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# Development and validation of HPTLC method for the analysis of Olmutinib in Bulk drug

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**Abstract :** This paper describes a simple, precise, rapid and accurate high- performance thin layer chromatographic (HPTLC) method for determination of Olmutinib in bulk drug. Chroma to graphic separation was per formed on aluminium plates precoated with silicagel60$F_{254}$ as the stationary phase using solvent system consisted of chloro form: methanol: (9:1v/v). After the application of bands using CAMAG Automatic TLC Sampler 4, the plate was developed in the solvent system up to 70 mm in CAMAGT win Trough Chamber. This solvent system was found to give compact spot for Olmutinib with Rfvalue of 0.32±0.02. The spots were scanned at 267.68nm. The calibration curves were linear with co-relation coefficient of 0.995 for Olmutinib. Linear regression analysis showed good linearity in the concentration range of 100-1100 ng per spot. The method was validated in terms of Precision, specificity, and Linearity. The average recovery of the standards in the samples was found to be 99.65% at the same time we have checked the C.V. values of Reproducibility, intra-day and inter-day tabulated further. The proposed method can be successfully applied to determine the drug content of bulk drug.
**Keyword :** HPTLC, Olmutinib, silica gel, Linearity, Specificity, Precision.

## 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]



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In the present study, we developed a novel analytical method and validation of first derivative method for Olmutinib in bulk using UV spectroscopy. The method was validated in accordance with the guidelines of the International Conference on Harmonization (ICH).

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

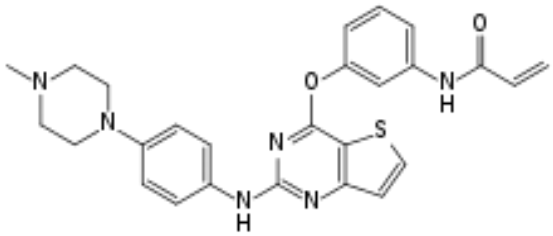

**Figure 01: Chemical Structure of Olmutinib**

## Experimental:

**Instruments and Apparatus :** HPTLC plates pre-coated with aluminium plate60$F_{254}$ plates (100×100 mm) with 250 µm thickness (E. Merk, Darmstadt, Germany), supplied by Anchrom technologist, Mumbai using a Camag Linomat V. Densitometry was carried out with a CAMAG TLC Scanner 3, fitted with a win-CATS 1.4.0 planar chromatography manager software. Samples were applied to the HPTLC plates using the spray-on technique of CAMAG LINOMAT V under nitrogen gas flow, and developed in a CAMAG 10 cm 10 cm twin troughchambers.

**Chemicals and reagents:**

The Olmutinib was supplied as a gift sample by Mylan laboratories Pvt. Ltd. Hyderabad (India). The pure drug obtained had 99.9% w/w assay value, and was used without further purification. All the solvents used were either analytical or HPLC grade.[8]

**ChromatographicConditions:**

Before analysis, HPTLC plates were cleaned by pre-development with methanol and activated at 110°C for 5 min for solvent removal. Analysis was performed on silica gel precoated aluminium plate 60 F254, 20 × 10 cm. The samples were applied to the plates as bands that were 6 mm wide and 10 mm a part by means of a Camag Linom at V sample applicator. Automatic spotter equipped with a 10$\mu$ L syringe and operated with settings of band length, 8 mm; distance between bands, 10 mm; distance from the plate edge,10mm; and distance from the bottom of the plate, 10 mm. The chamber saturation time with mobile phase was 20 min. The plates were developed in a Camag twin trough glass chamber with prior saturation of 30 min with the mobile phase. The mobile phase consisted of Chloroform: Methanol (9:1v/v). After development, plates were allowed to dry and were observed under Camag TLC Visualizer. Densitometric scanning was performed using a Camag TLC scanner 4 in the reflectance absorbance mode at 267.68nm[8, 9]for all measurements and operated by the WINCAT'S software version 2.0. The source of radiation used was a deuterium lamp emitting a UV spectrum between 190 and 900nm.

