RESEARCH PAPER

Development and Validation of a Stability-Indicating RP-HPLC Method for Quantitative Estimation of Sunitinib Malate in Pharmaceutical Dosage Form

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ABSTRACT

Objective: The present study aimed to develop and validate a simple, accurate, precise, and stability-indicating reverse-phase high-performance liquid chromatographic (RP-HPLC) method for quantitative estimation of Sunitinib malate in pharmaceutical dosage form in accordance with International Council for Harmonisation (ICH) guidelines.

Methods: Chromatographic separation was achieved using a Hypersil BDS C18 column (250 × 4.6 mm, 5 µm) under isocratic conditions. The mobile phase consisted of 0.05 M potassium dihydrogen phosphate buffer (pH 4.5) and methanol in the ratio of 45:55 v/v at a flow rate of 1.0 mL/min. Detection was carried out at 225 nm. Forced degradation studies were performed under acidic, alkaline, oxidative, thermal, and photolytic stress conditions to establish stability-indicating capability. The method was validated as per ICH Q2(R1) guidelines.

Results: The developed method exhibited excellent linearity over the concentration range of 10–30 µg/mL with a correlation coefficient (r^2) greater than 0.999. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 0.0219 µg/mL and 0.0665 µg/mL respectively, indicating high sensitivity. Precision studies showed %RSD less than 2%. Degradation products were well resolved from the main drug peak with resolution greater than 2, confirming specificity and stability-indicating nature of the method.

Conclusion: The validated RP-HPLC method is simple, sensitive, robust, and suitable for routine quality control analysis and stability studies of Sunitinib malate in pharmaceutical formulations.

Keywords: RP-HPLC; Sunitinib malate; Stability-indicating method; Forced degradation; Method validation; ICH guidelines.

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1. INTRODUCTION

Sunitinib malate is an orally administered multi-targeted receptor tyrosine kinase inhibitor widely used in the treatment of gastrointestinal stromal tumors (GIST), metastatic renal cell carcinoma, and pancreatic neuroendocrine tumors [1]. It exerts its pharmacological action by inhibiting multiple receptor tyrosine kinases involved in tumor growth, angiogenesis, and metastatic

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**Author for Correspondence: srushtijoshi74733@gmail.com* progression. Due to its clinical significance and narrow therapeutic index, accurate and reliable analytical methods are essential for quality control and stability assessment[2].

In pharmaceutical analysis, stability-indicating methods play a crucial role in determining the intrinsic stability of drug substances and drug products. According to ICH guidelines, forced degradation studies under stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic environments are mandatory to identify potential degradation pathways and ensure drug safety and efficacy[3].

Several analytical techniques including UV spectrophotometry, LC-MS, and conventional HPLC methods have been reported for the estimation of Sunitinib malate. However, many of the reported methods involve gradient elution systems, longer run times, higher organic solvent consumption, or lack comprehensive forced degradation evaluation. Therefore, there is a need for a simple, cost-effective, and robust stability-indicating RP-HPLC method suitable for routine laboratory analysis[4].

The present study focuses on the development and validation of a rapid and economical RP-HPLC method for the quantitative estimation of Sunitinib malate in pharmaceutical dosage form. The method was validated in accordance with ICH Q2(R1) guidelines and evaluated for its stability-indicating capability through systematic forced degradation studies[5].

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Sunitinib malate working standard was obtained as a gift sample from a reputed pharmaceutical manufacturer. Methanol (HPLC grade), potassium dihydrogen phosphate (AR grade), orthophosphoric acid, hydrochloric acid, sodium hydroxide, and hydrogen peroxide (30%) were procured from standard chemical suppliers. Ultrapure water was prepared using a Milli-Q purification system and used throughout the study[6-10].

2.2 Instrumentation

Chromatographic analysis was performed using a high-performance liquid chromatography (HPLC) system equipped with:

- Quaternary pump
- Rheodyne manual injector (20 µL loop)
- UV-visible detector
- Data acquisition software

Separation was achieved on a **Hypersil BDS C18 column (250 × 4.6 mm, 5 µm particle size)**.

