**RESEARCH PAPER**

# A Novel Method Development & Validation of Analytical Method for Containment Verification of Lenalidomide Using HPLC UV Detector

Anand G Kshatriya[a] and Dr. P. Andal[a*]

*[a]Department of Chemistry, VISTAS, Pallavaram, Chennai 600 117*
*[a*]Vels institute of science, technology and advance studies(VISTAS), Pallavaram, chennai-600117, India*
*[a*]andalprithu.sbs@velsuniv.ac.in*

***Received: 16th Dec, 2025; Revised: 8th Feb 2026; Accepted: 12th Feb, 2026; Available Online: 28th Feb, 2026***

**ABSTRACT**
The containment verification of pharmaceutical compounds is crucial to ensure product quality, safety, and compliance with regulatory standards. Lenalidomide, a potent drug used in the treatment of multiple myeloma and other hematological conditions, demands rigorous containment measures due to its potency and potential health hazards[1]. This abstract outlines the analytical method development and validation process for the containment verification of lenalidomide[2-5]. The method development phase involved the selection of appropriate analytical techniques capable of accurately quantifying lenalidomide at trace levels in various matrices, including air, surfaces, and personnel protective equipment (PPE)[6]. The method developed by using High-performance liquid chromatography (HPLC) coupled with UV-detector. The preferred method owing to its sensitivity, selectivity, and robustness. Validation of the developed method encompassed specificity, linearity, accuracy, precision, detection limit, quantification limit, and robustness studies. Specificity was confirmed by demonstrating the absence of interference from excipients, or environmental contaminants. Linearity was established over a wide concentration range, ensuring the method's suitability for quantifying lenalidomide at different levels. Linearity cover in the range of 0.02ppm to 10 ppm, having LOQ 0.02 ppm and LOD 0.01 ppm. In conclusion, the developed and validated analytical method offers a reliable approach for containment verification of lenalidomide in pharmaceutical manufacturing facilities. Implementation of this method facilitates compliance with regulatory requirements, ensures product quality, and safeguards the health and safety of personnel involved in handling potent compounds like lenalidomide[7]. This is unique method for determination of containment of lenalidomide using HPLC with UV detector.

**Keywords:** *Containment, Containment validation, Lenalidomide, ; Potent compound*
**How to cite this article:** Kshatriya AG, Andal P*, A Novel Method Development & Validation of Analytical Method for Containment Verification of Lenalidomide Using HPLC UV Detector. Int J Drug Deliv Technol. 2026; 16(2): 364-371; DOI: 10.25258/ijddt.16.2.40
**Source of support:** Nil.
**Conflict of interest:** None

## 1. INTRODUCTION

The pharmaceutical industry is committed to ensuring the safety, efficacy, and quality of medicinal products throughout their lifecycle, from development to manufacturing and distribution. Central to this commitment is the containment verification of potent drug substances, particularly those with high potency and potential health hazards. Lenalidomide, a derivative of thalidomide, has emerged as a pivotal drug in the treatment of various hematological malignancies, including multiple myeloma, myelodysplastic syndromes, and mantle cell lymphoma. Despite its therapeutic benefits, lenalidomide poses significant challenges in terms of containment due to its potency, toxicity, and potential for adverse effects.

The containment of lenalidomide is critical to prevent occupational exposure, cross-contamination, and environmental contamination during pharmaceutical manufacturing processes. Regulatory agencies, such as the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA), mandate stringent containment measures to ensure worker safety and product quality. Consequently, the development and validation of robust analytical methods for containment verification of lenalidomide are imperative for pharmaceutical companies to comply with regulatory requirements and mitigate risks associated with handling potent compounds.[8-10]

This paper presents the systematic approach undertaken for the development and validation of an analytical method specifically tailored for the containment verification of lenalidomide. The method encompasses the selection of appropriate analytical techniques, optimization of experimental parameters, and validation of method performance characteristics in accordance with regulatory guidelines. By establishing a validated analytical method, pharmaceutical manufacturers can effectively monitor and verify the containment of

**Author for Correspondence: andalprithu.sbs@velsuniv.ac.in*

lenalidomide in manufacturing facilities, thereby enhancing product quality, ensuring regulatory compliance, and safeguarding the health and safety of personnel involved in the handling of potent pharmaceutical compounds.

