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Spectrophotometric Determination of Olmutinib in Bulk by Area under Curve and First Order Derivative Methods and its Validation as per ICH guidelines

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ABSTRACT

Abstract: A simple, precise and economical UV-spectrophotometric method has been developed for the estimation of Olmutinib from bulk. Two methods were developed First method (A) applied was area under curve (AUC) in which the area was integrated in wavelength from 262-272nm. Second method (B) was first order derivative spectrometric method. In this method absorbance at λmin=256.57nm, λmax=282.83nm and zero cross=267.68nm was measured. Calibration curves were plotted for the method by using instrumental response at selected wavelength and concentration of analyte in the solution. In both the methods, linearity was observed in the concentration range of 2-12µg/ml at the λmax=267.68nm. Accuracy and precision studies were carried out and results were satisfactorily obtained. The drug at each of the 80 %, 100 % and 120 % levels showed good recoveries that is in the range of 98.00 to 99.00% for both methods, hence it could be said that the method was accurate. Limit of detection (LOD) and limit of quantitation (LOQ) were determined for the method. The method was validated as per International Conference on Harmonization. All validation parameters were within the acceptable limit. The developed method was successfully applied to estimate the amount of Olmutinib in pharmaceutical formulation.

Keywords: UV spectroscopy, validation, Assay, Precision, % Recovery, Olmutinib.

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INTRODUCTION:

Epidermal growth factor binds with high affinity to epidermal growth factor receptor (EGFR) upon cell surface, activates the intrinsic protein-tyrosine kinase activity of receptor, initiating signal transduction cascade results in various biochemical changes in the cell - rises calcium levels, increased protein synthesis and increases expression of certain genes. Eg.gene of EGFR leads to DNA synthesis and cell proliferation. Mutations of EGFR expressions or activity results are in cancer.[1]

Olmutinib N-(3-((2-((4-(4-methylpiperazin-1-yl)phenyl)aminao)thieno[3,2-d]pyrimidin-4-yl)oxy)phenyl)acrylamide.

Olmutinib is an oral epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) of third generation which is being developed by Boehringer Ingelheim and Hanmi Pharmaceutical Co. Ltd to treat non-small-cell lung cancer (NSCLC). Olmutinib binds covalently and inhibits activating EGFR mutations and overcoming T790Mresistance mutation (mutation of epidermal growth factor receptor).[2, 3]

In the present study, we developed a novel analytical method and validation of first derivative method for Olmutinib in bulk using UV spectroscopy.

Literature survey reveals that a few spectrophotometric, RP-HPLC methods are reported for the estimation of Olmutinib in combination with other drugs. [4]

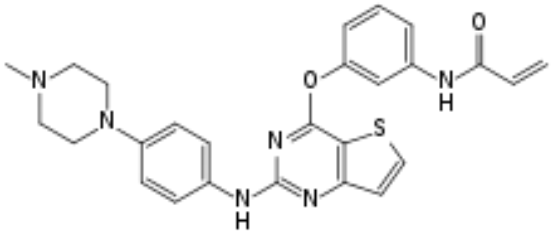

Fig 01: Chemical Structure of Olmutinib

MATERIALS AND METHODS:

The Olmutinib was kindly supplied as a gift sample by Mylan laboratories pvt.ltd., Hyderabad (India). All rest of chemicals used were of HPLC grade. A double beam UV-Visible spectrophotometer, (UV 1800, Shimadzu limited, Japan) having two matched cells with 1cm light path. A citizen analytical balance (Sartorius) was used for weighing the bulk sample.

Preparation of standard stock solutions:

Standard solution of Olmutinib was prepared by transferring accurately weighed 10 mg of drug into a 100ml volumetric flask and the volume was made up to 100ml using methanol as a solvent to get the concentration of 100µg/ml.

Selection of wavelength for analysis of Olmutinib:

Accurately pipette out 1.0 mL volume of standard stock solution of Olmutinib was transfer into a 10 mL volumetric flask, diluted up to a mark with methanol to give concentration of 10 µg/ml. The resulting solution was scanned in the UV range (200–400 nm) using Shimadzu UV-VIS spectrophotometer instrument. The maximum absorbance of solution was measured at the wavelength 267.68nm (Figure 2).

Preparation of calibration curve:

From the standard stock solution fresh aliquots were pipette out and suitably diluted with methanol to get final concentration in the range of 2-12 (µg/ml). The solutions were scanned under 200-400 nm wavelength range and a sharp peak was obtained at 267.68nm (figure 2). Calibration curve was plotted by taking absorbance on y-axis and concentration of solution on x-axis (figure 03 and 04). For both the methods A and B. The drug follows linearity in the concentration range 2-12µg/mL with a correlation coefficient of Method A value (R^2)0.999and Method B value (R^2) 0.997.

