IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
Volume 14, Issue 2 Series. I (February 2024), PP. 22-29
www.iosrphr.org

Development and validation of UV Method for Quantitative Estimation of Leniolisib in pharmaceutical dosage form

G. Sai Teja*, D. Archana, B. Srinu, SK. Abbas Ali, S. Siva Narayana Reddy, B. Divya, P. Srinivasa Babu, P. Ravi Sankar*

Department of Pharmaceutical Analysis, Vignan Pharmacy College, Vadlamudi, Guntur, A.P, India.
**Corresponding author's E-mail: banuman35@gmail.com*
Received 10 February 2024; Accepted 25 February 2024

ABSTRACT
In this investigation, a robust, cost-effective, and swift approach for determining Leniolisib in bulk and pharmaceutical formulations, following the guidelines set by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). The method employs double-beam UV Spectrophotometry and has undergone thorough validation to ensure its reliability. Leniolisib samples, dissolved in methanol, exhibited maximum absorbance at 222.1 nm. The method's linearity, validated within the concentration range of 5-30 µg/mL, adheres to Beer's law. Validation parameters such as precision, limit of detection (LOD), limit of quantification (LOQ), accuracy, robustness, and ruggedness were meticulously assessed. The method demonstrated high precision, low LOD and LOQ, indicating its sensitivity. Accuracy, evaluated through % recovery, ranged from 99.0 % to 100.1 %. Additionally, when applied to Leniolisib Tablets, the method showed a percentage assay exceeding 99.9 %, underscoring its accuracy and reliability in pharmaceutical dosage form analysis. In conclusion, the proposed UV Spectrophotometric method is proven to be precise, accurate, and reproducible, making it suitable for routine Leniolisib analysis in bulk and pharmaceutical formulations. Aligned with ICH guidelines, this method provides a practical and cost-effective alternative for Leniolisib quantification, contributing to the pharmaceutical analytical toolkit for research and quality control.
Keywords: Leniolisib, Method development, Validation, Ultraviolet Spectroscopy, Pharmaceutical Analysis, ICH Guidelines.

I. INTRODUCTION

Cancer is a leading cause of death worldwide (nearly one in six deaths according to the WHO). Breast Cancer Resistance Protein Inhibitor. In 2021, out of 10 million cancer-related deaths, breast cancer contributed 2.26 million cases. Leniolisib,[1] a novel, potent oral selective oral Phospho Inositide 3-kinase-delta PI3Kδ path way inhibitor. Leniolisib interferes with the signalling pathways that drive cancer cell proliferation and, cell growth leading to cell death and tumour shrinkage[2-5]. It is crucial to develop a new analytical method for the estimation of Lineolisib in bulk and pharmaceutical dosage forms to ensure safe, precise analysis, product quality. In March 2023, leniolisib (Joenja 70 mg) received its first approval for the treatment of immunodeficiency disorders. Activated PI3K-delta syndrome (also known as APDS) is a disorder that impairs the immune system. It is also used in the treatment of breast cancer and lymphoma [a type of blood cancer]. Symptoms are frequent upper respiratory tract infections, sinus infections, ear infections, bronchitis, swollen lymph nodes and pneumonia (lung infection). This breakthrough medication marks a significant step in forward in addressing both immune deficiency disorders and certain types of cancer. Upon conducting an extensive literature review, it has been identified that there is a lack of reported analytical techniques for the quantification of leniolisib in both its bulk form, pharmaceutical preparations, and biological fluids. In light of this gap in existing methodologies, we have chosen to devise a straightforward, accurate, precise, and dependable UV-spectrophotometric approach for the determination of leniolisib in pharmaceutical dosage forms. The chemical structure of leniolisib is shown in the Figure 1.

