Development and Validation of RP-HPLC Method for estimation of Lenalidomide in Pharmaceutical dosage form – A Comprehensive Review

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ABSTRACT

Lenalidomide is an immunomodulatory drug widely used in the treatment of hematological malignancies such as Multiple Myeloma and Myelodysplastic Syndrome. Accurate estimation of lenalidomide in pharmaceutical dosage forms is essential for quality control and ensuring therapeutic efficacy. Among the various analytical techniques available, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is considered one of the most reliable and widely used methods for drug analysis due to its high sensitivity, precision, and reproducibility. The present review discusses different approaches for the development and validation of RP-HPLC methods for the estimation of lenalidomide in pharmaceutical formulations. The review also highlights important chromatographic parameters such as mobile phase composition, column selection, detection wavelength, and flow rate that influence method performance. Furthermore, method validation parameters including linearity, accuracy, precision, specificity, robustness, limit of detection (LOD), and limit of quantification (LOQ) are discussed according to guidelines provided by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

KEYWORDS

Lenalidomide, RP-HPLC, Method Development, Method Validation, Pharmaceutical Analysis, Quality Control

1. INTRODUCTION

Lenalidomide is a synthetic derivative of Thalidomide and belongs to the class of immunomodulatory drugs. It exhibits antineoplastic, antiangiogenic, and immunomodulatory properties. Lenalidomide is commonly prescribed for the treatment of Multiple Myeloma and Myelodysplastic Syndrome. The quality, safety, and efficacy of pharmaceutical products depend largely on the accurate determination of active pharmaceutical ingredients (APIs).

In the pharmaceutical industry, the quantitative estimation of Active Pharmaceutical Ingredients (APIs) is a vital step in Quality Control (QC) and Quality Assurance (QA). While various methods such as UV-Visible spectrophotometry, titrimetric, and electrochemical methods exist, they often lack the specificity required to

distinguish the API from impurities, degradation products, or excipients. RP-HPLC allows for the separation and quantification of Lenalidomide with high precision and accuracy, making it the preferred method for regulatory compliance. .1,4,12

2. PHYSICOCHEMICAL PROFILE

Understanding the physicochemical properties of the analyte is the cornerstone of method development.

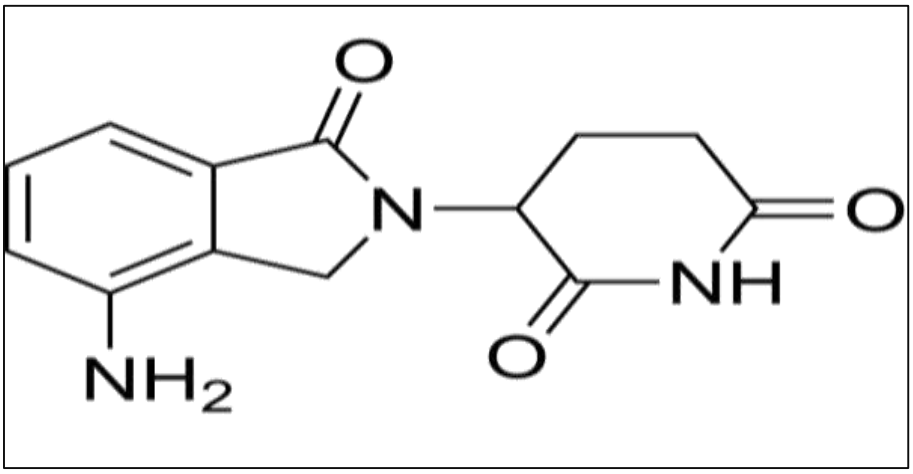

Fig.1 Lenalidomide Structure

- **Appearance:** White to off-white solid powder.
- **Molecular Formula:** $C_{13}H_{13}N_3O_3$
- **Molecular Weight:** 259.25 g/mol.
- **Solubility:** Poorly soluble in water and organic solvents; maximum solubility observed in 0.1N HCl (18 mg/mL).
- **Melting Point:** 265–268 °C.
- **logP:** Estimated at ~0.03, indicating low lipophilicity.

3. ANALYTICAL METHOD DEVELOPMENT STRATEGIES IN RP-HPLC

a) Drug Solubility

Lenalidomide is a white to pale yellow solid. Solubility studies are the first step to ensure complete sample extraction and mobile phase compatibility.