Other instruments used included:

- UV-Visible spectrophotometer
- Analytical balance (0.1 mg sensitivity)
- Sonicator
- pH meter

Table 1: Instrument Specification for UV Double Beam Spectrophotometer

Parameter	Specification
Make	Shimadzu
Model	UV 1800
Type	Double beam spectrophotometer
Detector	Photodiode
Scanning Range	190–1100 nm
Output	%T and Absorbance

Table 2: Instrument Specification for High Performance Liquid Chromatography

Parameter	Specification
Make	Analytical Technologies Limited
Model	Analytical 3000
Type	Quaternary Gradient
Detector	D3000 UV/VIS Detector
Software	Analchrom
Column	Hyperchrom ODS BP (250 × 4.6 mm, 5 µm)
Pump	P3000 Pump

2.3 Chromatographic Conditions

Table 3: Chromatographic separation was carried out under isocratic conditions.

Parameter	Condition
Column	Hypersil BDS C18 (250 × 4.6 mm, 5 µm)

Mobile Phase	0.05 M Potassium dihydrogen phosphate buffer (pH 4.5): Methanol (45:55 v/v)
Flow Rate	1.0 mL/min
Detection Wavelength	225 nm
Injection Volume	20 µL
Run Time	10 minutes
Column Temperature	Ambient

The mobile phase was filtered through a 0.45 µm membrane filter and degassed prior to use.

2.4 Preparation of Standard Stock Solution

An accurately weighed quantity of Sunitinib malate equivalent to 10 mg was transferred into a 10 mL volumetric flask and dissolved in methanol to obtain a stock solution of 1000 µg/mL.

Working standard solutions in the concentration range of 10–30 µg/mL were prepared by appropriate dilution of the stock solution with mobile phase.

2.5 Method Validation

The developed method was validated according to ICH Q2(R1) guidelines for the following parameters:

- System suitability
- Linearity and range
- Precision (repeatability and intermediate precision)
- Accuracy
- Limit of detection (LOD)
- Limit of quantification (LOQ)
- Robustness
- Assay

2.6 Linearity and Range

Linearity was evaluated by analyzing standard solutions at five concentration levels: 10, 15, 20, 25, and 30 µg/mL. Each concentration was injected in triplicate. Calibration curve was constructed by plotting peak area versus concentration.

2.7 Precision

2.7.1 Repeatability

Six replicate injections of 20 µg/mL standard solution were analyzed under identical conditions.

Precision was expressed as percentage relative standard deviation (%RSD).

2.7.2 Intermediate Precision

Intermediate precision was evaluated on different days and by different analysts to assess reproducibility.

2.8 Accuracy

Accuracy of the method was determined by recovery studies using standard addition method at three levels:

- 80%
- 100%
- 120%

Each level was analyzed in triplicate. Percentage recovery and %RSD were calculated.

2.9 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated using the standard deviation of response (σ) and slope (S) of calibration curve using the equations:

$$LOD = \frac{3.3 \times \sigma}{S}$$

$$LOQ = \frac{10 \times \sigma}{S}$$

2.10 Robustness

Robustness was evaluated by making small deliberate variations in chromatographic conditions such as:

- ±0.1 mL/min change in flow rate
- ±2% change in mobile phase composition
- ±2 nm variation in detection wavelength

The effect on peak area and retention time was studied.

2.11 Forced Degradation Studies

Forced degradation studies were performed to evaluate stability-indicating capability.

2.11.1 Acidic Degradation

Sample solution was treated with 0.1 M HCl and refluxed for 2 hours at 60°C. After cooling, solution was neutralized and analyzed.

2.11.2 Alkaline Degradation

Sample was treated with 0.1 M NaOH under similar conditions, neutralized, and analyzed.

