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METHOD DEVELOPMENT AND VALIDATION OF SORAFENIB IN BULK FORMULATION

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ABSTRACT

Background: Sorafenib, an oral multi-kinase inhibitor, is extensively utilized in oncology for its antineoplastic properties. Accurate quantification of Sorafenib in pharmaceutical formulations is critical for quality control and therapeutic efficacy. Ultra-performance liquid chromatography (UPLC) offers a sensitive and efficient analytical method for such evaluations. **Aim:** This study aimed to develop and validate a precise and robust UPLC method for the quantification of Sorafenib, focusing on accuracy, precision, linearity, and robustness. **Research Methodology:** The method development employed UPLC with a C18 column as the stationary phase. The mobile phase consisted of methanol and acetonitrile in a 55:45 ratio (%v/v). Prednisolone was used as an internal standard. Validation studies encompassed accuracy, precision, linearity, robustness, and ruggedness. Results demonstrated distinct retention times for Sorafenib (7.289 minutes) and the internal standard (2.639 minutes), with excellent resolution and a clear baseline. Accuracy ranged from 99.62% to 99.87%, while the linearity coefficient was 0.9985. **Conclusion:** The validated UPLC method proved to be robust, precise, and reproducible, demonstrating its suitability for routine analysis of Sorafenib in pharmaceutical formulations.

KEYWORDS: UPLC, Sorafenib, Validation.

INTRODUCTION

- Sorafenib, an oral tyrosine kinase inhibitor, has gained prominence for its efficacy in treating various malignancies, including hepatocellular carcinoma and renal cell carcinoma. Its pharmacological significance necessitates the development of reliable analytical methods for quality assurance and dosage accuracy. Analytical quantification plays a pivotal role in ensuring pharmaceutical safety and efficacy, particularly in highly potent anticancer drugs like Sorafenib.
- Modern analytical techniques such as ultra-performance liquid chromatography (UPLC) have revolutionized drug analysis by providing enhanced sensitivity, resolution, and reduced analysis time. UPLC, compared to high-performance liquid chromatography (HPLC), employs smaller particle sizes in the stationary phase, enabling higher efficiency and better separation of compounds. This study focuses on developing a validated UPLC method for the quantification of Sorafenib in pharmaceutical formulations, adhering to the guidelines of regulatory agencies such as the International Council for Harmonisation (ICH).
- The development and validation of a method for Sorafenib analysis require a systematic approach. Parameters such as accuracy, precision, linearity, limit of detection (LOD), and limit of quantitation (LOQ) are critical for assessing the method's robustness and reliability. Furthermore, factors like temperature, flow rate, and wavelength are optimized to ensure reproducibility and minimal interference.

METHODOLOGY

Chemicals and Reagents

Methanol, acetonitrile, and prednisolone (internal standard) of HPLC grade were procured. Sorafenib standard was obtained from a certified supplier. Mobile phases were prepared using methanol and acetonitrile in varying ratios.

Instrumentation

The analysis was conducted using an Acquity UPLC system equipped with a C18 column. Detection was performed using a UV detector set at optimal wavelengths determined during method development.

Method development

The chromatographic separation of Sorafenib was optimized using a mobile phase of methanol and acetonitrile in a 55:45 ratio. A flow rate of 1.2 mL/min at ambient temperature ensured adequate resolution. The retention times for Sorafenib and the internal standard were recorded as 7.289 minutes and 2.639 minutes, respectively.

Validation

1. **Accuracy:** Recovery studies at three levels (50%, 100%, and 150%) demonstrated a mean recovery rate of 99.74%, confirming method reliability.
2. **Precision:** Repeatability and intermediate precision were evaluated. The %RSD for method precision was 0.48%, indicating excellent consistency.
3. **Linearity:** Sorafenib showed linearity over a concentration range of 8–40 µg/mL with a correlation coefficient of 0.9985.
4. **Robustness:** Variations in flow rate, temperature, and wavelength exhibited minimal deviations, confirming method robustness.
5. **LOD and LOQ:** The LOD and LOQ values were determined as 0.838 µg/mL and 2.540 µg/mL, respectively. This methodology ensures that the UPLC method developed is both reliable and reproducible for pharmaceutical quality control applications.

RESULTS

Sorafenib	
Method development by UPLC	
System	UPLC
Stationary Phase	C18
"Mobile Phase"	"Methanol and Acetonitrile in the ratio of 55:45 %v/v"
Internal Standard	Prednisolone
Injection volume	20µl
Temperature	Ambient
Flow rate	1.2 mL/min
UV detection	240 nm
Retention Time	SORAFENIB– 7.289mins; Prednisolone – 2.639 mins
Inference	"Better resolution of the peaks with clear base line separation was found."

