Research Article

Development and Validation of UV Method for Quantitative Estimation of Olutasidenib in Pharmaceutical Dosage Form

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Received: 06-01-2024; **Revised:** 05-02-2024; **Accepted:** 10-03-2024; **Published on:** 15-03-2024.

ABSTRACT

Objective: The aim of this study is to develop and validate a straightforward, sensitive, precise, rapid, and cost-effective method for the determination of olutasidenib in both bulk and pharmaceutical formulations in accordance with ICH Guidelines.

Methods: A double-beam UV spectrophotometric method has been devised and validated, incorporating various parameters including linearity, precision, robustness, accuracy, and ruggedness. The maximum absorbance of Olutasidenib in acetonitrile was observed at 218.1 nm. The Beer's law was adhered to in the concentration range of 2.50-15 mcg/mL. Additionally, the recovery of Olutasidenib in tablet formulations fell within the range of 98.8-99.9 %, with a percentage assay of olutasidenib tablets exceeding 99.1 %.

Results: The proposed method demonstrates that olutasidenib in acetonitrile exhibits maximum absorbance at 218.1 nm. The Beer's law is applicable in the concentration range of 2.50-15 mcg/mL. The recovery of olutasidenib in tablet formulations falls within the range of 98.8-99.9 %, and the percentage assay of olutasidenib tablets is found to be over 99.1 %.

Conclusion: The developed method proves to be precise, accurate, and reproducible, making it suitable for the routine analysis of olutasidenib in both bulk and pharmaceutical dosage forms.

Keywords: Olutasidenib, Method development, Validation, Ultraviolet Spectroscopy.

INTRODUCTION

The chemical name for Olutasidenib[1] is 5-[[(1S)-1-(6-chloro-2-oxo-1H-quinolin-3-yl) ethyl]amino]-1-methyl-6-oxopyridine-2-carbonitrile. It has a molecular formula of $C_{18}H_{15}ClN_4O_2$ and a molecular weight of 354.8 g/mol. Cancer is a leading cause of death worldwide (nearly one in six deaths according to the WHO). Olutasidenib (Rezlidhia -150 mg) for relapsed or refractory Acute Myeloid Leukaemia[2] (AML) in adults with a specific gene mutant called Isocitrate Dehydrogenase-1 (IDH1)[3] Glioma. Generally, when combination of cytarabine and daunorubicin is used most often for AML. Particularly Olutasidenib is used for treating adults AML when the cancer has returned or has not improved after previous therapeutic option(s). Olutasidenib inhibiting the formation of the oncometabolite 2-hydroxyglutarate (2HG)[4-10].

This prevents 2HG-mediated signalling and leads to both an induction of cellular differentiation and an inhibition of cellular proliferation in tumour cells. A meticulous review of the literature indicates a scarcity of reported analytical methods for determining Olutasidenib in both its bulk form and pharmaceutical preparations. Consequently, we have chosen to formulate a straightforward, precise, and dependable UV spectrophotometric method with the aim of addressing this gap in the existing research. Figure 1 shows the mechanism and molecular structure of Olutasidenib.

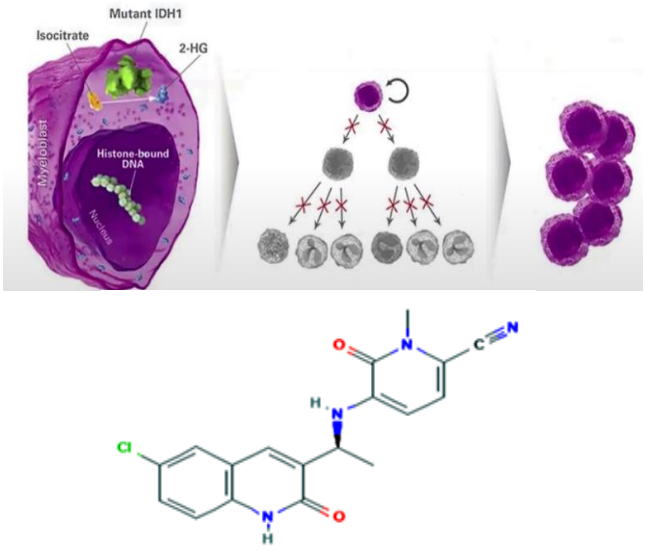


Figure 1: Mechanism of action and Molecular structure of Olutasidenib

MATERIALS AND METHODS

Instruments:

A double beam UV-3200 Labindia spectrophotometer containing two matched quartz cells with a one 1cm light path was taken for measuring of absorbance of Olutasidenib. Essae balance was used for weighing. Ultra Sonicator bath UCA 701 Unichrome was used in this present study.

