RP-HPLC Method Development and Validation for the Estimation of Midostaurin in Bulk and Pharmaceutical Dosage Form

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**ABSTRACT**
An elementary, Valid, speedy and decisive strategy was developed to determine Midostaurin quantitatively in a fixed dosage form. Effective Chromatographic separation of Midostaurin was achieved by using Hypersil C18 Column (250 mm X 4.6 mm internal diameter, five μm particle size) using a mobile phase composed of Methanol and Buffer in the proportion of 75:25(by volume). The Mobile phase was siphoned using a gradient HPLC system at a low rate of 1.0 ml/min, and quantification was based on peak area measurement at 270 nm. RT (Retention Time) for Midostaurin was 2.142 min, and dimensionality of Midostaurin was found to be linear with a statistic value of 0.999. The acceptance criteria of precision were relative variance should be less than 2.0%, and also the strategy showed precision 0.3 for Midostaurin, which shows that the tactic was precise. The full Recovery was found to be 99.96 %. Detection Limit and Quantitation Limit values for Midostaurin were found as 0.439 & 1.466. The exactness and authenticity were assessed by evaluation of validation parameters like linearity, precision, specificity, accuracy, LOD, LOQ values as per ICH guidelines. The proposed strategy has been applied to the formulation without additives interference and specific for the estimation of Midostaurin.

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**INTRODUCTION**
Midostaurin is chemically N-((9S,10R,11R,13R)-10-methoxy-9-methyl-1-oxo-2, 3, 10, 11, 12, 13-hexahydro-9,13-epoxy-1H,9H-diindolo(1,2,3-GH:3′,2′,1′-lm)pyrrolo[3,4j](1,7)benzodiazonin-11-yl)-n-methylbenzamide (as Rydapt) used for the treatment of Acute Myeloid Leukemia in adults (Ahmed et al., 2018; Valent et al., 2017) having molecular formula C_{35}H_{30}N_{4}O_{4} and Molecular Weight 570.649 g/mol (Kim, 2017). It is being used in the treatment of cancer, myelodysplastic syndrome (He et al., 2017) and systemic mastocytosis (Fischer et al., 2010) showing activity towards hematopoietic tumours. It mainly works by slowing the growth of cancer cells and certain immune system cells (van Andel et al., 2018). The chemical structure of Midostaurin is exhibited in Figure 1.

The current strategy focused on isocratic high-performance liquid chromatography method for the estimation of Midostaurin. After performing extensive literature Survey on chromatographic analysis of several dosage forms (Desai et al., 2019; Sangameshwar et al., 2019), an attempt was made to
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develop a new valid (Vidushi and Meenakshi, 2017), elementary, speedy and Decisive method for the estimation of Midostaurin in a fixed dosage form.

MATERIALS AND METHODS

Chemicals and Reagents
Nutech Labs Pvt Ltd kindly gifted Midostaurin, Hyderabad certified to contain acceptable purity limit and was used without any refinement. Solvents of HPLC grade were used in Chromatographic separation of Midostaurin. Rydapt capsules (label claim 25 mg of Midostaurin) of Novartis were utilized for analysis.

Instrument
Liquid Chromatography system used consists of Waters HPLC having Empower Software with 2695 separation module having PDA detector with universal loop injector of injection capacity 20 µL. The column used was Agilent C\textsubscript{18} Column, 5 µ (250 × 4.6 mm) at surrounding temperature. Several mobile phases were tested to search out the suitable conditions to separate the Drug.

Optimized Chromatographic conditions
The mobile phase having Methanol and Phosphate buffer having four pH in a proportion of 75:25 by volume was preferred because it ideally resolves the height with Retention Time (RT) of 2.142 minutes for Midostaurin, respectively. Standard Drug was scanned over a broad range of wavelength ranging from 200 nm to 390 nm, and wavelength was selected at 270 nm because of showing reasonably good response with characteristic UV Spectrum as exhibited in Figure 2.