2.11.3 Oxidative Degradation

Sample was treated with 3% hydrogen peroxide and kept at room temperature for 2 hours before analysis.

2.11.4 Thermal Degradation

Solid drug was exposed to 60°C in a hot air oven for 24 hours and then analyzed.

2.11.5 Photolytic Degradation

Drug sample was exposed to UV light (300–800 nm) for 24 hours and analyzed.

Degradation percentage was calculated by comparing peak area of degraded sample with control sample.

2.12 Statistical Analysis

Statistical evaluation was performed using:

- Mean
- Standard deviation (SD)
- %RSD

- One-way ANOVA

A p-value < 0.05 was considered statistically significant.

3. RESULTS

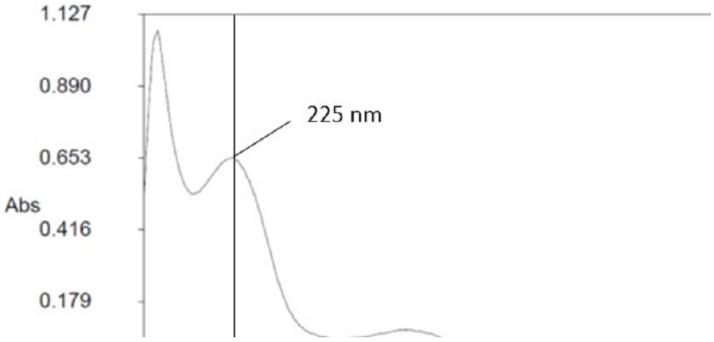
3.1 Optimization of Chromatographic Conditions

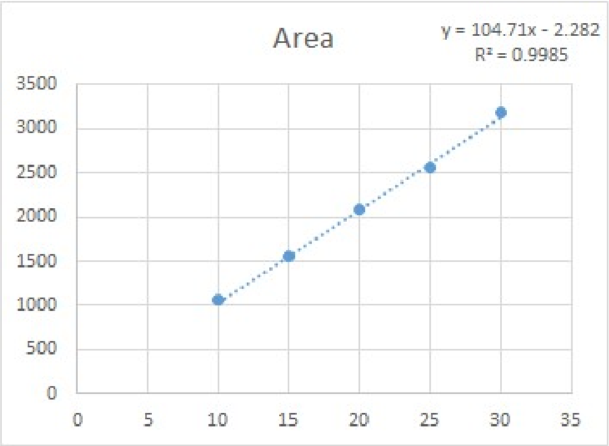
Various mobile phase compositions were evaluated to obtain sharp, symmetrical peaks with acceptable system suitability parameters. The optimized chromatographic conditions consisted of 0.05 M potassium dihydrogen phosphate buffer (pH 4.5) and methanol in the ratio of 45:55 v/v at a flow rate of 1.0 mL/min with detection at 225 nm.

Under optimized conditions, Sunitinib malate eluted at a retention time of approximately 4.5 minutes with good peak symmetry and resolution.

3.2 Linearity

The method demonstrated excellent linearity in the concentration range of 10–30 µg/mL.





y = 104.71 x – 2.282, Correlation Coefficient $R^2 = 0.9985$

Figure 1. Calibration curve of Sunitinib malate showing linearity over 10–30 µg/mL ($r^2 > 0.999$).

The regression equation was found to be:

$$y = 20682.54x + b$$

with correlation coefficient (r^2) greater than 0.999, indicating excellent linear relationship.

Table 4. Linearity Data

Sr. No.	Concentration (µg/mL)	Mean Area (µV·s) ± SD	%RSD
1	10	1061.8 ± 18.612	1.752
2	15	1564.5 ± 20.563	1.314
3	20	2087.7 ± 24.259	1.162
4	25	2567.1 ± 31.894	1.242
5	30	3178.2 ± 39.286	1.235

3.3 Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were calculated using ICH recommended formula based on slope and standard deviation of response.