**Preparation of Standard Solution:**

Accurately weighed and transferred 10 mg of API (Olmutinib) working standard into a 10 ml cleaned and dried volumetric flask and dissolved in 10 ml of chloroform: methanol(9:1)to it and sonicateit for 15 mins. The solution with the concentration of 1mg/ml is prepared. Further concentration of 0.1mg/ml is prepared by Pipetting out 1mL from the above prepared stock solution and transferring into 10mL of volumetric flask and volume is adjusted. The concentration of this solution is 0.1mg/ml. Pipette out 1ml of the above solution andadd9mlofmixtureofchloroform: methanol(9:1).The concentration of this solution is0.01mg/ml.[5,6]

**Preparation of sample solution:**

Tablets were accurately weighed and triturated to a fine powder. The average weight of the tablet powder was transferred into a 50ml standard volumetric flask. 40ml mixture of chloroform: methanol (9:1) was added to it and sonicated for 15mins. The concentration of this solution is 1mg/ml. Pipette out 1ml of the above solution in a 10 ml standard volumetric and adds 9 ml of mixture of chloroform: methanol (9:1). The concentration of this solution is 0.1mg/ml. Pipette out 1ml of the above solution in a 10 ml standard volumetric and add 9 ml of mixture of chloroform: methanol (1:4). The concentration of this solution is0.01mg/ml.[7,8]

**Method Validation**:

As per the ICH guidelines, the method validation parameters were checked for Identification, Precision, specificity, and Linearity. The present method was validated according to the ICHQ2(R1) guidelines. The linearity was determined by using working standard solutions between 100-1100 ng/spot. The spectrums of these solutions were recorded at wavelength 267.68nm A calibration curve was constructed by plotting by taking concentrations on X axis and absorbance of standards on Y-axis and regression equation was obtained. Repeatability study was carried out by preparing aminimum 6 determinations of standard solution of Olmutinib and analysed by Camag scanner and absorbance was recorded at 267.68 nm Relative standard deviation (%RSD) was calculated.

Precision (In intra-day and inter-day) with 6 concentrations of Olmutinib working standard were analyzed 3 on the same day and 3 on next day. The results were reported in terms of percentage relative standard deviation (%RSD). For LOD and LOQ nine sets of known concentrations (0.00-0.004 µg/spot) were prepared. Calibrationcurveswereplottedforeachset.LODandLOQwerecalculatedusing the regression equation and following formulaeas;

LOD = 3.3 SD/S LOQ =
10 SD/S

Where,

SD= is standard deviation of y-intercept of the calibration curves.
S= is mean slope of five calibration curves

The accuracy of the method was determined by calculating recoveries of Olmutinib by the standard addition method. Known amount of Olmutinib at 80 %, 100 %, 120 % were added to a pre quantified sample solution. Each level of accuracy was carried out in a triplicate. The mean of the recoveries at 80%, 100%, and 120% levels were estimated by applying the obtained values to the regression equation of the calibrationcurve.[8,9,10]

## Results and Discussion:

These method developed was proceeding with wavelength selection. The optimized wavelength was 267.68nm.In order to get the optimized HPTLC method various mobile phases were used. The mobile phase consisted of an aqueous solution of chloroform: methanol (9:1 v/v) was used and the Rf value was about 0.32. The specificity of the method was determined for presence of components that may be unexpected to be present. The absence of additional peaks in the chromatogram indicates non-interference of the excipients in the tablet dosage form. The linearity was determined in analyte concentration range of 100-1100 ng per spot. The calibration curve obtained by plotting concentration versus absorbance was linear and the correlation coefficient was found to be 0.995 for Olmutinib (Table 1, Fig. 2). The precision of the method was ascertained from determinations of peak areas of six replicates of sample solution. The repeatability, interday and intraday was calculated for Olmutinib(Table 2). The accuracy study was performed in 80%, 100% and 120%. The percentage recovery was determined and was found to be 99.12% (Tables 3).

The LOD and LOQ were calculated as 0.50 ng/spot and 2.9ng/spot respectively (Table 4).