Chemicals and reagents:

An analytically pure sample of Olutasidenib was obtained from Hetero Drugs Ltd., Hyderabad, and Telangana, India. Olutasidenib tablets containing 150 mg labeled claim. Olutasidenib tablets were used for this study. ACN and CH_3OH were procured from E. Merck specialties, private Ltd., Mumbai, India.

Selection of the solvent:

Plentiful trials were executed to find out the suitable solvent system for dissolving the Olutasidenib. The solvents such as acetonitrile, methanol, and triple distilled water were tried based on the solubility of the drug. Olutasidenib is soluble in solvents such as methanol and Acetonitrile.

Selection of detection wavelength:

Olutasidenib: A solution of 10 µg/mL was scanned against Acetonitrile blank in the range of 200-400 nm. The λ max was found to be 218.1 nm. (Figure 2)

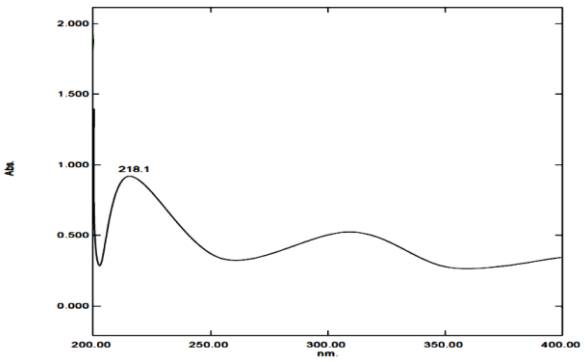


Figure 2: UV spectrum of Olutasidenib

Preparation of stock and working standard solution:

The pure drug of 10 mg was weighed and transferred in to a 100 mL volumetric flask. The drug was dissolved completely in Acetonitrile and made up to the final volume with the same solvent to get a stock solution of concentration 100 µg/mL. Aliquots of standard stock solution were pipette out 1 mL to 10 mL and diluted suitably with Acetonitrile to get the final concentration of standard solutions.

Selection of analytical concentration range:

Appropriate aliquots were pipette out from the standard stock solution in to a series of 10 mL volumetric flasks. The volume was made up to the mark with water to obtain a series of dilutions of concentration range, ranging from 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 µg/mL of Olutasidenib. (Table 1).

Absorbance of the above solutions were measured at 218 nm and converted to zero order spectra calibration curve of absorbance against concentration were plotted. The regression equation and correlation coefficient were determined. Beer Lambert's law was obeyed in the concentration range of 2.5-15.0 µg/mL for the method. (Figure 3 &3a)

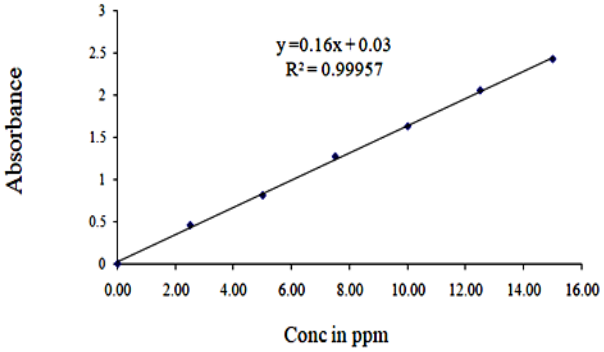
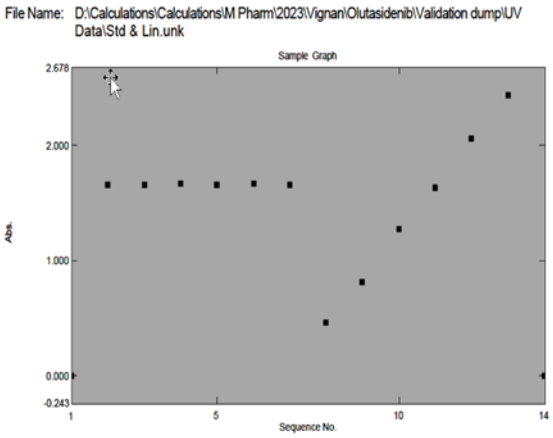


