Development of novel analytical bioanalytical methods of Sorafenib tosylate and its degradation and stability studies by RP-UFLC method.

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Abstract

Sorafenib tosylate (ST) is a kinase inhibitor drug approved for the treatment of primary kidney cancer, advanced liver cancer and in radioactive iodine resistant thyroid carcinoma. The efficacy and toxicity of the drug is dose dependent manner. A rapid and sensitive method for analytical and bioanalytical determination of sorafenib tosylate was developed and validated according to ICH guidelines. The drug was also studied for degradation and stability properties under different conditions. The analysis was carried out by using UFLC AD 20 with UV detection. The chromatographic peak was achieved at 2.6 min on a reversed-phase column 18 at 40 ºC by applying a gradient elution method with a methanol and acetonitrile in the ratio of 90:10 were used at 1.00 ml/min and was detected at 265 nm. Calibration curve is linear in the concentration range of 5-25 µg/ml (r2=0.9929). The %RSD of intraday and interday was within 2% and accuracy percentage ranged from 98.24 to 99.91. The results of newly developed UFLC method are more sensitive precise and accurate than previous reported methods using UV detection. This new analytical method is applied successfully for bioanalytical method development. The method is efficiently improved bioavailability and evidenced by validation parameters. In degradation study, percentage of degradation observed was less in all the conditions. In stability study, the drug was found to more stable. The developed method can be used for routine analysis of sorafenib tosylate in pharmaceutical formulation and in human plasma.

Keywords: Sorafenib tosylate, RP-UFLC, Bioanalytical study, Degradation study, Stability study.

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Introduction

Sorafenib tosylate (ST), is a drug approved for the treatment of kidney cancer through kinase inhibitor, liver cancer and radioactive iodine resistant advanced thyroid carcinoma [1].

ST is chemically known as 4-(4-{3-[4-chloro-3-(trifluoro methyl) phenyl] ureido} phenoxy)-N-2-methyl pyridine-2-carboxamide-4-methyl benzenesulfonate.

An acceptable tolerance and other dose related toxicities like fatigue and diarrhoea were exhibited in phase 1 clinical trials. In phase II clinical trials clinical activity of the drug in patients with hepatocellular carcinoma was demonstrated and in phase III better tolerance with minimal and manageable side effects was achieved [2]. ST was found to hinder cell proliferation, reduce cancer growth in clinical and preclinical trials [3]. The efficacy of ST is dose dependent and is the tolerability of sorafenib i.e., the anticancer activity and the inhibition of cell growth is dose-dependent. To reduce the adverse reactions in the phase III the dose of the drug was reduced in 26% of the subjects. Hence, the toxicity and efficacy of the drug is dose dependent and requires regular therapeutic monitoring which requires for determination of ST in biological matrix. Therefore, a simple and rapid method for analytical and bioanalytical determination of ST was developed and validated as per ICH guidelines [4].

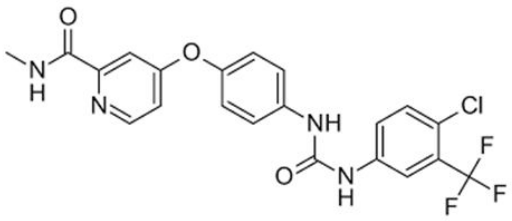

Figure 1: *Chemical structure of Sorafenib tosylate.*

Experimental

Analytical method development materials and reagents

Sorafenib tosylate pure drug (Table 1) was procured from Shilpa Medicare Limited (Bengaluru). Marketed formulation (Soranib 200 mg) manufactured by Cipla was procured. Pure water (HPLC grade, >18 MΩ・cm) was obtained by a Milli-Q water apparatus (Millipore (Milford, MA, USA). HPLC grade acetonitrile, HPLC grade methanol was procured from Merck laboratory and other chemicals used were of analytical grade.

