ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF BEDAQUILINE (BDQ) USING RP-HPLC

Abstract

The United States Food and Drug Administration (USFDA) approved bedaquiline (BDQ) in 2012 for the treatment of drug-resistant tuberculosis, which has become a serious global issue. For the measurement of bedaquiline in bulk medication, a reversed-phase high performance liquid chromatography (RP-HPLC) approach was devised and validated. The separation was obtained using a 10mM Ammonium acetate: methanol in the ratio 15:85 v/v (pH adjusted to 4.5 with OPA) as mobile phase at a flow rate of 1.0 ml/min on a Thermo C18 analytical column (250mm4.6 mm i.d.,5.0µ). A UV detector with a ~~232mm~~ 232nm focal length was used for detection. The entire chromatographic analysis time per sample was around 5.0 minutes, with emtricitabine eluting at roughly 3.225minutes retention time. The accuracy, precision, specificity, linearity, and sensitivity of the approach were all tested. Validation studies have shown that this HPLC approach is simple, specific, quick, dependable, and repeatable. The standard curve was linear over the concentration range of 5-25µg/ml with r^2 close to one (0.999). Bedaquiline had a limit of detection (LOD) of 0.3525µg/ml and a limit of quantitation (LOQ) of 0.95 25µg/ml, respectively. The suggested method's high recovery and low relative standard deviation show its usefulness for determining bedaquiline in pharmaceutical formulations.

Keywords: Bedaquiline, Analytical method development, ~~Reversed~~ Reversed-phase HPLC method, ICH guidelines, Method validation.

Introduction

Mycobacterium tuberculosis (TB) is a well-known bacterium, also known as a superbug, that is the cause of tuberculosis [1]. TB is a disease that affects not just the lungs but also other organs of the body. One-third of the world's population is thought to be infected with tuberculosis, with an annual prevalence of 1% of the entire population [2]. In 2016, 1.3 million deaths were reported out of a total of 10 million active tuberculosis cases. Over 95% of deaths occurred in developing nations, with 50% occurring in South Asian countries such as India, China, Pakistan, Indonesia, and the Philippines [3, 4]. The USFDA initially approved bedaquiline (BDQ), a quinolone derivative, as a novel chemical for the treatment of M. tuberculosis in 2012. By 2015, all developing nations had embraced BDQ, and it is already being used to treat multidrug-resistant tuberculosis (MDRTB) alone or in

combination with other antibiotics [5-7]. Chemically it is [(1R, 2S)-1-(6-~~bromo~~Bromo-2 methoxy-3-quinolinyl)-4-(dimethylamino)-2-(1-naphthalenyl)-1-phenyl-2-butanol (Fig. 1). BDQ kills the superbug by blocking the ATP synthase enzyme, which is essential for energy generation. The cytochrome P450 enzyme CYP3A4 is responsible for BDQ metabolism, which produces M2 as the main metabolite with 6 times reduced activity [8]. BDQ pills are now available in 100 mg strength, which must be taken four times per day for the first two weeks of treatment, followed by 200 mg three times per week with a minimum 48-hour break between doses for the remainder of treatment. The medicine should be prescribed under tight supervision, with the specific advice to save BDQ for situations where an effective TB regimen is not available [9, 10]. The Biopharmaceutical Classification System (BCS) classifies BDQ as having poor water solubility. When taken orally, its bioavailability/dissolution rate is hampered (restricted) [11]. According to the FDA, the official dissolution media for BDQ is 0.01 N HCl, while methanol is usually employed as a solvent to generate the stock solution during analytical technique development. As a result, BDQ must remain stable in official dissolving media and methanol for the duration of the investigation. Spectrophotometric method [12], forced degradation research [13], simultaneous estimates [14, 15], bio-analytical HPLC/MS methods [16, 17], chiral analysis [18, 19], pharmacokinetic study [20, 21], and simultaneous bio-analytical analysis [22] have all been documented in the literature. This work covers the invention and validation of a ~~reversed~~ reversed-phase HPLC method for estimating BDQ in bulk medicines that is reliable, simple, resilient, and saves time and money. According to ICH criteria [23], the developed approach was validated.

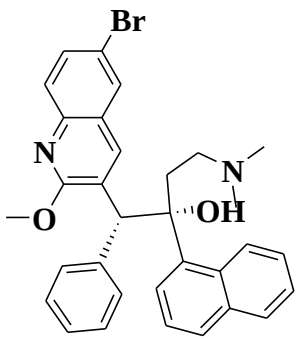


Figure 1 Chemical structure of Bedaquiline

Materials and Methods

Instrumentation

Waters model 784 liquid chromatographic system with a manual injector, Waters 515 binary pump for constant flow and constant pressure delivery, and UV-Visible detector connected to

Data Ace software for managing the instruments and processing the obtained data. The weighing was done using a Citizen Scale (I) Pvt. Ltd. Digital Micro Balance (CX-265).

