Available online on 30.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

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Analytical method development and validation of olmutinib bulk drug as per ICH Q2 guidelines by using RP-HPLC Method

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ABSTRACT

RP-HPLC is fast, simple, sensitive, precise, and reproducible (liquid chromatography) method, developed and validated to analyseolmutinib bulk dosage form. Using C-18 HPLC column separation was carried out. This was maintained at ambient temperature. During separation mobile phase consist of methanol (100 v/v) was delivered at a rate of 1mL/min. Using UV detector analysis was carried out at the wavelength 267.68 nm. RP-HPLC method was validated by using various parameters like, precision, limit of quantitation (LOQ), linearity and robustness. The RP-HPLC method was found to be linear over the concentration ranges from 50-100 µg/mL (r^2 =0.999). Retention time for bulk olmutinib was found to be 9.349 min. LOQ of method was 5.8540 µg/mL and LOD 3.0536µg/mL. Thus, the developed RP-HPLC method was found to be robust and rugged which can be applied for the regular analysis of olmutinib in the bulk as well as pharmaceutical dosage form.

Keywords: C18, RP-HPLC, Methanol, Olmutinib

Article Info: Received 20 June 2019; Review Completed 16 Aug 2019; Accepted 22 Aug 2019; Available online 30 Aug 2019

Cite this article as:

Khandare BS, Musle AC, Arole SS, Popalghat PV, Analytical method development and validation of olmutinib bulk drug as per ICH Q2 guidelines by using RP-HPLC Method, Journal of Drug Delivery and Therapeutics. 2019; 9(4-A):608-611
http://dx.doi.org/10.22270/jddt.v9i4-A.3527

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INTRODUCTION:

Boehringer Ingelheim and Hanmi Pharmaceutical Co. Ltd developed an oral third generation epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) OlmutinibN-(3-((2-((4-(4-methylpiperazin-1-yl)phenyl)aminao)thieno[3,2-d]pyrimidin-4-yl)oxy)phenyl)acrylamide, for the treatment of non-small cell lung cancer(NSCLC). With high affinity epidermal growth factor binds to epidermal growth factor receptor (EGFR) on cell surface, activating the intrinsic protein-tyrosine kinase activity of receptor, initiates signal transduction cascade which results in various biochemical changes in the cell - rises calcium levels, increased protein synthesis and increases expression of certain genes.Olmutinib was granted breakthrough therapy designated in NSCLC by the US FDA on December 2015. Olmutinib received its first global approval in South Korea for the treatment of patients with locally advanced or metastatic EGFR T790M mutation-positive NSCLC on May 2016.

Eg. EGFR gene leads to DNA synthesis and cell proliferation. The Mutations of EGFR expression or activity results in cancer. Olmutinib binds covalently and inhibits activating EGFR mutations and overcoming T790M resistance mutation (mutation of epidermal growth factor receptor). [1-4]

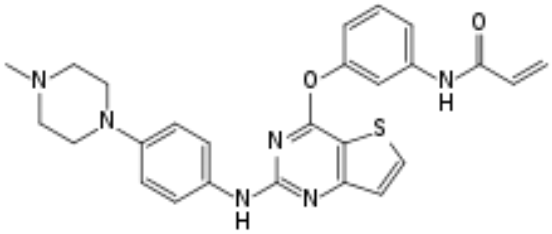

Fig 01: Chemical Structure of Olmutinib

Literature survey reveals that a few spectrophotometric, RP-HPLC methods are reported for the estimation of Olmutinib in combination with other drugs. Main purpose of this work is to develop a RP-HPLC method for the determination of olmutinib in bulk form to provide more scope in further research study on the drug and pharma industry.

MATERIALS AND METHOD:

Chemicals:

Olmutinib was received as a gift sample from Mylan Laboratories Limited, Hyderabad, India. HPLC grade

methanol and double distilled water as solvent was used for the other purpose.

Instrumentation:

The chromatographic technique was performed on a Shimadzu LC-2010C_{HT} Liquid chromatography with UV-visible detector and LC-Solution software, reversed phase C18 column (Inerstil ODS-3V 5 um 250×4.6 mm), Shimadzu analytical balance AY-220, Vacuum micro filtration unit with 0.45µ membrane filter was used in the study. In Double beam UV-visible spectrophotometer (UV-probe 2.32 software). [5]

Determination of working wavelength (λmax)

10 mg of olmutinib was weighed and transferred in to 100 mL volumetric flask and dissolved in methanol and then make a dilution of that stock solution. Prepare 10 µg/mL solution by diluting 1 mL to 10 mL with methanol. Wavelength of maximum absorption for 10 µg/mL solution of the bulk drug in methanol was scanned using UV-Visible spectrophotometer within the range of 200-400 nm wavelength with methanol as reference. The absorption curve shows at 267.68 nm for olmutinib bulk. [6-8]

Chromatographic condition:

Mobile phase for this developed method methanol 100% and it was filtered through a 0.45µmmembrane filter degassed with a helium spurge for 30min and pumped from the respective solvent reservoir to the column inerstil C18 column (250×4.6 mm) at flow rate 1.0 mL/min.This HPLC developed method run time was set at 10 min and column temperature was maintained at RT. Then prior to inject the bulk drug solution in to the column, column was equilibrated for at least 30 min with the same mobile phase flowing through the system.The eluent was monitored at 267.68 nm. Using "LC-Solution" software data was stored and analysed. [9-11]

Selection of mobile phase:

At the beginning solution of bulk drug olmutinib was injected into the HPLC system and run in various solvent system. Various mobile phase methanol, water, acetonitrile, and phosphate buffer in different ratio were tried and finally methanol (100%) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for bulk olmutinib. [18]

Evaluation of analytical methods:

Linearity:

For the linearity study were prepared suitable dilution (ranging from 50-100 µg /mL) of standard stock solution using mobile phase. Though linear response was obtained at lower concentrations for olmutinib and higher concentration range was used to improve signal to noise ratio. Linearity was determined by analysing five working standard solutions over the concentration range of 50-100 µg /mL for olmutinib. [18]

Limit of detection (LOD):

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula,

$LOD = 3.3\sigma/s$

Where, σ = standard deviation of the response

S = slope of the calibration curve.

