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Optimization of stability indicating RP-HPLC method for the estimation of an anti-cancer drug Sorafenib Tosylate in pure and pharmaceutical dosage form

Ramesh Jayaprakash*, Dr. Senthil Kumar Natesan

Department of Pharmaceutical Analysis

JKK Munirajah Medical Research Foundation's- Annai JKK Sampoorani Ammal College of Pharmacy, Komarapalayam, Namakkal -DT, Tamilnadu, India.

Affiliated to: The Tamilnadu DR. M.G.R. Medical University, Chennai- 600 032

Corresponding author: Ramesh Jayaprakash

Email: rameshj1974@gmail.com

ABSTRACT

A simple, specific and precise stability indicating RP- HPLC method has been developed and validated for the estimation of sorafenib tosylate in tablet dosage from using Phenomenex Luna- C_{18} column (4.5 x 250 mm; 5 µm particle size) as a stationary phase, methanol: acetonitrile: water (65:25: 10 v/v/v) as a mobile phase, flow rate of 1 mL/min and detection was carried out at 248 nm. The retention time of sorafenib was 2.887 minute. RP- HPLC method was developed with linearity range of 20-120 µg/mL of sorafenib tosylate. The correlation coefficient was found to be 0.9997 for sorafenib tosylate. The assay results obtained in good agreement with the corresponding labeled amount by developed method within range of 99.15% - 101.58%. Accuracy, precision, LOD, LOQ, specificity, robustness and ruggedness were met all the acceptance criteria for the validation of analytical method as per ICH Q2 (R1) guideline. This method can be conveniently used to detect the possible degradation product in the dosage form of sorafenib tosylate during stability studies (acidic, alkaline, oxidative, thermal and photolytic). The method proved to be effective on application to a stressed marketed tablet formulation.

Keywords: Analytical method development, Validation, Stability indicating, Sorafenib tosylate

INTRODUCTION

Sorafenib tosylate is chemically 4-[4-[[4-chloro-3-(trifluoromethyl) phenyl] ureido} phenoxy) - N - 2 - methyl-pyridine - 2 – carboximide 4 – methylbenzenesulfonate. The drug was approved for the treatment of primary kidney cancer and advanced primary liver cancer. Sorafenib tosylate was estimated by RP-HPLC in human serum [1, 2]. It was estimated in bulk and in tablets by RP-HPLC [3,4], HPTLC [5], UV method [6,7] and by LC-MS/MS [8]. But to the best our

knowledge, there is no stability-indicating method reported for sorafenib tosylate. The present work aims to develop a simple, precise, and accurate stability indicating RP-HPLC method for the estimation of sorafenib tosylate in pure and in its tablet formulation through stress studies under a variety of ICH recommended test conditions [9] and to develop a validated stability-indicating assay method [10,11].

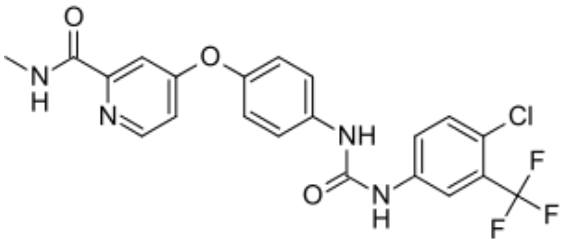

Fig 1. Sorafenib

EXPERIMENTAL

Instrument & apparatus

The HPLC experiment was carried out in Hitachi HPLC system equipped with Phenomenex Luna- C_{18} column (4.5 x 250 mm; 5 µm particle sizes), auto sampler L-2200, pump L-2130, detector PDA L-2455, software D-2000 Elite HSM (English) was used for analysis.

Chemical & reagents

Standard Sorafenib tosylate was procured as a gift sample from Spectrum Labs Limited, Hyderabad, India. The reagents utilized for analysis are HPLC grade methanol, HPLC grade acetonitrile, HPLC grade water.

