RP-HPLC method for analytical method development and validation of multi-kinase inhibitor

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Abstract

This study was set out to create an RP-HPLC system that is effective, sensitive, picky, precise, accurate and practical. For this, a UV detection technique for detecting Sorafenib tosylate-loaded solid lipid nanoparticles has been developed and validated. To improve the procedure, many parameters were used [pH and Column]. The chromatographic separation was carried out using a Shimadzu prominence-i LC-2030C and a C8 short column [5 m 4.6 x 100 mm]. With a runtime of 10 minutes, 10 mL injection volume was maintained at 1 mL/min flow rate. The mobile phase used in the study was a mixture of 70:30 methanol: 0.1% formic acid in water. The effluent was detected at 261nm using a UV detector. Drug Entrapment Efficiency [DEE] and Drug Loading [DL] for ST from the extracted SLNs matrix were found to be 86.9% and 19%, respectively. The developed analytical method exhibited a linearity range of 1-64g/ml and an R2 value of 0.998. 0.88 g/ml detection limit [LOD] and 1.0 g/ml limit of quantification [LOQ], and 0.88 g/ml detection limit [LOQ]. Using ICH Q2 [R1] guidelines, the proposed technique was evaluated, and it was shown to be accurate, linear, robust, and specific. Using the devised analytical method, drug release, drug loading, and drug entrapment effectiveness were all studied.

Keywords: Sorafenib tosylate, RP-HPLC, Solid Lipid Nanoparticles [SLNs], UV detection, LOD, LOQ.

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Introduction

The US FDA has approved the anticancer medication sorafenib tosylate BAY-43-9006 for the treatment of untreatable hepatocellular carcinoma and advanced renal cell carcinoma. It is marketed as Nexavar®, by Bayer [1-2]. Its chemical name is 4-[4-[4-chloro-3-[trifluoromethyl] phenyl] ureidophenoxy]-N-2-methylpyridine-2-carboxamide4-methylbenzenesulfonate, also known as sorafenib tosylate. It is an effective oral multi-kinase inhibitor for the treatment of cancer. By inhibiting autophosphorylation of several cell surface tyrosine - kinases involved in regulating cell growth and differentiation, such as intracellular participants in the signal transduction cascade of the mitogen-activated protein kinase [MAPK], vascular endothelial growth

factor receptors [VEGFR-1,2, and 3] and platelet-derived growth factor receptors, SORA demonstrates anti-angiogenic and anti-tumour activity.[3] However, due to SFN's poor water solubility, substantial hepatic first-pass impact, and high efflux by the permeability-glycoprotein [P-gp], which ultimately results in its poor [8.43%] and irregular oral bioavailability, clinical application of SFN is restricted[4].

When compared to traditional colloidal carriers including emulsions, liposomes, and polymeric micro- and nanoparticles, solid lipid nanoparticles [SLN] developed in 1991, offer an alternative solution [5]. Solid lipid-based nanoparticles are receiving a lot of attention as a potential colloidal drug carrier for intravenous applications [6] They have been suggested as an alternate particle carrier system. SLN are physiological lipid-based sub-micron colloidal carriers with a size range of 50 to 1000 nm that are distributed in water or an aqueous surfactant solution. Due of their potential to enhance the efficacy of pharmaceuticals, SLN are appealing due to their distinctive qualities, which include the phase interaction at the interface, high drug loading, large surface area, and small size [7-8]. As an alternative to polymers and identical to an oil-in-water emulsion for parenteral nutrition, solid lipid nanoparticles are one of the unique possible colloidal carrier systems. The solid lipid has been used in place of the emulsion's liquid lipid. They have a number of benefits, including superior biocompatibility, minimal toxicity, and improved lipophilic drug delivery via solid lipid nanoparticles [9-10]
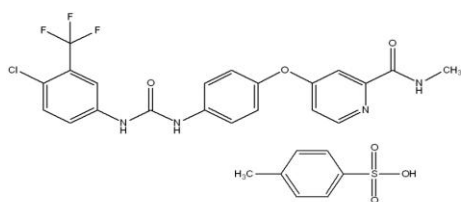
Figure 1. Chemical structure of Sorafenib tosylate

