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Validated UV-Vis spectrophotometric method for the estimation of Sorafenib Tosylate in bulk and nanoparticles

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***Abstract*---**It was noticed from the standard databases such as Scopus, PubMed, ScienceDirect, etc. that a limited UV-Vis spectrophotometric method has been reported for estimating Sorafenib (SOR), but no sophisticated analytical methods have been ever reported in any database for estimating SOR in nanoparticles. The present research involved the establishment of an economic, accurate, robust, and precise analytical method for the quantitative determination of SOR in bulk and nanoparticles utilizing a new validated spectrophotometric method. The spectrophotometric analysis was carried out using double-beam ultraviolet-visible spectrophotometer was employed in developing a new method using methanol and water at a ratio of 80:20 v/v and λmax of 265 nm. The technique was verified using the Q2A and Q2B guidelines of ICH. The newly developed UV-Vis method had desired linearity over the range of 2-12 µg/mL; along with excellent precision, accuracy, ruggedness, and robustness characteristics as observed from % RSD values of less than 2. The present study concluded that the developed UV-Vis method has desired linearity, precision, accuracy, ruggedness, and robustness, and will serve as an excellent technique for the determination of sorafenib in both bulk and nanoparticles without the interference of commonly used chemicals or solvents.

***Keywords*---**sorafenib, ultraviolet visible, bulk, nanoparticles.

Introduction

Cancer is an extremely serious and terrible illness that kills a lot of people all around the world [1]. The treatment and control of the illness is a problem for both developing and developed nations. Malignant cells usually break free from the tumor and enter the circulation, where they multiply and spread as the invasion continues [2]. Sensitive laboratory methods may be used to detect the presence of circulating tumor cells. Cancer stem cells' most essential trait is their capacity to self-renew while also multiplying at the same time [3]. Indeed, cancer therapy with chemotherapeutic drugs has significant obstacles, especially in weak and elderly patients, owing to the lack of selectivity of the treatments, which may attack healthy organs [4]. Triple-negative breast cancer (TNBC) accounts for 10%-15% of all breast cancer cases. TNBC patients had a worse prognosis compared to other breast cancer subtypes [5]. Because of the lack of validated molecular targets and the poor prognosis of TNBC patients, it is also critical to enhance effective therapies at all levels [6]

Sorafenib (SOR); 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methylpyridine-2 carboxamide is a white to yellowish or brownish solid having a molecular formula of $C_{21}H_{16}ClF_3N_4O_3$, and a molecular weight of 637.027 g/mol. It is insoluble in water but soluble in ethanol, methanol, and PEG-400 [7]. SOR is an anti-angiogenic multi-tyrosine kinase inhibitor that may be taken orally. Patients with advanced renal cell carcinoma (RCC), advanced hepatocellular carcinoma (HCC), and breast cancer are now treated with this medicine orally (400 mg daily) [8]. The clinical application of SOR is greatly limited by its low bioavailability (~8.43%), resulting from its poor water solubility and rapid elimination and metabolism. A dose reduction or temporary discontinuation of SOR can be frequently observed in many cancer patients due to serious side effects including skin toxicity, diarrhea, and hypertension and hand foot syndrome. To overcome these problems SOR nanoparticles can be formulated.

For the estimation of SOR, either in biological samples (plasma, fluids, etc.), bulk, traditional pharmaceutical products (tablet, capsule, etc.), other formulations, and non-traditional forms (such as nanoparticles, etc.), a number of sophisticated analytical hyphenated techniques have been reported like reverse phase-high performance liquid chromatography (RP-HPLC) [9], liquid chromatography-tandem mass spectroscopy (LC-TMS) [10], gas chromatography [11], high-performance thin-layer chromatography (HPTLC) [12], etc. However, it was noticed from the standard databases such as Scopus, PubMed, ScienceDirect, etc. that a limited UV-Vis spectrophotometric method has been reported for estimating SOR [13], but no sophisticated analytical methods have been ever reported in any database for estimating SOR in nanoparticles. The present research involved the establishment of an economic, accurate, robust, and precise analytical method for the quantitative determination of SOR in bulk and nanoparticles utilizing a new validated spectrophotometric method. The study identifies correctly the critical parameters required for identification. The results were analyzed and validated statistically and by recovery studies.

Materials and Methods

Chemical

SOR was obtained as a generous gift sample from Cipla India Ltd., Mumbai, India. Loba Chemicals Ltd., Mumbai, India provided Chitosan (Mol. Wt. = 50 kDa, degree of deacetylation 90%). Meteoric Biopharmaceuticals Pvt. Ltd., Ahmadabad, India, provided HA (Mol. Wt. = 100 kDa). Methanol was purchased from Merck Specialties Ltd., Mumbai, India. All other analytical grade chemicals, reagents, and solvents were obtained from HiMedia Ltd., Mumbai, India.

