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INNOVATIONAL PUBLISHER

INNOVATIONAL JOURNAL OF CHEMISTRY

Innovational Journal of Chemistry 1(2) (2016) 63-71

DEVELOPEMENT AND METHOD VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION AND FORCED DEGRADATION OF SORAFENIB TOSYLATE IN BULK AND PHARMACEUTICAL DOSAGE FORM

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Received 08 June 2016; Revise 10 July 2016; Accepted 15 July 2016

Abstract:

The present work describes development and validation of a specific, sensitive, precise and force degradation high performance liquid chromatographic method of analysis of sorafenib tosylate, as bulk drug and marketed formulation. The separation was achieved by using a mobile phase of methanol: water (80:20 v/v) on a thermo BDS hypersil C18 column (250 × 4.6 mm i.d.5 µm) at ambient temperature at flow rate of 1.0 ml/min. The detection was done at 268 nm. The retention time of sorafenib tosylate was 4.515 min. This method was successively applied to pharmaceutical dosage form of sorafenib tosylate was subjected to stress conditions of hydrolysis, oxidation, alkaline and acidic degradation. The degraded products were well resolved from the pure drug with significantly different retention time values. Linearity was found to be in the range of 20-100µg/ml with significantly high value of correlation coefficient. The method was validated for precision, robustness and recovery. The limits of detection and quantitation were 0.48µg/ml and 0.4466µg/ml respectively.

Keywords: Sorafenib Tosylate, marketed formulation (nexavar), Stress degradation, Validation, ICH guidelines.

Introduction:

Sorafenib tosylate is a multikinase inhibitor approved for cancer treatment that blocks the receptor tyrosine kinases VEGFR (Vascular Endothelial Growth Factor Receptor) and PDGFR (Platelet Derived Growth Factor Receptor) and the RAF serine/threonine kinases along the RAF/MEK/ERK pathway.Sorafenib indicated for the treatment of patients with advanced renal cell carcinoma who have failed prior interferon-alpha or interleukin-2 based therapy ortreatment of patients with hepatocellular carcinoma are considered unsuitable forSuch therapy. Sorafenib tosylate 4-[4-({[4-chloro-3-trifluoromethyl) phenyl]carbamoyl} amino)phenoxy] -N-methylpyridine-2-carboxamide Its molecular weight is 637.027 g/mol and the empirical formula is C21H16ClF3N4O3 . Sorafenib is white crystalline powder .The International Conference on Harmonization (ICH) guideline entitled 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 and thus generates degradation products that can be studied to determine the stability of the molecule. The aim of present work is to develop an accurate, specific, and reproducible stability indicating HPLC method for determination of sorafenib tosylate in presence of degradation products formed under different stress conditions[1-20].

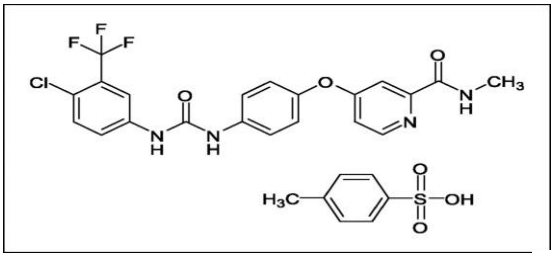

Fig: 1. Structure of Sorafenib tosylate {4-[4-({[4-chloro-3-(trifluoromethyl) phenyl] carbamoyl} amino) phenoxy]-N-methylpyridine-2-carboxamide}.

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Materials & Reagents:-
Quality approved reference standards of Sorafenib Tosylate were provided by Reliance Laboratory, Mumbai, and that contains Sorafenib Tosylate 99.70% as potency. The details of reference standards are given in Table. All chemicals and reagents used were of HPLC grade for analysis and were purchased from reputed organizations and details of reference std given in Table 1.