Materials and Methods

Instrument, Chemical and Reagents

Sorafenib tosylate standard was procured from Natco Pharmaceuticals limited. Sorafenib tosylate marketed formulation manufactured by Bayer pharmaceutical [Brand name-Nexavar®] was procured from local market. HPLC grade Methanol obtained from Merck Pharmaceutical Ltd and Formic acid from Amco International was used as the diluent for preparation of the solutions. Compritol ATO and Gelucire 44/14 reagent are of Analytical grade obtained from Gattefosse. The chromatographic separation was carried out in HPLC Prominence – I – Series 2030C + autosampler and the analyte was detected using UV detector at 261 nm.

Chromatographic conditions

The study was performed by carrying out separation using Phenomenex luna CN C8 short column [100mm X 4.60 mm 5μm] at a wavelength of 261 nm detected by UV spectrophotometer. Methanol: 0.1% Formic Acid in water [70:30] was utilized as the mobile phase by maintaining injection volume of 10μl at a flow rate of 1mL/min. The run time was set to 10 mins and retention time was found to be 6.8 mins.

Standard stock preparation

Primary stock solutions of Sorafenib tosylate were made in methanol at a concentration of [1000 g/mL each]. These solutions were kept in the refrigerator at 4°C [0.5°C] and covered with aluminium foil until they were analysed. Primary standard samples were diluted appropriately with mobile phase to make secondary stock solutions.

Preparation of Sorafenib tosylate [ST] loaded Solid Lipid Nanoparticles [SLNs]

Hot melt homogenization was used to make the ST-loaded SLNs using Compritol ATO and Gelucire 44/14 as a solid lipid mix with Tween 80 as a surfactant. Briefly, specified amount of lipid mix [5% w/v] was melted at 60 °C and ST was added to the molten lipid mix to form a clear lipid phase. The aqueous phase contains Tween 80 [1%] dissolved in H_2O and heated at 60 °C. Slowly, with continuous homogenization, the molten lipid phase received a heated aqueous phase. [Homogenizer, IKA T 25 ULTRA-TURRAX®] at 15000 rpm for 30 min. The hot emulsion was then subsequently cooled to obtain SLNs.

Optimization of Chromatographic Conditions

Reverse phase – HPLC technique was developed for the quantification of ST Loaded Solid lipid nanoparticle. The method was further validated following Q2 [R1] ICH guidelines. Melting point estimation and absorption maximum [λ max] were used to determine the structural integrity of the ST. The melting point was found to be 199–211 °C and ST showed λ max at 261 nm. The effect of process variables on chromatographic resolution, such as stationary phase, column, mobile phase composition, flow rate, and detector temperature, were investigated throughout the optimization study. In order to attain the optimal peak shape and elution time, the chromatographic conditions were adjusted.

In first trial, ACN and Acetate buffer [pH-4.5] in the ratio of 60:40 was used at a flow rate of 1mL/min. However, it was found that the peak structure was absolutely poor. Hence, the MP was altered to reduce the retention time.

In the next trial, Methanol: water with 0.1% formic acid in the ratio of 70:30 was taken and adjusted to a flow rate of 1 mL/min. This exhibited a retention time of 6.8 mins and tailing factor within acceptable limits. Hence, this optimized method was used in the estimation of Sorafenib tosylate.