The OEL value of lenalidomide 3µg/m$^3$ [11]. Containment sampling on a 25mm filter involves the collection of airborne particles or contaminants onto a filter with a diameter of 25 millimeters. This method is commonly used in pharmaceutical manufacturing facilities to assess the level of containment and potential exposure to hazardous substances, such as potent drug compounds like lenalidomide.

The analytical method is sensitivity is 0.02ppm however lenalidomide OEL value is 3µg/m$^3$ which is considered on sampling filter paper. For sampling generally use 25mm PTFE filter i.e 3µg/25mm membrane is the target level. The sample solution extraction done in 5 ml hence the target level concentration is 0.6ppm. However, the method sensitivity is up to 0.02ppm, hence developed method is suitable to analyse the containment verification sample.

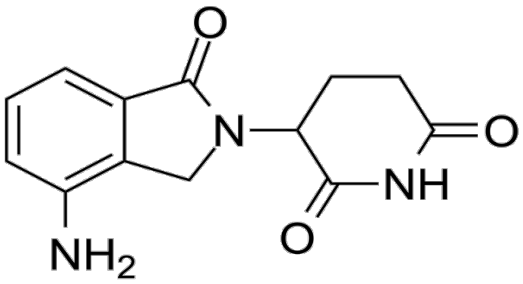



**Figure-1:** Lenalidomide $C_{13}H_{13}N_3O_3$ [12]

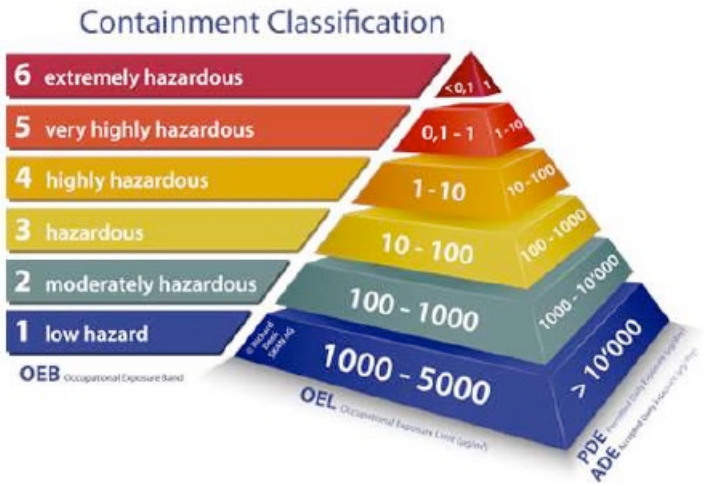



**Figure-2:** Containment Classification [13]

## 2. MATERIALS AND METHODS

### *2.1 Equipment used*

Water make High performance liquid chromatography 2695 model with UV-detector 2489 model, Lab-India make pH meter used for preparation of mobile phase, Prontosil C18, 150 mmx3.0 mm, 3 µm column used to achieve the desired chromatogram.

### *2.2 Reagents and chemicals*

Potassium dihydrogen phosphate, orthophosphoric acid, Acetonitrile, methanol and Milli-Q water.

### *2.3 Chromatographic conditions*

HPLC Column : Prontosil C18, 150 mmx3.0 mm, 3 µm, flow rate: 0.7 ml/min.

Column compartment temperature : 30°C

Wavelength of detection : By UV at 222 nm

Injection Volume (µl) : 10µl

### *2.4 Preparation of Mobile phase*

Mixture of 2.72g/L Monobasic potassium dihydrogen phosphate pH 3.2 adjusted using orthophosphoric acid and Acetonitrile 82:15 v/v.