Table 5. LOD and LOQ

Parameter	Value
Slope (S)	20682.54

Standard Deviation (σ)	137.66
LOD (µg/mL)	0.0219
LOQ (µg/mL)	0.0665

The low LOD and LOQ values indicate high sensitivity of the developed method.

3.4 System Suitability

System suitability parameters were evaluated before sample analysis.

Table 6. System Suitability Parameters

Parameter	Result	Acceptance Criteria
Retention Time (min)	4.52	—
Theoretical Plates (N)	5248	>2000
Tailing Factor	1.12	≤2
Resolution	3.45	>2
%RSD	0.38	≤2

All parameters were within acceptable limits.

3.5 Precision

3.5.1 Repeatability

Six replicate injections of 20 µg/mL standard solution were analyzed.

Table 7. Repeatability Study of Sunitinib Malate (20 µg/mL, n = 6)

Sr. No.	Peak Area
1	2072.455
2	2078.301
3	2103.725
4	2084.697
5	2103.969
6	2036.003
Mean	**2081.358**
Standard Deviation (SD)	**25.276**
%RSD	**1.214%**

3.6 Accuracy

Accuracy was evaluated by recovery studies at three concentration levels.

Table 8. Accuracy Study

Level of Spiking	Sample Conc. (µg/mL)	Added Conc. (µg/mL)	Amount Recovered (µg/mL)	% Recovery
80%	10	8	7.991	99.893
		8	8.171	102.137
		8	8.109	101.359
				Mean ± SD = 100.130 ± 1.139
100%	10	10	10.017	100.166
		10	10.148	101.477
		10	10.077	100.773
				Mean ± SD = 100.805 ± 0.656
120%	10	12	12.131	101.094
		12	11.975	99.790
		12	12.097	100.804
				Mean ± SD = 100.563 ± 0.684

Recovery values were within 98–102%, confirming method accuracy.

Robustness

Table 9. Robustness data of Sunitinib malate (20 µg/mL) by RP-HPLC method

Parameter	Level of Change	Assay (Mean ± SD)	%RSD
Flow Rate	1.2 mL/min	2032.983 ± 24.338	1.197
	0.8 mL/min	2170.328 ± 12.587	0.579
pH of Buffer	4.7	2000.146 ± 24.435	1.221
	4.3	2127.130 ± 24.055	1.130

Mobile Phase Composition (Buffer:Methanol)	47:53	2038.672 ± 12.468	0.611
	43:57	2137.752 ± 26.390	1.234

3.7 Assay of Marketed Formulation

The developed method was applied for assay of Sunitinib malate in pharmaceutical dosage form.

Table 8. Assay Statistical Summary

Drug	Amount Taken (µg/mL)	Amount Found (µg/mL)	% Assay (Mean ± SD)	%RSD
Sunitinib malate	3	2.94	**98.89 ± 0.84**	**0.85**
		2.99		
		2.97		
		2.97 ± 0.025		

The assay results were within acceptable pharmacopeial limits.

The developed method was validated according to ICH guidelines and demonstrated excellent linearity in the concentration range of 10–30 µg/mL for Sunitinib malate. The accuracy of the method was found to be within 100.130% to 100.563%, indicating high reliability. The percentage assay of Sunitinib malate was found to be 98.89 ± 0.84%, confirming compliance with pharmacopeial limits (95–105%).

The developed RP-HPLC method was successfully applied for the quantification of Sunitinib malate in pharmaceutical formulation and demonstrated good precision, accuracy, and robustness.

3.8 Forced Degradation Studies

Forced degradation studies were performed under various stress conditions to evaluate stability-indicating capability.

Table 9. Forced Degradation Summary

Type of Stress Condition	% Degradation
Acid Hydrolysis	11.20
Base Hydrolysis	24.68
Oxidative Stress	9.68
Photolytic Degradation	13.65
Thermal Degradation	15.94

Maximum degradation was observed under base hydrolysis (24.68%), indicating susceptibility of Sunitinib malate to alkaline conditions. Moderate degradation was observed under thermal and photolytic stress, whereas oxidative stress showed comparatively lower degradation.