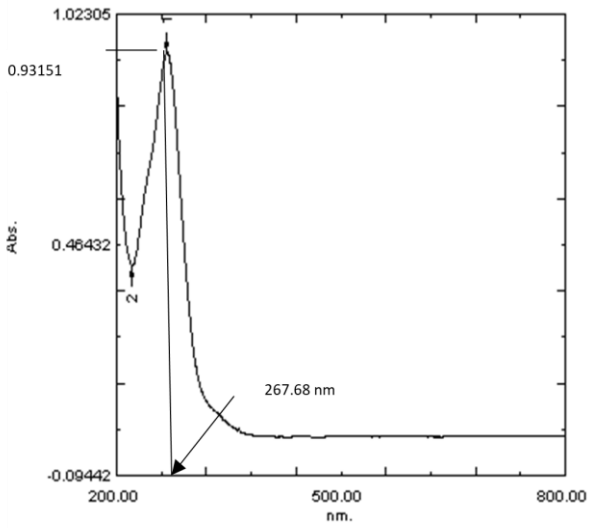

Fig 02: Determination of C_{max} of Olmutinib std. stock solution

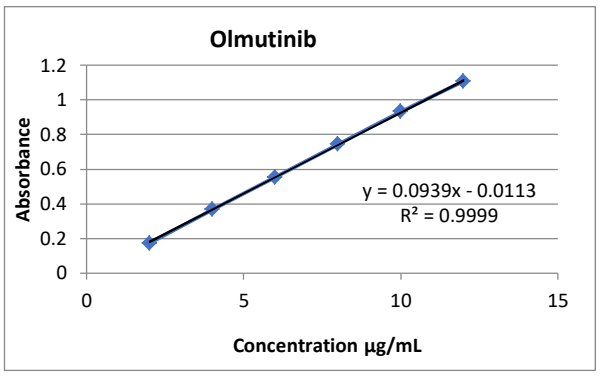

Fig 03: Calibration curve AUC of Olmutinib

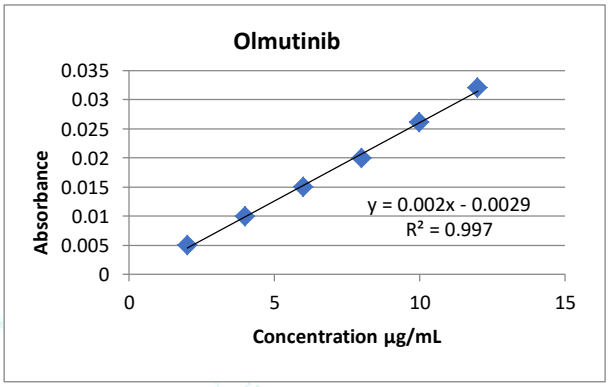

Fig 04: Calibration curve First order derivative of Olmutinib

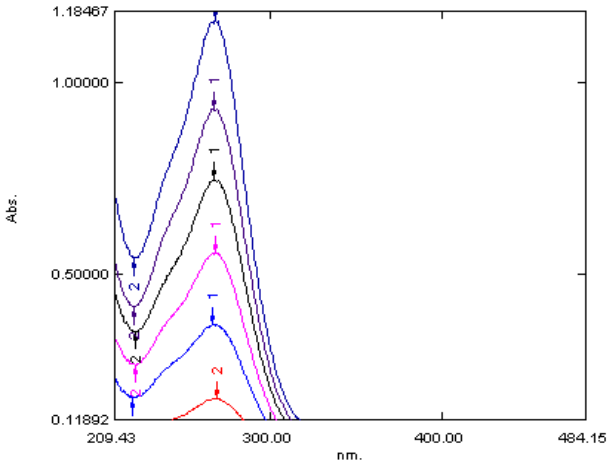

Fig 05: Overlay of Olmutinib showing linearity as increasing concentration.

