*ejpmr, 2025, 12(5), 226-233*

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF SUNITINIB MALATE IN BULK AND MARKETED PHARMACEUTICAL DOSAGE FORMS

**Madireddy Mamata***

Associate Professor & HOD, Department of Pharmaceutical Analysis, Trinity College of Pharmaceutical Sciences, Bandarikunta, Peddapalli, Telangana (St), India, 505172.

***Corresponding Author: Madireddy Mamata**

Associate Professor & HOD, Department of Pharmaceutical Analysis, Trinity College of Pharmaceutical Sciences, Bandarikunta, Peddapalli, Telangana (St), India, 505172.

**Article Received on 04/03/2025** **Article Revised on 24/03/2025** **Article Accepted on 14/04/2025**

## ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Sunitinib malate in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm column with Methanol and DMSO in the ratio of 60:40 as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 430nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 6.0 min. The retention time of Sunitinib was found to be 2.570min. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 0.8 and 0.24ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.79%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%.The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Sunitinib in bulk and marketed pharmaceutical dosage form dosage form.

**KEYWORD:-** Sunitinib, RP-HPLC, Validation, Accuracy, Precision, Robustness, ICH Guidelines.

## INTRODUCTION

Sunitinib is an anti-cancer medication. It is a small-molecule, multi-targeted receptor tyrosine kinase (RTK) inhibitor that was approved by the FDA for the treatment of renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumor (GIST) in January 2006. Sunitinib was the first cancer drug simultaneously approved for two different indications. Sunitinib is approved for the treatment of metastatic renal cell carcinoma. Renal cell carcinoma is generally resistant to chemotherapy or radiation. Before RTKs, metastatic disease could only be treated with the cytokines interferon alpha (IFNα) or interleukin-2. However, these agents demonstrated low rates of efficacy (5%-20%). Sunitinib inhibits cellular signalling by targeting multiple receptor tyrosine kinases (RTKs). These include all receptors for platelet-derived growth factor (PDGF-Rs) and vascular endothelial growth factor receptors (VEGFRs), which play a role in both tumor angiogenesis and tumor cell proliferation. The simultaneous inhibition of these targets therefore reduces tumor vascularization and triggers cancer cell apoptosis and thus results in tumor shrinkage. The Chemical Structure of Sunitinib is shown in following Fig-1.

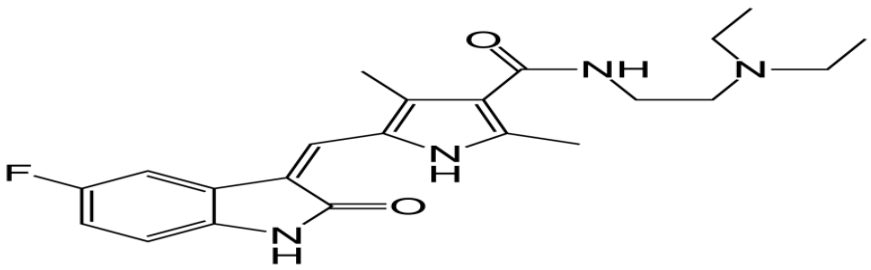


**Fig. 1: Chemical structure of sunitinib.**

Literature survey revealed that very few analytical methods have been reported for the estimation of Sunitinib in pure drug and pharmaceutical dosage forms using liquid chromatography. The aim of the present work is to develop a validated simple, precise and accurate RP-HPLC method with UV detection for the determination of Sunitinib in bulk and pharmaceutical dosage form.

