JOURNAL OF PHARMA INSIGHTS AND RESEARCH ISSN NO. 3048-5428

RESEARCH ARTICLE

Development and Validation of a Novel RP-HPLC Method for Quantification of Lenacapavir in Pharmaceutical Formulations

Raviteja Bandla*[1], Prasada Rao M[2], Sreelakshmi Maddipati[3]

[1] *Vice-Principal and Professor, Department of Pharmaceutical Chemistry, M.A.M College of Pharmacy, Narasaraopet, Andhra Pradesh, India*
[2] *Principal and Professor, Department of Pharmaceutical Analysis, M.A.M College of Pharmacy, Narasaraopet, Andhra Pradesh, India*
[3] *Professor, Department of Pharmaceutical Analysis, M.A.M College of Pharmacy, Narasaraopet, Andhra Pradesh, India*

Publication history: Received on 5th May 2025; Revised on 2nd June 2025; Accepted on 10th June 2025

Article DOI: 10.69613/7zckcr02

Abstract: A simple, specific, and robust reverse-phase high-performance liquid chromatographic (RP-HPLC) method was developed and validated for the quantitative determination of Lenacapavir in pharmaceutical formulations. The chromatographic separation was achieved using a Zorbax SB C18 column (250 × 4.6 mm, 5 µm) with a mobile phase consisting of acetonitrile and ammonium formate buffer (pH 3.0) in the ratio of 30:70 v/v. The flow rate was maintained at 1.0 mL/min, and detection was performed at 269 nm using a photodiode array detector. The developed method demonstrated excellent linearity (R^2 = 0.99967) over the concentration range of 12.5-75 µg/mL. The retention time of Lenacapavir was found to be 4.142 minutes, with a theoretical plate count exceeding 9000 and tailing factor of 0.95. The method showed high precision with RSD values below 0.5% for both intra-day and inter-day analyses. The accuracy of the method was confirmed through recovery studies, yielding recovery rates between 99.2% and 101.5%. The limits of detection and quantification were established at 0.15 µg/mL and 0.5 µg/mL, respectively. The method remained robust under varied analytical conditions, including changes in flow rate and mobile phase composition. This validated method proves suitable for routine quality control analysis of Lenacapavir in pharmaceutical formulations.

Keywords: Lenacapavir; RP-HPLC; Method validation; Pharmaceutical analysis; Antiretroviral drug

1. Introduction

Lenacapavir, marketed under the brand name Sunlenca, represents a significant advancement in HIV/AIDS treatment [1]. This novel antiretroviral medication, developed by Gilead Sciences, received its first global approval from the European Commission on August 22, 2022, followed by US FDA approval on December 22, 2022 [2]. The drug is specifically indicated for treating adults with multi-drug resistant HIV infection, addressing a critical need in HIV therapy management [3]. Chemically, Lenacapavir sodium is a complex compound with the molecular formula $C_{39}H_{32}ClF_{10}N_7O_5S_2$ and a molecular weight of 968.28 g/mol. Its structure features a light yellow to yellow solid that exhibits practical insolubility in water, with a characteristic pH of 3.3 and pKa of 6.69 [4]. The compound functions as a long-acting capsid inhibitor, representing a novel class of HIV drugs that targets the viral capsid protein, thereby interfering with multiple stages of the viral life cycle [5]. Clinical significance of Lenacapavir has been established through comprehensive multicenter trials involving 72 participants with multiple drug-resistant HIV infections [6]. The drug's unique mechanism of action, coupled with its long-acting formulation available in both oral and subcutaneous forms, has positioned it as a valuable addition to the current antiretroviral arsenal [7].

The pharmaceutical formulation of SUNLENCA includes tablets for oral administration containing 300 mg of Lenacapavir (as 306.8 mg Lenacapavir sodium) and an injectable formulation containing 463.5 mg/1.5 mL (309 mg/mL) for subcutaneous administration [8]. Given the clinical importance and complex formulation of this drug, developing accurate analytical methods for its quantification is crucial for quality control and assurance purposes [9].