Table 1: Details of Reference standards

Drug sample	Purity%
Sorafenib Tosylate	99.70%

Apparatus and Instrumentation:-
The development and validation of the method was performed on HPLC system System: HPLC Binary Gradient System, Model no. HPLC 3000 Series. The detector consisted of a UV-Visible spectrophotometer operated at 268 nm. The pump used was P-3000-M Reciprocating (40MPa). The column used was Thermo scientific Hypersil BDS C18 (250mm x 4.6ID, Particle size: 5 micron). The software used was HPLC Workstation.

HPLC Analysis:-

Optimization of HPLC method:-
Optimization of method started with mobile phase of methanol, water (80:20) with different pH values. A further trial was done with water, combinations and the column used was C18. The final chromatographic conditions set for the method were mobile phase of water and methanol 80:20 v/v, pH adjusted to 3.0 with O-phosphoric acid with flow rate of 1 mL/min and temperature of 40 0C. The column used was Hypersil BDS C-18 (250X4.6 mm) 5µ with 20µl injection volume and detection was carried out at 268 nm.

Preparation of Solutions for HPLC method:-
a) Preparation of Buffer solution (0.02 M Phosphate Buffer)
About 2.75 grams of KH2PO4 was accurately weighed and transferred into a 1000mL beaker, and dissolved in HPLC water. Finally pH was adjusted to 3.0 with o-phosphoric acid and volume was made up to mark with HPLC grade water.
b) Preparation of Mobile Phase
The mobile phase was prepared by adding methanol 80 ml (80%), 20 mL of water HPLC grade (20%) The pH of the solution was adjusted to 3.0 with O-phosphoric acid the contents were degassed in ultrasonic water bath for 10 minutes. The solution was further filtered by using 0.45 µ filter.
c) Preparation of Standard stock solution
Standard stock solution of Sorafenib (400µg/mL):
An accurately weighed quantity of powder equivalent to 40.0 mg of Sorafenib was transferred to 100 mL volumetric flask. The drug was dissolved and diluted up to the mark with methanol
d) Working standard preparation
Working standard solution containing 40µg/mL of Sorafenib was prepared by diluting 1mL stock solution of each of Sorafenib, transferred to 100 mL volumetric flask. The final Volume made with mobile phase & mixed we

RESULTS AND DISCUSSION:-
Selection of Wavelength for detection:-
In UV analysis Sorafenib Tosylate showed at 268 nm. Hence 268 nm was selected as the analytical wavelength for further analysis.

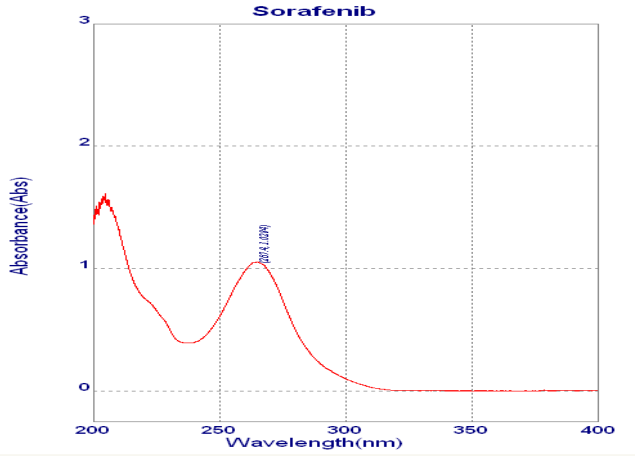

Fig 2. Overlain UV spectra of Sorafenib Tosylate (max nm)in methanol and water showing at 268 nm.

HPLC Method Development:-
Different diluents were tried in order to find the best conditions for determination of sorafenib tosylate like methanol and water in 80:20 v/v ratio. Mobile phase consisting of Methanol and water (80: 20 v/v) gave a sharp peak of sorafenib tosylate and this system was optimized. It was observed that the developed chromatographic condition provides better separation of sorafenib tosylate (4.51 5min) shown in Fig.3.