Preparation of Diluent

The diluent was prepared by mixing 65 mL of methanol, 25 mL of acetonitrile and 10 mL of water and the resulting solution was sonicated for 15 min and it was used as diluent.

Preparation of standard stock solution

Quantity of sorafenib tosylate equivalent to 200 mg was weighed and transferred in to a 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 80 µg/mL. 10 µL of the final solution were injected into the system and the chromatograms were recorded.

Preparation of sample stock solution

Tablet powder equivalent to 200 mg of sorafenib tosylate was weighed and transferred in to 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 80 µg/mL. 10 µL of the final solution were injected into the system and the chromatograms were recorded.

METHOD VALIDATION

Linearity

Linearity study was carried at six different concentration levels ranging from 20-120 µg/mL of sorafenib tosylate was prepared. The response of the drug was found to be linear in the selected concentration range.

Accuracy

Accuracy was calculated by addition of standard drugs to reanalyzed sample at 3 different concentration levels and computing percentage recoveries. Standard limit of % recovery study is 98 - 102 % as per ICH guideline. From the studies it was concluded that % recovery study of sorafenib tosylate complies with standard limit of ICH guideline.

Precision

Repeatability

Solution containing 80 µg / mL of sorafenib tosylate was prepared. Prepared solution was analyzed six times in same day as per the proposed method

Intermediate precision

Intraday precision

Solution containing 80 µg / mL of sorafenib tosylate was prepared from their respective standard stock solution. Analysis was replicated for 3 different times within same day.

Intraday precision

Solution containing 80 µg / mL of sorafenib tosylate was prepared from their respective standard stock solution. Analysis was replicated for 3 different days.

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

The Limit of Detection and Limit of Quantification of the developed method were calculated from the standard deviation of the y-intercepts and slop of the calibration curve of sorafenib tosylate using the formula as given below.

Limit of Detection = 3.3 α / S

Limit of Quantitation = 10 α / S

Where α is the standard deviation of the y – intercepts and S is the slop of the calibration curve.

Robustness

As per ICH, the prepared solution was analyzed as per proposed method with small but deliberate change in chromatographic conditions as listed below:

- Change in flow rate
- Change in mobile phase composition
- Change in nanometer
- Change in temperature

System suitability parameters

System suitability tests were carried out on standard stock solution of sorafenib tosylate (80 µg / mL) and these solutions were injected under optimized chromatographic condition. Various parameters like asymmetry factor, theoretical plates, repeatability of peak area and retention time were checked.

Analysis of Pharmaceutical Formulation

Twenty tablets were weighed accurately and their average weight was determined. The tablets were crushed to fine powder and from the triturate; tablet powder equivalent to 200 mg of sorafenib tosylate was weighed and transferred in to 100 mL volumetric flask, 30 mL of diluent was added and sonicated for 15 min and the volume was made up to the mark with diluent. From this solution further dilution was made to get the final concentration of 80 µg/mL. 10 µL of the prepared solution was injected into the system and the chromatogram was recorded. Concentration of sorafenib tosylate was computed by putting value of the peak areas in respective standard regression equation obtained from calibration curve.

Forced degradation study

Forced degradation studies were performed to evaluate the stability indicating properties (Specificity) of the proposed method. Sorafenib tosylate was subjected to neutral, acid, base, oxidation, thermal and photo degradation to ensure the effective separation of degradation peaks and main peak.

Control sample

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further pipette 10 mL of the above stock solution and transferred to 100 mL volumetric flask and made up to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate and 10 µL of the solutions were injected in to the system and the chromatograms were recorded.

