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Development and Validation of Novel Stability-Indicating RP-HPLC Method for Determination of Bedaquilline Fumarate in Tablet Dosage Form

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ABSTRACT

A fast, accurate, and precise stability-indicating HPLC analytical method has been developed and validated for the quantitative analysis of Bedaquilline in the tablet dosage form. The goal of this study was to explore the degradation of Bedaquilline behavior under ICH-recommended stress conditions, employing a newly designed stability-indicating HPLC method and validating it. We provide a simple and fast analytical method that can be utilized as a quality control tool for the determination of Bedaquiiline as bulk and in its formulation. The method was developed on an HPLC column (250 mm × 4.6 mm) with Methanol: Ammonium acetate buffer (pH 4) (90: 10, v/v) as a mobile phase using a simple isocratic elution technique. The developed method was successfully validated as per ICH Q2 (R1) guidelines. The developed method was found to be linear within the range of 5-30 µg mL^{-1} (R^2 =0.998), precise as % RSD for Inter-day and Intra-day Precision were found to be < 2 %, accurate as % recovery value in accuracy study was found to be in the range of 98-102 % and sensitive as the limit of detection and limit of quantitation were found to be 0.17 µg mL^{-1} and 0.53 µg mL^{-1} respectively.

1.0 INTRODUCTION

Bedaquilline, chemically, 1-(6-Bromo-2methyl-quinolin-3-yl)-4dimethylamino-2-napthalen-1-yl-1-phenylbutan-2-ol (Figure 1) is a bactericidal antimycobacterial drug. [1] It is the first drug to be approved with a well-defined mechanism of action, for tuberculosis after 1998. [2] It is used to treat active tuberculosis. Specifically, it is used to treat multi-drug-resistant tuberculosis (MDR-TB) along with other medications for tuberculosis. [3, 4]

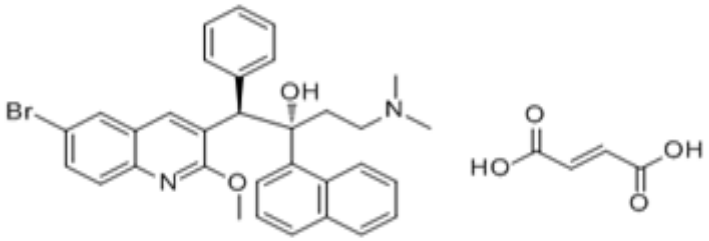

Figure No 1: Structure of Bedaquilline fumarate

An extensive literature survey concerning quantitative estimation of bedaquilline revealed that analytical methods such as UV spectrophotometry [5] and high-performance liquid chromatography [6-8] were reported for its determination in human plasma and/or pharmaceutical formulations either as a single drug or in combination with other drugs. To the best of our knowledge, no reports were available in the literature for the determination of bedaquilline in tablet dosage form by the stability-indicating RP-HPLC method. Based on this information, we have developed a simple, precise, accurate, and sensitive high-performance liquid chromatography method for the estimation of bedaquilline following ICH guidelines. The current study focuses on the degradation behavior of bulk drugs upon exposure to stress conditions such as hydrolysis, oxidative, thermal, and photolytic and its validation as per ICH guidelines. [9, 10]

2.0 MATERIALS AND METHODS

2.1 Chemicals and reagents

Working standard Bedaquilline was obtained as a gift sample from Ajanta Pharma Ltd. (Aurangabad, India). The pharmaceutical tablet dosage form Sirturo containing 100 mg of Bedaquilline was procured from a local pharmacy. Methanol (HPLC grade), and Ammonium Acetate (AR Grade) were obtained from Merck specialties Pvt. Ltd. (Mumbai, India).

2.2 HPLC instrumentation

The samples were analyzed using the HPLC system (JASCO), model PU 2080 plus pump with Rheodyne sample injection port (20 µL). The study was performed using Brava TM BDS C8 PN: 5154151 columns and detection was carried out with a PDA detector (MD 2010) with Borwin chromatography software (version 1.5) and quantification at 229 nm wavelength. The mobile phase was optimized which contain methanol: ammonium acetate buffer (90: 10, v/v) at the flow rate of 1 mL min^{-1}, employed in isocratic mode. The study involved other instruments like UV-Visible Spectrophotometer (SHIMADZU UV-1780), Photo stability chamber (Neutronic), Vacuum pump (JET-VAC-J1), and hot air oven (Kumar Laboratory Oven).