Table 1: *Properties of Sorafenib tosylate.*

Molecular formula	$C_28H_24ClF_3N_4O_6S$
Molecular Weight	637 g/mol
Melting Point	205.6 °C
Color/Form	White solid
Solubility	Practically insoluble in aqueous media, slightly soluble in methanol and soluble in PEG

Preparation of stock solution, calibration standards and sample solutions

About 10 mg of ST was weighed and transferred to a 10 ml volumetric flask containing 5 ml of methanol. The solution was sonicated and the volume was made up to 10 ml with methanol to obtain 1000 µg/ml. The series of dilutions were made to get concentration range of 5-25 µg/ml from the stock solution in a mixture of acetonitrile and methanol (90:10) and the solutions were filtered and used for analysis.

The sample solution was prepared by taking 26.5 mg of marketed formulation (Soranib tablet) containing 10 mg of the drug and was transferred to a 10 ml volumetric flask it was dissolved and sonicated, the volume was made up to 10 ml with methanol obtaining 1000 µg/ml. From the stock sample solution dilutions were made to obtain 15 µg/ml.

Sample preparation and extraction for bioanalytical study

For quantification of ST in human plasma, liquid-liquid extraction method was performed using optimized conditions as described below. Each aliquot of plasma (100 µl) in an Eppendorf tube spiked with ST solutions (calibration standards and quality control samples) and the mixture was vertexed for 1 min. After vortexing the volume was made upto 0.4 ml with methanol and was centrifuged at 10000 rpm for 5 mins to precipitate sample proteins and the supernatant was collected in separate Eppendorf tube in which 1.5 ml of methanol was added and vertexed for 30 s and again centrifuged at 10000 rpm for 5 mins to obtain organic layer. The lower organic layer was collected and evaporated to dryness at 45 ºC and reconstituted with 100 µl of mobile phase and subjected for centrifugation for 3 more minutes at 10000 rpm and 10 µl of the final sample were injected in the chromatographic system.

Instruments and chromatographic conditions

Analysis was carried out by using UFLC Shimadzu LC-20AD PROMINENCE equipped with UV detector. The separation was done using Phenomenex luna C18 column. A gradient elution was applied using a mobile phase containing acetonitrile and methanol (90: 10 v/v) and the flow rate was set to 1.00 ml/min. The mobile phase was degassed ultrasonically for 20 min before use. The injection volume was 10 µl and run time was 10 minutes. The analysis was carried out at 265 nm wavelength.

Validation

In the present validation parameters as system suitability, selectivity, linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ), robustness, absolute recovery and degradation and stability study were considered for ST in sample and in biological matrix. For validation, the guideline for analytical method, ICH guidelines and for bioanalytical method, Food and Drug Administration (FDA) guidance for industry on bioanalytical method validation [6] criteria were followed.

Linearity

The linearity was assessed by injecting a series of standards ranging from 5-25 µg/ml. The peak area of different concentrations was plotted against concentrations of the standard solutions.

System suitability

The standard solution was injected for 5 times into the chromatographic system to test the system suitability parameters such as theoretical plates and tailing factor and was found that the parameters are within acceptance criteria.

Precision

The reproducibility of the method was analysed by multiple sampling of the standard. Reproducibility was demonstrated by carrying out interday and intraday precision and average % Coefficient was calculated.

Accuracy

Accuracy of a method is the proximity of the obtained value to the true value of the sample. The accuracy of the method was checked by spiking 50, 100 and 150% of the sample to the standard and injected to the chromatographic system and the % recovery of the drug is calculated.

LOD and LOQ

The limit of detection and limit of quantification of the method is calculated using the formula LOD=3.3*N÷S where N is the standard deviation and S is the slope and LOQ=10*N÷S can be used to calculate LOQ where N is the standard deviation and S is the slope.

Robustness

Robustness of a method is drug ability to remain unchanged by minor changes and also the efficiency of the method. The robustness of the current method was tested by changing the wavelength and the mobile phase ratio.

Degradation Studies

The drug was subjected to various conditions to study its stability under accelerated conditions. Degradation studies were carried by subjecting the drug to various stress conditions like acidic, basic, oxidative and photolytic.

Acid hydrolysis

Acid hydrolysis was carried out by subjecting the drug to 0.1N hydrochloric acid for 1 hour and the solution was neutralized by 0.1N sodium hydroxide and filtered and injected into the chromatographic system.