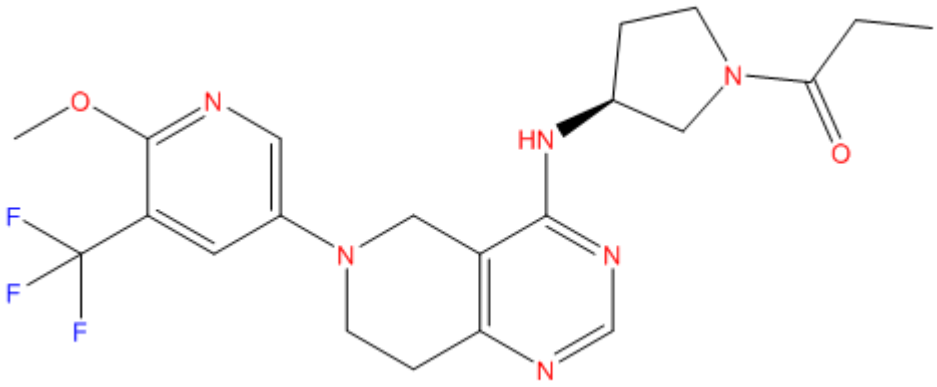


Fig No. 1: Chemical structure of Leniolisib

II. MATERIALS AND METHODS

Instruments

A double beam ELICO SL 210 UV spectrophotometer containing two matched quartz cells with a one cm light path was taken for measuring of absorbance of Leniolisib. Essae vibra AJ (0.1 mg sensitivity) balance was used for weighing. Ultra Sonicator bath Model no – 91250, PCI Ltd., Mumbai was used in this present study.

Chemicals and reagents

An analytically pure sample of Leniolisib was obtained from Hetero Drugs Ltd., Hyderabad, and Telangana, India. Leniolisib Tablets containing 70 mg labeled claim. Leniolisib Tablets were used for this study. ACN and CH_3OH were procured from E. Merck specialties, private Ltd., Mumbai, India.

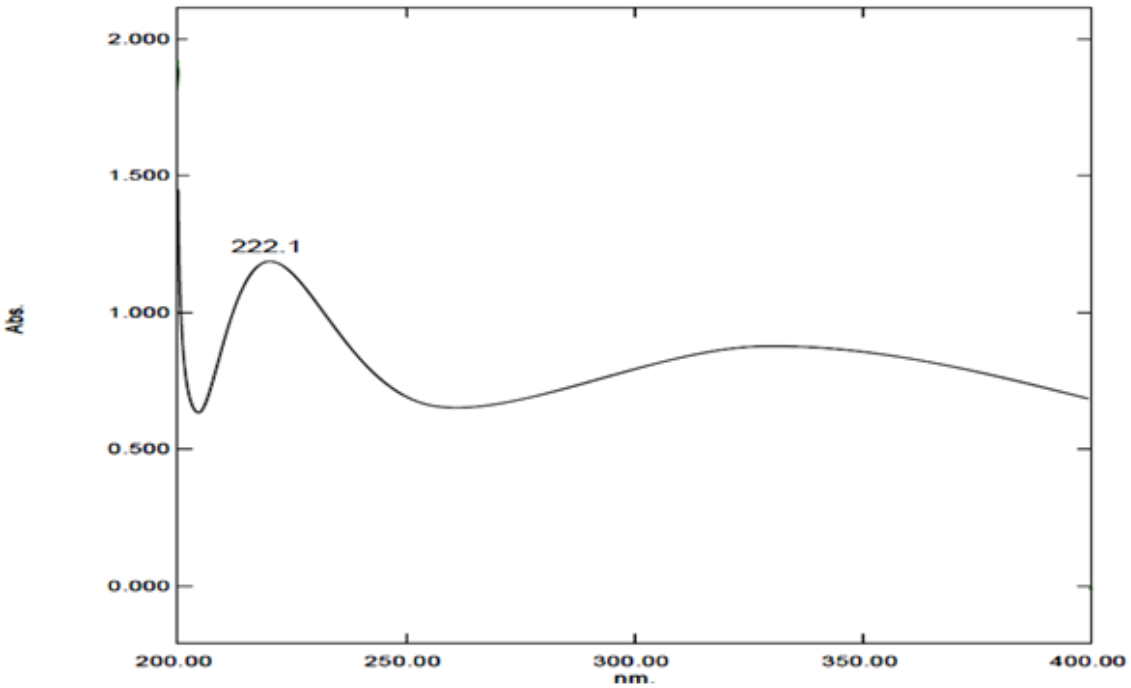


Figure 2: UV spectrum of Leniolisib

Selection of solvent:

Solubility of the drug was checked in different solvents and the drug was found to be soluble in Acetonitrile. From the solubility analysis, Acetonitrile was selected as solubilising agent for method development.