2.3 Selection of analytical wavelength

A solution of 10 µg mL^{-1} was prepared from a standard stock solution (1000 µg mL^{-1}) and scanned over 200-400 nm in UV Spectrophotometer. The maximum absorbance was shown at 229 nm. Hence it was selected as the analytical wavelength. The UV spectrum is given in Figure 2.

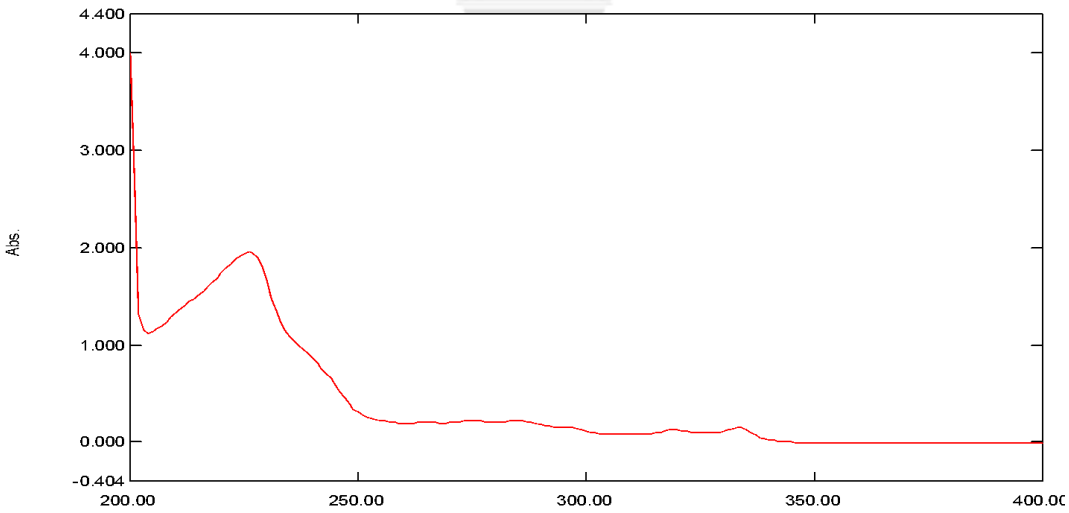

Figure No 2: UV spectrum of Bedaquilline (10 µg mL^{-1})

2.4 Stock solution and working standard preparation

An accurately 10 mg of Bedaquilline was weighed and transferred to a 10 mL volumetric flask, and the volume was made up to 10 mL with methanol to get a standard stock solution

of Bedaquilline (1000 µg mL^{-1}). From the standard stock solution, the working standard solution was prepared using the mobile phase as the final diluent.

2.5 Preparation of mobile phase

The mobile phase was prepared by mixing Ammonium acetate buffer (pH 4 adjusted with glacial acetic acid) and Methanol in the ratio of (10: 90, v/v). It was then filtered through 0.45 µm membrane filter paper using filtration assembly and then sonicated in an ultrasonic water bath for 15 min.

2.6 Preparation of sample solution

Twenty tablets were weighed accurately and powdered. A quantity of tablet powder equivalent to 10 mg of Bedaquilline was weighed and transferred to a 100 mL volumetric flask containing about 60 mL of methanol and ultrasonicated for 15 min and volume was made up to the mark with the Methanol. The solution was filtered through Whatman paper No. 41. One mL of this solution was transferred to a 10 mL calibrated volumetric flask and the volume was made up to the mark with the methanol to get a solution of concentration 10 µg mL^{-1} for Bedaquilline. After setting the chromatographic conditions, the tablet sample solution was injected, a chromatogram was obtained and the peak areas were recorded. The injections were repeated six times and the amount of each drug present per tablet was estimated from the respective calibration curve. The % assay was found to be 99.83 ± 0.76 (mean ± S.D.).

2.7 Stress degradation studies

The effect of different environmental factors on drug stability and quality must be checked. Thus, the bulk drug was subjected to various stress conditions for varying periods, using various strengths of reagents. Conditions were optimized to achieve degradation within limits specified by ICH guidelines. Bedaquilline standard was exposed to hydrolytic, oxidative, photolytic, and thermal stress conditions. All studies were done at 25 µg mL^{-1} concentration. The hydrolytic studies were carried out by treatment of a stock solution of a drug separately with 0.1 N HCl and 0.1 N NaOH at room temperature for 1 h. The stressed samples of acid and alkali were neutralized with NaOH and HCl, respectively to furnish the final concentration of 25 µg mL^{-1}. The oxidative degradation was carried out in 3 % H_2O_2 at room temperature for 1 h and the sample was diluted to obtain a 25 µg mL^{-1} solution. Thermal

stress degradation was performed by keeping the drug in the oven at 100ºC for a period of 1 h. Photolytic degradation studies were carried out by exposure of the drug to UV light.