Neutral degradation studies

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the

solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further pipette 10 mL of the above stock solution and transferred to 100 mL volumetric flask and made up to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate and the solution was refluxed in water bath for 30 minutes at 80^0C and 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

Acid degradation studies

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. 10 mL of the above stock solution was transferred to 100 mL volumetric flask to that 10 mL of 1 N hydrochloric acid was added and refluxed for 30 minutes at 80^0C. The resulting solution was diluted to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate . 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

Alkaline degradation studies

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. 10 mL of the above stock solution was transferred to 100 mL volumetric flask to that 10 mL of 1 N sodium hydroxide was added and refluxed for 30 minutes at 80^0C. The resulting solution was diluted to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate . 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

Oxidative degradation studies

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. 10 mL of the above stock solution was transferred to 100 mL volumetric flask to that 10 mL of 3 % hydrogen peroxide (H_2O_2) was added and refluxed for 30 minutes at 80^0C. The resulting solution was diluted to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate . 10 µL of the refluxed solutions were injected in to the system and the chromatograms were recorded.

Thermal degradation studies

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further pipette 10 mL of the above stock solution and transferred to 100 mL volumetric flask and made up to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate and the solution was placed in oven at 80^0C for 48 hours. 10 µL of the solutions were injected in to the system and the chromatograms were recorded.

Photolytic degradation studies

A quantity tablet powder equivalent to 200 mg of sorafenib tosylate was accurately weighed and transferred to 100 mL volumetric flask and it is dissolved in 10 mL of the diluent. Sonicated the solution for few minutes to dissolve the drug completely. Then it is filtered through 0.45 µ filter and the volume was made up to 100 mL with diluent. Further pipette 10 mL of the above stock solution and transferred to 100 mL volumetric flask and made up to 100 mL with diluent. From the above resulting solution pipette out 4 mL and made up to 10 mL with diluent to get the final concentration of 80 µg/mL of sorafenib tosylate

and the solution was exposed to UV light by keeping the volumetric flask in UV chamber for 7 days. 10 µL of the solutions were injected in to the system and the chromatograms were recorded.

RESULT AND DISCUSSION

An exertion has been made for a simple, rapid, accurate and precise stability indicating analytical method based on RP- HPLC using PDA detection was developed and validated for assay determination of sorafenib tosylate in pure form and in tablet dosage formulation. The analytical conditions were selected keeping in mind the chemical nature of sorafenib tosylate. The development trials were taken using different mobile phase with different composition. The column selection has been done on the basis of back pressure, peak shape, theoretical plates and day -to- day reproducibility of the retention time. After evaluating all these factors, Phenomenex Luna- C_{18} column (4.5 x 250 mm; 5 µm) was found to be giving satisfactory results. The selection of mobile phase was done based on chemical structure of the drug. Best results were obtained with methanol: acetonitrile: water 65:25: 10 v/v/v (Table I). The selection of mobile phase, methanol: acetonitrile: water (65:25: 10 v/v/v) was chosen to reduce the longer retention time and to attain good peak shape.

Hence, the Separation was carried out on Phenomenex Luna- C_{18} column (4.5 x 250 mm; 5 µm) column using a mobile phase consisting of methanol: acetonitrile: water (65:25: 10 v/v/v). The flow rate 1 mL/min and the injection volume was 10µL. The detection was carried out at 248 nm. The peak retention time of sorafenib tosylate was found to be 2.887 minutes. A representative chromatogram of standard and sample were shown in Figure 2(a) and (b).

Table I: Optimized Chromatographic Condition for the Estimation of Sorafenib Tosylate

Parameter	Condition
Mobile phase	Methanol: acetonitrile: water (65:25: 10 v/v/v)
Diluent	Mobile phase
Column	Phenomenex Luna- C_{18} column (4.5 x 250 mm; 5 µm)
Column temperature	30^0C
Detection wavelength	248 nm
Injection volume	10µL
Flow rate	1 mL/min
Run time	8 min

