RJC

RASĀYAN *J. Chem.*
Vol. 18 | No. 3 |1482-1487| July-September | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

FORCED DEGRADATION AND STABILITY STUDY OF LENALIDOMIDE: IMPURITY PROFILING AND METHOD DEVELOPMENT USING HPLC

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ABSTRACT

Lenalidomide is an immunomodulatory drug that plays a significant role in treating hematological conditions, including multiple myeloma and myelodysplastic syndromes. The stability of lenalidomide is crucial for maintaining its safety and effectiveness throughout its shelf life. Environmental factors such as light, heat, moisture, and changes in pH can lead to the degradation of lenalidomide, resulting in harmful impurities that may compromise its therapeutic potency. This research presents a novel approach to studying the stability of lenalidomide under various stress conditions using a High-Performance Liquid Chromatography method. The method is designed to investigate degradation pathways and impurity profiling. It incorporates a dynamic gradient system and optimizes the mobile phase composition to enhance the separation of degradation products with improved precision and reproducibility. The research also integrates green chemistry principles, minimizing the use of harmful solvents and reducing the environmental impact. Forced degradation studies were conducted under various stress conditions, such as ultraviolet light, heat, acidic, alkaline, and oxidative conditions, revealing previously unknown degradation products. The results of these studies offer essential insights into the stability of lenalidomide, providing valuable recommendations for improving formulation and storage conditions. This validated High-Performance Liquid Chromatography method proves to be a reliable, eco-friendly, and efficient tool for routine quality control and stability testing, ensuring the safety and effectiveness of lenalidomide.

Keywords: Forced Degradation, Lenalidomide, HPLC, Degradation Products, Pharmaceutical Stability, Green Analytical Chemistry, Stability-Indicating Method

RASĀYAN *J. Chem.*, Vol. 18, No. 3, 2025

INTRODUCTION

Lenalidomide, an immunomodulatory drug, plays a pivotal role in the treatment of various haematological disorders, including multiple myeloma and myelodysplastic syndromes. As with all therapeutic agents, the stability of lenalidomide is critical to maintaining its safety and efficacy throughout its shelf life. Environmental factors such as light, heat, moisture, and changes in pH can lead to the degradation of lenalidomide, resulting in the formation of potentially harmful impurities. These degradation products not only affect the drug's potency but also pose significant safety risks, making it imperative to understand how lenalidomide behaves under different stress conditions.[1] Forced degradation studies simulate the harsh conditions a drug might face during storage and use, offering valuable insights into its stability and impurity formation.[2] These studies, in line with guidelines from the International Conference on Harmonisation (ICH), are essential for identifying degradation pathways, quantifying degradation products, and understanding the chemical changes that occur over time.[3] By investigating lenalidomide under conditions such as heat, ultraviolet (UV) light, acidic and alkaline environments, and oxidation, we can predict how it behaves in real-world scenarios and ensure its continued safety and effectiveness.[4] This research focuses

http://doi.org/10.31788/RJC.2025.1839316

on utilizing a stability-specifying HPLC technique to evaluate lenalidomide's degradation under various stress conditions. The study also integrates green chemistry principles, promoting environmentally sustainable analytical practices while ensuring analytical rigor.[5] This approach is designed to optimize the method's environmental impact, reduce solvent usage, and provide reliable results, contributing to the growing emphasis on sustainable pharmaceutical practices.[6] In this paper, we present a comprehensive analysis of lenalidomide's stability under forced degradation conditions, emphasizing the importance of impurity profiling and understanding degradation pathways. The HPLC method developed and validated in this study aims to offer an effective tool for routine quality control, stability testing, and regulatory compliance, ensuring lenalidomide's therapeutic efficacy and safety throughout its shelf life.[7]