➢ **Validation studies for sorafenib**

Accuracy procedure

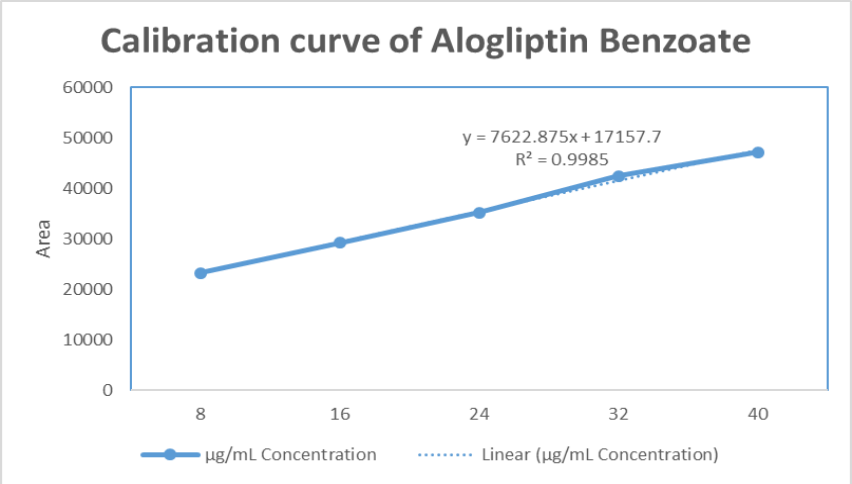
Sorafenib						
Level %	**Amount added (µg/ml)**	**Amount found (µg/ml)**	**% Recovery**	**Mean recovery (%)**	**Std.Dev**	**% RSD**
50	08.09	08.06	99.62	99.74	0.12503	0.13%
100	16.18	16.16	99.87			
150	24.27	24.21	99.75			

Method precision

Replicate		***Sorafenib***	
S. No.	**Concentration Taken (µg/ml)**	**Area**	**%LC**
1	16.18	29234	99.98%
2		29318	99.70%
3		29421	99.35%
4		29521	99.01%
5		29556	99.90%
6		29581	98.81%
Average			99.45%
Std.Dev			0.4813
% RSD			0.48%
Standard weight			16.18mcg
Standard potency			99.80%

Linearity

Sorafenib		
Linearity level	**Concentration in µg/mL**	**Area**
1	8 µg/mL	23261
2	16 µg/mL	29231
3	24 µg/mL	35187
4	32 µg/mL	42432
5	40 µg/mL	47152
Correlation co-efficient	0.9985	
Slope	7622.875	
Intercept	17157.7	



Robustness

Robustness studies			
Parameter	**Value**	**Peak area**	**% RSD**
Flow Rate	Low	29541	0.02%
	Actual	29546	
	Plus	29551	
Temperature	Low	29392	0.05%
	Actual	29406	
	Plus	28420	
Wavelength	Low	29604	0.01%
	Actual	29609	
	Plus	29612	

Ruggedness

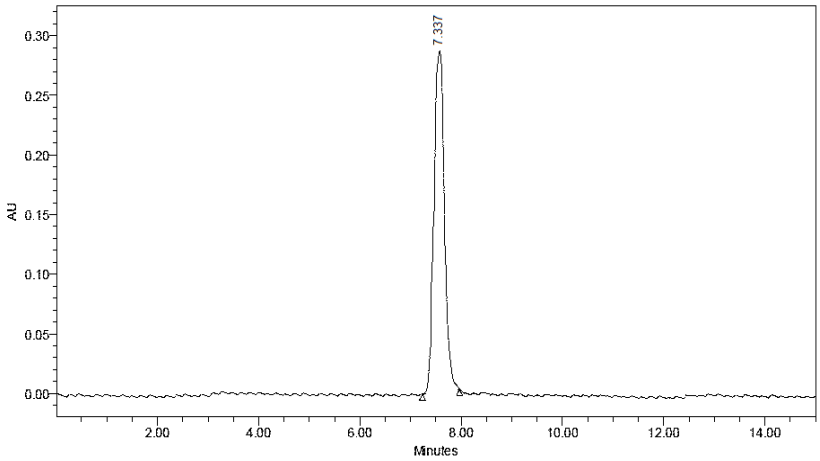
Sorafenib			
Ruggedness			
Parameter	**Peak area**	**% RSD**	**%LC**
Intraday precision	29326	0.33%	98.97%
	29453		99.54%
	29519		99.31%
Inter day precision	29371	0.28%	99.81%
	29434		99.60%
	29532		99.27%
Instrument:1 Acquity UPLC Waters, 2695H	29548	0.02%	99.22%
	29554		99.20%
	29541		99.24%
Instrument:2 Agilent Technologies,1290	29546	0.01%	99.22%
	29552		99.20%
	29547		99.22%
Average			99.31

Std.Dev		0.225
%RSD		0.23%

LOD and LOQ
LOD
LOD= 3.3*(1936.58/ 7622.875)
LOD= 3.3*(0.2540485)
LOD= 0.83836(µg/ml)

LOQ
LOQ=10*(SD/S)
LOQ= 10*(1936.58/ 7622.875)
LOQ= 2.54048(µg/ml)

Assay studies
Sample control



Evaluation of methods
Assay studies

➢ **Analysis of sorafenib**

Conditions	Sample amount (µg/ml)	Peak Area	% claim	% Degradation
Sample control	16.18	29521	99.33%	-

$$\%\ Assay = \frac{AT}{AS} \times \frac{W1}{100} \times \frac{1}{25} \times \frac{100}{W2} \times \frac{25}{1} \times \frac{AW}{LC} \times P$$

Sample control (Sorafenib)
% Assay = 99.33%

CONCLUSION
The validated UPLC method proved to be robust, precise, and reproducible, demonstrating its suitability for routine analysis of Sorafenib in pharmaceutical formulations.

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