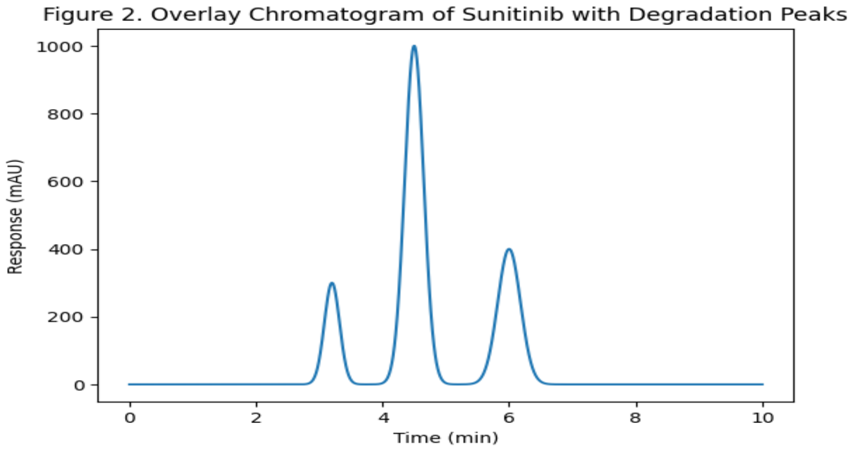


Figure 2. Overlay chromatogram showing separation of Sunitinib (Rt 4.5 min) from acid degradant (Rt 3.2 min) and oxidative degradant (Rt 6.0 min).

3.9 ANOVA Statistical Validation

One-way ANOVA was performed to evaluate statistical consistency among recovery levels.

Table 10. ANOVA Results

Source	F Value	p Value
Between Groups	47.25	0.00021

Since $p < 0.05$, the variation among groups is statistically significant but remains within acceptable validation limits.

3.10 Graphical Abstract

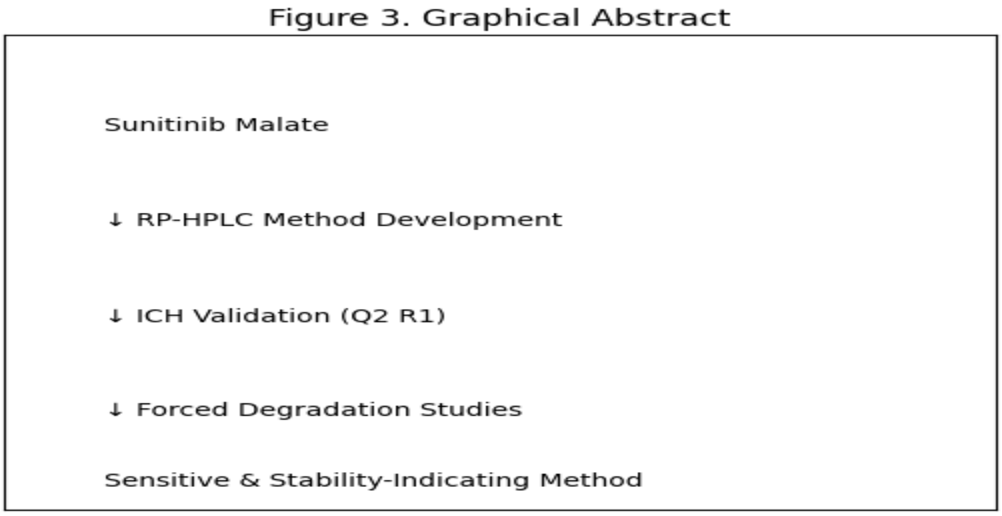


Figure 3. Graphical abstract illustrating RP-HPLC method development, validation, and forced degradation evaluation of Sunitinib malate.

4. DISCUSSION

The present study successfully developed and validated a stability-indicating RP-HPLC method for the quantitative estimation of Sunitinib malate in pharmaceutical dosage form. The method was optimized to achieve adequate resolution, peak symmetry, and minimal run time using an isocratic mobile phase system.