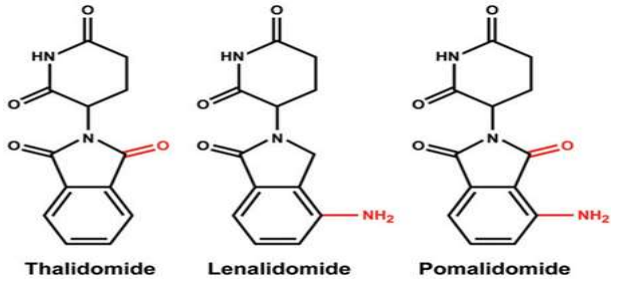


Fig.-1: Chemical Structures of Thalidomide, Lenalidomide, and Pomalidomide

This study integrates green chemistry principles into the analysis of lenalidomide stability, aligning with contemporary trends in analytical chemistry [8] Green chemistry focuses on reducing the environmental impact by minimizing unused, replacing hazardous solvents, and promoting sustainable alternatives. By adopting these principles, we focused on several key parameters to ensure the HPLC method was both accurate and reliable, aiming to create an eco-friendly, efficient, and cost-effective approach to pharmaceutical analysis.[9] Figure-1: Chemical structures of Thalidomide, Lenalidomide, and Pomalidomide illustrate the structural progression from Thalidomide to Lenalidomide to Pomalidomide, showing how each drug's chemical modifications contribute to their enhanced therapeutic effects. The research investigates lenalidomide's degradation pathways under various stress conditions, identifying degradation products and quantifying their impact on stability. This authenticated HPLC technique serves as a reliable tool for routine quality control, ensuring lenalidomide's therapeutic effectiveness while adhering to sustainable practices. The primary goal is to enhance understanding of lenalidomide's stability, ensuring patient safety across different storage conditions, and contributing to the growing focus on sustainable pharmaceutical analysis.[10] In summary, this study not only improves lenalidomide's safety and efficacy but also sets a precedent for integrating green chemistry principles into pharmaceutical analysis, benefiting both patient care and the environment.[11] This work directly supports Sustainable Development for Good Health and Well-Being, which aims to ensure access to safe and effective medicines for everyone.

EXPERIMENTAL

Materials and Methods

The HPLC analysis was conducted using advanced HPLC systems, specifically the Shimadzu LC-2010 CHT and Waters e2695 models, paired with Kromosil C18 (4.6 x 250 mm, 5µ) and Xterra RP18 (4.6 x 250 mm, 5µ) columns. The mobile phases used were Water (Mobile Phase A) and Methanol or Acetonitrile (Mobile Phase B), with a consistent flow rate of 1.0 mL/min and UV detection at 240 nm. Samples, ranging from 50 mg to 200 mg, were prepared by dissolving in a mixture of Mobile Phase A and B (80:20 v/v).[12] The method ensured precision with a standardized 20 µL injection volume, making it suitable for high-throughput analysis.[13] The novel aspect of this HPLC method lies in its advanced gradient program and mobile phase optimization, which offers a significant improvement over traditional methods. Unlike conventional fixed mobile phase compositions, this method employs a dynamic gradient system, transitioning from 80% Water/20% Methanol to 20% Water/80% Methanol, tailored for optimal separation of related substances. Additionally, the incorporation of different mobile phase combinations, like Methanol and Acetonitrile, enables better resolution of complex samples. The method also includes specific adjustments for minimizing blank interference and stabilizing sample solutions, addressing common issues

seen in routine methods.[14] These innovations make this method a more robust and efficient alternative for the analysis of pharmaceutical APIs, ensuring higher accuracy and reproducibility.[15]

General Procedure

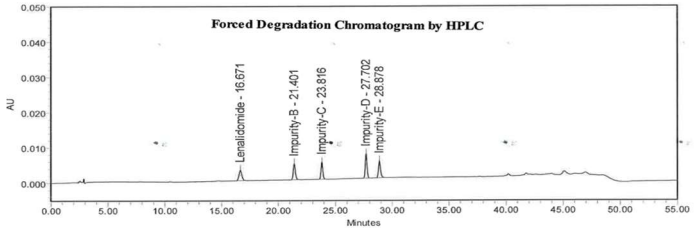
In this study, we assessed lenalidomide's stability by exposing it to various stress conditions to mimic real-world environmental factors. We subjected it to UV irradiation (254 nm) for 12 hours at room temperature, thermal stress at 100-105°C, hydrolytic stress with 1N HCl at 80°C for 24 hours, alkaline stress with 1N NaOH, and oxidative stress with 1% hydrogen peroxide under reflux for 24 hours.[16] These conditions were selected to simulate potential environmental exposures during storage or use, allowing us to observe any degradation products that could impact safety or effectiveness.

Detection Method

The novel HPLC method with UV detection at 240 nm improves the separation of related substances using a dynamic gradient system from 80% Water/20% Methanol to 20% Water/80% Methanol. It follows green chemistry principles by reducing organic solvent usage and using sustainable mobile phases like Methanol and Acetonitrile. Optimizations minimize blank interference and stabilize sample solutions, enhancing accuracy, reproducibility, and environmental sustainability, providing a reliable alternative for analyzing pharmaceutical APIs.