Determination of λ_{max}

A solution of Leniolisib 20 µg/mL was scanned against acetonitrile blank in the range of 200-400 nm. The λ_{max} was found to be 222.1 nm**.**

Preparation of standard solution

Leniolisib 20 mg was accurately weighed and transferred into a 100 mL volumetric flask. The drug was fully dissolved in Acetonitrile, and the solution was adjusted to the final volume with the same solvent, resulting in a stock solution with a concentration of 200 µg/mL. Aliquots ranging from 1 mL to 10 mL were then pipetted from the standard stock solution and appropriately diluted with acetonitrile to achieve the desired final concentrations for the standard solutions.

Preparation of calibration curve

Aliquots were carefully pipetted from the standard stock solution into a set of 10 mL volumetric flasks. The volumes were then adjusted with water to reach the mark, creating a range of dilutions with concentrations of 5, 10, 15, 20, 25, and 30 µg/mL of Leniolisib. The absorbance of these solutions was measured at 222.1 nm, and the data was used to construct a calibration curve, plotting absorbance against concentration in a zero-order spectra.

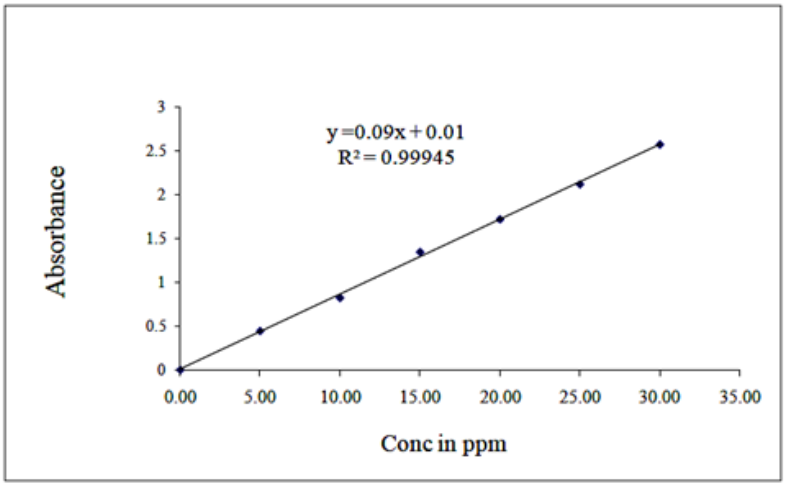
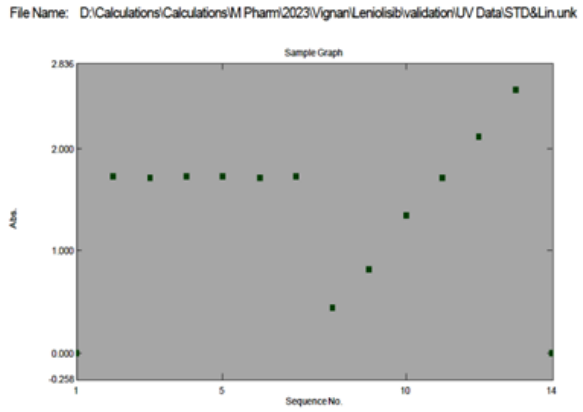


Figure 3: Calibration Curve for Leniolisib

Table 1: Calibration data of Leniolisib

Leniolisib		
S.No	**Concentration (µg/mL)**	**Absorbance**
1	5.00	0.444
2	10.00	0.823
3	15.00	1.349
4	20.00	1.723
5	25.00	2.123
6	30.00	2.578



Sample Table

	Sample ID	WL222.0
1 *	Blank	0.000
2	Standard 1	1.729
3	Standard 2	1.722
4	Standard 3	1.731
5	Standard 4	1.726
6	Standard 5	1.720
7	Standard 6	1.728
8	Linearity 1	0.444
9	Linearity 2	0.823
10	Linearity 3	1.349
11	Linearity 4	1.723
12	Linearity 5	2.123
13	Linearity 6	2.578
14	Blank 1	0.000
15		

