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Original Article

DEVELOPMENT AND VALIDATION FOR DETERMINATION OF LENACAPAVIR BY UV-SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, rapid, accurate, and economical UV-Spectrophotometric method has been developed for determination of Lenacapavir in bulk and pharmaceutical formulation. The λmax of Lenacapavir in water was found to be 281 nm. The drug follows linearity in the concentration range 25-125 ppm with a correlation coefficient value of 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated was 99.19% and was found to be in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels, i.e., 50%, 100% and 150%. The % recovery was found to be in the range of 98.54-99.98%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intraday; interday variations, and repeatability. The % RSD value < 2 indicates that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts.

Keywords: *Lenacapavir, UV-Spectrophotometry, Analytical Method Validation, ICH Guidelines.*

INTRODUCTION

Lenacapavir is chemically sodium [4-chloro-7-[2-[(1S)-2-(3,5-difluorophenyl)-1-[[2-[(2S,4R)-5,5-difluoro-9-(trifluoro methyl)-7,8-diazatricyclonona-1(6),8-dien-7-yl]acetyl]amino] ethyl]-6-(3-methyl-3-methylsulfonylbut-1-ynyl)-3-pyridinyl]-1-(2,2,2-trifluoroethyl)indazol-3-yl]-methylsulfonylazanide[1]. It is an antiretroviral medication used to treat andpreventHIV/AIDS. It is taken by mouth or by subcutaneous injection[2, 3]. Lenacapavir is a human immunodeficiency virus type 1 (HIV-1) capsid inhibitor [2, 4].

While some of the research publications referred from different Techniques like LC-MS methods[5], RP-HPLC techniques [6-8]. This study establishes a validated UV- Spectrophotometry method per ICH Q2(R1) guidelines [9, 10].

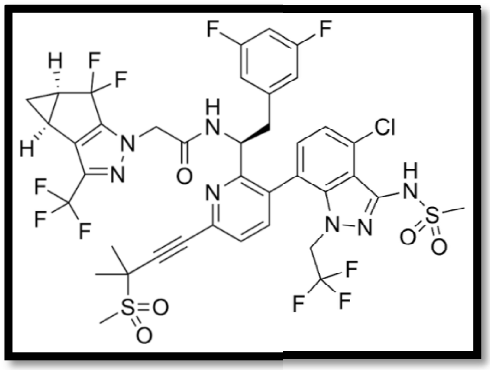

Fig. 1: Chemical Structure of Lenacapavir

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MATERIALS AND METHODS:

Table No. 1: Instruments Used

S. No.	Instrument	Model
1	UV/VIS spectrophotometer	HP UV 8453
2	pH meter	Adwa – AD 1020
3	Weighing machine	Afcoset ER-200A
4	Pipettes and Burettes	Borosil
5	Beakers	Borosil

Table No. 2: Chemicals Used

S. No.	Chemical	Company Name
1	Lenacapavir	Hetero labaroties
2	Distilled Water	Qualigens
3	Methanol	Fisher scientific
4	Ortho phosphoric Acid	Fisher scientific

UV Method Development:

Preparation of 0.1% Ortho-phosphoric acid Buffer:

Pipetted 1ml of ortho phosphoric acid in 1000 ml HPLC water.

Diluent Preparation: 0.1% OPA and Methanol (10:90)

Preparation of the Lenacapavir Standard & Sample Solution:

Standard Solution Preparation: Accurately weigh and transfer 100 mg of Lenacapavir working standard into a 10 mL clean dry volumetric flask add Methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of Lenacapavir of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Lenacapavir)

Sample Solution Preparation: Accurately weigh and transfer 100 mg of Lenacapavir (325.2mg of lyophilsed powder) sample into a 10 mL clean dry volumetric flask add small amount of Methanol and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44 micron Injection filter. (Stock solution)

Further pipette 0.75 ml of Lenacapavir of the above stock solution into a 25ml volumetric flask and dilute up to the mark with diluent. (75 ppm Lenacapavir)

Procedure: Keep standard, sample into the UV system and measure the absorbance for the Lenacapavir peaks and calculate the %Assay by using the formulae.

Calculation:

$$\%\ Assay = \frac{AT}{AS} * \frac{WS}{DS} * \frac{DT}{WT} * \frac{Average\ weight}{Label\ Claim} * \frac{P}{100} * 100$$

Where:

AT=Average absorbance counts of sample preparation; AS=Average absorbance counts of standard preparation; WS=Weight of working standard taken in mg; DS=Dilution of working standard in ml; DT=Dilution of sample in ml; WT=Weight of sample taken in mg; P=Percentage purity of working standard; LC=Label Claim of Lenacapavir and Valsartan mg/ml.

