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Original article

Method Development and Validation of Sunitinib in Bulk and Capsule Dosage Form and its Stability Studies by UV Spectrometric Method

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ABSTRACT:
A simple, Accurate, Precise spectrophotometric method was developed and validated for the estimation of sunitinib in bulk and its capsule dosage form. Further this study is designed to validate the developed methods as per ICH guidelines. The quantification process was performed on UV-spectrophotometer. Different analytical performance parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ) repeatability, stability studies were determined according to ICH guidelines. The solutions of standard and sample were prepared in methanol after suitable dilutions. 100µg/ml of the Sunitinib was prepared and scanned in the UV visible range 800 to 200nm. In the quantitative determination of the drug was carried at selected wavelength 431nm and the linearity range was formed to be 0.1 to 10µg/ml and r2 (0.9997). LOD and LOQ for sunitinib was found to be 0.011048µg/ml and 0.3348µg/ml. The proposed method can be adopted for routine quality control for estimation of drug in formulation.
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Keywords: Sunitinib, spectrophotometric method, validation, linearity, precision.

1. INTRODUCTION

Sunitinibmalate, *N*-(2-diethylaminoethyl)-5-[(*Z*)-(5-fluoro-2-oxo-1*H*-indol-3-ylidene)methyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide, is an oral anticancer drug, which is marketed under the trade name Sutent(r) by Pfizer, Inc., New York. This drug is a novel multitargeted tyrosine kinase inhibitor with antitumor and antiangiogenic activities for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST) . Sunitinib malate is an oral, potent multi-targeted TKI that displays antitumor and antiangiogenic actions. It is reported to inhibit VEGFR-1, VEGFR-2, VEGFR-3, KIT (stem-cell factor [SCF] receptor), PDGFR-a, and PDGFR-b in both cellular and biochemical evaluations. All these receptors are involved in the angiogenesis of well-differentiated primitive neuro-ectodermal tumors (PNETs). In vitro, sunitinib malate was described to persuade apoptosis of endothelial cells of the human umbilical vein. Moreover, it prohibits FMS-like tyrosine kinase 3, the receptor of glial cell line originated neurotrophic factor, and receptor of colony-stimulating factor-1 (CSF-1R). The chemical structure of sunitinib malate is shown in FIG 1 [1, 2].

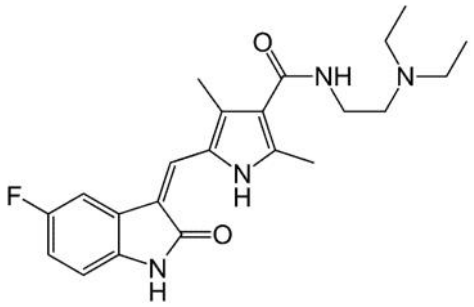

Fig: 1 Structure of Sunitinib

2. MATERIALS &METHODS

Chemicals and reagents:

A pure sample of sunitinib Monohydrate was obtained from Hetero drugs private ltd, Hyderabad, India. The marketed formulation of sutent-50 mg capsules was procured from a local pharmacy store. HPLC grade of acetonitrile was procured from Thermo Fisher Scientific India Pvt. Ltd., Mumbai, India. HPLC grade water and methanol were purchased from Merck Specialties Pvt.Ltd, Mumbai, Ind

Instrumentation:

UV-Visible Spectrophotometer (Elico SL-210) Shimadzu electronic balance (AX-200) was used for weighing purposes. Ultra Sonicator (PCI Ltd., Mumbai) was used for the preparation and degassing of samples.

UV spectrophotometric method:

Selection of solvent: Sunitinib is tested for its solubility in different solvents. The solution of Sunitinib was prepared in methanol, Acetonitrile, and water. UV spectra of each were recorded and scanned between 200 – 800nm. Among these solvents, methanol gave a good response. Hence, methanol was selected as a solvent for further studies [3, 4].