**Table no.1: Results for Linearity of Olmutinib**

| Concentration | Absorbance |
|---|---|
| 100 | 0.195 |
| 300 | 0.290 |
| 500 | 0.340 |
| 700 | 0.447 |
| 900 | 0.522 |
| 1100 | 0.605 |

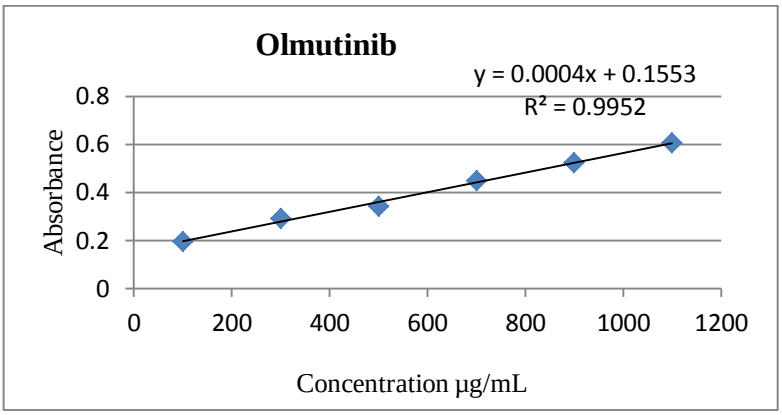


**Figure 2: Calibration Curve of Olmutinib**

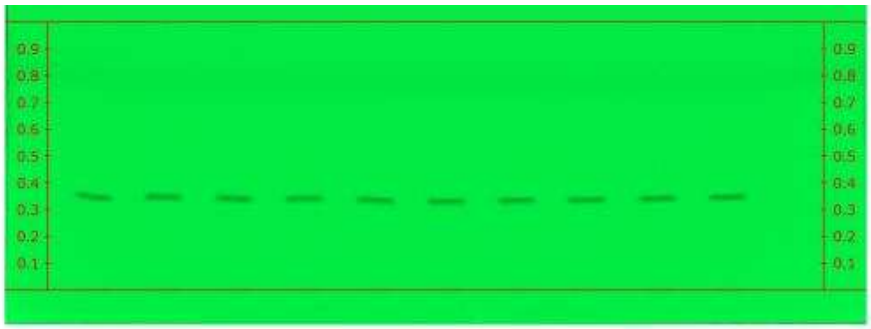

**Figure 3: Graph of Rf value olmutinib band run sample**

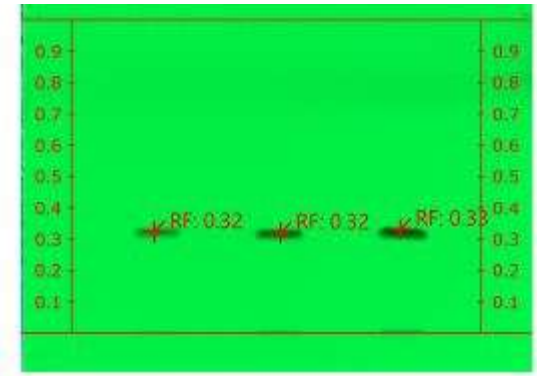


**Figure 4: Graph of Rf value olmutinib band**

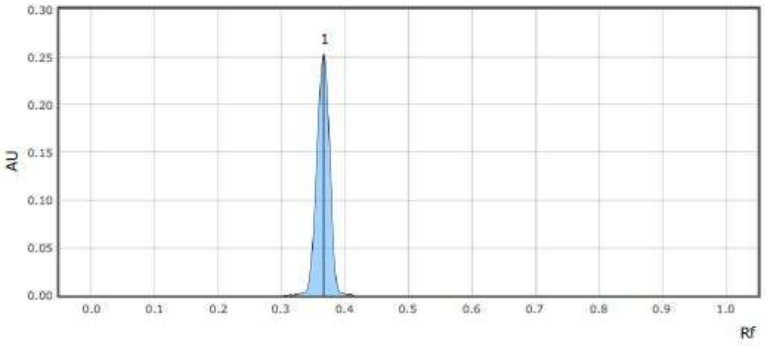


**Figure 5: Method developed Rf value Olmutinib peak**

**Table no. 2: Results for Precision of Olmutinib**

| **Repeatability** | | | | |
|---|---|---|---|---|
| **Rf value** | **Peak Area** | **Deviation** | **CV** | **Average Area** |
| 0.347 | 0.00988 | 2.41% | **1.94%** | **0.010** |
| 0.337 | 0.00969 | 0.47% | | |
| 0.334 | 0.00940 | -2.50% | | |
| 0.324 | 0.00952 | -1.33% | | |
| 0.326 | 0.00974 | 0.96% | | |
| **Intraday** | | | | |
| 0.360 | 0.00655 | 1.66% | **2.18%** | **0.006** |
| 0.355 | 0.00623 | -3.29% | | |
| 0.353 | 0.00641 | -0.54% | | |
| 0.345 | 0.00644 | -0.08% | | |
| 0.348 | 0.00659 | 2.25% | | |
| **Interday** | | | | |
| 0.315 | 0.00970 | 1.19% | **1.38%** | **0.010** |
| 0.308 | 0.00947 | -1.24% | | |
| 0.305 | 0.00955 | 0.36% | | |
| 0.308 | 0.00946 | -1.27% | | |