Base hydrolysis

Base hydrolysis was carried out by subjecting the drug to 0.1N sodium hydroxide for 1 hour and the solution was neutralized by 0.1N hydrochloric acid, filtered and injected into the chromatographic system.

Oxidative degradation

Oxidative degradation was studied by exposing the drug to 3% hydrogen peroxide and the solution was filtered and injected into the chromatographic system.

Photolysis

Photolytic degradation by keeping the drug under UV light and the solution was filtered and injected into the chromatographic system.

Bioanalytical method

Calibration Curve

The calibration curve was plotted by injecting the sample consisting of the matrix sample and the samples contain 5-25 µg/ml of analyte in it and a graph with concentration versus peak area was plotted.

Sensitivity

The sensitivity of the method was identified by injecting of 6 aliquots of LLOQ and the percentage deviation from nominal concentration and %CV was determined.

Selectivity

The selectivity of the method is its ability to separate the target analyte among the other constituents in the matrix. Sensitivity of the developed method was determined by analyzing plasma from two different batches. The blank plasma response and the LLOQ concentration was compared and assessed.

Accuracy and Precision

To determine the precision of the method five replicates of each quality control standard was taken and the precision was determined.

Recovery

The samples are spiked and different quality control levels are analyzed by injecting them into the chromatographic system and the percentage recovery was quantified by comparing the peak areas with different concentration levels LLOQ, LOQ, MQC, HQC and UQC.

Stability studies

The stability study was carried out for LQC and HQC quality control levels for its bench top stability, freeze thaw stability and long term stability.

Bench top stability

LQC and HQC quality controls were prepared and kept in room temperature at bench top for duration of 4 hours and were injected into the chromatographic system and the responses were compared with the freshly prepared samples and mean percentage change was determined.

Freeze thaw stability

The samples are analyzed after exposing them to three freeze thaw cycles. The analyzed samples are compared with the responses from the standard calibration curve and the mean percentage change was determined.

Long term stability

The long term stability of the sample is analyzed by keeping the samples in freeze state for a period of about 10 days and the response is compared with the standard calibration curve and the mean percentage change was determined.

Results

Analytical method development

The developed HPLC method was found to a simple, precise and accurate for ST in bulk, tablet formulation and in human plasma samples for the quantification. The determination of the appropriate chromatographic conditions was instigated by various mobile phases. First, several mobile phases composed of methanol and formic acid buffer phase at the proportions of 50:50, methanol and ammonium formate buffer were at the proportions of 50:50 and methanol and sodium phosphate buffer at 50:50 (v/v) were tested, but it was observed that 100 µg/ml of ST was injected and the obtained peak has tailing and therefore further trials were performed. Finally, ACN and MeOH was used for carrying out the analysis and to increase the peak resolution the mobile phase ratio, injection volume and the flow rate were adjusted. The final optimized method was carried out using ACN and MeOH in the ratio of 90:10

and at the flow rate 1 ml/min and the injection volume was 10 µl. Detection of drug was carried out at 265 nm using UV detector. In this mobile phase, good resolution peak at the retention time 2.6 min was observed.

Method validation

The validation was carried out for various considerations such as linearity, system suitability, precision, accuracy robustness (ICH guidelines). The final optimized method was carried out using ACN and MeOH in the ratio of 90:10. The standard solution (10 µl) was injected into the chromatographic system and the detection of drug was carried out at 265 nm using UV detector. The elution time was found at 2.6 minutes and the blank was given in figure 2, sample was given in figure 3 and standard chromatogram was given in figure 4. The developed method was validated for various validation parameters like linearity, system suitability, precision, accuracy robustness according to ICH guidelines [5].