Figure 1a: Standard and Linearity Data Pertaining to Lineolisib

III. RESULTS AND DISSCUSSION

Method development and validation[6-12]

Leniolisib 20 mg pure drug was carefully weighed and transferred into a 100 mL volumetric flask. The drug was fully dissolved in Acetonitrile and adjusted to the final volume with the same solvent, resulting in a stock solution with a concentration of 200 µg/mL. Subsequently, 1 ml to 10 ml aliquots were pipetted from the standard stock solution and appropriately diluted with acetonitrile to achieve final concentrations for standard solutions. For further dilutions, suitable aliquots were pipetted from the standard stock solution into a series of 10 mL volumetric flasks. The volume was then adjusted to the mark with water, creating a range of dilutions with concentrations ranging from 5, 10, 15, 20, 25, to 30 µg/mL of Leniolisib. The absorbance of these solutions was measured at 222 nm and converted into zero-order spectra. A calibration curve was plotted by correlating absorbance against concentration, and the regression equation and correlation coefficient were determined.

The method demonstrated adherence to Beer Lambert's law within the concentration range of 5-30 µg/mL. Validation of the UV spectrophotometric method was conducted in accordance with ICH guidelines, assessing parameters such as linearity, accuracy, precision, robustness, and ruggedness.

Linearity

Fresh aliquots were prepared from standard stock solution ranging from 5-30 µg/mL and the absorbance values of each concentration was recorded at 222 nm for zero order using acetonitrile as blank. The drug shows linearity between 5-30 µg/mL for method. The correlation co efficient was found to be 0.999 for method.

Table 2: Linearity of Leniolisib

S. No	Leniolisib	
	Conc.(µg/mL)	Absorbance
1	5.00	0.444
2	10.00	0.823
3	15.00	1.349
4	20.00	1.723
5	25.00	2.123
6	30.00	2.578
Regression	y = 0.09x+0.01	
Slope	0.09	
Intercept	0.01	
R^2	0.99945	

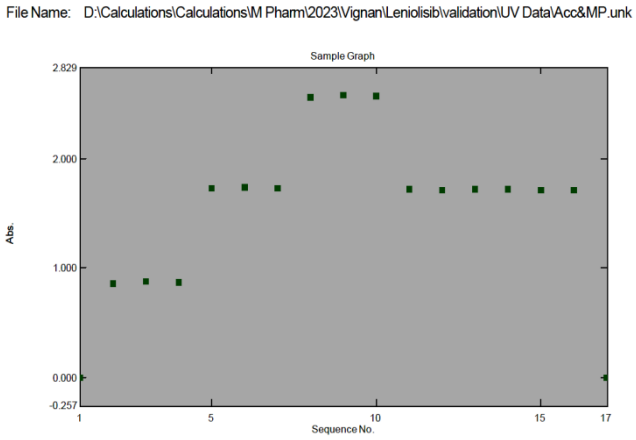
Accuracy

Accuracy of the developed method was confirmed by performing recovery studies at three different concentration ranges 50 %, 100 %, 150 % each one in triplicate and the accuracy was indicated by % recovery.

The % RSD for accuracy of Leniolisib in the method was found to be less than 2. The % recovery was in the range of 99.7 %. According to ICH guidelines[13] the statistical results were within the acceptance range.

Table 3: Accuracy data of UV Method

Method	Amount of µg/mL		% of drug added	% Recovered	% Mean Recovery
	LC	Pure drug			
Method	70	10	50	99.9	99.7
		20	100	100.1	
		30	150	99.0	



Sample Table

	Sample ID	WL222.0
1	Blank	0.000
2	Accuracy 50% 1	0.856
3	Accuracy 50% 2	0.871
4	Accuracy 50% 3	0.869
5	Accuracy 100% 1	1.726
6	Accuracy 100% 2	1.730
7	Accuracy 100% 3	1.728
8	Accuracy 150%1	2.555
9	Accuracy 150%2	2.572
10	Accuracy 150%3	2.563
11	Method Precision 1	1.716
12	Method Precision 2	1.710
13	Method Precision 3	1.719
14	Method Precision 4	1.714
15	Method Precision 5	1.708
16	Method Precision 6	1.704
17	Blank 1	0.000