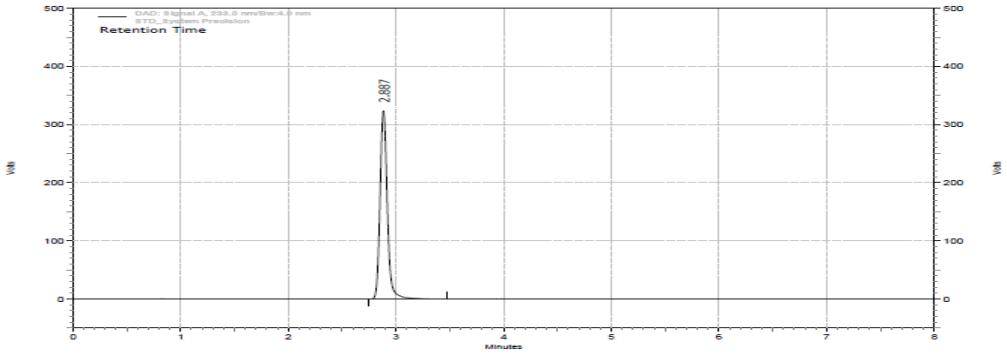

Fig 2 (a). Typical chromatogram of sorafenib tosylate standard drug

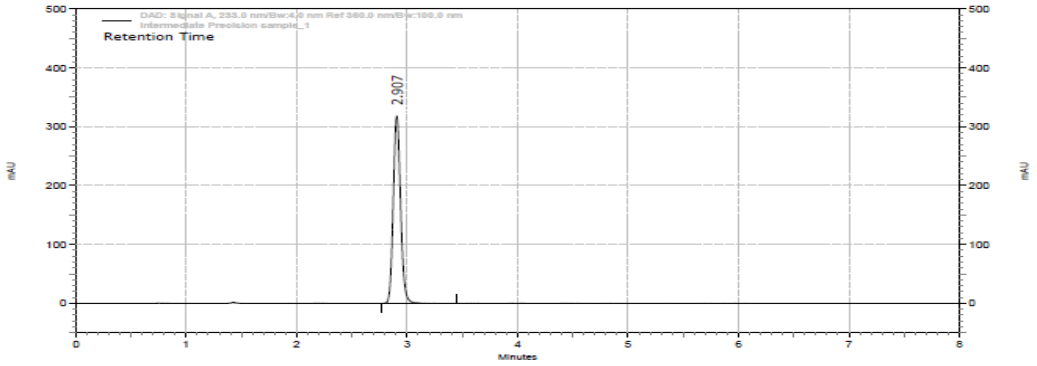

Fig 2 (b). Typical chromatogram of sorafenib tosylate sample drug

Method validation

The proposed RP-HPLC method was validated as per ICH guidelines.

Linearity

For linearity of six points calibration curve were obtained in a concentration ranges from 20-120 µg/mL of sorafenib tosylate. The response of the drug was found to be linear in the selected concentration range the correlation co-efficient for sorafenib tosylate was 0.9997. The Figure 3 shows the linearity plot of sorafenib tosylate. A representative data of linearity is shown in Table II.

Table II: Linearity Data of Sorafenib Tosylate

Concentration (µg/mL)	Mean peak area (n = 6)
20	48462025
40	98406779
60	153783209
80	197857918
100	247352497
120	296963067
Slope	**2478788.00**
y- intercept	**247790.44**
Correlation coefficient	**0.9997**

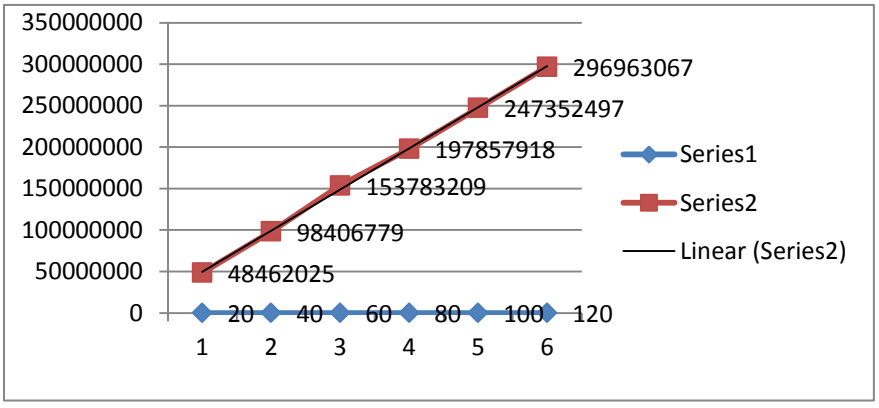

Fig 3. Linearity Plot of Sorafenib Tosylate

Accuracy (Standard addition Method)

