Section A-Research paper

Analytical method development and validation of RP-HPLC method for quantification of Sorafenib tosylate loaded solid Lipid Nanoparticles

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ABSTRACT:
This study set out to create an RP-HPLC system that was 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 10 minute runtime, a 10 mL injection volume, and a 1 mL/min flow rate, the mobile phase is a mixture of 70:30 methanol:0.1% formic acid in water. The effluent is 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 has 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

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. By Bayer, it is marketed as Nexavar®. Its chemical name is 4-(4-[4-chloro-3-(trifluoromethyl)phenyl]ureidophenoxy)-N-2-methylpyridine-2-carboxamide4-methylbenzenesulfonate, also known as sorafenib tosylate.[1] It is an effective oral multikinase 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-tumor activity 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.[2]

Solid lipid Nanoparticles:

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. Solid lipid-based nanoparticles are receiving a lot of attention as a potential colloidal drug carrier for intravenous applications. As an alternate particle carrier system, they have been suggested. 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. Because 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. 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.[3]

Sorafenib Tosylate:

Molecular Structure:

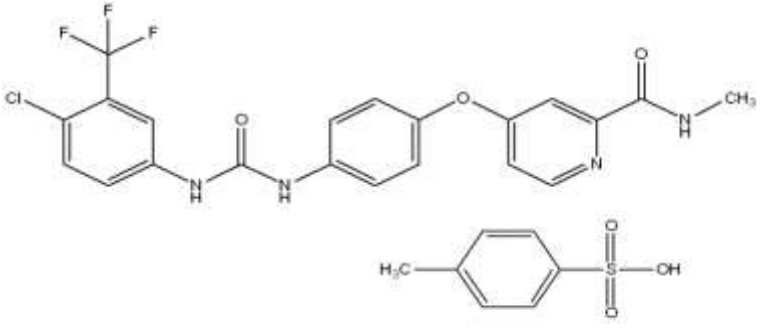


Figure 1. Chemical structure of Teneligliptin

Parameters	Descriptions
Name	Sorafenib tosylate

IUPAC Nomenclature	4-(4-{3-[4-Chloro-3-(trifluoromethyl)phenyl]ureido}phenoxy)N2methylpyridine-2-carboxamide 4-methylbenzenesulfonate.
Synonyms	4-(4-(3-(4-Chloro-3-trifluoromethylphenyl) ureido)phenoxy)pyridine-2-carboxylic acid methyamide-4-methylbenzenesulfonate;BAY 545-9085;BAY 673472; Nexavar, Sorafenib(Tosylate).
Molecular weight	637.0 Da
Molecular formula	$C_{28}H_{24}ClF_3N_4O_6S$
Physical characteristics	
Colour and nature	solid colur ranging from white to yellowish to darkish
Solubility	At 200 mg/ml, soluble in DMSO. In aqueous fluids, it is practically insoluble; however, it is mildly soluble in ethanol, Methanol, and PEG 400. 10-20 M is the maximum solubility in ordinary water. Buffers, serum, and other additives can change the aqueous solubility.
pKa	2.03 and 11.55
Log P	4.1.
Melting point	199–211 °C
Refractive index	1.626
Pharmacological category	Chemotherapeutic agent
Biopharmaceutics class	BCS and BDDCS class II compound which demonstrate a poor solubility (25 ng/mL in deionized water) and high permeability.

MATERIALS AND METHODS:

Chemical and Reagents:

SFT 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.

Table:1 Chromatographic conditions for (Sorafenib tosylate).

Column	Phenomenex luna CN C8 short column (100mm X 4.60 mm 5µm)
Wavelength	261nm
Flow rate	1mL/min
Detector	UV

Injection volume	10µl
mobile phase.	Methanol: 0.1% Formic Acid in water (70:30)
Run time	10 min
Retention time	6.8 min

Standard stock prepration:
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.