Method Validation [11-17]

Linearity:

Those calibration curves and equations of Sorafenib tosylate were calculated by graphing the drug's peak area vs. the concentrations of the components. Proposed approach was found to be linear for the compound among 1-64g/ml. The results were assessed using linear regression analysis on seven various concentrations with Sorafenib tosylate with in specified range [1, 2, 4, 8, 16, 32, 64g/mL]

Precision:

The repeatability and intermediate precision of the analytical approach were used to assess its precision. The same day and under its identical research conditions, five different preparations of Sorafenib tosylate standard solutions with a 50 g/mL concentration were injected for determination of intra-day repeatability, Intermediate precision [inter-day] was examined in the same laboratory with two separate analysts on different days, using the same LC instrument. The assays' percent RSD was determined.

Accuracy:

The percentage recovery of known concentrations of Sorafenib tosylate spiked in three separate working standards was used to determine accuracy. [1 ,8 & 64 μg/ml]. For each concentration, analysis was done in triplicate, and the percent recovery and percent RSD were determined.

LOD and LOQ:

Using standard deviation data and the slope of the linearity curve, the lower limit of detection [LOD] and lower limit of quantification [LOQ] were determined.

LOD = 3.3 X σ/S & LOQ = 10 X σ /S, where σ = standard deviation, y intercept;

S= linearity curve slope.

System suitability:

Six injections of Sorafenib tosylate standard solution at a concentration of 50 μg/mL were performed before and during the analysis to ensure the chromatographic system's performance and reproducibility. Retention time, peak size, peak symmetry. The tailing factor, theoretical plate number, and tailing factor were all calculated.

Stress studies:

Sorafenib tosylate was subjected to various stress settings in order to assess its degradation behaviour. At 80˚C for two hours, for acid hydrolysis, Sorafenib tosylate was treated with 0.1 N HCl and for basic hydrolysis, use 0.1 N NaOH. Oxidative experiments were conducted in 15 percent H_2O_2 at 80 ˚C for two hours. Before HPLC injection, samples were neutralised to verify that the pH of the sample was neutral. The samples were again dilute with mobile phase before being analysed using HPLC.

Robustness:

Robustness was assessed by varying two parameters independently. The mobile phase's flow rate [1mL/min], and the ratio of mobile phase constituted by a mixture of Methanol, formic acid [1%] and water [70:30] For this study, the analysis was performed using a 50 g/mL concentration and all Filtered samples were injected in triplicate through a 0.45m PVDF membrane filter [Millipore].

In vitro drug release studies

A dialysis bag technique [Dialysis membrane MW cutoff 12-14 kDa, Himedia, India] was used to relate the release rate of ST solution and ST loaded SLNs in dissolution medium - phosphate buffered saline [PBS] of 7.4 pH at room temperature, 37±0.5ºC. 2ml sample were withdrawn with predetermined time such as 0.5, 1, 2, 4, 6, 8, 12, 24 and 48 hrs. The sink conditions were maintained by substituting the withdrawn sample with the PBS buffer. The withdrawn samples were then filtered by membrane filters [0.45μm] and analyzed by developed and validated RP-HPLC procedure.

Results and Discussion

Method Development

Reverse phase – HPLC technique was developed for the quantification of ST Loaded Solid lipid nanoparticle. The method was further validated following Q2 [R1] ICH guidelines. Melting point estimation and absorption maximum [λ max] were used to determine the structural integrity of the ST. The study was performed by carrying out separation using Phenomenex luna CN C8 short column [100mm X 4.60 mm 5μm] at a wavelength of 261 nm detected

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Optimization of HPLC Method

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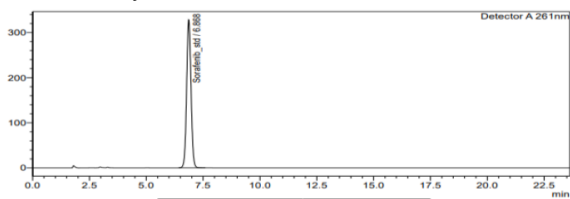

Figure 1: Chromatogram of standard Sorafenib tosylate

Method validation

Linearity:

The compound's proposed method was determined to be linear between 1.0-64 µg. mL-1. The data were assessed by calculating a calibration curve of concentration (x axis) versus absorbance for seven various concentrations (1- 64 g/mL) of Sorafenib tosylate in the chosen range (y axis). Regression equation was y = 112652x + 335171. ST peak area and concentration (g/mL) are represented by y and x, respectively (Figure 5). The optical characteristics were determined, including the coefficient of regression (R2 = 0.998), slope (112652), and Y-intercept (335171)
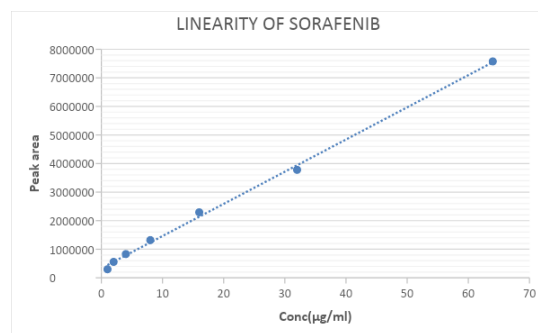

Figure 2: Linearity Curve for Sorafenib tosylate

Table 1: Peak Area for each concentration

Concentration	Peak area
1	298812
2	557081
4	830537
8	1319152
16	2292201
32	3779340
64	7575913

Precision:

The precision was calculated using five distinct standard concentration solutions. Interday & intraday precision was determined by injecting 50 µg/mL concentration solution of Sorafenib tosylate five times. Table 3 shows the interday as well as intraday precision values for method precision. Table 4 shows precision values for system.

Table.2: Sorafenib tosylate intraday precision study for method

METHOD PRECISION						
Injection	RT	Tailing	Plate count	Peak Area	Drug in mcg	Drug in %
1	6.685	1.007	2963	5867799	49.112559	98.22512
2	6.688	1.019	3068	5929743	49.6624294	99.32486
3	6.686	1.029	3100	5984526	50.1487324	100.2975
4	6.685	1.016	2984	5886824	49.281442	98.56288
5	6.686	1.005	2960	5928989	49.6557362	99.31147
Aver	6.6	1.02	301	59195	49.572	99.144

[23]

age	86		5	76.2	1798	366
Std dev.	0.0001			0.717395		
RSD %	0.002			0.723586		
Limit	1.0	2.0		2.0		90-110%

Accuracy:

The accuracy is obtained using the standard addition method. the inclusion of pre-quantified standard at 1%, 8%, & 64% into the known concentrations of a test sample (three replicates).

LOD and LOQ:

The slope of the linearity curve and standard deviation values are used to determine LOD (0.88 g/ml) and LOQ (1.0 g/ml). Table 6 enlists the value obtained for LOQ and LOD.

Table 3: Results for LOQ and LOD

	Concentration (µg/mL)
LOD	0.88
LOQ	1.0

System suitability:

The system suitability test was performed on chromatograms acquired under optimal conditions to evaluate several parameters such as theoretical plates, tailing, and resolution. The proposed approach obtained theoretical plates (>2000) while the entire analysis time was less than 10 minutes. Sorafenib tosylate retention time was 6.6 min. Table displays the system suitability results.

Table 4: sorafenib tosylate system suitability

Analyte	Conc µg/mL	RT	Theoretical Plate	Tailing Factor	HETP (mm)
Sorafenib tosylate	50	6.652	3099	1.017	48.404

Stress studies:

Following table summarizes all stability study results

- **Acid hydrolysis:** Acid degradation experiments revealed that 15.74% of Sorafenib tosylate drug was degraded.

- **Base hydrolysis:** Base degradation experiments revealed that 12.95% of Sorafenib tosylate was degraded.
- **Peroxide hydrolysis:** This experiment revealed that 19.10% of Sorafenib tosylate drug was degraded.

