

DEVELOPMENT AND VALIDATION OF STABILITY-INDICATING ASSAY METHOD FOR ESTIMATION OF SORAFENIB TOSYLATE IN PURE AND PHARMACEUTICAL DOSAGE FORM BY HPTLC

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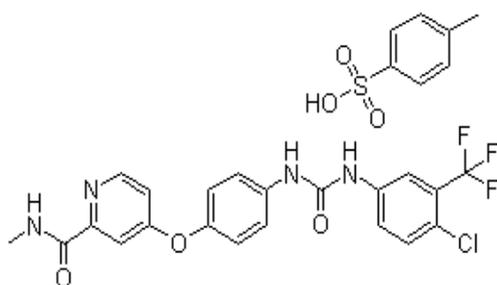
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ABSTRACT

A simple, specific and rapid validated stability indicating HPTLC method has been developed for estimation of Sorafenib Tosylate in bulk and pharmaceutical dosage form. The separation was carried out on Merck TLC aluminium sheets precoated with silica gel 60F₂₅₄ were used for chromatographic separation. Combination of toluene: ethyl acetate (3:7 v/v) was selected as the mobile phase. Detection was done by UV absorbance mode at wavelength 265nm. Sorafenib Tosylate showed well defined and sharp peak at R_f 0.45 ± 0.02. The calibration curve of drug was found linear in the concentration range 200–1000 ng/band for Sorafenib Tosylate. The developed method was precise, robust and the % RSD was found less than 2%. Stress degradation study includes acid and base hydrolysis, oxidation, thermal and photolytic stress conditions in accordance with International Conference on Harmonization (ICH) guidelines Q1 (R2). The proposed method can be used for routine quality control analysis of bulk drug and marketed formulation containing Sorafenib Tosylate.

KEYWORDS: Sorafenib Tosylate, HPTLC, Validation, Stability.**INTRODUCTION**

Sorafenib Tosylate belongs to Anticancer drugs category. Chemically Sorafenib Tosylate is 4-(4-{3-[4-Chloro-3-(trifluoromethyl) phenyl]ureido}phenoxy) N-2 methylpyridine-2-carboxamide 4-methylbenzene sulfonate. Sorafenib Tosylate is not official in IP/BP/USP.^[1] It is a drug approved for the treatment of primary kidney cancer (advanced renal cell carcinoma) and advanced primary liver cancer.^[2] It is freely soluble in methanol.^[3]

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

Literature survey revealed that there are few analytical methods reported for the determination of sorafenib Tosylate. Sorafenib Tosylate was estimated in bulk and in tablets by RP-HPLC^[4-6], it was estimated by RP-HPLC in human serum^[7,8], Bioavailability and

pharmacokinetics of Sorafenib in rat^[9], UV method^[10,11], and by LC-MS/MS.^[12] It revealed only one Stability indicating HPTLC method for the estimation of Sorafenib Tosylate. There is an increase tendency towards the development of stability indicating assay, using the approach of stress testing as mentioned in the ICH guideline. So, there was a need for the development of a simple, rapid, economic and sensitive assay of Sorafenib Tosylate. The proposed method is cheaper and simpler than other spectroscopic and chromatographic methods.

MATERIAL AND METHOD**Material**

Analytical standard sample of Sorafenib Tosylate was procured from Cipla Ltd. Mumbai as a gift sample. Methanol, Toluene and Ethyl acetate were used for the study.

Chromatographic Conditions and Instrumentation

The HPTLC system (CAMAG) consisting of Linomat 5 connected to a nitrogen cylinder, a twin trough glass chamber (20cm×10cm CAMAG) was used for linear ascending development of TLC plate under 10 min saturation time with stainless steel lid, a derivatization chamber & a plate heater. Pre-coated silica gel 60 F₂₅₄ TLC plates (10×10cm with 0.2mm layer thickness,

Merck, Germany) were used as the stationary phase. The Mobile phase composed of Toluene : Ethyl acetate (3:7v/v). TLC plates were prewashed with 10 ml of methanol and activated at 80°C for 10 min prior to sample application. The standard solution and working standard of Sorafenib Tosylate were spotted on plates as a band of size 6mm. The spotted plate dried after development and viewed under UV lamp for evaluation of the spot. Densitometric analysis was carried out using a TLC scanner IV with Win CATS software.

