INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

HPTLC DETERMINATION OF SORAFENIB TOSYLATE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM

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Article Received on: 18/11/11 Revised on: 09/01/12 Approved for publication: 31/01/12

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ABSTRACT
A simple and sensitive HPTLC method has been developed for the quantitative estimation of Sorafenib Tosylate in bulk and Pharmaceutical dosage form. Sorafenib Tosylate was chromatographed on silica Gel $^{60}F_{254}$ TLC plate using n-Hexane : isopropanol (8:2 v/v) as mobile phase. Sorafenib Tosylate showed Rf value 0.34±0.05 and scanned at 254 nm using Camag TLC Scanner 3. The method was validated in terms of linearity (100–500 ng/spot), precision (intra-day % RSD variation 0.07 to 0.39%, inter-day % RSD variation 0.5 to 1.2%), accuracy (98.88 to 100.815%) specificity, ruggedness, sensitivity and reproducibility. The limit of detection and limit of quantitation for Sorafenib Tosylate were found to be 1.57 ng/spot and 4.75 ng/spot, respectively. The developed method was successfully used for the assay of Sorafenib Tosylate in bulk and Pharmaceutical dosage form.. it can be used for the routine quality control testing of marketed formulations.
Keywords: Sorafenib, Methanol, HPTLC, Pharmaceutical dosage form, ICH.

INTRODUCTION

Sorafenib is an orally administered tyrosine kinase inhibitor that exhibits antiangiogenic and antitumor activity. Sorafenib is an inhibitor of C-RAF, B-RAF, c-KIT, FLT-3, platelet-derived growth factor receptor (PDGFR), and vascular endothelial growth factor receptor (VEGFR) 1, 2, and 3, and is approved for the treatment of advanced renal cell carcinoma and hepatocellular carcinoma. Sorafenib is currently being investigated for the treatment of other solid tumor malignancies and acute myelogenous leukemia[6]. Sorafenib Tosylate is Chemically {4-[[4-chloro-3-(trifluromethyl)phenyl]carbamoylaminophenoxy]*N*-methyl-pyridine-2-carboxamide}. Sorafenib has demonstrated preclinical and clinical activity against several tumor types, either in monotherapy or in combination with other anti-cancer agents. Sorafenib at 400 mg twice daily (bid) was recently approved for the treatment of patients with advanced renal cell carcinoma or unserectable hepato-carcinoma[6].

The literature survey reveals the chromatographic methods are reported for estimation of sorafenib and its metabolites in human plasma, human serum, and urine. It revealed only one HPLC method for its determination in pharmaceuticals and few in human serum, human plasma[1-4]. In recent times, there is an increase tendency towards the development of stability-indicating assay, using the approach of stress testing as mentioned in the ICH guidelines[7].

So, there is a need for development of a simple, rapid, economic and sensitive assay of sorafenib. The proposed method is cheaper and simpler than other spectroscopic and chromatographic methods.

Sorafenib is not official in any pharmacopoeia. It also recommends carrying out of stress testing on the drug substance to establish its inherent stability characteristics and to support the suitability of the proposed analytical procedure. The stress testing encompasses the influence of temperature, humidity, light, oxidizing agent as well as susceptibility over a wide range of pH values[6].

The aims of this study were firstly optimize the chromatographic conditions for the measurement of sorafenib in pure and pharmaceutical dosage form and to validate the method.

MATERIALS AND METHODS

Materials

All chemicals and reagents used were of analytical grade and purchased from Qualigens Fine Chemicals, Mumbai, India.

HPTLC instrumentation

A Camag HPTLC system comprising of Camag Linnomate V automatic sample applicator, Hamilton syringe (100 µl), Camag TLC Scanner 3, Camag WinCATS software, Camag Twin-trough chamber (10x10 cm) and ultrasonicator were used during study.

Preparation of standard and sample solutions:

Sorafenib Tosylate (10 mg) was weighed accurately and transferred in 10 ml volumetric flask. It was dissolved in and diluted up to mark with methanol. The final solution contained 1000 ng of Sorafenib Tosylate per µl of the solution (S1). The solution (1 ml) was diluted further to 10 ml with the same solvent. The final solution contained 100 ng of Sorafenib Tosylate per µl of the solution (S2).