Figure 3: Linearity graph of Olutasidenib



Sample Table

	Sample ID	WL218.0
1 *	Blank	0.000
2	Std 1	1.658
3	Std 2	1.656
4	Std 3	1.665
5	Std 4	1.663
6	Std 5	1.668
7	Std 6	1.659
8	Linearity 1	0.459
9	Linearity 2	0.814
10	Linearity 3	1.277
11	Linearity 4	1.635
12	Linearity 5	2.063
13	Linearity 6	2.435
14	Blank 1	0.000
15		

Figure 3a: Standard and Linearity Data Pertaining to Olutasidenib

Table 1: Calibration data of Olutasidenib

S. No	Olutasidenib	
	Conc. (µg/mL)	Absorbance
1	2.50	0.459
2	5.00	0.814
3	7.50	1.277
4	10.00	1.635
5	12.50	2.063
6	15.00	2.435
Regression equation	y = 0.16x+0.03	
Slope	0.16	
Intercept	0.03	
R^2	0.9995	

RESULTS AND DISCUSSION

Method development & Validation[11-18]

Solvents, including acetonitrile and methanol, were assessed at a concentration of 2.5 mg/mL. Olutasidenib exhibited solubility and stability for a minimum of 1 hour at room temperature in both methanol and acetonitrile. Consequently, these solvents were selected for determining the optimal detection wavelength and working concentration of the standard. To assess the method's applicability to pharmaceutical formulations, an assay of Rezlidhia tablets (150 mg) was conducted at the established working concentration. The assay at 218.1 nm demonstrated a % recovery within the range of 98.8-99.9 % using the acetonitrile sonication method for 15 minutes. Thus, the method was refined and optimized.

The International Conference on Harmonization (ICH) has outlined guidelines, specifically Q2(R1), for the validation of analytical methods. This process involves establishing characteristic performance through laboratory studies and ensuring that the method meets the requirements for its intended analytical application. The UV spectrophotometric method developed in this study underwent comprehensive validation according to the guidelines. The validation encompassed parameters such as linearity, accuracy, system precision, intra-day precision, inter-day precision (or intermediate precision), ruggedness, and robustness, confirming the method's reliability and suitability for routine analysis.

Precision:

Precision of the method was demonstrated by intra-day and inter-day variation studies. In intra-day variation study, six solutions of 10µg/mL were prepared and analysed three times in a day and the respective absorbances were noted. The results were indicated by % RSD. In the inter-day variation study, six solutions of 10µg/mL were prepared and analysed three times for three consecutive days and the respective absorbances were noted. The results were indicated by % RSD. The %RSD for intraday and inter day precision of Olutasidenib in method was found to be less than 2. According to ICH[19] guidelines, the % RSD should less than 2 (within the acceptance criteria). (Table 2 & 3)

Table 2: Intraday precision of Olutasidenib

Analytical method	Method precision	Absorbance	% Assay
Method	1	1.673	99.4
	2	1.668	
	3	1.655	
	4	1.629	
	5	1.635	
	6	1.648	

Table 3: Inter day precision of Olutasidenib

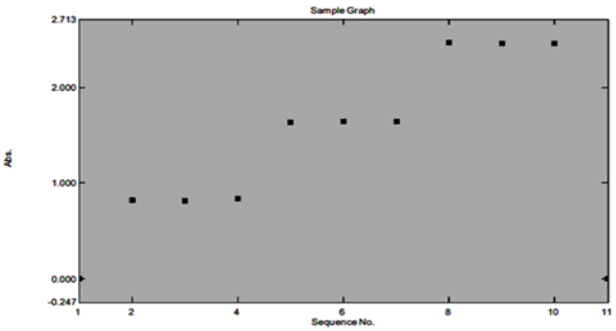
Analytical Method	Intermediate precision	Absorbance	% Assay
Method	1	1.659	99.4
	2	1.635	
	3	1.644	
	4	1.638	
	5	1.656	
	6	1.675	

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 Olutasidenib in the method was found to be less than 2. The % recovery was in the range of 98.9. According to ICH guidelines the statistical results were within the acceptance range. (Table 4)

Table 4: Accuracy data of UV Method

Method	Amount of µg/mL		% of drug added	% Recovered	% Mean Recovery
	Label claim	Pure drug			
Method	150 mg	5	50	99.0	99.2
		10	100	98.8	
		15	150	99.9	