Assay Results: (For Lenacapavir)

$$\frac{0.159}{0.158} * \frac{100}{100} * \frac{0.75}{10} * \frac{100}{325.2} * \frac{10}{0.75} * \frac{325.2}{100} * \frac{99.8}{100} * 100 = 100.43$$

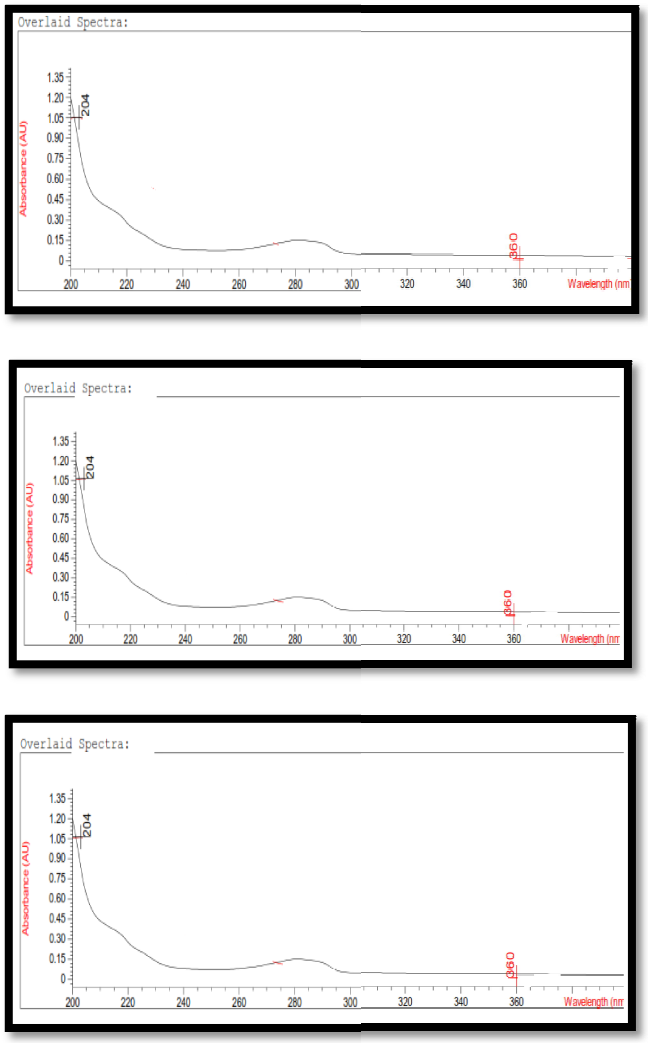

Fig. 2: Lenacapavir Standard Spectra's

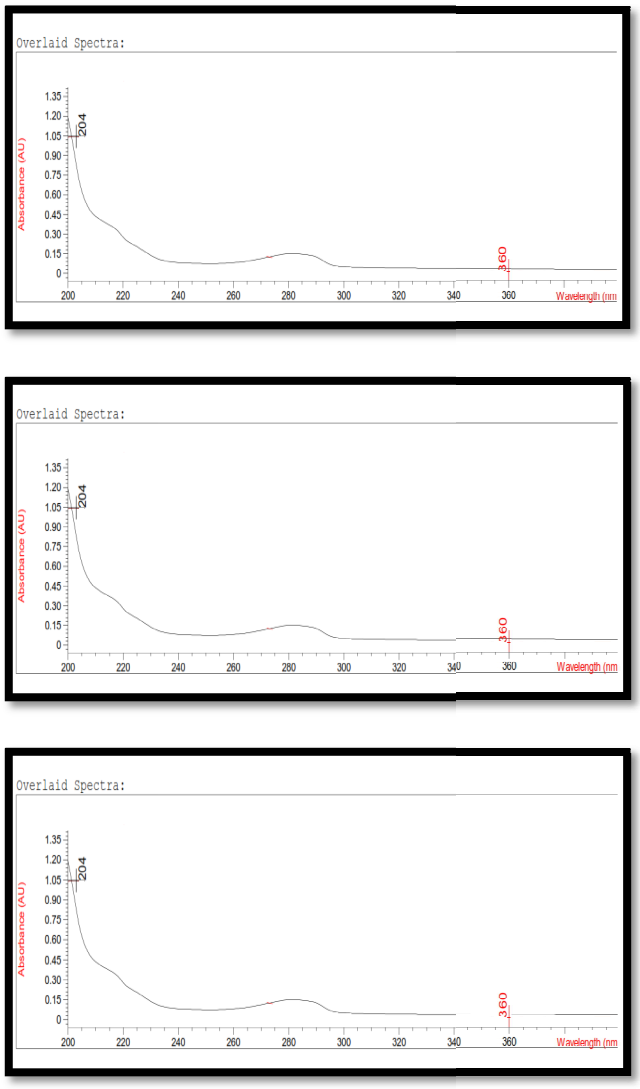

Fig. 3: Lenacapavir Sample Spectra's

Table No. 3: Standard and Sample

S. No.	Lenacapavir Standard at 281 nm	Lenacapavir Sample at 281 nm
1	0.159	0.158
2	0.158	0.159
3	0.158	0.158
4	0.158	0.159
5	0.159	0.159
6	0.158	0.159
Average	0.158	0.159
SD	0.001	0.001
% RSD	0.3	0.3

RESULTS AND DISCUSSIONS:

Method Validation:

1. Linearity:

Preparation of stock solution: Accurately weigh and transfer 100 mg of Lenacapavir working standard into a 100 mL clean dry volumetric flask add Methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent. And finally prepared different levels of Linearity Concentrations of 25 – 125 ppm

Table No. 4: Linearity Data of Lenacapavir

S. No.	Linearity Level	Concentration (µg/ml)	Absorbance at 281nm