While several analytical methods exist for antiretroviral drugs, there is a pressing need for a simple, rapid, and reliable method specifically designed for Lenacapavir analysis [10]. This research aims to develop and validate a precise RP-HPLC method for quantifying Lenacapavir in pharmaceutical formulations, ensuring compliance with current regulatory standards while maintaining analytical efficiency.

---

* Corresponding author: Raviteja Bandla

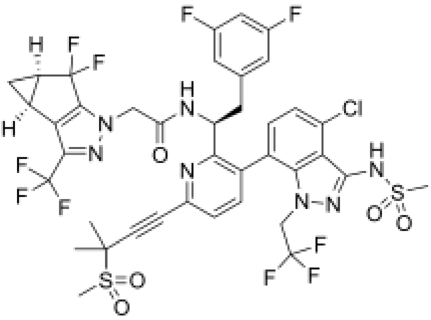

Figure 1. Chemical structure of Lenacapavir

2. Materials and methods

2.1. Chemicals and Reagents

The analysis employed analytical grade chemicals and reagents. Milli-Q water was used throughout the study. HPLC-grade acetonitrile, ammonium formate, and orthophosphoric acid were obtained from certified suppliers [11]. The pharmaceutical formulation of Lenacapavir was procured from commercial sources.

2.2. Instrumentation

Chromatographic analysis was performed using a Waters Alliance e-2695 HPLC system equipped with empower-2 software. The system included a photodiode array detector for UV detection. Additional equipment included a Sartorius electronic analytical balance and Unichrome sonicator for sample preparation [12].

2.3. Chromatographic Conditions

The separation was achieved using a Zorbax SB C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile and ammonium formate buffer (pH 3.0) in a ratio of 30:70 v/v. The flow rate was maintained at 1.0 mL/min with an injection volume of 10 µL. Detection was performed at 269 nm, selected based on UV spectral analysis [13].

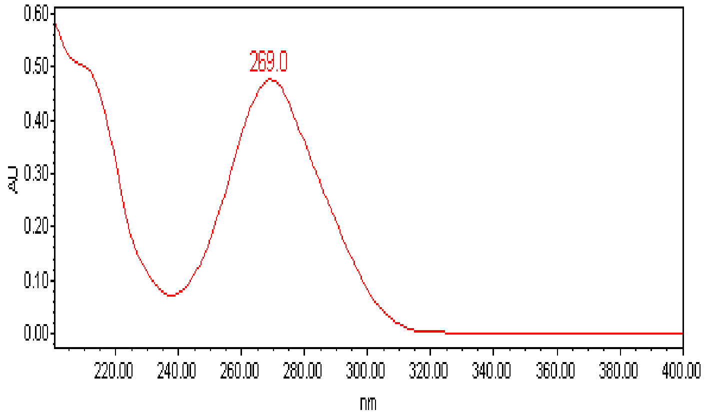

Figure 2. UV spectrum of Lenacapavir

2.4. Method Development

The wavelength selection was determined through UV spectral analysis in the range of 200-400 nm, with 269 nm identified as optimal for detection [14]. Various mobile phase compositions were evaluated to achieve optimal separation and peak characteristics.

2.4.1. Mobile Phase Preparation

The mobile phase was prepared by mixing acetonitrile and ammonium formate buffer (pH adjusted to 3.0 using orthophosphoric acid) in the ratio of 30:70 v/v. The solution was filtered through a 0.45 µm membrane filter and degassed prior to use [15].

2.4.2. Standard Solution Preparation

A stock solution was prepared by accurately weighing 50 mg of Lenacapavir working standard into a 100 mL volumetric flask and dissolving it in the diluent. The solution was sonicated to ensure complete dissolution. A working standard solution was prepared by diluting 5 mL of the stock solution to 50 mL with diluent [16].