Area under curve (Method A):

In area under curve method involves to calculation of integrated value of absorbance with selected wavelength. Area calculation calculated in bounded by the curve and horizontal axis. Horizontal axis represent baseline. Where, α is area of bounded portion by curve data and a straight line connecting the starting and end point and β is the area of portion bounded by straight line connecting starting and end point on curve data and horizontal axis, $\lambda_1$and λ_2 are wave length showing starting and end point from figure 6. In this AUC method area was integrated between the wavelength ranges from 262-272nm (figure 6). Also, calibration curves of Olmutinib was prepared in various concentration ranges i.e. 2-12µg/mL at their respective AUC range.[5,6]

$$\text{Area calculation } (\alpha + \beta) = \frac{\lambda 1}{\lambda 2} A d\lambda$$

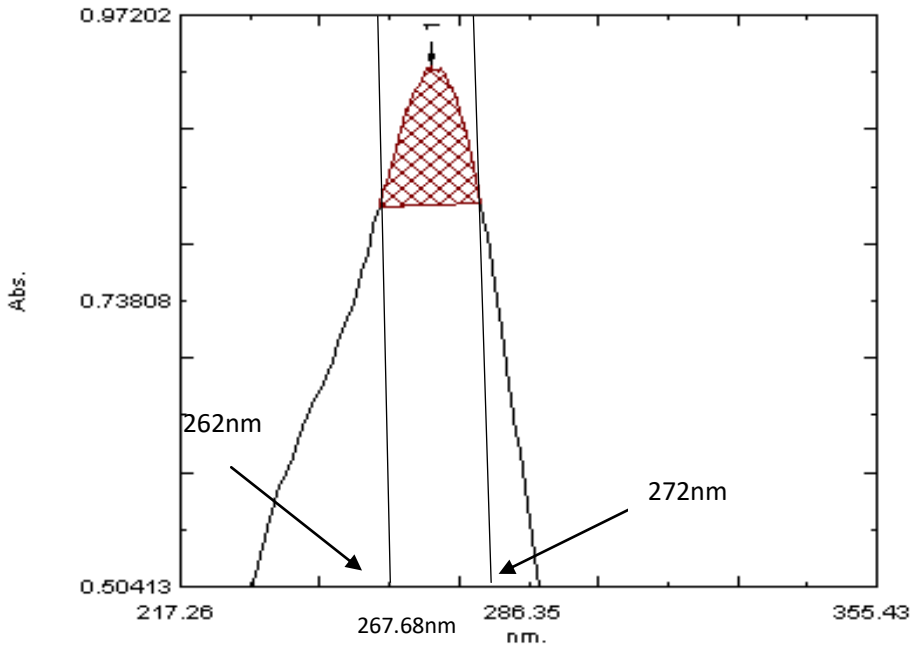

Fig 06: Area under curve graph of 10µg/mL Olmutinib

First Order Derivatives Spectrophotometry (Method B):

In this method solution of Olmutinib (2-12 µg/ml) were prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm. The absorption spectra obtained were derivatised from first fourth order. First order derivative spectra were selected for analysis of drug. From spectra of drug the absorbance was measured at λmax=2582.83 nm, λmin=256.57 nm and zero cross =267.68 nm, amplitude difference (dA) with respect to wavelength difference (dλ) was measured for the respective concentration of standard and was plotted against concentrations and regression equation was calculated.[7, 8]

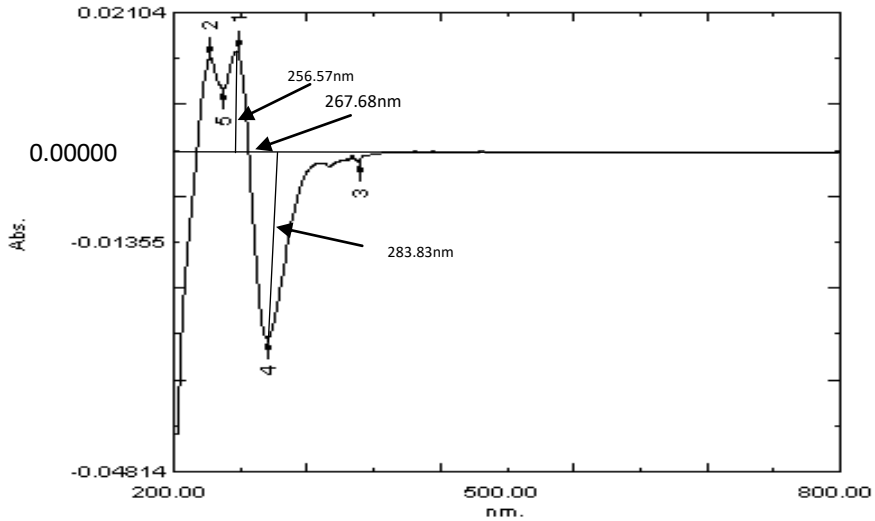

Fig 07: First Order Derivative spectra of Olmutinib

Validation of the developed method:

The objective of validation of analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline.[10, 11]

Table 01: Linearity results of Olmutinib in methanol

Concentration (µg/ml)	Method A (Area under curve)	Method B (First order derivative)
2	0.1723	0.00523
4	0.3655	0.0105
6	0.5534	0.0175
8	0.7430	0.0250
10	0.93151	0.0310
12	1.10882	0.0405

Table 02: Result of Precision

Precision	Method A (%RSD)	Method B (% RSD)
Repeatability	0.368	0.375
Intraday	0.546	0.548
Interday	0.784	0.872

Linearity:

Fresh aliquots were prepared from the stock solution (100µg/ml) in different concentrations. The samples were scanned in UV-visible spectrophotometer against reagent blank. It was found that the selected drug shows linearity between the 2-12µg/ml (Table 01) and fig 05.

