Use Of Rp-Hplc For The Analytical Method
Development Of Anti-Tubercular Drug- Bedaquiline

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Abstract

A rapid sensitive and accurate method for the quantitative and qualitative estimation of bedaquiline was developed using the reverse phase HPLC (RP HPLC) method using acetonitrile and 0.1% trifluoroacetic acid as mobile phase. The system’s suitability was established. The method was validated as per ICH guidelines. The system suitability was established with a good resolution of peak and retention time of 2.27min. The linearity was established at 242nm in the range of 30-180µg/ml, with a correlation coefficient of 0.9999. The limit of detection and the limit of quantification was found to be 3µg/ml and 12µg/ml respectively. The accuracy and precision of the process were found to be within the limit of acceptance calculated through relative standard deviation(RSD). The robustness of the process was established with a deliberate change in the injection flow rate, the content of the organic phase and the wavelength. The chromatographic method was found to be specific for the determination of the analyte in a photodegradation study of the known concentration of the sample. Hence it can be concluded that the overall process is simple, rapid, accurate and robust enough for the routine analysis of Bedaquiline in pharmaceutical formulation.

Keywords: Bedaquiline, RP-HPLC, Analytical validation, Accuracy, Precision, Robustness.

INTRODUCTION

Bedaquiline fumarate is an USFDA-approved diarylquinoline drug for the treatment of multidrug-resistant tuberculosis. It is used in combination with the first-line treatment of tuberculosis(Sarathy et al 2019). The diarylquinoline drug acts by inhibiting the mycobacterial enzyme ATP synthase. The IUPAC name of Bedaquiline is 1(R,2S)-(6-bromo2methoxyquinolin-3-yl)-4-(dimethylamino)-2-naphthalene-1-yl-1-phenylbutan-2-ol. The drug is highly protein bound and has a very long half-life of 5.5 months. The long half-life indicates the slow release of the drug in the plasma(Nagabushan et al 2014). In presence of the enzyme CYP3A4, the drug metabolizes to N-monodesmethyl metabolite which is 4 to 6 times less potent than the parent molecule. The drug is classified in the category II of the biopharmaceutical classification system and shows poor oral bioavailability. Currently, the drug is marketed in the form of tablets at a strength of 20mg or 100mg. The drug is prescribed under strict supervision. The official dissolution media for the preparation of the stock solution of the drug is 0.1N hydrochloric acid and methanol(Pardhi et al 2020). The researchers are adopting various novel techniques to improve its solubility and bioavailability. Therefore, a quick quantitative and qualitative analysis of the drug is very much essential. The analytical method should be simple, accurate, and reliable enough to estimate the drug even in meagre quantity. The present study focuses on the development of a new reversed phased HPLC method without the use of alcohol and aims to get a short retention time.

MATERIALS AND METHODS

Bedaquiline was procured from Clearsynth labs, Mumbai, India. HPLC grade acetonitrile, Trifluoroacetic acid, and water were purchased from Himedia labs, Bangalore.
INSTRUMENTATION AND CHROMATOGRAPHIC CONDITIONS

The liquid chromatographic system used in the present work consisted of Waters Alliance e-2695 quaternary pump system, the detector used was Waters 2998 Photodiode Array (PDA). EMPOWER 2.0 software was used for data acquisition and processing. The analysis was performed at ambient column oven temperature on Agilent, Symmetry C18 column (150mm × 4.6mm, 3.5μm particle size). The isocratic mode was employed for all the experiments.

The following chromatographic conditions were used for the analyte detection. PDA detector was set to analyze the analyte in the spectral range of 210 to 400nm. At 242nm the chromatogram was extracted. The mobile phase was consisted of acetonitrile and 0.1%v/v trifluoroacetic acid (50:50). A volume of 10μL was injected at a flow rate of 1.0ml/min for 5min at ambient column temperature.

PREPARATION OF CALIBRATION CURVE

A stock solution of the sample was prepared by dissolving 12mg of the sample in 10ml of the mobile phase. The stock was further diluted and calibration solutions were made in the range of 30-180µg/ml. An injection volume of 10μL was inserted into the column. The peak area was noted. Each trial was made in triplicate to eliminate the system error(Patan et al 2021).

METHOD VALIDATION

ICH Q2(R1) guidelines were followed to validate the analytical method. The process was established by estimation of system suitability, linearity, accuracy, precision, robustness, and quantification limit.

SYSTEM SUITABILITY

The system suitability was established by verification of resolution and reproducibility. A standard concentration of 120µg/ml was injected in six replicates. The retention time and peak area were determined. % RSD was calculated for both to assure system suitability as per ICH guidelines.

ESTIMATION OF LINEARITY

The calibration curve was made in the dilution range of 30-180µg/ml. The peak area was noted. Each trial was made in triplicate to eliminate the system error. The data were subjected to linear regression analysis. Linearity equation and regression coefficient were determined.