Preparation of Standard Stock Solution

Standard stock solution and working standard of Sorafenib Tosylate was prepared by dissolving 20 mg of pure drug in methanol. The prepared solution was sonicated for 10 minute. Then volume was made up to 10ml with Methanol to get concentration of 200 µg/ml.

Preparation of Sample Stock Solution

Twenty tablets were weighed accurately and crushed to fine powder. From the triturated tablet powder equivalent to 200 mg of Sorafenib Tosylate was weighed and transferred to 10ml volumetric flask and mixed with methanol. The solution was sonicated for 10 minute and filtered through Whatmann filter paper. Volume was made up to 10 ml with methanol.

Selection of Wavelength

From standard stock solutions of drugs were prepared separately in Methanol and scanned over the range of 200-400 nm and spectra was obtained individually. It was observed that Sorafenib Tosylate showed considerable absorbance at 265 nm respectively.

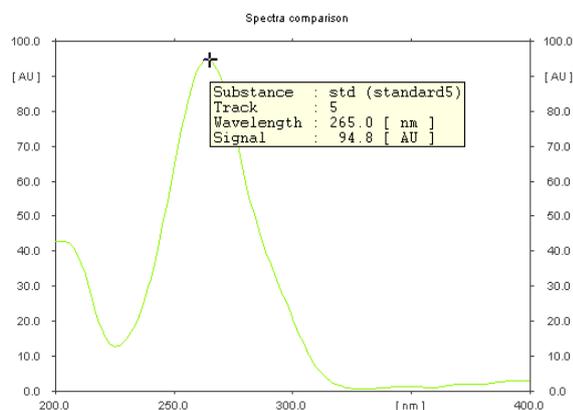


Fig. 2: Spectra of Sorafenib tosylate at 265nm.

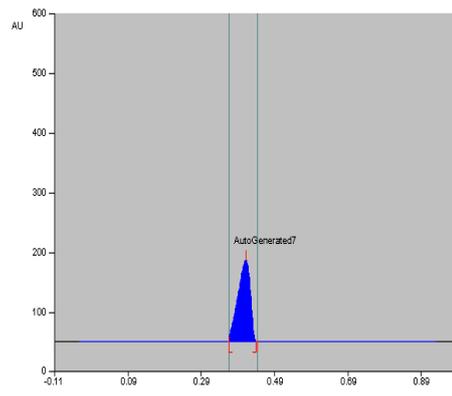


Fig. 3: Densitogram of standard solution of Sorafenib tosylate 400ng/band.

RESULTS AND DISCUSSION

METHOD VALIDATION

The method validation was carried out for various parameters in accordance with ICH guidelines.^[13-15]

Linearity

A standard stock solution of Sorafenib Tosylate was applied on TLC plate. The calibration curve was obtained in the range of 200-1200ng/spot. The plates was developed under optimized chromatographic conditions. The standard calibration curves were plotted as Peak area Vs concentration. The linearity of the calibration curve was calculated by linear regression analysis and the statistical data were calculated. The developed method was found to be linear in the range of 200-1200 ng/band for Sorafenib Tosylate shown in Figure 3. The equation of the calibration curve found for Sorafenib Tosylate was $Y=8.3079x+30.029$. The results of linearity studies were expressed in terms of the correlation coefficient. Values of correlation coefficient were found to be 0.997 for Sorafenib Tosylate.

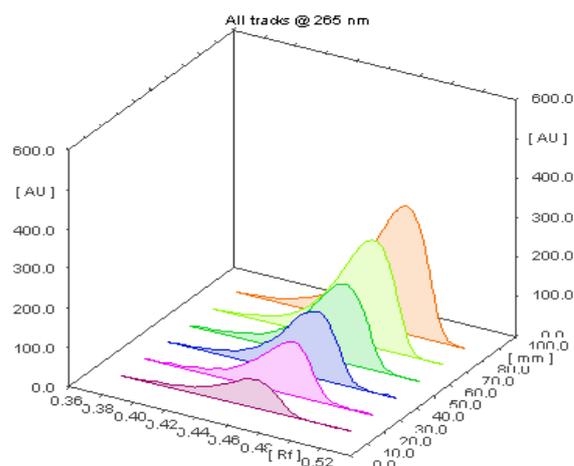


Fig. 4: 3D Spectra of Sorafenib tosylate using Wincats software.