3.0 RESULTS AND DISCUSSION

3.1 Method optimization

Analytical method development for estimation of Bedaquilline was started with preliminary trials using HPLC grade methanol, acetonitrile, and water mixed in different proportions as a mobile phase. The development was initiated through the use of the mobile phase which contained methanol: water, and acetonitrile: water in diverse ratios like 50: 50, 40: 60, and 60: 40. Finally, a solvent mixture composing methanol: ammonium acetate buffer (90: 10, v/v) was selected as an optimum mobile phase. The mobile phase utilized offered excellent resolution along with a sharp and well-resolved peak without any tailing. Short retention time with improved baseline stability as well as a low noise level was achieved. The retention time was found to be 3.00 ± 0.06 by the use of the proposed method. The optimized chromatographic conditions are summarized in Table 1. The representative chromatogram of the standard solution is shown in Figure 3.

Table No 1: Summary of optimized chromatographic conditions

Parameters	**Conditions used**
Stationary Phase	Brava TM BDS C8 PN: 5154151 (150 mm, 3 µ)
Mobile Phase	Methanol: Ammonium acetate buffer (90: 10, v/v)
Flow Rate	1 mL min^{-1}
Run Time	7 min
RT (min)	3.00 ± 0.06
Asymmetry	1.44
Plates (N)	>3609

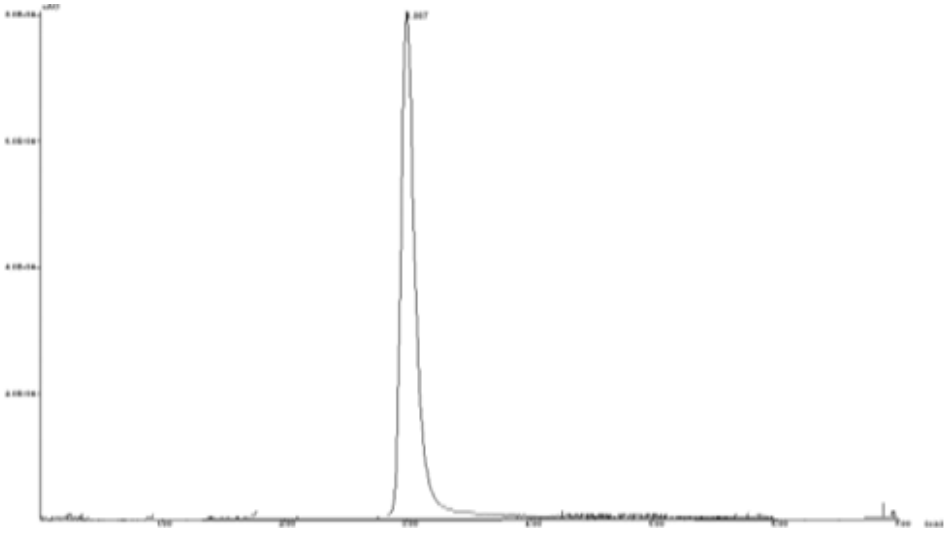

Figure no 3: Chromatogram of standard solution (25 µg mL^{-1}, RT = 3.00 ± 0.06)

3.2 Result of stress degradation studies

The stress degradation studies demonstrated that Bedaquilline was susceptible to hydrolytic, oxidative, and thermal stress conditions and found stable under photolytic stress conditions. The product was found to degrade significantly in oxidative, acid, and base hydrolysis degradation conditions. The degradation of the drug was observed without the appearance of a degradation product. The findings of stress degradation studies along with % degradation and % recovery are summarized in Table 2. The chromatograms of acid, alkali, and oxidative degradation are shown in 4, 5, and 6.