Mobile phase selection and preparation:
Sorafenib tosyalte is a lipophilic drug, For MP selection, many mobile phase combinations with varied ratios were examined. The standard Sorafenib tosyalte drug were mixed with a range of Mobile Phase mixes at different ratio and flow rates for peak optimization. Method was repeated until a sharp peak was attained. Methanol(B), 0.1 percent Formic acid in water(A) produced a sharp peak (70:30).

0.1% Formic acid prepration:
To make 0.1 percent formic acid preparation, mix 1 mL formic acid with 1000 mL HPLC grade water.

Formulation preparation procedure:
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.

RESULT AND DISCUSSION:

RP – HPLC technique for the quantification of ST Loaded in Solid lipid nanoparticle were produced. precision, accuracy and reliability of the methods are evaluated through validation studies adopting Q2 (R1) ICH guidelines. Melting point estimation and absorption maximum (λ max) were used to determine the structural integrity of the ST. melting point was found to be 199–211 °C ST showed λ max at 261 nm

Process parameter optimization for the RP-HPLC technique

The effects 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 process was adjusted. Table and Figure show the experimental

details.

Runs	Mobile phase	Flow rate	Observation
1	ACN and Acetate buffer (pH-4.5) 60:40 (v/v)	1 mL/min	• • The peak structure was absolutely poor. • The MP was altered to reduce the retention time.
2	methanol: Water with 0.1 percent formic acid (70:30).	1mL/min	• final optimized method for Sorafenib tosylate with 6.8 time for retention • The tailing factor was found to be within acceptable limits.

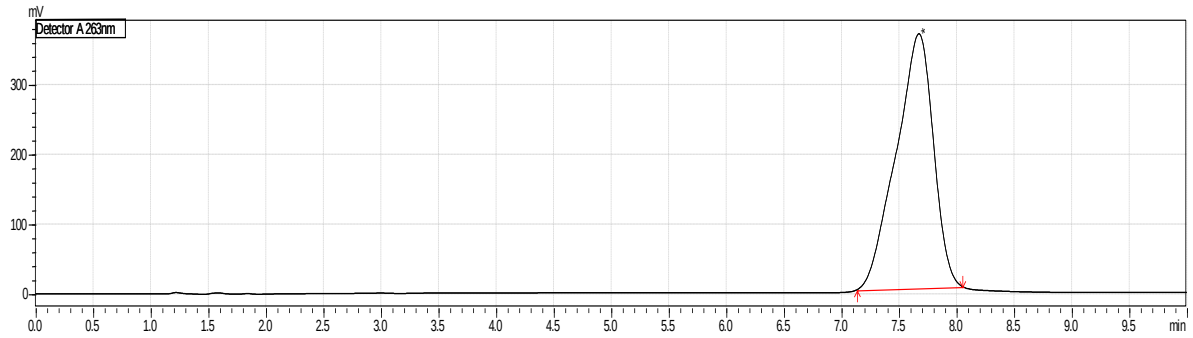


Figure.2: chromatogram of Sorafenib tosylate – Trail 1

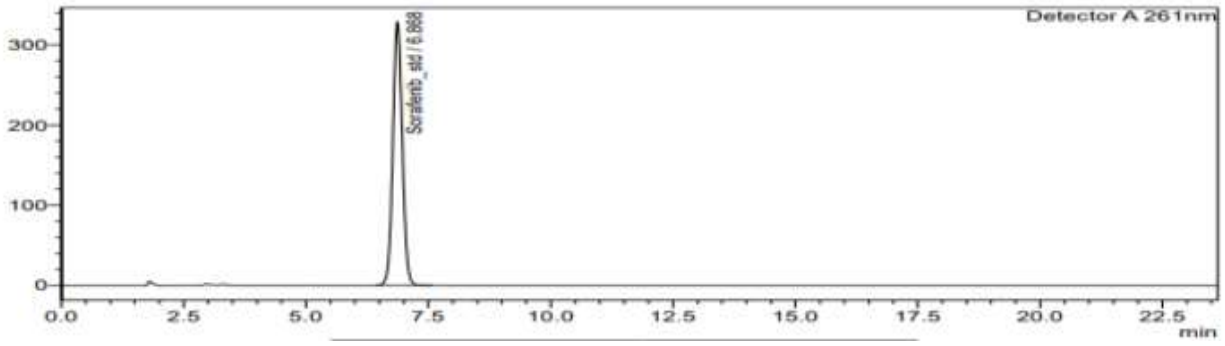


Figure.3: Trial two chromatogram of Sorafenib tosylate

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 concentration (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. The optical characteristics

were determined, including the coefficient of regression (R2 = 0.998), slope (112652), and Y-intercept (335171).