Sample Table

	Sample ID	WL218.0
1 *	Blank	0.000
2	Acc 50% 1	0.822
3	Acc 50% 2	0.816
4	Acc 50% 3	0.834
5	Acc 100% 1	1.633
6	Acc 100% 2	1.645
7	Acc 100% 3	1.648
8	Acc 150% 1	2.422
9	Acc 150% 2	2.458
10	Acc 150% 3	2.459
11	Blank 1	0.000
12		

Figure 4a: Accuracy pertaining to data of Olutasidenib

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 5)

Table 5: Robustness results

Parameter	Concentration 15 (µg/mL)	% Assay
Robustness Change in λ_{max} ± 5 nm)	λ+: 223 nm	100.2
	λ -: 213 nm	100.1

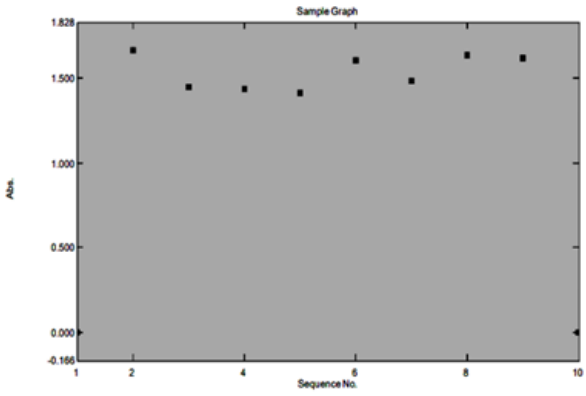
Ruggedness:

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 % RSD values for ruggedness of three replicates of Olutasidenib at a concentration of 10µg/mL was found to be within the acceptance limits.

Linearity:

Fresh aliquots were prepared from standard stock solution ranging from 2.5-15.0 µg/mL and the absorbance values of each concentration was recorded at 218.1 nm for zero order using acetonitrile as blank. The drug shows linearity between 2.5-15.0 µg/mL for method. The correlation co efficient was found to be 0.999 for method. Forced degradation pertaining to data of Olutasidenib is shown in Table 6 and Figure 6a.

Forced degradation:



Sample Table

	Sample ID	WL:218.0
1 *	Blank	0.000
2	Control Degradation	1.662
3	Acid Degradation	1.449
4	Alkali Degradation	1.439
5	Peroxide Degradation	1.413
6	Reduction Degradation	1.605
7	Thermal Degradation	1.483
8	Photolytic Degradation	1.636
9	Hydrolysis Degradation	1.619
10	Blank 1	0.000
11		

Figure 6a: Forced degradation pertaining to data of Olutasidenib

Table 6: Forced degradation Results of Olutasidenib

S. No	Degradation	Absorbance	% Degradation
1	Control	1.662	0
2	Acid	1.449	12.8
3	Alkali	1.439	13.4
4	Peroxide	1.413	15.0
5	Reduction	1.605	3.4
6	Thermal	1.483	10.8
7	Photolytic	1.636	1.6
8	Hydrolysis	1.619	2.6

Analysis of marketed formulation:

Weigh 17.47 mg of Olutasidenib sample and taken in a 100 mL volumetric flask and it was dissolved in acetonitrile and made up to the mark with same solvent. Then the solution was filtered using Whitman filter paper No.40. From this filtrate, dilute 1ml to 10 ml volumetric flask was made with water to obtain the desired concentration (10 µg/mL). These solutions were analysed in UV and the result was indicated by % assay. Table 7 shows the results of analysis of formulation and summary of validation of optical characteristics are shown in table 8.

Table 7: Results of analysis of Formulation by UV-Spectrophotometry

Wave Length nm	Label claim (mg/tab)	Standard absorbance	Test absorbance	Amount found (mg/mL)	% recovery
218	150	1.658	1.646	9.91	99.1

Table 8: Summary of Validation & Optical characteristics

Parameter	Results
Beer's law limit (µg/mL)	5.0-15.0
Linear regression equation	Y = 0.16x+0.03
Linearity indicated by correlation coefficient	0.99957
Precision indicated by % RSD	0.28
Intraday precision	1.06
Inter day precision	0.90
Accuracy indicated by % recovery	98.8-99.9 %
Robustness indicated by % recovery (W+, W-)	100.2/100.1
Ruggedness indicated by % recovery	99.4

CONCLUSION

The developed UV method for the estimation of Olutasidenib is simple, rapid, accurate, precise, robust and economical. The 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 non-interference 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 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 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 Olutasidenib.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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