Results obtained reveals that % recovery of sorafenib tosylate was found to be 98.02 – 100.34 (Table III)

Precision

The repeatability, % RSD was found to be 0.9334 for sorafenib tosylate. For intraday precision, % RSD was found to be 0.1988, for interday precision, % RSD was found to be 0.2588 (Table IV, V& VI).

Robustness

Variation in the flow rate, mobile phase, nanometer and temperature has been made to the analytical method in order to evaluate and measure the capacity of the method to remain unaffected by such variations. The % RSD was found to be less than 2. (Table VII).

Ruggedness

Ruggedness of the method was confirmed by the analysis of formulation was done by the different analysts (Table VIII).

Table III: Accuracy Data of Sorafenib Tosylate

Parameters	Amount present (µg/mL)	Amount added (µg/mL)	Amount found (µg/mL)	Amount recovered (µg/mL)	% Amount recovered
80%	80	64	143.94	63.94	98.47
			143.86	63.86	98.42
			143.60	63.6	98.02
100%	80	80	159.81	79.81	98.38
			159.80	79.80	98.36
			159.84	79.84	98.41
120%	80	96	177.15	97.15	100.34
			175.49	95.49	98.63
			176.44	96.44	99.61
Average					**98.73**
SD					**0.7340**
%RSD					**0.7434**
SE					**0.2447**
CI (Confidence Interval 99%)					**97.94 – 99.53**

Table IV: Repeatability data of Sorafenib Tosylate

Sample	Peak areas (Mean, n = 6)	SD	% RSD
Sorafenib Tosylate	201622298	**0.9391**	**0.9334**

Table V: Intra-day Precision Data of Sorafenib Tosylate

Parameter	Concentration (µg / mL)	Peak area (Mean, n = 6)	% Amount found (Mean, n = 6)	SD	%RSD
0 Hours	80	201431728	101.80	**0.2022**	**0.1988**
3 Hours		201269951	101.69		
6 Hours		201568914	101.87		

Table VI: Inter-day Precision Data of Sorafenib Tosylate

Parameter	Concentration (µg / mL)	Peak area (Mean, n = 6)	% Amount found (Mean, n = 6)	SD	%RSD
Day - I	80	201255017	101.71	**0.2632**	**0.2588**
Day - II		201199706	101.68		
Day - III		201326709	101.74		

Table VII: Robustness Data of Sorafenib Tosylate

Parameters	Retention time	Mean area (n = 6)	% Amount found (n = 6)	SD	%RSD
Flow minus (0.8 mL/min)	3.233	201646091	101.27	**0.3659**	**0.3612**
Flow plus (1.2 mL/min)	3.007	195248987	99.09	**0.3990**	**0.4027**
nm plus (250 nm)	2.907	197081299	99.74	**0.3045**	**0.3053**
nm minus (246nm)	2.907	195323652	99.00	**0.2879**	**0.2908**
Temperature plus (32^0C)	3.007	197943676	100.09	**0.1359**	**0.1358**
Temperature minus (28^0C)	2.907	197252152	99.84	**0.1750**	**0.1752**
Methanol (60)	3.204	196116774	99.18	**0.1934**	**0.1950**
Methanol (70)	2.907	198686649	100.42	**0.2050**	**0.2042**
Acetonitrile (20)	3.204	199254135	100.85	**0.1722**	**0.1707**
Acetonitrile (30)	3.233	194872921	98.61	**0.2805**	**0.2845**

Table VIII: Ruggedness Data of Sorafenib Tosylate

Parameter	Concentration (µg / mL)	Mean area (n = 6)	% Amount found*	SD	%RSD
Different Analyst	80	200124698	101.14	**0.8342**	**0.8247**

Limit of Detection and Limit of Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of sorafenib tosylate was determined by using standard deviation of the response and slope approach as defined in ICH guidelines (Table IX).