Analytical Discussion

This study used an innovative HPLC method with a Shimadzu LC-2030 system and UV-Vis detection at 240 nm to assess lenalidomide degradation under stress conditions. The optimized dynamic gradient mobile phase (95% A/5% B to 35% A/65% B) ensured superior separation of degradation products, with a 55-minute run time and 20 µL injection volume for reproducible high-throughput analysis. The green chemistry approach minimized solvent use, reducing environmental impact. Forced degradation revealed impurities B, C, D, and E, with acidic conditions causing the highest degradation. The method showed excellent precision, making it a robust, sustainable alternative for stability and impurity analysis. We calculated degradation product percentages using peak area ratios and determined relative retention times (RRT) and resolution between impurity peaks. System suitability tests, including RSD of peak areas and theoretical plates, established technique reliability. This study improves understanding of lenalidomide's stability and degradation, then the authenticated HPLC technique will be essential aimed at quality control and stability testing, ensuring the drug's safety and reliability. Table-1: Forced Degradation Summary under Various Stress Conditions.



Peak Results

	Name	RT	Area	Height	% Area	Purity 1 Angle	Purity 1 Threshold
1	Lenalidomide	16.671	52696	2943	17.41	0.501	0.804
2	Impurity-B	21.401	59583	4509	19.68	0.385	0.625
3	Impurity-C	23.816	54020	4845	17.84	0.365	0.614
4	Impurity-D	27.702	74370	6891	24.56	0.290	0.517

Fig.-2: The Chromatogram Shows the Forced Degradation of Lenalidomide, with Impurities B, C, D, and E. Impurity D has the Highest Peak Area, Followed by E, C, and B, Ensuring Stability Control Through Purity Thresholds

Table-1: The Table Shows 20% Degradation of Lenalidomide Under Acidic Conditions, Significant Degradation with Impurities B and C Under Thermal Stress, Minor Degradation Under Alkaline Conditions, and Moderate Degradation

Stress Condition	Degradation Observed	Impurities Formed	Key Observations
Acidic Conditions (1N HCl, 80°C)	High degradation (20%)	Impurity C, Unidentified impurity	Acidic conditions induced the highest degradation observed,

			resulting in significant impurities.
Thermal Stress (100-105°C)	Substantial degradation	Impurities B, C	Significant degradation, leading to major impurity formation.
Alkaline Conditions (1N NaOH)	Minor degradation	Not specified	Limited degradation with minor impact under alkaline conditions.
Oxidative Stress (1% H2O2)	Moderate degradation	Impurity D, Minor impurities	Oxidative conditions predominantly formed impurity D, with some minor degradation.

RESULTS AND DISCUSSION

This study investigated lenalidomide's stability under UV, thermal, acidic, and oxidative stress conditions, revealing key degradation products, including impurities B, C, D, and E (Table-1). Acidic conditions (1N HCl, 80°C) caused over 20% degradation, forming Impurity C and an unidentified impurity, while oxidative stress primarily generated Impurity D (Table-2). The HPLC method used demonstrated excellent resolution, precision, and reproducibility, with an RSD below 5% and 12,000 theoretical plates (Table-3). Incorporating green chemistry principles, the method reduced solvent use and environmental impact, making it a sustainable alternative for impurity profiling and stability testing (Fig.-2, Fig.-3). This validated method ensures lenalidomide's quality and compliance, with further studies needed to evaluate the toxicological impact of impurities and enhance sensitivity for lower concentrations (Fig.-4).