Table 2: RP-HPLC Method Developmental Trial- 4

Column	Mobile Phase	Wavelength and Flow Rate	Observation	Conclusion
Grace C18 (4.6ID x 250mm, Particle size: 5 micron)	Methanol: water pH3 (80:20 v/v)	268 nm and 1 ml/min.	Sharp peaks observed, peaks with proper resolution and acceptable system suitability test parameters	Mobile phase was optimized for further Studies. **ACCEPTED**

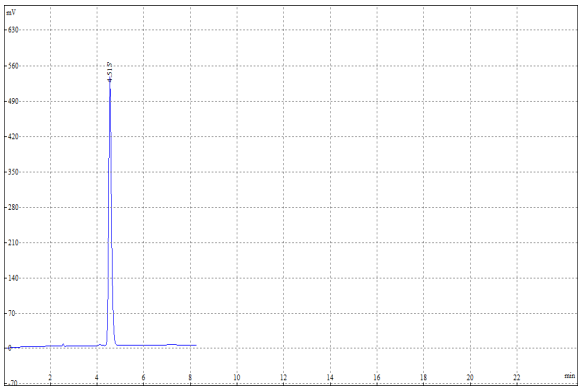

Fig.3 Chromatogram of Sorafenib Tosylate Std methanol: water (80:20 v/v), Grace C18 column.

The component is eluted with C18 column with different chromatographic conditions. Different mobile phases were tried in order to find the best conditions for separation of Sorafenib Tosylate in binary mixture. System suitability parameters obtained by trial 1 to 3, were not satisfactory and therefore, these chromatographic conditions were rejected for method development. Another trial provided significant system suitability parameters (Trial 4). Hence, these chromatographic conditions were selected for this method. The composition, flow rate of mobile phase and column, column temperature was suitably optimized for better separation of these drugs.

System suitability parameters of Optimized RP- HPLC method:-

System suitability tests were performed to verify the resolution and reproducibility of the chromatographic system. System suitability parameters for the optimized method were found to be within the acceptable limit. Results of system suitability are shown in Table 3.

Table 3: System suitability test parameters of optimized RP- HPLC method

Peak Name	RT (min)	Mean Peak Area	% RSD of Area	Tailing	Resolution	Theoretical Plates
SORAFENIB	4.515	4373905	1.1395	1.29	0	7194

Analysis of Marketed Formulation by RP-HPLC Method:-

The peaks at RT 4.515 ± 0.005 min was observed for Sorafenib Tosylate in the chromatogram of the drug sample extracted from Table (Fig.3). Experimental results of the amount of Sorafenib Tosylate in Sterile powder dosage form expressed as percentage of label claim were in good agreement with the label claims illustrating that there is no interference from any excipients, which are normally present in sterile dry powder. The drug content was found to be 398.18± 400mg for sorafenib. Results are presented in Table 4.

Table 4: Assay of marketed formulation

Drug	Label Claim(mg)	Amount Found (mg)	Mean % Drug Recovered ±SD*	% RSD*
SORAFENIB	400	398.1860911	99.54 ± 0.01727	0.017351