It is sparingly soluble in water, but exhibits better solubility in organic solvents like Dimethylformamide (DMF) and Dimethyl Sulfoxide (DMSO).

Most methods use a mixture of Methanol or Acetonitrile with aqueous buffers as a diluent to ensure the drug remains in solution throughout the analysis.1

b) Selection of Wavelength

A UV scan of Lenalidomide in the mobile phase typically shows significant absorption peaks at approximately 210 nm - 240 nm, and 250 nm.

210 nm is often selected for stability-indicating methods to capture degradation products, while 240-250 nm is used for routine assays to minimize baseline noise (Sharma & Singh, 2019).

c) Preparation of Solution and Linearity by UV

Before HPLC, UV spectrophotometry is used as a screening tool.

- **Standard Preparation:** A stock solution is prepared (e.g., 100 g/mL), and serial dilutions are made (2 -20 g/mL).
- **Linearity:** A calibration curve is plotted (Absorbance vs. Concentration). This establishes the initial concentration range for the more sensitive HPLC trials.

d) Selection of Stationary and Mobile Phase

- **Stationary Phase:** The C18 (Octadecylsilane) column is the preferred stationary phase due to the moderate polarity of the molecule, providing optimal retention and peak resolution (Patel & Patel, 2021).
- **Mobile Phase:** Based on the drug's pKa (2.31), an acidic buffer is required. Common selections include Phosphate buffer (pH 2.5–3.5**)** or Acetate buffer mixed with Acetonitrile (Raju & Venkatesham, 2023).

e) Selection and Optimization of Chromatographic Conditions

Optimization involves refining parameters to achieve high theoretical plates ($N > 2000$) and a low tailing factor ($T < 1.5$).

- **Trial & Error / AQbD:** Parameters like flow rate (0.8 - 1.2 mL/min) and organic ratio are adjusted. Modern methods use Quality by Design to find the "Design Space" where separation is most robust (Patel & Gandhi, 2023).

f) Preparation of Solution and Linearity study by RP-HPLC

Standard solutions are injected into the HPLC system.

- **Range:** Typically validated from **10 - 150 g/mL**.
- **Linearity:** The peak area is plotted against concentration. A correlation coefficient **(r^2) > 0.999** is expected (Singh & Kumar, 2022).

g) Validation of HPLC Method as per ICH Guidelines

Parameter	**ICH Requirement / Observation**
i. Range	Derived from the linearity study; usually 80-120 % of the target concentration.
ii. Linearity	Demonstrated by a linear regression line with minimal intercept.
iii. Specificity	Confirmed by absence of interference at the Rt of Lenalidomide from excipients or degradants
iv. Precision	**Repeatability** (Intraday) and **Intermediate Precision** (Interday) must show %RSD < 2.0.
v. Accuracy	Determined via recovery studies at 50%, 100%, and 150% levels; results should be 98 % - 102%
vi. LOQ	The lowest concentration that can be quantified with suitable precision and accuracy.
vii. LOD	The lowest detectable concentration (Signal-to-Noise ratio of 3:1).
viii. Robustness	Method remains unaffected by small variations in flow rate, pH, or temperature

Parameter	ICH Requirement / Observation
ix. Ruggedness	Consistency of results when performed by different analysts or on different days

1. Linearity

Linearity evaluates the ability of an analytical method to produce results that are directly proportional to the concentration of the analyte within a given range. In Reverse Phase High Performance Liquid Chromatography methods for lenalidomide, linearity is typically established by preparing a series of standard solutions and injecting them into the chromatographic system. A calibration curve is constructed by plotting peak area versus drug concentration, and the regression equation is calculated.

For lenalidomide analysis a linear concentration range suitable for pharmaceutical dosage forms with a correlation coefficient greater than **0.999**, indicating a strong linear relationship between concentration and peak area.

2. Specificity

Specificity refers to the ability of the analytical method to accurately measure the analyte in the presence of other components such as impurities, degradation products, and formulation excipients. In RP-HPLC analysis of Lenalidomide, specificity is evaluated by injecting blank samples, placebo formulations, and stressed samples into the chromatographic system.

According to studies the developed RP-HPLC method showed clear separation between lenalidomide and its excipients or degradation products.