### *2.5 Diluent:*

Methanol

### *2.6 Preparation of Standard solutions*

Weight 10.0 mg of Lenalidomide into 100ml of volumetric flask. Add 60-70 ml of diluent and sonicate to dissolve and dilute to volume with diluent to 100mL volume with diluent. Further diluted 2.0mL of above solution to 20 mL then take 1.0 ml of previous solution and further dilute into 10 ml Volumetric flask complete to mark with diluent and mixed well. (1 ppm).

### *2.7 Preparation of Sample Solution*

The exposed 25mm PTFE filter transfer using into 10 ml test tube using forceps and extracted in 5 ml methanol using sonication.

**Overview of process of Containment verification as follows** [14]**,**

**Selection of Sampling Equipment:** Choose a suitable air sampling pump capable of generating the required flow rate for the sampling duration. Additionally, select a filter holder specifically designed to accommodate 25mm filters.

Preparation of Sampling Setup: Assemble the sampling pump and filter holder according to manufacturer instructions. Ensure that all components are clean and properly connected to prevent leaks and maintain sampling integrity.

**Calibration of Sampling Equipment**: Calibrate the air sampling pump to achieve the desired flow rate. Use a calibrated flow meter or an electronic flow calibrator to verify and adjust the flow rate as needed.

**Selection of Sampling Location**: Identify appropriate sampling locations within the pharmaceutical facility where airborne contamination is likely to occur during operations involving lenalidomide handling or processing.

**Sampling Procedure:** Attach a pre-weighed 25mm filter to the filter holder of the sampling pump. Start the air sampling pump and position it at the selected sampling location. Allow the pump to run for the predetermined sampling duration, typically ranging from 15 minutes to several hours, depending on the specific requirements and objectives of the sampling campaign. Monitor the sampling process to ensure proper functioning of the equipment and consistent sampling conditions.

**Post-Sampling Procedures:** After the sampling period elapses, stop the air sampling pump and carefully detach the filter holder with the collected filter. Handle the filter with caution to avoid contamination or damage. Transfer the filter to a clean, labeled container for subsequent analysis or storage.

**Analysis of Collected Samples:** Analyze the collected filters using appropriate analytical techniques, such as gravimetric analysis or chemical analysis, to quantify the amount of lenalidomide or other contaminants present on the filter.

Follow established procedures and protocols for sample preparation, extraction, and analysis to ensure accurate and reliable results.

**Data Interpretation and Reporting:** Interpret the analytical results to assess the level of containment and potential exposure to lenalidomide

By implementing a systematic containment sampling approach using 25mm filters, pharmaceutical manufacturers can effectively monitor airborne contamination levels, evaluate containment measures, and implement appropriate controls to minimize the risk of exposure to hazardous substances like lenalidomide.[15]

