UV-VISIBLE SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION OF SUNITINIB MALATE IN BULK AND FORMULATION

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ABSTRACT

A simple and sensitive spectroscopic method in UV-visible region has been developed for the determination of Sunitinib malate in bulk and formulation. A method was performed with methanol which shows maximum absorbance at 432nm. The linearity was found to be $R^2 = 0.999$. The proposed methods have been successfully applied for the assay of the drug in pharmaceutical formulations. No interference was observe during the method development and validation of the drug. Results of analysis were validated statistically and thorough recovery studies.

KEYWORDS: UV-Visible Spectrophotometer, Sunitinib Malate, Methanol.

1. INTRODUCTION

SUTENT, an oral multi-kinase inhibitor targeting several receptor tyrosine kinases (RTK), is the malate salt of sunitinib. Sunitinib malate is described chemically as Butanedioic acid,hydroxy-,,(2S)-, compound with N-[2-(diethylamino)ethyl]-5-[(Z)-(5-fluoro-1,2-dihydro-2-oxo-3H-indol-3-ylidine)methyl]-2,4-dimethyl-1H-pyrrole-3-carboxamide(1:1). The molecular formula is $C_{22}H_{27}FN_4O_2\cdot C_4H_6O_5$ and the molecular weight is 532.6 Daltons. The chemical structure of sunitinib malate is:
Sunitinib malate is a yellow to orange powder with a pKa of 8.95. The solubility of sunitinib malate in aqueous media over the range pH 1.2 to pH 6.8 is in excess of 25 mg/mL. The log of the distribution coefficient (octanol/water) at pH 7 is 5.2.

**Clinical data**
Trade names: SUTENT
Routes: ORAL

**Chemical data**
FORMULA: $\text{C}_{22}\text{H}_{27}\text{FN}_{4}\text{O}_{2}$
MOL.MASS: 398.474 gm/mol, 532.561 gm/mol (malate)

5. METHODOLOGY
The Objective of this study is to validate the method used for determination of Assay of Sunitinib malate in Sunitinib malate tablets by U.V method.

**SUMMARY OF TEST METHOD**
I. Reagents and Standard – Sunitinib malate Tablet
   a. Sunitinib Working Standards
   b. Methanol

**Diluent**
Use the Methanol as Diluent.

**Instrument used**
LAB INDIA/UV-VISIBLE SPECTROPHOTOMETER [ELICO].
II. Preparation of the Sunitinib Standard & Sample Solution

i. Standard Solution Preparation

a. Accurately weigh and transfer 10mg of Sunitinib Working standard into a 100ml volumetric flask add about 70mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

b. Further pipette 3ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

ii. Sample preparation

Accurately weigh and transfer equivalent to 10mg of Tablet powder into a 100ml volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 3ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

6. RESULTS AND DISCUSSION

With standard scanning range from 200-400nm, it was found that \( \lambda_{\text{max}} \) is at 440nm.

Blank

![Scan Spectrum Curve](image_url)

Graph no. 6.1.
7. METHOD VALIDATION

7.1. PRECISION

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. The measured standard deviation can be subdivided into 3 categories: repeatability, intermediate precision and reproducibility.
Preparation of Standard Solution

Accurately weigh and transfer 10mg of Sunitinib working standard into a 100ml volumetric flask add about 70 mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 3ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Measure the absorbance of the Sunitinib standards at 281 nm for five times and calculate the %RSD. The %RSD for the five replicate absorbance was found to be within the specified limits.

SYSTEM Precision

Precision 2

Graph no. 7.1.

The results are summarized (for Sunitinib)

Table no.7.1.1.

<table>
<thead>
<tr>
<th>Reading</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reading-1</td>
<td>0.305</td>
</tr>
<tr>
<td>Reading-2</td>
<td>0.302</td>
</tr>
<tr>
<td>Reading-3</td>
<td>0.302</td>
</tr>
<tr>
<td>Reading-4</td>
<td>0.3</td>
</tr>
<tr>
<td>Reading-5</td>
<td>0.303</td>
</tr>
<tr>
<td>Average</td>
<td>0.3024</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.001817</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.600724</td>
</tr>
</tbody>
</table>
Acceptance Criteria
The % RSD for the area of five standard injections results should not be more than 2%

7.2. ACCURACY
Accuracy can also be described as the closeness of agreement between test results generated by the method and the true value.