| 0.311 | 0.00975 | 1.69% | | |

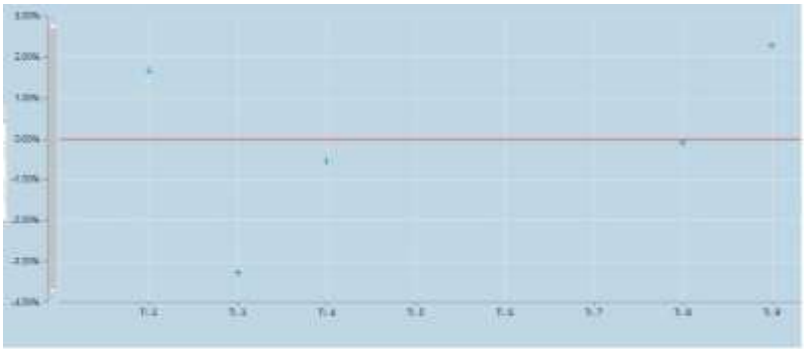

**Figure 6: Olmutinib graph of Intraday Precision**

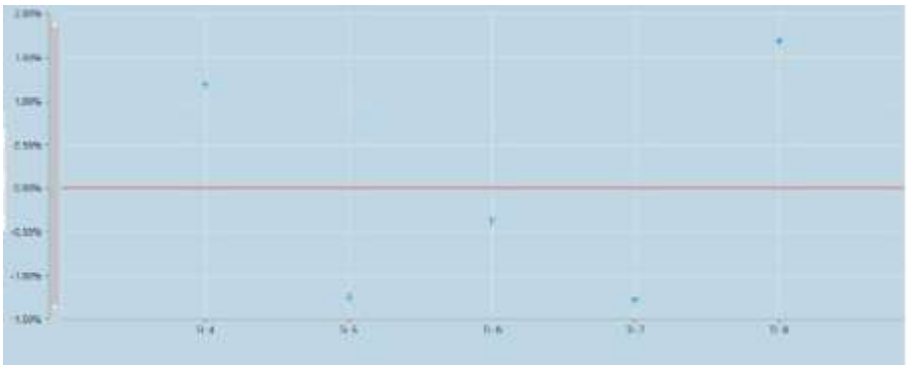

**Figure 7:Olmutinib graph of Interday Precision**

**Table no. 3: Results for Accuracy Studies of Olmutinib**

| Concentration taken in µg/spot (A) | Standard addition in µg/spot (B) | Total drug Concentration (µg/spot) (A+B) | Area | Average | Recovery | |
|---|---|---|---|---|---|---|
| | | | | | % | Mean |
| 100 | 80 | 180 | 5321 | 5354.33 | 106.411 | 99.12 |
| | | | 5332 | | | |
| | | | 5410 | | | |
| 100 | 100 | 200 | 5601 | 5706.66 | 100.590 | |
| | | | 5620 | | | |
| | | | 5899 | | | |
| 100 | 120 | 220 | 6259 | 6267.66 | 93.746 | |
| | | | 6255 | | | |
| | | | 6289 | | | |

**Table no. 4: Results for LOD and LOQ**

| Drug | LOD | LOQ |
|---|---|---|
| Olmutinib | 1.06 | 3.66 |

## Conclusion:

The developed HPTLC procedure was found to be precise, specific and accurate. Statistical analysis indicated that the method was reproducible and selective for the analysis of Olmutinib in bulk drug and without interference from excipients. Hence, this method can be easily and conveniently adopted for routine analysis of Olmutinib in bulk and its pharmaceutical dosage forms.

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