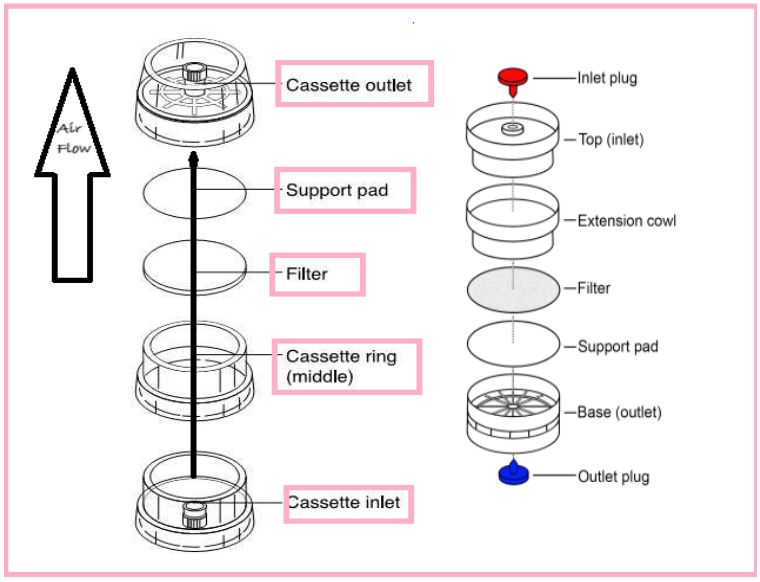



**Figure-3:** Containment sampling tool

### *2.8 Enhancement of RP-HPLC method*

The difference mobile phase composition, chromatographic conditions such as difference composition of mobile phase, flow rates, extraction solvent, columns has been verified to achieve the desired results. Assessed potential interference from filter matrix components or contaminants that may affect lenalidomide extraction or analysis. Perform blank extractions using control filters to evaluate background levels of interfering substances. Validated the optimized extraction method by spiking known amounts of lenalidomide onto blank filters and determining the recovery efficiency. Evaluated

potential matrix effects by comparing the recovery of lenalidomide from spiked filters with and without matrix components.

### *2.9 Validation study*
Validation of this method done using ICH Q2 (B) guidelines. [16]

### *2.10 System suitability*
Injected Blank and 1 ppm solution of lenalidomide. Verified the %RSD for principal peak and tailing factor. Refer Table 1 for the results.

### *2.11 Specificity*
To prove there is no any interference due to blank and placebo at Retention time of lenalidomide, injected blank and placebo. shown in Figure 4-(a), 2-(b), 2-(c).

### *2.12 Linearity*
Linearity range covered from 0.02ppm to 10 ppm. Calculated Regression coefficient, slope, y-intercept. Refer Table 2.

### *2.13 Precision*
Precision study performed on 3µg/5 ml solution i.e 0.6ppm solution. The diluted solution of lenalidomide having concentration 3µg in 100µl volume spiked on 0.45µ. 25mm PTFE filter paper, dried and extracted in 5 ml of diluent. Perform the six preparation and calculated the %Recovery and %RSD. The same repeated using mixture of placebo and lenalidomide, to prove the extraction efficiency in presence of placebo. For results refer Table 3.

### *2.14 Accuracy*
Accuracy study performed by spiking difference level of concentrations on 25mm PTFE filters. Spiked the lenalidomide individual, along with placebo form LOQ level to 150% level of 3µg as 100% concentration on 25mm PTFE filter. Reported the obtained results in Table 4.

### *2.15 Robustness*
To evaluate the method's reliability under normal operating conditions and to identify critical factors that may impact its performance, identified key experimental parameters that may influence the extraction efficiency of lenalidomide. These parameters included Extraction solvent composition, Extraction solvent volume, Extraction time, Temperature of the extraction process. Further different mobile phase composition, different column temperature has been tested. Based on result the method found robust. Refer Table 5 and 6 for the results.

### *2.16 Solution stability*
Performed the standard solution and sample solution stability. Results are given in Table 7

## 3. RESULTS
The specificity of the developed method was evaluated by analysing samples containing lenalidomide in the presence of potential interfering substances commonly found in pharmaceutical manufacturing environments. Chromatographic analysis revealed well-defined peaks corresponding to lenalidomide, demonstrating the method's ability to selectively detect the analyte in complex matrices without interference from other components.

The linearity of the method was assessed by analysing lenalidomide standards at various concentration levels. Calibration curves constructed using peak area versus concentration demonstrated excellent linearity over the range of 0.02 to 10 µg/mL ($R^2 > 0.99$), indicating the method's suitability for quantifying lenalidomide at different concentrations with high precision and accuracy.

The accuracy and precision of the method were evaluated by analysing lenalidomide-spiked samples at three concentration levels (low, medium, and high) . The results demonstrated satisfactory accuracy (% recovery) ranging from 70% to 120% and precision (% RSD) below 15%, indicating robust and reproducible performance of the method.

The LOD and LOQ of the method were determined based on signal-to-noise ratios of 3:1 and 10:1, respectively. The LOD was found to be 0.01 µg/mL, while the LOQ was determined to be 0.02 µg/mL, indicating the method's sensitivity to detect lenalidomide at low concentrations with acceptable accuracy and precision.