Reagents and chemicals

Dishman Pharmaceuticals and API, Ahmedabad, Gujarat, India, generously donated an analytically pure sample of BDQ, together with their analytical results. Rankem, RFCL Limited, New Delhi, India provided the methanol and acetonitrile. Central Drug House (P) Limited, New Delhi, India, provided ammonium acetate AR, sodium dihydrogen phosphate AR, and ortho-phosphoric acid AR grade. Advanced Micro Devices provided the 0.45m pump nylon filter (Ambala Cantt, India). All of the other chemicals were of analytical quality. The entire experiment was conducted with triple distilled water that was produced in-house.

Chromatographic conditions

The isocratic mobile phase was 10mM Ammonium acetate: methanol (15:85v/v) (pH adjusted to 4.5 with OPA), flowing at a constant flow rate of 1.0 ml/min through the column. Before use, the mobile phase was degassed and filtered using nylon 0.22µm membrane filters (30 min). The stationary phase was a Thermo (C-18) column (5 m, 250mm x 4.60mm). The detection wavelength for the UV-Visible detector was chosen based on the chromatographic parameter, sensitivity, and selectivity of the technique for pharmaceuticals.

Selection of Mobile Phase

Initially, different ratios of mobile phases were used to determine bedaquiline in bulk medication. The mobile phase found to be best suited for analysis was 10mM Ammonium acetate buffer: methanol in the ratio of 15:85v/v, taking into account system suitability parameters such as RT, Tailing factor, No. of theoretical plates, and HETP (pH adjusted with 4.5 with OPA). To remove particulate debris, the mobile phase was filtered through 0.45µmm filter paper and then degassed using sonication. For the analysis, a flow rate of 1.0 ml/min was used.

Selection of Diluent

The diluent used to prepare the sample was compatible with the mobile phase and had no effect on the analyte's retention or resolution. 6.8 pH Phosphate Buffer was employed as diluents after several trials.

Preparation of Stock Solution

Accurately weighed 10 mg API of BDQ was transferred into 10 ml volumetric flask separately and added 5ml of phosphate buffer pH 6.8 as diluents, sonicated for 20 minutes

and volume was made up to 10ml with phosphate buffer pH 6.8 to get the concentration of solution 1000µg/ml (Stock-A).

Preparation of Sub Stock Solution

5 ml of solution was taken from stock-A of both the drug and transferred into 50ml volumetric flask separately and diluted up to 50 ml with diluent (phosphate buffer pH 6.8) to give the concentration of 100µg/ml of BDQ respectively (Stock-B).

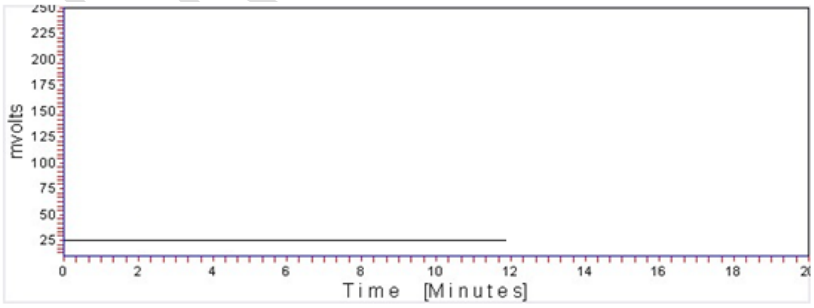
Preparation of Different Solution

0.5ml, 1.0ml, 1.5ml, 2.0ml, and 2.5ml of stock-B were taken separately in a 10 ml volumetric flask, and volume was made up to 10ml with (phosphate buffer pH 6.8). This gives the solutions of 5µg/ml, 10µg/ml, 15µg/ml, 20µg/ml and 25µg/ml, for BDQ.