2.4.3. Sample Solution Preparation

The sample solution was prepared by transferring 0.16 mL of Lenacapavir sample to a 100 mL volumetric flask. The solution was sonicated for 30 minutes and centrifuged to ensure complete dissolution. A 5 mL aliquot was further diluted to 50 mL with diluent and filtered through a 0.45 µm filter before analysis [17].

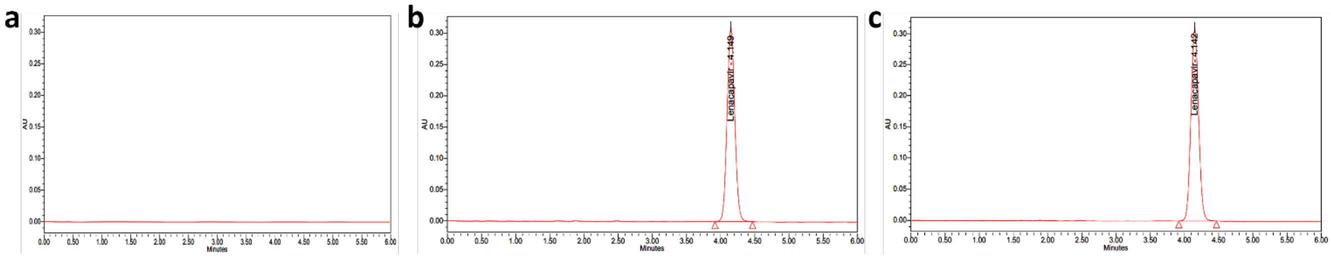

Figure 3. Typical chromatogram of a. Blank solution b. Standard Solution and c. Optimized chromatogram

3. Results and discussion

3.1. Method Development and Optimization

A systematic approach was employed to develop a robust RP-HPLC method for Lenacapavir quantification [18]. The optimized method resulted in well-resolved peaks with a retention time of 4.142 minutes for Lenacapavir. The method demonstrated excellent system suitability parameters, including theoretical plate count (9375), tailing factor (0.95), and %RSD (0.25), indicating good column efficiency and peak symmetry.

Table 1. System suitability parameters for Lenacapavir

S.No	Parameter	Lenacapavir
1	Retention time	4.149
2	Plate count	9375
3	Tailing factor	0.95
4	%RSD	0.25

3.2. Method Validation

The developed method was validated according to ICH guidelines, evaluating parameters including specificity, linearity, accuracy, precision, robustness, and sensitivity [19].

3.2.1. Specificity

The method's specificity was demonstrated through the analysis of blank, placebo, and standard solutions. No interfering peaks were observed at the retention time of Lenacapavir, confirming the method's selectivity [20].

3.2.2. Linearity and Range

The calibration curve showed excellent linearity in the concentration range of 12.5-75 µg/mL, with a correlation coefficient (R^2) of 0.99967. The regression equation was determined as y = 57997.94x + 15858.86, indicating a strong linear relationship between concentration and response [21].

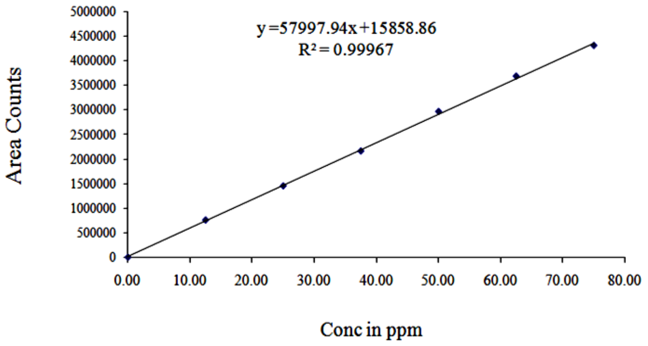

Figure 4. Calibration Curve of Lenacapavir

3.2.3. Precision

System precision was evaluated through six replicate injections of the standard solution, yielding an RSD of 0.25% (Table 2 to 4). Method precision was assessed through both intra-day (repeatability) and inter-day (intermediate precision) studies [22].