1	I	0	0
2	II	25	0.052
3	III	50	0.098
4	IV	75	0.145
5	V	100	0.188
6	VI	125	0.232
Correlation Coefficient			0.999

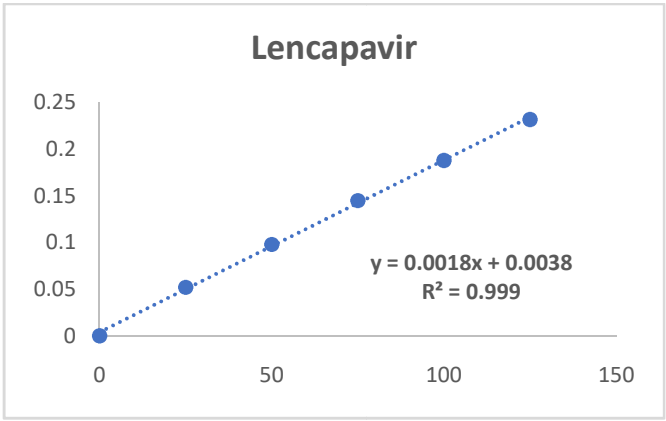

Fig. 4: Lenacapavir Correlation Coefficient Curve

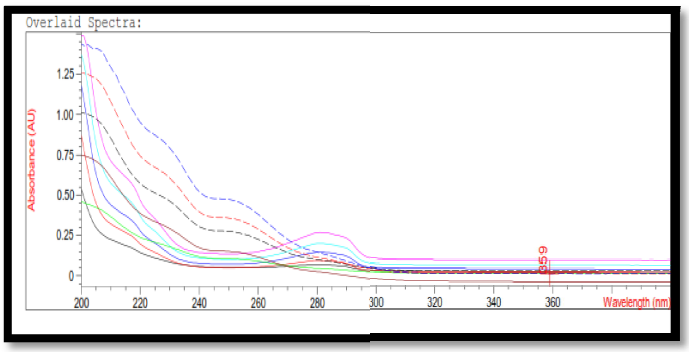

Fig. 5: Linearity Overlay

2. Precision:

Preparation of stock solution: Accurately weigh and transfer 100 mg of Lenacapavir working standard into a 10 mL clean dry

volumetric flask add Methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

Further pipette 0.75 ml of Lenacapavir of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Lenacapavir)

Table No. 5: Precision results are summarized for Lenacapavir

Precision	Lenacapavir at 281nm
Precision-1	0.159
Precision-2	0.157
Precision-3	0.159
Precision-4	0.159
Precision-5	0.158
Precision-6	0.158
Average	0.158
Standard Deviation	0.001
%RSD	0.5

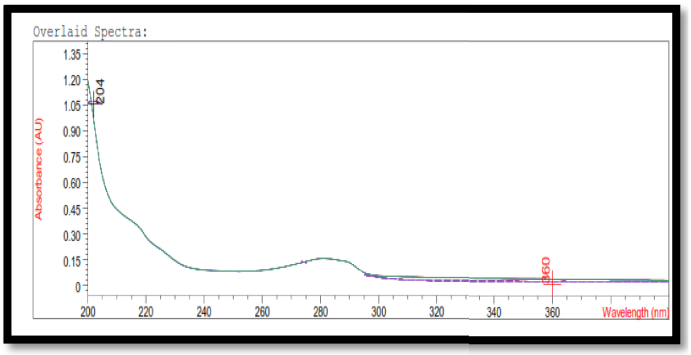

Fig. 6: Precision Overlay

2.1. Intermediate Precision / Ruggedness:

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day.