I. Preparation of standard stock solution
Accurately weigh and transfer 10mg of Sunitinib working standard into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 3ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

II. Preparation sample solutions
i. For preparation of 50% solution (With respect to target Assay concentration)
   a. Accurately weigh and transfer 5.0mg of Sunitinib sample into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).
   b. Further pipette 3ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

ii. For preparation of 100% solution (With respect to target Assay concentration)
   a. Accurately weigh and transfer 10.0mg of Sunitinib sample into a 50ml volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).
   b. Further pipette 3ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

iii. For preparation of 150% solution (With respect to target Assay concentration)
   a. Accurately weigh and transfer 15.0mg of Sunitinib sample into a 100ml volumetric flask add about 70ml of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).
   b. Further pipette 3.0ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.
III. Procedure

Measure the Absorbance of the standard solution, Accuracy -50%, Accuracy - 100% and Accuracy - 150% solutions at 281nm. Calculate the amount found and amount added for Sunitinib. Calculate the individual recovery and mean recovery values.

Fig. no. 7.2(a).

Accuracy 50% Sample 2

Graph no. 7.2.2.
Accuracy 100% Sample 2

![Graph no. 7.2.5.](image)

Accuracy 150% Sample 2

![Graph no. 7.2.8.](image)

The results are summarized (for Sunitinib)

**Table no. 7.2.1.**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Amount added (ppm)</th>
<th>Amount found (ppm)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% Sample 1</td>
<td>5</td>
<td>5.078</td>
<td>101.57</td>
</tr>
<tr>
<td>50% Sample 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50% Sample 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Sample 1</td>
<td>10</td>
<td>10.098</td>
<td>100.98</td>
</tr>
<tr>
<td>100% Sample 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% Sample 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150% Sample 1</td>
<td>15</td>
<td>15.17</td>
<td>101.17</td>
</tr>
<tr>
<td>150% Sample 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>150% Sample 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Acceptance Criteria
The % Recovery for each level should be between 98.0 to 102.0%.

7.3. LINEARITY
It gives us the relation between the concentration of the samples tested and absorbance they show. A linear slope should be obtained when the graph is drawn between the concentration and absorbance.

Fig. no. 7.3(a).

a. Preparation of stock solution
Accurately weigh and transfer 10mg of Sunitinib sample into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Fig.no.7.3(b).
Level – I: Preparation of 1µg/ml Sunitinib solution
Further pipette 1.0ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – II: Preparation of 2µg/ml Sunitinib solution
Further pipette 2.0ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – III: Preparation of 3µg/ml Sunitinib solution
Further pipette 3.0ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – IV: Preparation of 4µg/ml Sunitinib solution
Further pipette 4.0ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – V: Preparation of 5µg/ml Sunitinib solution
Further pipette 5.0ml of the Sunitinib stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

a. Procedure
- Measure the absorbance of the above levels at 430nm
- Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

Linearity overlay

Graph no. 7.3.1.
LINEARITY RESULTS: (for Sunitinib)

Table no. 7.3.1.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I (50%)</td>
<td>1µg/ml</td>
<td>0.137</td>
</tr>
<tr>
<td>2</td>
<td>II (75%)</td>
<td>2µg/ml</td>
<td>0.264</td>
</tr>
<tr>
<td>3</td>
<td>III (100%)</td>
<td>3µg/ml</td>
<td>0.391</td>
</tr>
<tr>
<td>4</td>
<td>IV (125%)</td>
<td>4µg/ml</td>
<td>0.499</td>
</tr>
<tr>
<td>5</td>
<td>V (150%)</td>
<td>5µg/ml</td>
<td>0.634</td>
</tr>
</tbody>
</table>

Correlation Coefficient 0.999

Acceptance Criteria

Correlation coefficient should be not less than 0.999.

![Absorbance Graph](https://example.com/graph.png)

Graph no. 7.3.7.

7.4. ROBUSTNESS

As part of the Robustness, deliberate change in the Wave length

a) The Wave length was varied at 428 nm to 432nm

Standard solution 3ppm of Sunitinib was prepared and analysed using the varied wave length along with method wave length.

b) The results are summarized

On evaluation of the above results, it can be concluded that the variation in wave length affected the method significantly. Hence it indicates that the method is robust even by change in the wave length ±1

The method is robust only in wave length condition.
7.5. RUGGEDNESS
Ruggedness is defined by the USP as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials.

Id precision 1

Graph no. 7.5.1: $\lambda_{\text{max}} = 432$nm, absorbance$ = 0.303$

Id precision 2

Graph no. 7.5.2: $\lambda_{\text{max}} = 432$nm, absorbance$ = 0.306$. 
Id precision 3

Graph no.7.5.3: $\Lambda_{max}=432\text{nm}$, absorbance $= 0.305$.