**Experimental**

**Table 1: List of instrument used.**

| S. No. | Instruments/ Equipments/ Apparatus |
|---|---|
| 1. | **HPLC** with Empower2 Software with Isocratic with UV-Visible Detector (Waters). |
| 2. | **T60-LAB INDIA** UV – Vis spectrophotometer |
| 3. | Electronic Balance **(SHIMADZU ATY224)** |
| 4. | Ultra Sonicator **(Wensar wuc-2L)** |
| 5. | Thermal Oven |
| 6. | Symmetry ODS RP $C_{18}$,5µm, 15mm x 4.6mm i.d. |
| 7. | $P^H$ Analyzer **(ELICO)** |
| 8. | Vacuum filtration kit **(BOROSIL)** |

**Table 2: List of chemicals used.**

| S. No. | Name | Specifications | | Manufacturer/Supplier |
|---|---|---|---|---|
| | | Purity | Grade | |
| 1. | Doubled distilled water | 99.9% | HPLC | Sd fine-Chem ltd; Mumbai |
| 2. | Methanol | 99.9% | HPLC | Loba Chem; Mumbai. |
| 3. | Dipotassium hydrogen orthophosphate | 96% | A.R. | Sd fine-Chem ltd; Mumbai |
| 4. | Acetonitrile | 99.9% | HPLC | Loba Chem; Mumbai. |
| 5. | Potassium dihydrogen orthophosphate | 99.9% | A.R. | Sd fine-Chem ltd; Mumbai |
| 6. | Sodium hydroxide | 99.9% | A.R. | Sd fine-Chem ltd; Mumbai |
| 7. | DMSO | 99.9% | A.R. | Loba Chem; Mumbai. |
| 8. | Hydrogen Peroxide | 99.9% | A.R. | Loba Chem; Mumbai. |

**Method development and its validation for sunitinib by RP-HPLC**

**Selection of wavelength**

The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis. It scanned in the UV spectrum in the range of 200 to 800nm. This has been performed to know the maxima of Sunitinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Sunitinib. The scanned UV spectrum is attached in the following page.

**Sample & Standard Preparation for the UV-Spectrophotometer Analysis**

25 mg of Sunitinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.[5]

**Optimization of chromatographic conditions:** The chromatographic conditions were optimized by different means.[6] (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

**Table 3: Summary of process optimization.**

| Column Used | Mobile Phase | Flow Rate | Wave length | Observation | Result |
|---|---|---|---|---|---|
| Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column. | Methanol : Acetonitrile = 40 : 60 | 1.0ml/min | 430nm | Very Low response | Method rejected |
| Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column. | Methanol : Acetonitrile = 55 : 45 | 1.0ml/min | 430nm | Low response | Method rejected |
| Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column. | Acetonitrile : Water = 50:50 | 1.0ml/min | 430nm | Tailing peaks | Method rejected |
| Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column. | Methanol : Water = 70:30 | 1.0ml/min | 430nm | Resolution was not good | Method rejected |
| Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, | Methanol: DMSO = 900:10 | 1.0ml/min | 430nm | Tailing peak | Method rejected |

| | | | | | |
|---|---|---|---|---|---|
| Column. | | | | | |
| Symmetry $C_{18,}$ ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column. | Methanol: DMSO = 60:40 | 1.0ml/min | 430nm | Nice peak | Method accepted |

**Preparation of mobile phase**

600ml of HPLC Grade Methanol and 400ml DMSO were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration.[7]

**Validation of analytical method**

The developed method was further validated as per ICH guidelines for accuracy, Precision, LOD, LOQ, specificity, sensitivity, and robustness.

## RESULTS AND DISCUSSION

**Optimization of analytical method**

**Selection of wavelength**

While scanning the Sunitinib solution we observed the maxima at 430nm. The UV spectrum has been recorded on T60-LAB INDIA make UV – Vis spectrophotometer model UV-2450.

**Summary of optimized chromatographic conditions**

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

**Table- 4: Summary of optimised chromatographic conditions.**

| | |
|---|---|
| Mobile phase | Methanol: DMSO = 60:40 |
| Column | Symmetry ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm, 5µm |
| Column Temperature | Ambient |
| Detection Wavelength | 430 nm |
| Flow rate | 1.0 ml/ min. |
| Run time | 06 min. |
| Temperature of Auto sampler | Ambient |
| Diluent | Mobile Phase |
| Injection Volume | 10µl |
| Type of Elution | Isocratic |
| Retention time | 2.570 minutes |