3. Precision

Precision measures the closeness of agreement among multiple measurements of the same sample under specified conditions. It is typically evaluated in terms of repeatability (intraday precision) and intermediate precision (interday precision).

In RP-HPLC analysis of lenalidomide, the %RSD values for replicate injections were less than **2 %**, indicating excellent reproducibility of the developed method.

4. Accuracy

Accuracy expresses the closeness between the measured value and the true value of the analyte. In pharmaceutical analysis, accuracy is usually evaluated through recovery studies.

For lenalidomide analysis, known amounts of the drug are added to the pre-analyzed formulation at different concentration levels, typically 50 %, 100 %, and 150 % of the target concentration. The samples are then analyzed using the developed RP-HPLC method, and the percentage recovery is calculated.

5. Limit of Detection (LOD)

The Limit of Detection (LOD) represents the lowest concentration of the analyte that can be detected but not necessarily quantified with precision. It is generally calculated based on the signal-to-noise ratio of approximately 3:1.

6. Limit of Quantification (LOQ)

The Limit of Quantification (LOQ**)** is defined as the lowest concentration of the analyte that can be quantitatively determined with acceptable precision and accuracy. LOQ is usually calculated based on a signal-to-noise ratio of approximately 10:1.

7. Robustness

Robustness assesses the reliability of the analytical method when small deliberate changes are made to chromatographic parameters. Such variations may include changes in mobile phase composition, flow rate, pH of the buffer, or column temperature.

8. Ruggedness

Ruggedness evaluates the reproducibility of the analytical method under different operating conditions such as different laboratories, instruments, analysts, or days. It ensures that the analytical method provides consistent results regardless of variations in experimental conditions.

4. Literature Review of Reported Methods

A comparison of various reported RP-HPLC methods for Metronidazole estimation is summarized below to highlight trends in chromatographic conditions.

Reference	Column (Stationary Phase)	Mobile Phase Composition	Flow Rate (mL/min)	Detection λ (nm)	Key Highlights
Prasad et al. (2019)	C18 (250 × 4.6 mm, 5 µm)	Acetate buffer (pH 5.0) : Methanol (85:15)	1	250	Isocratic; rapid estimation in bulk & formulation.
Patel & Patel (2021)	Shimadzu C18 (250 × 4.6 mm, 5 µm)	Phosphate buffer : Acetonitrile (55:45)	1	242	Stability-indicating; Rt approx. 2.5 min.
Subramanyam (2016)	Chiralpak IC (250 × 4.6 mm, 5 µm)	n-Hexane : Ethanol : IPA : Diethylamine	1	230	Stereoselective; separates enantiomers.
Raju & Venkat. (2023)	Kromasil C18 (150 × 4.6 mm, 5 µm)	Phosphate buffer (pH 2.5) : ACN (90:10)	1	210	High sensitivity; stability-indicating for capsules.
Sharma & Singh (2019)	X-Bridge C18 (150 × 4.6 mm, 3.5 µm)	Phosphate buffer (pH 3.6) : Methanol (Gradient)	0.8	210	AQbD-based; optimized for impurity resolution.
Mishra & Sahu (2021)	C18 (150 × 4.6 mm, 5 µm)	Water : Ethanol (60:40)	1	304	Green Chemistry; eco-friendly solvents used.
Bharath et al. (2023)	Inertsil ODS-3V (150 × 4.6 mm, 3 µm)	Phos. buffer (pH 3.0) : ACN : Water (Gradient)	1	210	Quantification of 13+ related substances.

Key Observations from Literature:

- Mobile Phase : Most validated methods employ Phosphate or Acetate buffers.
- pH Control: Lenalidomide has a pKa of approximately 2.31.

- Literature indicates that maintaining a mobile phase pH between 2.5 and 3.6 is critical for achieving sharp peak symmetry and consistent retention times.
- Wavelength Selection: 240 to 250 nm Preferred for routine dosage form assays to minimize baseline noise and interference from common tablet excipients.
- Run times is from 10 -15 minutes

5. CONCLUSION

The review of current literature confirms that RP-HPLC is an indispensable tool for the pharmaceutical analysis of Lenalidomide. While C18 columns and acidic phosphate buffers remain the standard, the integration of AQbD and green chemistry principles marks the next evolution in LND estimation. These methods not only ensure regulatory compliance but also provide the sensitivity required for modern impurity profiling and stability monitoring.

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