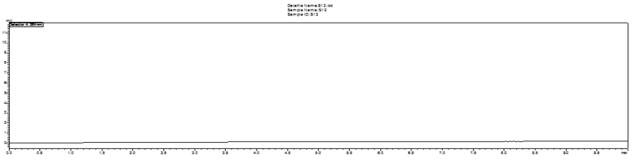

Figure 2: *Blank chromatogram.*

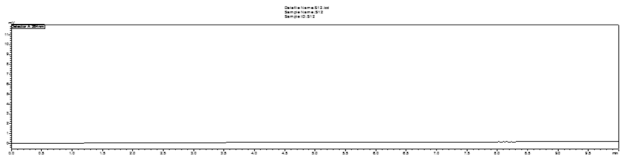

Figure 3: *Sample chromatogram Sorafenib tosylate (25 µg/ml).*

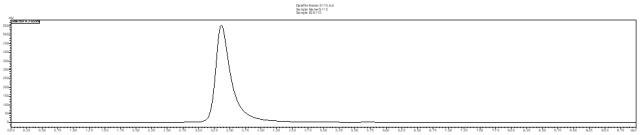

Figure 4: *Standard chromatogram of Sorafenib tosylate.*

The linearity was assessed for a concentration range of 5-25 µg/ml. The method was found to be linear and the r2 value was found to be 0.993. The standard calibration curve of peak area and concentration is given in figure 5 and table 2. The system suitability was determined by injecting the standard solution for 5 times into the chromatographic system. The tailing factor of the peaks obtained was found to be 1.837 and the theoretical plates were 2118 and found that the parameters are within acceptance criteria. The results are given in Table 2. The reproducibility of precision was carried out and the % RSD of interday precision was found to be 1.075 and intraday precision was 0.414. The results were found to be within the limits and are given in Table 3. In the accuracy study of the method was measured by spiking 50, 100 and 150% of the sample to the standard and injected to the chromatographic system and the % recovery of the drug was found to be 98.24, 99.64 and 99.91 respectively and is given in Table 4. The limit of detection (LOD) was found to be 0.32 µg/ml and limit of quantification (LOQ) of the method was found to be 1.27 µg/ml. The LOD and LOQ of the method is given in the Table 2. The robustness of the current method was tested by changing the wavelength and the mobile phase ratio and the %RSD were found to be 0.05 and 0.019 respectively. The results are given in Table 5.

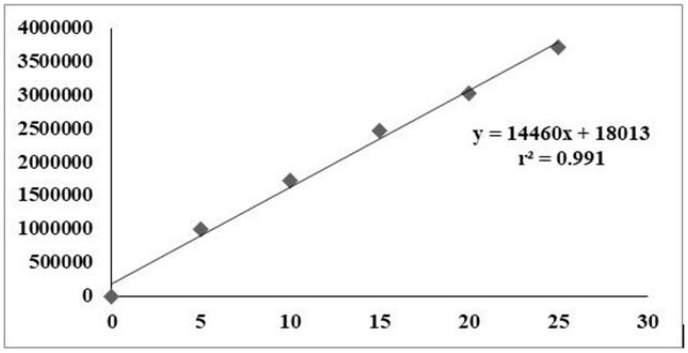

Figure 5: *Representative calibration curve of Sorafenib tosylate.*

Table 2: *Analytical linearity data of calibration curve of Sorafenib tosylate, system suitability and LOD, LOQ of the method.*

Concentration (µg/ml)	Peak Area
5	1007471
10	1726926
15	2464742
20	3022336
25	3704697
r2 (from the graph)	0.9929
Tailing factor (NMT 2.0)	1.837
Theoretical plates (NLT 2000.0)	2118
LOD	0.319 µg/ml
LOQ	1.274 µg/ml

Table 3: *Representative data of precision.*

S.No	Peak Area	
	Interday	**Intraday**
1	3773618	2474642
2	3667597	2491744
3	3743819	2499206
4	3738593	2478812
5	3753275	2479381
%RSD	1.075	0.414

Table 4: *Representative data of accuracy.*

Level of recovery	Amount of formulation	Amount of pure drug	Total amount of drug	Peak area	Difference	Percentage recovery	Mean
50	20	10	30	4625062	1602726	98.76	
	20	10	30	4619419	1597083	96.83	98.24
	20	10	30	4671352	1649016	99.14	
100	20	20	40	5772816	2750480	99.58	
	20	20	40	5855293	2832957	100.67	99.64
	20	20	40	5713641	2691305	98.69	
150	20	30	50	6432584	3410248	101.32	
	20	30	50	6365741	3343405	98.96	99.91
	20	30	50	6384368	3362032	99.47	