Repeatability:

The precision of the method was checked by repeatedly injecting (n=6) standard solutions of olmutinib (10µg/mL). Area under curve of each of these solutions was measured in the range of 262-272nm. Percentage relative standard deviation (%RSD) was calculated (Table 2).

In first order Spectrophotometry derivative method concentration of the solution was determined by measuring absorbance at λmin= 282.83nm, λmax=256.57nm and zero cross=267.68nm.

Intermediate precision (reproducibility):

The intra-day and inter-day precision of the proposed method was determined by analysing the corresponding responses 3 times on the same day and on 3 different days over a period of 1 week for 3 different concentrations of standard solutions of olmutinib(6, 8 and 10µg/mL).The results were reported in terms of relative standard deviation (%RSD). The results were tabulated in (Table 2).

Accuracy (Recovery studies):

The accuracy for the analytical procedure was determined at 80%, 100% and 120% levels of standard solution. Area under curve was measured in the range of 262-272nmalso first derivative measured in the range 256.57-282.83nm and results were expressed in terms of % recoveries. Three determinations at each level were performed and % RSD was calculated. The results were tabulated in (Table 3).

Table 03: Recovery Study of Olmutinib

Accuracy level	Method A		Method B	
	Mean % recovery	%RSD	Mean % recovery	%RSD
80%	98.03	0.630	99.09	0.825
100%	99.06	0.760	98.08	0.745
120%	98.29	0.648	98.25	0.798

Table 04: LOD and LOQ of Olmutinib

Method	Method A	Method B
LOD	0.3786	0.4958
LOQ	1.0523	1.7525

Limit of detection and Limit of Quantitation:

The objective of validation of analytical procedure is to demonstrate whether the procedure is suitable for its intended purpose. The proposed method was validated for various parameters such as Linearity, Accuracy, Precision, Limit of detection (LOD) and Limit of Quantitation (LOQ) according to ICH Q2 (R1) guideline. (Table 4)

RESULT AND DISCUSSION:

In spectroscopic technique was developed simple and specific method for the determination of Olmutinib in bulk form. The generated regression equations were,

Method A - $\int_{272}^{262} Ad\lambda 0.093x + 0.011$ $R^2 = 0.999$

Method B- $\int_{283}^{256} Ad\lambda 0.0.002x + 0.0029$ $R^2 = 0.997$

Where $\int_{272}^{262} A d\lambda$ is area under curve between 262-272 nm, $\frac{dA}{d\lambda}$is amplitude difference, x is concentration and R^2 is correlation coefficient. The R^2 values were 0.999 and 0.997 for Method A and B respectively indicated that developed methods were linear. The proposed methods were found to be precise as % RSD values for intraday as well as Interday precision were satisfactory. The drug at each of the 80 %, 100 % and 120 % levels showed good recoveries that is in the range of 98.00to 99.00% for both methods, hence it could be said that these methods were accurate. The LOD and LOQ were calculated as 0.3786µg/ml and 1.0523µg/ml for method A and 0.4958µg/ml and 1.7525µg/ml for method B respectively. Thus, the developed method is found to be robust and rugged which can be applied as a rapid tool for routine analysis of olmutinib in the bulk and in the pharmaceutical dosage form. The validation parameters for method A and method B are summarized in Table 05.

Table No. 05: Optical Parameters/ Summary of Olmutinib

Parameter	Result for Method A	Result for Method B
Range	262-272	256.57-282.83
Linearity range	2-12 (µg/mL)	2-12 (µg/mL)
Standard regression equation	0.093x+0.011	0.002x+0.0029
Correlation coefficient (R^2)	0.9997	0.994
Repeatability	0.368	0.375
Intraday	0.546	0.548
Interday	0.784	0.872
Accuracy (Mean % Recovery)	99.06	99.09
LOD	0.3786	0.4958
LOQ	1.0523	1.7525

CONCLUSION:

From the results and discussion, two spectrophotometric methods were developed and validated as per ICH guidelines Q2 (R1). In this paper described for the determination of Olmutinib in bulk is simple, sensitive and reproducible. The proposed methods can be successfully applied for Olmutinib without any interference in quality control.

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