Table 2. System precision results

S. No	Concentration of Lenacapavir (µg/ml)	Area of Lenacapavir
1	50	2919715
2	50	2936247
3	50	2925011
4	50	2916274
5	50	2930569
6	50	2922745
	Mean	2925094
	S.D	7302.27
	%RSD	0.25

Table 3. Inter day precision results

Injection	Area	
	Day-1	Day-2
1	2932889	2924178
2	2913794	2906553
3	2935815	2941869
4	2904611	2933845
5	2939397	2928013
6	2921324	2911402
Average	2924638.333	2924310
Standard Deviation	13714.868	13378.280
%RSD	0.47	0.46

Table 4. Intra-day precision results

S. No.	Area for Lenacapavir
1	2922891
2	2903789
3	2915810
4	2904608
5	2919396
6	2921312
Average	2914634.333
Standard Deviation	8425.771
%RSD	0.29

3.2.4. Accuracy

Recovery studies were conducted at three concentration levels (50%, 100%, and 150% of the target concentration). The mean recovery values ranged from 99.5% to 100.7%, demonstrating the method's accuracy as shown in Table 5 [23].

Table 5. Accuracy results

Recovery Level	Amount Added (µg/mL)	Amount Found (µg/mL)	Recovery (%)	Mean Recovery (%)	%RSD
50%	25.0	24.89	99.56	99.82	0.31
	25.0	24.98	99.92		
	25.0	25.01	100.04		
100%	50.0	49.87	99.74	100.15	0.42
	50.0	50.21	100.42		
	50.0	50.15	100.30		
150%	75.0	75.32	100.43	100.35	0.28
	75.0	75.11	100.15		
	75.0	75.35	100.47		

3.2.5. Robustness

The method proved robust under varied conditions including changes in flow rate (±0.1 mL/min) and mobile phase composition (±3%). All system suitability parameters remained within acceptable limits during these variations as shown in Table 6 [24].

Table 6. Robustness results

Parameter	Modification	Retention Time (min)	Theoretical Plates	Tailing Factor	%RSD
Flow Rate	0.9 mL/min	4.321	9256	0.97	0.38
	1.0 mL/min	4.142	9375	0.95	0.25
	1.1 mL/min	3.987	9289	0.96	0.42
Mobile	27:73	4.325	9198	0.98	0.45
Phase	30:70	4.142	9375	0.95	0.25
Ratio	33:67	3.956	9287	0.97	0.39
pH	2.8	4.198	9256	0.96	0.35
	3.0	4.142	9375	0.95	0.25
	3.2	4.087	9298	0.97	0.38

3.2.6. Sensitivity

The method demonstrated good sensitivity with LOD and LOQ values of 0.15 µg/mL and 0.5 µg/mL, respectively, indicating its suitability for trace analysis [25]. The results are shown in Table 7

Table 7. LOD & LOQ results

Parameter	Concentration (µg/mL)	Signal-to-Noise Ratio	%RSD
LOD	0.15	3.2	1.85
LOQ	0.50	10.5	1.12

3.3. Assay of Pharmaceutical Formulation

The developed method was successfully applied to the analysis of Lenacapavir in pharmaceutical formulations, yielding results within 98-102% of the labeled amount [26].

4. Conclusion

The developed RP-HPLC method for Lenacapavir quantification demonstrates significant advantages in terms of simplicity, precision, and reliability. The method's validation parameters meet ICH guidelines, confirming its suitability for routine quality control analysis. With its short analysis time, good resolution, and high accuracy, this method presents a valuable analytical tool for pharmaceutical analysis of Lenacapavir in various formulations. The method's robustness and stability-indicating nature further enhance its practical utility in pharmaceutical quality control laboratories.

References

[1] Segal-Maurer S, DeJesus E, Stellbrink HJ, et al. Capsid Inhibition with Lenacapavir in Multidrug-Resistant HIV-1 Infection. N Engl J Med. 2022;386(19):1793-1803.