Figure 3a: Accuracy and precision pertaining to data of Lineolisib

Precision

The precision of the method was verified through intra-day and inter-day variation studies. For the intra-day study, six solutions with a concentration of 20 µg/mL were prepared and analyzed three times within a single day, with the respective absorbances recorded. The results were expressed as % RSD. In the inter-day study, six solutions of 20 µg/mL were prepared and analyzed three times over three consecutive days, with the corresponding absorbances noted. The outcomes were presented as % RSD. The % RSD values for both intra-day and inter-day precision of Leniolisib in the method were found to be less than 2. In accordance with ICH guidelines[13], the % RSD should be less than 2, falling within the accepted criteria.

Table 4: Intra-day precision of Leniolisib

Analytical method	Method Precision	Absorbance	% Assay
Method	1	1.716	99.2
	2	1.710	
	3	1.719	
	4	1.714	
	5	1.708	
	6	1.704	

Table 5: Inter day precision of Leniolisib

Analytical method	Intermediate Precision	Absorbance	% Assay
Method	1	1.704	99.1
	2	1.713	
	3	1.718	

	4	1.707	
	5	1.721	
	6	1.700	

Robustness:
Robustness of the method was determined by carrying out the analysis at two different wavelengths (±5nm). The respective absorbances were noted and the results were indicated by % RSD. The % RSD values were found to be within the acceptance criteria.

Table 6: Robustness results

Parameter	Concentration 20 (µg/mL)	% Assay
		Method-A
Robustness Change in λ_{max} (± 5nm)	λ+: 227 nm	99.7
	λ -: 217 nm	99.9

Ruggedness

Ruggedness of the method was determined by carrying out the analysis by two different analysts and the respective absorbances were noted. The results were indicated by % RSD**.** The % ruggedness of three replicates of Leniolisib at a concentration of 20 µg/mL was found to be within the acceptance limits.

Forced Degradation Table 28: Forced degradation results of Leniolisib

S.No	Degradation	Absorbance	% degradation
1	CONTROL	1.728	0
2	ACID	1.518	12.1
3	ALKALI	1.496	13.4
4	PEROXIDE	1.459	15.6
5	REDUCTION	1.689	2.3
6	THERMAL	1.709	1.1
7	PHOTOLYTIC	1.552	10.2
8	HYDROLYSIS	1.708	1.2

Analysis of marketed formulation

The developed method was applied to analyze commercially available Lineolisib. The tablet was having the content of Lineolisib equivalent to 70 mg. Ten tablets were weighed and a weight equivalent to 70 mg was dissolved in acetonitril. By frequent shaking, volume was made up to mark with acetonitril. The solution was then filtered through Whatman filter paper #41. This filtrate was diluted suitability with solvent to get a solution of 5 µg/ml concentration. The absorbance was measured against the solution blank. The amount of Lineolisib was calculated from the calibration curve. The readings were taken in triplicate. The assay results are show

Table No.7: Assay of Leniolisib

Brand	Drug	Sample Area	Avg sample area (n=5)	Std. wt	Sample wt.	Label amount (mg)	Std purity	Amount found (µg/ml)	% assay
-	Leniolisib	3024237 3046948	3035593	7.0	15.6	70	99.9	7.0	100.0

Table 8: Summary of Validation & Optical characteristics

Parameter	Results
Beer's law limit (µg/mL) Linear regression equation Linearity indicated by correlation coefficient Precision indicated by %RSD Intraday precision Inter day precision Accuracy indicated by % recovery Robustness indicated by % recovery (WP/WM) Ruggedness indicated by % recovery	5-30 y=0.09x+0.01 0.999 0.25 0.32 0.38 99.0-100.1% 99.7/99.9 99.1

IV. DISCUSSION

An attempt has been made to develop a validated stability indicating UV method for the estimation of Leniolisib. The peak of Leniolisib was eluted at retention times of 3.978 min. Using Shimadzu 1700- UV Visible spectrophotometer the estimation of Leniolisib is done by using acetonitrile as a solubilising agent and solvent and the λ_{max} was found to be 222 nm for calibration curve method and first order derivative. The UV Spectrophotometric estimation uses Acetonitrile as solubilising agent, and in major proportions and validated according to ICH guidelines for linearity, sensitivity parameters, precision, accuracy, ruggedness and robustness and all the validation results were found well within the limits, indicating that the developed method was simple, rapid, accurate, precise, robust and economical. In this proposed UV method for the selected drug showed good linearity. Results for the recoveries of selected drugs were found to be within limits (98 – 102 %). These indicate that the proposed method was accurate for the analysis.