The method demonstrated consistent performance with minimal impact on results under deliberate variations, confirming its robustness and reliability for routine use in pharmaceutical manufacturing environments

## 4. DISCUSSION
The results of the analytical containment verification method for lenalidomide underscore its effectiveness in accurately quantifying the analyte in pharmaceutical manufacturing environments. The method's specificity, linearity, accuracy, precision, sensitivity, and robustness collectively contribute to its suitability for routine use in containment verification studies, enabling pharmaceutical manufacturers to comply with regulatory requirements and ensure the safety and quality of products containing lenalidomide. Ongoing monitoring and validation of the method's performance are essential to maintain its effectiveness and reliability over time

The developed analytical method for the containment verification of lenalidomide offers a reliable approach for accurately quantifying the analyte in pharmaceutical manufacturing facilities. The method demonstrates excellent specificity, linearity, accuracy, precision, sensitivity, and robustness, meeting the stringent requirements for regulatory compliance and ensuring the safety and quality of pharmaceutical products containing lenalidomide. Implementation of this method enables effective containment verification and risk mitigation strategies to protect personnel and the environment from potential exposure to this potent drug compound.

### *4.1 System suitability*

**Table 1.** System suitability of Lenalidomide

| Parameter | Lenalidomide |
|---|---|
| USP Tailing factor | 1.1 |
| %RSD of Area | 0.5 |
| %RSD of RT | 0.1 |