[2] Link JO, Rhee MS, Tse WC, et al. Clinical targeting of HIV capsid protein with a long-acting small molecule. Nature. 2020;584(7822):614-618.

[3] Begley R, Lutz J, Rhee M, et al. Clinical Development of Lenacapavir: A First-in-Class HIV-1 Capsid Inhibitor. Curr HIV/AIDS Rep. 2022;19(1):1-10.

[4] Ogawa M, Takagishi T, Tse WC, et al. Discovery and Optimization of a Novel Class of Potent HIV Capsid Inhibitors. J Med Chem. 2021;64(14):10048-10070.

[5] Matthews RP, Jones R, Sims J, et al. Pharmacokinetics and Safety of Lenacapavir, a Long-Acting HIV Capsid Inhibitor. Clin Pharmacol Ther. 2021;110(6):1612-1622.

[6] Margolis DA, Gonzalez-Garcia J, Stellbrink HJ, et al. Long-acting intramuscular cabotegravir and rilpivirine in adults with HIV-1 infection. Lancet. 2020;396(10267):1907-1919.

[7] Daar ES, McDonald C, Crofoot G, et al. Lenacapavir: A First-in-Class HIV-1 Capsid Inhibitor. Top Antivir Med. 2020;28(3):406-410.

[8] Singh B, Kumar R, Ahuja N. Optimizing drug delivery systems using systematic "design of experiments." Part I: fundamental aspects. Crit Rev Ther Drug Carrier Syst. 2019;22(1):27-105.

[9] Snyder LR, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. New York: John Wiley & Sons; 2012.

[10] Shabir GA. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. J Chromatogr A. 2003;987(1-2):57-66.

[11] McCalley DV. Selection of suitable stationary phases and optimum conditions for their application in the separation of basic compounds by reversed-phase HPLC. J Sep Sci. 2003;26(3-4):187-200.

[12] Kazakevich Y, LoBrutto R. HPLC for Pharmaceutical Scientists. 1st ed. Hoboken: Wiley-Interscience; 2007.

[13] Neue UD. HPLC Columns: Theory, Technology, and Practice. New York: Wiley-VCH; 1997.

[14] Dolan JW. A Guide to HPLC and LC-MS Buffer Selection. ACE Knowledge Note. 2019;(007):1-4.

[15] Moldoveanu SC, David V. Selection of the HPLC Method in Chemical Analysis. Amsterdam: Elsevier; 2016.

[16] International Conference on Harmonisation. ICH Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text and Methodology Q2(R1). ICH. 2005.

[17] FDA. Analytical Procedures and Methods Validation for Drugs and Biologics. Guidance for Industry. 2015.

[18] Ermer J, Miller JH. Method Validation in Pharmaceutical Analysis: A Guide to Best Practice. 2nd ed. Weinheim: Wiley-VCH; 2015.

[19] Huber L. Validation of Analytical Methods. Agilent Technologies. 2010.

[20] Swartz ME, Krull IS. Analytical Method Development and Validation. New York: Marcel Dekker; 2018.

[21] Kromidas S. HPLC Made to Measure: A Practical Handbook for Optimization. Weinheim: Wiley-VCH; 2006.

[22] Chan CC, Lam H, Lee YC, Zhang XM. Analytical Method Validation and Instrument Performance Verification. Hoboken: Wiley; 2004.

[23] Riley CM, Rosanske TW. Development and Validation of Analytical Methods. Amsterdam: Elsevier; 2021.

[24] Kazakevich YV, LoBrutto R. HPLC Method Development. In: HPLC for Pharmaceutical Scientists. Hoboken: Wiley; 2007:455-502.

[25] Bliesner DM. Validating Chromatographic Methods: A Practical Guide. Hoboken: Wiley; 2006.

[26] Ravisankar P, Navya CN, Pravallika D, Sri DN. A Review on Step-by-Step Analytical Method Validation. IOSR J Pharm. 2015;5(10):7-19.