Table No 2: Summary of stress degradation studies

Stress conditions/ duration	% Recovered	% Degradation
Acid / 0.1 N HCl/ Kept at RT for 1 h	79.50	20.49
Alkali /0.1 N NaOH/ Kept at RT for 1 h	82.94	18.05
Oxidative /3 % H_2O_2 / Kept at RT for 1 h	84.06	15.93
Dry heat/ 100ºC/ 1 h	83.47	16.52
Photolysis: UV light 200-watt h square $meter^{-1}$	99.68	stable

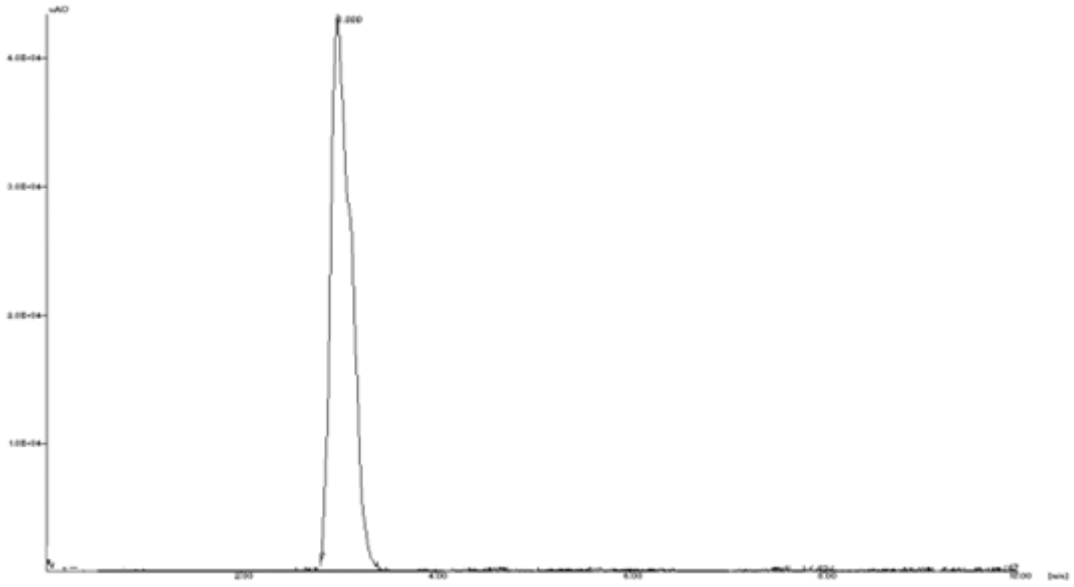

Figure no 4: Chromatogram after treatment with 0.1 N HCl kept at RT for 1 h

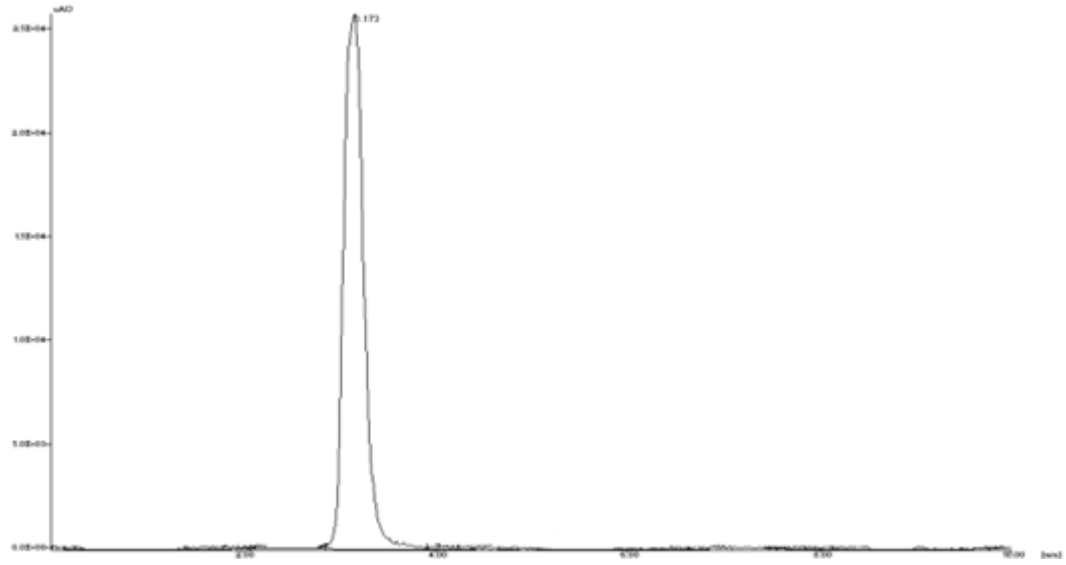

Figure no 5: Chromatogram after treatment with 0.1 N NaOH kept at RT for 1 h

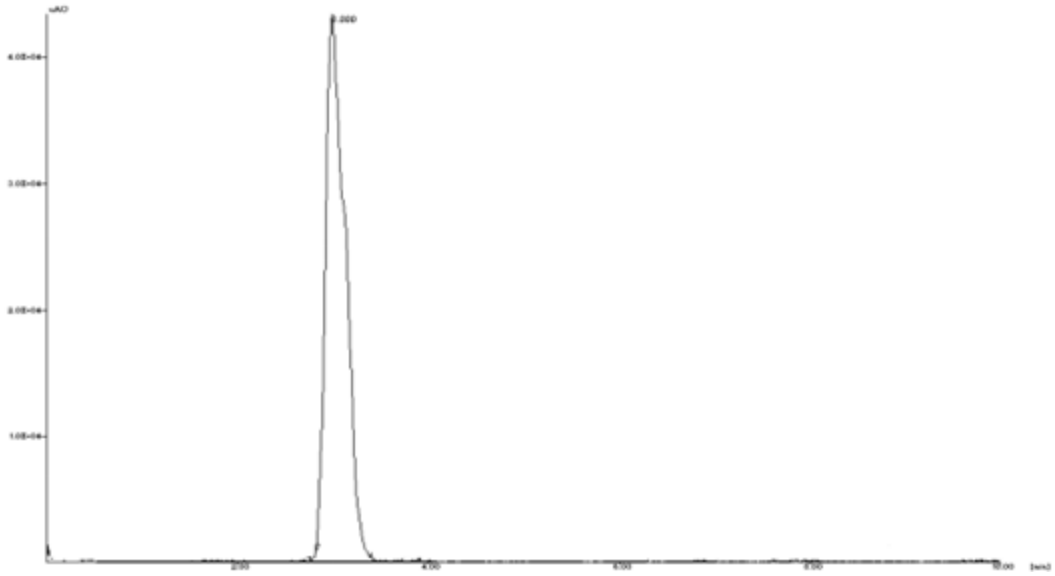

Figure no 6: Chromatogram after treatment with 3 % H_2O_2 kept at RT for 1 h

3.3 Analytical method validation

The method was validated for linearity, accuracy, and intra-day and inter-day precision, specificity, and robustness, following ICH guidelines.

3.3.1 Linearity

The linearity of the method was evaluated by linear regression analysis and the linearity of the drug was found in the concentration range of 5-30 µg mL^{-1}. Calibration standards were prepared by spiking the required volume of working standard (100 µg mL^{-1}) solution into different 10 mL volumetric flasks and volume made up with mobile phase to yield concentrations of 5, 10, 15, 20, 25, and 30 µg mL^{-1}. The calibration curve and overlay spectrum of linearity in the concentration range of 5-30 µg mL^{-1} are represented in Figures 7 and 8.