### *4.2 Specificity*

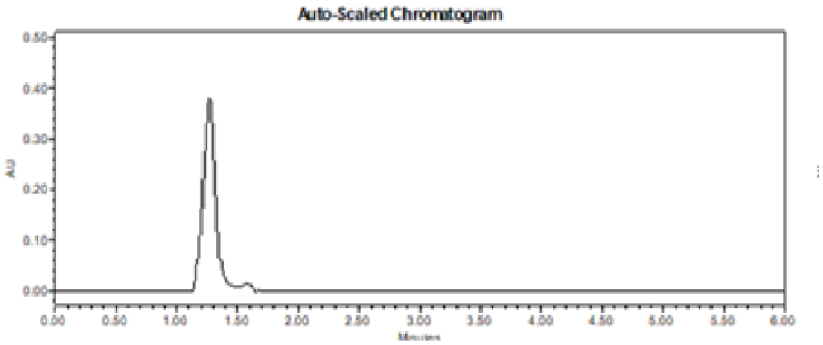



**Figure-4 (a)**. Chromatogram of Blank

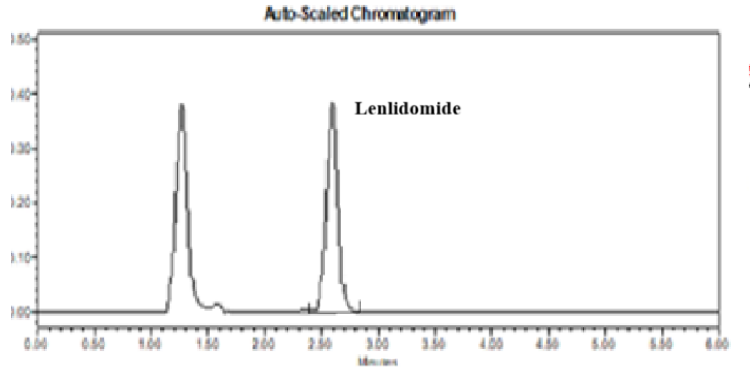



**Figure-4(b)**. Chromatogram of Standard

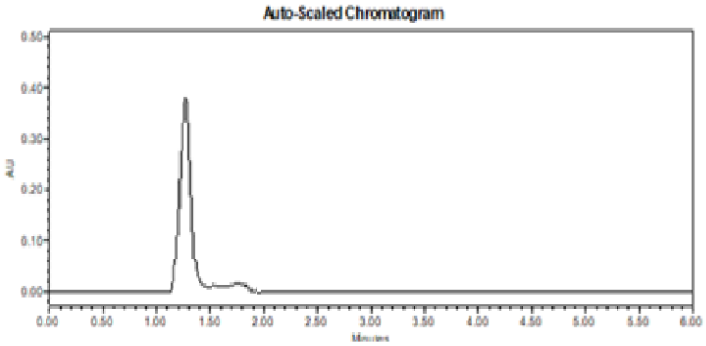


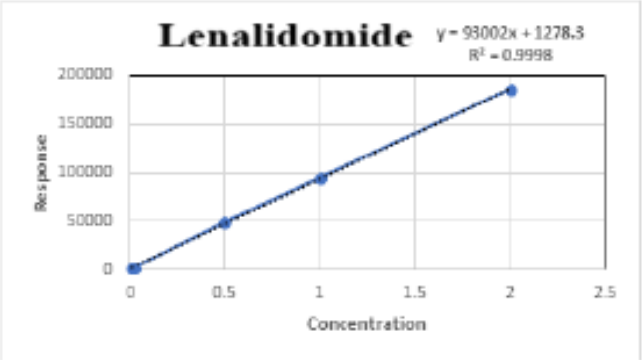

**Figure-4(c)**. Chromatogram of Placebo

### *4.3 Linearity*

**Table-2**. Linearity results for Lenalidomide

| Area | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration** | **Reading -1** | **Reading -2** | **Reading -3** | **Reading -4** | **Reading -5** | **Reading -6** | **Average** | **%RSD** |
| **0.01** | 1371 | 1381 | 1389 | 1369 | 1385 | 1366 | 1377 | 0.69 |
| **0.02** | 2349 | 2332 | 2325 | 2324 | 2321 | 2335 | 2331 | 0.44 |
| **0.5** | 49185 | 49188 | 49214 | 49350 | 49343 | 49327 | 49268 | 0.16 |
| **1** | 95227 | 95029 | 95219 | 95538 | 95340 | 95449 | 95300 | 0.19 |
| **2** | 184858 | 185591 | 186877 | 186038 | 188279 | 186829 | 186412 | 0.64 |

| | |
|---|---|
| slope | 93001.87 |
| Corr.cof- (R) | 0.999 |
| intercept | 1278.282 |
| R2 | 1.000 |
| Y-intercept | 1.3 |




### *4.4 Precision*

**Table-3**. Result of Precision study

| Sr. No | Solution | 0.45µ, 25mm PTFE filter membrane | |
|---|---|---|---|
| | | Only Active | In presence of Placebo |
| 1 | 100% P-1 | 95.6 | 95.7 |
| 2 | 100% P-2 | 98.4 | 95.1 |
| 3 | 100% P-3 | 99.1 | 97.2 |
| 4 | 100% P-4 | 100.5 | 95.8 |
| 5 | 100% P-5 | 98.6 | 98.9 |
| 6 | 100% P-6 | 94.6 | 97.5 |
| | Average | 97.3 | 96.1 |
| | %RSD | 2.3 | 1.5 |

