery studies for Sorafenib tosylate.**

Level (%)	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery
80	16	15.43	96.46
100	20	19.76	98.82
120	24	23.32	97.18

**Precision**

The intra-day and inter-day precision of HPLC method is shown in Table 5. Results expressed in terms of %RSD, which describes intra-day and inter-day variation of Sorafenib tosylate at concentration of 20 µg/ml (n= 6).

**Table 5: Intra-day and Inter-day precision.**

Sr. No.	Amount (µg/ml)	Intra-day	SD	% RSD	Inter-day	SD	% RSD
1	20	946477.76	17415.29	1.80	953070.03	12375.44	1.28
2	20	950911.62			964526.98		
3	20	962718.08			951824.98		
4	20	988078.01			981973.82		
5	20	954330.00			973492.02		
6	20	983070.30			954619.58		

**Limit of Detection (LOD) and Limit of Quantification (LOQ)**

The limit of detection and limit of quantification is the lowest concentration of analyte in a sample which can be detected and quantified with acceptable accuracy and precision. The LOD and LOQ of the developed method were calculated using the formula as given below.

$$\text{Limit of Detection} = 3.3 \cdot \sigma / S$$

$$\text{Limit of Quantitation} = 10 \cdot \sigma / S$$

Where,  $\sigma$  = standard deviation of the response at lowest concentration, S = slope of the calibration curve.

LOD and LOQ were found to be 0.47 µg/ml and 1.43 µg/ml respectively, which shows the sufficient sensitivity of the method.

**Robustness**

Peak area checked after flow rate variation, change in mobile phase composition and change in pH were well within the limit, indicating that the proposed method is robust under given set of defined experimental conditions.

**Solution stability**

The Standard stock solutions of Sorafenib tosylate were found to be stable for 48hrs if stored at room temperature.

**Table no. 6: Summary of validation study.**

Sr.No.	Validation Parameters	Sorafenib tosylate
1	Linearity Equation ( $r^2$ )	$Y = 84544X + 74681$ $r^2 = 0.9909$
2	Range	10-50 µg/ml
3	Precision (% RSD)	
	Interday	1.80
	Intraday	1.28
4	Accuracy	
	80%	96.46
	100%	98.82
	120%	97.18
5	Limit of Detection (µg/ml)	0.47
6	Limit of Quantitation (µg/ml)	1.43
7	Specificity	Specific
8	Robustness	Robust
9	Solution stability	Stable

**DISCUSSION**

The method was developed using isocratic system with run time 10 min., in Indian Pharmacopoeia the method was developed using gradient system with run time 45 min. As per literature survey degradation pattern in acid, base and oxidation area seen comparatively same, in other condition like photo degradation and thermal no degradation seen.

**CONCLUSION**

The developed method is simple, rapid and stability indicating. It may be used to monitor stability of Sorafenib tosylate.

**ACKNOWLEDGEMENTS**

Authors are thankful to the Principal and the management of AISSMS College of Pharmacy, Pune for providing the necessary facilities for research work and to NATCO pharma (Hyderabad, India) for providing API.

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