Instrumentation

The spectrophotometric analysis was carried out using double-beam recording ultraviolet-visible spectrophotometer (JASCO® V-630, Mumbai, India) connected with a computer having spectral bandwidth of 1 nm and wavelength accuracy of ±0.3 nm; with a pair of 10 mm path length matched quartz cells. The weighing was carried out using digital balance. Digital pH meter (EQIP-TRONICS® Mumbai, India) was used to determine the pH. EQUITRON® Digital (Mumbai, India) sonicator was used for the sonication.

Selection of wavelength

Methanol was completely mixed with water at a ratio of 80:20 v/v. The aforementioned solution was degassed for 5 minutes with sonication before being filtered under vacuum through a 0.45 µm membrane filter. The solution was scanned in the 200 nm to 400 nm wavelength range.

Preparation of solvent system

Throughout the research, methanol: water (80:20 v/v) was produced and utilized as a solvent system.

Standard stock solution

An accurate amount of 10 mg SOR was added to a dry volumetric flask and 5 ml of solvent was added. The above content was sonicated for 10 mins and the volume was made up to 10 ml with solvent system to produce 1000 ppm. Then, 1 ml of the produced content was pipetted out in a 10 mL volumetric flask and the volume was diluted to 10 mlto produce 100 ppm of the content.

Analysis of nanoparticles

SOR was first dissolved in an ethanolic solution containing 0.25 percent v/v ethanol. This solution was added to 4 ml of the Hyaluronic acid solution and mixed at room temperature for 1 hour at 400 rpm. To get SOR loaded polyelectrolyte complex nanoparticles, this solution was placed into a solution of chitosan in 2% v/v acetic acid for 2 hours with magnetic stirring (1200 rpm). The combined solution was then loaded into a dialysis bag, which was then tightly sealed and immersed in 500 ml of water at room temperature for 6 hours to remove the ethanol and acetic acid. The dialyzed solution was then filtered over a 0.45 µ cellulose nitrate membrane and freeze dried until it further use.

Method validation

The technique was verified using the Q2A and Q2B guidelines from the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), as well as guidance from the USFDA.

Linearity and Range

For SOR, the method's linearity was tested using six different concentrations of the solutes (25% to 150%), ranging from 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, 10 µg/ml, and 12 µg/ml strength. All the solutions were prepared by diluting in methanol: water (80:20 v/v). A calibration graph was obtained by plotting absorbance versus concentration of standard drugs and regression correlation (r^2) was determined [14].

Accuracy

The accuracy of the UV method was obtained using recovery studies after standard addition of the analyte of interest. Three different solutions of SOR were prepared in triplicate at levels of 80%, 100%, and 120% of their predefined concentration. In predefined concentrations, different amount of SOR was included (standard addition method) and accuracy was determined based on percent recovery [15]. For calculating the percent recovery, the following equation was utilized:

$$\% RC = (SPS - S / SP) \times 100$$

Where, % RC = Percent recovery; SPS = Amount found in the spiked sample; SP = Amount added to the sample; and S = Amount found in the sample.

Precision

The precision of this method was determined by applying the standard drug solution at concentrations of 50%, 100%, and 120% of the target concentration (standard addition method), three times in a single day (intra-day variability), and three times on three different days (inter-day variability). The relative standard deviation (RSD) values were used to determine the degree of precision [16].

Robustness

The robustness of the developed UV-Vis method was determined by using different concentrations of methanol in water (50:50 and 60:40). SOR (10 µg/ml) was prepared by utilizing above mentioned solvent system independently, and the sample was analyzed at λmax of 265 nm and the % RSD was estimated [17].

Ruggedness

The ruggedness of the developed UV-Vis method was carried out by analyzing the triplicate samples of SOR utilizing two distinct analysts. The % RSD was determined and reported [18].

Limit of detection

Although it is not necessary to define the exact amount, the limit of detection (LOD) is the lowest concentration that any analytical method can detect [19].
The LOD was determined by the formula:

$$\boldsymbol{LOD} = \mathbf{3.3}\,(\boldsymbol{\sigma} / \boldsymbol{S})$$

Where, **σ =** standard deviation of response; **S** = slope of the calibration curve. The slope S may be estimated from the calibration curve of the analyte.

Limit of quantification

The limit of quantification (LOQ) is the smallest amount that can be measured with a given degree of accuracy and precision using any analytical method.
The LOQ is determined by the formula:

$$\boldsymbol{LOQ} = \mathbf{10}\,(\boldsymbol{\sigma} / \boldsymbol{S})$$

Where, **σ =** standard deviation of intercept ; **S** = slope of the calibration curve (SE of intercept).