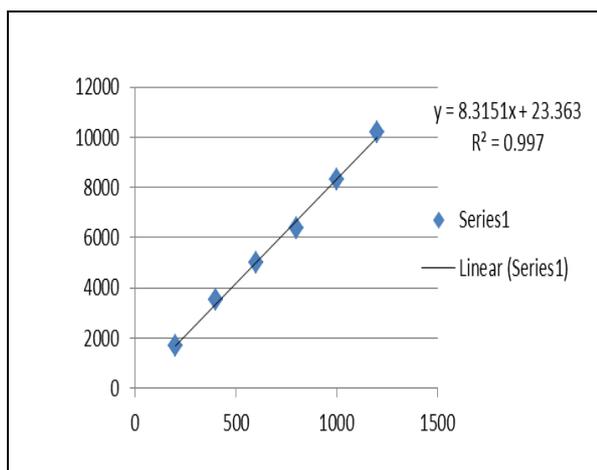


Fig. 5: Calibration curve for Sorafenib tosylate.

Precision

The precision of the method was demonstrated by Intraday and Interday precision studies. The interday and intraday study were determined using concentration 400ng/band for Sorafenib Tosylate. For intraday study, one standard concentration (400ng/band for Sorafenib Tosylate) was analyzed in same day with three different time interval. The interday study was tested by repeating the same concentrations on three consecutive days and percentage RSD was calculated for interday and intraday precision. The values of % RSD were found For intraday precision 0.6% and For interday precision 0.9% for Sorafenib Tosylate. The values of % RSD was found less than 2 indicating that the method was precise.

Table 3: Accuracy results of sorafenib tosylate at 265nm.

Sr. No.	Level %	Weight of tablet powder taken (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% recovery
1	80	40	16	15.77	98.59
2	100	40	20	19.93	99.65
3	120	40	24	24.1	100.5

Method sensitivity (limit of detection and limit of quantitation)

The limit of detection and the limit of quantitation is the lowest concentration of analyte in a sample which can be detected and quantified with acceptable accuracy and precision. LOD and LOQ were calculated using the regression equation. The LOD and LOQ were calculated as $3.3 \cdot \sigma / S$ and $10 \cdot \sigma / S$.

Where,

σ = is the standard deviation of the y- intercept,

S= is the slope of the calibration curve.

LOD of Sorafenib Tosylate = 19.9707ng/band

Table 1: Intra-day precision.

Sr. No	Conc. (ng/band)	Mean	%RSD
1	800	6367.68	0.6
2	800	6366.09	0.8
3	800	6372.33	0.4

Table 2: Inter-day precision.

Sr. No	Conc. (ng/band)	Mean peak area	%RSD
1	800	6346.74	0.7
2	800	6356.63	0.9
3	800	6322.46	1.1

Accuracy (Recovery study)

Recovery studies were performed by the standard addition method where known amount of standard drug were spiked to the analyzed dosage form in 3 different concentration levels. The amount of Sorafenib Tosylate was estimated by substituting values in the regression equation. The accuracy of the method was determined by % recovery studies at three different concentration levels 80, 100, 120%. The results of the recovery studies indicated that the method is accurate for estimation of Sorafenib Tosylate. The results obtained are shown in Table 1.

LOQ of Sorafenib Tosylate = 60.5174ng/band

Robustness

To analyze the robustness of the proposed method, small but deliberate variations in the optimized method parameters such as chamber saturation time, change in composition of the mobile phase, change in amount of mobile phase and the effect on peak area was noted. Effect of these changes on % drug content was evaluated in terms of SD and % RSD. Values of % RSD were found to be less than 2 which confirmed the robust nature of the developed method.

Table 4: Results of robustness study.

Sr. no.	Parameters	Robust condition	%RSD
1	Saturation time (10min) \pm 5min	5 min	0.85
		15 min	0.61
2	Amount of mobile phase (10ml) \pm 1 ml	9ml	1.1
		11ml	1.04

Analysis of Marketed Formulation

The marketed formulation was analyzed by the developed method. The R_f value were found to be $0.45 \pm$

0.02 for Sorafenib Tosylate. The % drug content (Table.5) was calculated and found to be 99.24 ± 1.18 for Sorafenib Tosylate.