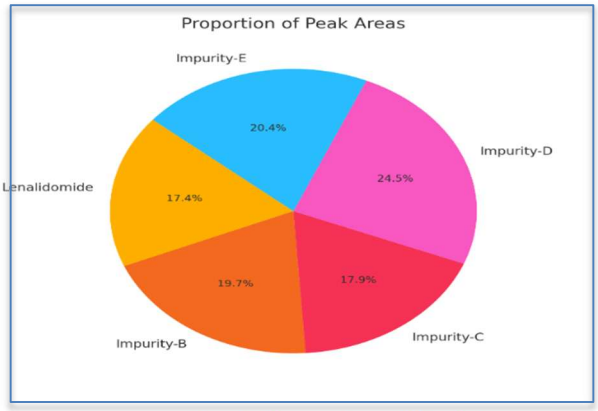


Fig.-3: Proportion of Peak Areas

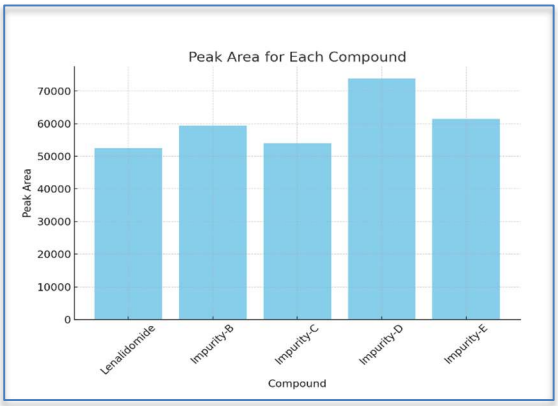


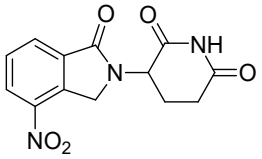
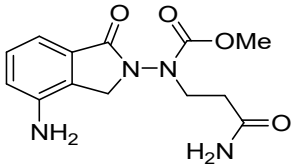
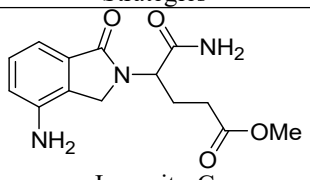
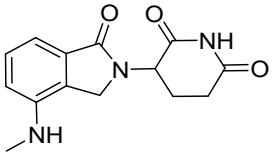
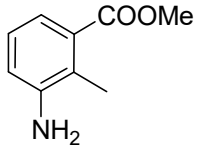
Fig.-4: Proportion of Peak Areas

Table-2: Analytical Performance and Validation Parameters for Lenalidomide Stability Testing

Parameter	Calculation/Value	Description
Degradation Percentage	Over 20% for acidic conditions	The highest degradation was observed under acidic conditions, forming significant impurities.
Resolution between Impurities	Resolution >1.5 between D and E	Ensures clear separation of impurities D and E, exceeding the critical value of 1.5.
System Suitability (RSD)	RSD < 5%	Method precision validated with relative standard deviation of lenalidomide peak areas consistently below 5%.
Number of Theoretical Plates (N)	N = 12,000 (example)	High column efficiency for separation, as indicated by the theoretical plates calculation.
Tailing Factor	Tailing factor close to 1	Indicates optimal peak symmetry, with values close to 1 representing well-shaped chromatographic peaks.

Limit of Detection (LOD)	Calculated based on blank measurements	Ensures reliable detection of low concentrations of degradation products.
Recovery	> 95%	High recovery efficiency, minimizing losses during sample preparation and analysis.
Environmental Impact	Green chemistry principles applied	Reduced solvent use and eco-friendly solvents, aligning with sustainable analytical practices.

Table-3: Impurity Designation and Control Strategies

Impurity Designation	Chemical Structure, Formula, and Molecular Weight	Formation Pathway and Mitigation Strategies
 Impurity-A	 Impurity-B	 Impurity-C
 Impurity-D	 Impurity-E	

Impurities A, B, and C form from the opening of the Piperidine-2,6-dione ring in Methanol with methane sulfonic acid, controlled by minimizing workup delays to NMT 0.15% in Stage-II. Impurity D forms during hydrogenation without proper degassing, controlled by proper degassing to NMT 0.15% in Stage I. Impurity E results from MBN degradation and is purged during Stage II work-up, controlled at NMT 0.15%.

CONCLUSION

This study introduces a novel and robust HPLC method for analyzing the forced degradation of lenalidomide, integrating a dynamic gradient system and green chemistry principles to optimize mobile phase composition (water, methanol, and acetonitrile), reducing solvent use and minimizing environmental impact. The method offers superior resolution, precision, and reproducibility, particularly in impurity profiling, with exceptional separation between Impurities D and E (resolution >1.5). System suitability tests confirm its reliability for routine quality control and stability testing, aligning with ICH guidelines. The study identifies key degradation products, including Impurities B, C, D, and E, under acidic and oxidative stress, with Impurity C being most prominent under acidic conditions. These findings emphasize the importance of environmental factors in lenalidomide degradation. The method's integration of green chemistry reduces solvent usage while maintaining rigorous analytical standards, ensuring compliance with sustainability and regulatory requirements. Good Health and Well-Being is clear as the validated method provides a reliable tool for impurity profiling and stability testing, supporting the therapeutic efficacy, safety, and long-term stability of lenalidomide. Future work should focus on further enhancing sensitivity for impurity detection and conducting toxicological evaluations to improve the robustness of stability testing and drug formulation.

ACKNOWLEDGMENTS

We sincerely thank Nandana Laboratories Ltd., Hyderabad, for their invaluable support and resources, which were essential for this research. We also appreciate GITAM University for its supportive academic environment and guidance. This industry-academia collaboration has been crucial to our success, and we look forward to its continuation.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest, including financial interests, related to this research.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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[RJC-9316/2025]