LIMIT OF DETECTION (LOD) AND LIMIT OF QUANTIFICATION (LOQ)

The limit of detection and limit of quantification gives an idea of the ability of the developed process to detect and quantify the minimum amount of the analyte in a given sample. It is calculated from the regression analysis data and can be estimated by the following equations(Armbruster et al 2008)

\[
LOD = 3.3 \times \frac{Standard\ error\ of\ Y\ intercept}{Slope\ of\ the\ calibration\ curve}
\]

\[
LOQ = 10 \times \frac{Standard\ error\ of\ Y\ intercept}{Slope\ of\ the\ calibration\ curve}
\]

ESTIMATION OF ACCURACY

Three different levels of target concentrations (50%,100%, and 150%) of the analyte were analyzed to determine the accuracy of the method. Each concentration was analyzed in triplicate. Percentage recovery and % RSD were calculated(Bhattacharyya et al 2019).

ESTIMATION OF PRECISION

The precision of the analytical method was established by injecting the analyte at a concentration of 120μg/ml in six replicates for 3 days. From the chromatogram, peak area was noted to calculate % label claim and % RSD. Intermediate precision was carried out on different days at different concentrations (50μg/ml, 100μg/ml, and 150 μg/ml) for three consecutive days. Each concentration was experimented with in triplicates. % RSD was estimated from the trials(Kavya et al 2019).
ESTIMATION OF ROBUSTNESS
The robustness of the process was established by varying the injection flow rate by ±10%, varying the composition of the organic phase by ±10%, and by varying the wavelength by ±5(Vani et al 2015).

DETERMINATION OF SPECIFICITY
The specificity was determined with a known concentration of bedaquiline (120µg/ml) that was subjected to direct sunlight for 12hours. The estimation was carried out to confirm none of the degradation products interfered with the analysis(Weich et al 2007).

RESULTS AND DISCUSSION
Preliminary trials were carried out to select the suitable solvent system for the method development. Trials were made with different ratios of acetonitrile and 0.1% orthophosphoric acid. The observations revealed the system suitability and retention time were not within the limit. Hence trials were made with acetonitrile and 0.1% trifluoroacetic acid in three different ratios (30:70, 40:60, and 50:50). The process was optimized with optimum system suitability, characteristics of the peak, and retention time at a solvent composition acetonitrile and 0.1% trifluoroacetic acid at 50:50. Hence, the analytical method of Bedaquiline was carried out in a mobile phase consisting of acetonitrile and 0.1% trifluoroacetic acid at a ratio of 1:1 in C18 column dimension of 150mm × 4.6 mm, and particle size of 3.5µm. The acceptable symmetric peak and resolution of Bedaquiline were obtained at a flow rate of 1ml/min and at a retention time of 2.27 min.

METHOD VALIDATION
The analytical chromatographic process to quantify and qualify Bedaquiline was validated as per regulatory guidelines. The developed process was checked for linearity, accuracy, precision, robustness, and specificity. The relative standard deviation was estimated for all the processes.

SYSTEM SUITABILITY
System suitability is a crucial part of the method development. The developed liquid chromatographic technique requires the generation of high-resolution good intensity peaks to establish the quality and reproducibility of the procedure. In the present study, 120µg/ml of the analyte was injected in six replicates. From the chromatograms, the peak area, retention time, capacity factor, USP plate count, and tailing were estimated and are presented in Table 1. All the values estimated for system suitability are found to be within the limit of acceptance. The system suitability chromatogram is depicted in Figure 1. Hence it can be deduced that the findings are suitable for the analytical development of Bedaquiline in the optimized chromatographic conditions.

Table 1. System suitability

<table>
<thead>
<tr>
<th>System suitability parameters</th>
<th>Limit of acceptance</th>
<th>Experimental results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity factor</td>
<td>≥2</td>
<td>2.27</td>
</tr>
<tr>
<td>Precision (for area) (n=6)</td>
<td>RSD≤2</td>
<td>0.49</td>
</tr>
<tr>
<td>USP tailing factor</td>
<td>≤2</td>
<td>1.05±0.008</td>
</tr>
<tr>
<td>USP plate count</td>
<td>&gt;2000</td>
<td>2621±5.04</td>
</tr>
<tr>
<td>Resolution</td>
<td>&gt;2</td>
<td>3.76</td>
</tr>
</tbody>
</table>
Figure 1. HPLC chromatogram for estimation of system suitability for bedaquiline

ESTIMATION OF LINEARITY
Linearity measurement gives the range within which accurate and precise results can be obtained. The linearity range was measured in a wide range between 25% to 150% of the target concentration (120µg/ml) i.e. 30 to 180 µg/ml. Linearity was obtained with the concentration and the peak area as shown in Figure 2. The data were subjected to simple linear regression analysis. The linearity was derived with the equation \(Y = 20325X + 21664\), with a correlation coefficient \((r^2)\) of 0.99998. The overall % RSD of the process was found to be 0.49.