Limit of Quantification (LOQ):

The limit quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula,

$LOQ = 10\sigma/s$

Where, σ = standard deviation of the response

S = slope of calibration curve

Accuracy:

Accuracy of this method was carried out using one set of different standard addition methods at different concentration levels80%, 100% and 120%, and then comparing the difference between the spiked value (theoretical value) and actual found value.[10,11]

Precision:

Five sets of aliquots with same concentration (90 µg /mL) were prepared and these solutions were analysed to record any intra and inter day variations in the results. The results obtained for Intra and inter day variations.

Robustness:

Robustness of the proposed method for olmutinibwas carried out by the slight variation in flow rate, temperature and mobile phase ratio. The percentage recovery and RSD were noted for olmutinib.

RESULT AND DISCUSSION:

Checking resolution of drug and materials:

The column was saturated with the mobile phase (indicated by constant back pressure at desired flow rate). Standard solution of olmutinib was injected to get the chromatogram. The retention time for olmutinib was found to be 9.349 min. It is shown in the Table 1.

Table 1 Resolution of bulk drug

Drug	Ret. Time	Area	Height	Theoretical plate	Tailing factor
Olmutinib	9.349	32687814	330510	3308.216	0.820

Linearity:

The data of the peak area vs drug concentration were evaluated by linear regression analysis as shown in the Table 2 and calibration curve obtained after plotting drug concentration vs area shown in the fig. 1. Linear regression analysis demonstrated that chromatograph response for the drug was highly linear (r^2=0.999) in the studied concentration range of 50-100 µg/mL. A typical chromatogram of olmutinib (50 µg/mL) shown in fig. 1.

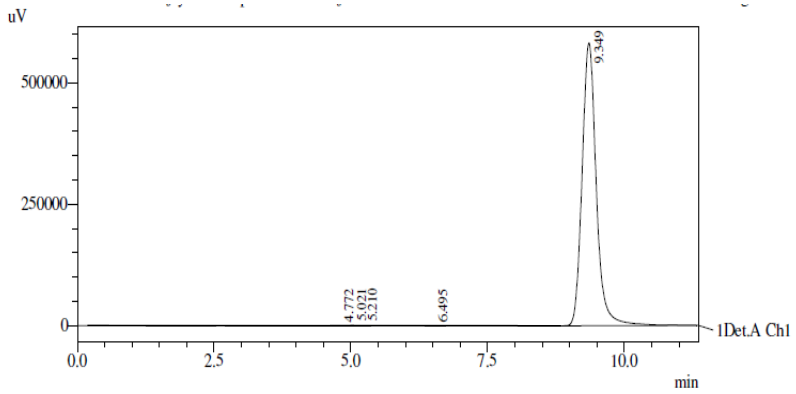

Fig.1. A typical chromatogram for olmutinib (50µg/mL)

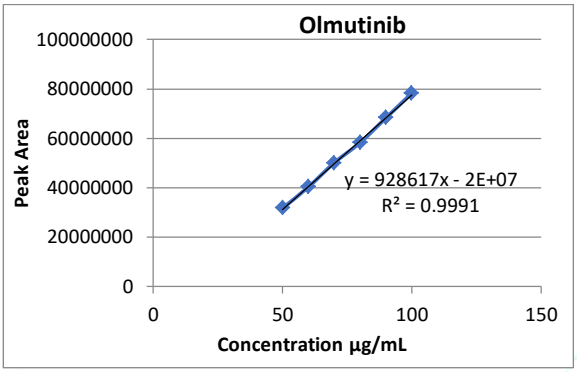

Fig. 2. Calibration curve of Olmutinib

Table 2. Calibration of Olmutinib

Sr no.	Concentration (µg /mL)	Peak area
1	50	31687814
2	60	40257278
3	70	50054020
4	80	58237139
5	90	68259861
6	100	78252828

Precision:

The result depicted in the table3a,3b indicated that the given method has sufficient precision as indicated by the corresponding values of %RSD ranging 0.15 and 0.18 for intraday as well as inter day studies respectively. The values of %RSD for both the studies are well below 1.0% constructing adequate precision.

Table. 3a. Intra-day Precision for Olmutinib

Concentration (µg /mL)	Peak area	Mean (n=5)	S.D.	%RSD
90	78252828			
90	79620844			
90	75528703	76528703	11622.96	0.15
90	70429855			
90	78069865			

Table. 3b. Inter-day Precision for Olmutinib

Concentration (µg /mL)	Peak area	Mean (n=5)	S.D.	%RSD
90	77252828			
90	78620844			
90	75528703	76145843	40217.53	0.18
90	74429855			
90	77069865			

Limit of detection and quantification:

Standard error and slope of linear data is used to predict LOD and LOQ of olmutinib and precision was established at the predict concentration. The result was shown in the table.4

Table 4 Limit of detection and Limit of quantification

Limit of detection	Limit of quantification
3.0536µg/mL	5.8540µg/mL

CONCLUSION:

From the results and discussion, RP-HPLC 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.

ACKNOWLEDGEMENTS:

The authors are grateful to, the Principal and the Management of Kasturi Shikshan Sanstha College of Pharmacy Shikrapur Pune. The authors are thankful to mylan laboratories pvt. ltd., Hyderabad (India) for providing gift sample.

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