Table IX: LOD and LOQ Data of Sorafenib Tosylate

Slope	Y-Intercept
2480445	-163207.85
2477594	215825.53
2479296	520276.10
2478792	246185.07
2478268	335633.5
2478330	332030.32
Average	**2478788**
SD	**227598.4**
LOD (µg/mL)	**0.3030**
LOQ (µg/mL)	**0.9181**

System suitability parameters

System suitability was established to determine the adequate reproducibility of the proposed method. Parameters including asymmetry factor, theoretical plates, repeatability of peak area and retention time was calculated (Table X).

Table X: System suitability parameters of Sorafenib Tosylate

Parameters	Results
Theoretical plates (N)	9599
Asymmetry factor	1.2
Retention time	2.887
% RSD of peak area	0.0805
% RSD of retention time	0.4962

Assay of marketed formulation

Percentage purity of sorafenib tosylate was found to be 100.61 (Table XI).

Table XI: Analysis of Sorafenib Tosylate in Marketed Formulation

S.No	Standard area	Sample area	Label Claim (mg)	Amount found (mg)	% Assay
1	198578162	200222571	200 mg	201.56	100.78
2	198667350	200800079		203.08	101.54
3	198732900	201187708		203.16	101.58
4	198524996	201686801		202.13	101.06
5	198412720	202294839		198.30	99.15
6	198578540	203541795		199.10	99.55
Average					**100.61**
SD					**1.029**
%RSD					**1.022**
SE					**0.4200**
CI (Confidence Interval 99%)					**99.06 – 102.15**

Forced degradation studies

From the degradation of these solutions under the stress condition gives us an idea about the origin of degrading products. Degradants did not show any interference with the elution of drug peaks. Hence, the method is stability indicating (Table XII).

Table XII: Forced Degradation Study Data of Sorafenib Tosylate

Parameters	R.T	Mean area (n = 6)	% Degradation	% of Active drug present after degradation
Control sample	3.307	202541795	-	-
Neutral sample	3.273	199874643	0.86%	98.68%
Acidic degradation	3.320	195937462	2.82%	96.73%
Alkaline degradation	3.247	194382721	3.58%	95.97%
Oxidative degradation	3.313	192842271	4.34%	95.21%
Thermal degradation	3.353	199782468	0.92%	98.63%
Photolytic degradation	3.280	199264532	1.17%	98.38%