V. CONCLUSION

The developed UV method for the estimation of selected drug is simple, rapid, accurate, precise, robust and economical. The mobile phase and solvents are simple to prepare and economical, reliable, sensitive and less time consuming. The sample recoveries were in good agreement with their respective label claims and they suggested noninterference of formulation recipients in the estimation and can be used in laboratories for the routine analysis of selected drug. Since the system validation parameters of UV method used for estimation of selected drug in pure and have shown satisfactory, accurate and reproducible results (without any interference of recipients) as well, it is deduced that the simple and short proposed methods be most useful for analysis purpose. The present work concluded that stability indicating assay method by UV was simple, accurate, precise, and

specific and has no interference with the placebo and degradation products. Hence these can be used for routine analysis of Leniolisib.

REFERENCES:

[1]. Duggan S, Al-Salama ZT. Leniolisib: First Approval. Drugs. 2023;83(10):943-948. doi: 10.1007/s40265-023-01895-4. PMID: 37256490.

[2]. Rao VK, Webster S, Šedivá A, et al. A randomized, placebo-controlled phase 3 trial of the PI3Kδ inhibitor leniolisib for activated PI3Kδ syndrome. Blood. 2023;141(9):971–83.

[3]. Newman H, Teachey DT. PI3king apart a rare disease with targeted therapy. Blood. 2023;141(9):963–4.

[4]. Bloomfield M, Klocperk A, Zachova R, Milota T, Kanderova V, Sediva A. Natural Course of Activated Phosphoinositide 3-Kinase Delta Syndrome in Childhood and Adolescence. Front Pediatr. 2021 Jul 19;9:697706. doi: 10.3389/fped.2021.697706. PMID: 34350147; PMCID: PMC8326455.

[5]. Rao VK, Webster S, Dalm V, et al. Effective "activated PI3Kδ syndrome"-targeted therapy with the PI3Kδ inhibitor leniolisib. Blood. 2017;130(21):2307–16.

[6]. Ravisankar P, Anusha S, Supriya K, Ajith Kumar U, Fundamental chromatographic parameters, Int. J. Pharm. Sci. Rev. Res. 2019; 55(2): 46-50.

[7]. Sharma Ajay, Sharma Rohit, Validation of analytical procedures: a comparison of ICH vs Pharmacopoeia (USP) and FDA, International research journal of Pharmacy 2012; 3(6): 39-42.

[8]. Lavanya Chowdary G, Ravisankar P, Akhil Kumar G, Mounika K, Srinivasa Babu, Analytical method validation parameters: An updated review. Int. J. Pharm. Sci. Rev.Res., 2020;61(2):1- 7.

[9]. Ravi Sankar P, Swathi Vaka, Srinivasa Babu Puttagunta, Shaheem Sulthana Md, Gousepeer SK, Current trends in performance of forced degradation studies and stability indicating studies of drugs, IOSR Journal of Pharmacy and Biological Sciences 2017; 12 (6): 17-36.

[10]. Ravi Sankar P, Sai Geethika A, Rachana G, Srinivasa Babu P, Bioanalytical method validation: A comprehensive review, Int. J. Pharm. Sci. Rev. Res 2019; 56(1): 50-58.

[11]. Gaurav Tiwari, Ruchi Tiwari, Bioanalytical method validation: An updated review, Pharm Methods 2010; 1(1):25-38.

[12]. Nandini Ch, Snehitha M. V, Meghana A, Sathvika K, Anvesh G, Shivani B, Srinivasa Babu, P. Ravi Sankar P, Recent advances and strategies for successful bioanalytical method development and validation: A Comprehensive Review, IOSR Journal of Pharmacy,2023;13(7):1-12.

[13]. Validation of analytical procedure: Methodology Q2B, ICH Harmonized Tripartite Guidelines 1996; 1-8.