Selection of wavelength (λmax): The stock solution was prepared for Sunitiniband was scanned in UV region (200 – 800nm). The peak was obtained at a wavelength of 431nm against methanol as blank. The wavelength of 431nm was selected for Sunitinibfor further studies. The overlain spectra was shown in the figure 2

Preparation of stock solution: Standard stock solution of Sunitinib was prepared by dissolving 0.1mg of drug in 10ml of methanol and was sonicated for proper dilution of drug in an ultra Sonicator for about 15mins. The concentration of the above-prepared solution of Sunitinibis 100µ/ml [5].

Dilutions: Appropriate known volumes of aliquots from the first dilution were transferred to separate 10ml volumetric flasks. The volume was adjusted to the mark with methanol to a series of concentrations in the range of 0.1-1µg/ml. The solution was scanned in the UV range 200-800nm [The absorbance of these solutions was recorded at 431nm and calibration curve was plotted, absorbance vs concentration as shown in Fig. 3 (B) [6, 7].

Linearity: Standard solutions of Sunitinib were prepared in the concentration range of 0.1–10µg/ml. The volume in each volumetric flask were made with methanol and mixed. Calibration curves were plotted by taking concentration on X-axis and absorbance on Y-axis. The correlation coefficient was found to be 0.9997 at 431nm. The slope was found to be 0.0706 and intercept was found to be 0.033 at 431nm. The overlain spectra of linearity is shown in Fig.3 (A)

Range: The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of the analyte in the sample (including these concentrations) for which it has been shown that the analytical procedure has a suitable level of precision, accuracy and linearity [8].

Accuracy: The accuracy studies were carried out at three different levels i.e., at 80%, 100% and 120% levels. To ensure the reliability of the above method recover studies were carried out by mixing a known quantity of the standard drug with the preanalyzed sample formulation and the contents were reanalyzed by the proposed method. The overlain spectra and calibration curve of marketed formulation was shown [9, 10, 11] in Fig 4 (A) (B) respectively.

Precision: The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision of the method was determined in terms of repeatability and intraday and interday precisions. The data was represented in table 3.

Intraday precision: Intraday precision was found by carrying out the analysis of the intermediate concentration for 10 times on the same day. Mean and standard deviation were then calculated.

Interday precision: Intraday precision was found by carrying out the analysis of the intermediate concentration for 10 times for 5 different days. Mean and standard deviation were then calculated.

Repeatability: Repeatability was found by carrying out the analysis of the intermediate concentration for 10 times on the same day for every 5 minutes. Mean and standard deviation were then calculated [12, 13].

Limit of Detection (LOD): The LOD of sunitinib was determined by using standard deviation of response and slope. The experimental LOD was found to be 0.11048

Limit of Quantification (LOQ): The LOQ of sunitinib was determined by using standard deviation of response and slope. The experimental LOQ was found to be 0.3348

Specificity: Specificity is the ability to access unequivocally the analyte in the presence of components that maybe expected to be present. Specificity is determined by analysing 0.5µg/ml concentration repeatedly and measuring the absorbance at 431nm wavelength. The specificity of the method for determination of sunitinib in tablet dosage form was determined by comparing the spectrum of capsule dosage form solution with that of standard solution. The sample spectrum was checked for any interference from the excipients. [14, 15]

Assay of formulation: Twenty sunitinib capsules each containing 50mg of sunitinib were weighed, average weight was calculated and powdered. A quantity equivalent to 50mg of sunitinib is weighed and transferred into 50ml volumetric flask and is dissolved in methanol to obtain final concentration of 1000µg/ml. The solution was further diluted with methanol to get a solution having concentration of 10µg/ml of sunitinib. This procedure was repeated at different concentrations of 0.2ppm and 0.6 ppm. The absorbance was measured at wavelength 431nm. A graph has been plotted with Absorbance on X-axis and Concentration on Y-axis. [16, 17]

Forced degradation studies:

Stress testing was suggested by ICH guidelines as a means to determine the intrinsic stability of drug substances. Acid degradation (2 N HCl, refluxed at 60 °C for 30 minutes), alkali degradation (2 N NaOH, refluxed at 60 °C for 30 minutes), peroxide degradation (20% hydrogen peroxide heated at 60 °C for 30 minutes), thermal degradation (samples arranged in a hot air oven at 105 °C for 6 hour), photolytic degradation (sample in an ultraviolet chamber for seven days), and hydrolysis were all applied to the solution

of the standard in these investigations. The peak areas of the so-stressed samples were determined and compared to the standard's peak areas. The data was represented in table 5, and the degradation chromatograms are represented in Fig 5.