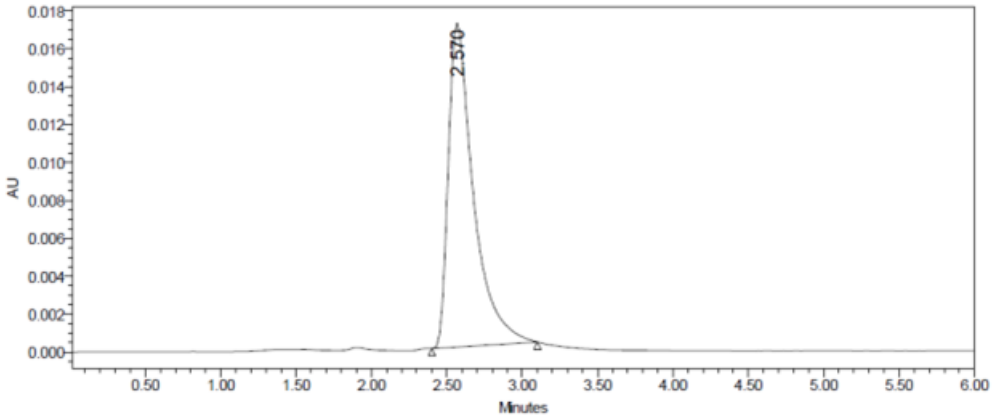


**Fig. 2: Chromatogram of sunitinib in optimized condition.**

**Observation:** The selected and optimized mobile phase was Methanol: DMSO = 60:40 and conditions optimized were flow rate (1.0 ml/minute), wavelength (430nm), Run time was 06 mins. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug.

**Method validation**

**1. Accuracy**

**Recovery study**

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Sunitinib were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation y = 19423x + 5444.4. The results were shown in table-5.

**Table 5: Readings of accuracy.**

| Conc. In ppm | Conc. Found | Peak Area | | % Recovery |
|---|---|---|---|---|
| 8 | 8.035 | 161523 | | 100.437 |
| 8 | 8.153 | 163815 | | 101.912 |
| 8 | 8.061 | 162023 | | 100.762 |
| | | | Avg. | 101.037 |
| | | | S.D | 0.775 |
| | | | %RSD | 0.767046 |
| **Conc. In ppm** | **Conc. Found** | **Peak Area** | | % Recovery |
| 10 | 9.930 | 198315 | | 99.30 |
| 10 | 10.033 | 200320 | | 100.33 |
| 10 | 10.044 | 200540 | | 100.44 |
| | | | Avg. | 100.0233 |
| | | | S.D | 0.628835 |
| | | | %RSD | 0.628688 |
| **Conc. In ppm** | **Conc. Found** | **Peak Area** | | % Recovery |
| 12 | 11.981 | 238151 | | 99.841 |
| 12 | 12.066 | 239819 | | 100.55 |
| 12 | 12.215 | 242712 | | 101.791 |
| | | | Avg. | 100.7273 |
| | | | S.D | 0.987021 |
| | | | %RSD | 0.979894 |

## 2. Precision

### 2.1. Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Sunitinib (API). The percent relative standard deviation was calculated for Sunitinib are presented in the table-6.

**Table 6: Readings of repeatability.**

| HPLC Injection Replicates of Sunitinib | Retention Time (Minutes) | Peak Area (AUC) |
|---|---|---|
| Replicate – 1 | 2.572 | 197236 |
| Replicate – 2 | 2.570 | 197762 |
| Replicate – 3 | 2.573 | 195969 |
| Replicate – 4 | 2.570 | 194724 |
| Replicate – 5 | 2.574 | 198327 |
| Replicate – 6 | 2.573 | 198711 |
| Average | | 197121.5 |
| Standard Deviation | | 1515.213 |
| % RSD | | 0.768667 |

**Observation:** The repeatability study which was conducted on the solution having the concentration of about 10µg/ml for Sunitinib (n =6) showed a RSD of 0.768667% for Sunitinib. It was concluded that the analytical technique showed good repeatability.

### 2.2. Intermediate Precision/Ruggedness

#### 2.2.1. Intra-Day & Inter-Day

The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Sunitinib revealed that the proposed method is precise.