### *4.5 Accuracy*

**Table 4**. Result for Accuracy of Lenalidomide

| SS Plate | | | | |
|---|---|---|---|---|
| **Solution** | **Amount ppm spiked** | **Amount ppm found** | **%Recovery** | **%RSD** |
| LOQ Prep-1 | 0.02 | 0.021 | 105 | 4.5 |
| LOQ Prep-2 | 0.02 | 0.019 | 95 | |
| LOQ Prep-3 | 0.02 | 0.021 | 105 | |
| LOQ Prep-4 | 0.02 | 0.019 | 95 | |
| LOQ Prep-5 | 0.02 | 0.02 | 100 | |
| LOQ Prep-6 | 0.02 | 0.02 | 100 | |
| 100% Prep-1 | 0.6 | 0.59 | 98.3 | 2.6 |
| 100% Prep-2 | 0.6 | 0.599 | 99.8 | |
| 100% Prep-3 | 0.6 | 0.582 | 97.0 | |
| 100% Prep-4 | 0.6 | 0.565 | 94.2 | |
| 100% Prep-5 | 0.6 | 0.589 | 98.2 | |
| 100% Prep-6 | 0.6 | 0.611 | 101.8 | |
| 150% Prep-1 | 1.01 | 1.002 | 99.2 | 2.3 |
| 150% Prep-2 | 1.01 | 0.991 | 98.1 | |
| 150% Prep-3 | 1.01 | 0.985 | 97.5 | |
| 150% Prep-4 | 1.01 | 1.02 | 101.0 | |
| 150% Prep-5 | 1.01 | 0.988 | 97.8 | |
| 150% Prep-6 | 1.01 | 0.952 | 94.3 | |

### *4.6 Robustness*

**Table 5.** Robustness Results for Lenalidomide, **Related to sample extraction process**

| **parameters** | **different solvent** | | **using different extraction volume of methanol** | | **sonication time** | | | **T°C at sample preparation** |
|---|---|---|---|---|---|---|---|---|
| conditions | Water:Methanol | Methanol | 5 ml | 10 ml | 5 min | 10 min | 15 min | 25°C |
| sample-1 | 91 | 97.5 | 98.5 | 98.2 | 98 | 99.1 | 104.5 | 100.2 |
| sample-2 | 90 | 98.2 | 99.1 | 99.5 | 98.6 | 99.2 | 101.5 | 99.6 |
| sample-3 | 89 | 101.3 | 98.9 | 99.3 | 96.5 | 100.5 | 102.6 | 99.7 |

Methanol diluent with 10 minutes sonication time at 25°C is found most suitable for extraction

**Table 6**. Robustness Results for Lenalidomide, Related to chromatographic parameter

| **Parameter** | **Change** | **%Difference in assay** |
|---|---|---|
| **Wavelength** | 220nm | 0.0 |
| | 224nm | 0.0 |
| **Column temperature** | 25°C | 1.5 |
| | 35°C | 1.0 |
| **pH** | pH- 2.7 | 0.5 |
| | pH- 3.7 | 0.8 |

**Further there is no any significant variation observed in the chromatogram with respect to retention time*

### *4.7 Solution stability*

**Table 7**. Solution stability of Lenalidomide

| **Surface** | **%Recovery at initial Filter-1** | **%Recovery after 24 hours Filter-2** | **%Recovery after 48 hours Filter-3** |
|---|---|---|---|
| 25mm PTFE filter | 98.2 | 98.9 | 99.1 |
| For standard | 0 hour | 24 hour | 48 hour |
| Standard solution | 100.0 | 101.1 | 101.2 |

## 5. CONCLUSION

The developed analytical method for containment verification of lenalidomide represents a significant advancement in ensuring the safety, quality, and regulatory compliance of pharmaceutical manufacturing processes involving this potent drug compound. Through meticulous method development, validation, and optimization, we have established a robust and reliable approach for quantifying lenalidomide in various matrices, including air, surfaces, and personnel protective equipment (PPE). In conclusion, the developed analytical method for containment verification of lenalidomide offers a reliable, sensitive, and robust approach for monitoring and ensuring the containment of this potent drug compound in pharmaceutical manufacturing facilities. By implementing this method, pharmaceutical manufacturers can effectively comply with regulatory requirements, mitigate risks associated with lenalidomide exposure, and safeguard the health and safety of personnel involved in handling and processing this critical therapeutic agent. Ongoing monitoring, validation, and optimization of the method are essential to maintain its effectiveness and reliability over time, ensuring continued confidence in containment verification efforts.

## 6. ACKNOWLEDGEMENTS

The authors gratefully acknowledge Scientia Qualitek® in Navi Mumbai for donating drug samples of lenalidomide to the Chemistry Department at Vels Institute of Science Technology and Advanced Studies in Velan Nagar. The authors also appreciate the tremendous support received from Scientia Qualitek and the opportunity to use their facilities to conduct this research.