Preparation of Buffer
Accurately weighed and transferred 3.464 g of Potassium Dihydrogen Phosphate into 1000 ml clean, dry volumetric flask. To this add 500 ml of HPLC water and sonicated for five minutes to dissolve it entirely and make up the volume to mark with HPLC water and pH was adjusted to four by addition of few drops of Orthophosphoric acid.

Preparation of Mobile Phase
Accurately measured 750 millilitres of Methanol (75%) and 250 millilitres of phosphate buffer (25%) were mixed and kept for sonication in inaudible water tub for 10 minutes and after sonication filter the above solution using 0.45 µ membrane filter under vacuum prior its use and used as a diluent

Standard Stock Preparation
Accurately weighed and transferred 10 mg of Midostaurin working standard into 100 millilitres

Figure 1: Chemical Structure of Midostaurin

Figure 2: Overlain UV spectrum of Midostaurin

Figure 3: Standard Solution Chromatogram

Figure 4: Sample solution Chromatogram

Figure 5: Linearity Curve of Midostaurin
### Table 1: Linearity Data of Midostaurin

<table>
<thead>
<tr>
<th>S.No</th>
<th>Linearity Level</th>
<th>Concentration (ppm)</th>
<th>Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>20</td>
<td>1646252</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>40</td>
<td>3272416</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>60</td>
<td>4838743</td>
</tr>
<tr>
<td>IV</td>
<td>4</td>
<td>80</td>
<td>6475024</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>100</td>
<td>8312151</td>
</tr>
</tbody>
</table>

Correlation Coefficient: 0.999

### Table 2: Accuracy Report of Midostaurin

<table>
<thead>
<tr>
<th>% Concentration</th>
<th>Average Area</th>
<th>Amount Added (mg)</th>
<th>Amount found (mg)</th>
<th>% Recovery</th>
<th>Mean Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>1143519</td>
<td>5</td>
<td>4.99</td>
<td>99.81%</td>
<td>99.63%</td>
</tr>
<tr>
<td>100%</td>
<td>2938342</td>
<td>10</td>
<td>9.88</td>
<td>99.08%</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>4452758</td>
<td>15</td>
<td>15.0</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Precision Report of Midostaurin

<table>
<thead>
<tr>
<th>Peak Name</th>
<th>RT</th>
<th>Area</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Midostaurin</td>
<td>2.185</td>
<td>824170</td>
<td>158772</td>
</tr>
<tr>
<td>2 Midostaurin</td>
<td>2.191</td>
<td>826053</td>
<td>157336</td>
</tr>
<tr>
<td>3 Midostaurin</td>
<td>2.204</td>
<td>823442</td>
<td>156124</td>
</tr>
<tr>
<td>4 Midostaurin</td>
<td>2.207</td>
<td>818967</td>
<td>155674</td>
</tr>
<tr>
<td>5 Midostaurin</td>
<td>2.210</td>
<td>823476</td>
<td>156033</td>
</tr>
</tbody>
</table>

Mean: 823221.9
Std.Dev.: 2604.2
%RSD: 0.3

### Table 4: Results of Robustness

<table>
<thead>
<tr>
<th>S.No</th>
<th>Flow Rate (ml/min)</th>
<th>System Suitability Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plate Count</td>
</tr>
<tr>
<td>I</td>
<td>0.8</td>
<td>4517</td>
</tr>
<tr>
<td>II</td>
<td>1.0</td>
<td>4343</td>
</tr>
<tr>
<td>III</td>
<td>1.2</td>
<td>4209</td>
</tr>
</tbody>
</table>