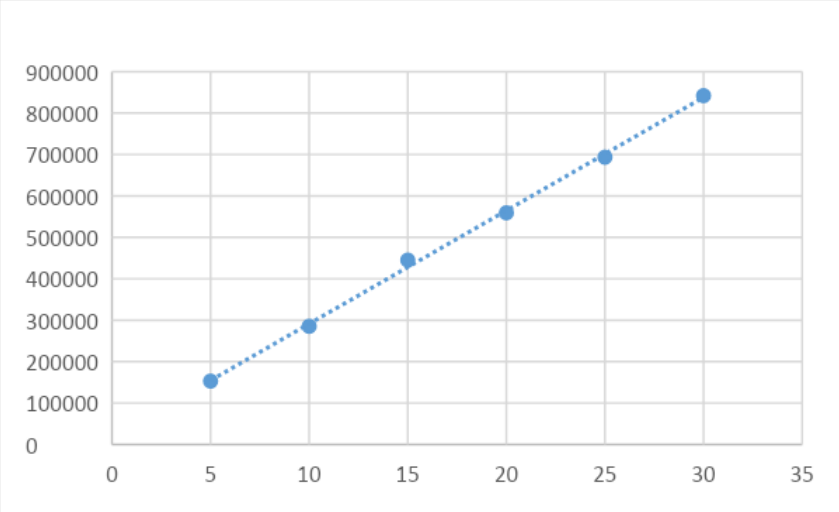

Figure no 7: Calibration curve for Bedaquilline

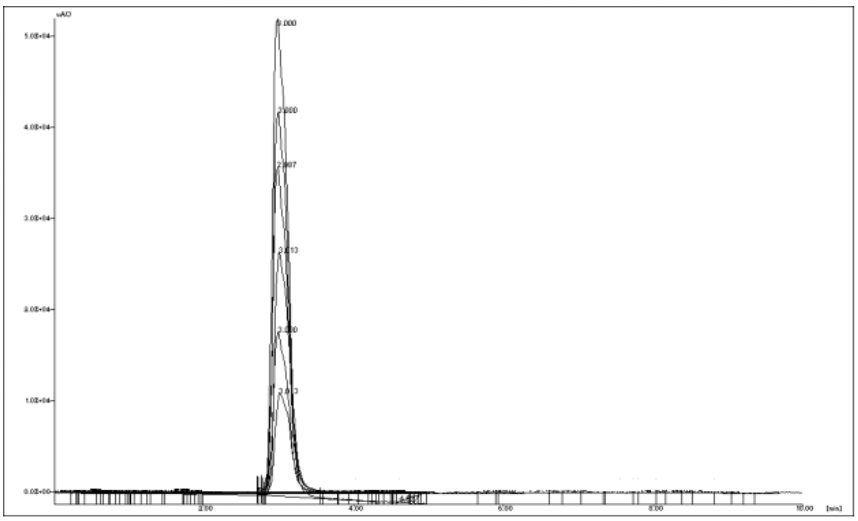

Figure no 8: Linear Overlay spectrum of linearity (5-30 µg mL^{-1})

3.3.2 Precision

Intra-day and inter-day variation studies were carried out to find out the precision of the method. In the Intra-day studies, 3 replicates of 3 different concentrations were analyzed on the same day and percentage R.S.D. was calculated. For the inter-day variation studies, 3 replicates of 3 different concentrations were analyzed on three consecutive days and percentage R.S.D. was calculated. The results obtained are summarized in Tables 3 and 4.

Table no 3: Intraday precision studies

Concentration (µg mL^{-1})	Average Area	Recovered concentration (µg mL^{-1})	% R.S.D.*
10	288955	09.91	0.62
15	428488	15.01	0.41
20	563928	19.96	0.27

*n = 3

Table no 4: Interday precision studies

Concentration (µg mL^{-1})	Average Area	Recovered concentration (µg mL^{-1})	% R.S.D.*
10	291198	09.99	1.43
15	430454	15.08	1.08
20	565086	20.01	0.82

*n = 3

3.3.3 Limit of detection (LOD) and Limit of quantitation (LOQ)

LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively; where σ is the standard deviation of the response (y-intercept) and S is the slope of the calibration plot. The LOD and LOQ values were found to be 0.17 µg mL^{-1} and 0.53 µg mL^{-1} respectively.

3.3.4 Accuracy

Method accuracy was determined by performing recovery studies by the method of standard addition. It involved the addition of standard drug solution to pre-analyzed sample solution at three different levels 80 %, 100 %, and 120 %. A sample concentration of 10 µg mL^{-1} from tablet solution was used for analysis. The results obtained demonstrated the accuracy of the method for the determination of drugs in the tablet dosage form. The results are summarized in Table 5.

Table no 5: Recovery studies

Drug	Basic sample concentration (µg mL^{-1})	Concentration added (µg mL^{-1})	Concentration found (µg mL^{-1})	% Recovery	% R.S.D.*
Bedaquilline	10	8	18.12	100.71	1.11
	10	10	19.98	99.93	1.00
	10	12	22.09	100.42	0.45

*Average of three determinations, R.S.D. is the relative standard deviation.

3.3.5 Specificity

Method specificity was confirmed by peak purity profiling studies. The peak purity values were found to be ≥993, indicating no interference of any other impurity or matrix.

3.3.6 Robustness

The deliberate variations in method parameters were done to ascertain the robustness of the developed procedure. The varied parameters were changed in mobile phase composition (± 1% methanol), flow rate (± 0.1 mL min^{-1}), and the effect on the peak areas of the drug was noted. The method was found to be robust as there were no marked changes in the chromatograms.

4.0 CONCLUSION

Stability demonstrating the RP-HPLC method for the estimation of Bedaquilline in tablet dosage form was established and validated as per the ICH guidelines. This developed RP-HPLC method was validated according to ICH guidelines in terms of linearity, precision, accuracy, and robust and stability studies. All validation parameters were found to be within the acceptable limit following ICH guidelines. The developed method is simple, sensitive, rapid, linear, precise, rugged, accurate, and robust. Consequently, the developed method can be used for assessing the stability of Bedaquilline in bulk drugs and pharmaceutical dosage forms.

5.0 ACKNOWLEDGEMENTS

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