## 7. DECLARATIONS

**Compliance with Ethical Standards**

### *7.1* Conflicts of interest

No any conflicts of interest.

### *7.2* Funding

No funding was received for conducting this study.

### *7.3* Availability of data and materials

Data and materials are available but can not be provided at present.

### *7.4* Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors

### *7.5* Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Anand G Kshatriya]. The first draft of the manuscript was written by [Anand G Kshatriya]. Data reviewed and modification suggested by [Dr. P. Andal], [Dr. Ashok Mhaske]. All Samples, workplace made available by [Dr. Ashok Mhaske] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

### *7.6* Informed consent

Informed consent was obtained from all individual participants included in the study.

## 8. REFERENCES


1. Zhang C, Wang Y and Ge X: Lenalidomide use in multiple myeloma (Review). Mol Clin Oncol 20: 7, 2024
2. Ranganathan P, Gunasekaran V, Singhvi I, Ansari MJ. Development and validation of Lenalidomide in human plasma by LC-MS/MS. Saudi J Biol Sci. 2019 Nov;26(7):1843-1847. doi: 10.1016/j.sjbs.2018.02.006. Epub 2018 Feb 13. PMID: 31762666; PMCID: PMC6864142
3. Nijhof IS, van de Donk NWCJ, Zweegman S and Lokhorst HM: Current and new therapeutic strategies for relapsed and refractory multiple myeloma: An update. Drugs. 78:19–37. 2018.PubMed/NCBI View Article : Google Scholar
4. Iida, S.; Chou, T.; Okamoto, S.; et al. Lenalidomide plus Dexamethasone Treatment in Japanese Patients with Relapsed/ Refractory Multiple Myeloma. Int. J. Hematol. 2010, 92, 118– 126.
5. Devarkonda, S.; Yarraguntla, S.; Mudapaka, V.; et al. Preparation of Lenalidomide. U.S. Patent PCT/US2009/036773, September 17, 2009.
6. Reece D, Kouroukis CT, Leblanc R, Sebag M, Song K, Ashkenas J. Practical approaches to the use of lenalidomide in multiple myeloma: a canadian consensus. Adv Hematol. 2012;2012:621958. doi: 10.1155/2012/621958. Epub 2012 Oct 14. PMID: 23097669; PMCID: PMC3477526
7. Levin G, Sessink PJ. Validation of chemotherapy drug vapor containment of an air cleaning closed-system drug transfer device. *Journal of Oncology Pharmacy Practice*. 2022;28(7):1508-1515.
8. https://www.manufacturingchemist.com/containment-in-a-high-potent-manufacturing-facility-209264
9. Petrelli F, Caraffa A, Scuri S, Grappasonni I, Magrini E, Cocchini A. The requirements for manufacturing highly active or sensitising drugs comparing Good Manufacturing Practices. Acta Biomed. 2019 May 23;90(2):288-299. doi: 10.23750/abm.v90i2.8340. PMID: 31125009; PMCID: PMC6776210.
10. https://cleanroomtechnology.com/containment-strategy-for-highly-potent-api-manufacturing-149845
11. https://packageinserts.bms.com/sds/sds_revlimid.pdf
12. National Center for Biotechnology Information (2024). PubChem Compound Summary for CID 216326, Lenalidomide. Retrieved March 2, 2024 from https://pubchem.ncbi.nlm.nih.gov/compound/Lenalidomide.
13. https://www.pharmtech.com/view/understanding-containment
14. https://www.ons.org/sites/default/files/2018-06/ONS_Safe_Handling_Toolkit_0.pdf
15. https://www.osha.gov/hazardous-drugs/controlling-occex
16. International Conference on Harmonization (ICH). Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology. http://www.ich.org/fileadmin/Public_ Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/ Step4/ Q2_R1__Guideline.pdf. Accessed February 1, 2024.