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

Table.2: Linearity of Sorafenib tosylate

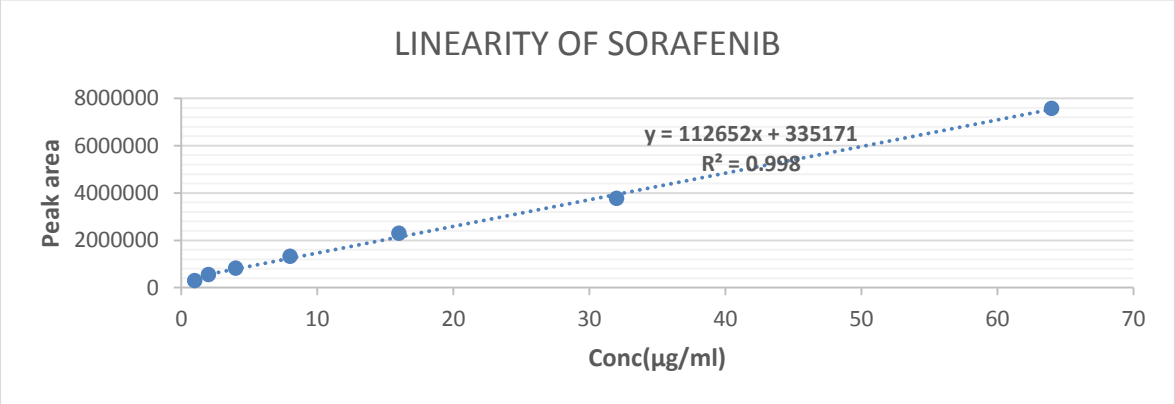


Figure.4: Sorafenib tosylate Linearity Curve

Precision:

The Precision repeat measurements on interday and intraday variations are determined by the precision measurement. 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 shows the interday as well as intraday precision values.

Table.3: Sorafenib tosylate intraday precision study

Method precision:

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
Average	**6.686**	**1.02**	**3015**	**5919576.2**	**49.5721798**	**99.144366**

Std dev.	**0.001**			0.717395		
RSD %	**0.02**			0.723586		
Limit	1.0	2.0		2.0		90-110%

System precision:

System precision						
Injection	RT	Tailing	Plate count	Peak Area	Drug in mcg	Drug in %
1	6.668	1.008	2987	5998976	50.2770035	100.554
2	6.668	1.010	2980	5976772	50.0799009	100.1598
3	6.665	1.017	2996	5899878	49.397321	98.79464
4	6.677	1.008	3023	5886978	49.2828809	98.56562
5	6.680	1.018	3050	5998989	50.2771189	100.5542
Average	**6.672**	**1.01**	**3007**	**5952318.6**	**49.86284468**	**99.725652**
Std dev.	**0.007**			0.868754		
RSD %	**0.10**			0.871144		
Limit	1.0	2.0		2.0		90-110%

Analyte	Nominal (µg/ml)	conc.	Found (µg/ml)	%Accuracy	Average% Bias	% RSD
LQC	1	0.98123424		98.12342	1.876576	1.041349
MQC	8	7.87382973		98.42287	1.577128	0.850748

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).