Table 5: Results of analysis of tablet formulation.

Sr.No	Weight of tablet powder taken (mg)	Amount of pure drug estimated (mg)	% label claim	Mean % label claim	SD	%RSD
1	40	19.85	100.8	100.49	0.94	0.93
2	40	19.50	101			
3	40	19.90	99.24			
4	40	20.1	99.50			
5	40	20.0	101.7			
6	40	19.8	100.75			

Stress Degradation Studies

Stress degradation studies were carried on the standard drug to examine stability indicating properties of the developed method. The API were subjected to acid, base and neutral hydrolysis, oxidative, thermal and photolytic degradation to ensure the effective separation of degradation peaks and main peak as per ICH guidelines.

Acid induced degradation

In acidic stress degradation 20 mg of Sorafenib Tosylate were transferred to 10 ml dried volumetric flask and then dissolved in 3 ml of 0.1 N HCL. The mixtures was refluxed at 80°C for zero to three hours and the volume was made up with methanol. The resulting solution was applied on TLC plates and analyzed by developed method.

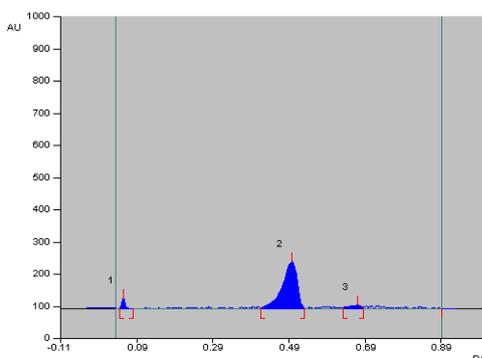


Fig. 6: HPTLC densitogram of Sorafenib tosylate in acidic stress condition.

Base Induced degradation

In alkaline stress degradation 20 mg of Sorafenib Tosylate were transferred to 10 ml dried volumetric flask and then dissolved in 3 ml of 0.1 N NaOH. The mixtures was refluxed at 80°C for zero to three hours and the volume was made up with methanol. The resulting solution was applied on TLC plates and analyzed by developed method.

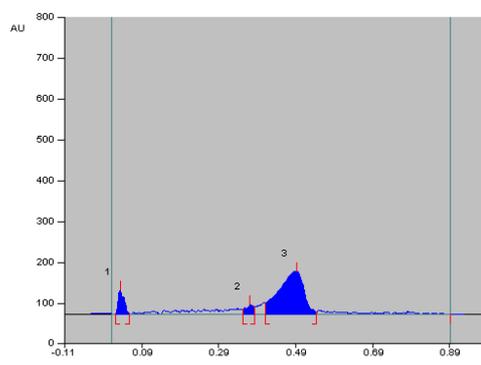


Fig. 7: HPTLC densitogram of Sorafenib tosylate in alkaline stress condition.

Oxidative stress degradation

In oxidative stress degradation 20 mg of Sorafenib Tosylate were transferred to 10 ml dried volumetric flask and then dissolved in 3ml of 3% H_2O_2 . The mixtures was refluxed at 80°C for zero to three hours and the volume was made up with methanol. The resulting solution was applied on TLC plates and analyzed by developed method.

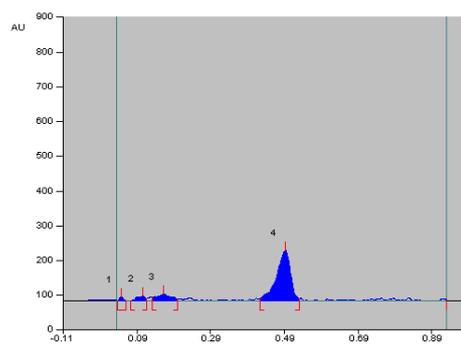


Fig. 8: HPTLC densitogram of Sorafenib tosylate in oxidative stress condition.

Hydrolytic stress degradation

In hydrolytic stress degradation 20 mg of Sorafenib Tosylate were transferred to 10 ml dried volumetric flask and then dissolved in 3ml distilled water. The mixtures was refluxed at 80°C for zero to three hours and the volume was made up with methanol. The resulting

solution was applied on TLC plates and analyzed by developed method.