Preparation of stock solution: Accurately weigh and transfer 100 mg of Lenacapavir working standard into a 10ml clean dry volumetric flask add Methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of Lenacapavir of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Lenacapavir)

Table No. 6: Intermediate Precision results are summarized for Lenacapavir

Precision	Lenacapavir at 281nm
Precision-1	0.157
Precision-2	0.157
Precision-3	0.154
Precision-4	0.155
Precision-5	0.157
Precision-6	0.156
Average	0.156
Standard Deviation	0.001
% RSD	0.8

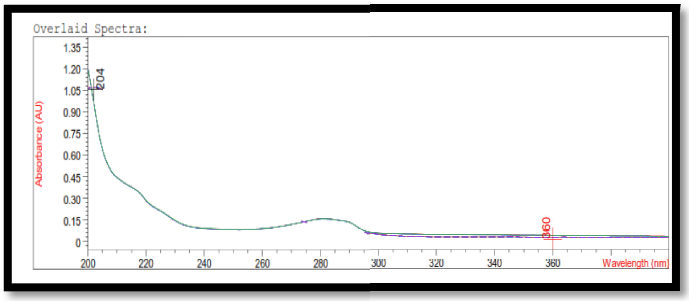

Fig. 7: Intermediate Precision Overlay

2.2. Method Precision:

To evaluate the method precision six individual samples solutions were prepared and calculate the % of Assay.

Preparation of standard solution: Accurately weigh and transfer 100 mg of Lenacapavir working standard into a 10 mL clean dry volumetric flask add Methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of Lenacapavir of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Lenacapavir)

Table No.7: Precision results for Lenacapavir

Precision	Lenacapavir at 281nm(%Assay)
Precision-1	100.94
Precision-2	100.46
Precision-3	100.34
Precision-4	99.77
Precision-5	100.37
Precision-6	99.80
Average	100.28
Standard Deviation	0.44
%RSD	0.44

3. Accuracy:

Preparation of Standard stock solution: Accurately weigh and transfer 100 mg of Lenacapavir working standard into a 10 mL clean dry volumetric flask add Methanol and sonicate to dissolve

it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of Lenacapavir of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent. (75 ppm Lenacapavir)

Preparation Sample solutions:

For preparation of 50%, 100% & 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 50 mg, 100 mg & 150 mg of Lenacapavir working standard into a 10 mL clean dry volumetric flask separately then add Methanol and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette 0.75 ml of Lenacapavir of the above stock solution into separatethree 10 ml volumetric flasks and dilute up to the mark with diluent. (37.5 ppm, 75 ppm & 112.5 ppmLenacapavir)

Table No.8: Accuracy results are summarized for Lenacapavir

%Concentration (at specification Level)	Abs	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	0.079	50	49.9	99.80	
100%	0.159	100	100.4	100.43	
150%	0.238	150	150.3	100.22	100.15

4. Robustness:

As part of the Robustness, deliberate change in the Wave length

a. The Wave length was varied ±3nm.

Standard solution 75 ppm of Lenacapavir was prepared and analysed using the varied wavelength along with method wave length.

Table No. 9: Robustness results are summarized for Lenacapavir

S. No.	Lenacapavir	
	Wavelength(nm)	Absorbance
1	279	0.148
2	281	0.152
3	283	0.155

5. Detection Limit and Quantitation Limit (LOD & LOQ):

The limits are commonly associated with the signal to noise ratio (S/N). In the case of LOD, analysts often use S/N (signal to noise ratio) of 2:1 or 3:1, while a S/N of 10:1 is often considered to be necessary for the LOQ. Typically, the signal is measured from the base line to peak apex and divided by the peak-to-peak noise, which is determined from the blank plasma injection.

$$LOQ = 10\ \sigma / S \qquad LOD = 3.3\ \sigma / S$$

Where, σ - Standard deviation from response S - Slope from calibration curve.

Table No.10: Detection Limit and Quantitation Limit Data of Lenacapavir

Parameters	Lenacapavir
LOD	0.210 µg/mL
LOQ	0.467 µg/mL

6. Degradation Studies:

Preparation of Standard stock solution: Accurately weigh 100 mg Lenacapavir sample into a 10 ml clean dry volumetric flask add about 30 ml of Diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.44-micron Injection filter.