3. RESULTS AND DISCUSSION

The analysis of sunitinib was achieved by using double beam UV-Visible spectrophotometer. The linearity was checked in different concentrations and Beer's law was obeyed in the concentration range of 0.1-10µg/ml for sunitinib. The slope, intercept and correlation coefficient values of sunitinib are 0.0706, 0.033 and 0.9995 respectively. The precision studies were carried in terms of intraday, interday and repeatability. The percentage relative standard deviation (%RSD) values were found to be less than 2, which indicate that the method is precise. The recovery studies were carried out to ensure that reproducibility and reliability of the method by adding known amount of standard drugs and analysis was carried out as per formulation procedure. The recovery values were within the limits indicating that the method is accurate. The robustness and ruggedness were carried out and was found to be within the limits. LOD and LOQ were carried out according to ICH guidelines and were found to be 0.11048 and 0.3348 [18, 19, 20].

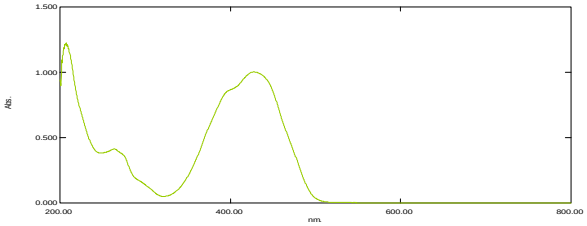

Fig 2: Overla in spectra of Sunitinib inmethanol

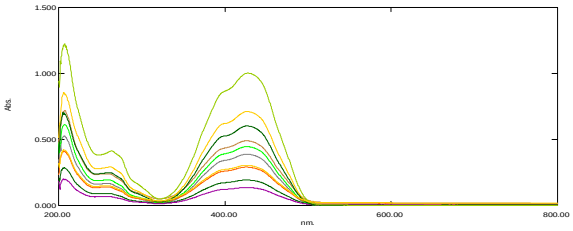

Fig 3(A): Overla in inearity spectra

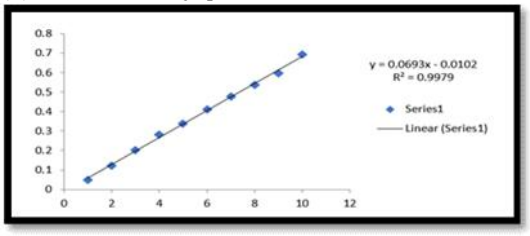

Fig 3(B): Calibration Curve

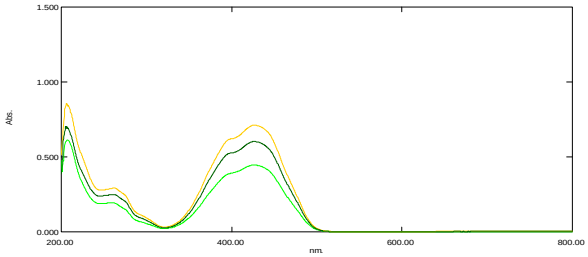

Fig 4 (A): Accuracy of Sunitinib (zero order derivatives)