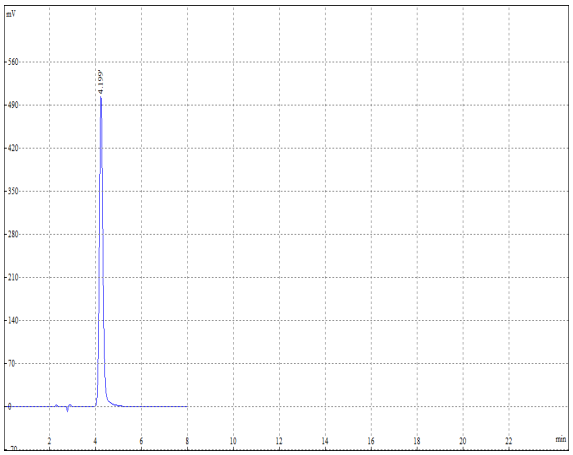

Fig.4: Chromatogram of marketed formulation of Sorafenib Tosylate tR= 8.02 min

Validation of RP-HPLC Method

Linearity and Range

To achieve linearity, stock solution containing Sorafenib Tosylate (1000 ppm) was prepared. Sorafenib Tosylate stock solution was diluted to yield concentration in the range of 20-100 µg/ml for Sorafenib Tosylate. The correlation co-efficient for Sorafenib Tosylate was 0.998 respectively. Linearity was evaluated for a set of five standard working solutions containing 20-100 µg/ml Sorafenib Tosylate respectively. The linearity of calibration graphs and adherence of the system to Beer's law was validated by determining correlation coefficient. The results of linearity for the Sorafenib Tosylate are presented in Table 5.

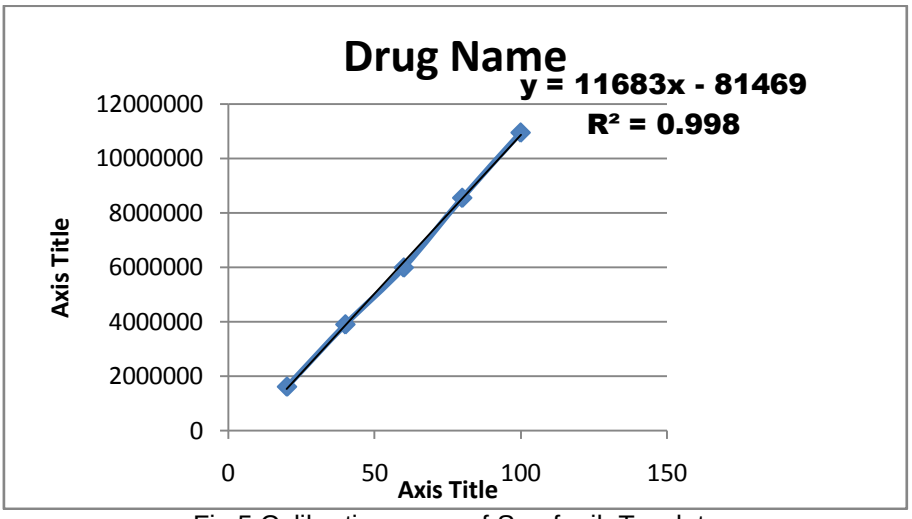

Fig.5 Calibration curve of Sorafenib Tosylate

Table 5: Linear regression data for calibration curves of Sorafenib Tosylate

Parameters	Results
Linearity range µg/ml	20-100
r^2	0.998
Slope	11683
Intercept	81469

Limit of Detection and Limit of Quantitation:-

The LOD values were found to be 0.48 µg/mol for Sorafenib Tosylate respectively. The LOQ values were found to be 0.4466 µg/ml. These results suggest that method is sensitive.

Precision Method

Precision (Repeatability):-

In repeatability, six different standard solutions were prepared each having concentration of 40µg/ml of Sorafenib Tosylate. The sequence of injections is presented in Table 4. The response of each of these solutions was measured and percentage relative standard deviation (% RSD) was calculated. It was in acceptable limit, indicates the reproducibility of the method.

Intermediate Precision:-

Intraday and Interlay precision was evaluated by assaying six samples, prepared as described in the sample preparation. Inter-day and Intraday precision were carried out in following concentration range 60, 80, 100 µg/ml of Sorafenib Tosylate respectively. Different time intervals in the same day and at the same time on different day's studies were carried out. The variation in the results was analysed and statistically validated. The % relative standard deviation (%RSD) and mean

area values for inter-day and intra-day precision for Sorafenib Tosylate shown in Table 5. The results obtained shows the method reproducibility which were within the given limit.

Acceptance Criteria:

The % RSD for the area of 6 sample injections results should not be more than 2%.