	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

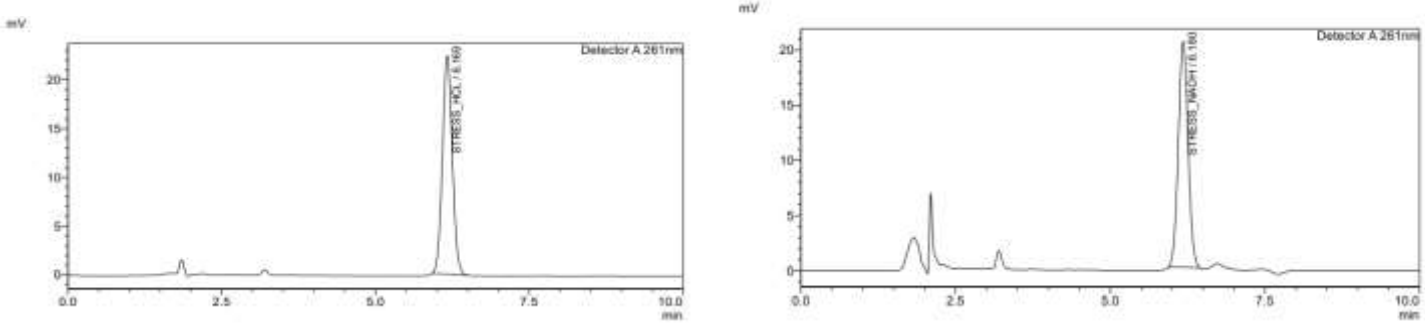
Stress studies:

Following table summarizes all stability study results

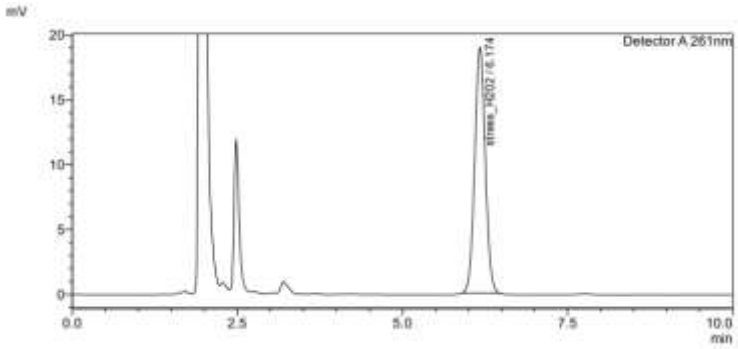
- **Acid hydrolysis:** Acid degradation experiments revealed that 15.74% of Sorafenib tosylate drug was degraded.
- **Base hydrolysis:** Base degradation experiments revealed that 12.95% of Sorafenib tosylate was degraded.
- **Peroxide hydrolysis:** This experiments revealed that 19.10% of Sorafenib 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 %**		



A) 0.1N HCL,2Hrs **B) 0.1N NAOH,2 hrs**



C) 15% H2O2,2 hrs

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.

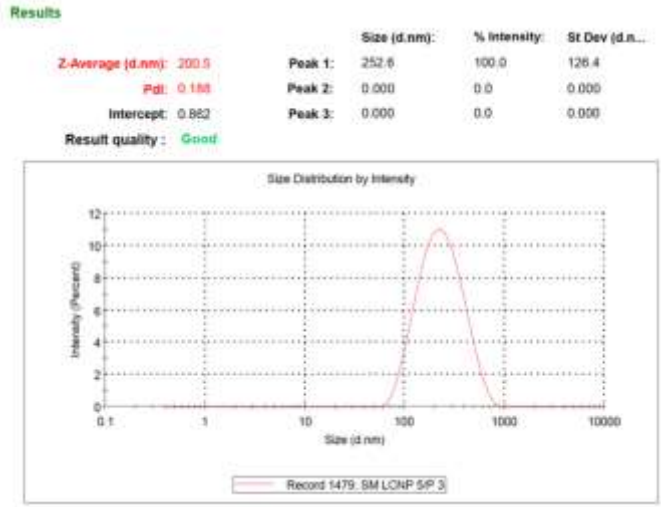


Fig.5 : Mono-dispersity of the SLNs

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.