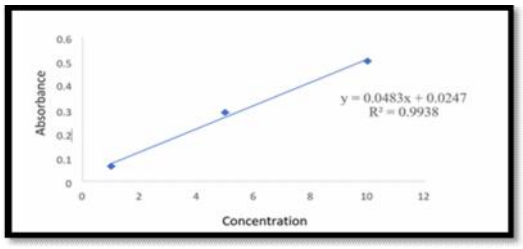

Fig 4 (B): Calibration curve of marketed product

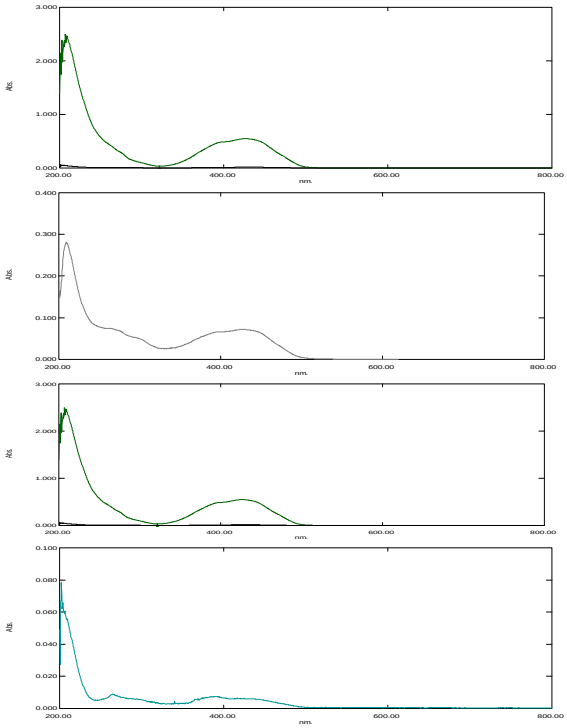

Fig 5: Typical chromatograms obtained during the forced degradation studies (A; Acid degradation, B; Base degradation, C; Neutral degradation, D; Photolytic degradation)

Table 1: Linearity data of Sunitinib (zero order derivative)

s.no	Concentration	Absorbance
1	0.1	0.090
2	0.2	0.168
3	0.3	0.248
4	0.4	0.323
5	0.5	0.408
6	0.6	0.489

7	**0.7**	**0.564**
8	**0.8**	**0.650**
9	**0.9**	**0.724**
10	**10**	**0.809**
Slope: 0.0693x **Intercept: 0.0102** **$R^{2:}$0.979**		

Table 2: Accuracy results for Sunitinib (zero order derivative)

Concentration (µg/ml)	Absorbance at 431 nm	Mean	Amount recovered	% Recovery
0.5	0.408	0.407	1.0 (µg/ml)	100.2%
0.5	0.407			
0.5	0.408			
0.6	0.489	0.488	1.2 (µg/ml)	100.3%
0.6	0.488			
0.6	0.489			
0.7	0.564	0.564	0.9 (µg/ml)	99.5%
0.7	0.564			
0.7	0.564			

Table 3: Repeatability of Sunitinib (zero order derivative)

Concentration (µg/ml)	Absorbance
0.2(µg/ml)	0.168
0.2 (µg/ml)	0.169
0.2 (µg/ml)	0.168
0.2 (µg/ml)	0.168
0.2 (µg/ml)	0.168
0.2 (µg/ml)	0.168
Mean	0.168
Standard deviation	0.0004
% RSD	0.23%

Table 4: Ruggedness of Sunitinib (zero order derivative)

Parameter	Concentration (µg/ml)	Absorbance	Mean	Standard deviation	Relative standard deviation
Analyst 1	0.5µg/ml	0.564	0.564	0.0004	0.07%
Analyst 2	0.5µg/ml	0.564			
Instrument 1	0.5µg/ml	0.564			
Instrument 2	0.5µg/ml	0.565			
Lab 1	0.5µg/ml	0.564			
Lab 2	0.5µg/ml	0.564			

Table 5: Degradation studies of Sunitinib

S.No.	Degradation condition	% Degraded
1	Acid	-
2	Alkaline	-
3	Neutral	13%
4	Photolytic	-
5	Dry heat	-
6	Oxidative	-

4. CONCLUSION

The estimation of Sunitinib was done by UV-method. In UV method the methanol is selected as the solvent. In this method Sunitinib was quantified at 431nm. The elevation of obtained values suggests that the proposed UV spectrophotometric method provided simple, precise and accurate quantitative analytical method for the determination of Sunitinib in dosage form. After validating proposed method as per ICH guidelines and correlating the obtained values with the standard values, satisfactory results were obtained. The sample recoveries in all formulations were in good agreement with their respective label claims and they suggested no interference off or mulation excipients in the estimation. The drug stability studies were performed according to ICH guidelines and were found to be stable. Hence, the method can be easily and conveniently adopted for routine estimation of Sunitinib in capsule dosage form. The method does not involve any tedious procedural steps, do not require any extra reagents or longer analysis time and a very simple instruments are required.

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