Table 6: Results for method precision (Repeatability)

Drug	Concentration of drug (µg/ml)	Means *	% RSD*
Sorafenib	40	3865561±4292.210	0.1109619

Table 7: Result for Intraday and Interlay precision

Precision	Amount of Drug (µg/ml)	Mean Area ± SD		% RSD*	
		Intraday	Interlay	Intraday	Interday
Sorafenib	50	5906004 ± 11382.42	5604314± 7830.159	0.192727	0.139717
	100	8487778 ± 12834.63	8248639 ± 64551.13	0.151213	0.154736
	150	10773107± 76558.05	10667217 ± 61645.83	0.71064	0.5779

Recovery Studies

The accuracy of the analytical method was established in triplicate across its range. The results of recovery experiments are given in Table 27and 28. The results indicate that the recovery of Sorafenib Tosylate ranges from 99.14% to 99.97 % respectively. The value of % relative standard deviation Sorafenib Tosylate was found to be 0.6144 to 1.4710 respectively. The recovery of Sorafenib Tosylate by proposed method is satisfactory as % relative standard deviation is not more than ±2.0% and means recovery lies between 98.0 and 102.0%

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102.0% and the % relative standard deviation is not more than ± 2.0%

Table 8: Accuracy data of Sorafenib Tosylate

Sample No.	Spiked level	Peak Area	Amount spiked (mg)	Amount Recovered (mg)	% Recovery	Mean % Recovery	% R.S.D.*
1	50%	58044787	20	31.96	98.60	99.14	0.6142
2	50%	5812821	20	32.08	99.01		
3	50%	5828058	20	32.12	99.80		
1	100%	7739040	40	39.48	99.14	99.36	0.1966
2	100%	7751438	40	39.61	99.46		
3	100%	7752803	40	39.57	99.46		
1	150%	9625407	60	47.53	98.50	99.97	1.4710
2	150%	9710827	60	47.52	99.96		
3	150%	9796622	60	47.56	101.44		

Robustness:

Robustness of the method was carried out by deliberately made small changes in the flow rate, change in pH, and organic phase composition in mobile phase. One parameter was change at one time. The retention time and the % R.S.D for the peak area from the five replicate injections of Sorafenib Tosylate standard was found to be within the acceptable limits, illustrating the robustness of the method. Results of robustness study are presented in Table 9.

Acceptance criteria: System suitability should pass with changed conditions

Table 9: Robustness studies of Sorafenib Tosylate

Change in flow rate(1ml/min)	**Flow rate(ml/min)**	**Average***	**Std Dev**	**%RSD**
Sorafenib Tosylate	0.8 ml/min	5950845	6345.546	0.106633
	1.2 ml /min	5881414	6289.17	0.106933
Change in mobile phase composition Methanol: water (80:20)v/v	**Mobile phase(80:20)v/v**	**Average***	**Std Dev**	**%RSD**
Sorafenib Tosylate	(78:22)v/v	5884885	1014.50	0.017239
	(82:18)v/v	5984654	636.5477	0.010636
Change in pH(2.8) ±0.2	**Change in pH**	**Average***	**Std Dev**	**%RSD**
Sorafenib Tosylate	3	5852189	735.1796	0.012562
	2.6	5942020	613.1071	0.225957

Table 10: Summary of the Validation Parameters for RP-HPLC method

Parameter (Unit)		**Results**
Linearity range (µg/ ml)		20-100
Correlation Coefficient		0.998
Slope		11683
Intercept		81469
% Recovery		99.14% to 99.97%
Precision %RSD	Intraday	92727 to 0.71064
	Intraday	0.139717 to 0.57791
Robustness		Robust
LOD		0.480 µg / ml
LOQ		0.4466 µg/ ml

Stress degradation studies:

Stress studies were carried out on the drug sample according to ICH guideline Q1A (R2). The Sorafenib Tosylate was subjected to different stress conditions like hydrolysis, oxidation, thermal stress, photo and UV light. The stress conditions were optimized in such way that the drug will degrade at least 20-30%.