**Intra Day/Day-1/Analyst-1**

**Table 7: Results of intermediate precision analyst 1 for sunitinib.**

| S. No. | Peak Name | RT | Area (µV*sec) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|
| 1 | Sunitinib | 2.580 | 206587 | 3102 | 1.16 |
| 2 | Sunitinib | 2.597 | 206859 | 2986 | 1.18 |
| 3 | Sunitinib | 2.581 | 207854 | 3054 | 1.13 |
| 4 | Sunitinib | 2.573 | 208965 | 3154 | 1.14 |
| 5 | Sunitinib | 2.590 | 206547 | 3157 | 1.12 |
| 6 | Sunitinib | 2.572 | 209865 | 3268 | 1.18 |

| | | | | | |
|---|---|---|---|---|---|
| Mean | | | 207779.5 | | |
| Std. Dev. | | | 1381.9336 | | |
| % RSD | | | 0.665 | | |

**Inter Day/Day-2/Analyst-2**

**Table 8: Results of Intermediate Precision Analyst 2 for Sunitinib.**

| S. No. | Peak Name | RT | Area (µV*sec) | USP Plate count | USP Tailing |
|---|---|---|---|---|---|
| 1 | Sunitinib | 2.580 | 215263 | 3215 | 1.17 |
| 2 | Sunitinib | 2.597 | 214235 | 3652 | 1.19 |
| 3 | Sunitinib | 2.581 | 213254 | 3496 | 1.15 |
| 4 | Sunitinib | 2.573 | 212367 | 3258 | 1.16 |
| 5 | Sunitinib | 2.590 | 213698 | 3365 | 1.17 |
| 6 | Sunitinib | 2.572 | 217456 | 3524 | 1.14 |
| Mean | | | 214378.8 | | |
| Std. Dev. | | | 1791.516 | | |
| % RSD | | | 0.835678 | | |

**Observation:** Intraday and interday studies[16] show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength was found to be precise.

### 3. Linearity & Range

The calibration curve showed good linearity in the range of 6 – 14 µg/ml, for Sunitinib (API) with correlation coefficient ($r^2$) of 0.999 (Fig-4). A typical calibration curve has the regression equation of y = 19423x + 5444.4 for Sunitinib.

**Table 9: Linearity results.**

| CONC.(µg/ml) | MEAN AUC (n=6) |
|---|---|
| 0ppm | 0 |
| 6ppm | 129013 |
| 8ppm | 166523 |
| 10ppm | 198315 |
| 12ppm | 234151 |
| 14ppm | 275819 |

**Linearity Plot**

The plot of Concentration (x) versus the Average Peak Area (y) data of Sunitinib is a straight line.

Y = mx + c

Slope (m) = 19423

Intercept (c) = 5444.4

Correlation Coefficient (r) = 0.99

**Validation criteria:** The response linearity is verified if the Correlation Coefficient is 0.99 or greater.

**Conclusion:** Correlation Coefficient (r) is 0.99, and the intercept is 5444.4. These values meet the validation criteria.

### 4. Specificity

The system suitability for specificity was carried out to determine whether there was any interference of any impurities in the retention time of the analytical peak.