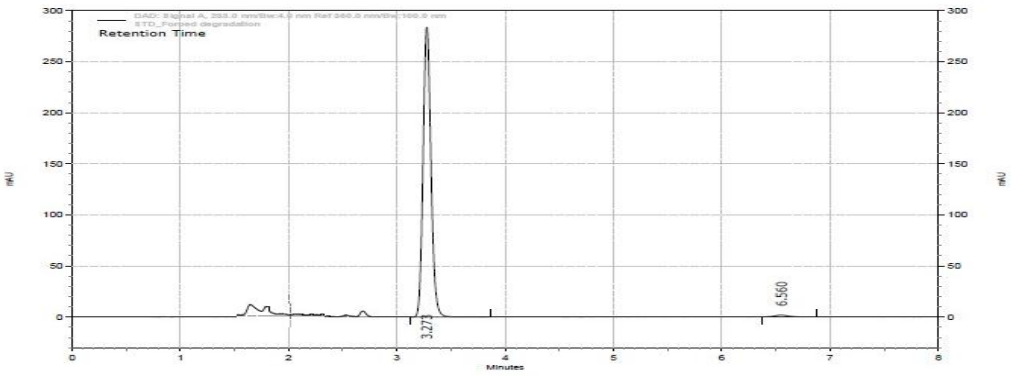

Fig 4: Neutral Degraded Chromatogram of Sorafenib Tosylate

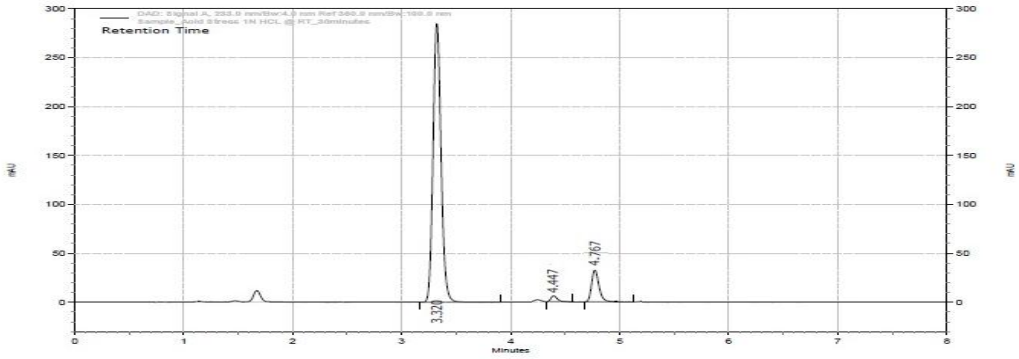

Fig 5: Acid Degraded Chromatogram of Sorafenib Tosylate

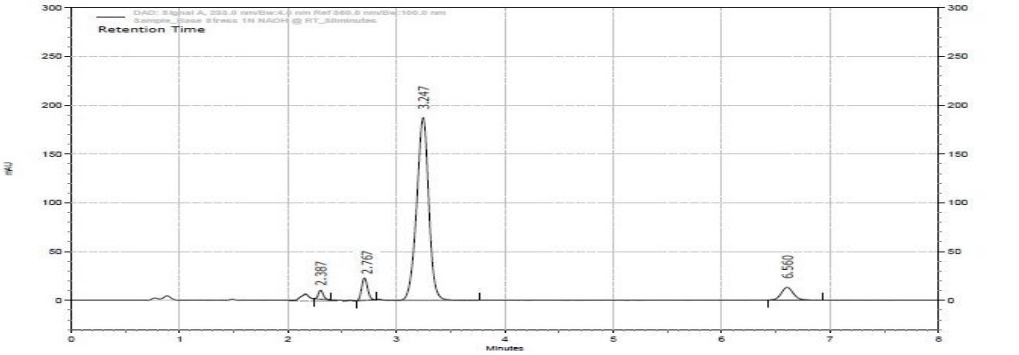

Fig 6: Alkali Degraded Chromatogram of Sorafenib Tosylate

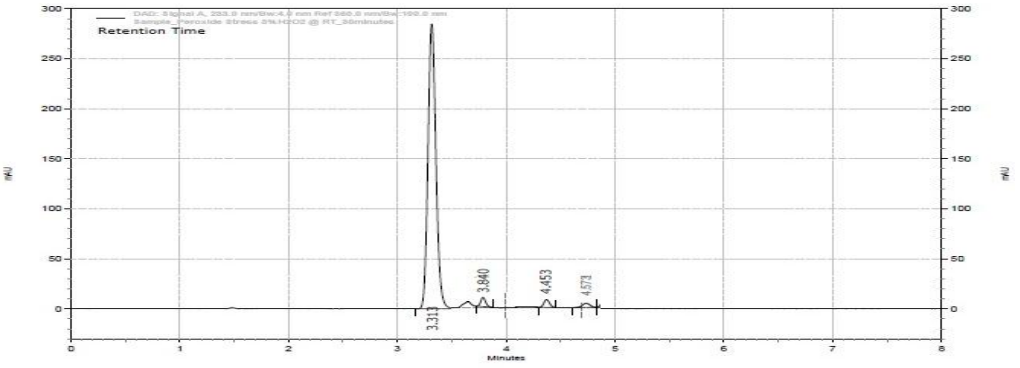

Fig 7: Peroxide Degraded Chromatogram of Sorafenib Tosylate

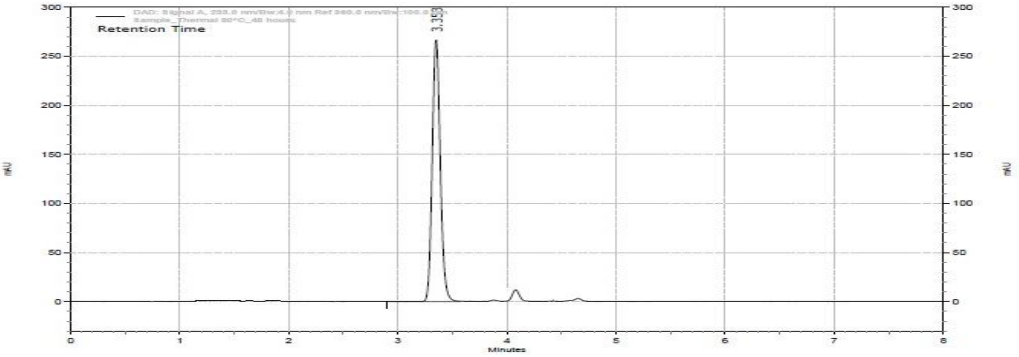

Fig 8: Thermal Degraded Chromatogram of Sorafenib Tosylate

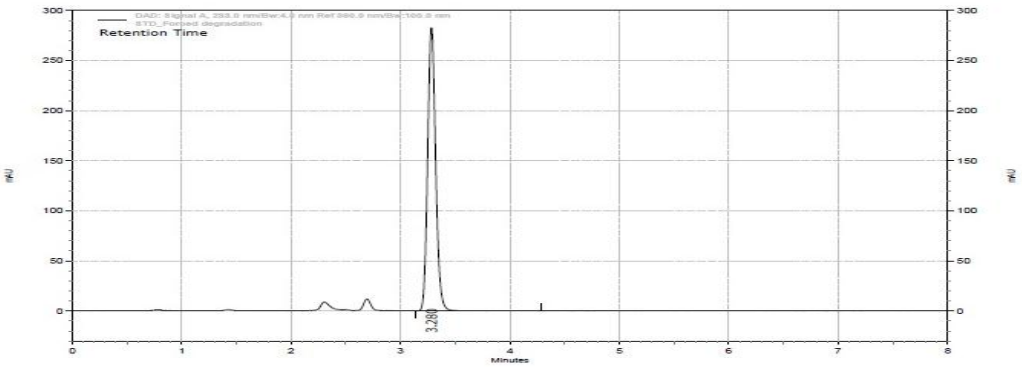

Fig 9: Photolytic Degraded Chromatogram of Sorafenib Tosylate

CONCLUSION

A simple, accurate and precise stability indicating RP – HPLC method has been developed for estimation of sorafenib tosylate in tablet dosage form. The method was validated for various parameters like specificity, linearity, LOD, LOQ, robustness, precision, accuracy. Linearity of the developed method was 0.9997, range was found to be 20 -120 µg/mL of sorafenib tosylate. The % RSD was found to be less than 2 for repeatability, intraday precision, intermediate precision, robustness and ruggedness. Forced degradation study of drug was carried out according to ICH guideline Q1A (R2). In degradation study it was found that the sorafenib tosylate was more susceptible under stress condition. So, the degradation study by the RP-HPLC 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. There is no interference of any degradants at the retention time of the main peak indicates that the developed method is stability indicating. The proposed method can be used as an alternative method for the analysis of sorafenib tosylate in its formulation.

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Disclosure of interest

The authors that they have no conflicts of interest concerning this article.

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