Figure 2. Calibration graph for bedaquiline

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION
LOD and LOQ were determined from the value of slope and standard error of Y-intercept derived from the regression analysis of linearity data (Aluri et al 2022). The LOD and LOQ were estimated to be 3µg/ml and 12µg/ml respectively. The findings of LOD and LOQ signify the sensitivity of the process.

ESTIMATION OF ACCURACY
Accuracy was estimated at 50%, 100%, and 150% levels of the target concentration of 120µg/ml. The analysis was carried out in triplicates to determine the drug content in the samples. The accuracy of the process was estimated through the findings of % recovery. % RSD was found to be 0.5. The results are tabulated in Table 2.
**Table 2.** Accuracy of the process to detect analyte at three different levels

<table>
<thead>
<tr>
<th>Levels</th>
<th>Peak Area ±SD</th>
<th>% Recovery ±SD</th>
<th>%RSD Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>123340.7±4552</td>
<td>101.1±0.377</td>
<td>0.5</td>
</tr>
<tr>
<td>100%</td>
<td>2441124.3±2571</td>
<td>100.1±1.053</td>
<td></td>
</tr>
<tr>
<td>150%</td>
<td>3681191±1311</td>
<td>100.6±0.358</td>
<td></td>
</tr>
</tbody>
</table>

All data are the mean of three trials ± Standard deviation (SD)

**ESTIMATION OF PRECISION**

The intraday precision was calculated for 3 days at the target level. The % label claim was found to be 99.3, 100.4, and 101.4 respectively on the consecutive days. The % RSD was found to be less than 1 for all the days. The data is represented in

**Table 3.**

<table>
<thead>
<tr>
<th>Injection No.</th>
<th>Intraday precision at the target level (120µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Average Peak area ±SD</td>
<td>2421541 ± 2678</td>
</tr>
<tr>
<td>% Label Claim</td>
<td>99.3±0.93</td>
</tr>
<tr>
<td>RSD</td>
<td>0.94</td>
</tr>
</tbody>
</table>

All data are the mean of three trials ± Standard deviation (SD)

The intermediate precision was estimated at different levels and is tabulated in Table 4. The results revealed that the % label claim in each level was accurate and it was further proved by % RSD which was less than 2 in each case.

**Table 4.** Intermediate precision at different levels

<table>
<thead>
<tr>
<th>Intermediate Precision</th>
<th>Average peak area at different levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 %</td>
</tr>
<tr>
<td>Average Peak area ± SD</td>
<td>1238364 ±3481</td>
</tr>
<tr>
<td>% Label claim</td>
<td>101.5±1.023</td>
</tr>
<tr>
<td>%RSD</td>
<td>1</td>
</tr>
</tbody>
</table>

All data are the mean of three trials ± Standard deviation (SD)

Hence the intraday and intermediate precision study proved the repeatability and correctness of the developed process.

**ESTIMATION OF ROBUSTNESS**

The robustness of the developed process was determined at three different conditions. The actual flow rate was 1 ml/min, it was varied for 1.1ml/min and 0.9ml/min. The usual organic phase and aqueous buffer phase for the developed process were in the ratio of 50:50, robustness was varied by changing the ratio from 55:45 to 45:55. The wavelength was varied from 242± 5nm. The three trials were made at the target concentration for all three conditions. The percentage recovery and %RSD are summarized in Table 5.
Table 5. Robustness of the method by varying the key parameters

<table>
<thead>
<tr>
<th>Conditions</th>
<th>% Recovery ± SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow Plus (1.1ml/min)</td>
<td>99.7±0.945</td>
<td>0.95</td>
</tr>
<tr>
<td>Flow Minus(0.9ml/min)</td>
<td>100.1±0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Organic:Buffer (55:45)</td>
<td>99.6±0.954</td>
<td>0.96</td>
</tr>
<tr>
<td>Organic:Buffer (45:55)</td>
<td>100.4±0.872</td>
<td>0.87</td>
</tr>
<tr>
<td>Wavelength (247nm)</td>
<td>100.1±0.675</td>
<td>0.67</td>
</tr>
<tr>
<td>Wavelength (237nm)</td>
<td>99.76±0.84</td>
<td>0.84</td>
</tr>
</tbody>
</table>

All data are the mean of three trials ± Standard deviation (SD)
The deliberate changes in the key parameters did not affect the performance of the process to quantify the analyte, therefore the reliability of the process was established.

ESTIMATION OF SPECIFICITY

A known concentration of sample was subjected to photo degradation under direct sunlight. The degradative product showed various peaks, whereas the non-degraded product of the analyte was detected at the same retention time as shown in Figure 1. Hence it proves the specificity of the process.

Figure 3. HPLC Chromatogram for determination of specificity.

CONCLUSION

The proposed chromatographic method was found to be accurate and sensitive enough for future application for qualitative and quantitative estimation of Bedaquiline in various pharmaceutical formulations as a routine control analysis method.

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