### Table 5: Effect of change in mobile phase Composition

<table>
<thead>
<tr>
<th>S. No</th>
<th>Mobile phase composition change</th>
<th>Plate Count</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>&lt;5%</td>
<td>4623</td>
<td>1.6</td>
</tr>
<tr>
<td>II</td>
<td>*Actual</td>
<td>4543</td>
<td>1.6</td>
</tr>
<tr>
<td>III</td>
<td>&gt;5%</td>
<td>4864</td>
<td>1.6</td>
</tr>
</tbody>
</table>
clean dry volumetric flask and add 70 ml of diluent and degassed for five min to dissolve Midostaurin standard entirely and make volume up to the mark with the identical solvent which gives 100 µg/ml of Midostaurin (Stock solution). From the above solution, 4.0 ml was pipetted out into a ten-millilitre volumetric flask and then makeup to the mark with diluent. The chromatogram was exhibited in Figure 3.

Sample Preparation
A portion of powder equivalent to the weight of 10 mg was accurately weighed and transferred into100 millilitre volumetric flask and diluent of 70 ml was added and degassed for five min to dissolve it completely and makeup to the mark with identical solvent and then the solution was filtered through a 0.45 µ membrane filter. From the above-filtered solution, 4.0 ml was pipetted out into a 10-millilitre volumetric flask and then make up to the final volume with diluent. Similarly, the sample was further diluted to get the required concentration The Chromatogram was exhibited in Figure 4.

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Midostaurin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Label claim (mg)</td>
<td>25</td>
</tr>
<tr>
<td>Drug found</td>
<td>24.77</td>
</tr>
<tr>
<td>% Accuracy</td>
<td>98-102</td>
</tr>
</tbody>
</table>

Preparation of Calibration Curves by HPLC
Serial dilutions of Midostaurin ranging from 10-100 µg/ml were made, and their chromatograms were recorded. Height space of Drug was calculated, and also the individual activity curve was planned against quantitative of the area underneath the curve, and their respective concentrations and results are reported in Table 1.

HPLC Method Validation
The Developed method was validated for Linearity, Accuracy, Specificity, Precision, LOD, LOQ parameters as described in ICH Guidelines.

Linearity and Range
One-dimensionality in a strategy is its ability to induce to take a glance at results and was constructed using the mean areas at their respective concentrations over a given range. Linearity for Midostaurin was within the range of 10 to 100µg/ml, respectively. The coefficient of correlation value for the calibration plot of Midostaurin was 0.999, which shows good linearity for the Drug. The Linearity curve was exhibited in Figure 5.

Accuracy
The Certainty of an approach is that the intimacy of the measured worth to actuality worth for the sample. The proportion recovery of Midostaurin was found to be 99.81%, 99.08% and 100.02% for accuracy 50%, 100% and 150% samples respectively. The mean recovery for Midostaurin is 99.63. The %RSD of the sample was found to be below two and results were tabulated in Table 2.

Precision
The precision of the strategy was evaluated by performing repeatability on the same day and inter-day studies. The Percentage Relative Standard Deviation of each study was calculated and was found to be less than 2% showing the strategy was precise, and the results were shown in Table 3.

LOD & LOQ
Limit of Detection, Limit of Quantitation values of the method were 0.439 and 1.466, respectively. The results obtained are within limits.

Robustness and Ruggedness
Robustness and Ruggedness studies were carried out by injecting five replicate injections of Midostaurin on different days and variations was calculated in terms of percentage relative variance which was found to be less than 2%, and results were reported in Tables 4 and 5.

Recovery studies
Midostaurin standard addition method is performed at 50,100,150 % levels, and interference of formulation additives was tested. The Recovery was calculated based on the amount of Drug found, and results are reported in Table 6.

CONCLUSIONS
Different trials were carried out to determine the optimized chromatographic conditions and an initial attempt was performed by utilizing a low proportion of organic solvents for the elution of compound by reducing the retention time of the compound. The proposed chromatographic conditions achieve acceptable results. The proposed method is easy, speedy and measurably substantial. During the analysis of Drug, no interfering peak was found within the chromatogram, indicating that there is no excipient interference.
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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

REFERENCES