Acid Degradation

10 mg of pure drug was transferred to round bottom flask. To this, 10 ml of 0.1M HCL was added and this reaction mixture kept aside for 12 hrs at room temperature ,from that 0.12 ml solution is diluted to 10 ml with diluents to obtain concentration of 120 µg/ml. This solution was mixed well and was filtered through 0.45µm filter (Nylon filter) and injected onto column under optimized RP-HPLC conditions

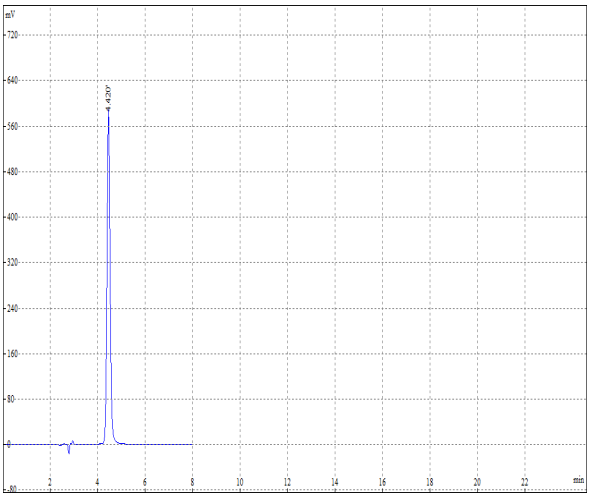

Figure 5: Chromatogram of acid degradation (0.1M HCl, 12hrs at 80°C)

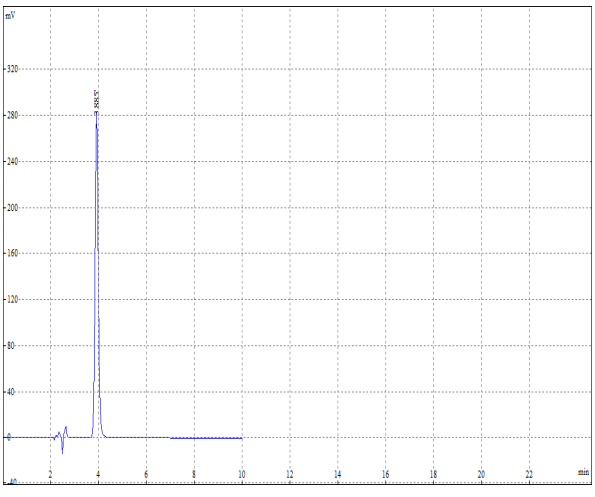

Figure 7: Chromatogram of base degradation (0.1M NaOH, 12hrs at 80°C)

Base induced degradation:
Sorafenib Tosylate was treated with 0.1M NAOH solution at 80^0C for 12hrs showed no degradation

Hydrogen Peroxide Degradation:
Sorafenib Tosylate was treated with hydrogen peroxide (3%) solution at room temperature for 15 min.

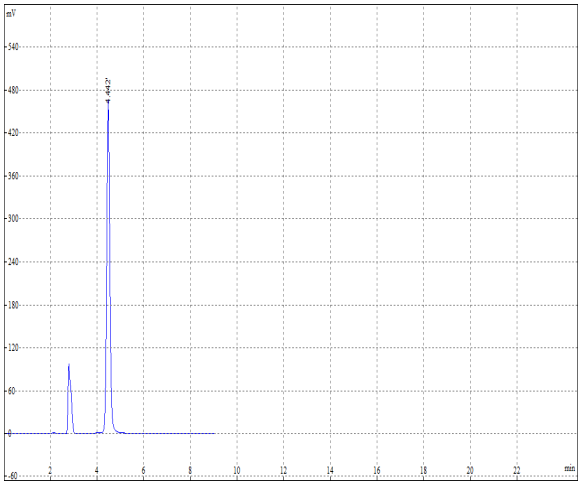

Figure 8: Chromatogram of peroxide (3% H2O2, 15 min)

Thermal degradation:
Accurately weighed 50mg of Sorafenib tosylate evenly spread in separate Petridis and kept in oven at 1050C for about 24 hours showed no degradation.