Twenty tablets were weighed accurately and ground to fine powder. Weight equivalent to 10 mg of Sorafenib Tosylate was transferred to conical flask and mixed with methanol. The solution was sonicated for 15 min. The extracts were filtered through Whatmann filter paper No. 41 and residue was washed with methanol. The extracts and washing were pooled and transferred to a 100 ml volumetric flask and volume was made up to 100 ml with methanol. Required dilutions were made to get 100 ng/µl of Sorafenib Tosylate.

HPTLC method and chromatographic conditions

The samples were spotted in the form of bands of width 6mm with a Camag microliter syringe on precoated silica gel aluminium Plate $^{60}F_{254}$ (20 cm×10 cm with 0.2 mm thickness, E. Merck, Germany) using a Camag Linomat IV (Switzerland). Space between two bands was 20 mm. The slit dimension was kept 6mm×0.30mm micro, 5 mm/s scanning speed was employed. The mobile phase consisted of *n*-Hexane: isopropanol (8:2, v/v). Linear ascending development was carried out in twin trough glass chamber

saturated with mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature. The length of chromatogram run was approximately 65 mm. Subsequent to the development; TLC plate was dried with the help of IR light for 5 min. Densitometric scanning was performed on Camag TLC scanner III using Win CATS software in the absorbance mode at 254 nm. The source of radiation utilized was deuterium lamp.

Stability

Sorafenib Tosylate is a stable substance and no sign of degradation is observed after 24 months of storage under ICH long term or accelerated conditions (12 months). The active substance was found to be resistant to heat, oxidation and hydrolysis. ICH light stability studies have been performed on sorafenib tosylate in the solid state and it was concluded to be stable. When dissolved in methanol it was shown to be slightly sensitive to light. Stability data was provided on three pilot scale batches of sorafenib tosylate micronized packaged in polyamide/polyethylene (PA/PE) bags[5].

RESULTS AND DISCUSSION

Chromatographic conditions

A typical HPTLC chromatogram for Sorafenib Tosylate is shown in fig no.1 and R_F value was 0.34.

Validation Procedures

The method validation was carried out according to the recommendations for analytical method validation.

Linearity

Aliquots of 1, 2, 3, 4 and 5 µl of standard solution of Sorafenib Tosylate was applied on the TLC plate (100 ng/ml of drug). TLC plate was dried, developed and analyzed photometrically as described earlier. The standard calibration curve was generated and The plot of peak area against concentration of Sorafenib Tosylate was found to be linear in the range of 100 to 500 ng/µl with correlation coefficient of 0.9965. The calibration data of Sorafenib Tosylate is given in Table 1 and The calibration curve of linearity of sorafenib is shown in fig 2.

Limit of detection (LOD) and limit of quantitation (LOQ)

LOD and LOQ were determined from standard deviation and slope method as per ICH guideline, for Sorafenib Tosylate LOD was found to be 1.57 ng/µl and LOQ was found to be 4.75 ng/µl.

Accuracy

To study accuracy of the method, recovery experiment was carried out by spiked concentrations. A known quantity of drug substance corresponding to 50, 100 and 150% was spiked, to determine accuracy (recovery) against 100% working standard. The accuracy was expressed as the percentage of analytes recovered by the assay. The results indicate the method is highly accurate for determination of the Sorafenib Tosylate. Table 2 lists the recoveries of the drugs from a series of spiked concentrations.

Precision

Repeatability of sample application and measurement of peak area were carried out using three replicates of three (100, 200 and 300 ng per spot of Sorafenib Tosylate). The relative standard deviation for each spot for Intraday precision is 0.39, 0.28 and 0.08, respectively. For inter-day precision for each spot is 1.22, 0.57 and 0.54, respectively. Results obtained for precision study are shown in Table 3.

Ruggedness

The ruggedness test of analytical assay method is defined as degree of reproducibility of assay results obtained by the successful applications of the assay over time and among multiple laboratories and analyst. The result of ruggedness testing is reported in Table 4.

Reproducibility

The reproducibility of sample application was assessed by spotting drug solution (1µl) six times on HPTLC plate then development of plate and recording peak height and for the spots. The % RSD for R_F value and peak area values of Sorafenib Tosylate were found to be 0.86 and 0.34 respectively and are shown in Table 5.