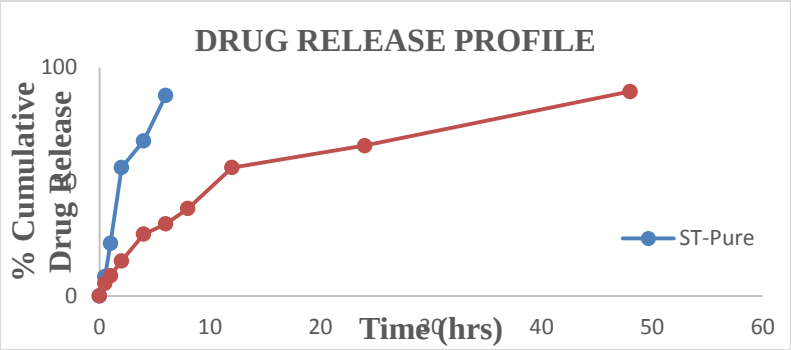


Fig.6: Drug release profile

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 tosyalte. The established method can be utilised with ease to quantify the drug in a variety of lipid-based nanoformulations, 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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CONFLICT OF INTEREST

There was no conflict of interest between the authors.

REFERENCES:

[1] US Food and Drug Administration, (n.d.). NEXAVAR (Sorafenib) Package Insert. 2010.

[2] S.M. Wilhelm, et al., BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, Cancer Res. 64 (19) (2004) 7099–7109.
[3] S. Yang, B. Zhang, X. Gong, T. Wang, Y. Liu, N. Zhang, In vivo biodistribution, biocompatibility, and efficacy of sorafenib-loaded lipid-based nanosuspensions evaluated experimentally in cancer, Int. J. Nanomed. 11 (2016) 2329.
[4] Drug Bank (n.d.) Drug Bank Accession Number is DB00398. http://www. drugbank.ca. (Accessed Feb 28, 2018).
[5] PubChem, (n.d.) PubChem CID: 216239, pubchem.ncbi.nlm.nih.gov (Accessed Feb 28, 2018).
[6] M. Moore, et al., Phase I study to determine the safety and pharmacokinetics of the novel Raf kinase and VEGFR inhibitor BAY 43-9006, administered for 28 days on/ 7 days off in patients with advanced, refractory solid tumors, Ann. Oncol. 16 (10) (2005) 1688–1694.
[7] R.C. Kane, et al., Sorafenib for the treatment of advanced renal cell carcinoma, Clin. Cancer Res. 12 (24) (2006) 7271–7278.
[8] A.M. Filppula, P.J. Neuvonen, J.T. Backman, In vitro assessment of time-dependent inhibitory effects on CYP2C8 and CYP3A activity by fourteen protein kinase inhibitors, Drug Metab. Dispos. 42 (7) (2014) 1202–1209.
[9] L.V. Moretti, R.O. Montalvo, Elevated international normalized ratio associated with concurrent use of sorafenib and warfarin, Am. J. Health Syst. Pharm. 66 (23) (2009) 2123–2125.
[10] B. Blanchet, B. Billemont, J. Cramard et al., "Validation of an HPLC-UV method for sorafenib determination in human plasma and application to cancer patients in routine clinical practice," Journal of Pharmaceutical and Biomedical Analysis , vol. 49, no. 4, pp. 1109–1114, 2009.
[11] W. J. Heinz, K. Kahle, A. Helle-Beyersdorf et al., "Highperformance liquid chromatographic method for the determination of sorafenib in human serum and peritoneal liquid," Cancer Chemotherapy and Pharmacology, vol. 68, no. 1, pp. 239–245, 2011.
[12] S.M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, C. Chen, X. Zhang, P. Vincent, M. McHugh, BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, Cancer Res. 64 (2004) 7099–7109.
[13] B. Escudier, T. Eisen, W.M. Stadler, C. Szczylik, S. Oudard, M. Staehler, S. Negrier, C. Chevreau, A.A. Desai, F. Rolland, Sorafenib for treatment of renal cell carcinoma: final efficacy and safety results of the phase III treatment approaches in renal cancer global evaluation trial, J. Clin. Oncol. 27 (2009) 3312–3318.