6.1. Hydrolytic degradation under acidic condition: Pipette 0.75 ml of above solution into a 10 ml volumetric flask and 3 ml of 0.1N HCl was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1 N NaOH and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and scan the Solution at 281 nm.

6.2. Hydrolytic degradation under alkaline condition: Pipette 0.75 ml of above solution into a 10 ml volumetric flask and add 3 ml of 0.1N NaOH was added. Then, the volumetric flask was kept at 60ºC for 6 hours and then neutralized with 0.1N HCl and make up to 10ml with diluent. Filter the solution with 0.22 microns syringe filters and scan the Solution at 281 nm.

6.3. Oxidative degradation: Pipette 0.75 ml above stock solution into a 10ml volumetric flask add 3 ml 3% w/v of hydrogen peroxide added in 10 ml of volumetric flask and the volume was made up to the mark with diluent. The volumetric flask was then kept at room temperature for 15 min. Filter the solution with 0.45 microns syringe filters and scan the Solution at 281 nm.

6.4. Thermal induced degradation: Lenacapavir sample was taken in petridish and kept in Hot air oven at 1100 C for 24 hours. Then the sample was taken and diluted with diluents and scan the Solution at 281 nm and analysed.

6.5. Photo degradation: Pipette 10 ml above stock solution into a 100ml volumetric flask and expose to sunlight for 24hrs and the volume was made up to the mark with diluent. Filter the solution with 0.45 microns syringe filters and scan the Solution at 281 nm.

Table No. 11: Degradation Data of Lenacapavir

	Absorbance		% Degraded
Standard	0.156	100	
Acid	0.150	96.15	3.85
Base	0.146	93.59	2.56
Peroxide	0.147	94.23	5.77
Thermal	0.152	97.44	2.56
Photo	0.152	97.44	2.56

CONCLUSION:

A new novel simple, sensitive analytical method was developed and validated for determination of analytical method was validated according to USFDA guidelines. All the parameters validated were within the acceptable criteria and UV-spectrophotometric method has been developed for determination of lenacapavir in bulk and pharmaceutical formulation. The λmax of lenacapavir in water was found to be 281 nm. The drug follows linearity in the concentration range 25-125 ppm with a correlation coefficient value of 0.999. The proposed method was applied to pharmaceutical formulation and % amount of drug estimated was 99.19% and was found to be in good agreement with the label claim. The accuracy of the method was checked by recovery experiment performed at three different levels, i.e., 50%, 100% & 150%. The % recovery was found to be in the range of 98.54-99.98%. The low values of % RSD are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as an intraday; interday variations, and repeatability. The % RSD value < 2 indicates that the method is precise. Ruggedness of the proposed method was studied with the help of two analysts.

As the sensitivity of spectrophotometry of this method is high, this method can be applied for pharmacokinetic studies of newly developed formulations.

Reference:

1. https://pubchem.ncbi.nlm.nih.gov/compound/Lenacapavir-Sodium#section=3D-Status.
2. Sunlenca-lenacapavir sodium tablet, film coated Sunlenca-lenacapavir sodium kit. DailyMed. 21 December 2022. Archived from the original on 21 January 2023. Retrieved 21 January 2023.
3. Sunlenca EPAR. European Medicines Agency (EMA). 22 June 2022. Archived from the original on 26 August 2022. Retrieved 25 August 2022.
4. Yeztugo-lenacapavir sodium tablet, film coated; Yeztugo-lenacapavir sodium kit. Daily Med. 18 June 2025. Retrieved 6 July 2025.
5. Gupta V, et al. J Chromatogr B. 2020; 1145:122102.
6. Snyder LR, et al. Practical HPLC Method Development. Wiley; 2012. (p. 203-217).
7. Prasanth VG, et al. Development and Validation of a Reverse-Phase HPLC Method for Estimation of Lenacapavir in Pure and Pharmaceutical Formulations. Entire Res Int Multi-Discip Res J, 2025; 17(2): 1-7.
8. Raviteja Bandla, et al. Development and Validation of a Novel RP-HPLC Method for Quantification of Lenacapavir in Pharmaceutical Formulations. J Pharm Insights and Res, 2025; 3(4): 40-45. DOI: 10.69613/7zckcr02.
9. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1). International Conference on Harmonization, Geneva, 2005; p. 8-15.
10. EMA Guideline. CPMP/ICH/381/95. 1995; p. 3-9.

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