CONCLUSION

The results indicate that the developed HPTLC method is simple, accurate, precise and specific, for estimation of Sorafenib Tosylate in bulk and its formulation. The developed method was validated based on ICH guidelines[7]. Statistical analysis proves that the method is repeatable and selective for the analysis of Sorafenib Tosylate in bulk drug and Pharmaceutical dosage form. The method can be used to determine the purity of the drug available from the various sources by detecting the related impurities.

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TABLE 1: CALIBRATION DATA OF SORAFENIB TOSYLATE BY HPTLC METHOD

Sr. No.	Concentration (ng/µl)	Peak Area
1	100	1038.6
2	200	1893.1
3	300	2690.5
4	400	3477.1
5	500	4211.3

TABLE 2: RECOVERY STUDIES FOR SORAFENIB TOSYLATE BY PROPOSED METHOD

Sr No.	Initial amount (ng/µl)	Amount added (%)	Amount recovered (ng/µl)	Recovery ± SD (%)	% RSD
1	300	50	457.29	100.81 ± 0.29	0.29
2	300	100	593.28	98.88 ± 0.11	0.11
3	300	150	744.52	99.27 ± 0.09	0.09

*Average of three determinations

TABLE 3: PRECISION RESULTS FOR SORAFENIB TOSYLATE BY HPTLC METHOD

Sr.No.	Concentration per spot	Intraday Precision Mean ± SD*	%RSD	Interday Precision Mean ± SD*	%RSD
1	100	1038.3 ± 4.05	0.39	1087.9 ± 13.36	1.22
2	200	1929.0 ± 5.46	0.28	2025.8 ± 11.46	0.56
3	300	2682.5 ± 2.07	0.07	2708.1 ± 14.68	0.54

*Avearge of three determinations

TABLE 4: RUGGEDNESS RESULTS OF SORAFENIB TOSYLATE BY HPTLC

Sample	Label claim	Analyst 1		Analyst 2	
		Amount found (mg)	% Recovery ± SD*	Amount found(mg)	% Recovery ± SD*
Soranib	200	202.84	101.42 ± 0.58	201.30	100.65 ± 1.08

*Average of six determinations

TABLE 5: REPRODUCIBILITY RESULTS OF SORAFENIB TOSYLATE BY HPTLC

Sr. No.	Area	R_F Value
1	1028.8	0.33
2	1039.8	0.34
3	1045.0	0.34
4	1053.5	0.33
5	1040.2	0.34
6	1051.5	0.34
Mean*	1043.1	0.3366
SD*	9.01	0.0051
%RSD	0.86	1.53

*indicates average of six readings

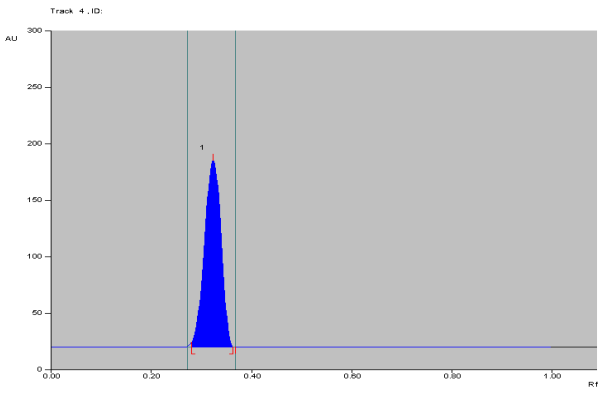


Figure 1: A typical HPTLC chromatogram of Sorafenib tosylate

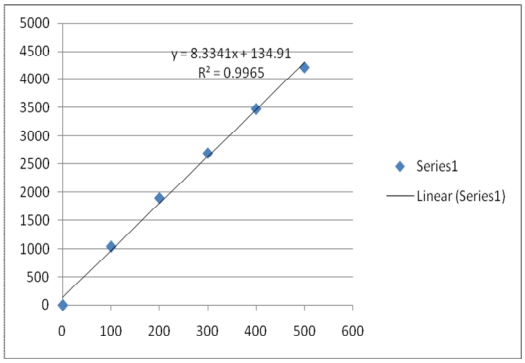


Figure 2: Calibration curve of Sorafenib tosylate by HPTLC method