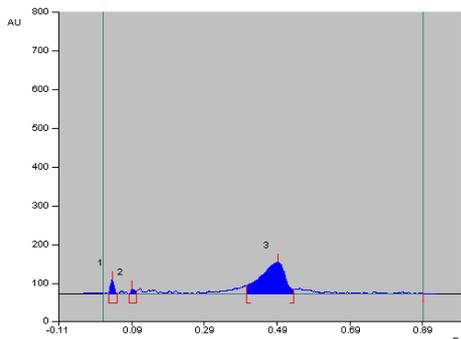


Fig. 9: HPTLC densitogram of Sorafenib tosylate in hydrolytic stress condition.

Photolytic stress degradation

In photolytic stress degradation Standard drug powder were exposed to UV light for 24 hrs. Appropriate dilutions were made with methanol and analyzed by developed method.

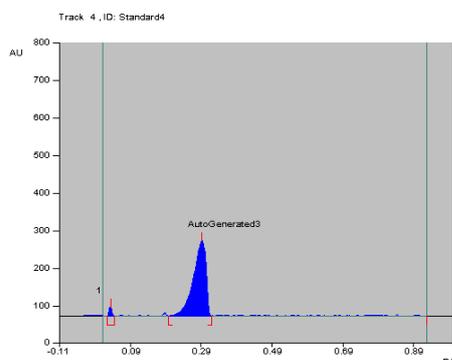


Fig. 10: HPTLC densitogram of Sorafenib tosylate in photolytic stress condition.

Thermal stress degradation

In thermal stress degradation Standard drug powder were placed in an oven at 80°C for 1 hr. Appropriate dilutions

were made with methanol and analyzed by developed method.

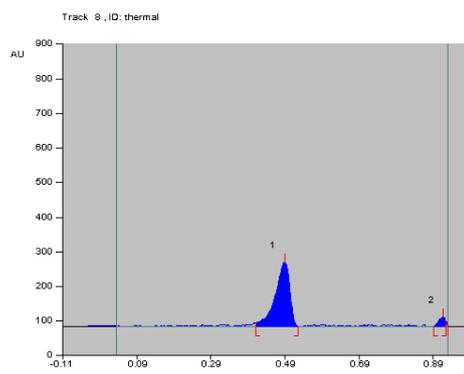


Fig. 11: HPTLC densitogram of Sorafenib tosylate in thermal stress condition.

Result of stress degradation Studies

The standard drug were subjected to acid, base and neutral hydrolysis, oxidative, thermal and photolytic degradation as per ICH guidelines. Results of stress Degradation Studies are presented in Table 6. Sorafenib Tosylate was found to be more susceptible to Neutral, oxidative and acidic condition and less susceptible to thermal and photolytic and alkaline stress condition. The peaks of the degradants in each condition were well resolved from main peak (800ng/band). In previous HPTLC^[1] paper it was mentioned that there was no separate peak for degradation product observed. Degradation peaks did not show any interference with the drug peaks and. Hence, the method is stability indicating.

Table 6: The results of the stress degradation studies of Sorafenib tosylate.

Sr. no.	Stress test Conditions	Solvents	Temp.	% recovery	% Degradation
1	Acidic	0.1 N HCL	80 ⁰ C	81.92	18.09
2	Alkaline	0.1 N NaOH	80 ⁰ C	88.52	11.48
3	Oxidative	3% H ₂ O ₂	80 ⁰ C	80.40	19.6
4	Hydrolytic	Distilled Water	80 ⁰ C	79.30	20.7
5	Thermal	--	80 ⁰ C	94.88	5.12
6	Photolytic	--	UV light	92.48	7.52

CONCLUSION

A simple, rapid and stability indicating HPTLC method has been developed for the estimation of Sorafenib Tosylate in bulk and tablet dosage form. The method was validated for various parameters. Stress degradation study was carried out in accordance with ICH guideline

Q1 (R2). In degradation study it was found that the Sorafenib Tosylate was more susceptible under Neutral, oxidative and acidic stress condition. So, the degradation study by the HPTLC method can be successfully applied for the estimation of this drug in dosage form. The peaks of the degradants in each condition were well resolved

from main peak. The proposed method can be used as an alternative method for the analysis of Sorafenib Tosylate in its formulation.

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