Table 5: Stress studies of the Sorafenib tosylate

Stress studies					
System Suitability & % Difference of Area Results for Sample Solution					
Time period (in Hrs)	RT	Tailing Factor	Plates	Area	% Degradation	S.D
HCl	6.169	1.047	6628	250727	15.7496	101.4992011
NaOH	6.180	1.039	6736	227160	12.9588	101.9176047
H2O2	6.174	1.039	6731	210883	19.10329	102.2065831
Limit	NMT 2.0	NLT 1000		± 2.0 %		

Application of developed method:

Particle Size and poly-dispersity index (PDI)

SLNs were analyzed for size of particle and PDI. The size of particle of the formulated ST loaded SLNs was found to be 200.5 nm with a PDI of 0.188 as represented in Figure that shows mono-dispersity of the SLNs.
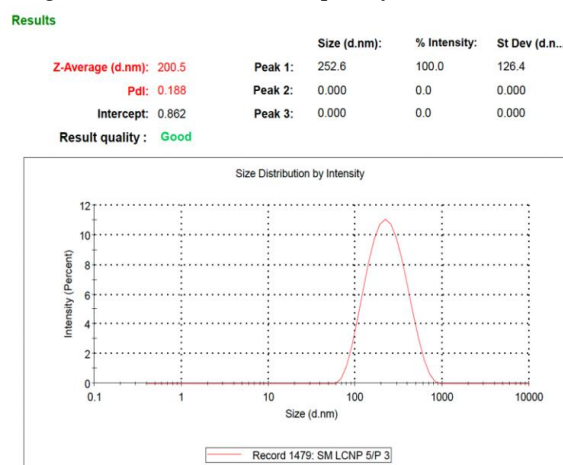

Figure 3: Mono-dispersity of the SLNs

Drug encapsulation parameters

From the recovered SLNs matrix, the percent Drug encapsulation efficiency and drug loading for ST were computed and determined to be 86.9% and 19%, respectively.

In vitro drug release studies

Both the ST-pure drug and the ST-loaded SLNs were evaluated for invitro release. The pure drug was released within 6 hours, or 87.8% purity. In case of ST loaded SLNs, drug released upto 56%, 65%, 89% within 12, 24 and 48 hrs. The designed and approved RP-HPLC analytical model, according to estimations, is capable of measuring the drug in SLNs and evaluating in vitro physico-chemical characteristic.
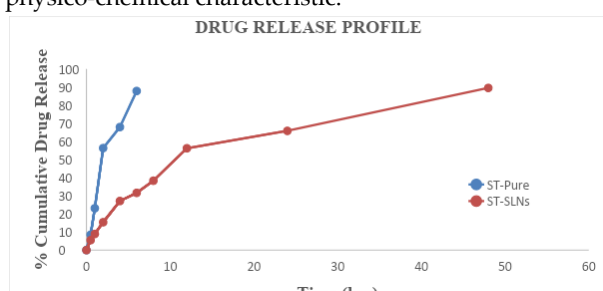

Figure 4: Drug release profile

Conclusion

According to ICH guidelines, the described RP-HPLC technique for measuring Sorafenib tosylate was developed and put through testing. The developed technique was evaluated for system compatibility, specificity, linearity, range, accuracy, and precision. Current approach for quantification of Sorafenib tosylate in solid lipid nanoparticles for determination of percent DEE, percent DL, and cumulative percent drug release analysis of SLNs was shown to be easy, quick, precise, and accurate. Absence of an interference signal at retention time illustrates the method's specificity, which uses a single sample preparation step to separate the drug from a complicated matrix. According to an in-vitro drug release research, the SLNs formulation enhances the solubility and stability of Sorafenib tosylate. The established method can be utilized with ease to quantify the drug in a variety of lipid-based Nano formulations, including lipid drug conjugates, polymer-lipid hybrid nanoparticles, and others, for both in vitro and in vivo experiments. In addition, pharmacokinetic and Pharmacodynamic studies of the SLN formulation of Sorafenib tosylate will be conducted in vitro and in vivo.

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