Table 5: *Representative data of robustness.*

Mobilephase	Concn (µg/ml)	Peak Area	Mobilephase	Concn (µg/ml)	Peak Area
	20	3035271		20	2983417
	20	3043746		20	3017591
ACN: MeOH (90:10 v/v)	20	3104138		20	3009439
	AVG	3061052	ACN: MeOH (80:20 v/v)	AVG	3003482
	STDV	30662.47		STDV	17848.72
	%RSD	0.17		%RSD	0.05
Flow rate	Concn (µg/ml)	Peak area	Flow rate	Concn (µg/ml)	Peak area
1.0 ml/min	20	3027391	0.7 ml/min	20	3075962
	20	3106328		20	3163752
	20	3172162		20	3048133
	AVG	3101960		AVG	3095949
	STDV	72484.26		STDV	60345.24
	%RSD	0.02		%RSD	0.019

Degradation studies

The drug was subjected for degradation studies under various conditions like acidic, basic, oxidative and photolytic conditions to study its stability under accelerated conditions and the % degradation was calculated. The drug was observed to degrade by 12% in acidic condition, 8% in basic condition, 17% by oxidative degradation and was found to be stable under UV light (Figures 6-8, Table 6).

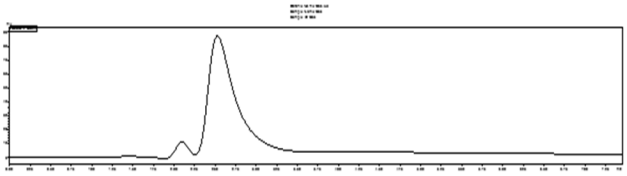

Figure 6: *Chromatogram showing acid degradation.*

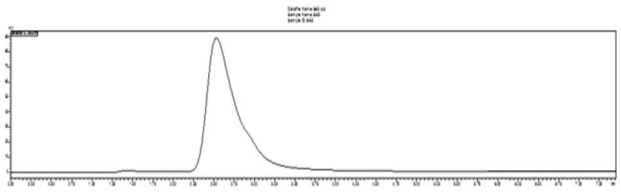

Figure 7: *Chromatogram showing basic degradation.*

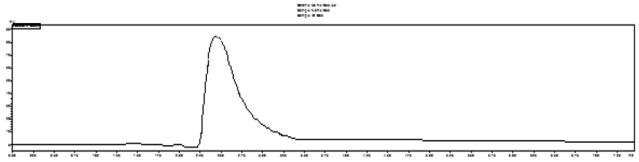

Figure 8: *Chromatogram showing oxidative degradation.*

Table 6: *Representative data degradation studies of Sorafenib tosylate in various conditions.*

Degradation Condition	Peak Area	Percentage Degradation
Acid hydrolysis	2986056	12%
Basic hydrolysis	3019917	8%
Oxidative degradation	2970960	17%
Photolysis	3106574	-

Bioanalytical method development and validation

The drug was spiked into the human plasma and was injected into the chromatographic system and validated for the parameters like precision, selectivity, sensitivity and recovery. The blank and sample peaks were shown in figures 9 and 10. The calibration curve was plotted for a concentration range of 5-25 µg/ml.

The method was linear with an r2 value of 0.9942. The results for the calibration curve are given in the Table 7 and figure 11. The sensitivity of the method was determined by%CV to assess the sensitivity of the developed method and was sensitive.

The selectivity of the method was assessed and was found that no interference from blank plasma was observed in the retention time of the drug and hence the method is selective. Accuracy and precision were determined by five replicates of each quality control standard.

The mean nominal concentration and %CV were within limits and are given in the Table 8.

The % recovery of different concentration levels LLOQ, LOQ, MQC, HQC and UQC was found to be 78.16%, 79.61%, 77.60%, 75.54% and 79.30% respectively. The percentage recovery data of each quality control was given in the Table 9.