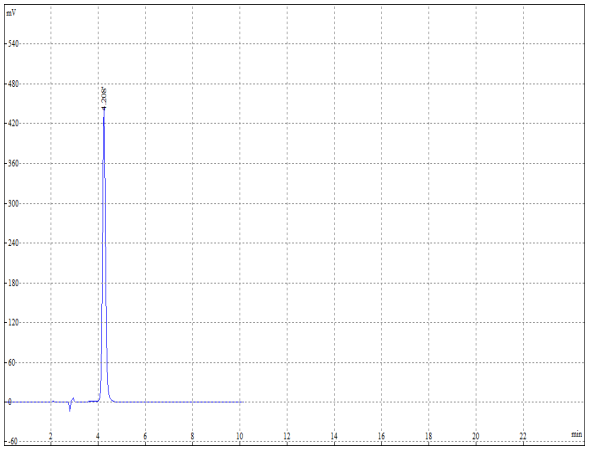

Figure 8: Chromatogram of Dry heat degradation (24hrs at 105°C)

Photolytic degradation:
The Sorafenib Tosylate powder and solutions of both were prepared and exposed to light to determine the irradiation of light on the stability of solution and powder form of drugs. Approximately 100mg of drug powder and 1mg/ml solution were spread on a glass dish in a layer that was less than 2mm thickness and were placed in a light cabinet and exposed to UV light for 12hrs. After 12hrs the samples are removed and diluted with diluents to get a concentration of 200µg/ml solution and then injected.

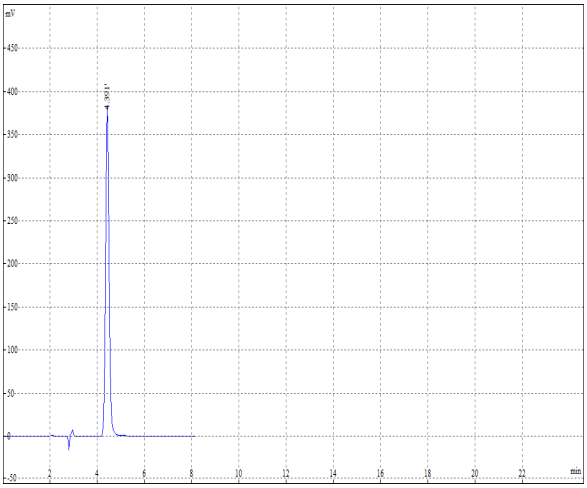

Figure 9: Chromatogram of photolytic degradation (HPLC grade water, 12hrs at 80°C)

Table 11: Results of forced degradation studies:

Stress Condition	Time(hrs)	Retention Time
As such	12hrs	4.515
Acid Hydrolysis (0.1 N, at RT)	12hrs	4.420
Base Hydrolysis (0.1N at RT)	12hrs	3.885
Oxidation (5% H_2O_2 at RT)	12hrs	4.442
Photolysis(UV Light and sunlight)	12hrs	4.391
Thermal (at 80^0c)	12hrs	4.208

Conclusion:-
The present work represents the report that deals with simultaneous analysis of Sorafenib Tosylate in bulk and pharmaceutical dosage forms using RP-HPLC. It can be concluded from the results that the proposed method is simple, accurate, robust and precise. Forced degradation is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions and thus generates

degradation products that can be studied to determine the stability of the molecule; this method was validated as per ICH guidelines. Thus, it can be used for routine quality control studies for assay of Sorafenib Tosylate.

Acknowledgement:-

The Authors are thankful to Principal, S.N.D. College of Pharmacy Babhulgaon, Yeola for providing platform and facility for to conduct search work. Also thankful Mr. Jadhav sir , Assistant Professor, S.N.D. College of Pharmacy Babhulgaon, Yeola for providing valuable assistance for the analysis and handling of instruments. The authors also thank the anonymous reviewers whose valuable comments and suggestions greatly helped in improving the manuscript.

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