7.6. LOD: (Limit of Detection)
The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified.

**Calculation**
LOD = 3*Standard deviation/Slope=value
LOD = 3*0.001817/0.122 = 0.044µg/ml

7.7. LOQ: (limit of Quantitation)
The limit of quantitation is the minimum concentration of the sample that produces quantitative measurements.

**Calculation**
LOQ = 10*standard deviation/slope value
LOQ = 10*0.001817/0.122 = 0.153µg/ml

7.8. ASSAY

Assay % =

<table>
<thead>
<tr>
<th>AT</th>
<th>X</th>
<th>WS</th>
<th>X</th>
<th>DT</th>
<th>X</th>
<th>P</th>
<th>X</th>
<th>Avg. Wt</th>
<th>X</th>
<th>100</th>
<th>Label Claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS</td>
<td></td>
<td>DS</td>
<td></td>
<td>WT</td>
<td></td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Where
AT = Peak Area of Sunitinib obtained with test preparation
AS = Peak Area of Sunitinib obtained with standard preparation
WS = Weight of working standard taken in mg
WT = Weight of sample taken in mg
DS = Dilution of Standard solution
DT = Dilution of sample solution
P = Percentage purity of working standard.

Assay Results: (Sunitinib)

\[
\begin{array}{ccccccc}
& AT & WS & DT & P & \text{Avg. Wt} & \text{Label Claim} \\
\hline
\text{AS} & \text{DS} & \text{WT} & 100 & & & \\
\end{array}
\]

Procedure
Measure the absorbance of the Sunitinib standard and sample at 281 nm and calculate the %Assay by using the formulae.

SAMPLE AND STANDARD DETAILS [Table no. 7.8.1]

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Samples</th>
<th>B.NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sunitinib tablets 50mg</td>
<td>_</td>
</tr>
<tr>
<td>2</td>
<td>Sunitinib working standards</td>
<td>--</td>
</tr>
</tbody>
</table>

8. TABLE
OPTICAL CHARACTERISTICS AND PRECISION DATA OF SUNITINIB MALATE WITH METHANOL

Table no. 8.1.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lamda max (λ_{max})</td>
<td>432 nm</td>
</tr>
<tr>
<td>Beer’s Law (μg/ml)</td>
<td>50% - 150%</td>
</tr>
<tr>
<td>Regression equation</td>
<td>Y = 0.122X + 0.016</td>
</tr>
<tr>
<td>Slope (M)</td>
<td>0.0122</td>
</tr>
<tr>
<td>Intercept (C)</td>
<td>0.016</td>
</tr>
<tr>
<td>Correlation coefficient (R^2)</td>
<td>0.999</td>
</tr>
<tr>
<td>%RSD</td>
<td>0.6007</td>
</tr>
<tr>
<td>LOD</td>
<td>0.044μg/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>0.153μg/ml</td>
</tr>
<tr>
<td>Assay</td>
<td>99.8%</td>
</tr>
</tbody>
</table>
9. SUMMARY
A method development is quantitative method in which unknown concentrations of a given species by absorption spectrophotometry was determined. A choice of absorption band was selected at which absorbance measurements were made when several absorption bands of suitable absorptivity are present and at the selected absorption band the highest wavelength was detected i.e., at 432nm.

Various validation parameters have been calculated and their limits were justified. The values obtained through linearity and precision were within Beer-Lambert Law’s range. A total of eight methods were performed as per the ICH guidelines. The correlation coefficient was found to be 0.6 and limit of detection, limit of quantitation were below 10.

Different analyst performed the validation procedures and found that the results were not affected majorly. Different pH conditions were adopted and the results obtained showed no differentiations.

We were thus successful in developing a precise, accurate and simple methods for validating the active pharmaceutical ingredient, SUNITINIB MALATE, in its respective formulation.

10. CONCLUSION
A simple UV-Visible spectrophotometric method was developed for the determination of Sunitinib malate in pure and its dosage form using methanol. The absorbance formed was maximum at wavelength 432nm against the corresponding blank.

All the above methods were simple, precise and rapid for detection of Sunitinib malate in its pure and dosage form. The statistical parameter and recovery study data clearly indicates reproducibility and accuracy of the method. These can thus be conveniently adopted for routine analysis of Sunitinib malate in pure as well as in its dosage form.

11. ACKNOWLEDGEMENT
We are thankful to the staff of the Deccan school of Pharmacy for their help and kind cooperation for the guidance and immense encouragement given during the course of this project work.
11. BIBLIOGRAPHY


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15. FDA approves sutent for rare type of pancreatic cancer.