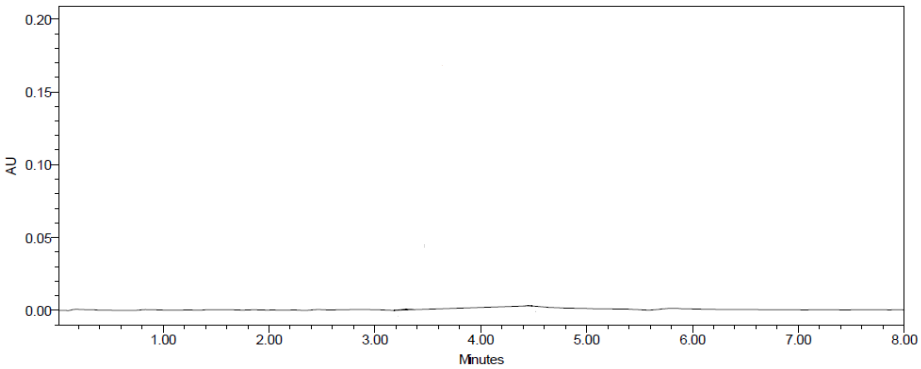


**Fig. 5: Chromatogram for blank solution.**

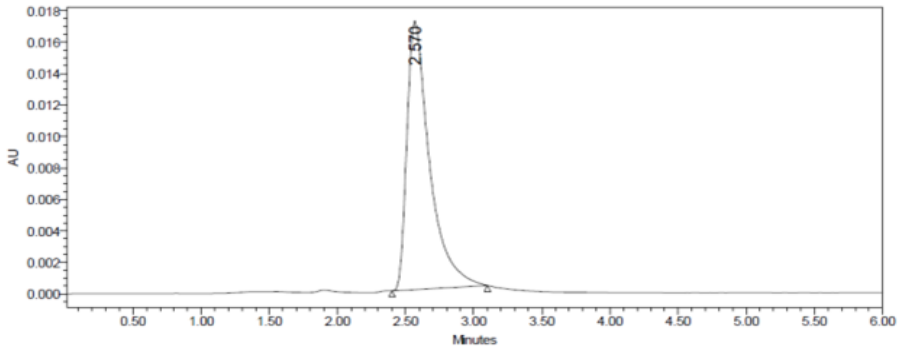


**Fig. 6: Chromatogram of sunitinib standard solution.**

**Observation:** The study was performed by injecting blank and standard into the system. There was no interference of any peak in the blank with the retention time of the analytical peaks.

**5. Method robustness**

Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection (±2nm) & organic phase in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-10, % RSD < 2%) the developed RP-HPLC method for the analysis of Sunitinib (API).

**Table 10: Results for robustness for sunitinib.**

| Parameter used for sample analysis | Peak area | Retention time | Theoretical plates | Tailing factor |
|---|---|---|---|---|
| Actual Flow rate of 1.0 mL/min | 203654 | 2.570 | 2915 | 1.16 |
| Less Flow rate of 0.9 mL/min | 265876 | 2.573 | 3652 | 1.19 |
| More Flow rate of 1.1 mL/min | 298653 | 2.631 | 3854 | 1.20 |
| Less Organic Phase | 315874 | 2.590 | 3945 | 1.17 |
| More Organic Phase | 326985 | 2.602 | 3487 | 1.19 |

**6. LOD & LOQ**

**LOD:** The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

**LOD = 3.3 × σ / s**

**LOQ:** The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

**LOQ = 10×σ/S**

**Observation:** The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

**7. System suitability parameter**

System suitability was carried out with six injections of solution of 100% concentration having 10µg/ml of Avapritinib in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table-11.

**Table 11: Data of system suitability parameter.**

| S. No. | Parameter | Limit | Result |
|---|---|---|---|
| 1 | Asymmetry | T ≤ 2 | Sunitinib=0.23 |
| 2 | Theoretical plate | N > 2000 | Sunitinib=2987 |
| 3 | Tailing Factor | T<2 | Sunitinib=1.17 |

**8. Estimation of sunitinib in pharmaceutical dosage form**

Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after to decide the normal weight. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 m) and sonicated to degas. The arrangement arranged was infused in five reproduces into the HPLC framework and the perceptions were recorded.

A copy infusion of the standard arrangement was additionally infused into the HPLC framework and the peak regions were recorded. The information is appeared in Table-12.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:
AT = Peak Area of medication acquired with test arrangement
AS = Peak Area of medication acquired with standard arrangement
WS = Weight of working standard taken in mg
WT = Weight of test taken in mg
DS = Dilution of Standard arrangement
DT = Dilution of test arrangement
P = Percentage virtue of working standard

**Table 12: Recovery data for estimation of sunitinib in suninat 50mg capsule.**

| Brand Name of Sunitinib | Labelled amount of Drug (mg) | Mean (± SD) amount (mg) found by the proposed method (n=6) | Assay % (± SD) |
|---|---|---|---|
| Suninat 50 Capsules | 50mg | 49.81 (± 0.458) | 99.63 (± 0.368) |

## RESULT AND DISCUSSION

The amount of drug in Suninat was found to be 49.81 (± 0.458) mg/tab for Sunitinib & % assay was 99.63 %.

## SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Sunitinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS ($C_{18}$) RP Column, 250 mm x 4.6 mm, 5µm Column was preferred because using this column peak shape, resolution and absorbance were good. Mobile phase & diluent for preparation of various samples were finalized after studying the solubility of API in different solvents of our disposal (Methanol, Acetonitrile, dichloromethane, water, 0.1N NaOH, 0.1NHCl). Further, a stream rate of 1.0 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is amazingly, one more reasonable strategy for measure and dependability related debasement examines which can help in the investigation of Sunitinib in various details.

## BIBLIOGRAPHY


1. https://go.drugbank.com/drugs/DB01268
2. https://pubchem.ncbi.nlm.nih.gov/compound/Sunitinib
3. https://en.wikipedia.org/wiki/Sunitinib
4. Morgan, David J., "Fraction collector (Post on Flickr)". *Flickr*. Retrieved, 2015; 28.
5. Karger, Barry L. "HPLC: Early and Recent Perspectives". Journal of Chemical Education, *1997;* 74, 45: 74-45.
6. Ettre, C. "Milestones in Chromatography: The Birth of Partition Chromatography" (PDF). LCGC, *2016* and 2001; 19(5): 506–512.
7. Martin, A J P; Synge, R L M, "Separation of the higher mono amino-acids by counter-current liquid-liquid extraction: the amino-acid composition of wool". Biochemical Journal, 1941; 35(1–2): 91–121.
8. Lindsay, S.; Kealey, D., High performance liquid chromatography. Wiley. *from review* Hung, L. B.; Parcher, J. F.; Shores, J. C.; Ward, E. H. "Theoretical and experimental foundation for surface-coverage programming in gas–solid chromatography with an adsorbable carrier gas". J. Am. Chem. Soc, 1987, 1988; 110(11): 1090–1096.
9. Journal of Pharmaceutical and Biomedical Analysis, 1999; 21, 2: 371–382, 1.
10. Tropical Journal of Pharmaceutical Research, © Pharmacotherapy Group, 2009; 8(5): 449-454.
11. Rabi Sankar, Instrumental Method of Analysis, P-18-6, P-18-3.
12. Validation of analytical procedures, methodology, ICH harmonized tripartite guideline, 1996; 108.
13. B.K. Sharma, Instrumental Methods of Chemical Analysis, 75-78, 113-115.
14. ICH Topic Q 2 (R1) Validation of Analytical Procedures: Text and Methodology.
15. Mohsen padervand etal, RP HPLC determination of sunitinib malate using UV detector, its isomerization study, method development and validation, Journal of analytical chemistry, 72: 567-574.
16. M. Mamatha, G. Mounikha, Ch. Sampath; method development and validation for the simultaneous estimation of metal ozone and spiranolactone for rp hplc method, ijpbs, 2023; 12(3): 142-147, ISSN 2321-3272.
17. Santhosh Illendula, T. Sumanjali, D. Sandhya, N. Lavanya & KNV Rao; Method development & validation of Afatinib in bulk & pharmaceutical dosage form by UV spectroscopic method, Indo American journal of pharmaceutical sciences IAJPS, 2018; 05(03): 1569-1575.
18. Madireddy Mamatha and Anil Middha; a new separation technique for method development and validation of rosuvastatin and micronized

fenofibrate in its pure and pharmaceutical dosage form, World journal of pharmaceutical research, 6, 16: 1658-1676, ISSN 2277-7105.
19. Santhosh Illendula, A. Navyasree, CH. Ganesh, K.Sneha & KNV Rao; Method development and validation of Axitinib in bulk and pharmaceutical dosage form by UV spectroscopic method, Indo American journal of pharmaceutical sciences IAJPS, 2019; 06(03): 6221-6227.
20. Kishan patel, Most reliable, specific and validated method of sunitinib malate for its related compounds including process impurities.
21. Santhosh Illendula, Shailesh sharma; UPLC method development and validation of acalabrutinib in bulk and pharmaceutical dosage form, International journal of chemical and biochemical sciences IJCBS, 2024; 25(14): 397-411.