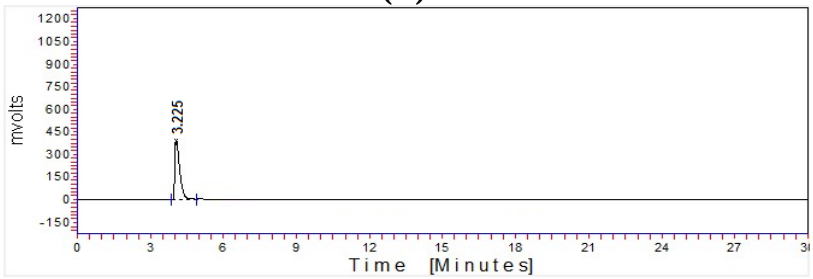
Results and discussion

Chromatography

After multiple trials with various proportions and pH values of methanol, isopropyl alcohol, acetonitrile, water, and buffer solutions, the mobile phase was chosen. To achieve maximum separation and sensitivity, a mobile phase consisting of 10mM Ammonium acetate buffer: methanol in the ratio of 15:85v/v (pH adjusted to 4.5 with OPA) was chosen. Flow rates of 0.5 to 1.5 minutes were investigated. With a flow rate of 1 ml/min, the signal-to-noise ratio was excellent and the separation time was reasonable. Using a reversed-phase C_{18} column, the retention times for BDQ ~~was~~ were observed to be 3.225 ± 0.002min. The total time of analysis was less than 5 min. The maximum absorption of BDQ was detected at 232nm and this wavelength was chosen for the analysis Figure 2.



(A)



(B)

Figure 2 Chromatograms of (A) Blank mobile phase (B) bedaquiline (15µg/ml) as reference substances

System suitability

The number of theoretical plates, HETP, and peak tailing are all determined as system suitability characteristics. Table 1 displays the results collected. BDQ had a theoretical plate count of 2667.333.

Table 1 Results of system suitability parameters

Parameters	Bedaquiline
AUC*	526.593
No. of Theoretical Plates	2667.333
Tailing Factor*	1.278
Retention time*	3.225±0.002
Calibration range (µg/ml)	5-25

*Each value is the mean ± SD of six determinations

Linearity

The calibration curve was linear over the concentration range of 5-25µg/ml for BDQ. The linearity was represented by a linear regression equation as follows:

Y (BDQ) = 50.02conc+11.52 (r^2 = 0.999)

Accuracy

To calculate the accuracy of the devised approach to pre ~~analysed~~ analyzed sample solution, recovery studies were conducted. A specific concentration of standard medication (80 percent, 100 percent, and 120 percent) was added, and the recovery was measured. At all three levels, the percentage RSD was found to be less than 2, indicating good recovery at 80, 100, and 120 percent, respectively. Table 2 shows how each level was created in three different ways.

Table 2 Results of recovery study

% Level	% Mean±SD*
	Bedaquiline
80%	99.31±0.352
100%	98.96±0.383
120%	99.19±0.339

***** Value of three replicate and three concentrations.

Precision

Repeatability

The repeatability of five dilutions in three repetitions was tested on the same day, and the results were found to be within acceptable limits (RSD 2), as shown in Table 3.

Intermediate precision

On two distinct days and by two analysts, five dilutions in three replicates were tested for day-to-day and analyst-to-analyst variability, and the results were found to be within acceptable limits (RSD 2), as shown in Table 3.

Robustness

Small but controlled fluctuations in the concentration of the mobile phase were made ~~in accordance with~~ under ICH guidelines to test the method's ability to stay unaffected. The ratio of mobile phase was changed from, 10mM Ammonium acetate: methanol (15:85% v/v) to (20:80 % v/v), and the method is found robust as RSD is again found < 2.0 table 3.

Table 3 Statistical data for precision and robustness

Statistical parameter	Bedaquiline		
	Mean*	S.D*	R.S.D*
Repeatability	99.229	0.081	0.082
Intermediate Precision (I) (A day to day)	99.419	0.081	0.081
(II) Analyst to Analyst	99.065	0.047	0.047
Robustness	98.757	0.119	0.120

*Mean of 15 determinations (three replicates at five concentration levels)

Detection Limit and Quantitation Limit

The LOD and LOQ of the developed method were calculated based on the standard deviation of response and slope of the linearity curve Table 4.

Table 4 LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Bedaquiline	0.35	0.95

Conclusion

The proposed HPLC method was validated according to the ICH Q2B Guidelines and found to be suitable for routine quantitative analysis of BDQ by HPLC in a pharmaceutical dosage form. The linearity, precision, accuracy, and specificity values were all found to be within acceptable levels. The approach allows for selective BDQ quantification. The proposed method was extremely repeatable, dependable, fast, robust, and precise. As a result, with a high percentage of recovery and run ~~duration of less~~fewer than five minutes, it can be used for regular BDQ determination in pharmaceutical dosage forms.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in our area of research and country. There is ~~absolutely~~ no conflict of interest between the authors and producers of

the products because we do not intend to use these products as an avenue for any litigation but ~~for~~ the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

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