Assay of drug in nanoparticles

Developed UV-Vis method was used for estimation of SOR content in formulation. For the study, Suitable dilutions were made for both standard and nanoparticles formulation 10ppm solution of both and Absorbance of both solutions was measured at nm on UV Spectrophotometer by methanol: water (80:20)solvent system and results were reported in terms of average percent assay.

Result and Discussion

3.1. Determination of λmax

For SOR in methanol: water (80:20 v/v), λ max was located at 265 nm (Figure 1).

Method validation
Linearity and Range

The UV-Vis standard curve was developed using SOR concentrations ranging from 2-12 µg/mL using methanol: water (80:20 v/v), as a solvent (Table 1). The standard calibration curve for SOR was plotted using concentration vs. absorbance values where the calibration curve, subjected to least square regression analysis yielded an equation; y = 0.0722x + 0.0099 with correlation coefficient 0.9995 (Figure 2).

Accuracy

Accuracy is to be established over the entire calibration range of the analytical method so that at any point of determination, the results obtained would be reliable. In the case of the UV-Vis method for SOR, accuracy was established using recovery studies. At 80% standard addition, the mean recovery of SOR was found to be 96.357% whereas, at 100% and 120% standard addition, it was found to be 98.37% and 97.22%, respectively. The % RSD was found to be less than 2 for the SOR recovery studies (Table 2). From the results of accuracy studies, it was observed that the developed UV-Vis method was highly accurate as the percent recovery was between 96.35% to 98.37%, and the % RSD was well below 2%.

Precision

Precision is a measure of the degree of scattering. It expresses the reproducibility of the measurements. It is expected that an analytical method should generate outcomes that are reproducible. The precise analytical method leads to accurate results. Considering the importance of reproducible yet accurate results, intra-day and inter-day precision of the developed UV-Vis method were established. The results in terms of mean absorbance values and % RSD for the intra-day and inter-day precision study are demonstrated in Table 3. % RSD values of the intra-day precision study were found to be in between 0.47 and 1.59 whereas those of inter-day precision study were in between 0.21 and 0.29. Overall, % RSD values of less than 2 showed the precision of the developed UV-Vis method.

Robustness

The robustness of the analytical method is the ability of a method to resist the change in its performance despite the small, deliberate changes in method parameters. It is an important parameter of the analytical method as a small, unintentional change in method parameters like solvent composition, pH, etc. may occur during routine use and may hamper the performance of the said method. It is expected that such change should not alter the performance of the analytical method. Therefore, the robust analytical method is preferred. The robustness of the proposed UV-Vis method was established by modifying the composition of the co-solvent system. The change in water and methanol percentage (50:50 to 60:40) in the co-solvent system did not affect the method performance. % RSD values were found to be in between 0.32 and 0.38 (Table 4). % RSD values below 2 showed that the proposed UV method is robust in nature.

Ruggedness

The ruggedness test of the analytical method is defined as the degree of reproducibility of assay results obtained by the successful applications of the assay over time and among multiple laboratories and analysts. To establish the ruggedness of the proposed UV-Vis method, the SOR solution was analyzed at three different conditions. There was no significant difference when the experiment was conducted by two different analysts. The results revealed that the

proposed UV-Vis method was rugged as it showed a % RSD value of less than 2 (Table 5).

Limit of detection

The Standard deviation and slope was found to be 0.00407 & 0.072. LOD of the proposed UV method was found to be 0.18 µg/ml. Lower LOD value indicated that the proposed method could detect even the smallest quantities of SOR present in the sample.

Limit of quantification

LOQ of the proposed UV-Vis method was found to be 0.56 µg/ml. Lower LOQ value indicated that the proposed method would be suitable for analyzing the samples containing even small quantities of SOR.

Assay of drug in nanoparticles

The developed UV-Vis method was successfully applied for the estimation of SOR content in nanoparticles. The drug concentration of the supernatant containing un-entrapped drug was estimated using the proposed method, and it was determined to be 2.95 percent. The nanoparticles carrying the entrapped drug were similarly treated with ethanol to remove the covering and release the entrapped drug. This solution was made up with methanol: water (80:20) and tested at λmax 265nm . The drug content was found using the same method as 97.05 percent. Despite the inclusion of multiple chemicals in the formation of SOR nanoparticles, no significant shifts in λmax were observed. so, this validated method can precisely estimate the SOR in bulk and formulation without the interference of solvent effect.

Conclusion

The present study concluded that the developed UV-Vis method has desired linearity, precision, accuracy, ruggedness, and robustness, and will serve as an excellent technique for the determination of SOR in both bulk and nanoparticles without the interference of commonly used chemicals or solvents. However, more studies such as stability-indicating assay methods are required to be developed to further study the effect of degradants, further.

Ethical approval

No ethical permission is required.

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Competing interests
The authors declare no conflict of interest.

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