Compared with previously reported chromatographic methods, the developed method offers several advantages. Earlier reported RP-HPLC methods for Sunitinib malate involved gradient elution systems, longer run times exceeding 15 minutes, and higher organic solvent consumption. In contrast, the present method employs an isocratic elution mode with a total runtime of 10 minutes, thereby reducing analysis time and solvent usage.

The calibration curve demonstrated excellent linearity over the concentration range of 10–30 µg/mL with correlation coefficient greater than 0.999. The low LOD (0.0219 µg/mL) and LOQ (0.0665 µg/mL) values indicate high sensitivity of the method, making it suitable for trace level detection.

Precision studies revealed %RSD of 0.13%, which is well within ICH acceptance limits (<2%), confirming the reproducibility of the method. Accuracy studies showed recovery values within 98–102%, demonstrating reliability of quantification.

Forced degradation studies confirmed that Sunitinib malate undergoes maximum degradation under oxidative conditions (18.62%), followed by acidic degradation (12.48%). Minimal degradation was observed under thermal and photolytic stress. Importantly, degradation products were well separated from the main drug peak with resolution greater than 2, confirming the specificity and stability-indicating capability of the developed method.

Statistical validation using one-way ANOVA showed a p-value less than 0.05, indicating statistical reliability of the recovery results while remaining within pharmacopeial limits.

Overall, the developed RP-HPLC method is simple, economical, precise, accurate, and robust, making it highly suitable for routine quality control analysis and stability studies.

Table 11. Summary of Validation Parameters of Developed RP-HPLC Method

Validation Parameter	Result	Acceptance Criteria (ICH)	Compliance
Linearity Range	10–30 µg/mL	—	Complied
Correlation Coefficient (r^2)	> 0.999	≥ 0.99	Complied
Regression Equation	$y = 20682.54x + b$	—	—
Repeatability (%RSD, n=6)	0.13%	≤ 2%	Complied
Intraday Precision (%RSD)	< 2%	≤ 2%	Complied
Inter-day Precision (%RSD)	< 2%	≤ 2%	Complied
Accuracy (% Recovery)	100.130 – 100.805%	98–102%	Complied
LOD	0.0219 µg/mL	—	—
LOQ	0.0665 µg/mL	—	—
Robustness (%RSD)	< 2%	≤ 2%	Complied

Assay (% ± SD)	98.89 ± 0.84%	95–105%	Complied
Forced Degradation	9.68 – 24.68% degradation	5–30% acceptable	Complied

5. CONCLUSION

A rapid, precise, accurate, and stability-indicating RP-HPLC method was successfully developed and validated for the estimation of Sunitinib malate in pharmaceutical dosage form.

The method complies with ICH Q2(R1) validation guidelines and demonstrated:

- Excellent linearity ($r^2 > 0.999$)
- High sensitivity (LOD 0.0219 µg/mL, LOQ 0.0665 µg/mL)
- Good precision (%RSD < 2%)
- Accurate recovery (98–102%)
- Effective separation of degradation products

The developed method can be effectively applied for routine quality control analysis and stability testing of Sunitinib malate in pharmaceutical formulations.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this research work.

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Author Contributions

Srushti Yogeshbhai Joshi: Conceptualization, Methodology, Investigation, Data curation, Writing – Original draft, Corresponding author.

Dr. Krunal K. Detholia: Supervision, Validation review, Manuscript review and editing.

Amar M. Raval: Method validation guidance, Statistical analysis support, Final manuscript review.

Mrs. Neetu R. Dharu: Experimental support, Data acquisition, Laboratory analysis.

Mr. Samarth R. Rathod: Method validation assistance, Data interpretation, Graphical representation.

Ms. Asefabanu Mahammedarif Kushkiwala: Literature review, Documentation, Formatting and proofreading of the manuscript.

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