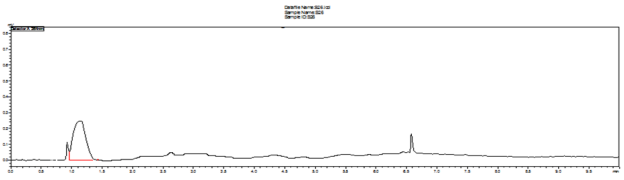

Figure 9: *Blank chromatogram in plasma.*

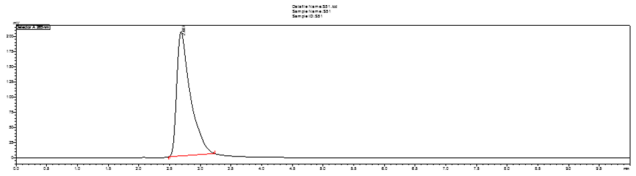

Figure 10: *Sample chromatogram in plasma.*

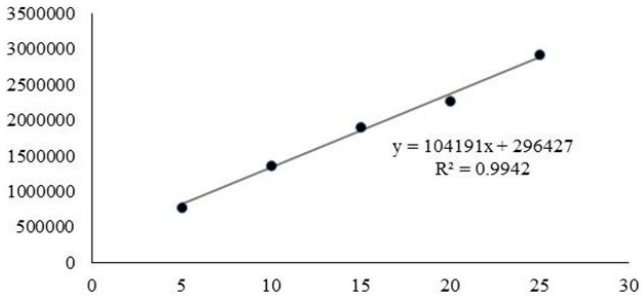

Figure 11: *Calibration curve for bioanalytical standard.*

Table 9: *Representative data of recovery study.*

Concn	Analytical peak area	Bioanalytical peak Area	Recovery percentage
5	1007471	787531	78.16%
10	1726926	1374912	79.61%
15	2464742	1912647	77.60%
20	3022336	2283179	75.54%
25	3704697	2938164	79.30%

Stability studies

The stability of the sample was assessed in different conditions and the %CV and mean recovered concentration was carried out and was found that the drug was stable in the assessed conditions. The stability data is given in Table 10.

Table 10: *Representative data of stability study of drug in human plasma under various conditions.*

Stability	Standards	Concn	Mean recovered concn	SD	%CV
Bench top	LQC	10	9.462	0.026	0.273
	HQC	20	19.928	0.378	1.896
Freeze thaw	LQC	10	9.248	0.182	1.969
	HQC	20	18.659	0.248	1.329
Long term	LQC	10	8.984	0.051	0.572
	HQC	20	19.365	0.096	0.49

Discussion

The systematic method was used to develop UFLC method for ST and was found to be very effective and accurate, precise for routine analysis. The chromatographic conditions were determined by using various mobile phases. Several mobile phases were tried and composed of methanol and formic acid buffer, methanol and ammonium formate buffer, methanol and sodium phosphate buffer at 50: 50 v/v and found tailing peaks in all tested mobile phases. Finally, ACN and MeOH was used as mobile phase in the ratio of 90:10 and observed better peak and used for method development and validation of ST. In validation, the developed method proved higher resolution of peak, accurate, precise, sensitive and selective for the determination of drug. Moreover, in degradation study, lower percentage of drug degradation was observed but not found any identified product. The bioanalytical method was developed in human plasma and validated. The developed method was linear, precise, sensitive and selective. The calculated %CV were within limits and the recovery of different concentration levels such as LLOQ, LOQ, MQC, HQC and UQC were in good percentage [6]. The stability of the analyte was evaluated in different conditions and the %CV and mean recovered concentration was determined. The analyte was stable in the tested conditions.

Conclusion

The developed analytical and bioanalytical method for the estimation of Sorafenib tosylate in bulk, tablet formulation and human plasma was found a simple, accurate and precise and validated according to the ICH guidelines. The developed analytical method was found to be rapid, sensitive and is useful for routine analysis of sorafenib tosylate in formulation as well as in human plasma.

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